

MODULATION OF LONG-TERM POTENTIATION IN THE HIPPOCAMPUS

By

ZHENG XIE

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Department of Pharmacology & Therapeutics.

The University of British Columbia
Vancouver, Canada

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ABSTRACT

Brief trains of high-frequency stimulation of monosynaptic excitatory pathways in the hippocampus can cause a long-term synaptic potentiation (LTP) that can last for hours *in vitro* or weeks *in vivo*. This activity-mediated LTP is thought to be involved in certain forms of learning and memory.

Although mechanisms underlying the induction and maintenance of LTP process have been extensively investigated, factors that modulate LTP remain unresolved. Suppression of γ -aminobutyric acid (GABA) -ergic inhibition by GABA antagonists can facilitate LTP of the excitatory postsynaptic potential (EPSP) in the hippocampus. It is, however, unclear whether long-term changes in GABAergic inhibition occur after a tetanic stimulation, and if so, how such a change affects LTP of the EPSP. In this project, experiments were conducted to determine if the CA1 neuronal inhibitory postsynaptic potentials (IPSPs) were affected following a high frequency stimulation. Somatostatin co-exists with GABA in some interneurons in the CA1 area. Since the peptide might be co-released with GABA, experiments were carried out to determine if somatostatin modifies GABAergic IPSPs. Whether the peptide could modulate the induction of LTP of the EPSP was also tested. Previous studies from this laboratory showed that substances collected from the hippocampus or the neocortex during a tetanic stimulation could induce LTP if applied on hippocampal slices. It is generally believed that the induction of LTP requires the activation of postsynaptic NMDA receptors and that the maintenance of LTP is at least in part due to a presynaptic mechanism. It is possible that a release of substances from the postsynaptic cells or the nearby glia during a tetanic stimulation of the hippocampal afferents could result in a retrograde interaction of these substances with the presynaptic terminals leading to changes that sustain LTP.

In the present study, these substances were further characterized, and the mechanisms of their release as well as LTP-inducing action elucidated. Reports in the literature suggest that a deficiency in α -tocopherol (vitamin E) leads to enhancement in lipid peroxide content in the hippocampus as well as impairment of spatial learning. Oxygen free radicals have also been shown to accelerate the decay of established LTP. Studies were, therefore, initiated to examine if the vitamin causes LTP and if its deficiency leads to impairment of LTP-induction. EPSPs and IPSPs were recorded from CA1 neurons in guinea pig hippocampal slices in response to stratum radiatum stimulation.

In control CA1 neurons, tetanic stimulation of the stratum radiatum caused LTP of the EPSP and the fast IPSP without changing the slow IPSP. If BAPTA (a Ca^{2+} chelator) or K-252b (a PKC inhibitor) was injected into the CA1 neurons, LTP of the EPSP did not occur. However, in these drug-injected neurons LTP of the fast IPSP was enhanced, and LTP of the slow IPSP occurred after tetanus. With the potentiation of the IPSPs in the drug-injected neurons, the shape of the EPSP was distorted in most neurons. These findings indicate that LTP occurs not only at excitatory synapses but also at inhibitory synapses. The tetanus-induced increases in intracellular free Ca^{2+} and PKC activity potentiate the EPSP, while decreasing LTP of the IPSPs so that the distortion of the EPSP by the IPSPs is minimized for a better expression of LTP of the EPSP.

Somatostatin hyperpolarized the CA1 neurons and decreased the input resistance. The peptide depressed both the fast IPSP and the slow IPSP, without changing the EPSP. These actions of somatostatin were not due to interactions of the peptide with GABA_A or GABA_B receptors. However, the activation of GABA_B receptors by baclofen, reduced the somatostatin-induced hyperpolarization of the CA1 neurons. The suppression of the GABA_A receptor-mediated IPSP by somatostatin appears to be through a postsynaptic action of

the agent. It is concluded that interactions between somatostatin and GABAergic responses may be related to the peptide- and GABA-receptors being coupled to the same channels or to the sharing of the same second messenger systems for effects. By modulating the IPSPs, somatostatin could logically interfere with the induction of LTP of the EPSP. However, application of somatostatin failed to facilitate or block LTP.

Samples were collected from the rabbit neocortex during a tetanic (50 Hz, 5 s) stimulation (tetanized neocortical sample, TNS). Application of TNS caused LTP of the EPSP and population spike without changing the membrane potential or the input resistance of the CA1 neurons. The TNS-induced LTP required activation of afferents. TNS also induced a short-term potentiation (STP) of the fast IPSP without changing the slow IPSP. Since the TNS- and tetanus-induced LTPs occluded each other, these two LTPs may share some common mechanisms. Different fractions of TNS (<3 kDa, 3-10 kDa and >50 kDa) were able to cause LTP. APV did not block the TNS-induced LTP. However, PKC inhibitors such as sphingosine and K-252b blocked the TNS-induced LTP. TNS failed to induce LTP in Ca^{2+} -chelated CA1 neurons. If TNS was collected from the rabbits pretreated with MK-801 (i.p.), a non-competitive NMDA antagonist, this TNS failed to induce LTP. Gel electrophoresis of the substances in TNS revealed the presence of an acidic protein with a molecular weight of about 69 kDa. It, therefore, appears that NMDA receptor activation is required for the release but not for the LTP-inducing action of substances in TNS.

α -Tocopherol phosphate (referred to as α -tocopherol) induced a slowly developing LTP of the EPSP without changing the fast and slow IPSPs, and the electrical properties of the CA1 neurons. The agent failed to induce a further potentiation of the EPSP during a pre-established tetanus-induced LTP. The α -tocopherol-induced LTP was decreased by AP3 (an ACPD antagonist) but not by

APV (a NMDA antagonist). Furthermore, chelation of postsynaptic free Ca^{2+} with BAPTA or inhibition of PKC by sphingosine and K-252b prevented the α -tocopherol-induced LTP. Sodium ascorbate (a water soluble antioxidant) failed to induce LTP. DMSO (a lipid soluble antioxidant) was able to potentiate the EPSP as long as the application of the agent continued, but the EPSP quickly returned to the pre-application level once the application of the agent was stopped. In hippocampal slices obtained from vitamin E deficient rats, both tetanic stimulation and α -tocopherol failed to induce LTP of the EPSP in the CA1 neurons. It is possible that the structure and the function of the membrane or certain receptors on neurons are affected by the lack of α -tocopherol in the vitamin deficient rats and, therefore, an acute application of α -tocopherol may not be able to correct the changes induced by the long-term vitamin E deficiency.

In conclusion, various mechanisms that modulate LTP of the EPSP were examined in guinea pig hippocampal slices. It appears that the elevation of postsynaptic $[\text{Ca}^{2+}]$ and the activation of PKC are needed not only to cause LTP of the EPSP but also to diminish LTP of the IPSPs so that the expression of LTP of the EPSP is not distorted. Somatostatin suppresses GABAergic IPSPs through mechanisms other than to interfere with the amino acid receptors and the peptide appears not to affect LTP of the EPSP. Tetanic stimulation of the neocortical surface causes a release of LTP-inducing substances whose release but not action, depends on NMDA receptor activation. While vitamin E appears to induce LTP, animals with the vitamin deficiency appear to have a diminished ability to induce LTP.

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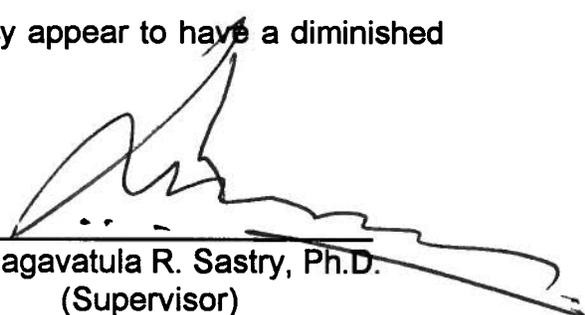

Bhagavatula R. Sastry, Ph.D.
(Supervisor)

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LIST OF ABBREVIATIONS

ABBREVIATION	WORD
ACPD	(±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid
AMPA	(RS)- α -amino-3-hydroxy-5-methyl-4-soxazolepropionic acid
AP3	L(+)-2-amino-3-phosphonopropionic acid
APV	DL-2-amino-phosphonovalerate
AHP	Afterhyperpolarization
ATP	Adenosine triphosphate
BAPTA	1,2-bis(2-aminophenoxy)ethane- <i>N',N',N',N'</i> -tetraacetic acid
cAMP	Adenosine 3':5"-cyclic phosphate
CA	Cornu ammonis
CaMKII	Ca ²⁺ /calmodulin-dependent protein kinase II
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione
DG	Dentate gyrus
EPSP	Excitatory postsynaptic potential
GABA	γ -aminobutyric acid
GAD	Glutamic acid decarboxylase
H-7	1-(5-isoquinolylsulfonyl)-2-methyl-piperazine
IPSP	Inhibitory postsynaptic potential
K-252b	9-carboxylic acid derivative of [8R*,9S*,11S*]-[-]-9-hydroxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1H,8H,11H-2,7b,11a-triazadibenzo [a,g] cycloocta [cde] trinden-1-one
LTP	Long-term potentiation
MK-801	[+]-5-methyl-10,11-dihydro-5H-dibenzo (a,b) cyclohepten-5,10-imine maleate
NMDA	N-methyl-D-aspartate
PC-12 cells	Rat adrenal pheochromocytoma cells
PKA	Protein kinase A
PKC	Protein kinase C
PP	Perforant pathway
QX-314	<i>N</i> -(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide
Sch	Schaffer collaterals
SS	Somatostatin
STP	Short-term potentiation
TTX	Tetrodotoxin
TNS	Tetanized neocortical sample
UNS	Untetanized neocortical sample
TUNS	UNS collected after a previous tetanic stimulation

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DEDICATION

Dedicated to my mother and father.

1. INTRODUCTION

Brief trains of high-frequency stimulation of monosynaptic excitatory pathways in the hippocampus can cause an abrupt and sustained increase in synaptic efficiency that can last for hours *in vitro* or weeks *in vivo*. This activity-dependent enhancement of synaptic transmission is referred to as long-term potentiation (LTP) (Bliss and Lomo, 1973). This LTP is characterized as an input specific long-term enhancement in the excitatory postsynaptic potentials (EPSP) of a single neuron or in the field potentials of a population of neurons. LTP is thought to be a strong candidate for the cellular mechanisms underlying learning and memory because it is consistent with the "Hebbian rule" that a synaptic modification for learning and memory occurs as a consequence of coincidence between pre- and post-synaptic activity (Hebb, 1949). There is growing evidence that LTP is correlated with some forms of learning and memory (Silva et al., 1992a,b; Grant et al., 1992; Bolhuis and Reid, 1992; Davis et al., 1992; Watanabe et al., 1992). In the last decade, efforts have been focused on elucidating the mechanisms underlying the induction and maintenance of LTP. It is generally believed that the induction of LTP is primarily mediated through postsynaptic mechanisms (McNaughton, 1982; Malenka et al., 1988). In the CA1 regions of the hippocampus the induction of the LTP appears to involve the activation of the *N*-methyl-D-aspartate (NMDA) receptors (Collingridge et al., 1983). Whether the maintenance of LTP is due to presynaptic (increase in neurotransmitter release) and/or postsynaptic (change in receptor/channel complex or increase in receptor number) mechanisms is debatable (Bliss and Collingridge, 1993; Otani and Ben-Ari, 1993). The LTP process, including the induction and maintenance, is known to be influenced by a variety of factors such as neurotransmitters, neuromodulators and

neurohormones (Otani and Ben-Ari, 1993). The major objective of the studies reported in this thesis was to examine factors which are involved in the modulation of the LTP process. A knowledge of how these factors modulate the LTP process may lead to a better understanding of the mechanisms of LTP.

1.1. LTP and endogenous substances

The current view on LTP in hippocampal CA1 region is that tetanic stimulation activates postsynaptic NMDA receptors which allow Ca^{2+} influx through NMDA channels, (Collingridge et al., 1983). The rise of intracellular free Ca^{2+} concentration triggers the activation of a number of protein kinases which probably leads to postsynaptic and/or presynaptic modifications and subsequently the induction of LTP (Otani and Ben-Ari, 1993). If presynaptic mechanisms such as increases in neurotransmitter release are responsible for the maintenance of LTP, interactions between the postsynaptic cell and the presynaptic terminals must occur. In addition to tetanic stimulation of afferents, direct depolarization of the CA1 neurons with intracellular current injection, when paired with stimulation of afferents, can also induce LTP of the EPSP (Sastry et al., 1986; Gustafsson and Wigstrom, 1986; Kelso et al., 1986). Depolarization of the postsynaptic neurons not only facilitates the opening of NMDA channels by removing Mg^{2+} block (Mayer et al., 1984, Gustafsson et al., 1987), but also causes release of some substances, such as proteins into extracellular fluid (Duffy et al., 1981; Nystrom et al., 1986; Sastry et al., 1988a, Chirwa and Sastry, 1986). It has been speculated that these released substances can interact with the presynaptic terminals leading to the induction of LTP. Therefore, retrograde messengers have been proposed to describe the substances which are released from the postsynaptic cells during tetanic stimulation, or injection of depolarizing current pulse and act on the presynaptic terminals. (Sastry et al., 1986; Bliss et al., 1986). Retrograde messengers presumably play a key role in mediating the

interactions between the postsynaptic cell and the presynaptic terminals during the development of LTP. Direct evidence for existence of retrograde messengers is lacking at the present time. Ideally, retrograde messengers should be released from postsynaptic cells and act quickly on the presynaptic terminals leading to the induction of LTP. Arachidonic acid and nitric oxide have been proposed to serve as retrograde messengers for LTP (Williams et al., 1989; Bohme et al., 1991; Schuman and Madison, 1991). Both agents are highly diffusible and are able to induce LTP (Williams et al., 1989; Bohme et al., 1991; Zhuo et al., 1993). However, unlike tetanic stimulation which induces LTP very rapidly, arachidonic acid (30 min application) induces a slowly developing LTP (Williams et al., 1989). Nitric oxide can only induce LTP in hippocampal CA1 neurons when paired with weak tetanus of afferents (Zhuo et al., 1993). Furthermore, nitric oxide synthesis has not yet been found in the CA1 neurons (Bredt et al., 1991). It appears that the notion that arachidonic acid and nitric oxide act as retrograde messengers for LTP is questionable. Previous studies in our laboratory first demonstrated that endogenous substances collected from hippocampus (Chirwa and Sastry, 1986) and neocortex (Sastry et al., 1988a) during tetanic stimulation *in vivo*, when applied to the hippocampal slices, induce LTP in these slices. Like tetanic stimulation, the endogenous substances can induce a rapidly developing LTP in the CA1 neurons of the hippocampus (Sastry et al., 1988a). These substances can also act as trophic factors to enhance neurite growth in cultured PC-12 cells (Sastry et al., 1988a). If these endogenous substances are released from the postsynaptic cells during tetanic stimulation, they may serve as retrograde messengers in LTP. In the present study, experiments were conducted to determine the possible mechanisms underlying the release and LTP-inducing action of the endogenous substances

collected from rabbit neocortex during tetanic stimulation in guinea pig hippocampal slices.

1.2. LTP and α -tocopherol

Although LTP has been widely studied for the last two decades, the physiological significance of LTP is unclear. LTP is thought to underlie the cellular mechanisms for learning and memory although clear evidence for this is lacking at present. Investigations on effects of factors which are involved in the memory process and diseases associated with loss of memory such as Alzheimer's disease on LTP will help us to better understand the physiological significance of LTP. α -Tocopherol (vitamin E) is a major antioxidant in the biological system (Tappel, 1962). α -Tocopherol, which can scavenge free radicals attacking from outside of the membrane and within the membrane, is essential for the maintenance of normal structure and function of the human nervous system (Tappel, 1962; Sokol, 1989). The vitamin has been suggested to be involved in spatial learning and memory in animals (Moriyama et al., 1990). In vitamin E deficient rats, impairment of spatial learning ability is associated with an increase in lipid peroxide contents of the hippocampus (Moriyama et al., 1990). Intracerebral administration of liposomes containing α -tocopherol has been demonstrated to facilitate the recovery of learning ability in rats with brain injury (Stein et al., 1991). In cultured central neurons, α -tocopherol has been shown to act as a growth-inducing factor which supports the survival of the neurons and enhances neurite growth (Nakajima et al., 1991; Sato et al., 1993). Changes in α -tocopherol and free radical levels have been found in patients with Alzheimer's disease (Jeandel et al., 1989). It thus appears that α -tocopherol and free radicals have a role in certain types of learning and memory. Since LTP is thought to underlie the cellular mechanisms for learning and memory, it is logical to speculate that α -tocopherol and free radicals are probably involved in

LTP. Recently, free radicals have been found to facilitate the decay of LTP (Pellmar et al., 1991). Therefore, it is of interest to determine whether α -tocopherol plays a role in LTP. In the present studies, experiments were conducted to examine the role of α -tocopherol in LTP using hippocampal slices obtained from both control animals and vitamin E deficient animals.

1.3. LTP and GABAergic inhibition

GABAergic inhibition is known to play an important role in LTP. Blockade of GABA_A receptors facilitates the induction of LTP because suppression of the GABA_A receptor-mediated fast inhibitory postsynaptic potentials (IPSP) allows the NMDA channels to open more readily (Wigstrom and Gustafsson, 1983). The role of GABA_B receptors in LTP was not clear until recently (Davies et al., 1991; Mott and Lewis, 1991). Presynaptic GABA_B receptors have been reported to regulate the release of GABA from presynaptic terminals through a negative-feedback mechanism (Davies et al., 1990). Activation of presynaptic GABA_B receptors has been suggested to facilitate the induction of LTP by suppressing GABA release and reducing the IPSPs (Davies et al., 1991; Mott and Lewis, 1991). The GABA_B receptor-mediated slow IPSP occurs in the apical dendrites close to the site where the EPSP occurs. Phaclofen, a postsynaptic GABA_B antagonist, has been shown to facilitate the induction of LTP (Olpe and Karlsson, 1990). It is thus apparent that changes in GABAergic inhibition have modulatory effects on the induction of LTP of the EPSP. Under normal conditions, factors that alter the GABAergic inhibition should also affect the induction of LTP.

1.3.1. LTP and IPSPs

Stimulation of the stratum radiatum not only evokes EPSPs in the CA1 neurons, but also induces fast and slow IPSPs in these neurons. While long-term changes in the EPSP after tetanic stimulation have been widely studied, the

long-term changes in the IPSPs have been somewhat ignored and are less clear. Tetanic stimulation has been reported to cause an increase, a decrease, or no change in the IPSPs (Abraham et al., 1987). The inconsistent results are partly due to the IPSPs in the CA1 neurons consisting of monosynaptic and polysynaptic components. Protein kinase C (PKC) activation and Ca^{2+} influx are known to occur during the induction of LTP in the CA1 neurons (Malinow et al., 1989; Malenka et al., 1989). PKC activation and the rise of intracellular Ca^{2+} concentration have been reported to have significant effects on IPSPs in the CA1 neurons (Baraban et al., 1985; Dutar and Nicoll, 1988b; Chen et al., 1990). Since changes in IPSPs have been shown to have significant modulatory effects on LTP, the present studies were conducted to examine the long-term effects of tetanic stimulation on IPSPs and the role of the IPSPs changes in LTP.

1.3.2. LTP and somatostatin

Somatostatin (SS), a peptide that co-exists with GABA in the inhibitory terminals, has been shown to depress the GABA receptor-mediated IPSPs in the CA1 neurons (Scharfman and Schwartzkroin, 1989). The mechanisms underlying the action of SS on the IPSPs are not clear. SS has also been reported to hyperpolarize the CA1 neurons (Pittman and Siggins, 1981). Depression of the IPSPs and hyperpolarization of the CA1 neurons can modulate the induction of LTP of the EPSP as previously discussed (Section 1.3). If SS and GABA are co-released from the same presynaptic terminals during tetanic stimulation, the actions of SS on the IPSPs and the membrane potential can affect the induction of LTP of the EPSP in the CA1 neurons. Furthermore, depletion of SS in the CNS has been shown to impair animal spatial learning ability, which is thought to be related to hippocampal LTP (Haroutunian et al., 1987). Therefore, it would be of interest to determine the role of this endogenous peptide in LTP and the mechanisms of the interactions

between SS and GABA in the CA1 neurons. Experiments were conducted to examine these issues.

2. THE HIPPOCAMPAL FORMATION

A comprehensive literature review on the hippocampal formation is useful for better interpretation of the electrophysiological signals of the hippocampus. Various nomenclatures have been used to describe the subdivisions of the hippocampus. The terminology introduced by Cajal (1911), Lorente de No (1934) and Blackstad (1956) is widely used in the literature and has been consistently employed in this review. The updated information in anatomical studies has been included in the discussion. (Teyler and DiScenna, 1984; Schwerdtfeger, 1984; Swanson et al. 1987; Amaral and Witter, 1989; Lopes Da Silva et al., 1990; Bronen, 1992).

2.1. General anatomy of the hippocampal formation

The cortex is divided into two basic types; the allocortex and the neocortex, during ontogenic development (Chronister and White, 1975). The neocortex, also called the isocortex, is a homogenous unit which completely separates from the mantle layer. The allocortex is a heterogeneous unit which fails to cleave completely from the mantle layer during development (Filimonoff, 1947). The regions which lie between the allocortex and the neocortex are called periallocortex. These are the peripalaeocortical claustral region, the entorhinal region, the presubicular region, the retrosplenial region and the periarchicortical cingulate region (Lorente de No, 1934; Stephan, 1975; Chronister and White, 1975). The allocortex is separated into palaeocortex and archicortex. The palaeocortex is composed of the olfactory bulb and accessory olfactory bulb, the retrobulbar region, the periamygdalar region, the olfactory tubercle, the septum, the diagonal region and the prepiriform region (Schwerdtfeger, 1984). The archicortex consists of the subiculum, cornu

ammonis, dentate gyrus, precommissural hippocampus and supracommissural hippocampus (Chronister and White, 1975; Schwerdtfeger, 1984; Teyler and DiSenna, 1984). Note the terms "archicortex", "hippocampus" and "hippocampal formation" are synonymous (Schwerdtfeger, 1984; Teyler and DiScenna, 1984; Lopes Da Silva et al., 1990). In this review, the hippocampal formation or the hippocampus refers to the unit consisting of the cornu ammonis, the dentate gyrus, the subicular complex and the entorhinal cortex (Swanson et al., 1987; Amaral and Witter, 1989).

2.2. Topography and cytoarchitecture of the hippocampus

The hippocampus is a convoluted symmetrical structure, forming the medial margin of the cortical hemisphere and is located on the medial wall of the lateral ventricle. The appearance of the curved and intertwined simple cortical layers account for the name hippocampus, meaning seahorse (Knowles, 1992). The three-dimensional shape of the hippocampus is relatively complex. The longitudinal axis, generally referred to as the septotemporal axis, bends in a C-shaped manner from the septal nuclei rostradorsally to the incipient temporal lobe caudoventrally. The transverse axis is oriented perpendicular to the septotemporal axis (Fig. 1). A transverse section of the hippocampus reveals two C-shaped interdigitating archicortical fields: the cornu ammonis and the dentate gyrus (Fig. 2) (Teyler and DiScenna, 1984; Amaral and Witter, 1989; Witter, 1989).

2.2.1. The dentate gyrus

The dentate gyrus consists of a densely packed cell layer, with granule cells as the major cell type. The C-shaped cell layer is named the stratum granulosum. The apical dendrites of granule cells orient away from the centre of

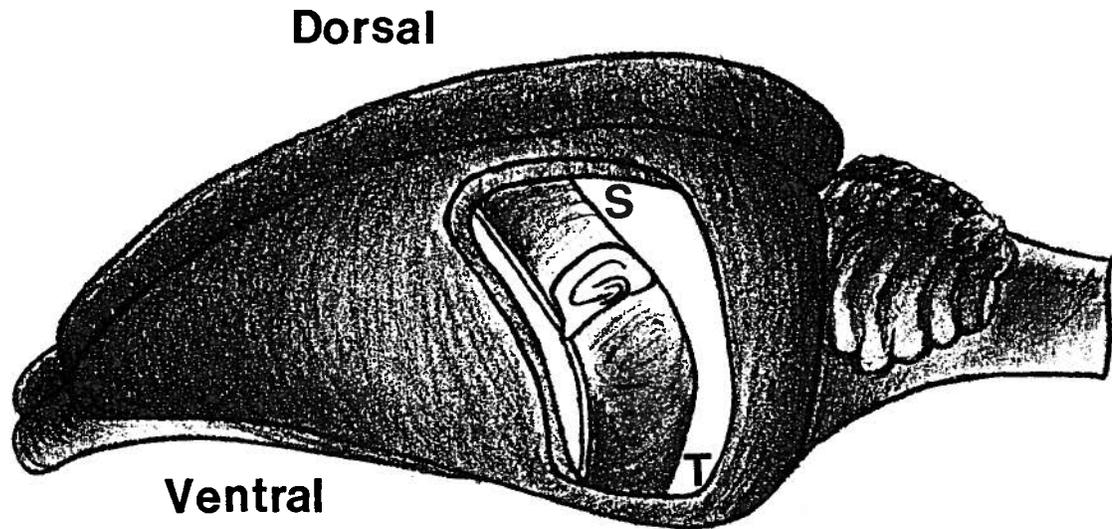


Figure 1 Overview of the hippocampal formation.

The drawing shows the position of the hippocampal formation in guinea pig brain with part of the neocortex overlying the hippocampus removed. Note the hippocampus is a C-shaped structure with its longitudinal axis running from the septal (S) nuclei rostrally to the temporal (T) lobe caudally. The transverse axis of the hippocampus is oriented perpendicular to the longitudinal axis. (Modified from Andersen et al., 1971a)

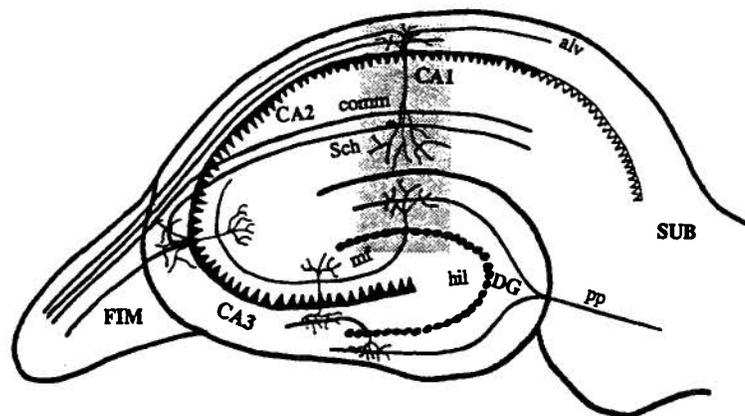
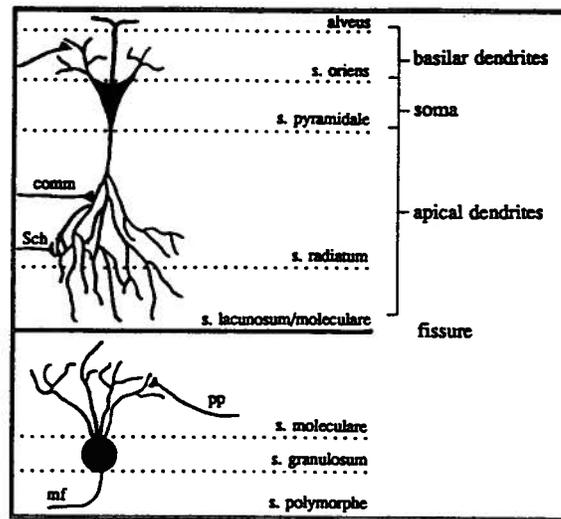


Figure 2 Schematic representation of a transverse section of the hippocampus. The major structures and afferents of the hippocampal formation are illustrated in a transverse section of the hippocampus. Note the dentate gyrus (DG) and the cornu ammonis (CA) are two major structures in the hippocampal formation. The hilus (hil) is a transition zone between the dentate gyrus and the cornu ammonis. The cornu ammonis contains a densely packed pyramidal cell layer; and the dentate gyrus consists of a densely packed granule cell layer. The perforant path (pp) is a major extrinsic input to the hippocampus innervating the granule cells. The axons of the granule cells, the mossy fibres (mf), make synapse with the pyramidal cells in CA3 field. The CA3 pyramidal cells give off the Schaffer collaterals (Sch) to innervate CA1 pyramidal cells. The commissural (comm) input originates from the contralateral hippocampus. The axons of CA1 pyramidal cells enter the alveus (alv) and then project into the subiculum (SUB) and the fimbria (FIM). The shadow in the transverse section is enlarged in the inset to show the arrangement of the layers of the hippocampus.

the "C" and project into the stratum moleculare layer that lies directly adjacent to the hippocampal fissure. The axons of granule cells, termed mossy fibres, project toward the centre of the "C" (Cajal, 1911; Lorente de No, 1934). Because of the curvature of the dentate regions around part of the cornu ammonis, the dentate granular layer is divided into a suprapyramidal layer (upper blade) and an infrapyramidal layer (lower blade) (Chronister and White, 1975; Teyler and DiScenna, 1984). The field between the two blades is called hilar region, which contains several layers of polymorphic cells (Cajal, 1911; Lorente de No, 1934). Opinions are split on whether the hilar region belongs to the dentate gyrus or the cornu ammonis (Cajal, 1911; Lorente de No, 1934; Blackstad, 1956). Blackstad (1956) considered it as the third layer of the dentate gyrus. Amaral (1978) described at least 21 different cell types in the hilus of rat dentate gyrus, including basket cells and mossy cells. His studies confirmed that the cells in the area are most closely related to the dentate gyrus. The hilar region may serve as a transition zone between the dentate gyrus and the cornu ammonis.

2.2.2. The cornu ammonis field

The cornu ammonis, also called the hippocampus proper or Ammon's horn, was divided into four subfields: CA1, CA2, CA3 and CA4 by Lorente de No (1934). The CA4 field corresponding to the polymorphic zone of the dentate gyrus as described by Cajal (1911) and Blackstad (1956), has been discussed in the previous section (section 2.2.1).

The cornu ammonis, composed predominantly of pyramidal cells, forms the other C-shaped cell layer of the hippocampus (Lorente de No, 1934; Teyler and DiScenna, 1984). The pyramidal cell layer is termed the stratum pyramidale. The pyramidal cells contain both apical and basal dendrites. These dendrites, together with the axons and their collaterals of the pyramidal cells,

form another five layers in the cornu ammonis field. There are two layers on the basal dendrite side of the pyramidal cell layer. The stratum oriens lies next to the stratum pyramidale. It mainly contains the tufts of the basal dendrites of the pyramidal cells, the collaterals of the axons of CA3 pyramidal cells and cell bodies of some interneurons. The alveus is situated next to the stratum oriens and marks the outer boundary of the cornu ammonis. This layer consists of the axons of pyramidal cells and the incoming fibres. Three layers lie on the apical dendrite side of the pyramidal cell layer. The stratum radiatum is situated next to the stratum pyramidale and mainly contains several fibre systems such as the Schaffer collaterals, and some sparse cell bodies. The stratum moleculare lies directly adjacent to the hippocampal fissure. It contains predominantly fibres and dendritic terminals. The stratum lacunosum is situated between the stratum radiatum and the stratum moleculare. This layer consists mainly of bundles of parallel fibres (Cajal, 1911; Lorente de No, 1934; Lopes Da Silva, 1990) (Fig. 2). Some authors combine the stratum moleculare and the stratum lacunosum into the stratum lacunosum-moleculare (Hjorth-Simonsen, 1977). In the CA3 field, an additional layer, which mainly contains the mossy fibres from the granule cells, lies between the stratum radiatum and the stratum pyramidale. This layer is called the stratum lucidum.

2.2.3. The subicular complex

The subicular complex can be divided into three subdivisions: the subiculum, presubiculum and parasubiculum (Amaral and Witter, 1989). The subiculum is considered to constitute the major output structure of the hippocampus. The subiculum consists of three major layers: a deep, thick layer of pyramidal cells with similar structure of the CA1 pyramidal cells; an intermediate cell-sparse layer that is more or less continuous with the stratum radiatum of the cornu ammonis; a molecular layer, which is continuous with the

presubiculum on one side and the CA1 field on the other. Fibres from the entorhinal area (the perforant path) run transversely through the subiculum to end in the molecular layer of the cornu ammonis and the dentate gyrus (Lorente de No, 1934; Swanson et al. 1987).

2.2.4. The entorhinal cortex

The entorhinal cortex is usually divided into two parts: a lateral and a medial part (Lorente de No, 1934; Hjorth-Simonsen, 1972b; Hjorth-Simonsen and Jeune, 1972). While the lateral entorhinal cortex sends its fibres mainly to the septal hippocampus, the medial entorhinal cortex projects to the temporal hippocampus (Witter et al. 1989a,b; Germroth et al., 1991). In the entorhinal cortex, two spinous types of neurons with "long-axon cylinders projecting to the white matter", the spiny stellate cells (the "star" cells) and the pyramidal cells, were described by Cajal (1911) and Lorente de No (1934).

2.3. Cell morphology of the hippocampus

2.3.1. Dentate gyrus granule cells

Granule cells are the major neurons in the dentate gyrus. The granule cells were first described by Golgi (1886). Further details were given by Sala (1892), Schaffer (1892) and Cajal (1893). These cells form a compact layer, called the stratum granulosum. The body of these neurons is oval shaped and about 20 by 15 μm in size. The granule cells are highly polarized in rodents. The dendrites of these neurons extend into the stratum moleculare where they receive the input of the entorhinal cortex through the perforant path fibres. In general, spines are confined to segments beyond the first branch of the stem dendrites (Williams and Matthysse, 1983). In humans and monkeys, a significant population of granule cells has basal dendrites in the hilus. Some basal dendrites curve up into the molecular layer where they have similar morphology as the apical dendrites. Other basal dendrites in the hilus are

shorter, thinner and only have a few side branches (Seress and Mrzljak, 1987). The granule cells give rise to distinctive axons, the mossy fibres, which collateralize in the polymorphic layer before entering the CA3 field where they form en passant synapses on the proximal dendrites of the pyramidal cells. The mossy fibres are mainly organized in a lamellar fashion (Amaral and Witter, 1989).

2.3.2. *Cornu ammonis pyramidal cells*

Pyramidal cells are densely packed in the stratum pyramidale. The pear-shaped pyramidal cell body is on an average 40 by 20 μm in size. However, the size of pyramidal cells varies in different subfields of the cornu ammonis. The CA1 field has the smallest pyramidal cells whereas the CA3 field has the largest cells (Lorente de No, 1934). All these pyramidal cells possess both apical and basal dendrites.

There are morphological differences among the subfields of cornu ammonis. In the CA1 field, the basal dendrites of pyramidal cells extend into the stratum oriens in a bush-like fashion. The apical dendrites travel for some distance in the stratum radiatum before they ramify. The distal apical dendrites extend into the stratum lacunosum-moleculare. A common characteristic of CA1 pyramidal cells is that their shafts have many fine side branches in the stratum radiatum which do not appear in the CA2 and CA3 fields (Lorente de No, 1934). The axons of CA1 pyramidal cells are thin. They arise from the basal side and reach out to the stratum orien and the alveus. Some axons also give off several collaterals, which ramify in the stratum radiatum. The CA1 field is further divided into three subfields: (1) CA1a lies next to the subiculum and contains some cells belonging to the subiculum; (2) CA1b contains the smallest pyramidal cells of cornu ammonis; (3) CA1c pyramidal cells have smooth dendrites with many side

branches and are bigger than the CA1b cells (Lorente de No, 1934). However, the borders of these CA1 subfields are not very clear.

The CA2 field makes up a small portion of the pyramidal cell layer. The CA2 pyramidal cells are bigger than the CA1 cells. They are characterized by possessing dendrites similar to those of CA3, but without the thick thorns. The stratum radiatum in the CA2 field is the thinnest among the subfields of cornu ammonis. The axons there do not have a Schaffer collateral, but have several collaterals for the longitudinal association path. These axons have long horizontal collaterals in the stratum oriens (Lorente de No, 1934).

In the CA3 field, the pyramidal cells are the biggest in cornu ammonis. They are characterized as described by Cajal (1911). The ascending shafts from apical dendrites do not have side branches in the stratum radiatum, but generally divide into two or more vertical branches which ascend to the stratum moleculare. The initial part of the shaft has numerous thick thorns, which make contact with the mossy fibre. The basal dendrites ramify in the stratum oriens (Lorente de No, 1934). The axons of CA3 pyramidal cells are thick and highly collateralized. The axons arise from the basal pole of the CA3 pyramidal cells and cross the stratum oriens into the fimbria giving off collaterals. Some collaterals terminate within the CA3 field. Others termed Schaffer collaterals cross the stratum pyramidale and radiatum and enter the stratum lacunosum-moleculare where they constitute horizontal fibres. Schaffer collaterals are very thick and myelinated. They make contact with the CA1a and CA1b apical dendrites (Lorente de No, 1934). Lorente de No (1934) described that only some CA3 pyramidal cells have Schaffer collaterals. He further divided the CA3 field into three subfields (CA3a,b,c). The CA3a lies next to the CA2 field while the CA3c lies next to the hilus. The CA3b is situated between the CA3a and CA3c. The dendrites of CA3c pyramidal cells make contact with the mossy

fibres from both infrapyramidal and superpyramidal granule cells, whereas the dendrites of CA3a and CA3b pyramidal cells only make contact with the mossy fibres from superpyramidal granule cells. All CA3c pyramidal cells and half of CA3b pyramidal cells have Schaffer collaterals. The other half of CA3b and all CA3a pyramidal cells do not have Schaffer collaterals. They have one or two collaterals which extend to the stratum radiatum and constitute an associational pathway running in the CA3a and the CA2 fields. However, Ishizuka et al.(1990) have recently described collaterals from all portions of CA3 field can reach widespread regions of CA3, CA2 and CA1 transversely and longitudinally, a few fibres enter the subicular complex, but none enter the entorhinal cortex.

2.3.3. Hilar region mossy cells

Mossy cells are one of the most distinctive and common cell types in the hilus of the hippocampal dentate gyrus (Amaral,1978; Ribak et al. 1985). These cells are distinguishable from other neurons of the hilar region primarily by the cluster of complex spines ('thorny excrescences') located at several locations on the soma and proximal dendrites, on the distal dendrites which have more typical spines (Ribak et al. 1985). Mossy cells have a triangular or multipolar shaped soma with about 20 to 25 μm in size. Three to four primary dendrites arise from the soma and bifurcate once or more to produce an extensive dendritic arborization restricted, for the most part, to the hilus where they make contact with the mossy fibres (Ribak et al., 1985). The axons of the mossy cells bifurcate and project towards the fimbria and the molecular layer of the dentate gyrus.

2.3.4. Interneurons

The two principal cell types, the pyramidal cells of the cornu ammonis and the granule cells of the dentate gyrus, make up 96-98% of the neurophil of the hippocampus (Seress and Pokorny, 1981; Buzsaki, 1984). Interneurons are

found both in the principal cell layers and other strata of the structure (Cajal, 1911; Lorente de No, 1934). There are various types of interneurons based on their morphological characteristics, such as pyramidal, horizontal, fusiform, inverted fusiform and multipolar. The somata of these interneurons vary in size (10 to 50 μm). These cells have aspiny dendrites and locally arborizing axons (Ribak and Anderson, 1980; Buzsaki, 1984). The most well-studied interneurons are basket cells characterized by their axonal terminals forming basket-like structures around the somata of the target cells (Cajal, 1911; Lorente de No, 1934). These basket cells are distributed in both the stratum pyramidale and the stratum granulosum. These cells lie very close to the granule cells and the pyramidal cells. The cell bodies vary in shape from spherical to triangular and are on an average 30-50 μm in size. Several dendrites with few branches and spines extend from the soma. They show frequent swelling like a string pearls (Andersen et al., 1969). In the cornu ammonis field, most of the basket cell dendrites distribute in the stratum oriens while some extend into the stratum radiatum (Andersen et al. 1969). In the dentate gyrus, the dendrites of basket cells are found in all layers (Ribak and Seress, 1983). The axons of these cells are very thin. They form an extensive plexus in the pyramidal cells or the granule cells. Each basket cell may synapse with as many as 200 to 500 pyramidal cells or granule cells (Anderson et al. 1969; Buzsaki, 1984). The synaptic sites of axon terminals are on the somata and proximal dendrites of the target cells.

Another group of interneurons is localized at the stratum oriens/alveus (O/A) border. The somata of these cells are oblong-shaped and 20 to 30 μm in size. The longitudinal axis of the soma is parallel to the alvear surface. The dendrites are long and coarse, and almost parallel to the alvear fibres (Cajal, 1911; Lorente de No, 1934; Andersen, 1963). These axons extensively ramify in

the stratum oriens, pyramidale, and radiatum (Cajal 1911; Lorente de No, 1934) where they make contact with the basal dendrites, the somata and the proximal dendrites of pyramidal cells (Lacaille et al. 1987).

A distinct group of interneurons, termed the stratum lacunosum-moleculare (L-M) interneurons, are located in the stratum lacunosum-moleculare and the stratum radiatum (Kawaguchi and Hama, 1987; Lacaille and Schwartzkroin, 1988a,b). L-M somata are fusiform in shape and 15 to 25 μm in size. Their dendrites are aspiny and slightly varicose. They branch profusely in different strata of the hippocampus (Kunkel et al. 1988). Their axons project throughout the stratum lacunosum-moleculare and into the stratum radiatum. Some dendrites and axon collaterals can extend towards the hippocampal fissure and cross into the stratum moleculare of the dentate gyrus (Kunkel et al., 1988). The L-M interneuron axons make synaptic contact with the pyramidal cell dendrites in the stratum lacunosum-moleculare and the stratum radiatum, and the granule cell dendrites in the stratum moleculare of the dentate gyrus. The L-M interneuron axons also make synaptic contact with the dendrites of non-pyramidal neurons in the stratum radiatum (Kunkel et al, 1988).

2.4. Intrinsic hippocampal circuitry

The "lamellar hypothesis" was proposed by Andersen and his colleagues (1971a). They summarized the results of their physiological studies in the rabbit and concluded that "The hippocampal cortex seems to be organized in parallel lamellae ... By means of this lamellar organization, small strips of the hippocampal cortex may operate as independent functional units, although excitatory and inhibitory transverse connections may modify the behaviour of the fibre lamellae." However, subsequent and recent neuroanatomical investigations have demonstrated that the major intrinsic hippocampal projections are as extensive and highly organized in the longitudinal or

septotemporal axis of the hippocampus as in the transverse axis (Hjorth-Simonsen, 1973; Laurberg, 1979; Swanson et al., 1978; Amaral and Witter, 1989; Ishizuka, 1990). These studies have clearly shown that the hippocampal formation is a three dimensional cortical structure with important information processing taking place in both the transverse and longitudinal axes. In the following discussion, the major intrinsic pathways within the hippocampal formation are roughly divided into transverse (lamellar circuits), longitudinal (septotemporal pathways) and local circuits. It is obvious that these circuits are highly interdependent.

2.4.1. Transverse circuit

The major excitatory pathway within the hippocampus enters from the entorhinal cortex via the perforant pathway across the hippocampal fissure to the granule cells of the dentate gyrus. The dentate granule cells distribute their mossy fibres to CA3 pyramidal cells (Cajal 1911; Lorente de No, 1934). The proximal portions of CA3 pyramidal cells (CA3c) are reached by the mossy fibres which originated in both the infrapyramidal and suprapyramidal blades. Only the mossy fibres which arise from the suprapyramidal blade, make contact with more distal portions of the CA3 field (CA3a and CA3b) (Lorente de No, 1934; Blackstad et al. 1970; Chronister and White, 1975; Witter, 1989). All along their course, the mossy fibres make numerous synapses en passage with the dendrites they pass in the hilus and the CA3 field (Andersen et al., 1966; O'Keefe and Nadel, 1978). The mossy fibres which terminate on the basal dendrites of the proximal CA3 pyramidal cells arise mainly from the infrapyramidal blade of the dentate gyrus (Witter, 1989). The mossy fibres are mainly organized in a lamellar fashion. Bands of mossy fibres are principally oriented transverse to the longitudinal axis of the hippocampal formation and the

bands arising from each septotemporal level of the dentate gyrus only minimally overlap those arising from other septotemporal levels (Amaral and Witter, 1989).

The CA3 pyramidal cells receive inputs not only from the mossy fibres, but also directly from the perforant path fibres. In turn, the axons of the CA3 pyramidal cells divide, with one branch entering the fimbria and going to the septum, while other branches remain within the hippocampus. Among the latter, Schaffer collaterals are the most prominent projection that arise from the CA3 pyramidal cells. The Schaffer collaterals run into the stratum radiatum and the stratum oriens of the CA1 field (Lorente de No, 1934; Hjorth-Simonsen, 1973). The Schaffer collaterals synapse with the apical dendrites in the stratum radiatum and the basal dendrites in the stratum oriens in the CA1 field. The CA1 pyramidal cell axons enter the alveus and project caudally into the subiculum and rostrally into the lateral septal nuclei and the prefrontal cortex. The connections between the CA3 and the CA1 neurons, and the connections from the CA1 to the subiculum show a columnar organization perpendicular to the cell layer (Witter, 1989). Proximal parts of CA3 and CA1 distribute fibres to distal parts of CA1 and the subiculum, respectively, while the more distal parts of CA3 and CA1 interact with more proximal parts of CA1 and the subiculum, respectively (Witter, 1989; Ishizuka et al., 1990).

2.4.2. Longitudinal pathway

The intrinsic connections between the various subfields of the hippocampus show the major hippocampal projections are as extensive and highly organized in the longitudinal or septotemporal axis of the hippocampus as in the transverse axis (Amaral and Witter, 1989; Ishizuka et al.; 1990). The perforant path from the entorhinal cortex to the granule cells spreads for at least millimeters along the longitudinal axis of the hippocampus. Certain neurons in the polymorphic layer of the dentate gyrus preferentially send fibres over lengthy

longitudinal distances to the granule cells (Hjorth-Simonsen and Laurberg, 1977; Knowles, 1992). The mossy fibres which arise from the granule cells, are the only projection organized in a lamellar fashion. On the other hand, the CA3 projections within the CA3 field and to the CA1 field are equally extensive in the transverse and longitudinal axes (Swanson et al., 1978; Amaral and Witter, 1989; Ishizuka, et al., 1990; Knowles, 1992). The Schaffer collateral system serves not only as a serial link from the CA3 field to the CA2 and CA1 fields, but also as a longitudinal association pathway within the CA3 field and along the CA2 and CA1 fields (Hjorth -Simonsen and Laurberg,1977; Knowles, 1992). Lorente de No (1934) described that the associational connections link different septotemporal levels of the hippocampus whereas the CA3 and CA1 projection link the two fields at one septotemporal level. His description seems to be oversimplified.

2.4.3. Local circuits

Within the subfields of the cornu ammonis and the dentate gyrus, interneurons and principal cells form two local synaptic circuits, the feed-forward and the recurrent inhibitory circuits (Andersen et al., 1969; Buzsaki, 1984). These inhibitory circuits play a crucial role in the hippocampal function and are discussed further in section 4.2.7 (inhibitory circuitry).

Granule cells send extensive collaterals not only to many interneurons, but also to the mossy cells in the hilus. The axon collaterals of the mossy cells project into the inner molecular layer of the dentate where they make contact with the dendrites of the granule cells. These axon collaterals also make contact with interneurons in the hilus of the dentate gyrus which in turn contact the granule cells (Schwartzkroin et al. 1990).

Both CA3 and CA1 pyramidal cells send excitatory axon collaterals to several classes of interneurons that inhibit the pyramidal cells (Knowles and

Schwartzkroin, 1981a, b). The CA3 pyramidal cells also have extensive local axon collaterals, the CA3 associational collaterals, which project along the longitudinal axis and excite neighbouring CA3 pyramidal cells (Ishizuka et al., 1990).

2.4.4. Commissural afferents

Extensive neural fibres connect the two hippocampi, crossing the midline in the ventral and dorsal hippocampal commissures (psalteria). The majority of the crossed hippocampal fibres appear to course through the ventral commissure, with only a few traveling through the dorsal commissure (O'Keefe and Nadel, 1978). Two major components of the projections have been described by Blackstad (1956) and Swanson et al. (1978). One projection arises from the dentate gyrus and ends on the inner one-third of the dendrites of the granule cells of the contralateral dentate gyrus, homotopic to the ipsilateral projection. The second projection arises from the CA3 field and ends in the stratum radiatum and the stratum oriens of the contralateral CA1, also homotopic to the ipsilateral projection. The origin of the cells of the contralateral projection to the dentate gyrus molecular layer has been controversial. Recent studies have shown that the mossy cells of the hilus are the major neurons which project to the inner molecular layer of the contralateral dentate gyrus, homotopic to the ipsilateral projection (Lauberg and Sorensen, 1981; Schwartzkroin et al., 1990; Ribak et al., 1985). The commissural connections of the hippocampal formation in the monkey is less than in the rat (Gottlieb and Cowan, 1973; Rosene and Van Hoesen, 1987; Knowles, 1992). The significance of this difference is not very clear. It may reflect a growing functional linkage between the hippocampal formation and increasingly lateralized cerebral cortex of primates.

2.5. Extrinsic afferents to the hippocampus

The hippocampus receives extrinsic afferents from a variety of other structures; including primarily the entorhinal cortex, the medial septal area and several brain-stem sites.

2.5.1. Entorhinal afferents

The perforant path is the major avenue of entorhinal afferents to the hippocampus. As previously described, the entorhinal cortex is usually divided into at least two parts; a lateral and a medial part (Lorente de No, 1934; Hjorth-Simonsen and Jeune, 1972; Amaral and Witter, 1989). While the lateral entorhinal cortical neurons send their fibres mainly to the septal hippocampus, the medial entorhinal neurons project into the temporal hippocampus (Witter et al., 1989). Whereas fibres from the lateral entorhinal cortex mainly terminate in the outer one-third of the molecular layer of the dentate gyrus, fibres from the medial entorhinal cortex preferentially distribute to the middle one-third of the molecular layer (Wyss, 1981; Witter, 1989; Steward, 1976). This differentiation between the lateral and the medial components of the perforant pathway is more prominent in rats and cats than in monkeys (Hjorth-Simonsen, 1972; Steward, 1976; Wyss, 1981; Witter et al., 1989). In rats, the fibres coming from the lateral entorhinal cortex prefer the suprapyramidal blade of the dentate gyrus, whereas the medial parts project to both the suprapyramidal and infrapyramidal blades (Witter, 1989). The entorhinal cortex also directly projects into the CA1, CA3 and the subiculum fields (Gottlieb and Cowan, 1972; Steward, 1976; Witter et al., 1988; Witter et al., 1989). The perforant path originates from layer I-III of the entorhinal cortex (Steward and Scoville, 1976). The projection into the dentate gyrus arises mainly from layer II of the entorhinal cortex whereas the projection to the CA1 field arises from the layer III of the entorhinal cortex (Steward and

Scoville, 1976). A few neurons in the layer V also send their axons to the hippocampus (Kohler, 1985).

2.5.2. Septal afferents

Daitz and Powell (1954) first demonstrated that neurons in the medial septal nucleus and the nucleus of the diagonal band (together known as the medial septal complex), project through the fimbria to the hippocampus in rats, rabbits and monkeys. The septo-hippocampal pathway appears to be crucial for the initiation or maintenance of θ rhythm activity in the hippocampus (Petsche et al., 1962; Andersen et al. 1979; Mitchel and Ranck, 1982). This projection also provides the major source of extrinsic cholinergic fibres to the hippocampus although some septo-hippocampal projection neurons are not cholinergic. (Lewis et al., 1967; Swanson et al., 1987). Some septo-hippocampal projection neurons in the medial septal nucleus and diagonal band contain glutamic acid decarboxylase (GAD), a synthesizing enzyme for the neurotransmitter GABA. Therefore, these neurons are presumably GABAergic (Kohler et al, 1984; Swanson et al., 1987). The neurons in the medial septal complex innervate all fields of the hippocampus (Swanson and Cowan, 1976, 1978; Swanson et al., 1987). Septal fibres to the dentate gyrus course primarily through the fimbria and reach the hilar region and a thin zone of the molecular layer, just superficial to the granule layer (Rose et al., 1976; Swanson et al., 1987). The septal fibres project into the cornu ammonis ending predominantly in the stratum radiatum and the stratum oriens of the CA3 field, and in the stratum oriens of the CA1 field (Nyakas, et al., 1987; Swanson et al., 1987). The input to the CA1 field is less dense than the input to the CA3 field, and appears to course predominantly through the dorsal fornix. The septal fibres also project to the subiculum. The target cells of the septo-hippocampal projection are pyramidal and granule cells as well as interneurons (O'Keefe and Nadel, 1978).

2.5.3. Other afferents

There are several other projections from different parts of the brain to the hippocampus. The major inputs from the thalamus, including one from the anterior thalamic nuclei and one from the midline nuclei, terminate in the CA1 field and the subiculum. The projections from hypothalamus end in the dentate gyrus, the subiculum, as well as the CA3 field (Swanson et al., 1987; Schwerdtfeger, 1984). The raphe nuclei give rise to serotonergic input to the hippocampus from the midbrain. These serotonergic fibres from the raphe nuclei innervate all fields of the hippocampus (The densest innervation occurs in the dentate hilar area) (Kohler, 1982; Swanson et al., 1987). The hippocampus also receive dopaminergic inputs from the ventral tegmental area and the substantia nigra, the pars compacta and the central linear nucleus (Swanson, 1982; Swanson et al., 1987). These dopaminergic fibres terminate in the subiculum entorhinal area, the dentate gyrus and the cornu ammonis (Swanson, 1982, Scatton et al., 1980; Swanson et al, 1987). All fields of the hippocampus receive noradrenergic input from the locus ceruleus of the pons (Blackstad et al. 1967; Swanson et al. 1987). The densest noradrenergic innervation is the hilar region of the dentate gyrus (Koda et al., 1978; Swanson et al., 1987).

2.6. Extrinsic efferents from the hippocampus

The hippocampus projects to a series of parahippocampal and isocortical structures, including the entorhinal cortex, the septum, the nucleus accumbens, the amygdaloid complex, and the hypothalamus. The fornix-fimbria system and the entorhinal area are the two main output pathways of the hippocampus.

2.6.1. Fornix Fimbria system

The rostrally directed efferent fibres of the hippocampus and its adjacent areas gather together in the fimbria and the dorsal fornix in which the fibres join at the columns of the fornix (O'Keefe and Nadel, 1978). The main fibres in the

fornix continue rostrally to penetrate the septal-fimbrial nucleus at the caudal parts of the septal region where the fibres split into two components to form the post-commissural and pre-commissural fornices. The latter originates primarily in the hippocampus, and the former arises mainly from adjacent allocortical areas (O'Keefe and Nadel, 1978).

The pre-commissural fornix arises from neurons throughout the longitudinal axis of the cornu ammonis and the subiculum and distributes to most of the nuclei of the septal area, including the lateral septum, the diagonal band of Broca, and the bed nucleus of the anterior commissure. The fibres also terminate in the lateral pre-optic region and the lateral hypothalamus (Swanson and Cowan, 1977; O'Keefe and Nadel, 1978; Swanson et al., 1987).

The post-commissural fornix divides into two components. One component arises from the pre- and parasubiculum and projects to the thalamus (Chronister and White, 1975; Swanson and Cowan, 1975; O'Keefe and Nadel, 1978). The other derives from the subiculum and projects to the mammillary bodies and rostral brain stem (Meibach and Siegel, 1975; O'Keefe and Nadel, 1978).

2.6.2. Entorhinal projection

A direct projection that originates in the CA3 field of primarily ventral hippocampus and terminates in layer IV of the entorhinal area, has been described by Hjorth-Simonsen (1971). Later, Swanson et al. (1978) described that another projection into the entorhinal cortex originating from the CA1 field. Several other efferent projections have also been reported by Swanson et al. (1978). They are as follows: the CA3 fibres directly project to the cingulate cortex; the subiculum fibres project to the perirhinal cortex and the medial frontal cortex; and the CA1 fibres project to the perirhinal cortex.

3. ELECTROPHYSIOLOGY OF HIPPOCAMPAL NEURONS

3.1. *Characteristic of hippocampal neuron*

The use of slice preparations has provided a great deal of information about the physiology of hippocampal neurons. The experimental data obtained from hippocampal slices are gratifyingly similar to results obtained from the intact animal (Kandel and Spencer, 1961; Kandel et al., 1961; Spencer and Kandel et al., 1961a, b; Schwartzkroin, 1975). Careful studies of the electrophysiological properties of the CA1, CA3 pyramidal cells and the dentate granule cells have been performed in hippocampal slices (Brown et al., 1981; Johnston, 1981; Brown and Johnston, 1983; Durand et al., 1983; Turner and Schwartzkroin, 1983).

The resting membrane potentials of hippocampal pyramidal and granule cells are on an average -50 to -70 mV and -55 to -80 mV, respectively. The input resistances calculated from the slope resistance within the linear range of the current-voltage (I-V) curves are 20-45 M Ω for pyramidal cells and 40-60 M Ω for granule cells (Brown et al., 1981; Turner and Schwartzkoin, 1983; Lambert and Jones, 1990). The membrane constants, which are the time for membrane potential to reach 1-1/e of their peak voltage, are on an average 25-35 msec for CA3 pyramidal neurons, 12-16 msec for CA1 pyramidal neurons, and 9-11 msec for granule cells (Brown et al., 1981; Durand et al., 1983; Turner and Schwartzkroin, 1983; Schwartzkroin and Mueller, 1987). Assuming a constant specific membrane capacitance, the differences in the membrane time constants suggest that there are proportionate differences in the specific membrane resistivity in the different types of hippocampal neurons. Based on the equivalent cylinder modeling theory (Rall, 1969; Rall, 1974; Rall, 1977), the electrotonic length of the equivalent dendritic cylinder and the conductance ratio of the dendrites to the soma have been estimated. The average estimated value

of the electrotonic length and the dendrite to soma conductance ratio of hippocampal neurons are 0.8-1 and 1.1-1.5, respectively (Brown et al, 1981; Johnston, 1981; Burand et al., 1983; Schwatzkroin and Mueller, 1987). However, using the multipolar cylinder model, Glenn (1988) suggested that the electrotonic length of neuron dendrites was overestimated. In fact, he found that the value of the electrotonic length was only 1/3 of the previous value.

Recently, the patch clamp technique has been applied to the slice preparation (Edward et al., 1989; Blanton et al., 1989; Randall et al., 1990). High input resistances (200 M Ω to 5 G Ω) of hippocampal neurons have been observed using whole cell patch clamp recording (Edward et al., 1989; Randall et al., 1990). Similar mean resting membrane potentials have been observed between conventional intracellular recording and whole cell recording. The membrane time constants are usually longer when using the whole cell recording compared to the intracellular recording (Edward et al., 1989). The discrepancy between the values obtained from two methods has not been satisfactorily explained (Edward et al., 1989; Strom, 1990a).

3.1.1. Bursting activity of hippocampal neurons

Hippocampal pyramidal cells can fire spikes spontaneously, or during intracellular depolarizing current pulses. There are significant differences in the mode of spike discharge between CA1 and CA3 pyramidal cells (Masukawa et al., 1982; Wong et al., 1979). Spontaneous burst generation occurs more commonly in CA3 neurons than in CA1 neurons. CA1 cells produce accommodating trains of spikes when depolarizing current pulses are injected intracellularly. In contrast, CA3 pyramidal cells can readily generate burst discharges in response to intracellular depolarizing current pulses (Wong et al., 1979; Schwatzkroin et al., 1990). The spike frequency accommodation in CA1 neurons is due to the activation of several K⁺ currents, primarily the Ca²⁺-

dependent K^+ currents. The event in CA3 cells is due to the activation of at least one type of Ca^{2+} current (Wong and Prince, 1978; Traub, 1982). The burst discharge property of CA3 cells contributes to the role of the CA3 regions as a "pacemaker" for internal discharge in the hippocampus and as "booster" for amplifying incoming excitatory signals from afferent pathways. Granule cells do not fire spontaneous bursts of spikes. This is probably due to the high threshold required for spike generation in these cells.

3.2. Hippocampal interneurons

Hippocampal interneurons can modulate the hippocampal input and the principal cells. These interneurons play a significant role in the hippocampal function. However, the electrophysiological characteristics of the hippocampal interneurons are not as well known as those of hippocampal pyramidal cells and granule cells. Three types of interneurons in the CA1 region, basket cells, oriens/alveus (O/A) interneurons, and lacunosum-moleculare (L-M) interneurons are discussed here. The resting membrane potentials and the input resistances of basket cells are on an average -50 to -70 mV and 30 to 50 M Ω , respectively. Basket cells display properties that clearly distinguish them from pyramidal and granule cells. Basket cells have a very brief action potential (average 0.8 ms), a large spike after hyperpolarization (AHP) (5 to 10 mV, 10 - 30 ms) and a short membrane time constant (approximately 3 ms). At resting membrane potential, basket cells generate spontaneous spikes at a high rate (5 - 25 Hz) and display a constant barrage of EPSPs. Basket cells also fire non-decrementing bursts of spikes in response to intracellular depolarizing pulses (Kawaguchi and Hama, 1987; Lacaille et al. 1989; Schwartzkron and Kunkel, 1985, Schwartzkron and Mathers, 1978).

O/A interneurons located at the stratum oriens-alveus borders have electrophysiological properties quite similar to those of basket cells (Lacaille et

al., 1987). The resting membrane potentials and the input resistances of O/A interneurons are on an average -50 to -70 mV and 30 to 65 M Ω , respectively (Lacaille and Williams, 1990). The action potentials are brief (approximately 1 ms) and the time constants are fast (4-6 ms). O/A interneurons have a large AHP (7 to 13 mV, 10-30 ms) following a spike. These cells generate spontaneous spike firing at high frequency (10-27 Hz) and receive a constant barrage of EPSPs; and they fire in non-accommodating bursts of action potentials in response to intracellular depolarizing current pulses.

Both basket cells and O/A interneurons show GABA-like immunoreactivity. These interneurons receive excitatory inputs from Schaffer collaterals, commissural fibres as well as the alvear fibres (Schwartzkroin and Kunkel, 1985; Lacaille et al., 1989). In turn, they provide feed-forward and feedback inhibition to pyramidal neurons (Lacaille et al. 1989).

The third type of interneurons in the CA1 field are called L-M interneurons which are located at the border between the stratum lacunosum-moleculare and the stratum radiatum. L-M interneurons display some distinctive membrane properties (Kawaguchi and Hama, 1987) and share some similar electrophysiological properties with other interneurons. The membrane potentials and the input resistances of L-M interneurons are -50 to -70 mV and 40 and 70 M Ω , respectively. L-M interneurons have a prominent AHP (7 to 14 mV, 30-34 ms) following an action potential and no spike frequency accommodation during depolarizing current pulses. The action potential of L-M interneurons is longer (approximately 2 ms) than that of basket cells or O/A interneurons. In addition, the membrane time constant of L-M interneurons is slower (8.6 ms) than that of basket cells or O/A interneurons. Unlike basket cells and O/A interneurons, the majority of L-M interneurons do not fire action potentials spontaneously; some L-M interneurons fire action potentials

spontaneously at low frequency (below 10 Hz). L-M also generate little spontaneous synaptic activity (Lacaille et al. 1989; Lacaille and Schwartzkroin, 1988).

L-M interneurons also display GABA-like immunoreactivity. These interneurons receive excitatory inputs from Schaffer collaterals and commissural fibres. Unlike basket cells and O/A interneurons which provide both feed-forward and feedback inhibition to pyramidal cells, L-M interneurons only give feed-forward inhibition to pyramidal neurons (Lacaille et al., 1989).

3.3. Membrane ionic currents of hippocampal pyramidal neurons

Multiple ionic channels co-exist in the membrane of the hippocampal neurons. Different ionic currents, mediated through the opening of voltage-gated and/or non-voltage-gated channels, play different roles in the functioning of the hippocampal neurons.

3.3.1. Na⁺ currents

Two types of Na⁺ currents have been found in hippocampal neurons: a fast current I_{Na} (fast) and a slowly inactivating current I_{Na} (slow) (Brown et al., 1990). The fast Na⁺ current behaves as an orthodox Hodgkin-Huxley current with an activation threshold of -60 mV and a time to peak of about 0.9 ms at 0 mV (Sah et al., 1988a; Brown et al., 1990). It is responsible for the depolarizing phase of the cell action potential. Inactivation is complete throughout the activation range. This current has been observed in both the soma and the dendrites of the hippocampal neurons (Benardo et al., 1982; Sah et al., 1988a; Huganard et al., 1989). The current is blocked by extracellular application of tetrodotoxin (TTX) and intracellular injection of QX-222 or QX-314 (Schwartzkroin and Slawsky, 1977; Connors and Prince, 1982; Schwartzkroin and Mueller, 1987).

The slowly inactivating Na^+ current has an activation threshold of about 5 to 10 mV positive to the resting potential and is triggered and sustained by depolarizing prepotentials (Lanthorn et al., 1984b; French and Gage, 1985). This current may be confined to the soma of the hippocampal neurons (Benardo et al., 1982). It is responsible for the repetitive firing of action potentials and acts as a "pacemaker" current for the hippocampal activity (Brown et al., 1990). The current is also TTX-sensitive and blocked by QX-314 (Benardo et al., 1982).

3.3.2. Ca^{2+} currents

There are at least four types of voltage-dependent calcium channels presently recognized in the hippocampal neurons. They are the high-threshold, sustained current (L-type), the low-threshold transient current (T-type), the high threshold inactivating current (N-type) (Ozawa et al., 1989; Takahashi et al., 1989; Fisher et al., 1990; Brown et al., 1990; Mogul and Fox, 1991) and the high-threshold ω -agatoxin IVA-sensitive (ω -Aga-IVA) current (P-type) (Hillman et al., 1991; Mintz et al., 1992).

The L-type Ca^{2+} current is a high-threshold and long-lasting inward current, which is activated at potentials between -20 mV and -10 mV and inactivated at potentials between -20 mV and -60 mV (Ozawa et al., 1989; Mogul and Fox, 1991). The inactivation rate of the L-type Ca^{2+} current is very slow (time constant > 500 ms at 0 mV) (Ozawa et al., 1989; Tsien et al., 1988). At the single channel level, the L-type conductance is 25-27 pS. The L-type current can be enhanced by Ba^{2+} and blocked by Co^{2+} , Cd^{2+} , Ni^{2+} (Fisher et al., 1990; Mogul and Fox, 1991). The current is blocked by verapamil and by dihydropyridine Ca^{2+} antagonists, such as nimodipine, nifedipine and PY108-068, and is enhanced by dihydropyridine agonist, BAY K8644 (Brown and Griffith 1983a,b, Brown et al., 1984; Tsien et al., 1988; Brown et al., 1990). ω -

Conotoxin suppresses the L-type more strongly than the N-type Ca^{2+} currents (Takahashi et al., 1989).

Another high threshold Ca^{2+} current, called N-type current, inactivates slowly, but much faster than the L-type current. The N-type current is activated at -20 mV or more positive and inactivated at -30 mV to -110 mV, decaying from a maintained depolarization with a time constant of 30-36 ms at 0 mV (Ozawa et al., 1989). The single channel conductance of the N-type channel is 13-15 pS (Brown et al., 1990; Tsien et al., 1988). The N-type current is enhanced by Ba^{2+} and blocked by Cd^{2+} and Ni^{2+} . This current is also blocked by ω -conotoxin. Dihydropyridine Ca^{2+} antagonists, nicardipine suppresses the N-type current in hippocampal neurons, but not in the peripheral neurons (Takahashi et al., 1989).

The third type of Ca^{2+} current, called the T-type current, is a low-threshold transient inward current. The T-type current is activated by depolarization beyond about -60 mV from holding potential down to -100 mV, and is completely inactivated at -40 to -50 mV (Brown et al., 1990, Takahashi et al., 1989). The time constant of inactivation of the T-type current is approximately 20 ms at -40 mV. The single channel conductance is 7-8 pS. Unlike the L-type and the N-type current, the T-type current is reduced or not changed on substituting Ba^{2+} for Ca^{2+} (Brown et al., 1990). The T-type current is blocked by Ni^{2+} and Cd^{2+} . It is sensitive to dihydropyridine Ca^{2+} antagonists such as nicardipine. However, the current is resistant to ω -conotoxin (Takahashi et al., 1989). The T-type current may contribute to spontaneous depolarization waves and acts as a generators of pacemaker activity (Tsien et al., 1988).

The fourth type of Ca^{2+} current, the P-type current, was first described in cerebellar Purkinje cells (Llinas et al., 1989). The P-current was later found in a variety of central and peripheral neurons, including hippocampal pyramidal neurons (Hillman et al., 1991; Mintz and Adams, 1992). The density of P-type

Ca^{2+} channels is lower in hippocampal pyramidal cells than cerebellar Purkinje cells (Hillman et al., 1991). The P-type current is characterized by being sensitive to ω -Aga-IVA but not to ω -conotoxin or dihydropyridine types of blocker (Llinas et al. 1989; Mintz et al., 1992). The P-current has a high activation voltages (-20 mV or more positive) with little inactivation (Llinas et al., 1992). The P-type current is enhanced by Ba^{2+} and blocked by Cd^{2+} and Co^{2+} . The P-type channels have been suggested to be involved in the release of neurotransmitters, such as glutamate (Turner et al., 1992).

Whether there are more than four types of Ca^{2+} channels in the hippocampal neurons is not very clear. Takahashi (1989) has reported a TTX-sensitive Ca^{2+} current, which differs from the L, N, P, T- type currents, in isolated hippocampal CA1 pyramidal neurons. This current is activated at more positive depolarizing pulses than around -65 mV from a holding potential of -100 mV, and reaches a peak value at about -35 mV. The inactivation rate is 7-10 ms at -35 mV. This current is suppressed by phenytoin and nifedipine, a dihydropyridine, and is resistant to ω -conotoxin. However, the identity of this current remains unknown at present. Recently, a "Q-type" Ca^{2+} current has been reported in cerebellar granule cells (Randall et al., 1993). The "Q-type" current is sensitive to ω -conotoxin-MV1C, but is insensitive to nimodipine, ω -conotoxin-GVIA or low concentrations (<30 nM) of ω -Aga-IVA. It is not clear whether "Q-type" channels also exist in the hippocampal pyramidal neurons.

Intradendritic recording has demonstrated that dendrites are probably the primary sites of Ca^{2+} -dependent bursts and Ca^{2+} spikes, particularly in CA1 neurons (Wong and Prince, 1979; Wong et al., 1979; Benardo et al., 1982). The dendritic Ca^{2+} spike is appreciatively larger than the somatic Ca^{2+} spike. On the other hand, the Ca^{2+} -dependent subthreshold depolarization appears to be restricted to the soma. Yaari et al. (1987) have shown that the low-threshold

transient current, which probably underlies the subthreshold depolarizations, is largely present in the soma of cultured rat embryonic neurons. The channels carrying the high-threshold currents are distributed in both soma and processes of hippocampal neurons.

3.3.3. K^+ currents

Multiple potassium (K^+) channels co-exist and function in hippocampal neurons. At least six types of voltage-dependent K^+ currents and two types of Ca^{2+} -dependent currents have presently been identified in hippocampal neurons. The voltage-dependent K^+ currents are as follows: delayed rectifier current (I_K); fast transient current (I_A); slowly inactivating delay current (I_D); M-current (I_M); fast inward rectifier current ($I_{K(IR)}$); and Q-current (I_Q). The former four types of K^+ currents are activated by depolarization whereas the latter two types of K^+ currents are activated by hyperpolarization. The Ca^{2+} -dependent K^+ currents include the fast Ca^{2+} -dependent K^+ current (I_C) and the slow Ca^{2+} -dependent K^+ current (I_{AHP}). They are activated by an influx of Ca^{2+} ions through voltage-gated Ca^{2+} channels (Brown et al., 1990; Storm, 1990b).

I_K corresponding broadly to the classical delayed rectifier current first described by Hodgkin and Huxley (1952) has been recorded in hippocampal neurons (Segal and Barker, 1984a; Numann et al., 1988; Madison et al., 1987a; Storm, 1990b). This current is activated by depolarization beyond -40 mV. It activates only during action potential. The activation is rather slow (time to peak, about 180 ms in acutely dissociated cells) (Numann et al., 1987). I_K is blocked by mM concentrations of external tetraethylammonium (TEA). It is resistant to 4-aminopyridine (4-AP) and external cesium (Cs^+). The single K^+ channels (15-20 pS) may underlie I_K in hippocampal neurons (Rogawsky, 1986; Brown et al., 1990). I_K has been found in both soma and dendrites of

hippocampal neurons (Masukawa and Hansen, 1987). The current may contribute to the repolarization of the action potential.

I_A is a prominent current which can be recorded from hippocampal neurons (Gustafsson et al., 1982; Zbicz and Weight, 1985). I_A is activated by depolarization beyond about -60 mV and it inactivates between -60 and -40 mV. The current activates very rapidly (time to peak within 5-10 ms) and inactivates with a time constant of 20-30 ms. I_A is blocked by a low mM concentration of 4-AP (Segal and Barker, 1984; Numann et al., 1987) and nM of dendrotoxin (DTX) (Harvey and Karlsson, 1980, 1982), but it is resistant to TEA (Segal and Barker, 1984a; Gustafsson et al., 1982; Storm, 1990b). Noradrenaline and acetylcholine have been reported to inhibit I_A (Sah et al., 1985; Nakajima et al., 1986; Storm, 1990). A single transient K^+ channel with the conductance of 15 pS has been found primarily in the soma of hippocampal neurons. I_A plays a major role in regulating the repetitive firing of neurons. It can delay the onset of firing up to 100ms (Gustafsson et al., 1982; Storm, 1988c). The current is also involved in repolarization of the action potential (Storm, 1988c, 1990a).

I_D has been found to co-exist with I_A in CA1 pyramidal cells (Storm, 1988a,b). I_D differs from I_A in that: (a) I_D has slow kinetics, particularly inactivation and recovery from inactivation; (b) it has more negative threshold for activation and inactivation; (c) it is more sensitive to 4-AP (Storm, 1990). I_D is activated by depolarization beyond -70 mV, within about 20 ms, and inactivates over several seconds. Inactivation starts at about -120 mV and is complete at -60 mV. I_D is blocked by mM concentration of 4-AP and nM DTX. It is insensitive to TEA, Ba^{2+} and Cs^+ (Storm, 1990b). The current can cause a long delay in the onset of firing in response to long-lasting depolarizing stimuli and keep the cell from depolarizing further. I_D contributes very little to the normal membrane potential and is only effective in cells with a highly negative resting potential.

The rapid activation of this current suggests that it may participate in spike repolarization (Storm, 1987a, 1988c, 1990b). Because of its slow recovery of inactivation (up to 20s), it enables the cell to "integrate" separate depolarizing inputs over several seconds. Therefore, the response of the cell not only reflects the immediate synaptic input, but also takes into account what happened in the preceding seconds (Storm, 1988a).

Hippocampal I_M was first reported in CA1 cells by Halliwell and Adams (1982) and later recorded in CA3 cells (Brown and Griffith, 1983b; Madison et al., 1987). The hippocampal I_M resembles closely the one in sympathetic ganglia (Brown and Adams, 1980; Adams et al., 1982a). It can be blocked by acetylcholine and other muscarinic agonists (Brown and Adams, 1980). I_M is activated by depolarization beyond about -60 mV. It activates and deactivates slowly (time constant: 50 ms) and does not inactivate (Adams et al., 1982). I_M is blocked by bath-application of Ba^{2+} and mM concentration of TEA, not by Cs^+ and 4-AP (Halliwell and Adam, 1982; Storm, 1989). This current is also blocked by serotonin (Colino and Halliwell, 1987, also see Andrade et al., 1986). On the other hand, somatostatin (SS-14 and SS-28) can enhance I_M (Moore et al., 1988; Watson and Pittman, 1988). I_M participates in an early phase of spike frequency accommodation (Madison and Nicoll, 1984). It is also involved in the medium AHP following a single spike or a burst of spikes (Storm, 1989). However, I_M contributes little to the resting membrane potential of hippocampal neurons unless the resting membrane potentials of the cells are more positive than -60 mV. I_M will form a component of outward rectification when these cells are depolarized, particularly for long duration.

$I_{K(IR)}$ is a fast inward rectifier current, which is activated by hyperpolarization beyond the resting potential and reaches the peak with 5 ms (Owen, 1987). It inactivates at potentials more negative than -100 mV (time

constant about 35 ms at -115mV). It is blocked by Cs^+ and Ba^{2+} (Brown, 1990b). A variety of neurotransmitters, including GABA (via GABA_B receptors), serotonin and adenosine can induce an inward rectifying K^+ current in hippocampal neurons (Newberry and Nicoll, 1985; Colino and Halliwell, 1987; Andrade and Nicoll, 1987).

I_Q is a slow mixed Na^+/K^+ inward rectifier current, which is activated by hyperpolarization beyond -80 mV (Halliwell and Adams, 1982). Activation is relatively slow (time constant about 100 ms at -82 mV), but accelerates with increasing hyperpolarization (time constant 37 ms at -130 mV) (Halliwell and Adams, 1982). I_Q is blocked by external Cs^+ but unaffected by Ba^{2+} . I_Q does not contribute to the normal resting potentials of hippocampal cells, but its activation serves to resist hyperpolarizing deviations from the resting potential, including a characteristic rebound depolarization during a hyperpolarizing current pulse, and hence stabilizes the membrane potential. Deactivation of I_Q contributes to the rebound depolarization and excitation following a hyperpolarizing pulse. I_Q contributes to spike after-hyperpolarization only when the spike is initiated from membrane potentials negative to the normal resting potential (Storm, 1989). As mentioned above, these six types of voltage-gated channels persist when Ca^{2+} -influx has been eliminated by Ca^{2+} -free medium, or by the Ca^{2+} channel blockers, Mn^{2+} or Cd^{2+} .

There are at least two types of K^+ currents which are Ca^{2+} -dependent. I_C is a "fast" and large Ca^{2+} -dependent K^+ -current, which is activated rapidly (within 1-2 ms) when Ca^{2+} ions flow through voltage-gated Ca^{2+} channels following their activation by a depolarization beyond -40 mV or during an action potential (Brown and Griffith, 1983b; Lancaster and Adams, 1986; Storm, 1987a; Lancaster and Nicoll, 1987). When the neuron is repolarized, I_C deactivates within 50-150 ms, depending on the voltage (Brown and Griffith, 1983b). The

activation of the current requires a relatively high concentration of internal Ca^{2+} (threshold $> 1 \mu\text{M}$) (Franciolin, 1988). I_C can be eliminated by Ca^{2+} -free medium and bath-application of Ca^{2+} -channel blockers Mn^{2+} , Cd^{2+} or Co^{2+} , and by injection of fast Ca^{2+} chelator BAPTA, but not by slow chelator EGTA (Lancaster and Nicoll, 1987; Storm 1987b; Madison et al., 1987a; Storm, 1989). I_C is also blocked by low concentration external TEA (1-10 mM) below that required to block the I_K , and by nM concentration charybdotoxin (CTX) (Lancaster and Nicoll, 1987; Storm, 1987c). Since I_C is strongly activated during a single action potential, its activation contributes to spike repolarization and generates the early phase of the spike after-hyperpolarization (Lancaster and Nicoll, 1987; Storm 1987a, b). I_C also has an influence on the initial part of the spike accommodation. At single channel level, I_C may be mediated through large-conductance (140-270 pS) Ca^{2+} -dependent K^+ channels, "BK" channels (Brett and Lancaster, 1985; Brett et al., 1986; Francilin, 1988; Ikemoto et al., 1989).

I_{AHP} rises slowly following Ca^{2+} entry, and peaks in 400-700 ms. It declines more slowly (time constant 1-1.6 s) on repolarization. Its decay is largely voltage-independent between -30 to -90 mV (Lancaster and Adams, 1986). I_{AHP} is activated by much lower Ca^{2+} concentrations (30 to 60 nM) than those required to activate I_C (Knopfel et al., 1989). It is not clear whether the slowness of I_{AHP} is partly intrinsic to the channels or whether it just reflects the time-course of the intracellular Ca^{2+} concentration or subsequent biochemical steps (Storm, 1990b). At single channel level, a class of small conductance (approximately 19 pS) Ca^{2+} -activated K^+ channels may mediate the I_{AHP} (Lancaster et al., 1987). I_{AHP} can be eliminated by Ca^{2+} -free medium and Ca^{2+} -channel blockers, Mn^{2+} , Cd^{2+} and Co^{2+} . Intracellular injection of BAPTA or EGTA also blocks the I_{AHP} (Lancaster and Nicoll, 1987). I_{AHP} differs from I_C

because I_{AHP} is resistant to TEA and CTX (Lancaster and Adams, 1986; Storm, 1987a; Lancaster and Nicoll, 1987). It is also insensitive to 4-AP in CA1 cells (Storm, 1990). In contrast to I_C , I_{AHP} can be regulated by a variety of neurotransmitters, acetylcholine and muscarinic agonists (via M_1 receptors) inhibit I_{AHP} (Cole and Nicoll, 1983; Madison et al., 1987a). I_{AHP} is about 10 fold more sensitive to muscarinic agonists than I_M (Madison et al., 1987a). Noradrenaline (via β_1 receptors) (Madison and Nicoll, 1982; Haas and Konnerth, 1983), histamine (via H_2 receptors) (Haas and Konnerth, 1983), serotonin (Andrade and Nicoll, 1987; Colino and Halliwell, 1987) and ACPD (via metabotropic glutamate receptors) (Charpak et al., 1990) can reduce I_{AHP} . These substances suppress I_{AHP} by blocking the K^+ current itself rather than affecting the Ca^{2+} transient (Knopfel et al., 1989). Adenosine (Haas and Greene, 1984) and dopamine (Benardo and Prince, 1982; Haas and Konnerth, 1983; also see Malenka and Nicoll, 1986) have been reported to enhance I_{AHP} . Activation of protein kinase C or cyclic AMP-dependent protein kinase also inhibit I_{AHP} (Baraban et al., 1985; Malenka et al., 1986a; Storm, 1990). I_{AHP} generates the long after-hyperpolarization following hippocampal action potentials. I_{AHP} provides a strong negative feedback control of the activity of the cells. It is activated by Ca^{2+} influx during the action potential and increases as the number of spikes increase (Lancaster and Adams, 1986). It tends to suppress further discharge by hyperpolarizing the cell and shunting depolarizing inputs. More importantly, I_{AHP} plays a major role in the spike frequency accommodation in hippocampal pyramidal cells (Madison and Nicoll, 1982, 1984).

3.3.4. Cl^- currents

$I_{Cl(V)}$ is a slow and voltage-dependent Cl^- current, which is activated by hyperpolarizing steps between -20 and -100 mV in the hippocampal pyramidal

cells (Madison et al., 1986). This current is not Ca^{2+} -dependent. It is blocked by Cd^{2+} and phorbol dibutyrate (Madison et al., 1986).

Another type of Cl^- current, the $I_{\text{Cl}(\text{CA})}$, is Ca^{2+} -dependent and voltage-insensitive (Owen et al., 1988). $I_{\text{Cl}(\text{CA})}$ is activated by $> 0.5 \text{ mM } \text{Ca}^{2+}$. It may be mediated through Ca^{2+} activated Cl^- channels of 20 pS conductance. This current may contribute to the long Ca^{2+} -dependent "tail currents" following Cl^- -loading coupled with suppression of Ca^{2+} -activated K^+ -currents (Brown and Griffith, 1983b).

3.3.5. "Leak" currents

"Leak" currents are the passive currents which remain around resting potential when voltage- and Ca^{2+} -gated currents are blocked. One of the "leak" currents is a voltage-insensitive K^+ current, which can be reduced by muscarinic agonists (Madison et al., 1987b; Benson et al., 1988). This effect may be mediated by a pertussis toxin insensitive GTP-binding protein (Brown et al., 1988). Some "leak" currents are mediated through Cl^- channels (Franciolini and Nonner, 1987; Franciolini and Petris, 1988; Owen et al., 1988). Both K^+ and Cl^- "leak" currents play an important role in stabilizing the membrane potential around -60 to -70 mV.

3.4. Evoked field potentials in the hippocampus

Due to the close packing and a parallel arrangement to each other of cells in the hippocampus, stimulation of excitatory afferents leads to a synchronous activation of a large population of neurons and generates large extracellular field potentials corresponding to EPSP activity (field EPSPs) and action potential discharge (population spike) (Andersen, 1975, Schwartzkroin and Mueller, 1987). In turn, the axon collaterals from the principal neurons activate a given interneuron population and generate field IPSPs. Moreover, direct stimulation of inhibitory interneurons affects a large number of principal neurons because their

axons ramify for hundreds of micrometers (Kunkel and Schwartzkroin, 1982). The discussion here will be focused on the field potentials in the CA1 regions. The field synaptic potentials can be generated by the stimulation of Schaffer collaterals or commissural afferents. The field EPSP recorded at the apical dendrites of the CA1 pyramidal cells is characterized as a large negative wave when the stratum radiatum is stimulated. The large negative wave actually represents a mixture of fields, generated largely by EPSPs from the apical dendrites and by some components of the IPSPs from the soma. This field EPSP can be interrupted by a positive peak when the stimulation strength is strong enough to cause the cells to discharge spikes. The positive peak may reflect a population spike generated in the somatic region. The negatively directed field EPSP is reversed in polarity to the positive wave, and the population spike manifests as a sharp negative wave when the recording electrode moves close to the CA1 cell body layer. The size of the population spike represents the number of synchronous firing cells. A negatively directed component recorded prior to the field EPSP and the population spike is the presynaptic volley. The presynaptic volley results from the extracellular currents surrounding the synchronously activated unmyelinated fibres running in the dendritic layer (Andersen et al., 1978) and is usually taken as an index of the number of fibres activated. Recently, field IPSPs have been analyzed in the presence of excitatory amino acid antagonists (Lambert et al., 1991b).

3.5. Ephaptic interactions

The large field potentials in the hippocampus are capable of influencing individual neuronal activity (Jefferys and Haas, 1982; Taylor and Dudek, 1982b; Turner et al., 1983). This phenomenon known as ephaptic interaction, is due to the effects of extracellular currents. The current flow associated with field potentials may discharge (or inhibit) neurons at the subliminal fringe of an active

population (Schwartzkroin and Mueller, 1987). Ephaptic interactions can act to recruit additional neurons into an already active population.

3.6. Electrotonic coupling

Electrophysiological and morphological evidence shows that electrotonic coupling exists among pyramidal cells and granule cells (MacVicar and Dudek, 1981, 1982; Taylor and Dudek, 1982a). Intracellular injection of Lucifer yellow, which does not cross chemical synaptic junctions, reveals gap junctions and the pattern of dye coupling in the hippocampus (MacVicar and Dudek, 1981). Anatomical ultrastructural studies suggest that gap junctions exist between interneurons (Kosaka, 1983; Schlander and Frotscher, 1986). Dudek et al. (1983) have demonstrated electrotonic coupling in a small percentage of cells by using antidromic stimuli to trigger "short-latency depolarization". The electrotonic coupling may facilitate or disrupt synchronous discharge produced by chemical synapses, depending on the relative strength and timing of chemical versus electrotonic connections (Traub et al., 1981; 1982; Traub et al., 1989; Dermietzel and Spray, 1993).

3.7. Excitatory postsynaptic potentials (EPSPs)

All major hippocampal afferents produce EPSPs in the hippocampal principal neurons (Andersen et al., 1966; 1975). Moreover, the afferents to hippocampal interneurons also generate EPSPs in these interneurons (Lacaille et al., 1987). Activation of the perforant path fibres produces EPSPs on the dendrites of granule cells (Blackstad, 1958; Hjorth-Simonsen and Jeune, 1972; Steward, 1976; McNaughton and Barnes, 1977). The unitary EPSP of granule cells is approximately 0.1 to 0.3 mV in amplitude (McNaughton et al., 1981). Activation of the mossy fibres generates EPSPs in the dendrites of CA3 pyramidal cells (Andersen and Lomo, 1966; Yamamoto, 1972). In CA1 field, excitatory inputs from Schaffer collaterals or commissural produce EPSPs in the

pyramidal dendrites (Andersen, 1960). The unitary EPSP is on average 0.15 mV in amplitude, probably produced by a single quantum of transmitter (Sayer et al., 1989; Andersen, 1990). EPSP has a reversal potential of approximately 0 mV. The EPSPs in response to distally located synapses are relatively slower than those elicited by proximally located synapses on the same cell. This is in agreement with the cable theory (Turner, 1988). EPSPs produced by neighbouring synapses can sum linearly or non-linearly with each other and with hyperpolarizing inhibitory potentials, depending on the space between the synapses and the timing of the synaptic input (Langeoen and Andersen, 1983). The summation effect will be greater for synapses contacting the same secondary dendrites than for more distributed dendritic contacts. L-Glutamate is the major excitatory neurotransmitter in the hippocampus. The CA1 pyramidal cell EPSPs are mediated by NMDA and non-NMDA receptors.

4. SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS

4.1. Excitatory neurotransmitters in the hippocampus

Glutamate and/or aspartate are thought to be the major excitatory neurotransmitters in the vertebrate central nervous system. In the hippocampus, all major fibre systems, including the perforant paths, the mossy fibre system, the Schaffer collateral/commissural system and the dentate associational/commissural system, are enriched in glutamate-like immunoreactivity (Storm-Mathisen et al., 1983; Liu et al., 1989; Bramham et al., 1990; Ottersen, 1991). These fibre systems also take up radiolabelled glutamate or aspartate (Taxt and Storm-Mathisen, 1984) and release glutamate and/or aspartate in a calcium dependent manner (Nadler et al., 1978; Hamberger et al., 1978; Ottersen, 1991). It has been shown that the reversal potential for glutamate-induced responses is similar to that for the natural

transmitter both in the Schaffer collateral system (Hablitz and Langmoen, 1982) and in the perforant paths (Crunelli et al., 1984).

As the major excitatory neurotransmitter in the hippocampus, glutamate has diverse actions in the hippocampal neurons and plays a very important role in the synaptic transmission of the hippocampus. The actions of glutamate are mediated by five different subtypes of glutamate receptors defined by the structural analogues of glutamate which have the highest specificity for each receptor type (Watkins et al., 1990; Zorumski and Thio, 1992; Barnes and Henley, 1992). These analogues of glutamate are N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), kainate, (\pm)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD) and L-2-amino-4-phosphonobutanoate (L-AP4). The ACPD receptor is referred to as the metabotropic glutamate receptor because it is linked to G-proteins and phosphoinositide metabolism (PI turnover). The AMPA and kainate receptors together are referred to as the non-NMDA receptors because these two receptors act very similarly in many brain areas. Autoradiographic studies have shown that the NMDA, AMPA, kainate and metabotropic receptors are dense in the molecular layer of the dentate gyrus, and the stratum oriens and the stratum radiatum of the CA1 and CA3 areas of the hippocampus (Young et al., 1991). NMDA and non-NMDA have been shown to be co-localized on the subsynaptic membrane (Bekkers and Stevens, 1989).

4.1.1. NMDA receptors and their role in synaptic transmission

NMDA receptor is the most well characterized glutamate receptor subtype because of its physiological properties and the development of NMDA receptor-specific antagonists. This receptor is a ligand-activated receptor-channel complex, which can be activated by glutamate or NMDA. L-Glutamate, L-aspartate and several sulphur amino acids, including L-homocysteic acid, are

potent endogenous NMDA receptor agonists (Mayer, 1991). The most potent and selective competitive NMDA antagonists are D-APV (Davies and Watkins, 1982), D-CPP and CPP (Davies et al., 1986; Olverman and Watkins, 1989). Noncompetitive antagonists, which apparently plug the NMDA channels in a voltage-dependent manner, include dissociative anaesthetics such as ketamine, PCP, TCP and MK-801, divalent cations such as Mg^{2+} and Zn^{2+} , and tricyclic antidepressants such as desipramine (Watkin et al., 1990; Mayer, 1991, MacDonald et al., 1991; Zorumski and Thio, 1992).

Unlike other ionotropic glutamate receptor channels which are permeable only to Na^+ and K^+ , NMDA receptor channels are highly permeable to Ca^{2+} . Activation of the NMDA receptor-channel complex allows Na^+ and Ca^{2+} influx, and K^+ efflux through the NMDA receptor channels (Mayer, 1991; Mayer and Miller, 1990). Single-channel studies have shown that NMDA receptor channels open for 5-10 ms on an average, with a main conductance state of 50 pS, and subconductance states of 10, 20, 30 and 40 pS (Cull-Candy and Usowicz, 1987; Jahr and Stevens, 1987; Ascher et al., 1988b, Cull-Candy et al., 1988). The main conductance state of the ion channel is preferentially opened by NMDA and shows significant permeability to Ca^{2+} (Ascher and Nowak, 1988b; Jahr and Stevens, 1987). Under the normal physiological conditions, the NMDA receptor-channel complex is regulated in a voltage-dependent manner by extracellular Mg^{2+} (Nowak et al., 1984; Mayer et al., 1984; Jahr and Steven, 1990; Mayer, 1991). The current-voltage (I-V) relationship for NMDA is highly nonlinear such that very little current flows at potentials negative to -70 mV, whereas beyond this point the current increases with depolarization. The I-V curve for NMDA becomes linear after removing Mg^{2+} from the recording medium (Nowak et al., 1984; Mayer et al., 1984; Jahr and Steven, 1990). At single channel level, Mg^{2+} has been found to block only the main conductance state (50 pS) in a voltage-

dependent manner (Nowak et al., 1984; Jahr and Stevens, 1987). The voltage-dependent blockade of NMDA receptor channels by extracellular Mg^{2+} may be due to the binding of the ion within the channel pore when the channel is open (Nowak et al., 1984; Mayer et al., 1984, Jahr and Stevens, 1990a). Recent evidence suggests that Mg^{2+} probably blocks the channels in both the open and closed conformations (Jahr and Stevens, 1990a,b). Since physiological level of Mg^{2+} (approximately 1 mM) in cerebrospinal fluid is much higher than the concentration that is required to have significant effect on NMDA channels, the synaptic transmission mediated through the NMDA receptors in the hippocampus is highly affected by the extracellular Mg^{2+} blockade of the NMDA channels under the normal physiological conditions. Johnson and Ascher (1990) have recently suggested that intracellular Mg^{2+} also blocks the NMDA receptor channels. However, the block would probably not influence the NMDA responses under normal physiological conditions because the concentration which is similar to the physiological intracellular Mg^{2+} levels (1-3 mM) has very little effect on the NMDA channels at voltages below 0 mV (Johnson, 1991).

Extracellular zinc, another divalent cation, has been shown to reversibly inhibit the NMDA-evoked responses. Unlike Mg^{2+} , Zn^{2+} non-competitively blocks the NMDA receptor-channel complex in a voltage-independent manner via a site presumed to be located on the extracellular domain of the complex (Peter et al., 1987; Westbrook and Mayer, 1987; Mayer et al., 1989b). Single channel analysis shows Zn^{2+} induces two effects on NMDA channels (Christine and Choi, 1990; Legendre and Westbrook, 1990). At low concentration (<10 mM), Zn^{2+} decreases the channel open probability by decreasing the channel opening frequency. At higher concentration, Zn^{2+} decreases the channel open time and burst duration, and reduces the single channel current amplitude. The effect of Zn^{2+} on the single channel current amplitude is voltage-dependent. It

has been suggested that Zn^{2+} may act at two separate sites in the NMDA complex (Christine and Choi, 1990; Legendre and Westbrook, 1990). Since Zn^{2+} is present in nerve terminals and synaptic vesicles in the hippocampus (Perez-Clausell and Danscher, 1985), it is possible that the ion can be released, together with glutamate, from the nerve terminals (Aniksztejn, 1987). Therefore, this ion may play a very important role in the hippocampal synaptic transmission. Zn^{2+} has been shown to reduce the NMDA receptor-mediated EPSPs as well as GABA receptor-mediated IPSPs in the hippocampus (Forsythe et al., 1988; Mayer and Vyklicky, 1989b).

Johnson and Ascher (1987) first reported that glycine strongly enhances the NMDA response, probably via an allosteric action at a site on the NMDA receptor-channel complex. Strychnine, a powerful antagonist of the inhibitory glycine receptor, did not inhibit the effect of glycine on the NMDA responses. Evidence has shown that the glycine binding sites located on the NMDA receptor-channel complex are distinct from the inhibitory glycine receptors (Langosch et al., 1990; Barnes and Henley, 1992). More recent studies suggest that glycine not only facilitates the NMDA responses, but also is essential for the NMDA receptor activation (Kleckner and Dingledine, 1988; Lerma et al., 1990; Sekiguchi, 1990; Vyklicky et al., 1990; Zorumski and Thio, 1992). Pharmacological and kinetic studies show that there are two binding sites for glycine on the NMDA receptor-channel complex. Occupation of these binding sites by glycine is required for the activation of the NMDA receptor by NMDA agonists (Benveniste et al., 1990; Javitt et al., 1990; Benveniste and Mayer, 1991; Clements and Westbrook, 1991). The glycine action on the NMDA receptor-channel complex can be antagonized by several drugs, including 7-chlorokynurenic acid (Kemp et al., 1988), CNQX, 3-amino-L-hydroxypyrrolid-2-

one (HA-996) and a series of derivatives of indole-2-carboxylic acid (Huettnner, 1989). HA-996 is also a partial agonist (Foster and Kemp, 1989).

The NMDA receptor-channel complex can be modulated by other agents, such as polyamines, sulfhydryl redox reagents and H⁺ ions. The endogenous polyamines, spermines and spermidine enhance NMDA responses in a voltage-independent fashion by binding a distinct site on the complex (Ransom and Stec, 1988; Williams et al., 1990, Scott, et al., 1993). Polyamines can enhance maximal responses to NMDA and glycine by increasing the affinity of the NMDA complex for glycine (McGurk et al., 1990). Polyamines also enhance the binding of non-competitive antagonists, such as MK-801 and TCP to the NMDA receptor channels (Ransom and Stec, 1988), and the binding of a competitive antagonist, CPP (Pullman and Powel, 1991). The disulfide reducing reagent dithiothreitol potentiates the NMDA responses whereas the disulfide oxidizing reagent 5, 5-dithio-bis-2-nitrobenzoic acid reduces NMDA responses in a voltage-independent manner by acting at a site that is distinct from the NMDA, glycine, polyamine binding sites (Aizenman et al., 1989; Reynold et al., 1990). Changes in the extracellular pH value can affect the NMDA receptor-channel complex (Tang et al., 1990; Vyklicky et al., 1990; Traynelis and Cull-Candy, 1990, 1991). Extracellular pH values greater and less than physiological pH enhance and depress NMDA currents, respectively. These effects are voltage-independent. Recent evidence suggests that changes in the extracellular pH value also change the potency of NMDA receptor competitive antagonists (Benveniste and Mayer, 1992). Other than the extracellular modulators, whole cell patch clamp studies show that intracellular factors are essential for maintaining the normal NMDA receptor function. During whole cell recording, the NMDA responses gradually "run down". The run down could be prevented if the recording electrode contained an ATP regenerating solution, suggesting that the loss of

similar factors is responsible for the irreversible decline in response (MacDonald et al., 1989).

Desensitization is a property shared by many ligand-gated ion channel-receptor complexes, including glutamate receptors. Three different modes of NMDA receptor desensitization have been observed. One type of NMDA receptor desensitization is voltage- and Ca^{2+} -dependent (Clark et al., 1990; Zorumski et al., 1989; Vyklicky et al., 1990). The rate and degree of desensitization increases with hyperpolarization and higher extracellular Ca^{2+} concentration (Clark et al., 1990). Intracellular injection of BAPTA reduces the Ca^{2+} -dependent desensitization (Mayer et al., 1991). With low extracellular Ca^{2+} , a second type of NMDA receptor desensitization, which is voltage-dependent and modulated by extracellular glycine, develops (Mayer, 1989b, Benveniste et al., 1990). The glycine-dependent NMDA receptor desensitization is reduced or inhibited by high concentrations of extracellular glycine (Mayer et al., 1991). The third form of NMDA receptor desensitization is Ca^{2+} - and glycine-independent (Sather et al., 1990; Shirasaki et al., 1990).

The degree which NMDA receptors contribute to synaptic transmission in the hippocampus is highly dependent on the extracellular Mg^{2+} concentration, the membrane potential and the state of synaptic inhibition. Under normal physiological conditions, the EPSP or EPSC mediated by NMDA receptors at the resting membrane potential in the hippocampal CA1 neurons is very small, if not totally absent (Collingridge et al., 1988a; Collingridge and Lester, 1989). The NMDA receptor-mediated component of the EPSP, which is APV-sensitive, appears when the cell is depolarized. The NMDA receptor-mediated component has a slow rise time (8-20 ms), a long duration (approximately 100-200 ms) and a reversal potential close to 0 mV (Collingridge et al., 1988a). The voltage-dependent NMDA component is due to the blockade of extracellular Mg^{2+} . The

NMDA component of the EPSP is affected by synaptic inhibition. The NMDA component can be observed at potentials near rest when the synaptic inhibition is blocked by GABA_A antagonists (picrotoxin or bicuculline) or reduced using voltage-clamp techniques (Collingridge et al., 1988a; Collingridge and Lester, 1989). The NMDA component is also frequency-dependent. Under normal physiological conditions, the NMDA component can be recorded during high-frequency stimulation (Herron et al., 1986; Collingridge, 1988b). It is suggested that during high-frequency stimulation, a neuron may become depolarized for a sufficient time to reduce the Mg²⁺ block of NMDA channels (Collingridge et al., 1988b).

4.1.2. Non-NMDA glutamate receptors

AMPA and kainate receptors together are generally referred to as non-NMDA receptors, which are readily distinguished pharmacologically from NMDA receptors. AMPA receptors are also called quisqualate receptors. However, quisqualate acts not only on the AMPA receptors, but also on the metabotropic glutamate receptors. AMPA appears to be much more selective to the AMPA receptors. Therefore, the ionotropic quisqualate receptors are named after AMPA in order to distinguish from the quisqualate-activated metabotropic glutamate receptors (Watkins et al., 1990; Zorumski and Thio, 1992). Autoradiographical studies show that the distribution of AMPA sites resembles that of NMDA sites; however, a higher level of binding is observed in the pyramidal cell layer (Monaghan et al., 1984a). Kainate binding sites are primarily confined to the stratum lucidum of CA3, the termination zone of the dentate granule cell-mossy fibres (Foster et al., 1981; Collingridge and Lester, 1989). L-Glutamate is the major endogenous neurotransmitter for the non-NMDA receptors. The concentrations of glutamate needed to activate physiological responses at non-NMDA receptors are 100 times greater than

those required at NMDA receptors (Patineau and Mayer, 1990). The most potent and selective competitive antagonists of the non-NMDA receptors are CNQX, DNQX and NBQX (Blake et al., 1988; Honore et al., 1988; Sheardown et al., 1990). At higher concentration, CNQX and DNQX may act as non-competitive NMDA antagonists by interacting with the glycine site (Birch et al., 1988; Harris and Miller, 1989; Yamada et al., 1989). NBQX is specific for non-NMDA receptors and also shows a 30 fold selectivity for AMPA receptors compared to kainate receptors (Sheardown et al., 1990).

The ion channels coupled to non-NMDA receptors are permeable nonselectively to monovalent cations (Na^+ and K^+), but exhibit a poor permeability to Ca^{2+} (Ascher and Nowak, 1988; Vylicky et al., 1988). The non-NMDA receptor channels are insensitive to Mg^{2+} (Nowak et al., 1984). Therefore, the I-V relationship for the two agonists is nearly linear over the range -90 to +30 mV. The reversal potential for AMPA or kainate is approximately 0 mV.

Non-NMDA receptors can be readily differentiated from NMDA receptors, but AMPA and kainate receptors are more difficult to differentiate from one another experimentally. AMPA and kainate show some competitive interactions, which may be explained by both agonists acting through the same receptor-channel complex with a common binding site (Lodge and Johnson, 1990, Barnes and Henley, 1992). However, independent actions of AMPA and kainate have also been observed (Perouansky and Grantyn, 1989; Mayer and Westbrook, 1987). It has been suggested that the diverse heterogeneous populations of binding sites for kainate and AMPA exist (Barnes and Henley, 1992). Several cloned non-NMDA receptor-channel complex subunits, when expressed alone or in combination with others, respond to both AMPA and kainate (Boulter et al., 1990; Dawson et al., 1990; Keinanen et al., 1990; Nakanishi et al., 1990).

However, the kainate selective non-NMDA receptor subunit has also been cloned (Egebjerg et al., 1991).

The channels coupled to AMPA and kainate receptors have multiple conductance states like those opened by NMDA (Jahr and Steven, 1987; Ascher and Nowak, 1988b; Cull-Candy et al., 1988; Cull-Candy and Usowicz, 1989). Activation of AMPA receptors preferentially opens a channel with conductance states of 8-15pS, while kainate receptor activation primarily opens the channel to smaller conductance states of 1-5 pS (Jahr and Stevens, 1987). Its mean open time of these conductances range from 0.5 to 5.3 ms. A high conductance (35 pS) activated by quisqualate, an AMPA receptor agonist, has been described in the hippocampal neurons by Tang et al. (1989). The mean open time is 3 to 8 ms. This conductance may underlie the generation of the fast AMPA-receptor-mediated EPSCs.

Desensitization occurs to both AMPA and kainate-activated responses. In dissociated and cultured hippocampal neurons, the response to glutamate and quisqualate desensitizes rapidly, while the response to kainate does not (Kiskin et al., 1986; MacDonald et al., 1987; Mayer and Vyklicky, 1989b; Trussell and Fischbach, 1989; Trussell et al., 1988). However, pretreatment with glutamate or with quisqualate substantially reduced the subsequent response to kainate (Kiskin et al., 1986; Trussell et al. 1988). Aniracetam (nootrophic agent), concanavalin A and wheat germ agglutinin (WGA) can reduce the desensitization of the non-NMDA receptors. Aniracetam appears to act by prolonging the AMPA channel open time (Tang et al., 1991; Vyklicky et al., 1991).

The non-NMDA responses have demonstrated "rundown" during whole-cell patch clamp recordings. This rundown can be prevented by the inclusion of

phosphorylation factors and activators of cAMP-dependent protein kinase in the patch electrode (Greengard et al., 1991; Wang et al., 1991).

Under normal physiological conditions, synaptic transmission at the resting membrane potential in the hippocampal CA1 pyramidal cells is primarily mediated by non-NMDA receptors. The non-NMDA receptors mediate a fast EPSP or EPSC component, which is voltage-independent over the range -100 to +40 mV and CNQX-sensitive (Hestrin et al., 1990a,b). The reversal potential of the EPSP or EPSC component is around 0 mV. The rise and the decay time constant of the non-NMDA component are 1-3 ms and 7-8 ms, respectively (Hestrin et al., 1990a,b). There is the possibility that the decay time constant of the fast EPSC may reflect the mean open time of the AMPA channel.

4.1.3. AP4 receptors

A fourth type of glutamate receptor has been proposed based on the antagonist actions of the L-glutamate analogue L-2-amino-4-phosphonobutyrate (L-AP4). The distribution of L-AP4 receptors is highly localized. In the hippocampus, L-AP4 receptors are located in the lateral perforant pathway (Koerner and Cotman, 1981) and in the guinea pig mossy fibre pathway (Yamamoto et al., 1983; Lanthorn et al., 1984). L-AP4 depresses the amplitude of the EPSC or EPSP in hippocampal neurons (Cotman et al., 1986; Forsyther and Clements, 1990). It has been suggested that the activation of presynaptic AP4 receptors by L-AP4 or L-glutamate depresses the release of glutamate from the presynaptic terminals (Cotman et al., 1986; Crowder et al., 1987; Gannon et al., 1989; Forsyther and Clements, 1990). The AP4 receptors may act as autoreceptors at the synaptic terminals. The mechanisms of this presynaptic inhibition are still not very clear. Recent evidence suggests that L-AP4 depresses glutamate release by inhibiting calcium influx via G-protein-coupled presynaptic AP4 receptors in cultured olfactory bulb neurons (Trombley and

Westbrook, 1992). Data on elucidating the precise binding site through which L-AP4 exerts its inhibitory effect on excitatory synaptic transmission are limited. This is because no selective agonists and antagonists are known to have sufficient affinity at the L-AP4 receptor that would enable the development of radioligand binding assays.

4.1.4. ACPD receptors

ACPD receptors are a distinct family of glutamate receptors, members of which are coupled to various second messenger systems through GTP binding proteins (G-proteins) (Schoepp and Conn, 1993; Tanabe et al., 1992, Desai et al., 1992). The G-protein-linked glutamate receptors, also referred to as metabotropic glutamate receptors (mGluRs), are selectively activated by (\pm)1-aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD) or its active isomer 1s,3R-ACPD (Schoepp and Conn, 1993; Conn and Desai, 1990; Palmer et al., 1989). ACPD receptors has been found in the CA1, CA3 and the dentate gyrus of the hippocampus (Young et al., 1991). Glutamate, quisqualate and ibotenate can activate ACPD receptors nonselectively. ACPD receptors were first described as the glutamate receptors that are coupled to phospholipase C (PLC) and phosphoinositide hydrolysis (Sladeczek et al, 1985; Nicoletti et al., 1985), ACPD receptors now have been demonstrated to couple with other second messenger systems which include the activation of phospholipase D (Boss and Conn, 1992) and changes in cAMP formation (Schoepp et al., 1992; Schoepp and Johnson, 1993; Casabona et al., 1992; Winder and Conn; 1992). Recent molecular studies have shown that ACPD receptors are a highly heterogeneous class of glutamate receptors that are coupled to multiple second messenger systems (Tanabe et al., 1992; Schoepp and Conn; 1993). L-AP3 is able to inhibit the ACPD-stimulated phosphoinositide hydrolysis in the hippocampal slices (Schoepp et al., 1991). However, it fails to prevent the inhibition of

forskolin-stimulated cAMP formation by 1s,3R-ACPD (Schoepp and Johnson, 1993; Casabona et al., 1992). Therefore, the ACPD receptors that are negatively linked to cAMP formation are distinct from the phosphoinositide hydrolysis-linked ACPD receptors.

ACPD receptors play a significant role in the modulation of ion channels and synaptic transmission in the hippocampus (Desai et al., 1992; Schoepp and Conn et al., 1993). Activation of ACPD receptors by ACPD receptor agonists depolarizes hippocampal neurons associated with an increase in the input resistance by reducing the voltage-dependent K^+ current (I_M). In addition, the activation of ACPD receptors blocks the spike frequency accommodation and the slow after-hyperpolarizing potential (AHP) by blocking the Ca^{2+} -dependent K^+ current (I_{AHP}), and delays the spike repolarization (Charpak et al., 1990; Stratton et al., 1989; Desai and Conn, 1991; Hu and Storm, 1991; Hu and Storm, 1992). These ACPD receptor-mediated actions enhance excitability of hippocampal neurons. Furthermore, the activation of ACPD receptors results in inhibition of a high-threshold Ca^{2+} current in cultured hippocampal neurons (Lester and Jahr, 1990, Swartz and Bean, 1992).

Several lines of evidence have shown that the activation of ACPD receptors by ACPD receptor agonists reduces both the NMDA receptor- and non-NMDA receptor-mediated EPSPs, and also suppresses the $GABA_A$ receptor-mediated fast IPSP and the $GABA_B$ receptor-mediated slow IPSP in the hippocampal CA1 neurons (Baskys and Malenka, 1991; Pacelli and Kelso, 1991). The mechanisms of the inhibition of synaptic transmission by ACPD receptor agonists are not clear. It has been suggested that the reduction of EPSPs by ACPD receptor agonists is probably due to the activation of presynaptic ACPD receptors which act as the autoreceptor, and the subsequent reduction of glutamate release (Baskys and Malenka, 1991). Recent evidence

suggests that the reduction of hippocampal IPSPs by ACPD agonists is at least partially mediated by a reduction in synaptic excitation of GABAergic interneurons (Desai and Conn, 1992).

L-AP3 selectively inhibits the ACPD-stimulated phosphoinositide hydrolysis, but fails to block both the ACPD agonist-induced actions on the excitability of hippocampal neurons (Hu and Storm, 1992) and the ACPD agonist-induced depression of hippocampal synaptic transmission (Goh and Musgrave, 1993). Boss et al. (1992) have recently shown that the ACPD agonist-induced phosphoinositide hydrolysis and the modulation of hippocampal pyramidal cell excitability do not undergo parallel developmental regulation. It is believed that the ACPD receptors that mediate the ACPD agonist-induced actions on hippocampal neuron excitability and synaptic transmission are either L-AP3 insensitive or distinct from the phosphoinositide hydrolysis-linked ACPD receptors. It is possible that different subtypes of ACPD receptors mediate different types of physiological responses by coupling to different second messenger systems. The discovery of more selective and potent antagonists of ACPD receptors will help to establish the functions of specific ACPD receptor subtypes. Recently, a new ACPD receptor antagonist, (+)- α -Methyl-4-carboxyphenylglycine ((+)-MCPG) has been suggested to be more selective than L-AP3 (Ito et al., 1992; Jane et al., 1993). However, the selectivity of this drug has been questioned by others (Chinestra et al., 1993).

Recent evidence has shown the involvement of ACPD receptors in synaptic plasticity (Behnisch et al., 1991; Ito and Sugiyama, 1991; Aniksztejn et al., 1992; Bortolotto and Collingridge, 1993). This action of the ACPD receptors will be further discussed in the chapter on LTP.

4.2. Inhibitory neurotransmitters in the hippocampus

γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain (Nicoll et al., 1990; Sivilotti and Nistri, 1992). Immunohistochemical studies show that the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) is concentrated in the nerve terminals of interneurons in the hippocampus (Ribak et al., 1978; Somogyi et al., 1983; Schwartzkroin and Mueller, 1987). The interneuron terminals containing GAD-like immunoreactivity make synaptic contacts with pyramidal and granule cell somata and dendrites (Ribak et al., 1978; Somogyi et al., 1983). There are at least two types of GABA receptors in the hippocampal neurons. They are GABA_A and GABA_B receptors. In the hippocampal CA1 field, GABA_A receptors are found in somata and dendrites of pyramidal cells while GABA_B receptors are mainly located in pyramidal dendrites and presynaptic terminals (Nicoll et al., 1990; Sivilotti and Nistri, 1992).

4.2.1. GABA_A receptors

The GABA_A receptor-channel complex comprises binding sites for GABA and for its allosteric modulators (Sivilotti and Nistri, 1992). Activation of GABA_A receptors by GABA opens Cl⁻ channels. Single channel analysis has shown that GABA activates a channel with a main conductance state of 20-30 pS and multiple subconductances (Segal and Barker, 1984a; Gary and Johnson, 1985; Edward et al., 1989; Sivilotti and Nistri, 1992). In addition to GABA, muscimol and isoguvacine, GABA_A agonists, can also selectively activate GABA_A receptors thereby opening the GABA_A channels (Segal and Barker, 1984b; Sivilotti and Nistri, 1992). Bicuculline and picrotoxinin are selective GABA_A antagonists. While bicuculline acts as a competitive GABA_A antagonist, picrotoxinin does not block GABA responses competitively. The binding site of picrotoxinin appears to be closely associated with the Cl⁻ channel of the GABA_A

receptor (Sieghart, 1992). It has been shown that picrotoxinin reduces the mean open time and the frequency of channel opening (Twyman et al., 1989). Penicillin also antagonizes GABA_A-mediated responses in a non-competitive manner (MacDonald and Barker, 1977; Pickles and Simmonds, 1980). The GABA_A receptor channels can be allosterically modulated by several classes of drugs, such as benzodiazepines, barbiturates and steroids. Benzodiazepines, such as diazepam, enhance the actions of GABA at the GABA_A receptor by increasing the frequency of the Cl⁻ channel opening (Study and Barker, 1981). Barbiturates, such as pentobarbital, enhance the actions of GABA by increasing the mean channel open time (Study and Barker, 1981; Sigel et al., 1990). At higher concentrations, barbiturates are able to enhance Cl⁻ conductance in the absence of GABA (Sigel et al., 1990; Sieghart, 1992). Like barbiturates, steroids, such as the anaesthetic alfaxalone, also enhance GABA-stimulated Cl⁻ conductance by prolonging the open time of the Cl⁻ channel (Barker et al., 1987; Peters et al., 1988). A run down of the GABA_A responses has been observed during whole cell recording. The run down can be prevented by including Mg²⁺-ATP in the low Ca²⁺ intracellular medium (Chen et al., 1990). It appears that the GABA_A receptor function is regulated by a phosphorylation process. This phosphorylation is not mediated by cAMP because analogues of cAMP decrease GABA_A responses (Tehrani et al., 1989).

4.2.2. GABA_B receptors

It has been found that GABA can presynaptically inhibit the release of other neurotransmitters. This action of GABA is insensitive to bicuculline and mimicked by the GABA analogue baclofen. The receptors mediating this GABA action have been classified as GABA_B receptors (Hill and Bowery, 1981). Baclofen is a selective agonist of this receptor. Electrophysiological evidence has shown that GABA_B receptors mediate not only the presynaptic actions of

GABA and baclofen, but also the postsynaptic actions of these drugs in the hippocampus (Newberry and Nicoll, 1984; Dutar and Nicoll, 1988a, b). Whether the postsynaptic and the presynaptic GABA_B receptors are homogenous, however, is still not clear (Dutar and Nicoll, 1988a,b; Thompson and Gahwiler, 1992; Solis and Nicoll, 1992a,b; Lambert et al., 1993; Potier and Dutar, 1993). CGP35348 and 2-hydroxy-saclofen can selectively antagonize both the presynaptic and the postsynaptic GABA_B responses. On the other hand, phaclofen, which can completely block the postsynaptic GABA_B responses, has little effect on the presynaptic GABA_B responses (Dutar and Nicoll, 1988a,b; Davies et al., 1990; Olpe et al., 1990; Solis and Nicoll, 1992a,b).

Activation of GABA_B receptors in the hippocampal pyramidal cells results in an increase in the membrane conductance for K⁺ (Newberry and Nicoll, 1984; 1985). The increase in K⁺ conductance by GABA_B agonists is blocked by pertussis toxin and the hydrolysis-resistant GDP analog, GDPβS (Nicoll, 1988; Nicoll et al., 1990; Thompson and Gahwiler, 1992; Potier and Dutar, 1993). It appears that the GABA_B receptor-mediated increase in K⁺ conductance is linked to a pertussis toxin-sensitive G protein. Evidence has shown that this G protein does not couple to intracellular second messengers, such as Ca²⁺ or cAMP, but is directly linked to the K⁺ channels (Andrade et al., 1986). Activation of protein kinase C with phorbol esters blocks the GABA_B receptor-mediated increase in K⁺ conductance (Baraban et al., 1985; Andrade et al., 1986). The effects of GABA_B agonists on voltage-dependent K⁺ currents have also been reported (Saint et al., 1990; Gage, 1992). GABA_B agonists can modulate a transient voltage-dependent K⁺ current, the A current, in hippocampal neurons. The effects of GABA_B agonists on Ca²⁺ currents are less clear than those on K⁺ currents in the hippocampal neurons. It has been suggested that the activation of GABA_B receptors by baclofen does not change sustained Ca²⁺ current in

hippocampal neurons (Gahwiler and Brown, 1985). However, Scholz and Miller (1991) have recently reported that activation of GABA_B receptors by baclofen inhibits a ω -CgTX-sensitive Ca²⁺ current in the soma of cultured hippocampal pyramidal cells through the activation of a pertussis toxin-sensitive G protein. More recently, Mintz and Bean (1993) have shown that the activation of GABA_B receptors in cerebellar Purkinje neurons by baclofen suppresses P-type Ca²⁺ currents through a G protein-mediated mechanism. It is not clear whether baclofen has similar effect on P-type Ca²⁺ channels in hippocampal pyramidal neurons. At single channel level, data on GABA_B receptors are limited. Premkumar et al. (1990) have recently reported that the GABA- or baclofen-induced K⁺ channels in cultured neurons have multiple conductance states with many smaller conductance states of 5-6 pS and a maximum conductance of 70 pS. The GABA_B agonist-induced K⁺ currents are blocked by GABA_B antagonists and suppressed by pertussis toxin. Whether the properties of the channels activated by applied GABA_B agonists on the soma of cultured neurons are the same as those properties of the channels coupled to GABA_B receptors in the dendrites remains to be determined.

The GABA_B receptors located in the presynaptic terminals are not as well characterized as the postsynaptic GABA_B receptors. While the actions of GABA and baclofen which are mediated by the presynaptic GABA_B receptors are blocked by GABA_B antagonists, such as 2-hydroxy-saclofen and CGP35348, pertussis toxin and protein kinase C, it is not very clear whether the actions of GABA and baclofen are mediated through the activation of K⁺ conductance, depression of Ca²⁺ current or other mechanisms (Nicoll et al., 1990; Sivilotti and Nistri, 1992; Thompson and Gahwiler, 1992; Thompson et al., 1993).

4.2.3. *GABA_A and GABA_B receptor-mediated responses*

GABA can elicit multiple types of responses in hippocampal pyramidal neurons (Andersen et al., 1980; Alger and Nicoll, 1982b; Nicoll et al., 1990). GABA evokes a hyperpolarization with a reversal potential of -70 mV in the soma. This hyperpolarization is due to an outward Cl⁻ current. The second GABA-evoked response is a depolarization in the dendrites. The induction of this depolarization requires a higher concentration of GABA. This depolarizing response is due to an inward Cl⁻ current and has an approximate reversal potential of -50 mV. GABA also evokes a bicuculline-sensitive fast hyperpolarization in the dendrites (Alger and Nicoll, 1982b; Newberry and Nicoll, 1984a,b). This hyperpolarization has a reversal potential of -70 mV, which is similar to the somatic hyperpolarization. The GABA-induced somatic hyperpolarization, dendritic depolarization and fast hyperpolarization are blocked by bicuculline and picrotoxinin, and are Cl⁻-mediated. It has been suggested that GABA_A receptors located on the main dendritic shafts mediate the dendritic fast hyperpolarization while the GABA_A receptors on the fine dendritic arbors are responsible for the dendritic depolarization. Nevertheless, the difference in the direction of Cl⁻ movement between the soma and the dendrites raises the questions whether GABA_A receptors are homogenous or a dendritic Cl⁻-uptake mechanism that reverses the electrochemical Cl⁻ gradient across dendritic membranes exists (Alger and Nicoll, 1982b; Misgeld et al., 1986; Sieghart, 1989). These questions remain to be answered. In addition to the GABA_A receptor-mediated fast hyperpolarization in the dendrites, a dendritic slow hyperpolarization can be elicited by GABA. This slow hyperpolarization is resistant to GABA_A antagonists and blocked by GABA_B antagonists. This hyperpolarization is K⁺-dependent. It is believed that this dendritic slow

hyperpolarization is mediated by GABA_B receptors. Baclofen can evoke a similar hyperpolarization (Dutar and Nicoll, 1988a, b).

4.2.4. GABA_A and GABA_B receptor-mediated IPSPs

Under normal physiological conditions, GABA receptors mediate three types of IPSPs in the hippocampal pyramidal neurons (Nicoll et al., 1990). A fast IPSP can be evoked by antidromic stimulation of pyramidal cell axons in the alveus. This fast antidromic IPSP with a latency to peak of 30-50 ms and a duration of 200-300 ms is mediated mainly by somatic GABA_A receptors (Alger and Nicoll, 1982a). This fast antidromic IPSP has a reversal potential of around -70 mV and is changed by altering the Cl⁻ gradient across the membrane (Spencer and Kandel, 1961c, Alger and Nicoll, 1982a, Dingledine and Langmoen, 1980). Orthodromic stimulation of afferents in the stratum radiatum induces a fast IPSP and a slow IPSP. The latency and duration of the orthodromic fast IPSP is very similar to the antidromic fast IPSP. The orthodromic IPSP is generated mainly on the dendrites of the pyramidal cells by activation of GABA_A receptors, but also has somatic component elicited via feedback and/or feedforward mechanisms (Alger and Nicoll, 1982a). The orthodromic slow IPSP has a slow latency to peak of 150-250 ms and a duration of 400-1500 ms (Alger and Nicoll, 1982a; Davies et al., 1990). This slow IPSP has a reversal potential of approximate -90 mV. This slow IPSP is due to an increase in K⁺ conductance by activation of GABA_B receptors. In the presence of 4-AP and pentobarbital, orthodromic stimulation of the stratum radiatum can evoke a depolarizing IPSP. This depolarizing IPSP has a reversal potential of -50 mV and is blocked by GABA_A antagonists (Alger and Nicoll et al., 1982a). The GABA_A receptors mediating this depolarizing IPSP are located in the dendrites area. The reasons for the absence of this depolarizing IPSP under normal conditions are not very clear. One hypothesis is that the powerful fast

and slow IPSPs mask the depolarizing IPSP because it has been shown that blockade of the slow IPSP by pertussis toxin reveals a depolarizing IPSP (Thalmann, 1988a,b).

Similar to GABA-induced GABA_A responses, the antidromic IPSP, the orthodromic fast IPSP and the depolarizing IPSP are blocked by GABA_A antagonists and altered by a change in extracellular or intracellular Cl⁻ concentration. The slow IPSP and the GABA_B agonist-induced dendritic hyperpolarization seem to be mediated by the same type of GABA_B receptors through similar mechanisms. Both are sensitive to pertussis toxin and blocked by the same type of GABA_B antagonists, Cs⁺, QX-314, and protein kinase C (Dutar and Nicoll, 1988a,b; Andrade, 1991; Lambert et al., 1989; Nathan et al., 1990). These GABA_B responses are also not affected by cAMP and intracellular injection of Ca²⁺ chelators (i.e., BAPTA and EGTA) (Alger, 1984; Newberry and Nicoll, 1984b; Hablitz and Thalmann, 1987).

4.2.5. Presynaptic GABA_B receptor-mediated actions

GABA_B receptors have been found not only at postsynaptic sites but also at both inhibitory and excitatory nerve terminals in the hippocampus (Bowerly et al., 1990; Nicoll et al., 1990; Thompson et al., 1993). While the postsynaptic GABA_B receptor-mediated responses are primarily due to an increase in K⁺ conductance, the mechanisms underlying the presynaptic GABA_B receptor-mediated the depression of both inhibitory and excitatory synaptic transmission are not clear. It is possible that the activation of presynaptic GABA_B receptors decreases Ca²⁺ influx by directly blocking voltage-gated conductances (Scholz and Miller, 1991) and/or by increasing K⁺ conductances (Thompson and Gahwiler, 1992; Saint et al., 1990). An reduction in Ca²⁺ influx in presynaptic terminals may lead to a depression in synaptic transmission. However, the current evidence is not sufficient to make a conclusion. It has been reported that

presynaptic and postsynaptic GABA_B receptors have distinct pharmacological and physiological properties (Dutar and Nicoll, 1988b). Phaclofen, a weak GABA_B antagonist, blocks the postsynaptic GABA_B receptor-mediated responses. However, this agent does not block the presynaptic GABA_B responses (Dutar and Nicoll, 1988b; Davies et al., 1990). Intraventricular injection of pertussis toxin blocks the hippocampal slow IPSP without affecting the presynaptic action of baclofen (Dutar and Nicoll, 1988b). Tetrahydroaminoacridine (THA), a K⁺ channel blocker, inhibits the actions of baclofen and GABA mediated through postsynaptic GABA_B receptor, and has no effect on the presynaptic GABA_B receptor-mediated responses (Lambert and Wilson, 1993). These lines of evidence seem to support the idea that presynaptic and postsynaptic GABA_B receptors are different subtypes of GABA_B receptors. However, the idea is not supported by several recent reports (Thompson and Gahwiler, 1992; Solis and Nicoll, 1992a). CGP35348 and 2-hydroxy-salcofen, more potent GABA_B antagonists, are able to block the postsynaptic GABA_B responses as well as presynaptic GABA_B responses (Davies et al., 1990; Thompson and Gahwiler, 1992; Solis and Nicoll, 1992a). These antagonists not only block the paired-pulse depression of IPSPs, which is believed to be mediated by the GABA_B receptors at the inhibitory nerve terminals, but also prevent the baclofen-induced EPSP depression, which is mediated by the GABA_B receptors at the excitatory nerve terminals. Direct injections of pertussis toxin into the hippocampus prevents both the postsynaptic and the presynaptic GABA_B receptor-mediated responses (Potier and Dutar, 1993). This result contradicts the previous finding reported by Dutar and Nicoll (1988b). The discrepancy between these two findings may be due to the site of pertussis toxin injection since the toxin diffuses poorly in nervous tissue (Van der Ploeg et al., 1991). Thus, pertussis toxin directly injected into the hippocampus

may cause a better effect on GABA_B receptors than that injected intraventricularly. Furthermore, protein kinase C activator, phorbol esters, also inhibits both the postsynaptic and the presynaptic GABA_B receptor-mediated responses (Thompson and Gähwiler, 1992; Dutar and Nicoll, 1988). It is apparent that the recent evidence seems to more favour the idea that the presynaptic and the postsynaptic GABA_B receptors are pharmacologically indistinguishable.

4.2.6. Spontaneous IPSPs

In addition to stimulation-induced IPSPs or IPSCs, spontaneous IPSPs or IPSCs have been observed in the hippocampal neurons (Alger and Nicoll, 1980; Collingridge et al., 1984; Miles and Wong, 1984). These spontaneous IPSPs are blocked by bicuculline and picrotoxin and are not sensitive to GABA_B antagonists. The spontaneous IPSPs are enhanced by pentobarbitone and suppressed by tetrodotoxin (TTX) (Alger and Nicoll, 1980). It is apparent that interneurons discharge is needed to generate these spontaneous IPSPs. Otis and Mody (1992) have recently showed that spontaneous IPSCs recorded in the presence of glutamate receptor blocker, CNQX and APV, are not affected by the application of baclofen and CGP35348. This evidence indicates that under normal conditions spontaneous transmitter release does not activate both presynaptic and postsynaptic GABA_B receptors.

Another type of spontaneous IPSPs or IPSCs, called miniature IPSPs or IPSCs, are observed in the presence of TTX and Cd²⁺ substitution of Ca²⁺ (Ropert et al., 1990; Otis et al., 1990). These miniature IPSCs are also blocked by bicuculline and picrotoxinin. The miniature IPSCs are much smaller and less frequent than the TTX-sensitive spontaneous IPSCs. It appears that GABA release continues even in the absence of firing interneurons (Ropert et al., 1990).

4.2.7. Inhibitory Circuitry

While the major afferents produce EPSPs in the hippocampal principle cells, the inhibitory potentials are very powerful and have ubiquitous features of hippocampal cell physiology (Kandel et al., 1961; Spencer and Kandel, 1968; Purpura et al., 1968). In the CA1 region, IPSPs can be generated through two different types of inhibitory pathways: a feedback (recurrent) and a feedforward circuit (Kandel et al., 1961; Dunwiddie et al., 1980; Buzsaki, 1984; Alger and Nicoll, 1982a).

The feedback inhibition is a circuit consisting of three elements. Firstly, an excitatory input directly arrives at the pyramidal cells. Secondly, the excitatory output of the pyramidal cells excites the inhibitory cells through recurrent axon collaterals. These inhibitory cells are mainly basket cells and O/A interneurons. Thirdly, the inhibitory cells may discharge and inhibit the pyramidal cells, including those which initially activated the interneurons (Buzsaki, 1984). The feedback inhibition is supported by studies that antidromic stimulation of alvear fibres produces prominent IPSPs in the pyramidal cells (Kandel et al., 1961; Andersen et al., 1964a). The feedback IPSP initiated by an antidromic stimulation is mediated by GABA_A receptors and has a reversal potential of approximately -70 mV, resulting from an increase in Cl⁻ conductance (Spencer and Kandel, 1961c, Alger and Nicoll, 1982a, Dingledine and Langmoen, 1980). It has been suggested that feedback inhibitory fibres terminate primarily on the soma and the initial segment region of the pyramidal cells (Kandel et al., 1961; Andersen et al., 1964a; Gottlieb and Cowan, 1972; Schwartzkroin et al., 1982). However, these GABAergic terminals also make contact with the apical and basal dendrites of pyramidal neuron although the density of contacts on the dendrites is lower than that on the soma (Somogyi et al., 1983).

In a feedforward inhibition, orthodromic stimulation of the stratum radiatum or the stratum oriens directly excites the inhibitory cells, which in turn induces IPSPs (Alger and Nicoll, 1982a). Orthodromic stimulation produces a fast GABA_A receptor-mediated IPSP and a slow GABA_B receptor-mediated IPSP (Alger and Nicoll, 1982a; Alger, 1984). The fast IPSP has characteristics similar to the antidromic IPSP. The slow IPSP appears to be generated on the pyramidal cell dendrites (Leung, 1978; Lacaille et al., 1989). The inhibitory cells excited by orthodromic stimulation include basket cells, O/A interneurons and L-M interneurons.

L-M interneurons make contact primarily with the dendrites of the pyramidal cells while basket cells and O/A interneurons make synapses not only on the somata of pyramidal cells, but also on the basal and proximal apical dendrites (Lacaille et al., 1989). Basket cells and O/A interneurons mediate both feedforward and feedback inhibition whereas L-M interneurons mediate solely feedforward inhibition in the CA1 field of the hippocampus. It has been suggested that O/A interneurons and basket cells mediate the fast IPSP while L-M interneurons mediate the slow IPSP in the pyramidal cells (Alger and Nicoll, 1982a, b; Alger, 1984; Newberry and Nicoll, 1985; Lacaille et al., 1989; Williams and Lacaille, 1992).

4.3. Other neurotransmitters in the hippocampus

Several other neurotransmitters have been found in the hippocampus (Nicoll et al., 1990; Schwartzkroin and Mueller, 1987; Swanson et al., 1987). These neurotransmitters are primarily localized in the nerve terminals of extrinsic projections from other areas of the brain. Most of these neurotransmitters are not as well studied as glutamate and GABA in the hippocampus.

4.3.1. Acetylcholine

Acetylcholine (ACh) is a major neurotransmitter in the CNS. The major cholinergic fibres in the hippocampus are from the septum (Lewis et al., 1967, Swanson et al., 1987). ACh plays a very important role in hippocampal function, such as θ rhythm activity (Petsche et al., 1962; Andersen et al., 1979).

Both muscarinic and nicotinic receptors have been found in the hippocampus (Kuhar and Yamamura, 1976; Marks and Collins, 1982; Schwartzkroin and Mueller, 1987; Swanson et al., 1987). However, most of the cholinergic responses in the hippocampus seem to be mediated by muscarinic receptors. Application of ACh or carbachol, an ACh analogue, generally induces a slow depolarization associated with an increase in the input resistance of hippocampal pyramidal cells (Benardo and Prince, 1982; Dodd, et al., 1981; Segal, 1982; Cole and Nicoll, 1983, 1984). It has been suggested that the ACh-evoked depolarization is due to the blockade of a voltage-independent leak K^+ current (Benardo and Prince, 1982; Benson et al., 1988; Madison et al., 1987a) and a voltage-dependent K^+ current, the M current (Halliwell and Adams, 1982). The I_M is probably mediated by M_2 receptors (Nicoll et al., 1990). Activation of muscarinic receptors also decrease a Ca^{2+} -dependent K^+ current, the I_{AHP} (Benardo and Prince, 1982; Madison et al., 1987a; Muller et al., 1988) and a transient K^+ , the I_A (Nakajima et al., 1986). A decrease in Ca^{2+} currents by the activation of muscarinic receptors has also been reported (Gahwiler and Brown, 1987b). A transient hyperpolarization induced by ACh or carbachol has also been reported (Benardo and Prince, 1982; Haas, 1982). Muscarinic receptors have been suggested to mediate a presynaptic depression in the hippocampus (Hounsgaard, 1978; Valentino and Dingledine, 1981; Dutar and Nicoll, 1988c; Williams and Johnston, 1988, Pitler and Alger, 1992). The mechanisms of this presynaptic inhibition are not very clear.

4.3.2. Noradrenaline

Noradrenergic innervation is denser in the hilus of the dentate gyrus than in the CA1 and CA3 fields (Swanson et al., 1987). The noradrenergic terminals in the hippocampus are largely from the pontine nucleus locus coeruleus (Blackstad et al., 1967). In hippocampal pyramidal cells, one of the most significant effects of noradrenaline (NA) is the blockade of the slow AHP (I_{AHP}) (Haas and Konnerth, 1983; Madison and Nicoll, 1982,1986). This action of NA is mediated by activation of β_1 receptors, which is probably involved in a cAMP-dependent protein kinase (Nicoll et al., 1990). Application of NA produce a hyperpolarization of CA1 neurons and sometime a depolarization(Langmoen et al., 1981; Segal, 1981). The hyperpolarization is mediated by an activation of α receptors and the depolarization is mediated by β receptors (Nicoll et al., 1990). Andreasen and Lambert (1991) have recently reported that NA receptors participate in the regulation of GABAergic inhibition in hippocampal CA1 neurons.

4.3.3. Serotonin

Serotonergic fibres from the raphe nuclei innervate all fields of the hippocampus (Kohler, 1982; Swanson et al., 1987). Serotonin, also called 5-hydroxytryptamine (5-HT), primarily induces a hyperpolarization associated with a decrease in the input resistance of the hippocampal neurons (Segal, 1980; Jahnsen, 1980). This hyperpolarization is due to an increase in a K^+ conductance by the activation of 5-HT $_1A$ receptors (Segal and Gutnick, 1980; Andrade and Nicoll; 1987; Colino and Halliwell, 1987; Ropert, 1988). 5-HT $_1A$ receptors are coupled to K^+ channels through a pertussis toxin-sensitive G protein. These K^+ channels probably also couple to GABA $_B$ receptors (Andrade et al., 1986; Nicoll, 1988). In addition to the hyperpolarization, 5-HT-induces an depolarization which is probably due to a blockade of a leak K^+ conductance,

has also been reported (Jahnsen et al., 1980, Andrade and Nicoll, 1987, Colino and Halliwell, 1987). 5-HT can reduce I_{AHP} in hippocampal neurons (Andrade and Nicoll, 1987; Colino and Halliwell, 1987). The receptor subtype which mediates the depolarization and the blockade of AHP is not 5-HT_{1A} and remains to be determined.

4.3.4. Dopamine

It has been reported that hippocampus receives dopaminergic fibres from other areas of the brain such as substantia nigra (Swanson, 1982; Swanson et al., 1987). Dopamine plays a significant role in the functioning of the brain. It has been suggested that dopamine D1 receptors can facilitate release of transmitters like GABA in the midbrain. However, the functions of dopamine in the hippocampus are not very clear (Nicoll et al., 1990).

4.3.5. Histamine

Hippocampus receives histaminergic projections from the supramammillary region of the midbrain (Segal and Landis, 1974; Schwartzkroin and Mueller, 1987). Histamine evokes a hyperpolarization of hippocampal neurons. This effect is probably mediated by H₂ receptors and activation of a K⁺ conductance (Haas, 1981).

4.3.6. Adenosine

Adenosine is known to have multiple effects in the hippocampus. Adenosine induces a hyperpolarization of hippocampal pyramidal cells as a result of an enhancement of a K⁺ conductance (Okada and Ozawa, 1980; Segal, 1982; Gerber et al., 1989; Dunwiddie and Fredholm, 1989). Pertussis toxin can prevent the adenosine-induced hyperpolarization (Fredholm et al., 1989). It appears that adenosine, GABA_B and 5-HT_{1A} receptors are coupled to the same type of K⁺ conductance through a pertussis toxin-sensitive G protein. Another postsynaptic effect of adenosine is to enhance I_{AHP} (Greene and Haas, 1985;

Haas and Greene, 1984). Adenosine also depresses synaptic transmission presynaptically (Dunwiddie, 1984; Dunwiddie and Haas, 1985). The mechanisms for this presynaptic depression are still unknown. It has been reported that the presynaptic action of adenosine is not blocked by pertussis toxin, but inhibited by phorbol esters, PKC activators. However, postsynaptic actions of adenosine are blocked by both agents (Thompson and Gahwiler, 1992). Therefore, the mechanisms for presynaptic actions of adenosine may be different from those for postsynaptic actions. Adenosine can also modulate adenylate cyclase activity. However, the link between the biochemical and electrophysiological actions of adenosine are not clear (Nicoll et al., 1990).

4.4. Neuropeptides in the hippocampus

A number of neuropeptides have been found in the hippocampus. These neuropeptides include opioid peptides, somatostatin, substance P, neuropeptide Y, cholecystokinin, vasoactive intestinal peptide, thyrotropin releasing hormone, neurotensin, and oxytocin/vasopressin (Swanson et al., 1987). The functional role for most of these peptides in the hippocampus is unknown. However, some of these peptides such as opioid peptides and somatostatin have been found to play a significant role as a neurotransmitter or neuromodulator in the hippocampus.

4.4.1. Opioid peptides

Several opioid peptides, including enkephalins, β -endorphin and dynorphins, have been identified in the hippocampus (Swanson et al., 1987). Binding studies suggest the existence of several subtypes of opioid receptors in the brain. Three major opioid receptor subtypes are designated as μ (Mu), δ (Delta) and κ (Kappa) (Swanson et al., 1987). The antagonist naloxone has high, but variable, affinity to all of these receptors. Opioid peptides do not cause significant change in the membrane properties of hippocampal pyramidal

neurons (Dingledine, 1985). On the other hand, opioid peptides induce a hyperpolarization of hippocampal interneurons, which is due to an increase in a K^+ conductance (Nicoll, 1986). This hyperpolarization results in an inhibition of interneuronal firing (Madison and Nicoll, 1988; Wimpey et al., 1990). This effect of opioid peptides is mediated by μ receptors, which couple to the K^+ channels through a pertussis toxin-sensitive G protein (Nicoll et al., 1990; Thompson et al., 1993). The inhibition of interneurons by opioids reduces GABA release from the GABAergic interneurons, which provide major inhibitory input to the pyramidal cells (Nicoll et al., 1990; Madison and Nicoll, 1988; Nicoll, 1986). Recent evidence has shown that opioids produce a decrease in miniature IPSP frequency and monosynaptic IPSP amplitude without changing the mean miniature IPSP amplitude in the pyramidal cells (Thompson et al., 1993). This action of opioid is prevented by the treatment of cultured neurons with pertussis toxin. Since opioid peptides reduce the GABAergic IPSPs by inhibiting the interneurons, these peptides indirectly increase the EPSP of the pyramidal cells (Nicoll et al., 1990). Opioid peptides increase the amplitude and duration, but not the slope of the EPSP. In CA3 field, dynorphin has been found in the glutamatergic mossy fibres (McGinty et al., 1983). Therefore, dynorphin may act as a co-transmitter in the mossy fibres. Actions of dynorphin are mediated by κ receptors. Dynorphin action at κ receptors inhibits a voltage-dependent, dihydropyridine-insensitive N-type current in dorsal root ganglion neurons (MacDonald and Wertz, 1986). Recently, Weisskopf et al. (1993) have demonstrated that dynorphin is co-released with glutamate from the mossy fibres. The release of dynorphin requires high frequency stimulation of mossy fibres. The synaptic release of dynorphin induced by tetanus causes heterosynaptic depression of the EPSP which can be reversed by application of naloxone. The released dynorphin may act on presynaptic κ receptors on the

mossy fibre terminals. The activation of these presynaptic κ receptors by dynorphin reduces glutamate release from the mossy fibres (Gannon and Terrian, 1991; Weisskopf et al., 1993). Wagner et al., (1992) have reported that κ -receptors located on perforant path terminals mediate the inhibition of excitatory synaptic transmission by κ -opioid agonists.

4.4.2. Somatostatin

Somatostatin was first found as a peptide that inhibits growth hormone secretion from the anterior pituitary (Brazeau et al., 1973). Recent evidence has shown that somatostatin plays a significant role as a neurotransmitter or neuromodulator in the brain (Epelbaum, 1986; Olpe et al., 1980). In the hippocampus, somatostatin-immunoreactivity is present in the CA1 and CA3 interneurons. Many of these interneurons are GABAergic interneurons which make contacts with pyramidal cells (Somogyi et al., 1984; Kunkel and Schwartzkroin, 1988). Somatostatin has diverse physiological actions in the CNS. Somatostatin induces a hyperpolarization associated with a reduction in the input resistance of the hippocampal pyramidal neurons (Pittman and Siggin, 1981). This hyperpolarization is due to an increase in a K^+ conductance by the activation of somatostatin receptors, which is probably coupled to pertussis toxin-sensitive G-protein. An increase in hippocampal I_M by somatostatin has also been reported (Moore et al., 1988). It has been suggested that different actions of somatostatin may be mediated by different receptor subtypes. More details will be discussed in the chapter on experimental tools.

4.4.3. Cholecystokinin

Cholecystokinin (CCK) was originally isolated from the gut. Cholecystokinin octapeptide (CCK_8) was later identified throughout the CNS (Dockray, 1976; Rehfeld, 1978). Immunohistochemical studies have shown the presence of CCK_8 -immunoreactivity in hippocampal neurons (Greenwood et al.,

1981). Some of the CCK₈-immunoreactivity appear in the GABAergic interneurons which synapse on the pyramidal cells of the hippocampus (Somogyi et al., 1984; Kosaka et al., 1985). CCK₈ has been reported to increase the excitability of hippocampal pyramidal neurons associated with a depolarization and a decrease in the input resistance of the neurons (Dodd and Kelly, 1981). Buckett and Saint have reported that CCK₈ blocks a voltage-dependent K⁺ conductance. This blockade of the voltage dependent K⁺ conductance may explain the increase of the hippocampal neuron excitability by CCK₈. CCK₈ has been found to increase synaptic transmission while CCK₈ antagonists have depressant effect on synaptic transmission (Jaffe et al., 1987). The authors have suggested that endogenous CCK₈ is released directly or indirectly by stimulation of Schaffer collateral-commissural fibres, thereby increasing the excitability of CA1 pyramidal cells. An inhibition of synaptic transmission in the hippocampus has also been reported. The discrepancy in these results is not clear. Nonetheless, further studies are needed to determine the physiological role of this endogenous peptide in the hippocampus.

4.4.4. Neuropeptide Y

Neuropeptide Y has been detected in the hippocampus (Swanson et al., 1987). Neuropeptide Y has no significant effect on the intrinsic properties of pyramidal cells (Colmers et al., 1987). However, neuropeptide Y can reduce the EPSP in the CA1 pyramidal cells through a presynaptic mechanism. It has been reported that neuropeptide Y inhibits a Ca²⁺ conductance by activating neuropeptide Y receptors at the presynaptic terminals that release glutamate (Colmers et al., 1988; Klapstein and Colmers, 1992). The neuropeptide Y receptors, which mediate the presynaptic inhibition, couple to a pertussis toxin-insensitive G protein (Colmers and Pittman, 1989).

4.4.5. Other neuropeptides

Other neuropeptides such as substance P, vasopressin/oxytocin, vasoactive intestinal peptide, angiotensin II and neurotensin have been detected in the hippocampus (Swanson et al., 1987). The functional role of these peptides in the hippocampus remains to be determined.

5. LONG-TERM POTENTIATION IN THE HIPPOCAMPUS

Long-term potentiation (LTP) was first reported in the hippocampal dentate area of anaesthetized rabbits by Lomo (1966) and later described in detail by Bliss and Lomo (1973), Bliss and Gardner-Medwin (1973). They demonstrated that brief trains of high-frequency stimulation of the perforant path induced a long-lasting potentiation of excitatory synaptic transmission in the dentate area of the rabbit hippocampus *in vivo*. Since then, LTP has been found in all excitatory pathways of the hippocampus *in vivo* as well as *in vitro* preparations. LTP has also been observed in other regions of the brain, including the neocortex (Tsumoto, 1992; Kirkwood et al., 1993), but LTP is most widely studied and best understood in the hippocampus, particularly in the CA1 field. Since LTP is thought to be involved in the mechanisms underlying at least some forms of learning and memory, it has been attracting researchers from around the world for the past two decades. Even though much progress has been made in characterizing the properties of LTP, the mechanisms underlying LTP remain controversial at present. The following discussion will be focused more on LTP in the CA1 area of the hippocampus since all the data in the present studies has been obtained from that area.

5.1. Characteristics and properties of LTP

5.1.1. Definition and classification of LTP

Brief trains of high-frequency stimulation to monosynaptic excitatory pathways in the hippocampus can induce an abrupt and persistent enhancement

of synaptic transmission *in vivo* as well as *in vitro*. This activity-dependent synaptic plasticity was first described in detail by Bliss and his colleagues in 1973. They observed the long-lasting potentiation of the population responses in the dentate area following repetitive stimulation (tetanus: 10-20 Hz for 10-15 sec or 100 Hz for 3-4 sec) of the perforant path fibres in rabbit hippocampus *in vivo*. The amplitude of the population spike and the field EPSP was increased to approximately 200-300 percent and 150-200 percent of the pre-tetanic responses, respectively. The latency of the population spike was reduced after tetanus. The potentiation of these field potentials could last for several hours in anaesthetized rabbits (Bliss and Lomo, 1973) and several weeks in the unanaesthetized rabbits (Bliss and Gardner-Medwin, 1973). The time course of tetanus-induced LTP seems to consist of two phases, an initial phase which declines within 20 min after tetanus and the second phase which occurs 20 to 30 min after tetanus and maintains stable. The first and second phases are also called decremental LTP and non-decremental LTP, respectively. Bliss and his colleagues also reported a phenomenon that the population spike did not always potentiate with corresponding changes in the field EPSP after tetanus. They explained that their observation was probably due to an increase in excitability of the granule cells by tetanus. Andersen et al. (1980) analyzed this phenomenon in the hippocampal slices and found that repetitive stimulation resulted in an increase in the amplitude of the population spike evoked by a given size of the field EPSP in addition to an increase in the size of the field EPSP. They called this form of LTP as EPSP-spike (E-S) potentiation. The E-S potentiation indicates an enhancement of cell body excitability or a reduction of spike threshold (Abraham et al., 1985, 1987; Kairiss et al., 1987; Tomasulo et al., 1991). The mechanisms underlying the E-P potentiation are unknown and believed to be distinct from the mechanisms of the synaptic LTP which the

potentiation of population spike is directly attributable to the EPSP potentiation, but not the change in neuronal excitability. The synaptic LTP has attracted much greater interest since it is clearly identified with potentiation at a particular group of tetanized synapses. The present discussion is also concentrated on the synaptic LTP, unless otherwise stated.

In addition to the increase in the amplitude of the field EPSP during LTP described by Bliss and his colleagues (1973), the slope of the field EPSP is also increased following tetanic stimulation (Alger and Teyler, 1976). In fact, the slope of the EPSP is a more efficient index for measuring the synaptic potentiation.

In a single hippocampal neuron, the slope and amplitude of the EPSP and/or the probability of spike discharge are increased following tetanic stimulation of the afferents (Schwartzkroin and Wester, 1975; Andersen et al., 1977; Andersen et al., 1980c). LTP of the EPSP is not associated with changes in the membrane potential, input resistance and excitability of the neuron (Andersen et al., 1977, 1980c; Barrionuevo et al., 1986). Barrionuevo et al. (1986) using the voltage clamp technique showed that the LTP of EPSP is associated with an increase in synaptic conductance. In some case, the probability of spike discharge is increased without the potentiation of the EPSP (Andersen et al., 1977). This is similar to the E-S potentiation in extracellular recording.

5.1.2. Distribution of LTP in the hippocampus

LTP occurs in all excitatory pathways in the hippocampus, which include the medial perforant path (Bliss and Lomo, 1973), the lateral perforant path (McNaughton et al., 1978), the mossy fibres (Alger and Teyler, 1976), the Schaffer collaterals (Schwartzkroin and Wester, 1975) and the commissural projection to the CA1 neurons (Buzsaki, 1980). A projection of perforant path to

the distal apical dendrites of CA1 also displays LTP (Doller and Weight, 1985). Excitatory afferents form synapses not only with pyramidal and granule cells but also with interneurons. Evidence has shown that tetanic stimulation of excitatory afferents can also induce LTP in the inhibitory interneurons in the CA1 field (Buzsaki and Eidelberg, 1982) and the dentate gyrus (Kairiss et al., 1987).

5.1.3. Homosynaptic and heterosynaptic LTP

In the CA1 field and the dentate gyrus, LTP occurs only in the tetanized pathway, but not in the non-tetanized pathways which converge on the same population of cells (Bliss et al., 1973; Andersen et al., 1977, Lynch et al., 1977; Sastry et al., 1986). This input-specificity of LTP is called homosynaptic LTP (McNaughton, 1983). A heterosynaptic depression has been reported in the non-tetanized pathways (Lynch et al., 1977; Alger et al., 1978). Unlike the CA1 field and the dentate gyrus where only homosynaptic LTP occurs, in the mossy fibre pathway of the CA3 field, LTP can occur in both tetanized and non-tetanized pathway (Yamamoto and Chujo, 1978; Misgeld et al., 1979). This is the so called heterosynaptic LTP. This heterosynaptic LTP is due to the polysynaptic component of the evoked responses in the CA3 field (Higashima and Yamamoto, 1985). Recently, Zalutsky and Nicoll (1992) have shown that LTP in the mossy fibre pathway also displays input specificity if the non-mossy fibre inputs are blocked.

5.1.4. Cooperativity of LTP

Cooperativity indicates the existence of an intensity threshold for the induction of LTP. The threshold for the induction reflects the need for a certain number of presynaptic fibres to be activated simultaneously (McNaughton et al., 1978). Weak tetanic stimulation induces a post-tetanic potentiation (PTP) that lasts for 2-5 min or short-term potentiation (STP) that lasts for 15-20 min, but not LTP (McNaughton et al., 1978; Malenka, 1991). Both high frequency and high

intensity of stimulation are required to induce LTP. However, frequency and stimulus strength can interact such that increasing one relatively decreases the requirement for the other. For example, a train with higher frequency and relative lower stimulus strength is as efficient as a train with higher stimulus and relative lower frequency to induce LTP. In the CA3 region, LTP in the mossy fibre pathway shows no apparent cooperativity (Zalutsky and Nicoll, 1992).

5.1.5. Associativity of LTP

McNaughton and his colleagues (1978) first examined associativity of LTP using two stimulating electrodes to activate two completely separate pathways converging on the same population of postsynaptic cells in hippocampus. One pathway was designated as the weak pathway which, when tetanized alone, could not produce LTP. The other pathway was designated as the strong pathway that produced LTP when tetanized. When both pathways were tetanized at the same time or almost at the same time, LTP occurred not only in the strong pathway but also in the weak pathway. This is so-called associative LTP. Sastry et al. (1986) showed that pairing single stimulation to afferents repetitively with depolarization of postsynaptic cells eventually induces LTP, further confirming associativity of LTP. Similar results have been reported by other investigators (Kelso et al., 1986; Wigstrom et al., 1986). The associative LTP is maximal when the weak pathway is activated at the same time as the conditioning trains to the strong input. However, LTP does not occur if the trains to the weak pathway are delayed longer than 100-200 msec (Kelso and Brown, 1986). This associative LTP can occur even when the two pathways are spatially separated by several hundred microns such as the two afferents terminating on the dendritic tree originating from opposite sides of the cell body (Gustafsson and Wigstrom, 1986). The interaction between the two pathways may be through the postsynaptic cell. The LTP induced by pairing the afferent

activation with postsynaptic depolarization is facilitated if picrotoxinin, a GABA_A antagonist, is present in the recording medium (Sastry et al., 1986). It is possible that the blockade of GABA inhibition allows the cell to be depolarized more readily. The induction of this LTP is not due to the high-frequency sodium spikes induced by depolarizing pulses because the LTP is not affected even when the sodium spikes are blocked by QX-222 or QX-314 (Kelso et al., 1986; Gustafsson et al., 1987). Malinow and Miller (1986) found that pairing hyperpolarization with the activation of afferents prevents the induction of LTP. Based on these results, associative LTP may be due to pairing the activation of the weak pathway with the postsynaptic cell depolarization which is induced by a tetanic stimulation to the strong pathway. Tetanizing to the weak pathway alone cannot depolarize the cell sufficiently to induce LTP. On the other hand, the interactions between the two pathways may also occur in presynaptic terminals (Goh and Sastry, 1985a; Sastry et al., 1986). A pairing of the conditioning trains of one pathway with the test stimulation of another pathway causes a postconditioning decrease in the excitability of the presynaptic terminals in the test pathway (Sastry et al., 1986). The associative effects are larger if two separate pathways terminate on the same dendritic tree. In contrast, the associative effects are smaller if two pathways terminate on the dendritic tree from opposite sides of the soma (Gustafsson and Wigstrom, 1986). Two pathways may interact more readily if they terminate on the same dendritic tree.

5.2. The induction of LTP

It is generally accepted that LTP consists of at least two parts: induction and maintenance. The induction is the initial sequence of events that triggers the process. The maintenance is the process underlying the persistence of the potentiated response. At present, the mechanisms underlying the induction of LTP seem to be better understood than those underlying the maintenance. The

associative property of LTP indicates that the both presynaptic and postsynaptic activity are required for the induction of LTP.

5.2.1. Involvement of glutamate receptors in LTP

The involvement of glutamate receptors in the induction of LTP has been determined basically by the use of antagonists of glutamate receptor subtypes. Collingridge et al. (1983) first suggested that activation of NMDA receptors plays a critical role in the induction of LTP because they showed that the induction of LTP in the CA1 field is blocked by APV, a selective NMDA antagonist. Since then, in addition to APV, several other NMDA antagonists, such as MK-801 (a NMDA channel blocker) and 7-chlorokynurenic acid (an antagonist at the allosteric glycine site), have also been reported to block the induction of LTP (Harris et al., 1984; Wigstrom and Gustafsson, 1984; Coan et al., 1987; Bashir et al., 1990). In the dentate gyrus, APV also blocks the induction of LTP in the perforant path (Morris et al., 1986). In the CA3 region, two forms of LTP occur. While the LTP in the associational/commissural pathway is sensitive to APV, the LTP in the mossy fibre pathway is not affected by APV (Harris and Cotman, 1986). The excitatory synaptic transmission in the mossy fibre pathway is primarily mediated by non-NMDA receptors (Monaghan and Cotman, 1985; Neuman et al., 1988). Other neurotransmitters may also be involved in the excitatory synaptic transmission in this pathway. APV has little effect on the basal excitatory synaptic responses. The properties of NMDA receptors make the involvement of NMDA receptors a suitable explanation for the properties of LTP. Strong tetanic stimulation or depolarizing current pulse is required for the depolarization of the neuron which removes the Mg^{2+} blockade. At the same time, activation of afferents releases glutamate which together with the depolarization of the postsynaptic cell can open up the NMDA channels. The activation of the weak pathway alone is not sufficient to remove the voltage-

dependent blockade of Mg^{2+} in the NMDA channels; the depolarization of the postsynaptic cell alone is also not able to activate the NMDA receptors. Therefore, only if both these postsynaptic and presynaptic events occur simultaneously, does the activation of NMDA receptors take place. It has been suggested that the activation of NMDA receptors allows Ca^{2+} influx which probably triggers a series of biochemical events and subsequently induces LTP. Brief exposure of the slices to Mg^{2+} free medium during low frequency stimulation of afferents can induce LTP (Coan and Collingridge, 1985; Wigstrom and Gustafsson, 1988). This potentiation induced by Mg^{2+} -free medium is blocked by APV. The evidence mentioned above appears to support the role of NMDA receptors in the induction of LTP. However, application of NMDA alone induces STP but not LTP (Collingridge et al., 1983; Kauer et al., 1988a). Recent evidence has shown that the activation of NMDA receptors by the application of NMDA (Huang et al., 1992b; Izumi et al., 1992) or weak tetanus (Huang et al., 1992b) before tetanic stimulation inhibits the induction of LTP in the CA1 region. It is obvious that the activation of NMDA receptors plays an important role in the induction of LTP. The different timing of this activation can lead to promotion or suppression of the induction of LTP. Furthermore, factors in addition to activation of NMDA receptors are needed for the induction of LTP. These factors can be presynaptic, postsynaptic or both. Recently, metabotropic glutamate receptors have been suggested to be involved in the induction of LTP. ACPD, a metabotropic glutamate receptor (ACPD receptor) agonist, induces a slowly developing LTP (Otani and Ben-Ari, 1991; Bortolotto and Collingridge, 1992, 1993). The ACPD-induced LTP is not blocked by APV (Bortolotto and Collingridge, 1993). ACPD fails to potentiate the EPSP if a tetanus-induced LTP established before drug application, and vice versa (Bortolotto and Collingridge, 1993). This result suggests that these two types of

LTP may share common mechanisms. AP3, an ACPD receptor antagonist, has been reported to block the induction of LTP in the hippocampus (Izumi et al., 1991; Behnisch et al., 1991). However, the effects of AP3 on LTP seem to be controversial because AP3 has also been reported to have no effect on the induction of LTP (Stanton et al., 1991; Musgrave et al., 1993). Moreover, AP3 is not a very selective and potent antagonist (Schoepp and Conn, 1993). Recently, Bashir et al. (1993) have shown that a more selective ACPD receptor antagonist, (RS)- α -methyl-4-carboxyphenylglycine (MCPG), blocks both tetanus- and ACPD-induced LTP. This result further supports the involvement of ACPD receptors in the induction of LTP. Musgrave et al. (1993) have demonstrated that co-application of ACPD and NMDA induces a LTP, which develops faster than the ACPD-induced LTP. The induction of LTP by ACPD alone or with NMDA is not required for the activation of afferents (Bortolotto and Collingridge, 1992; Musgrave et al., 1993). Whether these LTP are the same as tetanus-induced LTP remains to be determined. It appears that ACPD receptors play a major role in the induction of LTP. Synaptic activation of both NMDA and ACPD receptors is required for the induction of LTP.

In the CA1 region, an APV-insensitive LTP has also been reported (Grover and Teyler, 1990, 1992). Induction of this LTP, however, requires a higher frequency tetanic stimulation and is blocked by dihydropyridine, a voltage-dependent Ca^{2+} channel blocker.

In the CA3 region, the induction of LTP in the mossy fibre pathway is not blocked by APV, but by MCPG (Bashir et al., 1993). Activation of ACPD receptors is probably also needed for the induction of LTP in this pathway.

Activation of non-NMDA receptors is not required for the induction of LTP in the hippocampus because CNQX, a selective non-NMDA receptor blocker, does not block the induction of LTP (Kauer et al., 1988b).

5.2.2. *The role of Ca²⁺ in the induction of LTP*

Several lines of evidence suggest that Ca²⁺ plays an important role in the induction of LTP (Dunwiddie et al., 1978; Wigstrom et al., 1979; Izumi et al., 1987; Huang et al., 1988; Lynch et al., 1983; Malenka et al., 1988; Morishita and Sastry, 1991). Tetanic stimulation of afferents during a short period of exposure of slices to Ca²⁺-free (Dunwiddie et al., 1978; Wigstrom et al., 1979) or low Ca²⁺ medium (Dunwiddie and Lynch, 1979) fails to induce LTP. Izumi et al. (1987) has shown that extracellular Ca²⁺ is required not only during tetanus but also immediately after tetanus. An exposure to high Ca²⁺ medium has been reported to induce LTP (Turner et al., 1982). However, Huang et al. (1988) demonstrated that perfusion of high Ca²⁺ medium could only cause a short term potentiation which reverted following the return to control medium. The explanation for the difference between these two studies is not known. Nevertheless, these results support that the change in extracellular Ca²⁺ concentration can affect the induction of LTP. The mechanisms can be presynaptic, postsynaptic or both.

Direct injection of Ca²⁺ chelators such as EGTA, nitr-5 or BAPTA into the postsynaptic cell can block the induction of LTP in the drug-injected neuron without changing basal synaptic responses of that neuron (Lynch et al., 1983; Malenka et al., 1988; Morishita and Sastry, 1991). These results suggest that tetanic stimulation may induce a large increase in postsynaptic Ca²⁺ concentration which is critical for the induction of LTP. The increase in postsynaptic Ca²⁺ concentration can be due to a Ca²⁺ influx or a release of intracellular stores. It has been suggested that tetanic stimulation induces a large postsynaptic Ca²⁺ entry. Using Ca²⁺-imaging techniques, it has been shown that tetanic stimulation elevates Ca²⁺ within dendrites and spines (Regehr and Tank, 1990; Muller and Connor, 1991). The tetanus-induced rise in postsynaptic Ca²⁺ lasts for several minutes (Muller and Connor, 1991).

Whether this long period of increase in intracellular Ca^{2+} concentration by tetanus is required for the induction of LTP remains to be determined. Izumi et al. (1987) have reported that exposure of slice to Ca^{2+} -free medium for 5 min after tetanus inhibits the induction of LTP. In contrast, Malenka et al. (1992) have shown that the rise of intracellular Ca^{2+} for the induction of LTP is only needed for a few seconds by using photolabile Ca^{2+} buffer diazo-4. Whether an increase in postsynaptic Ca^{2+} concentration alone can induce LTP is another question needed to be answered. An release of Ca^{2+} into postsynaptic neurons by the caged Ca^{2+} compound nitr-5 has been suggested to be sufficient to produce a potentiation of synaptic transmission (Malenka et al., 1988). However, their conclusion has been questioned. First, this potentiation can only last for 20 to 70 min. Second, whether this potentiation by photolysis of nitr-5 is the same as the tetanus-induced LTP is not clear. Occlusion experiments have not been done.

The rise in postsynaptic Ca^{2+} concentration can be generated by three mechanisms. (1) The activation of NMDA receptors, which have been found in the dendrites and spines of CA1 neurons, by tetanus allows Ca^{2+} entry through NMDA channels (McDermott et al. 1986; Regher and Tank, 1990); (2) Strong depolarization induce the opening of voltage-dependent Ca^{2+} channels, which are also present in the dendrites, (Westenbroek et al., 1990); (3) Ca^{2+} releases from intracellular stores. In the CA1 region, synaptic activation of NMDA channels are apparent to allow Ca^{2+} entry which is critical for the induction of LTP. Under certain circumstances, such as strong tetanic stimulations which induce LTP in the presence of APV, voltage-gated Ca^{2+} channels mediate the Ca^{2+} entry (Grover and Teyler, 1990; 1992; Kullmann et al., 1992). Moreover, a brief application of tetraethylammonium (TEA), a K^{+} channel blocker, induces a Ca^{2+} -dependent NMDA-independent form of LTP

(Aniksztejn and Ben-Ari, 1991). This form of LTP is blocked by Ca^{2+} channel blockers such as flunarizine and nifedipine, or intracellular injection of Ca^{2+} chelator, BAPTA (Aniksztejn and Ben-Ari, 1991; Huang and Malenka, 1993). The voltage-dependent Ca^{2+} channels appear to mediate the induction of this TEA-induced LTP. Whether this TEA-induced LTP and tetanus-induced LTP have similar mechanisms are not very clear. In addition to the Ca^{2+} entry through NMDA or voltage-dependent channels, the release of Ca^{2+} from intracellular stores may also contribute to the rise of intracellular Ca^{2+} concentration. Thapsigargin, which depletes intracellular Ca^{2+} stores, also blocks the induction of LTP (Harvey and Collingridge, 1992). Furthermore, ACPD-induced LTP is probably also due to the increase in release of Ca^{2+} from intracellular stores (Bortolotto and Collingridge, 1993).

In the CA3 region, LTP in the associational-commissural pathway, but not the one in mossy fibre pathway, is Ca^{2+} -dependent (Zalutsky and Nicoll, 1990, but also see Williams and Johnston, 1989).

In summary, evidence has clearly shown that postsynaptic Ca^{2+} play a critical role in the induction of LTP in most of the pathways in the hippocampus. However, there is no convincing evidence to support that a rise in postsynaptic Ca^{2+} concentration is sufficient to trigger the induction of LTP. Whether changes of Ca^{2+} homeostasis in presynaptic terminals occur in the induction of LTP is unknown.

5.2.3. The role of protein kinases in the induction of LTP

The involvement of protein kinase C (PKC) was first reported by Routtenberg et al. (1985). They showed that a 47-kDa protein (referred to as F1, B50 or GAP-43) as a PKC substrate undergoes a LTP-associated phosphorylation (Akers and Routtenberg, 1985; Lovinger et al., 1985). Results from early studies suggest that the activation of PKC plays an important role in

the maintenance of LTP (Akers et al., 1986; Lovinger et al., 1987; Malinow et al., 1988; Malenka et al. 1989; Reymann et al., 1988a,b). Most of these studies are based on the use of PKC inhibitors such as H-7, polymyxin B, sphingosine. These inhibitors are able to facilitate the decay of LTP. Application of H-7 has been reported reversibly block the established-LTP (Malinow et al., 1988). However, the blockade of the established-LTP by H-7 has been questioned by others who demonstrated that H-7 can suppress the control responses as well (Muller et al., 1990; but also see Huang et al., 1992).

Recent studies have shown that PKC activity plays a significant role in the induction of LTP. Direct injection of PKC into the postsynaptic neurons induces a long-lasting potentiation (Hu et al., 1987). More recently, direct injection of a synthetic peptide PKC (19-31) which acts as a potent PKC antagonist, or of a combination of different types of PKC inhibitors into the postsynaptic cells has been found to block the induction of LTP (Malinow et al., 1989; Wang and Feng, 1992). Our results have also shown that intracellular injection of K-252b, a potent and relatively selective PKC inhibitor, blocks the induction of LTP (Xie and Sastry, 1991). Biochemical studies showed that an increase in cytosolic activity of PKC occurs in the first 2 min after the induction of LTP and disappears in 5 min (Otani et al., 1992). These results support the role of PKC in the induction of LTP.

Extracellular application of phorbol ester, a PKC activator, can induce a long-lasting potentiation (Malenka et al., 1986). However, this phorbol ester-induced long lasting potentiation is different from the tetanus-induced LTP because the latter does not occlude the former (Gustafsson et al. 1988; Kamiya et al., 1988; Muller et al., 1990). The phorbol ester-induced long-lasting potentiation is not sustained after complete washout of the drug. Therefore, the

experiment of phorbol ester cannot conclude the exact role of PKC in the LTP process.

The difficulty of studies on PKC involvement in LTP is due to the lack of selective PKC inhibitors. Controversy on this subject will not be over until a selective inhibitor is available. The available evidence suggests that postsynaptic PKC plays an important role in the induction of LTP. Activation of postsynaptic PKC probably converts STP to LTP. The role of PKC on the induction of LTP may be linked to its effect on NMDA receptor-mediated responses. A recent report has shown that PKC is able to potentiate the NMDA current by removing the voltage-dependent Mg^{2+} block of the NMDA channels in cultured trigeminal neurons (Chen and Huang, 1992). Activation of ACPD receptors has been shown to potentiate NMDA current through PKC in the CA1 pyramidal neurons (Aniksztejn et al., 1992). The role of PKC in the maintenance of LTP is no longer as certain as predicted previously. Whether PKC is persistently activated after the induction of LTP is not clear. A recent report suggests that activation of presynaptic PKC may last longer but no more than 60 min (Huang et al., 1992). Whether presynaptic PKC really contributes to the maintenance of LTP remains to be determined.

Ca^{2+} /calmodulin-dependent protein kinase II (CaM-KII), which is found abundantly in postsynaptic dendrites (Kelly et al., 1984; Kelly, 1991), has been shown to contribute to the induction of LTP (Mody et al., 1984; Reymann et al., 1988b; Malenka et al., 1989; Malinow et al., 1989). Injection of selective CaM-KII inhibitors, the synthetic peptides, into the postsynaptic cell blocks the induction of LTP with the STP intact (Malenka et al., 1989; Malinow et al., 1989). Bath application of calmidazolium, a CaM-KII inhibitor, blocks the induction of both STP and LTP (Reymann et al., 1988b). It is possible that presynaptic CaM-KII also plays a role in LTP. Recently, Silva et al. (1992a) have shown that LTP

is not observed in the hippocampus of α -CaM-KII mutant mice. This result further supports the role of CaM-KII in the induction of LTP. CaM-KII can regulate itself through an autophosphorylation mechanism whereby it becomes Ca^{2+} -independent following its initial activation (Saitoh and Schwartz, 1985; Miller and Kennedy, 1985; Kelly, 1991). Because of this property of CaM-KII, activation of CaM-KII may contribute to the later phase of LTP.

5.2.4. The role of GABA receptors in the induction of LTP

Under normal conditions, the activation of NMDA receptors is critical for the induction of LTP (Collingridge et al., 1983). Depolarization of postsynaptic cells by tetanic stimulation or injection of depolarizing current pulses removes the voltage-dependent Mg^{2+} block on NMDA channels. This is crucial for the NMDA channel opening as well as the induction of LTP. In contrast, hyperpolarization of postsynaptic neurons blocks the NMDA channel opening and the induction of LTP (Mayer et al., 1984; Malinow and Miller, 1986). Stimulation of afferents activates not only glutamatergic principle cells, but also GABAergic interneurons which in turn make synapses on the principle cells in the hippocampus. Activation of GABA receptor-mediated synaptic inhibition which hyperpolarizes the postsynaptic neurons has a significant effect on the activation of NMDA receptors and the induction of LTP. In the presence of GABA_A antagonists such as picrotoxinin, the induction of LTP can be facilitated (Wigstrom and Gustafsson, 1983). Disinhibition by picrotoxinin enhances the NMDA responses by removing the shunting effect produced by the GABA_A receptor-mediated hyperpolarization. In contrast, application of GABA prevents the induction of LTP (Scharfman and Sarvey, 1985). This effect of GABA is probably due to the GABA receptor-mediated hyperpolarization which makes the NMDA channels more difficult to open.

Recent evidence has shown that GABA_B receptors play an important role in the induction of LTP in both CA1 (Davies et al., 1991) and dentate gyrus of the hippocampus (Mott and Lewis, 1991). Baclofen, a GABA_B agonist, has been reported to facilitate the development of LTP of the population spike (Mott et al., 1990; Olpe and Karlsson et al., 1990). GABA_B receptor antagonists, 2-hydroxy-saclofen and CGP35348, have been found to block the induction of LTP (Davies et al., 1991; Mott and Lewis, et al., 1991). Presynaptic GABA_B receptors in the inhibitory terminals are believed to act as autoreceptors that appear to mediate the paired-pulse depression of the fast IPSP (Davies et al., 1990). This is supported by the ability of GABA_B antagonists such as 2-hydroxy-saclofen to reduce the paired-pulse depression of IPSPs (Davies et al., 1990). During tetanic stimulation, GABA release can be down-regulated by the activation of presynaptic GABA_B receptors. Subsequently, the postsynaptic GABA receptor-mediated hyperpolarization is reduced, the NMDA currents are enhanced, and the induction of LTP is facilitated. Application of phaclofen, a weak GABA_B antagonist, or a low concentration (100 μ M) of CGP-35348 only suppresses the postsynaptic GABA_B receptor-mediated responses with no significant effect on the presynaptic GABA_B receptor-mediated responses such as paired-pulse depression. However, phaclofen and the low concentration of CGP-35348 can facilitate the induction of LTP. This result suggests that postsynaptic GABA_B receptors, like postsynaptic GABA_A receptors, can modulate the induction of LTP as well.

5.3. *The maintenance of LTP*

The non-decremental phase of LTP is usually referred to as the maintenance of LTP which is stable and lasts for hours *in vitro* and days *in vivo*. The mechanisms underlying the maintenance of LTP are much less clear than those underlying the induction of LTP. Debates have been on the loci and the

nature of the maintenance of LTP. Whether the maintenance of LTP is due to presynaptic or postsynaptic changes is controversial. The review will be focused on the current progress on this subject.

5.3.1. Postsynaptic versus presynaptic mechanisms

Postsynaptic synaptic mechanisms include changes in postsynaptic receptor number or sensitivity, or changes in morphology of synaptic spines. An increase in postsynaptic glutamate receptor numbers has been suggested (Lynch and Baudry, 1984). Tetanic stimulation activates calpain, the Ca^{2+} -dependent proteolytic enzyme, which degrades cytoskeleton proteins and unmask the hidden postsynaptic receptors (Baudry et al., 1980, 1981; Lynch et al. 1982). However, whether LTP is associated with the activation of calpain is not very clear (Sastry, 1985; Oliver et al., 1990). In contrast, several studies have shown that LTP is not associated with an increase in transmitter binding (Sastry and Goh, 1984; Lynch et al., 1985; Kessler et al., 1991). Furthermore, an immunohistochemical study has shown the absence of calpain I and II in the region of axo-dendritic synapses in rat brain (Hamakudo et al., 1986). Increases in receptor sensitivity during the maintenance of LTP has been suggested. AMPA receptor sensitivity has been found to increase 20-30 min after tetanic stimulation. (Davies et al., 1989). PKC inhibitor, K-252b, prevents the increase in AMPA receptor sensitivity (Reymann et al., 1990). It is possible that protein kinases directly phosphorylate the receptors or ion channels. Furthermore, Kauer et al. (1988a,b) showed that the non-NMDA component of the EPSP, but not the NMDA component of the EPSP, increases during the maintenance of LTP. If an increase in neurotransmitter release from presynaptic terminals occurs during the maintenance of LTP, both NMDA and non-NMDA components of EPSPs should increase in a similar way. These results seem to support the idea that postsynaptic modification is responsible for the maintenance of LTP.

However, recent studies have shown that tetanic stimulation induces a persistent increase in both non-NMDA component and NMDA component of the EPSP (Bashir et al., 1991; Xie et al., 1992; Asztely et al., 1992). These results challenge the previous suggestion that postsynaptic mechanisms are responsible for the maintenance of LTP. Among these studies, Bashir et al., (1991) have demonstrated that the LTP of the NMDA component of the EPSP is similar in size to the LTP of the non-NMDA component. Asztely et al. (1992), however, have observed that the increase in the NMDA component is relatively smaller than that in the non-NMDA component. The explanation for the discrepancy between the two findings is unknown. It is more likely that both NMDA and non-NMDA components of the EPSP are potentiated during the maintenance of LTP. Since postsynaptic modifications occur during the maintenance of LTP, the degrees of increase in the NMDA and the non-NMDA components are different.

Another hypothesis for postsynaptic mechanisms is that changes in synaptic spine neck resistance after the induction of LTP may be responsible for the potentiation. An enlargement of synaptic spines after repetitive afferent stimulation has been reported (Chang and Greenough, 1984; Fifkova and van Hareveld, 1977; Desmond and Levy, 1983, 1988). Widening of the spine neck would decrease the resistance of spine neck and allow more current flow into the dendrites (Brown et al., 1988). So far, there is a lack of experimental evidence to support changes in spine shape having functional significance in LTP. In contrast, Jung et al. (1992) have recently shown that changes in spine neck resistance are not responsible for the maintenance of LTP.

Presynaptic changes have been suggested to be responsible for the maintenance of LTP. An increase in glutamate release during LTP in the hippocampus has been reported (Dolphin et al., 1982; Bliss et al., 1986, Lynch

et al., 1989a; Ghijsen et al., 1992). These studies showed that the glutamate concentration of the extracellular fluid increases after tetanic stimulation and remains elevated as long as LTP lasts. Although these results indicate that glutamate release is probably associated with LTP, they are not entirely conclusive that the increase of glutamate in the extracellular fluid is due to the release of glutamate from the tetanized terminals. It cannot be ruled out that glutamate is released from sources like glia, and glutamate uptake is decreased. Changes in excitability of presynaptic terminals during LTP have been reported (Sastry, 1982; Goh and Sastry, 1985a; Sastry et al. 1986). It is speculated that these changes in the excitability of presynaptic terminals may lead to an increase in neurotransmitter release. However, more direct evidence is needed to conclude that the increase of glutamate in the extracellular fluid is released from the presynaptic terminals.

The observation of the increase in glutamate release during LTP is not without controversy. A few other studies have detected no change or only transient increase in glutamate release after the induction of LTP in both the dentate gyrus and the CA1 region (Aniksztejn et al., 1989; Roisin et al., 1990). More recently, Klancnik et al.(1992) reported that high-frequency stimulation of Schaffer collateral-commissural fibres results in a significant increase in concentrations of cysteine sulphinic acid and homocysteic acid, but not other amino acids such as glutamate and aspartate. These discrepancies are probably due to the differences in the preparation and methods such as the frequency of tetanus used in the studies. If a sustained increase in glutamate release during LTP, indeed, occurs, it remains to be determined whether the increase is responsible for the maintenance of LTP or unrelated to LTP

Quantal analysis is developed and well-accepted now in studies on synaptic transmission at neuromuscular junctions (Del Castillo and Katz,

1954a,b; Katz, 1969). In general, the analysis provides estimates of the amplitude of response to a single quantum (v , quantal amplitude), the probability of release (p) and the number of release sites (N) based on the binomial distribution. The mean number of quanta released by a nerve impulse (m , mean quantal content) is equal to $N.p$. The mean amplitude of a single-fibre (unitary) postsynaptic potential (E) is thus equal to $v.m.$. In general, an increase in m indicates a presynaptic mechanism while a change in v suggests a postsynaptic mechanism.

Several methods of quantal analysis are commonly used. They are the direct method, the histogram method including deconvolution technique, the variance method and the method of failure (Redman, 1990; Voronin, 1993). The direct method is the technique that measures the quantal amplitude and quantal content directly. It is assumed that the amplitude of spontaneous miniature postsynaptic potentials or currents is equal to v . This method requires high signal resolution recording in order to measure small miniature current accurately. The histogram method is to estimate v by measuring the average interpeak distances. The histogram must include a sufficient number of trials ($N > 500$). The noise standard deviation (S_n) to v should be low. To minimize the problems created by background noise, the deconvolution technique based on a computer optimization algorithm is used to separate the underlying statistical features of the amplitude fluctuation from the background noise and the effects of random sampling (Edwards et al., 1976a, b; also see Voronin, 1993 for review). In the variance method, the mean amplitude of postsynaptic current or potential (E) and its variance (S^2) are measured. Based on a binomial model, the coefficient of variance (V) of the mean synaptic current is independent of postsynaptic parameters such as v using the equation $1/V^2 = Np/(1-p)$, where $1/V^2 = E^2/S^2$ (Malinow and Tsien, 1990; Bekkers and Stevens, 1990). A shift in

$1/V^2$ implicates a presynaptic change. The method of failure is to study small synaptic responses evoked with minimal stimulation which is only slightly stronger than the highest stimulus that gave all failures. This method works for the Poisson distribution. The equation is $m = \ln(N/N_0)$, where "N" and " N_0 " are number of trials and number of failure, respectively. A change in failure rate is interpreted as a presynaptic locus. Since all these methods discussed above are based on certain assumptions originally made for the neuromuscular junction, application of these methods to synapses in the CNS has met with many difficulties. Some of the assumptions for the neuromuscular junction, such as identical release probabilities for all release sites and identification of a single synaptic vesicle as the quantum, cannot be readily applied to synapses in the brain. Neurons in the CNS receive multiple inputs from many axons. It is not certain whether release probabilities from different sites are identical. Spontaneous miniature potentials, usually called minis which define the quanta in neuromuscular junctions, cannot be clearly characterized in the central neurons because they are too small to be well resolved with the noise level of the membrane potential. The variability of miniature potential size is much greater for the central neurons because the synaptic activities occur at a variety of location over the synaptic tree. The sites of origin of minis are always unclear (Redman, 1990; Stevens, 1993). Despite these problems of applying quantal analysis to central synapses, this technique has been used to analyze central synaptic transmission such as LTP (Voronin, 1983) and paired-pulse facilitation (Hess et al., 1987). The results of these studies have been largely compromised by the high noise level caused by the high resistance of microelectrode and the background synaptic activity. With the recent development of whole cell recording technique, which greatly improves the signal resolution, raises a new interest in applying quantal analysis to determine the locus of LTP.

Unfortunately, the findings obtained with this new technique do not resolve the issue on the locus of LTP, but rather make it more complicated because of the inconsistency of the results. Malinow and Tsien (1990) have observed changes in the synaptic variability and a decrease in the proportion of synaptic failure using the variance and failure methods in the slices. Bekkers and Stevens (1990) have shown an increase in release probability using the histogram, variance and failure methods in cultured neurons as well as in the slices. These authors have suggested that the locus of LTP is almost purely presynaptic even though a postsynaptic change is not completely ruled out. Malinow (1991) has recorded synaptic transmission between individual pre- and postsynaptic neurons in slices and his conclusion is in accord with those reports discussed above. However, these results have not convinced the other investigators who have come out with different results (Foster and McNaughton, 1991; Kullmann and Nicoll, 1992; Manabe et al., 1992). Foster and McNaughton (1991) have found a significant increase only in quantal size using the failure, variance and histogram methods and suggested a purely postsynaptic locus of LTP. Meanwhile, three different groups (Kullmann and Nicoll, 1992; Liao et al., 1992; Larkman et al., 1992) have shown that an increase in both quantal size and quantal content occurs during LTP. The latter three reports support the role of both presynaptic and postsynaptic modifications during LTP. There are some interesting findings reported by Voronin et al. (1992a, b, c) and Kuhnt et al., (1992). They have found that an increase in quantal content (m) is primarily responsible for the large LTP of EPSPs while an augmentation in quantal amplitude (a) is accounted for the small LTP of EPSPs. These authors suggests two types of synaptic mechanism for the maintenance of LTP. The change in quantal amplitude is saturated at about 10 to 30% increase in the post-tetanic amplitude above the pre-tetanic EPSP amplitude. The increase in quantal

content, on the other hand, contributes to the rest, the major part of LTP. Analyzing spontaneous miniature synaptic currents, Manabe et al. (1992) have found a significant increase in the spontaneous EPSC amplitude but not in the frequency during LTP or the application of NMDA in the hippocampal slices. The results implicate a postsynaptic modification in LTP. In contrast, Malgaroli and Tsien (1992) have shown that the application of glutamate induces long-term potentiation of the frequency of spontaneous EPSC without observing any changes in the amplitude of spontaneous EPSC in cultured hippocampal neurons. Their findings imply a presynaptic locus of LTP.

In summary, the application of quantal analysis for resolving the locus of LTP has not succeeded because of conflicting results and interpretations. Whether the technique designed for the study of neuromuscular junction is suitable for synapses in the CNS remains an opening question. Edwards, Larkman and their colleagues (Edwards et al., 1990; Edwards 1991; Larkman et al., 1991) hypothesized that the number of receptors available in the postsynaptic membrane of central synapses is limited and can be saturated by released transmitter from a single vesicle. Meanwhile, there are relatively unlimited postsynaptic receptors for released transmitters to act on in neuromuscular junctions. Therefore, the quantization of responses in central synapses is determined not only by the amount of transmitter released, but also by the availability of postsynaptic receptors. If this is indeed the case, the assumptions used in neuromuscular junctions cannot directly apply for synapses in the CNS. A more complete quantal analysis and a better understanding of central synapse structure and function are required to resolve the issue on the locus of LTP.

5.3.2. Signal transduction mechanisms

The transient increase in intracellular Ca^{2+} concentration during the induction of LTP may activate several second messenger systems which cause a series of biochemical events. These biochemical events may be responsible for the maintenance of LTP.

5.3.2.1. Protein kinases

The involvement of protein kinase C (PKC) and Ca^{2+} /calmodulin-dependent protein kinase (CaMKII) in LTP has been previously discussed in section 5.2.3. An increase in cytosolic activity of PKC occurs and lasts for a few minutes after tetanus (Otani et al., 1992). It has been suggested that presynaptic PKC activation may last for long periods (30 min to 1 hour) but still far shorter than the time course of LTP (Huang et al., 1992a). It is believed that activation of PKC after tetanus converts STP to LTP (Ben-Ari et al., 1992). It is possible that the activation of PKC after tetanus leads to a permanent change in receptors or ion channels, which causes LTP. However, it is not certain whether PKC activity is directly involved in the maintenance of LTP. An increase in Ca^{2+} -independent phosphorylation of a 17 kDa PKC substrate protein occurs 45-60 min after the induction of LTP (Klann et al., 1991). Recently, a Ca^{2+} -independent PKC-like activity in hippocampal cytosol fractions has been found to increase during LTP (Suzuki et al., 1992). The enhancement is blocked by calpain inhibitor and PKC inhibitor. However, how this kinase contributes to LTP is not known. Whether the activator-independent forms of PKC are involved in the maintenance of LTP remains to be determined.

CaMKII can be activated by the transient cytosolic Ca^{2+} increase during the induction of LTP (Malenka et al., 1989, Malinow et al., 1989). The kinase can remain active through an autophosphorylation mechanism. Whether the

autophosphorylated form of this kinase contributes to the maintenance of LTP is not known.

Protein kinase A (PKA), a cyclic adenosine monophosphate (cAMP)-dependent protein kinase, has recently been reported to play a role in the late stage of LTP (Frey et al., 1993; Matthies and Reymann, 1993). Application of PKA inhibitors such as H-8 or KT5720, or cAMP antagonist such as Rp-cAMPS suppresses the late stage (3 hours after the induction) of LTP. These agents only have a small effect on the early stage (30-60 min) of LTP. Application of Sp-cAMPS, a membrane-permeable analog of cAMP which activates PKA, induces an initial depression followed by a slowly developing LTP which is similar to the late stage of the tetanus-induced LTP (Frey et al. 1993). The tetanus-induced late stage LTP and the Sp-cAMP-induced LTP occlude on another. This result suggests that the two types of LTP may share a common mechanism. The Sp-cAMPS-induced LTP is blocked by anisomycin, a protein synthesis inhibitor, which also block the late stage of the tetanus-induced LTP. This finding implies that the activation of PKA may be related to protein synthesis. According to the report (Frey et al., 1993), cAMP level increases 1 min, but not 10 min, after tetanic stimulation (100 Hz for 1 s, 3 trains) which induces a longer lasting (more than 3 hours) LTP. A single tetanus (100 Hz, for 1s), which only induces early stage (1-3 hours) LTP, does not raise the cAMP level. A similar increase in cAMP level after tetanus in both the CA1 (Chetkovich et al., 1991) and the dentate gyrus (Stanton and Sarvey, 1985) has also been observed by others. The increase in cAMP is NMDA receptor-(Frey et al., 1993; Chetkovich et al., 1991) and dopamine (D1) receptor-dependent (Frey et al., 1993). The results demonstrate a possible role of cAMP and PKA in the maintenance of LTP. However, a number of questions remain to be answered. Whether a sustained increase in the activity of PKA is needed for LTP remains

to be determined. Whether the PKA inhibitors also block other protein kinases is not clear. Some inhibitors seem to suppress the basal synaptic transmission (Frey et al., 1993).

Protein tyrosine kinases (PTKs) have also been reported to be involved in LTP based on an inhibitor study (O'Dell et al., 1991). The PTK inhibitors, lavendustin A and genistein, block the induction of LTP with no effect on the normal synaptic transmission and established LTP. Recently, Grant et al. (1992) have reported the deficiency of LTP in mice with mutation in a tyrosine kinase gene, *fyn*.

5.3.2.2. *Protein synthesis*

Involvement of protein synthesis in LTP has been reported (Stanton and Sarvey, 1984; Krug et al., 1984; Otani et al., 1989; Frey et al., 1988, 1989; Frazeli et al., 1993). Most of these studies are based on the use of protein kinase inhibitors. Only the late stage of LTP is suppressed by anisomycin, a protein synthesis inhibitor (Frey et al., 1988; Otani et al., 1989). However, another protein synthesis inhibitor, cycloheximide, if applied for 30 min or more, blocks not only the maintenance but also the induction of LTP (Stanton and Sarvey, 1984; Deadwyler et al., 1987). Cycloheximide is probably more potent than anisomycin. Anisomycin is ineffective if applied 15 min after tetanic stimulation (Otani et al., 1989). Therefore, protein synthesis may occur very rapidly from already existing messenger RNAs (mRNAs), probably in postsynaptic sites such as cell bodies, shortly after tetanus (Otani and Abraham, 1989; Frey et al., 1989). The newly synthesized proteins may exert their effects later on. However, a recent report using gel electrophoresis has shown that protein synthesis after tetanic stimulation continues for more than 1 hour (Fazeli et al., 1993). It is apparent that the time course of protein synthesis requires further study. A similar fashion decay of LTP has been observed when LTP is

induced in synapses that are surgically isolated from the major site of protein synthesis in the cell body layer (Frey et al., 1989). Taken together, protein synthesis is probably required for the maintenance and/or the induction of LTP. Classes of newly synthesized protein during LTP have not been identified yet.

5.3.2.3. *Immediate early genes*

Immediate early genes (IEGs) are a class of genes that show rapid and transient but protein synthesis-independent increase in expression to extracellular signals such as growth factors, neurotransmitters or depolarization. Many IEGs code for transcription factors and may regulate the expression of other target genes. Several IEGs, including a c-fos-related gene (Dragunow et al., 1989, Jeffery et al., 1990), Jun-B and zif/268 (Cole et al., 1989; Wisden et al., 1990), have been shown to transiently increase transcription in the dentate gyrus following the induction of LTP by perforant path stimulation. In these studies, multiple trains of tetanic stimulation are used to induce long lasting (several hours or days) LTP. The increase in c-fos expression occurs only if animals are not under central anaesthesia during tetanic stimulation. Pentobarbital, which blocks the induction of long lasting LTP (Krug et al., 1984; Nikolaev et al., 1991), also blocks c-fos expression (Nikolaev et al., 1991). Dragunow et al. (1989) suggest that c-fos is likely to be related to the late stage of LTP. However, the correlation between the increase in c-fos and LTP has been questioned because c-fos expression does not always occur during LTP and vice versa. (Nikolaev et al., 1991; Kaczmarek , 1992).

An increase in zif/268 expression after LTP-inducing tetanic stimulation in anaesthetized and awake animals has been reported (Cole et al., 1989; Richardson et al., 1992). The increase in zif/268 probably occurs at postsynaptic sites. This increase is blocked by APV, a NMDA blocker (Wisden et al., 1990). The increase in zif/268 expression correlates better with the

maintenance than with the induction of LTP (Abraham et al., 1991; Richardson et al., 1992). Although the correlation between the expression of zif/268 and LTP is better than the one between the c-fos and LTP, direct evidence for the increase in zif/268 expression responsible for the maintenance of LTP is not available at present. A dissociation between LTP induction and zif/268 expression has also been reported (Schreiber et al., 1991). The increase in zif/268 occurs shortly after multiple train tetanus, reaches the peak in approximately 20 min and then returns to baseline within 2 hours (Richardson et al., 1992). It has been proposed that the IEG may not be directly involved in the late stage of LTP, but serves as a third messenger in the cascade of cellular and nuclear events that are responsible for the maintenance of LTP. In general, most of the current available data indicate that an increase in IEGs expression is associated with the late stage (several hours and days) of LTP. Whether this increase is responsible for LTP requires more experimental evidence.

5.3.2.4. Release of proteins

An increase in the release of proteins, including newly synthesized ones, into extracellular space in vitro (Duffy et al., 1981) and in vivo (Charriaut-Marlangue et al., 1988; Fazeli et al., 1988) has been observed after the induction of LTP. The presence of proteases in push-pull perfusates following the induction of LTP has been reported (Fazeli et al., 1990). Otani et al. (1992) have recently shown that the increase in protein release lasts for hours and is blocked by cycloheximide, a protein synthesis inhibitor. It is possible that the released proteins are involved in the maintenance of LTP. Fluid collected from guinea pig hippocampal (Chirwa and Sastry, 1986) or rabbit neocortical surface (Sastry et al., 1988a) during tetanic stimulation induces LTP and enhances neurite growth in PC-12 cells. Some components released into the fluid may be proteins (Sastry et al. 1988a,b; Xie et al., 1991), and this issue will be discussed

later. At present, there is clear evidence that the increase in protein release into the extracellular fluid is associated with LTP. Whether these proteins are directly involved in LTP is unknown.

5.3.3. Possible retrograde messengers

Based on the current available data, the induction of LTP is mainly postsynaptic while the maintenance of LTP is, at least in part, presynaptic. If this is true, some forms of communication between the postsynaptic neuron and the presynaptic terminal must occur. Therefore, the hypothesis of retrograde messengers has been proposed (Sastry et al., 1986; Bliss et al., 1986; Bliss et al. 1988). Malinow and Tsien (1990) have found that LTP occurs only if pairing presynaptic stimuli with postsynaptic depolarization is given within 30 min after gaining whole-cell access. The finding suggests that some kinds of diffusible cytoplasmic substances which are critical for the induction of LTP, are lost during the recording and these substances may be retrograde messengers. Ideally, retrograde messengers should be released from the postsynaptic cells during tetanic stimulation. The released substances should be highly diffusible and be able to act rapidly on the presynaptic terminals to induce transmitter release. There are not many substances which meet these requirements. In recent years, several substances, including arachidonic acid, nitric oxide, and carbon monoxide, have been proposed as retrograde messengers. In addition, K^+ , amino acids and proteins released from postsynaptic cells during tetanic stimulation are also candidates for retrograde messengers.

5.3.3.1. Arachidonic acid

Arachidonic acid, an unsaturated fatty acid, has been found to induce a slowly developing LTP (Williams et al., 1989). This LTP is activity-dependent (Williams et al., 1989; also see Drapeau et al., 1990). The arachidonic acid-induced LTP is not blocked by APV (Williams et al., 1989). However, recent

studies have shown that the arachidonic acid-induced LTP is blocked by APV (O'Dell et al., 1991) and arachidonic acid can potentiate NMDA currents (Miller et al. 1992). Nordihydroguaiaretic acid (NDGA), an inhibitor of arachidonic acid production and metabolism, has been reported to block the induction (Lynch et al., 1989; Williams and Bliss, 1989) and the maintenance of LTP (1989). An increase in glutamate release from hippocampal synaptosomes by arachidonic acid (Lynch and Voss, 1990) and an increase in arachidonic acid concentration in the postsynaptic membrane after LTP (Clements et al., 1991) have been seen. These findings further support the involvement of arachidonic acid in LTP. However, whether arachidonic acid acts as a retrograde messenger is doubtful. The effect of arachidonic acid on EPSPs is slow to develop. In contrast, the tetanus-induced LTP develops very rapidly. Furthermore, arachidonic acid can directly potentiate NMDA currents (Miller et al., 1992) and inhibit glutamate uptake in glial cells (Barbour et al., 1989; Martin et al., 1991a) suggesting that the actions of arachidonic acid on postsynaptic and glial cells may also be responsible for the arachidonic acid-induced LTP.

5.3.3.2. *Nitric oxide and carbon monoxide*

Nitric oxide (NO), which is derived from arginine in a reaction catalyzed by NO synthase has recently been reported to be involved in LTP (Bohme et al., 1991; Schuman and Madison, 1991; O'Dell et al., 1991). NO is highly diffusible and considered to be a strong candidate as a retrograde messenger for LTP. Bath application of NO synthase inhibitors such as N-methyl-L-arginine (NMLA) and L-nitroarginine (LNA), or NO scavenger, hemoglobin (Hb) inhibits the induction of LTP (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991a; Haley et al., 1992; also see Errington et al., 1991). However, NO inhibitors have no effect on the established LTP (Haley et al., 1992). Injection of NO synthase inhibitors into the postsynaptic neuron also prevent the LTP

induced by pairing postsynaptic depolarization with presynaptic stimulation (Schuman and Madison, 1991). On the other hand, sodium nitroprusside, which release NO, can induce LTP (Bohme et al., 1991). Furthermore, direct application of NO to hippocampal slices paired with a weak tetanus induces an input-specific LTP, which is blocked by hemoglobin, but not by APV and nifedipine, a Ca^{2+} channel blocker (Zhuo et al., 1993). This LTP develops rapidly and occludes the tetanus-induced LTP. Tetanic stimulation and NMDA have been reported to activate NO synthase in the hippocampus (East and Garthwaite, 1991). Most of the data discussed above seem to favour the notion that NO, indeed, is a retrograde messenger for LTP in the hippocampus. However, several lines of evidence undermine the hypothesis of NO as a retrograde messengers for LTP in the hippocampus. First, histochemical evidence for NO synthase (NOS-I) in CA1 pyramidal cells is not available (Bredt et al., 1991; Vincent and Kimura, 1992, but also see Schweizer et al., 1993). Second, it is not clear whether NO can directly increase glutamate release from presynaptic terminals. Third, injection of NO inhibitor into the postsynaptic neuron does not prevent the tetanus-induced LTP (Schuman and Madison, 1991). Fourth, the application of NO paired with low frequency stimulation does not induce LTP, but LTD (Zhuo et al., 1993). Fifth, the selectivity of NO inhibitors and hemoglobin has also been questioned. Therefore, conclusion should not be made until the answers for these questions are found.

Recently, carbon monoxide (CO) has been brought forward as a potential retrograde messenger for LTP (Zhuo et al., 1993; Stevens and Wang, 1993). Much like NO, CO is a highly diffusible agent. Moreover, the heme oxygenase which produces CO is clearly present in hippocampal pyramidal cells (Verma et al., 1993). Bath application of CO to hippocampal slices when paired with a weak tetanus or even low frequency stimulation induces an input-specific LTP

which is not blocked by APV and nifedipine (Zhuo et al., 1993). The heme oxygenase inhibitors such as zinc protoporphyrin IX (ZnPP) prevent the induction of LTP induced by a strong tetanus (Zhuo et al., 1993; Stevens and Wang et al., 1993). Furthermore, ZnPP can reverse the established LTP without affecting the control pathway (Stevens and Wang, 1993). At present, it is not known whether tetanic stimulation is able to activate heme oxygenase. Based on the available results, CO appears to be a very attractive candidate to serve as a retrograde messenger for LTP in hippocampus. However, it is too early to conclude the role of CO in LTP because more studies on this agent are needed.

5.3.3.3. *Neurotrophic factors*

Neurotrophic factors have been suggested to be involved in LTP (Sastry et al., 1988b). Application of nerve growth factor (NGF) when paired with weak tetanus induces LTP (Sastry et al., 1988b; also see Tancredi et al., 1993). Recently, LTP has been reportedly associated with an increase in neurotrophin levels in the stimulation region in hippocampal slices (Patterson et al., 1992). The evidence further supports the involvement of neurotrophic factors in LTP. Other neurotrophic factors such as epidermal growth factor (EGF) and fibroblast growth factor (FGF) have also been shown to convert STP to LTP or to facilitate LTP (Terlau and Seifert, 1989; Abe et al., 1991; 1992; Ishiyama et al., 1991; Hisajima et al., 1992). It is believed that neurotrophic factors such as NGF are released from postsynaptic neurons and induce biochemical and morphological changes in the presynaptic terminals (Hendry et al., 1974; Springer and Loy, 1985; Thoenen, 1991; Patterson and Nawa, 1993). Substances collected from guinea pig hippocampus (Chirwa and Sastry, 1986) or rabbit neocortical surface (Sastry et al., 1988a) during tetanic stimulation, when applied to guinea pig hippocampal slices, induce LTP. These substances also enhance neurite growth in cultured PC-12 cells (Sastry et al., 1988a). Saccharin which interferes

with NGF binding and decreases neurite growth (Ishii, 1982) prevents not only the tetanus-induced LTP, but also the substances-induced LTP (Morishita et al., 1992). Saccharin also blocks the enhancement of neurite growth in PC-12 cells produced by the substances. It is possible that these substances released from postsynaptic cells during tetanic stimulation act as neurotrophic factors and enhance transmitter release from presynaptic terminals. Further details on the effect and characteristics of these substances will be discussed in the results and discussion of this thesis.

5.3.3.4. Released K^+

Released K^+ from postsynaptic cells during tetanic stimulation is also a candidate to serve as a retrograde messenger for LTP. This ion is highly diffusible and induces effects very rapidly. Elevation of extracellular K^+ in the absence (May et al., 1987) and the presence of extracellular Ca^{2+} (Ballyk and Goh, 1992; Fleck et al., 1992) has been demonstrated to induce LTP. The mechanisms for the K^+ -induced LTP are not known. Ionophoretic application of K^+ or baclofen to dendritic zone in the CA1 field when paired with weak tetanus also induces LTP (Ballyk and Goh, 1992). It is possible that an increase in synaptic K^+ level induced by tetanic stimulation causes further changes around the synapses, including the presynaptic terminals. ACPD receptors have been implicated to be involved in the maintenance of LTP. Presynaptic ACPD receptors mediate a positive feed back of glutamate exocytosis (Herrero et al., 1992). The coupling between phospholipase C (PLC) and ACPD receptors is potentiated by extracellular K^+ (Irving et al., 1992). However, it is not sufficient evidence to decide what kind of role extracellular K^+ plays in LTP. Furthermore, K^+ can be released from sources other than postsynaptic cell such as glia. In fact, involvement of glia in LTP has been suggested (Sastry et al., 1988c)

5.4. Modulatory factors on LTP

Both endogenous glutamate and GABA play a critical role in controlling LTP in hippocampus. However, many other neurotransmitters have modulatory effects on LTP. Therefore, a brief discussion is provided here on the modifying role of neurotransmitters in LTP

Acetylcholine (ACh) has been reported to induce a NMDA receptor-dependent LTP in the CA1 region (Markram and Segal, 1990a). The effect of ACh is probably due to the NMDA potentiation by ACh (Markram and Segal, 1990b). Carbacol, an ACh receptor agonist, has shown to potentiate NMDA currents (Otani and Ben-Ari, 1993).

Noradrenaline (NA) has been shown to induce LTP in the dentate gyrus (Neuman and Harley, 1983). This LTP is linked to NMDA receptor activation (Burgard et al., 1989; Dahl and Sarvey, 1990). In the CA1 region, NA induces LTP of the population spike, but not LTP of the EPSP (Heginbotham and Dunwiddie, 1991).

Serotonin has been recently demonstrated to block the LTP induced by primed burst stimulation but not the LTP induced by strong tetanus (Corradetti et al., 1992, also see Bliss et al., 1983).

Dopamine has been found to increase after tetanic stimulation (Frey et al., 1990). Furthermore, Frey et al. (1991) have shown that D₁ receptor antagonist blocks the maintenance of LTP.

In summary, LTP can be modulated by other neurotransmitters. The mechanisms for the effect of these neurotransmitters on LTP are far less known than those for glutamate and GABA

Several neuropeptides, including somatostatin (Matsuoka et al., 1991a, b), arginine-vasopressin (AVP₄₋₈) (Rong et al., 1993) and dynorphin (Weisskopf et al., 1993) have been shown to be involved in LTP. Somatostatin has been

found to be co-localized with GABA in the CA1 and CA3 interneurons. Since the peptide and GABA can be co-released from the inhibitory terminals by afferent stimulation, it is of interest to understand how the peptide interacts with GABA and modulates LTP.

Recently, free radicals have also been shown to facilitate the decay of LTP in the CA1 area of the hippocampus (Pellmar et al., 1991). Both free radicals and antioxidants have been shown to modulate NMDA receptor function. Whether antioxidants, which remove free radicals, can also modulate LTP will be discussed later.

5.5. LTP in hippocampal interneurons

Orthodromic stimulation in CA1 field activates not only the afferents to pyramidal cells, but also the afferents to interneurons which, in turn, provide inhibition to pyramidal cells through the feedforward system. Therefore, changes in interneurons after tetanus directly affect the LTP in CA1 neurons. LTP in CA1 interneurons has been reported in vitro (Taube and Schwartzkroin, 1987; Abraham et al., 1987) and in vivo (Buzsaki and Eidelberg, 1982; Kairiss et al., 1987). However, IPSPs recorded from CA1 neurons show inconsistent changes after tetanic stimulation of the stratum radiatum (Abraham et al., 1987). IPSPs of CA1 neurons have been shown to increase, decrease or not change after tetanus (Abraham et al., 1987). The IPSPs in the CA1 neurons usually represent a mixture of feedforward and feedback inhibition. Changes in pyramidal neurons properties directly alter the expression of IPSPs. Therefore, the changes in the IPSPs of CA1 neurons do not directly reflect the changes in interneuron EPSP. Recently, Reece and Redman (1992) and Rai et al. (1993) have shown that LTP in CA1 interneurons can be induced by pairing weak tetanus and postsynaptic depolarization. This result is consistent with the previous findings reported by others (Buzsaki and Eidelberg, 1982; Taube and

Schwartzkroin, 1987). However, the mechanisms for interneuron LTP is little known. This is partly due to the difficulties of studies on interneurons.

5.6. The physiological significance of LTP

LTP has attracted the interest of many neuroscientists because it is a phenomenon of use-dependent modification in synapses of mammalian brain that is thought to be the cellular mechanism of learning and memory. Hebb (1949) postulated that the synapses linking two cells are strengthened when coincident activity of two cells occurs. This synaptic strengthening, he suggested, forms the foundation for learning and memory. Several lines of evidence support LTP as the cellular mechanism for learning and memory. First, the characteristics of LTP such as input-specificity, cooperativity and associativity are consistent with Hebb's rule. LTP can be induced by θ rhythms (Larson et al., 1986; Larson and Lynch, 1989), which occur during exploratory or learning activities (O'Keefe and Nadel, 1978; Vanderwolf, 1969). Second, behavioural studies have shown that some learning behaviours induce LTP or LTP-like changes in synaptic responses in the hippocampus (Sharp et al., 1985; Green and Greenough, 1986; Wilson et al., 1986; Skelton et al., 1987). Prior learning enhances the LTP induced later in the same brain (Bergis et al., 1990). A prior induction of LTP also affects the animal's later spatial learning (Castro et al., 1989; McNaughton et al., 1986; Berger, 1984). Third, recent studies have demonstrated that pharmacological agents such as APV, which block the induction of LTP in hippocampus, also impair spatial learning and memory of animals (Davis et al., 1992; Morris et al., 1986; Bolhuis and Reid, 1992). Genetic studies have shown that mice with mutations in certain genes, exhibit impaired spatial learning as well as impaired LTP (Silva et al., 1992a,b; Grant et al., 1992). These results suggest that LTP is correlated with certain types of learning and memory. However, although these lines of evidence are consistent

with the idea that LTP underlies the cellular mechanisms for certain forms of learning and memory, there is lack of direct evidence to make any firm conclusion at present. Experimentally, it is difficult to study isolated "learning and memory" in animals. Pharmacological agents and gene mutation not only affect learning and memory, but also may induce general changes in the structure and the function of the brain. If LTP underlies learning and memory at the cellular level, further understanding in the mechanisms of LTP will help to devise rational treatments for neurological disorders such as Alzheimer's disease, which are related to learning and memory.

5.7. Summary

LTP has been extensively studied by neuroscientists around the world since its discovery in hippocampus two decades ago. Among LTPs in different parts of the brains, the LTP in hippocampus is most well studied. Much progress has been made in elucidating the mechanisms underlying the induction and maintenance of LTP in recent years. However, many fundamental issues on LTP have not been resolved. The induction of LTP is primarily mediated through postsynaptic mechanisms. In most cases, the activation of NMDA is necessary for the induction. The Ca^{2+} influx through NMDA channels activates a number of enzymes which trigger a series of biochemical and morphological changes in the synapses. This would result in the induction of LTP. In addition to the NMDA receptor activation, a presynaptic element is obviously involved in the induction of LTP because the induction of LTP is presynaptic activity-dependent. Based on currently available data, the maintenance of LTP is likely due to both presynaptic and postsynaptic changes. The questions are what are these changes and how these changes occur. Many investigators favour the notion that LTP is due to an increase in neurotransmitter release from presynaptic terminals, changes in postsynaptic receptor sensitivity and/or number, and/or

changes in spine structure. These changes can be caused by the activation of protein kinases which induce phosphorylations of protein molecules in receptors or ion channels. However, more convincing evidence is required to conclude that these changes, indeed, occur and are responsible for the maintenance of LTP. Increases in protein synthesis and gene expression seem to be associated with LTP. Whether these increases are responsible for LTP remains to be determined. The search for retrograde messengers is one of the hottest topics in the field of LTP for the last few years. Nitric oxide, arachidonic acid, neurotrophic factors are among the strongest candidates. So far, none of them has been proved. GABAergic input is known to have influence in LTP, but the role of GABA_B receptors in LTP has been basically unknown until recently. In many studies of LTP, GABAergic inhibition is blocked to minimize the influence of the IPSPs on the EPSP. However, under normal physiological conditions, changes in GABAergic inhibition cannot be ignored. Understanding the changes of GABA receptor-mediated IPSPs during LTP will help to resolve some of the issues involved in LTP. Some endogenous neuropeptides are co-released with GABA or glutamate from presynaptic terminals. Endogenous peptides, such as somatostatin, AVP₄₋₈ and dynorphin, have been shown to have modulatory effects on LTP. Interactions between neuropeptides and neurotransmitters may occur during LTP. What kinds of roles do endogenous neuropeptides play in LTP is largely unknown. Finally, the physiological significance of LTP is not very clear. LTP is thought to underlie the cellular mechanism for learning and memory. If this is the case, agents, which affect learning and memory, should also affect LTP. Studies of the effects of these agents on LTP will lead to a better understanding of the physiological significance of LTP.

5.8 *Rationale and specific aims*

As noted in the review on LTP, many questions on LTP remain unanswered. The main goal in the present studies is to examine various mechanisms that modulate LTP. Briefly, the present investigations focus on three major subjects: (1) the role of endogenously released substances in LTP, (2) the influence of changes in GABAergic inhibition on LTP of the EPSP, (3) the role of α -tocopherol in LTP.

5.8.1. *The role of released substances in LTP*

It has been reported that proteins are released during tetanic stimulation or LTP (Duffy et al., 1981; Charriault-Marlangue et al., 1988). It is, however, not known whether these released proteins are involved in LTP. Previous studies in our laboratory found that substances collected from the guinea pig hippocampus (Chirwa and Sastry, 1986) and rabbit neocortex (Sastry et al., 1988a) during tetanic stimulation, when applied on guinea pig hippocampal slices, could induce LTP of the population spike in these slices. Since these substances are diffusible, it is possible that they act as retrograde messengers for LTP. It has been hypothesized that retrograde messengers are released from postsynaptic cells and cause presynaptic modifications which lead to LTP (Bliss et al., 1986; Sastry et al., 1986). If some of these substances are retrograde messengers for LTP, they could be released from postsynaptic cells through a NMDA receptor-dependent mechanism, and subsequently cause presynaptic modifications. However, the substances are not characterized and the mechanisms underlying their release and actions have not been examined. Experiments were, therefore, designed to address the following questions: (1) Does the release of these substances require the activation of NMDA receptors? (2) Do these substances enhance the excitatory transmission or do they also affect the inhibitory transmission? (3) Do the LTP-inducing actions of the substances involve

presynaptic or/and postsynaptic elements? (4) What are the molecular weights of the LTP-inducing substances and are proteins present?

5.8.2. *The influence of GABAergic inhibition on LTP*

Studies based on the effect of GABA receptor antagonists on LTP indicate that GABAergic inhibition has significant influence on the induction of LTP (Wigstrom and Gustafsson, 1983; Davies et al., 1991; Mott and Lewis, 1991). In many studies of LTP of the EPSP, the fast and the slow IPSPs are therefore blocked to minimize the effects of IPSP on the EPSP. However, under physiological conditions, stimulation of the stratum radiatum evokes not only EPSP but also GABA receptor-mediated fast and slow IPSPs in the CA1 region. It is known that tetanic stimulation induces LTP of the EPSP. However, post-tetanic changes in the IPSPs are controversial. If post-tetanic changes in the IPSPs occur, these changes may affect the expression of LTP of the EPSP. Therefore, the main objective of this study is to examine whether tetanic stimulation induces long-term changes in the IPSPs, and if so, whether these changes modulate the expression of LTP of the EPSP. Since tetanic stimulation is believed to induce LTP of the EPSP through an increase in postsynaptic free Ca^{2+} and PKC activity (Lynch et al., 1983; Malinow et al., 1989), and since increases in intracellular free Ca^{2+} and PKC activity have been shown to have significant effects on the IPSPs (Baraban et al., 1985; Dutar and Nicoll, 1988b; Chen et al., 1990), BAPTA (Ca^{2+} chelator) and K-252b (PKC inhibitor) were used as tools to determine whether changes in intracellular free Ca^{2+} and PKC activity would affect post-tetanic changes in the IPSPs. These experiments were designed to answer the following questions: (1) Is there any long-term change in the IPSPs after tetanic stimulation of the stratum radiatum, and if so, are there any differences between the fast and the slow IPSPs? (2) Do changes in postsynaptic free Ca^{2+} and PKC activity affect post-tetanic IPSPs? (3) Are post-

tetanic changes in the IPSPs dependent on glutamatergic transmission? and (4) Do post-tetanic IPSPs have any effect on the expression of LTP of the EPSP?

Somatostatin (SS), a peptide which is co-localized in some of GABAergic neurons and their terminals in the CA1 area, may be co-released with GABA from the same terminals by tetanic stimulation. At present, it is unknown whether this peptide has effects on LTP of the EPSP in CA1 neurons. Application of SS has been shown to depress both the fast and slow IPSPs through an unknown mechanism (Scharfman and Schwartzkroin, 1989). If interactions between SS and GABA occur at the same synapses, the peptide may modify LTP of the EPSP as well. If SS, like picrotoxinin (GABA_A blocker) or phaclofen (GABA_B blocker), blocks the fast and slow IPSPs, this peptide may facilitate the induction of LTP of the EPSP. On the other hand, SS may prevent the induction of LTP of the EPSP since it causes a hyperpolarization of the neurons (Pittman and Siggins, 1981). Therefore, it is important to determine how SS interacts with GABAergic inhibition and whether SS can influence LTP of the EPSP. Experiments were conducted to address the following issues: (1) How does SS modulate GABAergic inhibition? and (2) Does SS affect LTP of the EPSP?

5.8.3 *The role of α -tocopherol in LTP*

Free radicals and antioxidants have been implicated in adversely affecting the memory process in diseases such as Alzheimer's. α -Tocopherol, a major lipid soluble antioxidant in the biological systems, has been shown to be involved in spatial learning in rats (Moriyama et al., 1990) and its levels in the brain are lower in Alzheimer's disease (Jeandel et al., 1989). In cultured neurons, α -tocopherol can act as a neurite-inducing factor (Nakjima et al., 1991); and such factors have been implicated in LTP (Sastry et al., 1988b). Meanwhile, free radicals have been shown to facilitate the decay of LTP (Pellmar et al.,

1991). These lines of evidence raise the possibility that α -tocopherol may be involved in learning and memory as well as LTP. Therefore, the role of α -tocopherol in LTP was examined. Experiments were conducted to resolve the following issues: (1) Does α -tocopherol induce LTP of the EPSP? (2) If the agent induces LTP, is this LTP similar to the tetanus-induced LTP? (3) Is the ability to induce LTP impaired in hippocampal slices obtained from vitamin E deficient animals?

6. PHARMACOLOGICAL TOOLS FOR EXPERIMENTS

6.1. Somatostatin

Somatostatin (SS or SS-14) is a 14-amino acid containing peptide originally isolated from the hypothalamus and inhibits growth hormone secretion from the anterior pituitary (Brazeau et al., 1973). Several lines of evidence have shown that this peptide acts as a neurotransmitter in the CNS (Olpe et al., 1980; Epelbaum, 1986). SS and its precursor (SS-28) can induce multiple physiological actions in the brain, including the modulation of Ca^{2+} and K^{+} conductances, neuronal cell firing and neurotransmitter release (Epelbaum, 1986; Inoue and Yoshii, 1992). The discussion here is focused on the actions of SS in the hippocampus.

6.1.1. Distribution of somatostatin in hippocampus

Immunohistochemical studies have revealed the presence of SS binding sites and SS-like immunoreactivity in mammalian hippocampus and several other areas of the brain (Bennet-Clarke et al., 1980; Kohler and Chan-Palay, 1982). In the hippocampus, SS-immunoreactivity are present in some interneurons in the stratum oriens and the stratum radiatum of the CA1 and CA3 fields, and the hilus of the dentate gyrus (Johansson et al., 1984; Somogyi et al., 1984; Kosaka et al., 1988). Most of the SS-containing neurons are a subpopulation of GABAergic interneurons (Somogyi et al., 1984; Kosaka et al.,

1988). The highest densities of SS receptors are found in the stratum oriens and stratum radiatum of the CA1 field. Somewhat lower densities are found in the CA3 field. The pyramidal cell layer and the stratum lacunosum-moleculare of the CA field have very few SS receptors (Swanson et al., 1987). Recent studies have revealed that SS receptor subtypes exist in the brain (Raynor and Reisine, 1992; Bell and Reisine, 1993). Four types of SS receptors have been cloned. (Bell and Reisine, 1993). But the function of these cloned SS receptors remains to be defined. Two of the better known SS receptors are the SS₁ and SS₂ receptors. The SS₁ receptors have selectively high affinity for the agonist MK678 while the SS₂ receptors have high affinity for the agonist CGP 23996 with no affinity for MK678. Both SS₁ and SS₂ receptors have been found in the CA1 field. The dentate gyrus expresses primarily SS₁ receptors (Martin et al. 1991b; Raynor and Reisine, 1992).

6.1.2. Actions of somatostatin on hippocampal neurons

The electrophysiological data on the effects of SS have been quite confusing in the past (Delfs and Dichter, 1985). This is probably due to the data obtained from different types of cells and the existence of multiple SS receptor subtypes. In the hippocampus, SS has been reported to induce a depolarization (Dodd and Kelly, 1978) or a hyperpolarization (Pittman and Siggins, 1981) of the CA1 neurons. Scharfman and Schwartzkroin (1988) have shown that SS depolarizes the pyramidal cells when applied directly on the soma. When applied to the dendrites, particularly the distal dendrites, SS can induce a hyperpolarization of the CA1 neurons. These results implicate that different subtypes of SS receptors may exist in different area of the neurons. Bath application of SS consistently induces a hyperpolarization of the CA1 neurons, associated with a reduction in the input resistance (Pittman and Siggins, 1981; Scharfman and Schwartzkroin, 1988; Moore et al., 1988). The SS-induced

hyperpolarization is due to an increase in K^+ conductance (Pittman and Siggins, 1989; Twery et al., 1991). In addition, SS also enhances the noninactivating, voltage-dependent K^+ current (I_M) in the hippocampal CA1 neurons. The SS-induced increase in I_M is probably mediated by arachidonic acid metabolites (Schweitzer et al., 1990). Whether the effects of SS are mediated through different subtypes of SS receptors is not certain. It has been reported that the effects of somatostatin on the resting K^+ conductance are pertussis toxin-sensitive while the augmentation of I_M is pertussis toxin-insensitive (Schweitzer et al., 1989). It is known that SS_1 receptors couple to G proteins while SS_2 receptors do not (Raynor and Reisine, 1992). Therefore, it is possible that different subtypes of SS receptors mediate the effects of SS on the resting K^+ conductance and I_M . The effects of SS on Ca^{2+} currents in hippocampal neurons are not clear. However, SS has been reported to reduce Ca^{2+} currents in neocortical neurons (Wang et al., 1990) and other types of cells (Chen et al., 1990). Since SS and GABA are co-localized in some interneurons in the CA1 area of the hippocampus (Somogyi et al., 1984), interactions between SS and GABA may be relevant to the role of SS in epilepsy as well as LTP. It has been reported that SS depresses the GABA receptor-mediated IPSPs in hippocampal CA1 neurons (Scharfman and Schwartzkoin, 1989). The mechanisms of the interactions between SS and GABA are however not very clear.

6.1.3. The functional roles of somatostatin

Several lines of evidence implicate that somatostatin may be involved in learning and memory as well as LTP (Schettini, 1991; Haroutunian et al., 1987; Matsuoka et al., 1991a, b). The intracerebroventricular (i.c.v.) administration of SS significantly increases the active avoidance behaviour of the animals (Vecsei and Widerlov, 1988). Cysteamine can produce a rapid, relatively selective, but reversible, depletion of central SS stores in animal (Sagar et al., 1982).

Cysteamine-induced depletion of SS in the CNS results in a significant attenuation of passive avoidance retention test performance of the drug-treated rats (Haroutunian et al., 1987). LTP of the mossy fibre pathway in the hippocampus obtained from cysteamine-treated guinea pigs is significantly smaller than the LTP in the hippocampus obtained from the control guinea pigs (Matsuoka et al., 1991b). SS has also been reported to enhance LTP in the mossy-fibre pathway of the CA3 field. The mechanisms of the effects of SS on LTP are not clear. However, these reports implicate that somatostatin may play an important role in some forms of learning and memory. The effects of SS on LTP of the EPSP in the CA1 field have not been reported. SS may play a role in LTP in the CA1 field because of the interactions between SS and GABA.

Involvement of SS in Alzheimer's disease has been reported (Davies and Terry, 1981; Ferrier et al., 1983; Beal, 1990). In patients with Alzheimer's disease, SS levels are significantly decreased in several areas of the brain (Schettini, 1991; Bissette and Myers, 1992). The decrease in SS levels is more significant than the decrease in the levels of other neuropeptides in the brains of patients with Alzheimer's disease (Ferrier et al., 1983). The SS receptor binding in several parts of the brain, including the hippocampus, is significantly decreased with aging (Sato et al., 1991). These results indicate that SS is likely to be involved in the memory process. However, the exact role of SS in Alzheimer's disease and aging is not clear.

SS has been reported to be involved in epilepsy (Riekkinen and Pitkanen, 1990). Changes in SS levels in the frontal cortex and the hippocampus following single seizures have been reported (Sperk and Widman, 1985). In cerebrospinal fluid (CSF), SS level is elevated in the kindling model of epilepsy (Kato et al., 1983; Pitkanen et al., 1987). In epileptic patients, SS binding sites change in number (Riekkinen and Pitkanen, 1990). The real role of SS in

epilepsy is not clear. The effects of SS on GABAergic inhibition may play a role in epilepsy.

6.2. *α -Tocopherol*

α -Tocopherol (vitamin E) is also called the "antisterility vitamin" because it was originally found to be required to sustain a normal pregnancy in female rats (Evans and Bishop, 1922). α -Tocopherol is distributed throughout the tissues of animals and humans. Further studies have indicated that α -tocopherol, as an integral part of the cell membrane, is essential for the maintenance of normal structure and function of many organ systems, including the human nervous system (Tappel, 1962; Sokol, 1988, 1989). A prolonged deficiency of α -tocopherol results in a number of degenerative neurological syndromes in humans and experimental animals (Muller et al., 1983; Sokol, 1988, 1989). α -Tocopherol is a major lipid-soluble antioxidant which can scavenge free radicals attacking from outside of the membrane and within the membrane, and stabilize the biological membrane (Tappel, 1962; Witting, 1980; Erin et al., 1984; Lucy, 1972). α -Tocopherol can also modulate the metabolism of arachidonic acid cascade (Reddy et al., 1988; Tran and Chan, 1988).

6.2.1. *Physicochemical properties*

α -Tocopherol, 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol or 5,7,8-trimethyl tocol, the most active vitamin E factor, was first isolated from wheat germ oil (Evans et al., 1936). α -Tocopherol is a liposoluble compound and liquid at room temperature. The melting point of α -tocopherol is 2.5-3.5 °C. α -Tocopherol is insoluble in water. It is soluble in oils, ethanol, acetone and dimethyl sulfoxide (DMSO). However, the disodium salt of α -tocopherol phosphate is soluble in water while possesses the activity of α -tocopherol. α -Tocopherol is stable to heat and alkalis in the absence of oxygen. It can be slowly oxidized by atmospheric oxygen.

6.2.2. Antioxidant properties

A free radical is a molecule with an unpaired electron in its outer orbit. The unpaired electron makes the molecule unstable and highly reactive, especially towards biological molecules such as lipids, protein and DNA. Free radicals can be produced in a variety of cellular processes in biological systems. Increases in oxygen-derived free radicals have been implicated in pathogenesis of many diseases.

Free radicals and active oxygen compounds such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO^{\cdot}) are normally generated by cellular metabolism. Under normal physiological conditions, intrinsic enzyme systems and antioxidants keep the free radical levels in check (Demopoulos et al., 1982; Fridovich, 1978; Halliwell, 1987). Increases in these free radicals occur when intrinsic enzymes and antioxidants decrease or/and production of free radicals increase under certain pathological conditions such as ischemic injury and Alzheimer's disease.

α -Tocopherol is a major lipid-soluble antioxidant present in biological membranes and prevents lipid peroxidation of membranes (Tappel, 1962; Witting, 1980; Burton and Ingold, 1989). α -Tocopherol reacts directly with peroxy radicals, an intermediate of lipid peroxidation, and forms a very stable tocopheroxyl radical. Subsequently, it stops the free radical chain reaction. Therefore, α -tocopherol successfully protects reactive substrates, such as polyunsaturated fat, in membranes (Tappel, 1962). α -Tocopherol can also scavenge other reactive oxy-radicals, such as $O_2^{\cdot-}$, HO^{\cdot} . It is apparent that α -tocopherol plays a critical role in the maintenance of the structure and function of the membrane. Deficiency of α -tocopherol leads to membrane damage.

6.2.3. *The functional role of α -tocopherol in the CNS*

A severe and prolonged deficiency of α -tocopherol in humans and animals results in a series of neurological syndromes, including ataxia, hyporeflexia, proprioceptive loss, ophthalmoplegia and retinal pigmentation (Sokol, 1989). Neuropathological lesions include a degeneration of the axons in several parts of the brain. Treatment of patient with pharmacological doses of α -tocopherol prevents the development of neurological syndromes or causes improvement (Sokol, 1989).

Several diseases, including Alzheimer's disease, and normal aging (Wartanowicz et al., 1984) have been reported to be associated with decreases in α -tocopherol and other antioxidant levels, and increases in several free radicals contents (Wartanowicz et al., 1984; Carney et al., 1991; Jeandel et al., 1989; Smith et al., 1991). Whether these changes in α -tocopherol and free radicals are directly related to Alzheimer's disease and normal aging is not clear. Both Alzheimer's disease and aging are associated with deterioration of memory. Behavioural changes of α -tocopherol deficient rats have been observed (Ichitani et al., 1991; Moriyama et al., 1989, 1990). In α -tocopherol deficient rats, impairment of spatial learning ability associated with an increase in lipid peroxide content in hippocampus has been observed (Moriyama et al., 1990). In these reports, the experimental rats showed no significant changes in body weight, locomotor activity when the learning tests were performed. These results implicate that the learning impairment in α -tocopherol rats may be partly related to the increase of lipid peroxide content in hippocampus. α -Tocopherol has been shown to support the survival of cultured neurons and to enhance neurite growth (Nakajima et al., 1991; Sato et al., 1993). Recently, free radicals have been reported to facilitate the decay of LTP in the hippocampus (Pellmar et al., 1991). These findings demonstrate that α -tocopherol and free radicals are

probably related to certain types of learning and memory. However, clear evidence is needed to clarify this matter.

7. METHODS AND MATERIALS

7.1. Animals

Male Hartley guinea pigs, male Wistar rats and New Zealand White rabbits were obtained from the Animal Care Centre of the University of British Columbia. Once a week, 5-6 guinea pigs (150-200 g, approximately 20 day old) were received from the centre and placed in a wire cage in the animal room of the Department of Pharmacology & Therapeutics for less than a week before being used for experiments. These guinea pigs had free access to guinea pig chow and water. Two rabbits (either sex; approximately 2-3 kg; 42-56 days old) were received each time and 2-3 times a week. Each of these rabbits was kept in a big wire cage and fed with rabbit chow in the animal room. Three sets of sixteen male rats (50-100 g, approximately 10-14 days old) were obtained for vitamin E deficient experiments. Each set of rats was divided into two groups (8 in each group) and placed in separate cages. One group was fed on a vitamin E control diet while the other group was fed on a vitamin E deficient diet. These diets were obtained from Harlan Teklad (Madison, Wisconsin). With the exception of vitamin E, the components of vitamin E deficient diet are the same as those of vitamin E control diet. The first set of rats was fed on these diets for one month before being used for experiments. The second and third sets of rats were fed for two and three months, respectively. Different rooms were used for guinea pigs, rats and rabbits. The temperature and humidity of these animal rooms were controlled at 22-23 °C and 50-55%, respectively.

7.2. Perfusion media

The standard physiological medium was prepared daily. This medium contained (in mM) NaCl 120, KCl 3.1, NaHCO₃ 26, NaH₂PO₄ 1.8, MgCl₂ 2.0,

CaCl₂ 2.0 and dextrose 10.0. The pH of the medium was 7.4 when bubbled with carbogen (95% O₂ and 5% CO₂). The hippocampal slices were perfused with this standard medium unless otherwise stated.

In some experiments in which picrotoxinin (20 μM) was added to the medium, the concentration of CaCl₂ and MgCl₂ was increased to 4 mM and NaH₂PO₄ was omitted. The increase in the concentration of Ca²⁺ and Mg²⁺ in this picrotoxinin-containing medium was to minimize epileptiform activity which can be induced by adding picrotoxinin. Exclusion of NaH₂PO₄ in this medium was to avoid precipitation that may result from the increase in CaCl₂. The modification did not change the pH of the medium.

In the Mg²⁺-free medium, Mg²⁺ was omitted from the standard medium while the other components of the standard medium were unchanged.

Drugs used in the experiments were usually first prepared as concentrated stock solutions once or twice a week. These stock solutions were stored in the refrigerator. The concentrated stock solutions were diluted with the standard medium to the desired concentrations just before a experiment. Drugs prepared in this manner included D(-)-2-amino-5-phosphonovalerate (D-APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), N-methyl-D-aspartate (NMDA), L-glutamate, picrotoxinin (PTX), tetrodotoxin (TTX), (-)-baclofen, 2-OH-saclofen, phaclofen, sphingosine, K-252b, somatostatin-14 (SS) and α-tocopherol phosphate (disodium salt). In some experiments, NaH₂PO₄ was omitted from the standard medium when α-tocopherol phosphate was added. CNQX, 2-OH-saclofen and phaclofen were purchased from Tocris Neuramin. K-252b was obtained from Dr. A. Mori of Kyowa Hakko Kogyo Ltd. Japan. All other drugs were from Sigma.

The standard medium and the drug-containing media were contained in separate 50 ml barrels (Fig. 3). A tube from each of the barrels was connected

to a common manifold, and the single outlet of the manifold was connected to the slice chamber via a connecting tube. A reservoir (1L) containing the standard medium was placed above the barrels. This reservoir was connected to the main barrel which also contained the standard medium. The media in all the barrels and the reservoir were oxygenated via separate lines which were connected to a carbogen cylinder. The lines from the reservoir to the main barrel and from the main barrel to the slice chamber were always open except during the period of drug application. The flow rate was 1.5-2 ml/min. A suction line was connected to the outlet of the slice chamber so that the flow rate in the chamber was always constant. When drugs were applied, the line from the main barrel was closed quickly with a clip, and the line from the drug-containing barrel was opened simultaneously. The same procedure was applied for the termination of the application.

7.3. Slice Chamber

The slice chamber was fully described by Panaboina and Sastry (1984) from this laboratory and is illustrated in Figure 3. The chamber contained three main components. They are as follows: a plexiglass stage, a circular chamber drilled into the top surface of the plexiglass stage, and an aluminum temperature-regulating bar which was located underneath the chamber and could control the temperature of the chamber according to the need of the experiments.

7.4. Preparation of slices

Transversely sectioned hippocampal slices (450 μm thick) were obtained from male guinea pigs, with a few exceptions in which male rats were used. The animal was gently placed on the top of an ice pack (to lower the animal's metabolic demand) in a dessicator. The animal was anaesthetized with a mixture of halothane (2%) and carbogen (95% O_2 , 5% CO_2) introduced into the

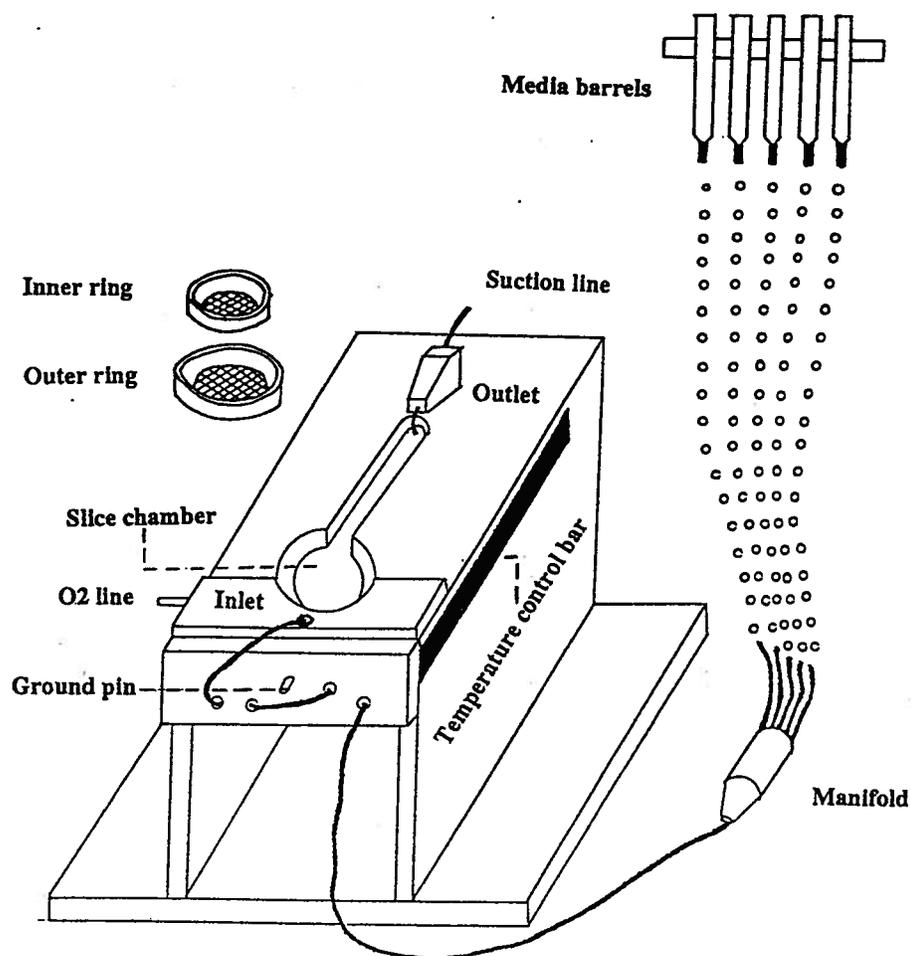


Figure 3 Slice chamber and perfusion system for maintaining hippocampal slices.

The slice chamber and perfusion system used in the present study is shown in this sketch. Note that the transversely sectioned hippocampal slices were placed between the meshes of the inner and outer rings which were inserted in the slice chamber. Oxygenated standard medium contained in one of the media barrels coursed through the manifold to the slice chamber where the slices were incubated. A suction line was used to maintain the constant flow rate and medium volume in the chamber.

dessicator. The animal was put on the operation table once the stage of surgical anaesthesia was reached. The skull of the animal was exposed by sagittal cut of the skin covering the top of the head with a scalpel. A small hole was made at the base of the skull. A pair of small scissors was inserted into the hole and cut through the skull along the sagittal suture. Then, the plates of the skull and dura were carefully removed and the brain was exposed. The brain was carefully taken out, placed on dissecting paper and quickly soaked with cold (4 °C) oxygenated standard medium. The hippocampus from one side of the brain were dissected free and placed on the cutting platform of a McIlwain tissue chopper where the hippocampus was transversely cut (450 μm thick of each section). The sliced hippocampus was then transferred to a plate containing cold oxygenated medium, where each section of the hippocampus was separated with a fine stainless steel spatula. Two to three slices obtained from the middle-portion of the hippocampus were placed between two nylon meshes to minimize movement and quickly transferred to the slice chamber, where the slices were submerged in the oxygenated medium. A carbogen line was placed at the edge of the slice chamber to introduce carbogen to the atmosphere above the slices. The procedure from the start of surgery to the placement of the slices in the chamber was completed within 3 min. The slices in the chamber were allowed to equilibrate with the standard medium for at least 1 hour prior to recording. Only those slices with well-defined border for cell body layers were used for experiments. The slices prepared in this manner could usually support normal physiological responses for more than 12 hours. However, most experiments lasted no more than 4 hours. Only one slice was used per animal in most experiments. In some experiments in which picrotoxinin was present in the perfusing medium, the CA3 region of the hippocampal slices was cut to minimize

the epileptiform activity of CA1 neurons caused by spontaneous activity of CA3 neurons.

7.5. Recording and stimulating systems

7.5.1. Amplifiers

Extracellular responses, including field EPSP and population spike, were amplified 1000 times with a DAM-5A differential pre-amplifier (World Precision Instruments). Intracellular responses were recorded with an Axoclamp 2A (Axon Instruments) in bridge current clamp mode. Intracellular EPSPs and IPSPs were amplified 10 times. The amplified field potentials and intracellular responses were fed into the Data Precision 6000 waveform analyzer. The current and voltage outputs from Axoclamp 2A were also connected to a chart recorder and an oscilloscope.

7.5.2. Recording electrodes

Extracellular recording electrodes were prepared from standard-wall borosilicate tubing (outer wall diameter, 1.20 mm; inner wall diameter, 0.69 mm: Sutter Instrument Co.). The microelectrodes were pulled on a PE-2 vertical puller (Narishige Scientific Instruments) and filled with 4 M NaCl. The resistances of these electrodes were 1 to 3 M Ω . Intracellular recording electrodes were made from standard-wall borosilicate tubing (outer wall diameter, 1.00 mm; inner wall diameter, 0.58 mm: Sutter Instrument Co.). These electrodes were pulled on a Flaming/Brown (model P-87) micropipette puller. The recording electrodes were filled with one of the following: 4 M potassium acetate; 4 M potassium acetate plus 0.1 M 1,2-bis(2-aminophenoxy)ethane-*N,N',N',N'*-tetraacetic acid (BAPTA) (Sigma); 4 M potassium acetate plus 100 mM QX-314 (Astra Pharmaceuticals, Sweden); 4 M potassium acetate plus 5 μ M K-252b (Kyowa Hakko Kogyo LTD.). The resistances of these electrodes were 80-120 M Ω .

7.5.3. Stimulating units

A Grass S88 stimulator (Grass Instrument Co.) was used to generate currents. This stimulator had two channels and each channel was connected to a photoelectric stimulus isolation unit (Grass Instrument Co.). One unit coupled to the Axoclamp was used as an external DC command generator. The DC current could be injected through recording electrodes. Another unit was connected to a stimulating electrode.

Bipolar concentric stimulating electrodes (SNEX-100) were obtained from Rhodes Medical Instruments. These electrodes had a small tip (0.1 mm in diameter), which could minimize the tissue damage caused by the placement of the electrodes. The resistances of these electrodes were approximately 1 M Ω , and electrodes were replaced if their resistances were higher than 5 M Ω .

7.5.4. Recording units

Extracellular field potentials were recorded with a DATA Precision 6000 waveform analyzer, where the slope and amplitude of the field potentials were measured. The intracellular EPSPs and IPSPs were recorded and analyzed with the DATA 6000 analyzer. The membrane potentials and the input resistances of neurons were continuously monitored on an oscilloscope (Tektronix Inc.) and on the chart paper of a polygraph (Grass Instrument Co.) or a HP7470-A graphics plotter (Hewlett-Packard). In some experiments, responses were recorded with a video cassette recorder (Toshiba) and used for later analysis.

7.6. Application of drugs

Bath application was used in most experiments of the present studies (see the section 7.2). K-252, BAPTA and QX-314 were applied into the CA1 neurons through recording electrodes. Hyperpolarizing current pulses (0.2-0.5 nA, 200 ms) were used to inject K-252b (an inhibitor of PKC) and BAPTA (a Ca²⁺ chelator) into the neurons. The blockade of afterhyperpolarization and the

loss of spike accommodation were used as the indices of chelation of the intracellular $[Ca^{2+}]$ in the neurons. Depolarizing current pulses (0.1-0.5 nA, 200 ms) were employed to aid the release of QX-314 into the cells. The blockade of the Na^+ spike and the slow IPSP were used to assess the effects of QX-314 in the neurons (Nathan et al., 1990).

7.7. Collection of endogenous samples

Each rabbit was anaesthetized with 1.5% halothane and its neocortex exposed (see Fig. 4). One 8-mm diameter cup was placed on the neocortical surface of each cerebral hemisphere. A ring-shaped stimulating electrode with 7-mm loop diameter and a suction line were placed in each cup. Micromanipulators were used to control both suction lines which collected samples into a common collection bottle that was kept on dry ice. Oxygenated medium (0.1 ml), used for perfusing guinea pig hippocampal slices, was added into each cup every 5 min. At the end of each 5 min incubation period, the neocortical surface was tetanized (bipolar pulses of 0.5 ms duration at 50 Hz for 5s, 30V) and the tetanized neocortical sample (TNS) was collected from the suction lines. This procedure was repeated until the desired volume of the TNS was collected. The same procedure was used to collect the untetanzed neocortical sample (UNS) except the collection took place without any tetanic stimulation. In some experiments, a single tetanic stimulation was given to the rabbit neocortical surface prior to any collection. After the termination of this tetanus, samples were collected at 5 min intervals without further tetanic stimulation. These samples will be referred to as TUNS to indicate that they were from previously tetanized neocortex that was untetanzed during the collection of the sample. Some TNS were collected from rabbits pretreated with MK-801 ([(+)-5-methyl-10,11-dihydro-5H-dibenzo(a,b)cyclohepten-5,10-imine maleate]) (Merck, Sharp & Dohme), a non-competitive NMDA receptor blocker.

In these rabbits, MK-801 (1 μ g/kg) was injected i.p. in two divided doses before the collection of samples (0.5 + 0.5 μ g/kg, with a 1h interval between injections). All samples were stored at -80 °C in the freezer immediately after each collection. The samples were taken out from the freezer and allowed to melt at room temperature just before the application of the samples.

7.8. Separation of endogenous samples

In some experiments, the tetanized neocortical samples (TNS) were separated into different fractions according to their molecular weight. Five different fractions were obtained: <3,000, 3,000-10,000, 10,000-25,000, 25,000-50,000 and >50,000 kDa. Centriflo membrane cones type CF 25 and type CF 50A from Amicon were used for fractionating molecular weights of 25,000 and 50,000 kDa, respectively. The samples were centrifuged at a relative centrifugal force of 1000 g at 4°C for 30 min. For the 10,000 mol. wt fraction, contricon-10 microconcentrators from Amicon were used. The samples were centrifuged at a relative centrifugal force of 5000 g at 4 °C for 1 hour. For the 3000 mol. wt fraction, centricon-3 microconcentrators from Amicon were used. These samples were centrifuged at a relative centrifugal force of 7500 g at 4°C for 2 hours.

7.9. Gel electrophoresis

One-dimensional (1-D) sodium dodecyl sulfate (SDS) gel electrophoresis was performed to analyze the presence of peptides in the neocortical samples. The procedure used was similar to that reported by Charriault-Marlangue et al. (1988). Briefly, 100 μ l of samples were mixed with 50 μ l of sample buffer consisting of 3% SDS, 10% sucrose, 3 mM EDTA, and 20 μ g/ml Bromophenol Blue in 30 mM triza hydrochloride (Tris-HCl) buffer (pH 8.0). The mixture was then incubated at 37°C for 20 min to reduce sulfhydryl groups. The

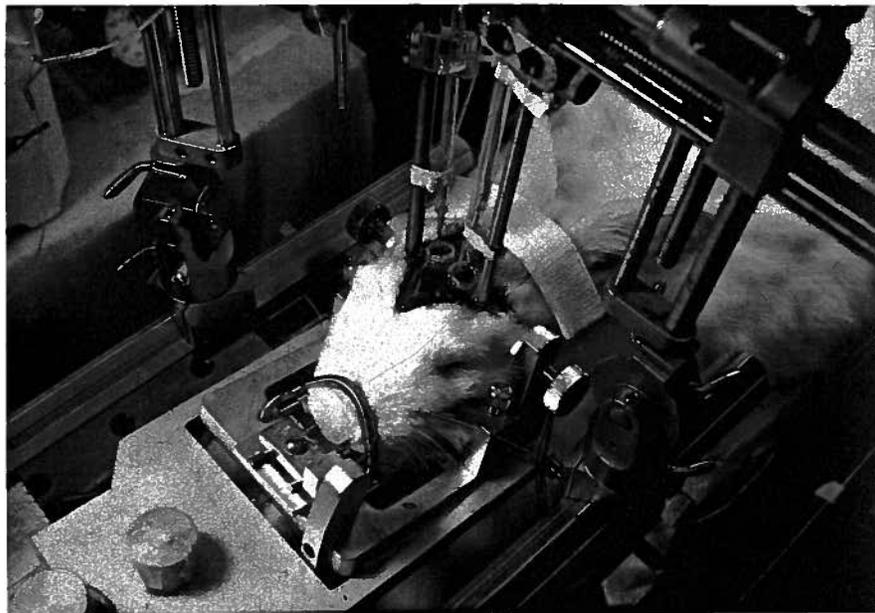


Figure 4 Preparation for collection of samples from rabbit neocortical surface in vivo.

polyacrylamide gel was composed of 95% acrylamide and 5% N,N'-methylene-bis-acrylamide with 0.1% SDS. Then, 50 μ l samples were applied to the gel slots and electrophoresis was performed at 200 mA in 50 mM Tris-HCl buffer containing 0.1% SDS pH (8.0). After electrophoresis, Coomassie Blue dye was used for gel staining. Six protein standards (Sigma) were used; namely, α -lactalbumin (mol. wt 14,400), trypsin inhibitor (20,000), carbonic anhydrase (30,000), ovalbumin (43,000), albumin (67,000) and phosphorylase B (94,000). The two-dimensional (2-D) gel electrophoresis was performed by Protein Database Inc., Huntington Station, NY. Acrylamide gel slab (15%) was used. In these experiments, paired UNS and TNS were run with a sample population of n=4.

7.10. Extracellular recordings

Population spike and field EPSPs were recorded in the CA1 cell body layer and the dendrites area, respectively, in response to 0.1-0.2 Hz stimulation of the stratum radiatum at the CA1-CA2 junction with a bipolar stimulating electrode (Fig. 5 & 6). The amplitude and slope of the field potentials were recorded and analyzed with a DATA 6000 waveform analyzer. The stimulus strength was set so that the control population spike and field EPSP were about 50% of the maximum size, leaving room for potentiation. The control responses had to be stable for 20 to 30 min before any drug applications or tetanic stimulation. The amplitudes of control population spike and field EPSP were usually 1.0-1.5 mV and 0.5-1.0 mV, respectively.

7.11. Intracellular recordings

Intracellular responses were recorded in the CA1 neurons in response to 0.03-0.2 Hz stimulation of stratum radiatum near the CA1/CA2 junctions with a bipolar stimulating electrode (Fig. 5 & 6). Only those CA1 neurons with stable resting membrane potentials of -55 to -65 mV and input resistances greater than

25 M Ω , were used for these studies. The input resistances of neurons were monitored by injecting rectangular hyperpolarizing current pulses (0.1-0.2 nA, 200ms) through the recording electrode and measuring the plateau of the change in the membrane potential. The membrane voltage responses to different rectangular current commands (0.05-0.75 nA of hyperpolarizing and 0.05-0.1 nA of depolarizing current pulses of 200 ms) were measured to plot the current-voltage curves. The slope and amplitude of EPSPs and the amplitude of IPSPs were recorded and analyzed using a DATA 6000 waveform analyzer. The stimulation strengths were adjusted so that the control EPSPs or/and IPSPs were about half of their maximum size, leaving adequate room for potentiation. The control responses had to be stable for at least 20 min prior to any drug application or tetanic stimulation. The amplitudes of control responses were usually 5-10 mV for EPSPs and 2-5 mV for IPSPs. If drugs changed the membrane potential, DC currents were sometimes injected into the neurons to bring the membrane potential back to the control level when the records of EPSPs and IPSPs were taken so that comparisons could be made between the control and test synaptic responses at similar membrane potential.

7.12. Induction of LTP

LTP could be induced by tetanic stimulations or single stimulation paired with depolarization of the neurons. Repetitive direct injections of depolarizing current pulses (3-4 nA, 100-200 ms) into the CA1 neurons paired with stimulation (0.2 Hz) of the stratum radiatum could induced LTP (Sastry et al., 1986). The induction of this LTP usually required the presence of picrotoxinin in the perfusing medium. In the present studies, tetanic stimulations were used to induce LTP in most of the experiments (Fig. 6 & 7). The following three types of tetanic stimulations were used to induce LTP; (1) 400 Hz for 1 sec; (2) 100 Hz for 5 sec; (3) two trains of 100 Hz for 1 sec, 20 sec interval. During tetanic

stimulations, the stimulation strength was maintained the same as the control one unless otherwise stated.

7.13. Data analysis

Population spike, field EPSP, intracellular EPSP and IPSPs were recorded and analyzed in the DATA 6000 waveform analyzer. The initial slopes of the field EPSP and intracellular EPSP were computed by the DATA 6000 waveform and expressed as mV/msec. The amplitude of population spike was measured (Fig. 6). The amplitudes of EPSPs and IPSPs were the lengths measured from the baseline to each peaks of the responses (Fig. 6). Individual records in each experiment were quantified by averaging 4-8 consecutive responses. Individual records were taken every 5 to 10 min throughout each experiment. All the control records were averaged. The individual control and the post-drug or post-tetanic responses were then normalized as a percentage of this averaged record. Data were displayed in graphs or histograms, and expressed as mean \pm S.E.M. The following statistical tests were employed to analyze the data. Paired Student's *t*-test was used to compare two correlated samples such as the pre-drug and the post-drug responses. Two-tailed tests were used unless otherwise stated. For multisample comparisons in which only a single criterion was involved (e.g. mean of EPSP slope), one-way ANOVA was used. Duncan's multiple comparison test was applied as "a posterior" test, if one-way ANOVA first rejected a multisample hypothesis of equal means. The *P* value was chosen arbitrarily at <0.05 for all the statistical tests.

8. RESULTS

8.1. LTP and IPSPs

Stimulation of the stratum radiatum which evokes EPSPs in the CA1 neurons of the hippocampus, also induces the fast and slow inhibitory postsynaptic potentials (IPSPs) mediated through GABA_A and GABA_B receptors,

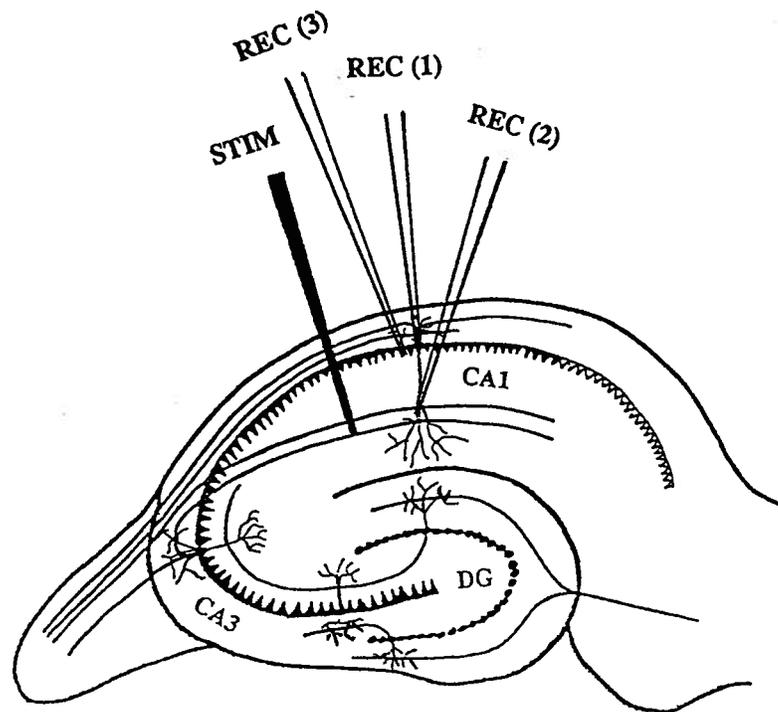


Figure 5 Schematic illustration for positioning of stimulating and recording electrodes in hippocampal slices.

Extracellular and intracellular responses in the CA1 area were evoked with the stimulating electrode (STIM) placed in the stratum radiatum near the CA1-CA2 junction. Extracellular EPSPs and population spike were recorded with the electrodes positioned in the apical dendritic area (REC2) and the cell body layer (REC1) of CA1 pyramidal cells, respectively. Intracellular EPSP and IPSPs were recorded with the electrode impaled in the soma of the CA1 neurons (REC3).

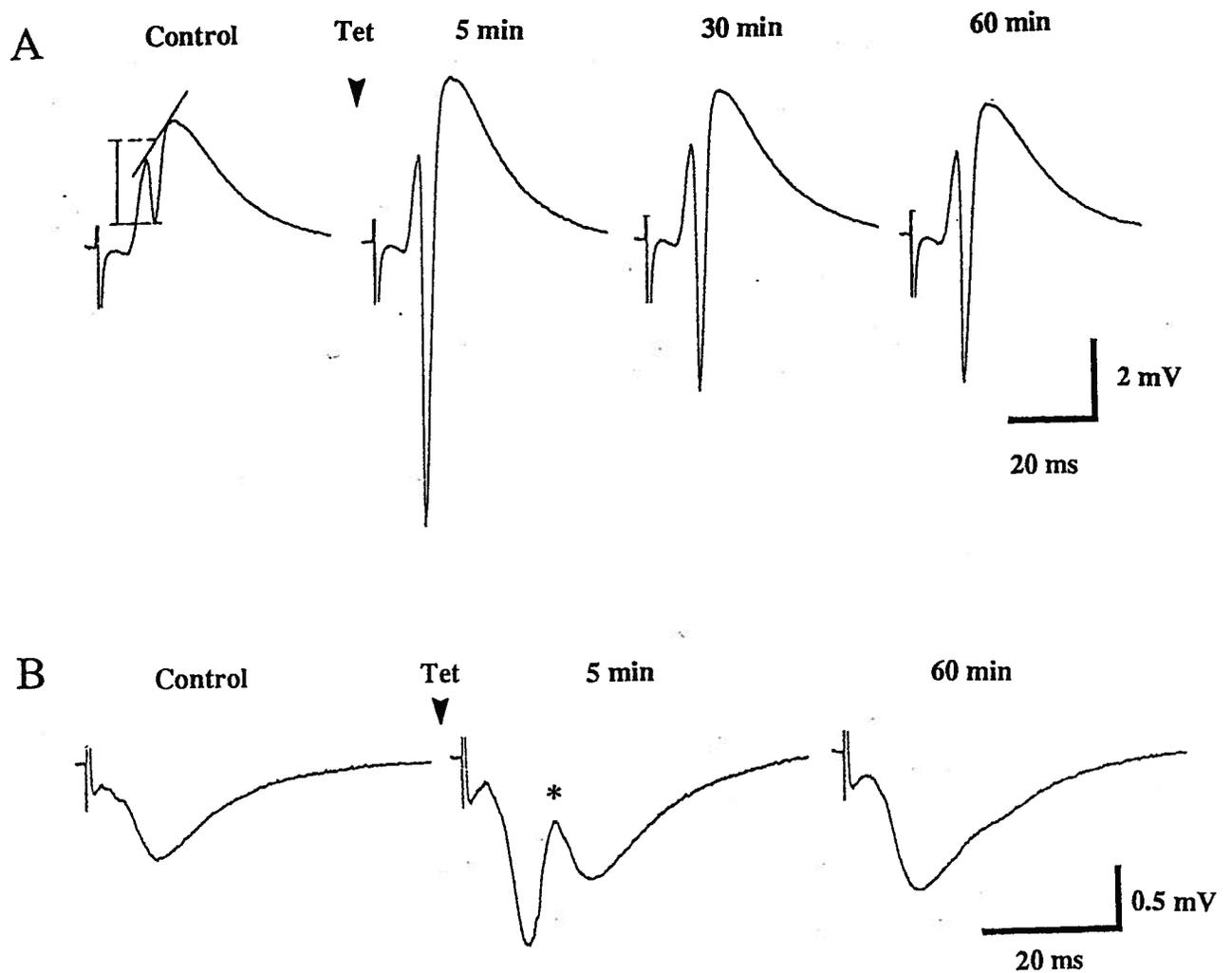


Figure 6 Representative field potentials and LTP in hippocampal CA1 area.

In **A**, population spikes recorded in CA1 cell body layer in response to stimulation of the stratum radiatum before and after tetanic stimulation are illustrated. The measurement of population spike amplitude is shown in the control record. Note the increase in the amplitude of post-tetanus records at 5, 30 and 60 min represents LTP of the population spike. In **B**, the control and post-tetanus extracellular EPSPs are illustrated. Note the positive spike (*) shown in the 5 min post-tetanus record represents a spike recorded in the apical dendritic area. The increase in the slope of extracellular EPSPs after tetanus indicates the occurrence of LTP of extracellular EPSPs.

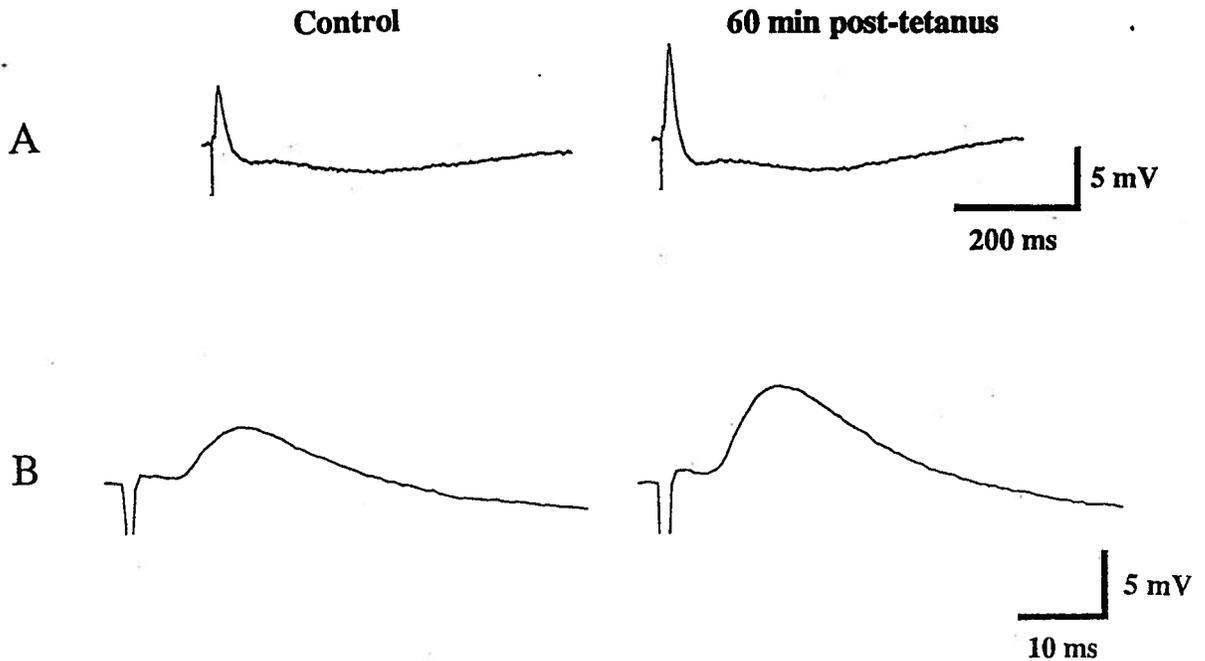


Figure 7 Characteristics of intracellular synaptic potentials and LTP in hippocampal CA1 cells.

In **A**, intracellular EPSP, fast and slow IPSPs evoked by stimulation of the stratum radiatum were recorded in the CA1 pyramidal neurons. In **B**, the EPSPs are shown in slow recording speed. Amplitude of EPSP, fast and slow IPSPs is measured from the baseline to the peak of each response. Note that the increase in the amplitude or slope of EPSP after tetanus indicates LTP of intracellular EPSP. Evaluation of LTP of fast and slow IPSP is based on the change in amplitude of these responses. Records in **A** and **B** were obtained from the same neuron with a resting membrane potential of -63 mV.

respectively (Fig. 7a). Tetanic stimulation of the stratum radiatum is known to induce LTP of the EPSP in the CA1 neurons of the hippocampus (Schwartzkroin and Wester, 1975). Transient depression or no change in IPSPs after a tetanic stimulation has been reported (McCarren and Alger, 1985; Thompson and Gahwiler, 1989). Inconsistent long-term changes of the IPSPs after tetanic stimulation have been reported by others (Misgeld et al., 1979; Abraham et al., 1987; Taube and Schwartzkroin, 1988). Following a tetanic stimulation of the stratum radiatum, IPSPs have been shown to be unchanged, increased, or decreased (Abraham et al., 1987). Most of these studies were focused on changes in the fast IPSP. In the present studies, experiments were conducted to examine whether long-term changes in the IPSPs, especially the slow IPSP, occur following tetanic stimulations. Whether such changes in the IPSPs affect LTP of the EPSP was also tested.

8.1.1. Tetanic stimulation and IPSPs

Intracellular EPSP, fast IPSP and slow IPSP in the CA1 neurons were recorded with electrodes containing 4 M potassium acetate in response to the stimulation of the stratum radiatum at 0.03-0.1 Hz. The IPSPs would get smaller at frequencies higher than 0.1 Hz (Ben-Ari et al., 1979; Davies et al., 1990). In control experiments, the EPSP, the fast and slow IPSPs were stable when evoked at 0.03-0.1 Hz and recorded for 60 min (responses at 60 min after the start of recording were expressed as a percentage of the averaged record taken during the first 10 min: EPSP: 105.15 ± 3.10 ; fast IPSP: 92.75 ± 6.67 ; slow IPSP: 97.87 ± 6.67 ; n=6).

After obtaining stable control responses for 20 min, tetanic stimulation (400 Hz for 1s; or 2 trains of 100 Hz for 1 s, 20 s interval) was applied to the stratum radiatum. Since the two protocols of tetanic stimulation induced similar results, data obtained from different tetanic stimulations were pooled together for

discussion in this and the subsequent sections. Tetanic stimulation induced LTP of the EPSP and the fast IPSP, but not of the slow IPSP in the CA1 neurons (Fig. 8B & C, n=8). The potentiation of the fast IPSP began at the first post-tetanic record and the enhancement persisted for at least 30 min when the recording was stopped. In three experiments in which the post-tetanus responses were followed for 60 min after tetanic stimulation, the potentiation of the EPSP and the fast IPSP was present throughout the 60 min period. In slices exposed to picrotoxinin (20 μ M), a tetanic stimulation produced LTP of the EPSP, but slightly depressed the slow IPSP (Fig. 9, n=8). The LTP of the EPSP was larger in the picrotoxinin-exposed slices than in the control slices (Fig. 8 & 9). These results indicate that the tetanic stimulation of the stratum radiatum not only induces LTP of the EPSP but also causes long-term changes in the fast IPSPs in CA1 neurons. It is possible that these long-term changes in the IPSPs may modify the expression of LTP of the EPSP.

8.1.2. Effects of Ca^{2+} chelator on IPSPs

Tetanic stimulation is known to induce a Ca^{2+} influx through membrane channels coupled to NMDA receptors on the CA1 neurons (Collingridge et al., 1983; Huang et al., 1988). The Ca^{2+} influx is believed to trigger a series of changes which are involved in the induction of LTP of the EPSP (Lynch et al., 1983; Malenka et al., 1988). A rise of intracellular Ca^{2+} concentration was suggested to destabilize the phosphorylation of the GABA_A receptor complex which decreases the fast IPSP (Chen et al., 1990). Experiments were, therefore, conducted to examine whether the tetanus-induced Ca^{2+} influx leads to long-term changes in the IPSPs using the Ca^{2+} chelator, BAPTA.

Intracellular EPSP, fast IPSP and slow IPSP were recorded with electrodes containing 4 M potassium acetate and 0.1 M BAPTA. BAPTA is a fast Ca^{2+} chelator. BAPTA was injected into the CA1 neurons by applying

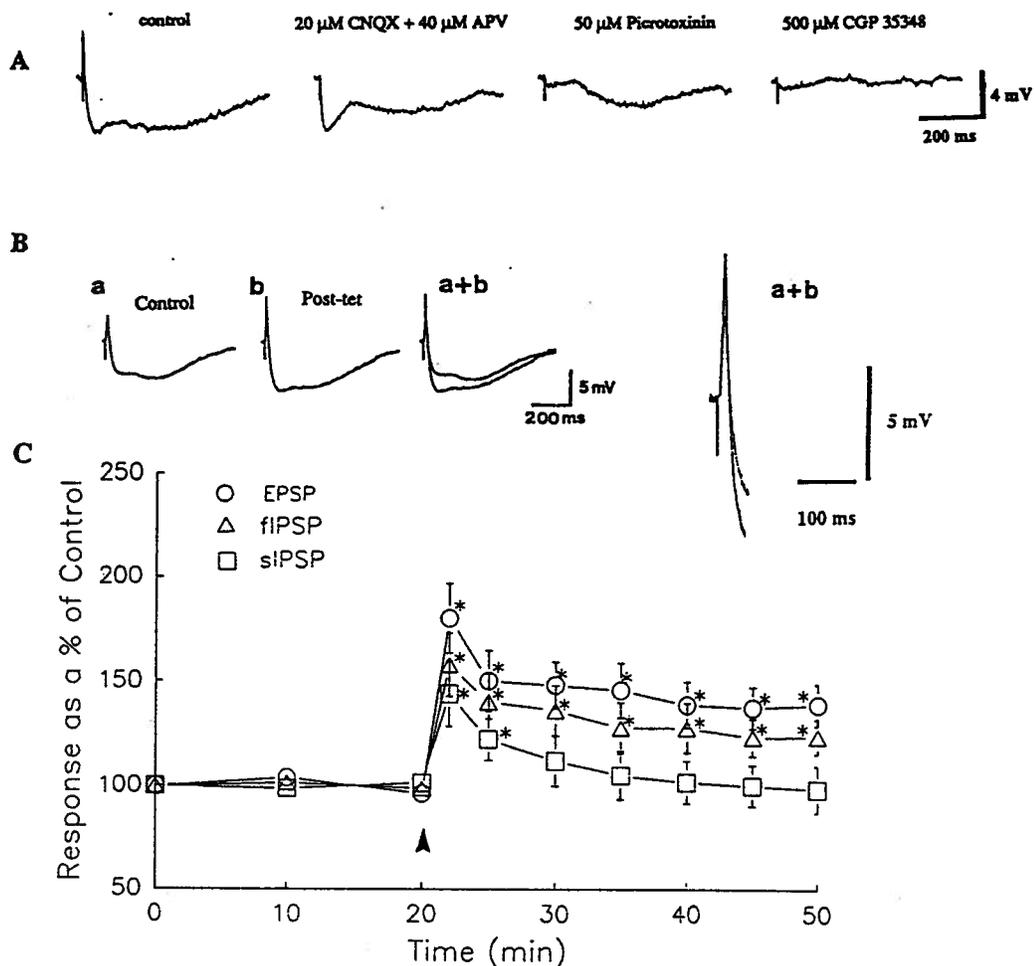


Figure 8 Pharmacologically isolated components of intracellular synaptic responses and the effects of tetanic stimulation on these components.

In **A**, an EPSP followed by a fast IPSP and a slow IPSP recorded in the CA1 cells in response to stimulation of the stratum radiatum is illustrated in the control record. The EPSP was abolished by 40 μ M APV and 20 μ M CNQX. The fast IPSP was blocked by 50 μ M picrotoxinin, and the slow IPSP was inhibited by 500 μ M CGP-35348. The membrane potential of this neuron was -60 mV. In **B**, changes in the EPSP and the fast and slow IPSPs induced by tetanic stimulation are shown. The record in **Bb** was taken 20 min after tetanic stimulation. The resting membrane potential of the cell was -61 mV. In **C**, graphs obtained from 8 neurons illustrate the effects of tetanic stimulation (arrow) on the EPSP, the fast and slow IPSPs. Note that the increase in the EPSP and the fast IPSP, but not in the slow IPSP, occurred after tetanic stimulation. All points on the graphs in this figure and subsequent figures represent the mean \pm S.E.M.. Recordings were made from one neuron per slice. Asterisk (*) indicates that post-tetanic responses are significantly different from the control responses using one-way ANOVA and Duncan's multiple comparison test. The P value was chosen arbitrarily at <0.05.

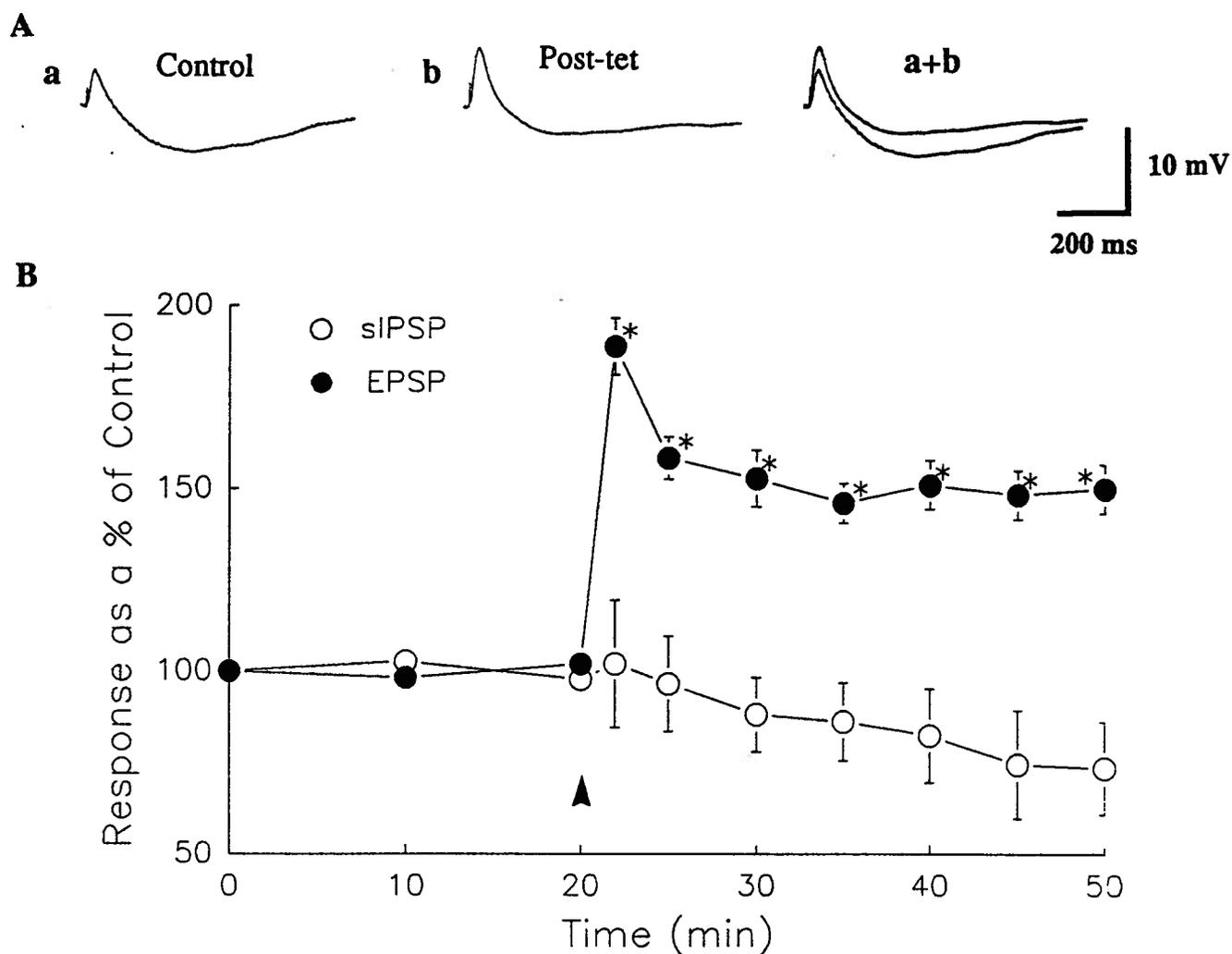


Figure 9 Changes in EPSP and slow IPSP induced by tetanic stimulation in the presence of picrotoxinin.

Changes in the EPSP and the slow IPSP caused by tetanic stimulation (arrow) are shown in **A** & **B**. The fast IPSP was blocked by 20 μ M picrotoxinin in these experiments. **A** was from an individual experiment. Post-tetanic responses were recorded 20 min after tetanic stimulation. The resting membrane potential of the neuron in **A** was -65 mV. The graphs shown in **B** were from data obtained from 8 neurons. Note that tetanic stimulation only induced LTP of the EPSP but not of the slow IPSP.

hyperpolarizing rectangular current pulses (0.2-0.5 nA, 200 ms) through the recording electrodes for 10 to 20 min. The blockade of after-spike hyperpolarization and the loss of spike accommodation were used as the indices of chelation of intracellular Ca^{2+} in the CA1 neurons (Fig. 10) (Strom, 1987c). Intracellular injection of BAPTA into the CA1 neurons did not change the control EPSP, fast and slow IPSPs. However, tetanic stimulation of the stratum radiatum did not induce LTP of the EPSP in BAPTA-injected CA1 neurons (Fig. 10, n=6). In contrast, the EPSP was slightly depressed after tetanic stimulation in 2 of 6 BAPTA-injected neurons. LTP of the fast IPSP and the slow IPSP occurred in these CA1 neurons after tetanic stimulation (Fig. 10, n=6). The LTP of the fast IPSP was significantly greater in BAPTA-injected CA1 neurons (Fig. 10B) than in control neurons (Fig. 8C). Like the potentiation of the fast IPSP, the potentiation of the slow IPSP, which did not occur in control CA1 neurons, began at the first post-tetanic record and was present until the experiment was terminated.

The slow IPSP was sometimes difficult to estimate accurately because the largely enhanced fast IPSP overlapped with the early phase of the slow IPSP. Therefore, some experiments were performed in slices exposed to picrotoxinin which completely blocked the fast IPSP. When picrotoxinin was present in the perfusing medium throughout the experiments, stimulation of the stratum radiatum evoked only EPSP and slow IPSP. Tetanic stimulation induced LTP of the slow IPSP but not of the EPSP in the BAPTA-injected neurons (Fig. 11, n=6).

The input resistance and the resting membrane potential of the CA1 neurons were not significantly changed during LTP of the fast and the slow IPSPs. The equilibrium potentials of the fast and the slow IPSPs measured 20 min post-tetanus were -70 to -75 mV and -90 to -95 mV, respectively (n=4, Fig. 15). These equilibrium potentials were not significantly different from the

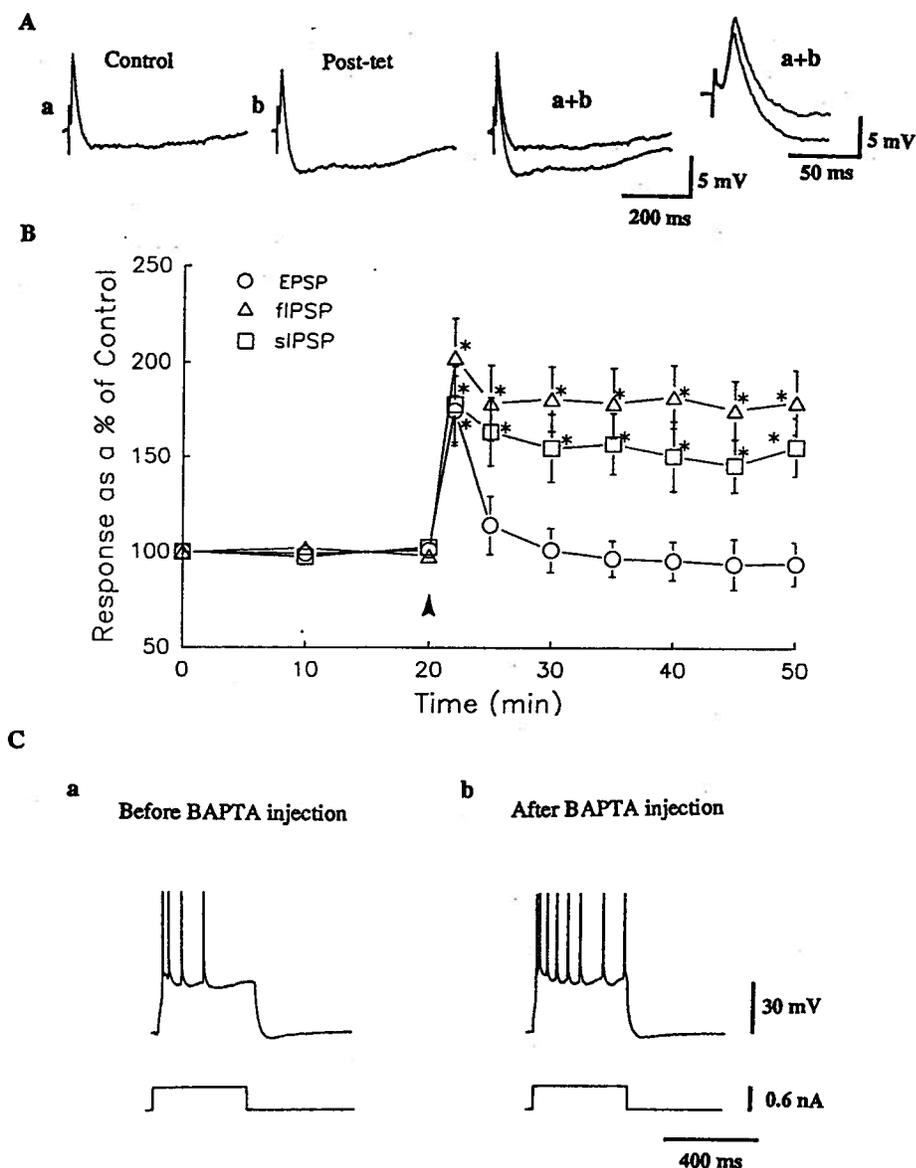


Figure 10 Effects of tetanic stimulation on evoked synaptic responses in BAPTA-injected neurons.

Changes in EPSP, fast and slow IPSPs caused by tetanic stimulation (arrow) in neurons injected with BAPTA are illustrated in **A** & **B**. **A** shows the records of an individual experiment. The post-tetanic responses were recorded 20 min after tetanic stimulation. The control and post-tetanic responses recorded with fast and slow speed were superimposed separately. The resting membrane potential of the neuron in **A** was -58 mV. In **B**, data obtained from 6 neurons are shown. Note that tetanic stimulation caused LTP of the fast and slow IPSPs but prevented LTP of the EPSP in neurons injected with BAPTA. **C** shows the ability of a depolarizing pulse to generate action potentials in CA1 neuron before (**Ca**) and after (**Cb**) BAPTA injection. Note that chelation of postsynaptic free Ca^{2+} reduced the spike accommodation in the CA1 neurons.

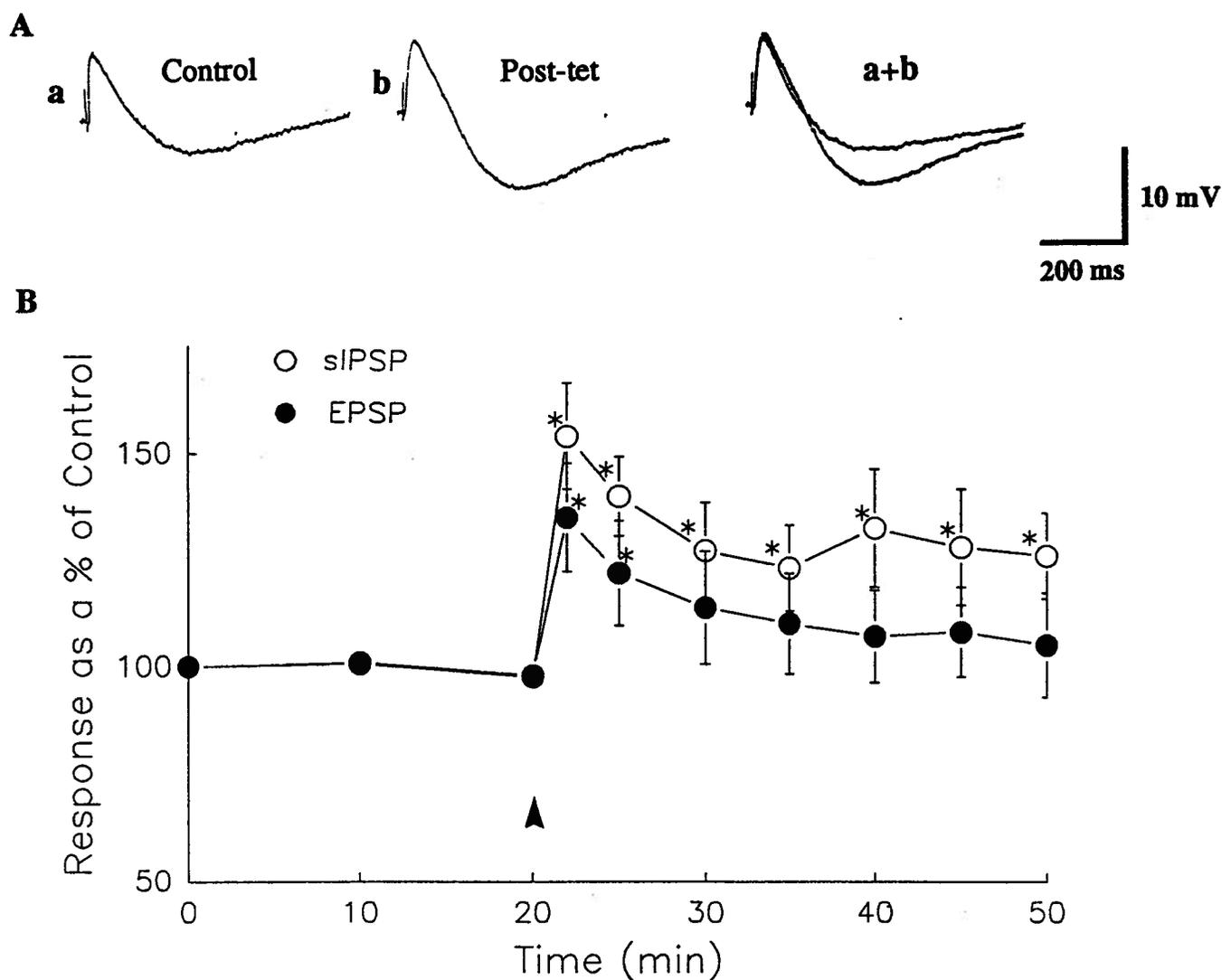


Figure 11 Effects of tetanic stimulation on EPSP and slow IPSP in BAPTA-injected neurons in the presence of picrotoxinin.

Post-tetanic changes of EPSP and slow IPSP in neurons injected with BAPTA are illustrated in A & B. In these experiments, the fast IPSP was blocked by picrotoxinin (20 μ M). Records from one experiment are shown in A. In B, data obtained from 6 neurons are shown. The resting membrane potential of the neuron in A was -67 mV, and post tetanic record was obtained 20 min after tetanic stimulation. Note that LTP of the slow IPSP but not of the EPSP in neurons injected with BAPTA occurred after tetanic stimulation.

equilibrium potentials of the control IPSPs. It appears that the potentiation of IPSPs was not due to changes in the equilibrium potentials of IPSPs.

The results are in agreement with those in literature that chelation of postsynaptic intracellular Ca^{2+} prevents the induction of LTP of the EPSP (Lynch et al., 1983; Malenka et al., 1988). These results also confirm the finding reported by Morishita and Sastry (1991). Morishita and Sastry suggested that the Ca^{2+} influx induced by tetanic stimulation suppresses the induction of LTP of the IPSPs and that the suppression of the IPSPs may lead to a better expression of LTP of the EPSP in the CA1 neurons. LTP of the slow IPSP occurs after tetanic stimulation only if the influx Ca^{2+} is sufficiently chelated.

8.1.3. Effects of protein kinase C inhibitor on IPSPs

Tetanic stimulation induces Ca^{2+} influx through the NMDA channels. The rise of intracellular Ca^{2+} concentration can trigger activation of several protein kinases which are involved in the LTP process. Protein kinase C (PKC) is one of the most studied protein kinases involved in LTP. Tetanic stimulation increases PKC activity (Otani et al. 1992), presumably through the increase of postsynaptic Ca^{2+} concentration. Activation of PKC is believed to be required for the induction and/or the maintenance of LTP (Lovinger et al., 1987; Malinow et al., 1989; Malenka et al., 1989). Drugs that increase the activation of PKC decrease the GABA_B receptor-mediated slow IPSP in CA1 neurons (Baraban et al., 1985; Dutar and Nicoll, 1988). It would be of interest to determine whether the increase in PKC activity leads to long-term changes in the IPSPs following tetanic stimulation.

PKC inhibitors like H-7, sphingosine and polymyxin B have been reported to block the induction of LTP (Malinow et al., 1988, 1989). These drugs are lipid soluble and can cross cell membranes making it difficult to decide their sites (presynaptic or postsynaptic) of the action. A potent PKC inhibitor, K-252b, was

used in the present experiments. K-252b cannot readily cross cell membranes (Kuroda et al. 1992). This property of K-252b was confirmed by the following experiments. In one set of experiments, K-252b (5 μ M) was added to the extracellular medium for 15-30 min and the stratum radiatum was tetanized 5 min after the start of drug application. K-252b did not block the induction of LTP of the EPSP in these experiments (Fig. 12B, n=6). The LTP induced in the presence of K-252b was not significantly different from the control LTP (Fig. 12 C, n=6). In another set of experiments, K-252b was injected into the CA1 neurons, through electrodes containing 4 M potassium acetate and 5 μ M K-252b, prior to the tetanic stimulation (by applying hyperpolarizing rectangular current pulses, 0.2-1 nA and 200 ms for 2 min). K-252b did not change the membrane potential, the input resistance and the control evoked synaptic responses. In these experiments, K-252b clearly blocked the induction of LTP of the EPSP (Fig. 12A, n=6). These results demonstrate that postsynaptic PKC activity is critical for the induction of LTP of the EPSP. Therefore, injections of K-252b into the CA1 neurons was used in subsequent experiments.

In K-252b-injected CA1 neurons, tetanic stimulation did not induce LTP of the EPSP but caused LTP of the fast and the slow IPSPs (Fig. 13, n=16). LTP of the fast IPSP was significantly greater in K-252b-injected CA1 neurons than in control neurons. The potentiation of the fast and the slow IPSPs was seen in the first post-tetanic record. In the slices exposed to picrotoxinin (20 μ M), tetanic stimulation clearly induced LTP of the slow IPSP, but not of the EPSP (Fig. 14, n=6). The equilibrium potentials of the control and the 20 min post-tetanic fast and slow IPSPs were not significantly different (n=4, Fig. 15).

Chelation of intracellular Ca^{2+} or inhibition of postsynaptic PKC activity blocked the induction of LTP of the EPSP but increased LTP of the fast and slow IPSPs. These results suggest that the increase in postsynaptic PKC activity

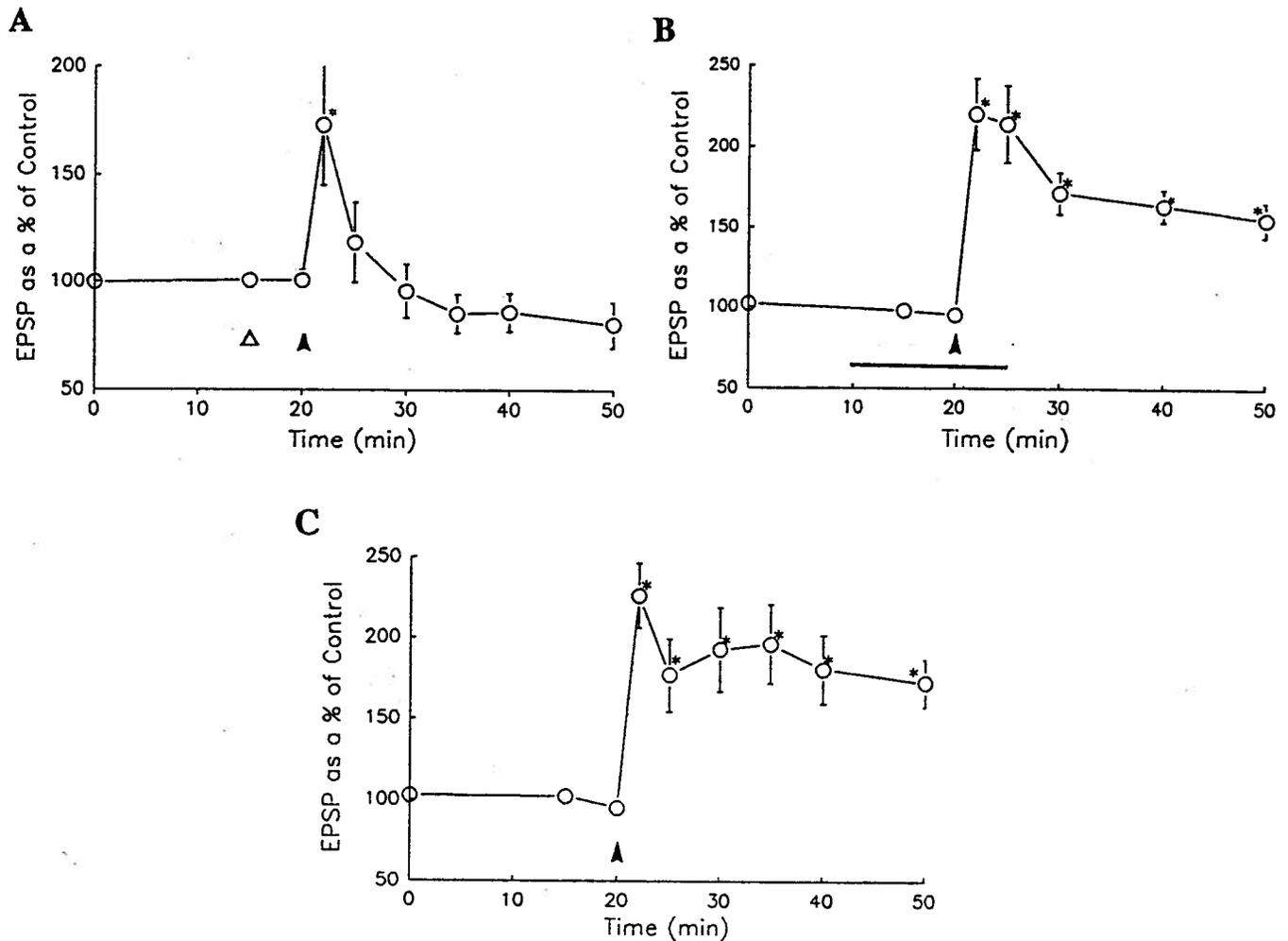


Figure 12 Actions of K-252b on the induction of LTP of the EPSP in CA1 neurons.

In **A**, K-252b was injected into CA1 neurons through recording electrodes containing 5 μ M K-252b. Effects of intracellularly injected K-252b on LTP of the EPSP induced by tetanic stimulation (arrow) are shown ($n=7$). Δ represents the time when K-252b was injected. Note that intracellularly injected K-252b did not affect control EPSP but blocked the induction of LTP of the EPSP. In **B**, the effects of extracellular application of K-252b (5 μ M, the long horizontal bar above the abscissa) on LTP are illustrated ($n=6$). In the presence of K-252 in the medium, tetanic stimulation (arrow) induced LTP of the EPSP which is not significantly different from the control LTP obtained without the presence of K-252b which is shown in **C** ($n=5$).

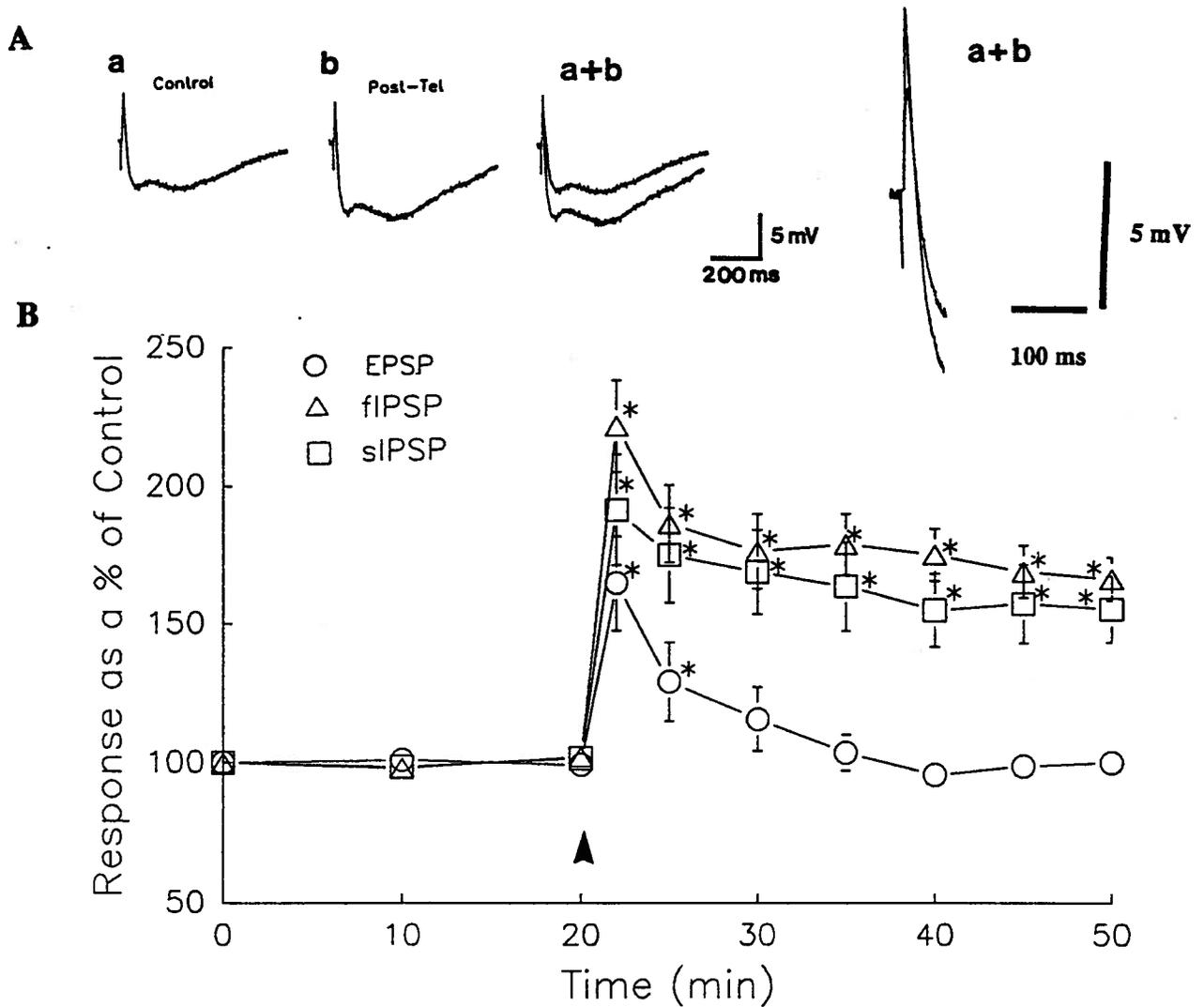


Figure 13 Actions of K-252b on changes in EPSP, fast and slow IPSPs induced by tetanic stimulation.

Post-tetanic changes of the EPSP, the fast and slow IPSPs in neurons injected with K-252b are illustrated in A & B. Tetanic stimulation (arrow) induced LTP of the fast and slow IPSPs but not of the EPSP. A shows the records from one experiment. B illustrates data obtained from 8 neurons in graphs. The resting membrane potential of the neuron in B was -60 mV and post-tetanic record was obtained 20 min after tetanic stimulation.

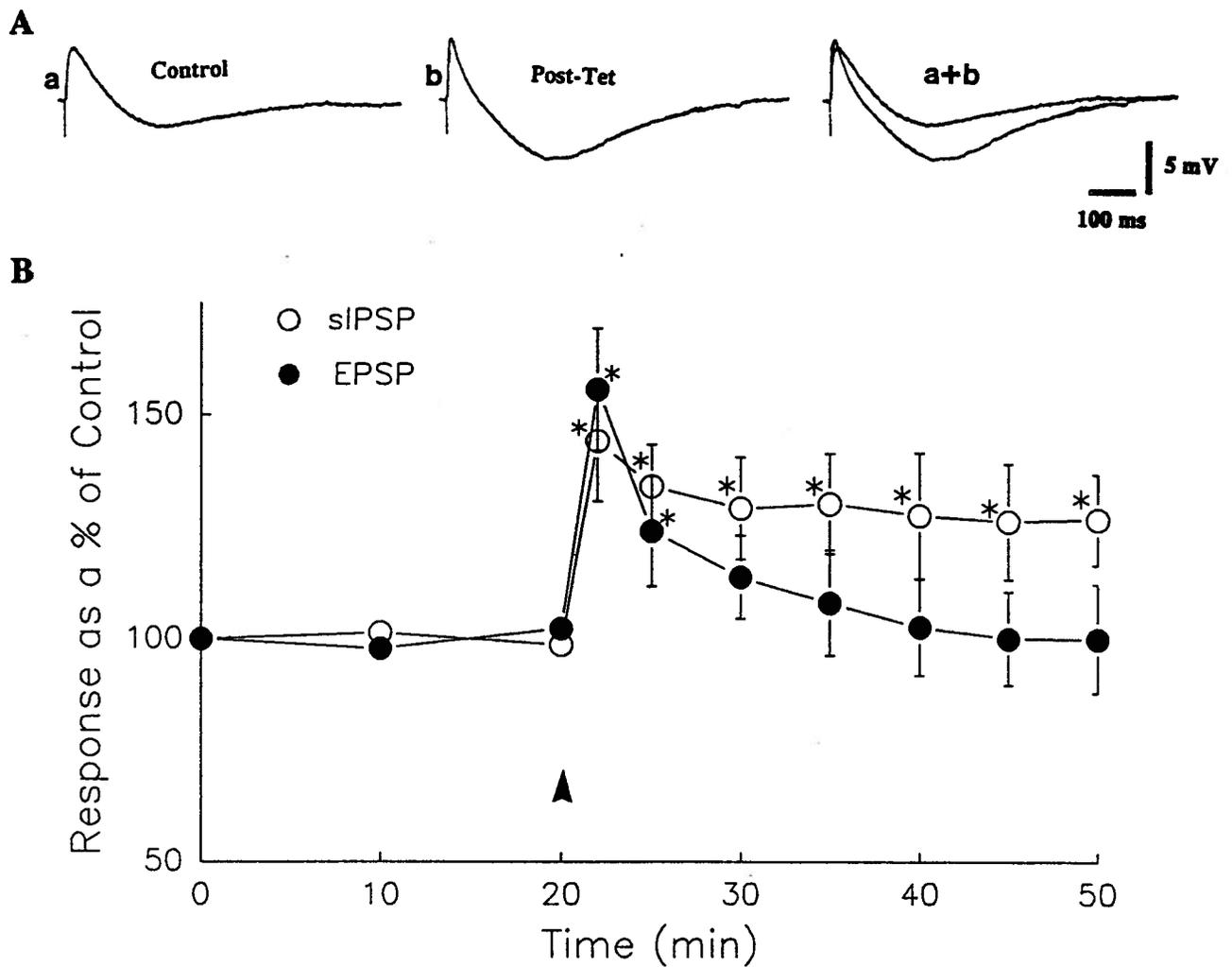


Figure 14 Effects of K-252b on post-tetanic changes of EPSP and slow IPSP in the presence of picrotoxinin.

Post-tetanic changes of the EPSP and the slow IPSP in neurons injected with K-252b are shown in A & B. In these experiments, the fast IPSP was blocked by picrotoxinin (20 μ M). A shows the records of an individual experiment, and B illustrates data obtained in 8 neurons injected with K-252b. Tetanic stimulation (arrow) induced LTP of the slow IPSP but not of the EPSP in neurons injected with K-252b. The resting membrane potential of the neuron in A was -67 mV and post-tetanic record was obtained 20 min after tetanic stimulation.

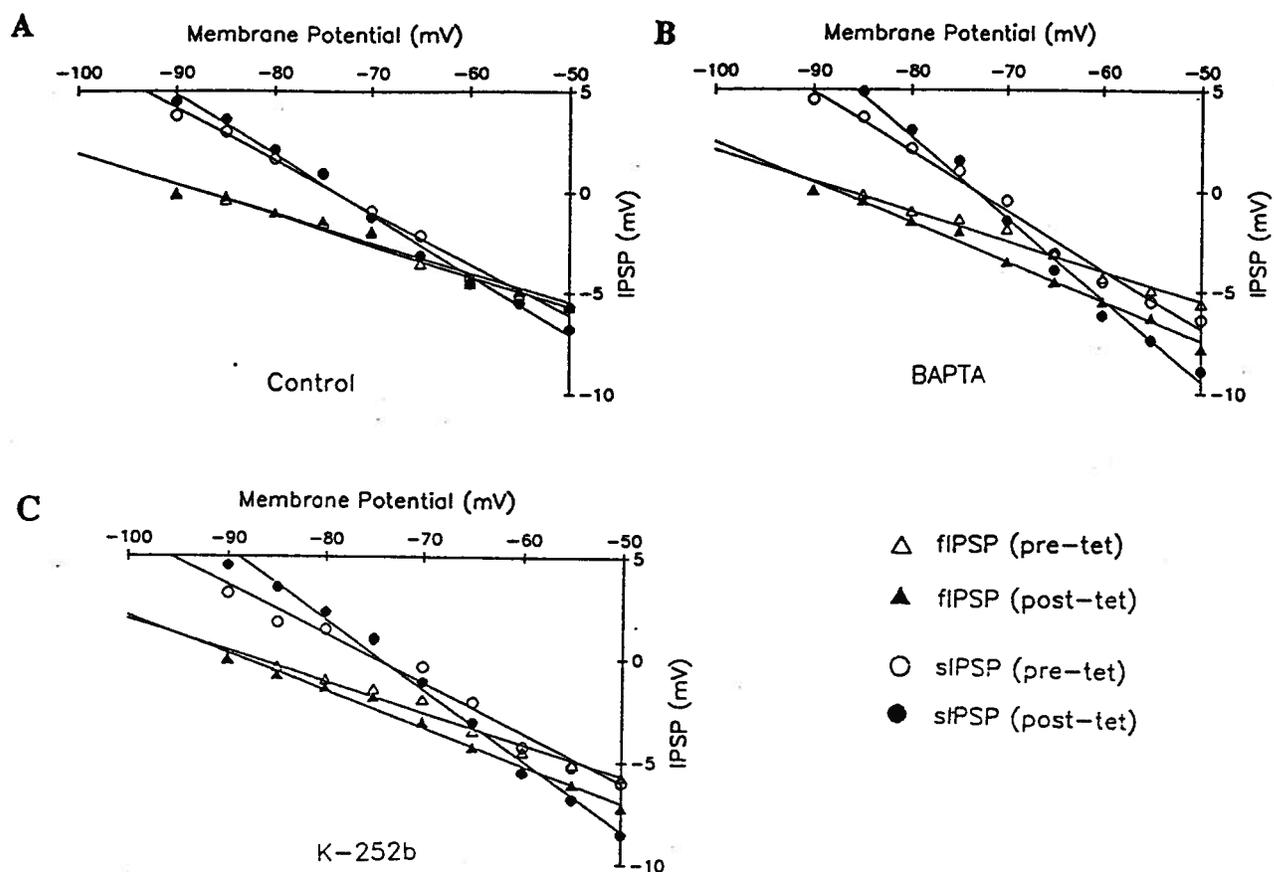


Figure 15 Equilibrium potentials for fast and slow IPSPs in control, BAPTA- and K-252b-injected neurons.

A, B, and C show equilibrium potentials for the fast IPSPs before and after tetanic stimulation, and for the slow IPSPs before and after tetanic stimulation in control (**A**), BAPTA- (**B**) and K-252b- (**C**) injected neurons. The post-tetanic data were obtained 20 min after tetanic stimulation. Note that equilibrium potentials for the fast and slow IPSPs in control, BAPTA- and K-252b injected neurons did not significantly change after tetanic stimulation. Equilibrium potentials for the fast and slow IPSPs were approximately -70 to -75 mV and -90 to -95 mV, respectively.

Table 1 Effects of intracellular injection of BAPTA and K-252b on post-tetanic EPSP, fast and slow IPSPs

	Control neurons	BAPTA-injected neurons	K-252b-injected neurons
EPSP	138.8 ± 10.0*	94.2 ± 11.4	99.8 ± 5.4
fIPSP	123.6 ± 8.4*	179.5 ± 17.0*	166.0 ± 7.8*
sIPSP	98.5 ± 11.3	155.6 ± 15.5*	155.0 ± 12.8*
No. of neurons	8	6	16

Data shown in this table and tables 2, 4 & 5 are responses (in height) 20 min post-tetanus as a percentage of control record (100%) and represent the mean ± S.E.M. Asterisk (*) indicates that the post-tetanus responses are significantly different from the control responses at a *P*-value of <0.05 using paired Student's *t*-test in this table and tables 2, 4 & 5.

Table 2 Effects of intracellular injection of BAPTA and K-252b on post-tetanic EPSP and slow IPSP in picrotoxinin-treated slices

	Control neurons	BAPTA-injected neurons	K-252b-injected neurons
EPSP	149.8 ± 9.7*	105.0 ± 12.0	99.9 ± 12.2
sIPSP	73.5 ± 12.6*	125.9 ± 10.1*	126.4 ± 10.1*
No. of neurons	8	6	6

Table 3 Effects of BAPTA and K-252b on post-tetanic EPSP duration

	Control neurons		BAPTA-injected neurons		K-252b-injected neurons	
	without PTX	with PTX	without PTX	with PTX	without PTX	with PTX
EPSP duration	108.8 ± 5.0	114.0 ± 3.9	67.3 ± 7.2	103.3 ± 4.9	79.8 ± 9.1	87.5 ± 6.8
No. of neurons	8	8	6	6	16	6

In slices exposed with picrotoxinin (PTX, 20 μM), fast IPSP was blocked.

depresses LTP of the IPSPs and allows a better expression of the LTP of the EPSP. LTP of the slow IPSP occurred only if postsynaptic PKC activity was inhibited or if intracellular Ca^{2+} was chelated. Tables 1 & 2 summarize the long-term changes of the IPSPs induced by tetanic stimulation and the effects of BAPTA and K-252b on the IPSPs in CA1 neurons.

8.1.4. Effects of IPSPs on EPSP

It is logical for me to think that the blockade of the fast IPSP can significantly increase the height and duration of the EPSP. In most BAPTA- or K-252b injected CA1 neurons, the potentiation of the fast IPSP induced by the tetanus is associated with a reduced height and duration of the EPSP (Fig. 10 & 13, Table 3). In control neurons, the slow IPSP was slightly depressed or not changed by tetanic stimulation. The potentiation of the post-tetanic slow IPSP occurred only in the BAPTA- and K-252b-injected neurons. Whether a blockade of the potentiation of the slow IPSP would change the shape of the EPSP was examined. In K-252b- (Fig. 16, n=8) and BAPTA- (Fig. 17, n=8) injected CA1 neurons, phaclofen (0.5-1 mM, applied for 10 min), a GABA_B antagonist, blocked the slow IPSP when applied before and after tetanic stimulation. The blockade of the potentiated slow IPSP by phaclofen is associated with a more significant change in the shape of the EPSP compared to the pre-tetanic slow IPSP (Fig. 16 & 17). The results indicate that the potentiated IPSPs significantly distort the shape of the EPSP in BAPTA- and K-252b-injected CA1 neurons.

8.1.5. Effects of APV on IPSPs

Multiple trains of tetanic stimulation (kindling) induce epileptic activity in CA1 neurons associated with NMDA receptor-mediated suppression of the IPSPs. APV, a NMDA antagonist, blocks the kindling-induced epileptic activity as well as the suppression of the IPSPs (Stelzer et al., 1987). It is possible that kindling causes a large Ca^{2+} influx through NMDA receptor-gated channels and

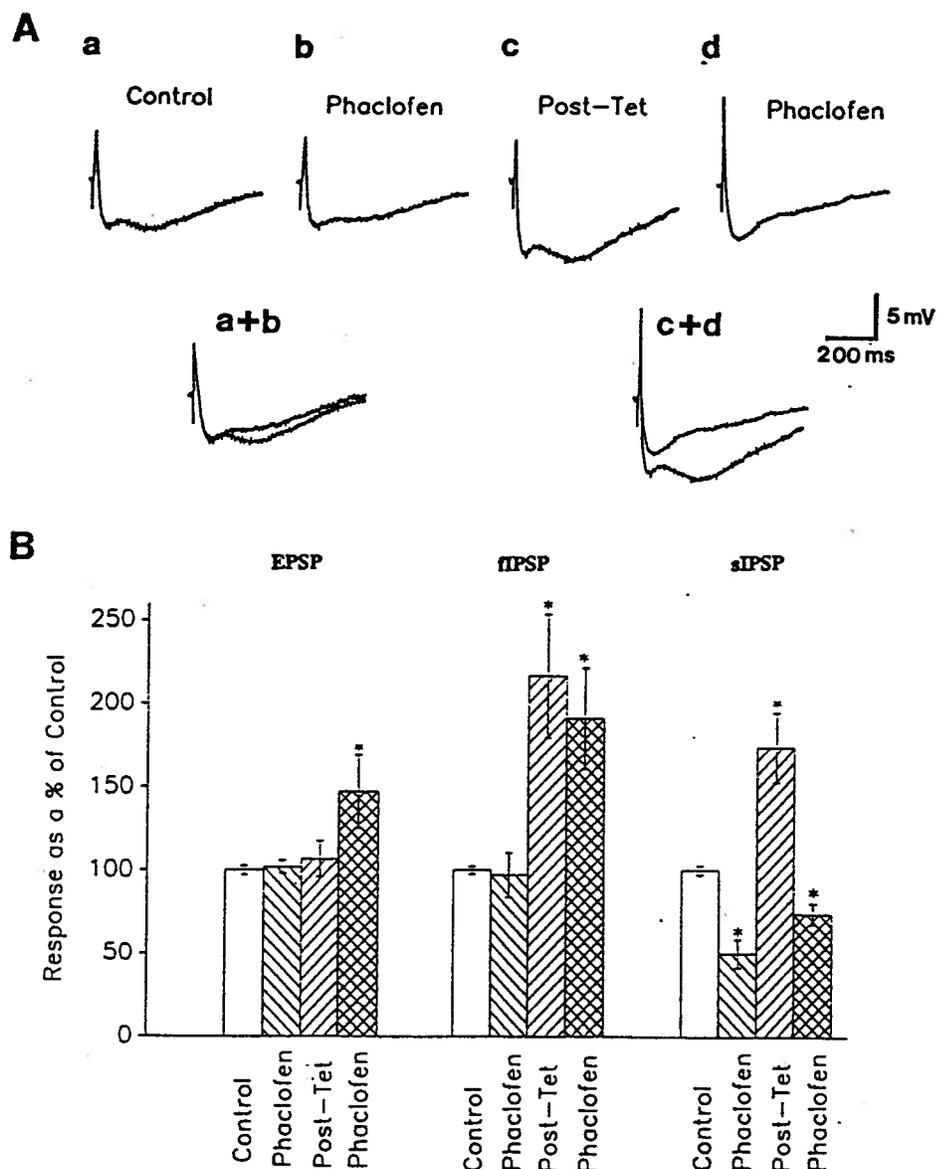


Figure 16 Effects of phaclofen on EPSP in K-252b-injected neurons before and after tetanic stimulation.

Phaclofen (500 μ M) was applied to slices before and 20 min after tetanic stimulation. **A** shows the records of an individual experiment. In **B**, data obtained from 8 neurons are summarized in histograms. Note that before tetanic stimulation, phaclofen suppressed the slow IPSP with no significant effect on the EPSP in K-252b injected neurons. Application of phaclofen 20 min after tetanic stimulation not only depressed the slow IPSP but also enhanced the amplitude of the EPSP in these neurons. Records shown in **B** were from the same neuron with a resting membrane potential of -63 mV.

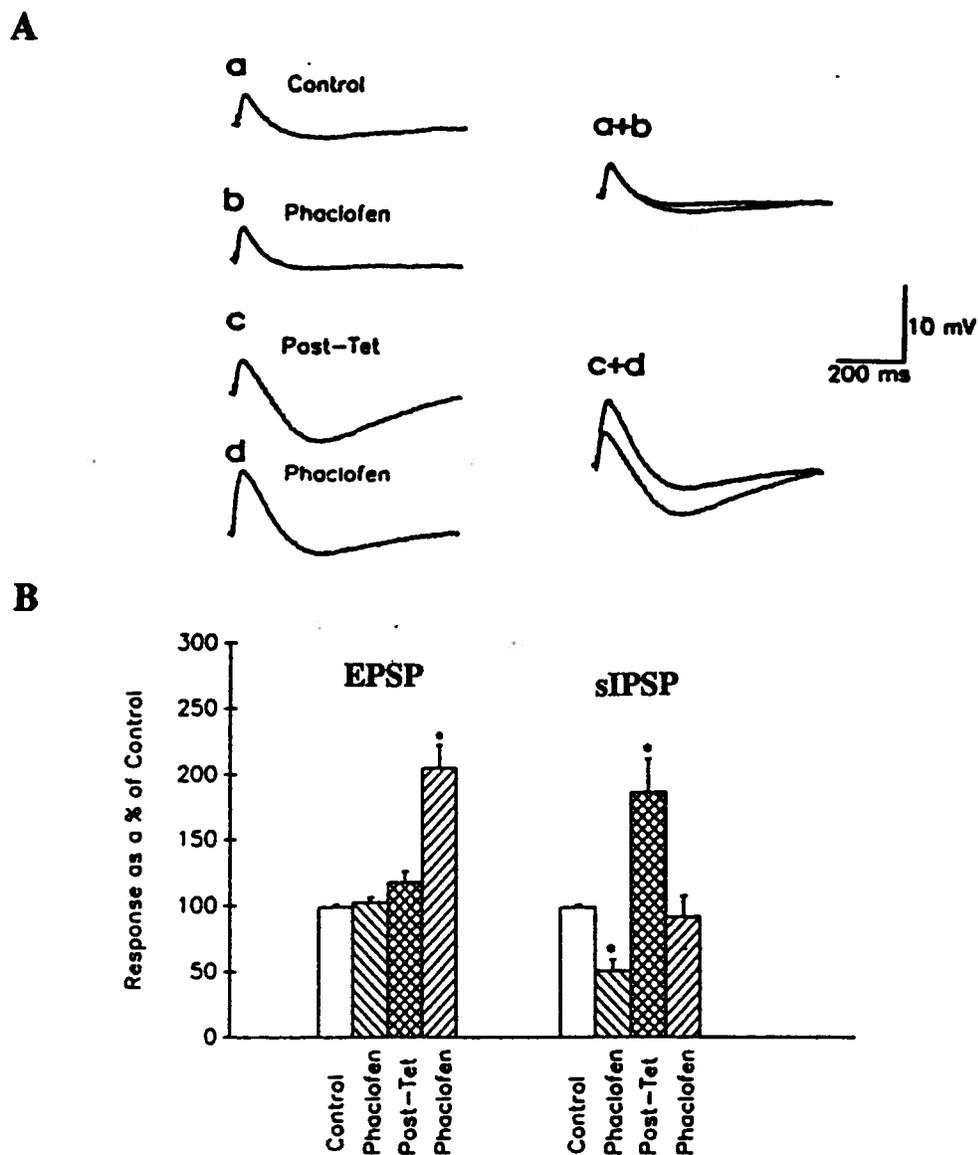


Figure 17 Effects of phaclofen on the EPSP in BAPTA-injected neurons before and after tetanic stimulation.

A and **B** show effects of phaclofen (500 μ M) on the EPSP in neurons injected with BAPTA. Phaclofen was applied before and 20 min after tetanic stimulation. In these experiments, slices were exposed to picrotoxinin (20 μ M) to block the fast IPSP. The records obtained from one individual experiment are shown in **A**, and data from 8 neurons are expressed in histograms in **B**. The membrane potential was -68 mV. Note that before tetanic stimulation, phaclofen blocked the slow IPSP without significantly affecting the EPSP. After tetanic stimulation, phaclofen not only suppressed the slow IPSP but also increased the amplitude of the EPSP.

subsequently triggers the activation of protein kinases. The suppression of the IPSPs induced by kindling may be due to the increase in postsynaptic Ca^{2+} concentration and the activation of PKC. In the present experiments, APV (40 μM) was applied to the picrotoxinin-free (Fig. 18) or the picrotoxinin-treated (Fig. 19) slices 5 min before tetanic stimulation and the drug application terminated after the tetanic stimulation. In control CA1 neurons, tetanic stimulation failed to induce LTP of the EPSP and caused a slight depression of the slow IPSP in the presence of APV (Fig. 18A,19A). In BAPTA- and K-252b- injected neurons, LTP of both the EPSP and the slow IPSP did not occur after tetanic stimulation in presence of APV (Fig. 18B & C, 19B & C). In slices not exposed to picrotoxinin, tetanic stimulation in presence of APV caused a LTP of the fast IPSP while APV prevented LTP of both the EPSP and the slow IPSP in control, BAPTA- or K-252b-injected neurons (Fig. 18A, B & C). This LTP of the fast IPSP is smaller in amplitude than the LTP obtained in BAPTA- and K-252b-injected neurons tested in the absence of APV. These results indicate that APV not only blocks the induction of LTP of the EPSP but also suppresses the potentiation of the IPSPs.

8.1.6. IPSPs in the absence of glutamatergic transmission

Under normal conditions, IPSPs evoked by tetanic stimulation of the stratum radiatum consist of both polysynaptic and monosynaptic components. The polysynaptic component of IPSPs sometimes was more prominent than the monosynaptic one although the stimulating electrode was placed in the stratum radiatum near the recording electrode. When glutamatergic transmission in the hippocampus was blocked by APV and CNQX, monosynaptic IPSPs could be recorded (Fig. 20A) (Davies et al., 1990). Under these conditions, changes in the monosynaptic IPSPs after tetanic stimulation were examined. When APV (40 μM) and CNQX (20 μM) were present in the perfusing medium throughout the experiment, tetanic stimulation induced a LTP of the fast IPSP and

Figure 18 Effects of APV on post-tetanic changes of IPSPs.

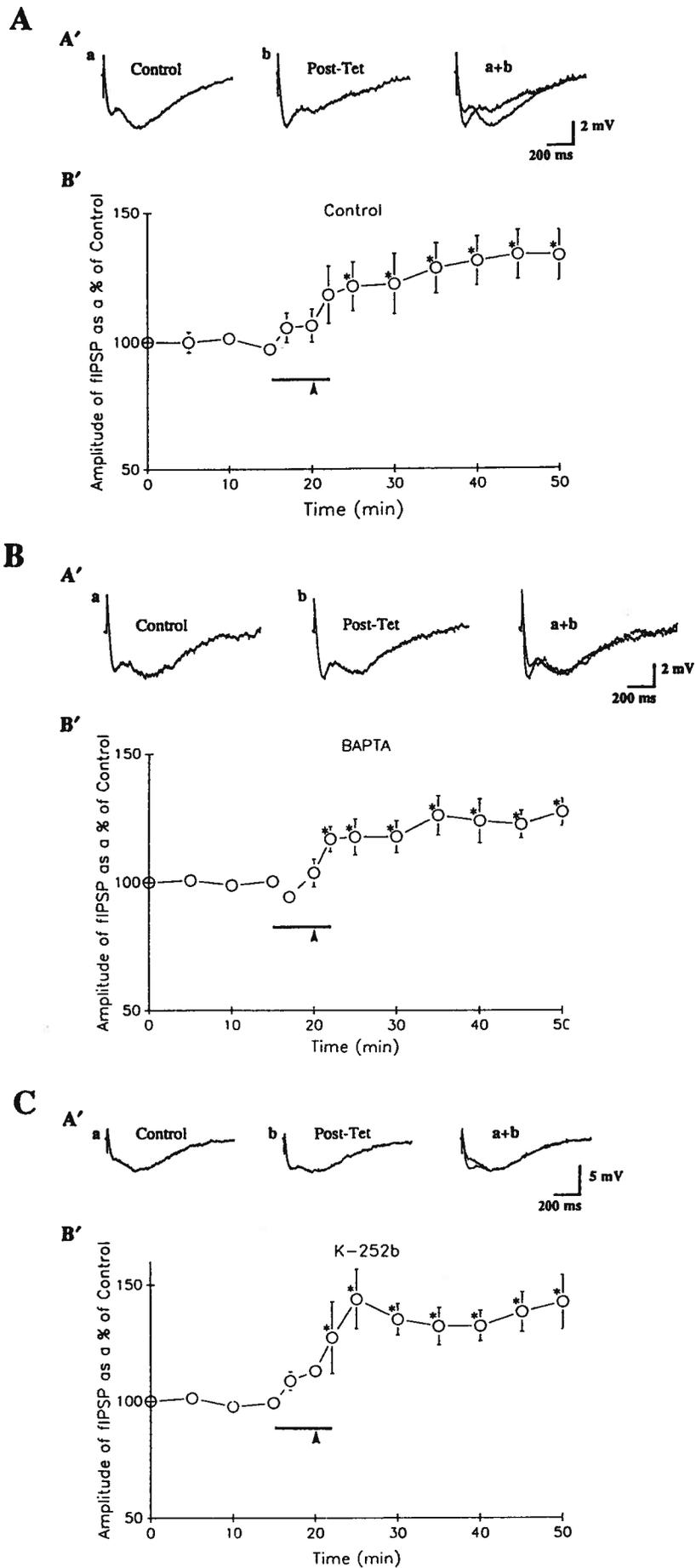


Figure 18 Effects of APV on post-tetanic changes of IPSPs.

Effects of APV on postsynaptic changes of the EPSP, fast and slow IPSPs are illustrated in **AB'** (control neurons, n=5), **BB'**(n=BAPTA-injected neurons, n=6) and **CB'**(K-252b-injected neurons, n=6). Tetanic stimulation (arrow) was given in the presence of APV (40 μ M, the horizontal line above the arrow). Individual records obtained from a control neuron (**AA'**), a BAPTA-injected neuron (**BA'**) and a K-252b injected neuron (**CA'**) are demonstrated. Post-tetanic responses were recorded 20 min after tetanus. Note that tetanic stimulation induced LTP of the fast IPSP but not of the EPSP and the slow IPSP in the presence of APV. In some BAPTA-injected neurons (**BA'**) and K-252b-injected neurons (**CA'**), the EPSP was suppressed after tetanic stimulation.

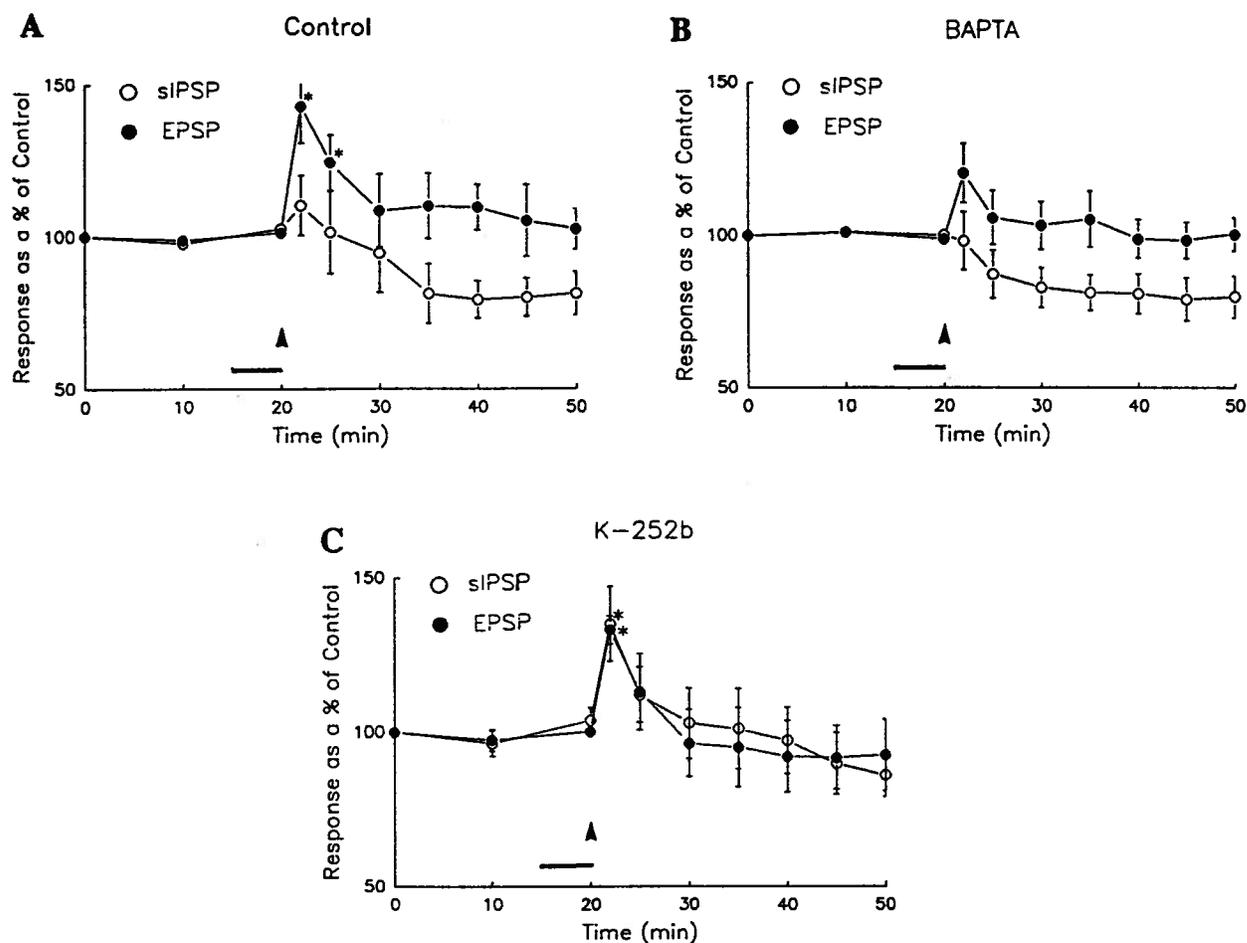


Figure 19 Effects of APV on post-tetanic changes of slow IPSP in the presence of picrotoxinin.

The ability of APV to block post-tetanic changes of the EPSP and the slow IPSP is illustrated in A, B & C. In these experiments, slices were exposed to 20 μ M picrotoxinin. In the presence of APV (the horizontal bar above the abscissa), tetanic stimulation (arrow) did not cause LTP of the EPSP and the slow IPSP in control (A, n=6), BAPTA- (B, n=8) and K-252b- (C, n=8) injected neurons.

an insignificant change in the slow IPSP of the control neurons (Fig. 20B, n=6). Similar results were obtained in BAPTA-injected (n=6) and K-252b-injected neurons (n=6) (Fig. 20C & D; see table 4). The tetanus-induced potentiation of the fast IPSP obtained in APV- and CNQX-treated slices was much smaller than the one observed in drug-free slices. In picrotoxinin-treated hippocampal slices, the isolated slow IPSP did not significantly change after tetanic stimulation in control neurons or BAPTA- and K-252b-injected neurons (Fig. 21, n=6 for each group; see table 5). These results indicate that long-term changes in the IPSPs following tetanic stimulation require glutamatergic transmission.

8.2. Effects of somatostatin on GABAergic inhibition and LTP

Somatostatin and GABA are co-localized in some interneurons of the CA1 area of the hippocampus. If the peptide and the amino acid are co-released from the same presynaptic terminals, interactions between the actions of somatostatin and the GABAergic responses on CA1 neurons may occur. Somatostatin has been reported to depress GABA receptor-mediated IPSPs in the hippocampal CA1 neurons (Scharfman and Schwartzkroin, 1989). Under normal conditions, changes in GABAergic inhibition can affect the induction of LTP of the EPSP (Wigstrom and Gustafsson, 1983; Davies et al., 1991; Mott and Lewis, 1991). The present experiments were conducted to examine the actions of somatostatin on the GABAergic synaptic transmission and LTP.

8.2.1. Effects of somatostatin on CA1 neurons

Different concentrations of somatostatin-14 (SS, 0.2-5 μ M) were applied to determine the appropriate concentration to induce significant and consistent effects in CA1 neurons. A concentration of 2 μ M SS, applied to slices for 2 min, was found to have reliable effects on the membrane potential, input resistance and evoked synaptic responses (n=8). This concentration was therefore used in the quantitative studies.

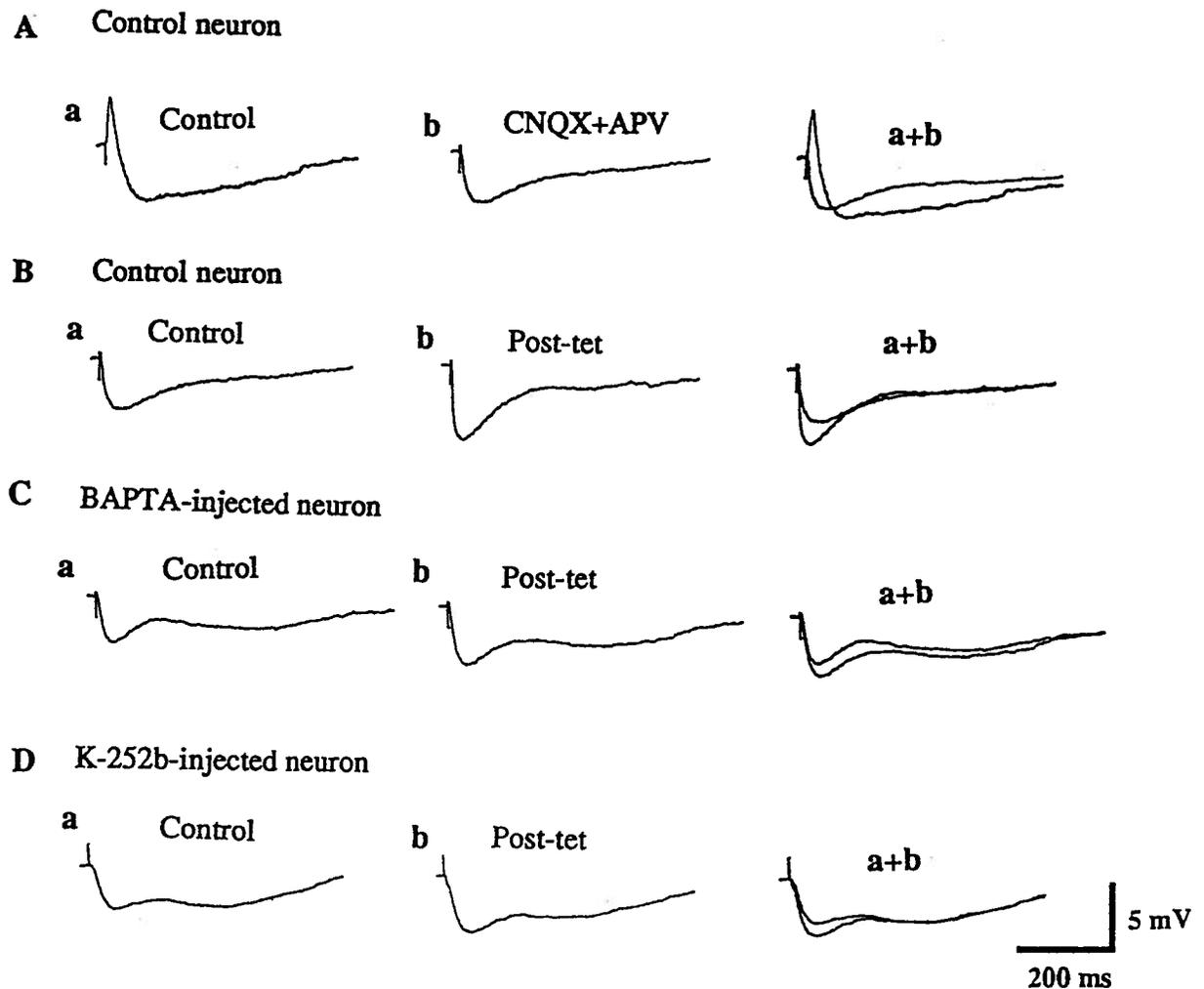


Figure 20 Post-tetanic changes of IPSPs in the presence of APV and CNQX. Monosynaptic IPSPs were obtained in the presence of APV (40 μ M) and CNQX (20 μ M). In A, the activation of monosynaptic IPSPs in CA1 neurons is illustrated. In B, C & D, post-tetanic changes of monosynaptic IPSPs in control (B), BAPTA- (C) and K-252b- (D)-injected neurons are shown. Note that tetanic stimulation induced an increase in the fast IPSP without significantly altering the slow IPSP in these neurons. A and B are from the same neuron with a resting membrane potential of -59 mV. C and D were from different neurons with a resting membrane potentials of -57 mV and -63 mV, respectively.

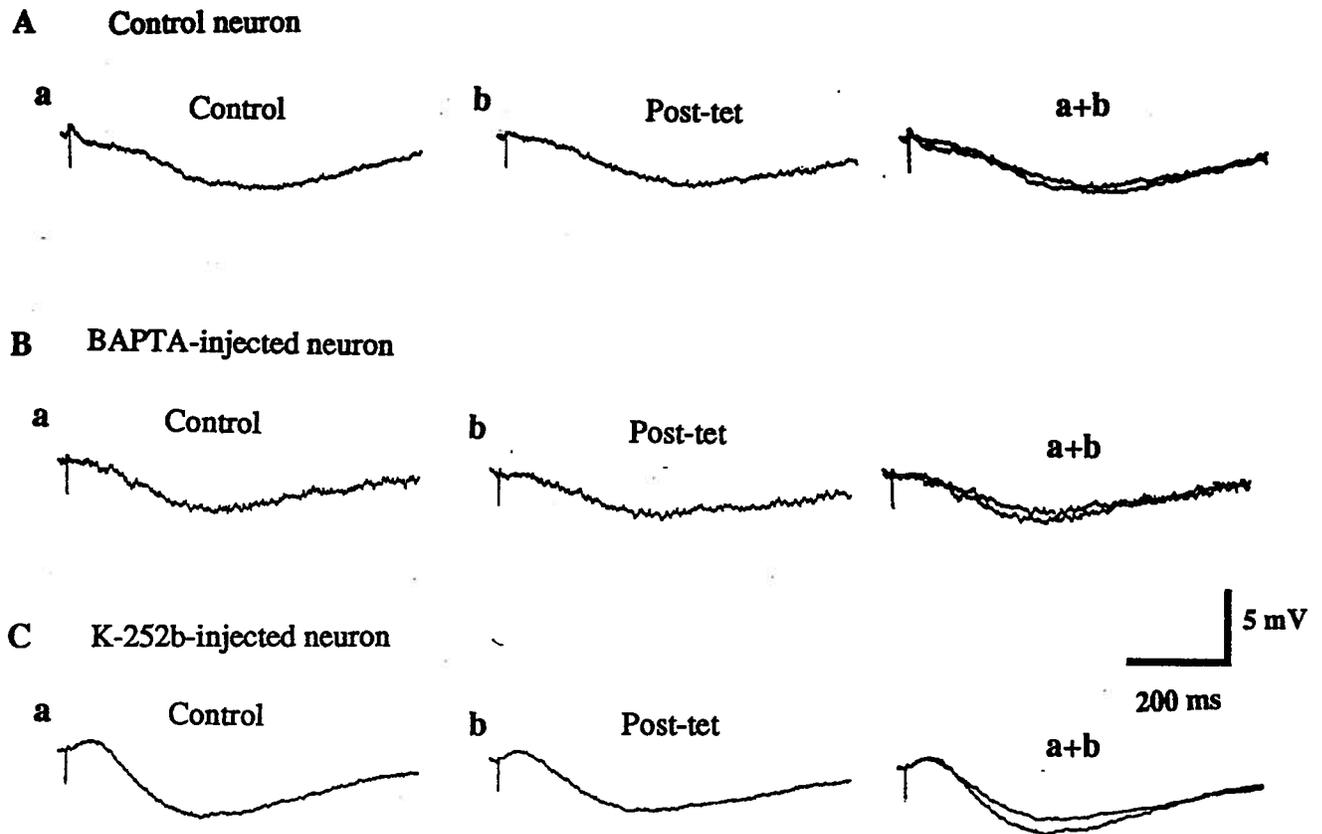


Figure 21 Post-tetanic changes of monosynaptic slow IPSP.

Isolated slow IPSP was obtained in the presence of APV ($40 \mu\text{M}$), CNQX ($20 \mu\text{M}$) and picrotoxinin ($20 \mu\text{M}$). Tetanic stimulation did not cause any significant changes in the monosynaptic slow IPSP in control (A), BAPTA- (B) and K-252b- (C) injected neurons. A, B and C were from different neurons with resting membrane potentials of -58 , -66 and -61 mV, respectively.

Table 4 Effects of intracellular injection of BAPTA and K-252b on post-tetanic fast and slow IPSPs in the presence of APV and CNQX

	Control neurons	BAPTA-injected neurons	K-252b-injected neurons
fIPSP	142.6 ± 6.3*	130.8 ± 9.9*	135.5 ± 11.1*
sIPSP	110.4 ± 10.9	115.0 ± 9.1	112.7 ± 10.1
No. of neurons	6	6	6

(see the footnote in table 1).

Table 5 Effects of intracellular injection of BAPTA and K-252b on post-tetanic slow IPSP in the presence of APV, CNQX and picrotoxinin

	Control neurons	BAPTA-injected neurons	K-252b-injected neurons
sIPSP	90.5 ± 9.1	88.1 ± 9.1	86.2 ± 8.2
No. of neurons	8	6	8

(see the footnote in table 1).

Application of SS induced a hyperpolarization of 7.49 ± 0.73 mV ($n=16$) of the hippocampal CA1 neurons associated with a decrease in the input resistance by 12.46 ± 1.10 M Ω ($n=16$) (Fig. 22A). SS significantly depressed the fast IPSP (response as a percentage of control: 31.50 ± 2.73 , $n=16$) and the slow IPSP (response as a percentage of control: 35.33 ± 2.10 , $n=16$) without significantly changing the EPSP (response as a percentage of control: 111.2 ± 7.83 , $n=16$) (Fig. 22B). These results are consistent with the reports in literature (Pittman and Siggins, 1981) that SS, when applied to the slices, consistently hyperpolarizes CA1 neurons and reduces the input resistance of the neurons.

8.2.2. Effects of somatostatin on GABA receptors

In order to determine whether SS interacts with GABA_A receptors, actions of SS were examined in the presence of picrotoxinin. In slices exposed to picrotoxinin (20 μ M), SS hyperpolarized the CA1 neurons by 7.32 ± 0.73 mV ($n=24$) and reduced the input resistance by 11.3 ± 1.13 M Ω ($n=24$) (Fig. 23A). SS also depressed the slow IPSP (response as a percentage of control: 36.50 ± 2.30 , $n=24$) without significantly changing the EPSP (response as a percentage of control: 105.20 ± 6.90 , $n=24$) (Fig. 23B).

To determine whether SS interacts with GABA_B receptors, SS was applied in the presence of phaclofen. Phaclofen (1 mM), which blocked the GABA_B receptor-mediated slow IPSP, significantly reduced the baclofen-induced hyperpolarization ($n=4$), but not the SS-induced hyperpolarization ($n=16$) (Fig. 24A & B). In the presence of phaclofen, SS induced a hyperpolarization of 7.58 ± 0.67 mV and a decrease in the input resistance by 12.6 ± 1.20 M Ω ($n=16$). The current-voltage (I-V) curves showed a decrease in the slope resistance in the presence of somatostatin (Fig. 24C) and a reversal potential of approximately -90 mV for the action of SS. Phaclofen did not change the SS-induced decrease in the slope resistance (Fig. 24C). 2-OH-Saclofen, which

blocks both presynaptic and postsynaptic GABA_B receptors (Davies et al., 1990; Dutar and Nicoll, 1988), did not change the actions of SS on the membrane potential, input resistance and fast IPSP in the CA1 neurons (n=4, Fig. 25). These results indicate that SS does not interact with GABA_A and postsynaptic & presynaptic GABA_B receptors.

8.2.3. Effects of somatostatin during the activation of GABA_B receptors

Baclofen-induced hyperpolarization is thought to be mediated by GABA_B receptors coupled to specific K⁺ channels through a pertussis toxin-(PTX) sensitive G protein (Andrade et al., 1986). It has been suggested that SS receptors are also coupled to K⁺ channels through a PTX-sensitive G protein (Schweitzer et al., 1989; Yatani et al., 1990). Whether GABA_B and SS receptors share the same type of K⁺ channels is, however, unclear. It has been reported in the literature that the hyperpolarizing action of baclofen is suppressed by SS (Twery and Gallagher, 1990). Experiments were conducted to determine whether the actions of SS would be affected by the activation of GABA_B receptors. Baclofen (20 μM) was applied to the slices prior to the application of SS. Baclofen induced a prolonged hyperpolarization of 15.5 ± 1.67 mV and a decrease in the input resistance by 20.3 ± 2.12 MΩ (n=6) of the CA1 neurons. SS was applied to the slices at the peak of the baclofen-induced hyperpolarization. SS did not induce further changes in the membrane potential and the input resistance of the CA1 neurons (n=6) (Fig. 26A & C). Even if the membrane potential of the neurons was adjusted to the pre-baclofen level by injecting a depolarizing current, SS did not have any further hyperpolarizing action (n=6) (Fig. 26B). These results indicate that the activation of GABA_B receptors results in a decrease in the action of SS on the CA1 neurons.

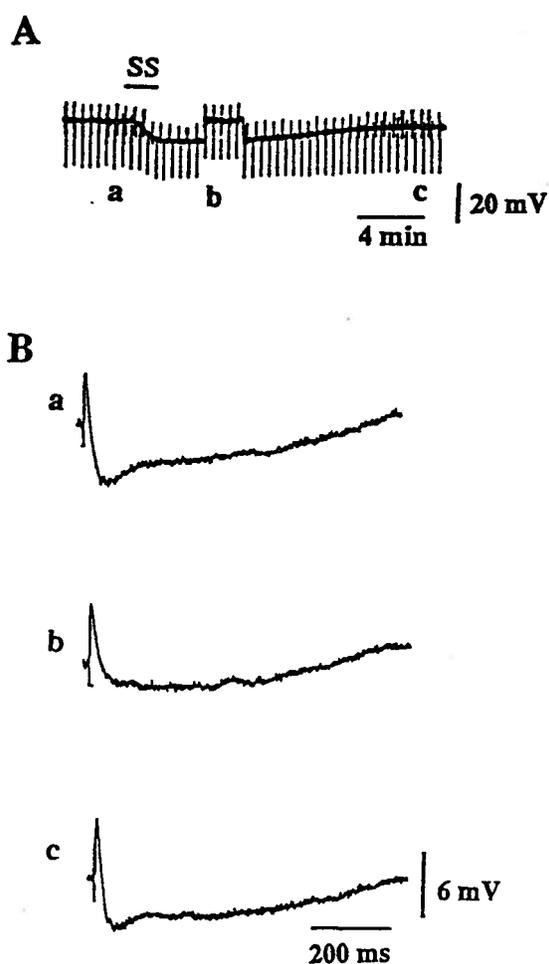


Figure 22 Effects of somatostatin on the membrane potential, input resistance and evoked synaptic responses in CA1 neurons of hippocampal slices. Somatostatin (SS, 2 μ M) was applied for 2 min. The input resistance was monitored by injecting hyperpolarizing current pulses throughout the experiment. **A** shows a hyperpolarization and a reduction of input resistance caused by somatostatin. **B** shows the effects of somatostatin on the EPSP, fast and slow IPSPs. The records of synaptic responses shown were taken during the control (a), at the peak of somatostatin-induced hyperpolarization while the membrane potential was adjusted to the control level by injection of a depolarizing current (b), and during a recovery from the actions of the peptide (c). Note that somatostatin suppressed the fast and slow IPSPs without significantly altering the height of the EPSP. In this figure and in figure 23, the small upward and downward deflections are due to the EPSP and the IPSPs, respectively, and the larger downward deflections are the voltage responses due to the hyperpolarizing intracellular current injections. The resting membrane potential of this neuron was -60 mV.

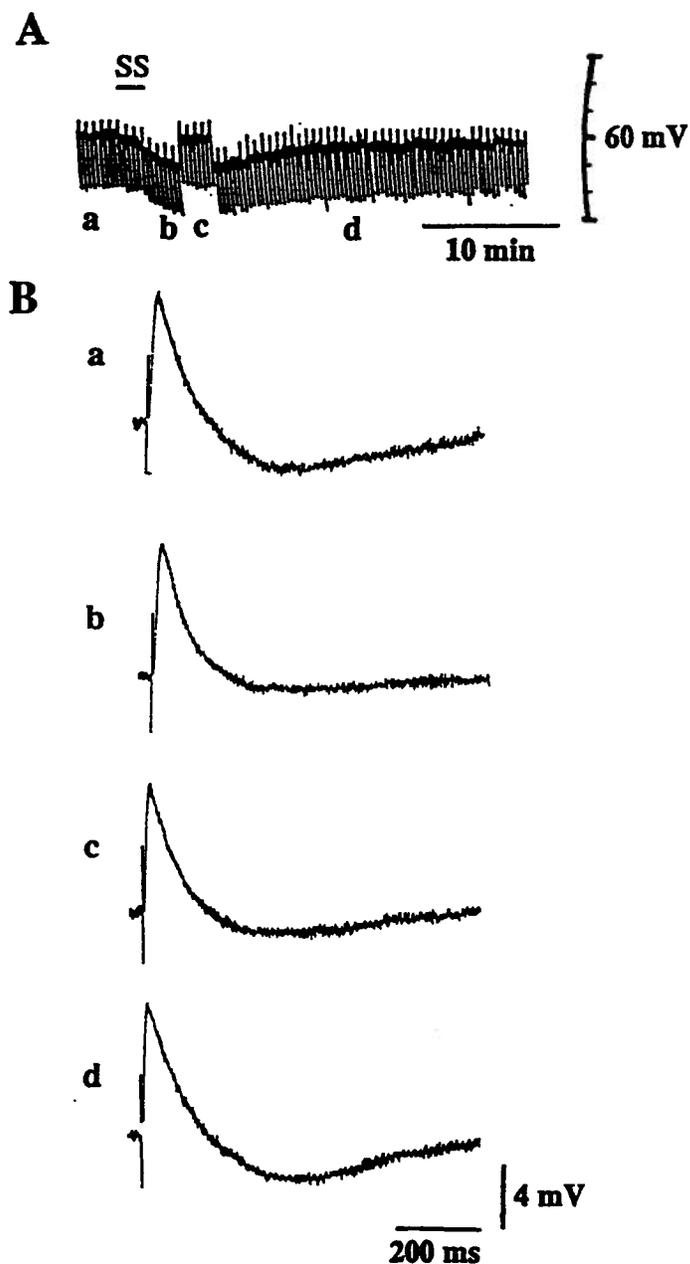


Figure 23 Actions of somatostatin in the presence of picrotoxinin. In the experiment, the hippocampal slice was superfused with picrotoxinin (20 μM)-containing medium to block the fast IPSP. **A** shows a hyperpolarization and a decrease in input resistance of the CA1 neuron induced by somatostatin (SS, 2 μM , applied for 2 min). In **B**, records of the synaptic transients taken during control (a), during the hyperpolarization induced by somatostatin (b), during a current-clamp of the neuron back to control membrane potential (c), and during a recovery from the action of the peptide (d). Note that somatostatin depressed the fast and slow IPSPs, but not the EPSP in the picrotoxinin-treated slices. The resting membrane potential of this neuron was -62 mV.

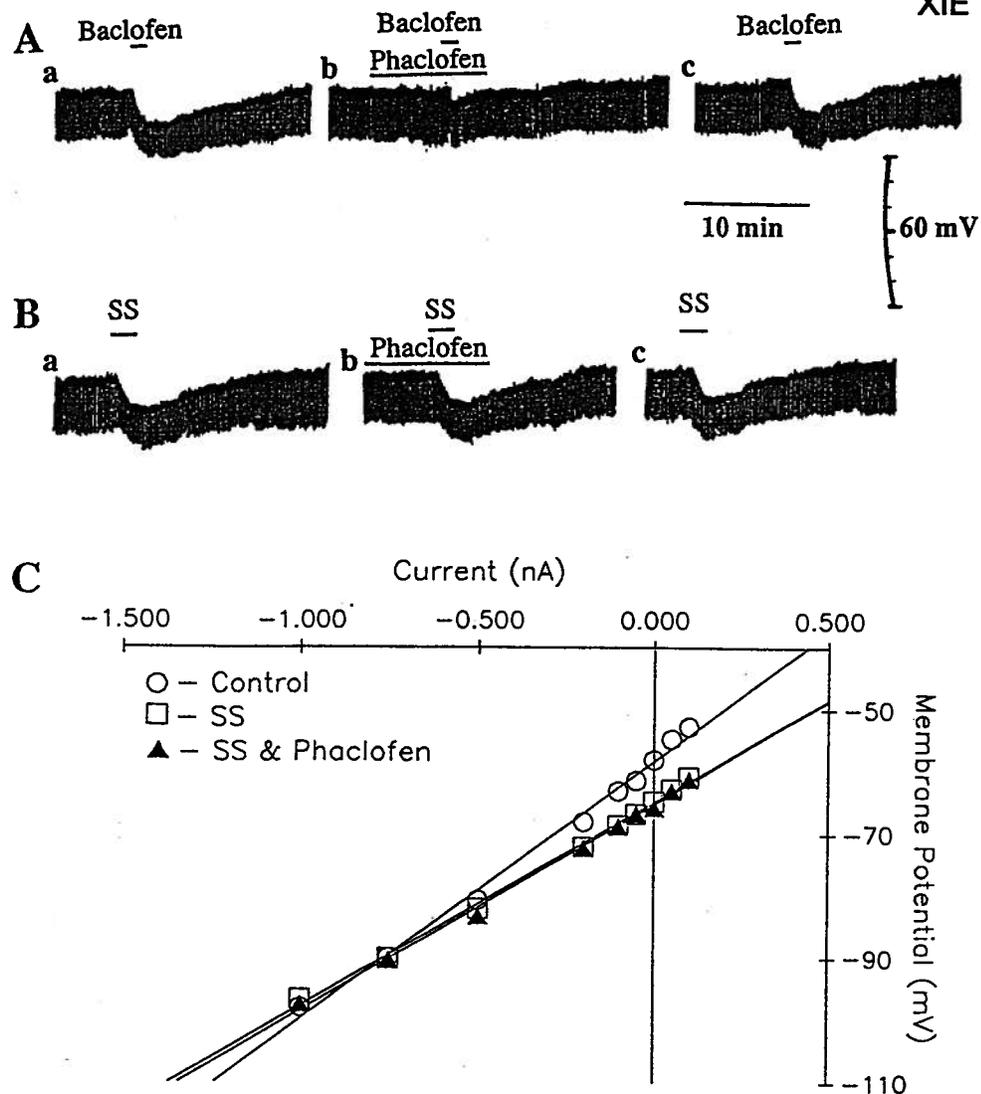


Figure 24 The effects of phaclofen on the hyperpolarizing actions of baclofen and somatostatin in picrotoxinin-treated slices.

In **Aa**: baclofen ($20 \mu\text{M}$, 1 min) induced a hyperpolarization and a decrease in the input resistance of the CA1 neuron. In **Ab**, the effects of baclofen on the membrane potential and the input resistance were significantly blocked in the presence of phaclofen (1 mM). In **Ac**, 20 min after the washout of phaclofen, baclofen's action recovered from the antagonism by phaclofen. **B** shows a similar interaction between somatostatin and baclofen; Note that phaclofen did not block the action of somatostatin. **A** and **B** were recorded from the same neuron with a resting membrane potential of -61 mV . In **C**, current-voltage curves for control, somatostatin and somatostatin in the presence of phaclofen, were plotted from a different CA1 neuron with the resting membrane potential of -58 mV . Note that the decrease in the slope resistance by somatostatin that was not significantly affected by phaclofen. The reversal potential for the hyperpolarizing action of somatostatin was close to -90 mV . Phaclofen alone did not significantly change the membrane potential and the input resistance of the neuron.

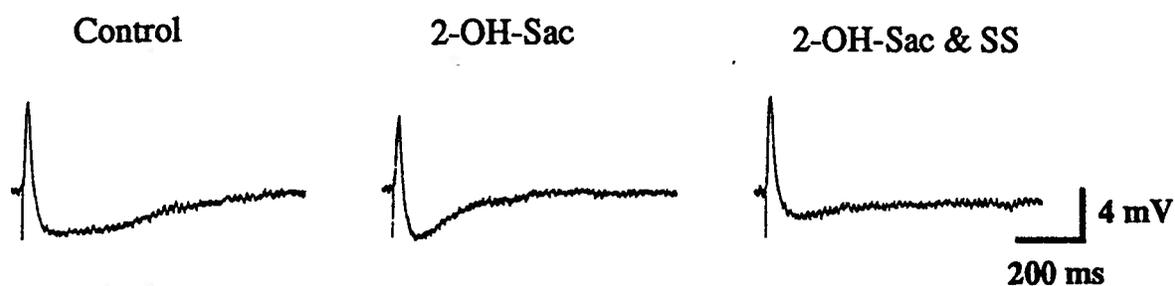


Figure 25 Effects of somatostatin on IPSPs in the presence of 2-OH-saclofen. Effects of 2-OH-saclofen (1 mM) on action of somatostatin are illustrated. Application of 2-OH-saclofen (1 mM) blocked the slow IPSP in the CA1 neuron. In the presence of 2-OH-saclofen, somatostatin (2 μ M) still suppressed the fast IPSP. Note that 2-OH-saclofen has been reported to block the presynaptic GABA_B receptor-mediated paired-pulse depression of the IPSPs in CA1 neurons (Davies et al., 1990).

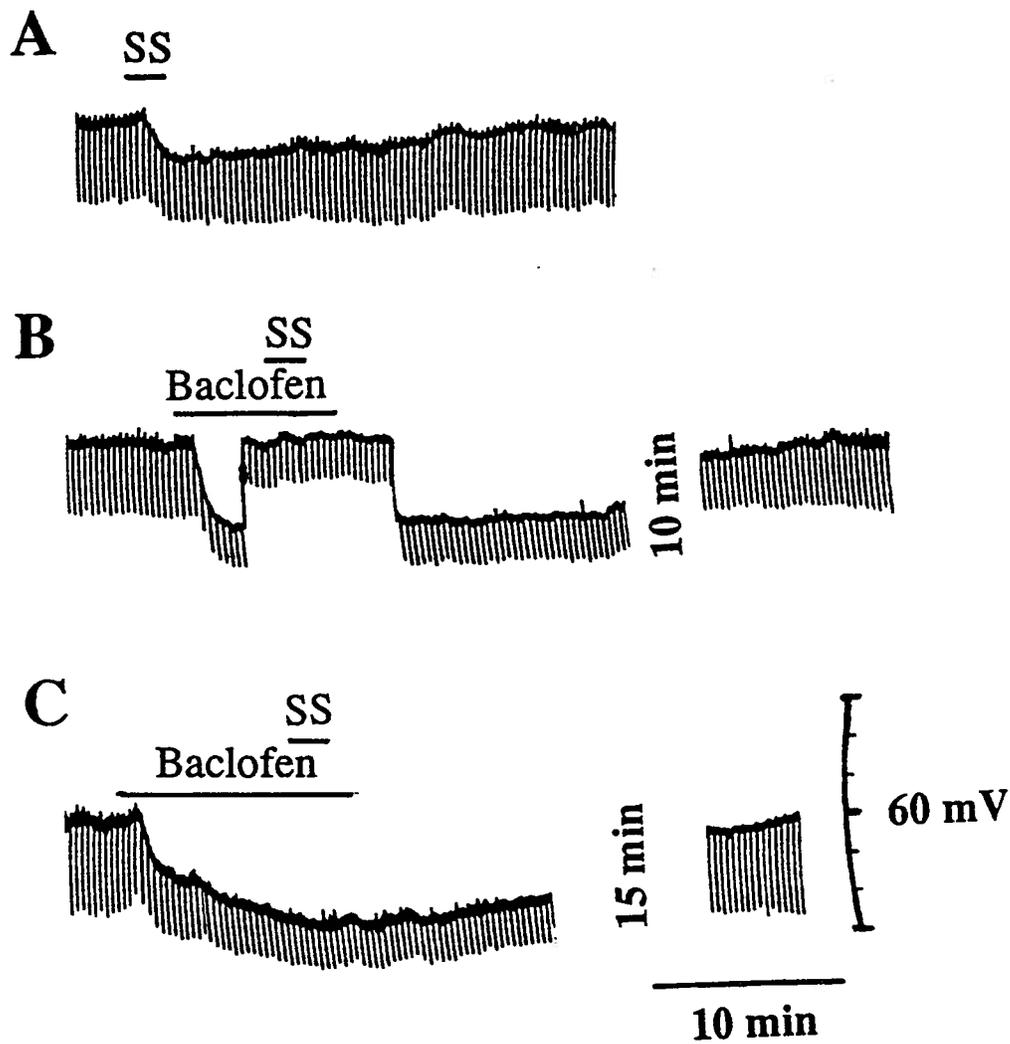


Figure 26 The interactions between baclofen and somatostatin.

A shows a hyperpolarization and a reduction in the input resistance induced by somatostatin ($2 \mu\text{M}$). **B** shows the blockade of the action of somatostatin by baclofen ($20 \mu\text{M}$) recorded from the same neuron as in **A**. Baclofen induced a prolonged hyperpolarization associated with a decrease in the input resistance. At the peak of the baclofen-induced hyperpolarization, the neuron was current-clamped to the pre-baclofen membrane potential when somatostatin was applied. Note that somatostatin did not induce any further hyperpolarization or a decrease in the input resistance of the neuron. **C** was taken from a different neuron, there was a lack of effect of somatostatin during the hyperpolarization induced by baclofen ($20 \mu\text{M}$). The resting membrane potentials of the neurons in **A** & **B** were -57 and -59 mV, respectively.

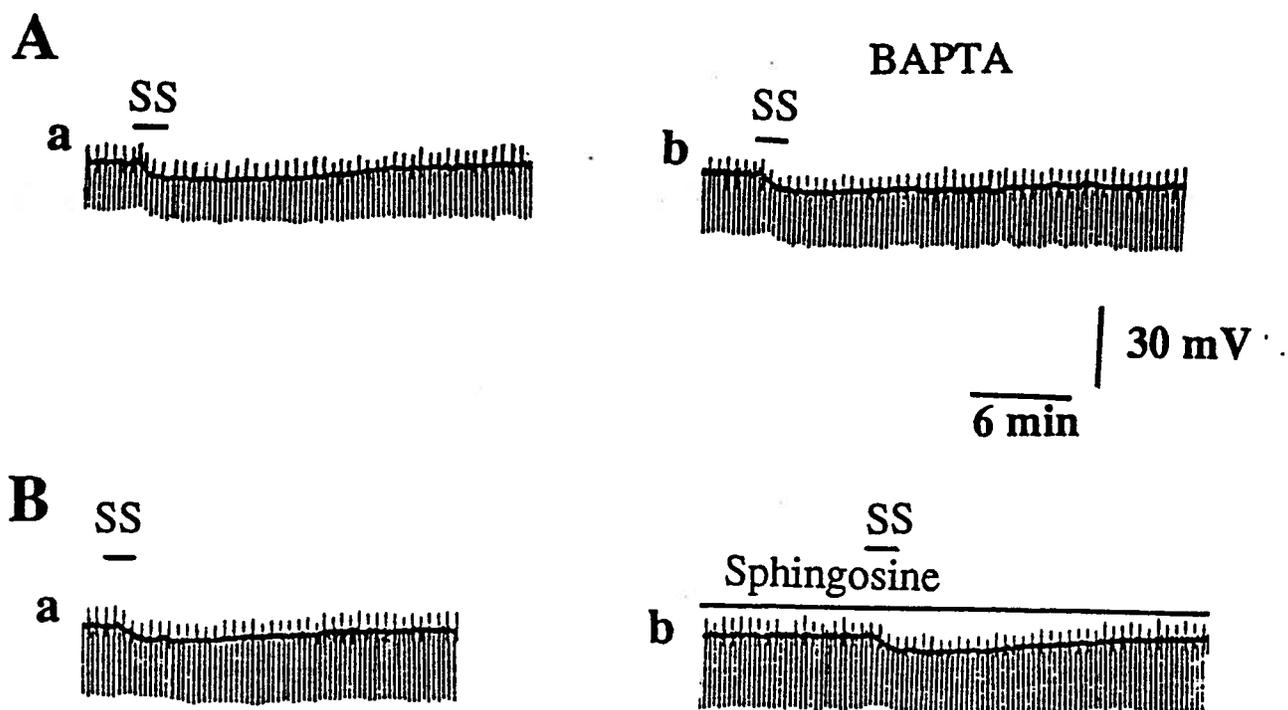


Figure 27 Possible role of protein kinase C and postsynaptic intracellular Ca^{2+} on hyperpolarizing action of somatostatin.

The hyperpolarization induced by somatostatin in control neurons (Aa, Ba), a neuron injected with BAPTA (Ab), and a neuron exposed to sphingosine ($30 \mu\text{M}$) (Bb). Note neither a chelation of the Ca^{2+} nor an inhibition of PKC interfered with the hyperpolarizing action of the peptide. Ba and Bb were from the same neuron. Aa (resting membrane potential: -64 mV), Ab (resting membrane potential: -62 mV) and B (resting membrane potential: -59 mV) were from three different neurons.

8.2.4. *The role of Ca²⁺ and PKC in the actions of somatostatin*

It has been suggested that changes in intracellular Ca²⁺ concentration and PKC activity can affect the GABA_B receptor-mediated responses (Baraban et al., 1985). Whether actions of somatostatin are affected by changes in intracellular Ca²⁺ concentration and PKC activity was examined in the present study.

BAPTA was injected into the CA1 neurons through recording electrodes, as previously described. SS induced a hyperpolarization of 7.13 ± 0.65 (n=6), a decrease in the input resistance by 12.9 ± 1.33 M Ω (n=6), a depression of the fast IPSP (response as a percentage of control: 33.50 ± 2.1 , n=6) and a suppression of the slow IPSP (response as a percentage of control: 36.66 ± 2.33 , n=6) while not significantly affecting the EPSP (response as a percentage of control: 107.00 ± 8.33 , n=6), in BAPTA-injected neurons (Fig. 27A). The effects of SS in the BAPTA-injected neurons were similar to those in the control neurons. The results indicate that chelation of postsynaptic Ca²⁺ does not affect the actions of SS.

In slices exposed to sphingosine (30 μ M), a PKC inhibitor, SS hyperpolarized the CA1 neurons by 7.77 ± 0.81 mV (n=4) and reduced the input resistance by 12.3 ± 1.33 M Ω (n=4). SS also depressed the fast IPSP (response as a percentage of control: 34.33 ± 2.99 , n=4) and the slow IPSP (response as a percentage of control: 37.11 ± 3.33 , n=4) without significantly changing the EPSP (response as percentage of control: 110.0 ± 10.33 , n=4) (Fig. 27B). Sphingosine did not change the membrane potential, input resistance, EPSP and IPSPs in the CA1 neurons. The findings suggest that the actions of SS are not due to changes in PKC activity.

8.2.5. Effects of QX-314 on the actions of somatostatin

QX-314, a derivative compound of lidocaine, has been found to block not only the GABA_B receptor-mediated slow IPSP (Nathan et al., 1990) and hyperpolarization (Andrade, 1991), but also the 5-HT_{1A} receptor-mediated hyperpolarization in the hippocampal CA1 neurons. These effects might be due to the blockade of a G protein-regulated K⁺ channels. Experiments were, therefore, conducted to determine whether QX-314 also affects the actions of SS in CA1 neurons.

QX-314 was allowed to diffuse into the CA1 neurons through recording electrodes. Since it usually took 20-30 min after the penetration of the cell for QX-314 to block the Na⁺ spike and suppress the slow IPSP, it was possible to examine the actions of SS during the early (5-15 min) and the late (35-65 min) stages of QX-314 diffusion into the cells. During the early stage of recording, when the Na⁺ spike and the slow IPSP were not blocked by QX-314, SS was able to cause a hyperpolarization of 6.98 ± 0.61 mV (n=12), a reduction in the input resistance by 10.03 ± 0.93 M Ω (n=12) (Fig. 28Aa) and a depression of the fast IPSP (response as a percentage of control: 44.19 ± 3.1 , n=12) without significantly changing the EPSP (response as a percentage of control: 107.23 ± 7.81 , n=12) (Fig. 28Bb). During the late stage of recording, SS could only induce very small hyperpolarization of 1.21 ± 0.10 mV (n=12), and decrease in the input resistance by 1.67 ± 0.07 M Ω (n=12) (Fig. 28Ab). More interestingly, QX-314 reduced the depressant effect of SS on the fast IPSP (response in SS as a percentage of control: 94.15 ± 5.5 , n=12) (Fig. 28Be). The baclofen-induced hyperpolarization of 1.72 ± 0.08 mV (n=8) and decrease in the input resistance by 1.88 ± 0.07 M Ω (n=8) were also much smaller during the late stage of recording in QX-314-injected cells (Fig. 28Ac). Baclofen, however, still significantly depressed the fast IPSP (response in baclofen as a percentage of

control: 22.13 ± 1.80 , $n=8$) during the late stage of recording (Fig. 28Bf). In these cells, the effect of SS on the slow IPSP could not be examined because QX-314, alone suppressed the slow IPSP. As reported in literature (Nathan et al., 1990), QX-314 by itself sometimes caused a slight increase in the input resistance and a small depolarization of the neurons. These results demonstrate that QX-314 blocks not only the baclofen-induced hyperpolarization but also the SS-induced hyperpolarization. However, QX-314 reduces the depressant effect of SS, but not of baclofen, on the fast IPSP, suggesting a post-synaptic locus of action for SS on the fast IPSP.

8.2.6. Effects of somatostatin on the induction of LTP

It is apparent that SS interacts with the GABAergic transmission in the CA1 neurons. GABAergic transmission is known to play an important role in modulating the induction of LTP. Experiments were, therefore, performed to examine whether SS affects the induction of LTP of the EPSP.

After stable control intracellular EPSP and field EPSP were obtained for 20 to 30 min, tetanic stimulation was given to the stratum radiatum during an application of SS ($2 \mu\text{M}$ for 2 min). The post-tetanus responses were monitored for 30-60 min. Two protocols of tetanic stimulations (2 trains of 100 Hz for 1 s, 20 s interval; 100 Hz for 0.5 s) were used in these experiments.

Since SS can induce a hyperpolarization of the CA1 neurons, the first set of experiments was conducted to determine whether SS blocks the induction of LTP by hyperpolarizing the neurons. In 6 slices, tetanic stimulation (2 trains of 100 Hz for 1 s, 20 s interval) in the presence of SS induced LTP of the field EPSP (field EPSP slope 30 min post-tetanus as a percentage of control: 190.75 ± 20.37 , $n=6$) (Fig. 29). This LTP was not significantly different from the LTP (EPSP slope 30 min post-tetanus as a percentage of control: 174.00 ± 12.53 , $n=6$) obtained in control experiments in which the tetanic stimulation was given

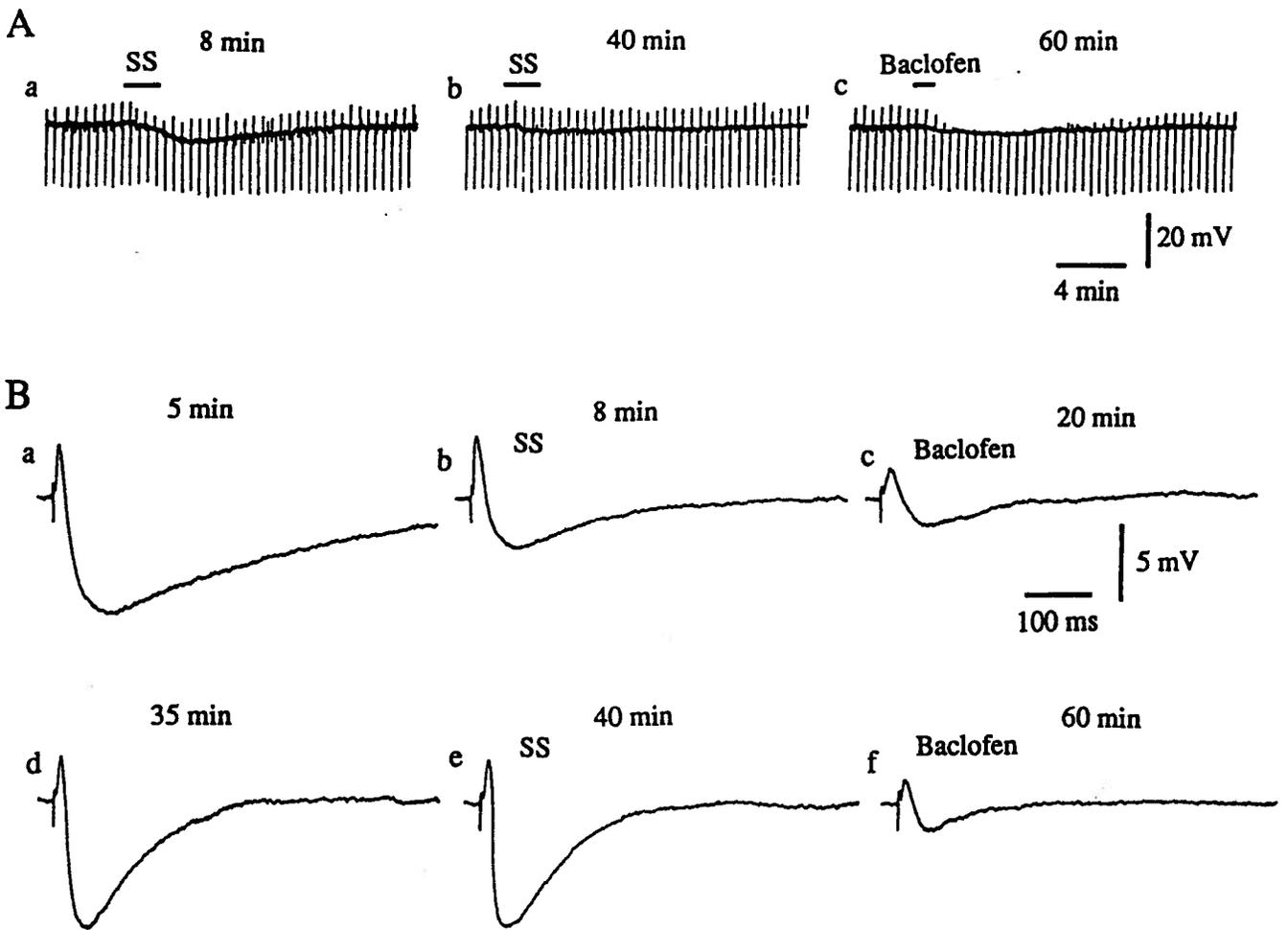


Figure 28 Effects of QX-314 on the actions of somatostatin

Figure 28 Effects of QX-314 on the actions of somatostatin.

The responses were recorded with a QX-314 (100 mM)-containing recording electrode. QX-314 was passively diffused into the cell through the recording electrode. It usually took 20-30 min to observe the blocking effects of QX-314 on the Na⁺ spike and the slow IPSP. **Aa**: the hyperpolarizing action of somatostatin (2 μM) was intact 8 min after the penetration of the neuron (when the Na⁺ spike and the slow IPSP were not blocked by QX-314). **Ab**: somatostatin was applied 40 min after the penetration of the cell when the Na⁺ spikes were blocked and the slow IPSP was suppressed by QX-314; Note that the hyperpolarizing action and the change in the input resistance caused by SS were greatly attenuated. **Ac**: the actions of baclofen (20 μM) 60 min after the penetration of the neuron. Note that baclofen produced little hyperpolarization of this neuron although it suppressed the synaptic transient (by activating the presynaptic GABA_B receptors?). The resting membrane potential of the neuron in **A** was -60 mV. **B** was taken from a different neuron recorded with a QX-314-filled electrode. Somatostatin (**Bb**) and baclofen (**Bc**) caused a depression of the synaptic transients during the early stages of recording. However, during the late stages of recording, baclofen (**Bf**) but not somatostatin (**Be**) continued to depress the EPSP and the fast IPSP. **Bb** and **Bc** were taken when the membrane potential of the neuron was current-clamped at the control level (-56 mV). In **Be** and **Bf**, the drugs did not significantly change the membrane potential. QX-314 started to suppress the action potential of the neuron in **B** 25 min after the penetration of the cell. The resting membrane potential of the neuron in **B** was -56 mV. The resting membrane potentials of the neurons in **A** & **B** were not significantly changed by QX-314.

without SS. Similar results were obtained for intracellularly recorded EPSPs. Tetanic stimulation (2 trains of 100 Hz for 1s, 20 s interval) in the presence of SS caused LTP of the EPSP (intracellular EPSP slope 30 min post-tetanus as a percentage of control: 162.48 ± 13.47 , $n=7$) (Fig. 30).

SS not only hyperpolarizes CA1 neurons, but also depresses the fast and slow IPSPs in these neurons. The blockade of the fast IPSPs facilitates the induction of LTP of the EPSP (Wigstrom and Gustafsson, 1983). In the experiments performed by Wigstrom and Gustafsson (1983), a weak tetanus was used to induce a short-term potentiation (of approximately 5 min duration) under normal conditions. However, this weak tetanus caused LTP when the GABA_A receptor-mediated inhibition was blocked by picrotoxinin (Wigstrom and Gustafsson, 1983). Similar protocol was used in the present study to determine whether SS can facilitate the induction of LTP. A weak tetanus (100 Hz, 0.5 s) was given in the presence of SS. During the application of SS, the membrane potentials of the CA1 neurons were current-clamped to control levels. Under this condition, this weak tetanus did not induce LTP but caused STP (intracellular EPSP slope 30 min post-tetanus as a percentage of control: 97.5 ± 8.02 , $n=6$) (Fig. 31). This STP was not different from the STP (98.20 ± 4.25 , $n=6$) obtained in the absence of SS. It, therefore, appears that SS does not facilitate the induction of LTP of the EPSP.

These present studies on SS indicate that the peptide does not have significant effects on the induction of LTP, but interacts with GABAergic inhibition.

8.3. LTP and endogenous substances

Stimulation of afferents when paired with the depolarization of postsynaptic cells induces LTP of the EPSP in CA1 neurons (Sastry et al., 1986; Kelso et al., 1986; Wigstrom et al., 1986). It is believed that the induction of LTP

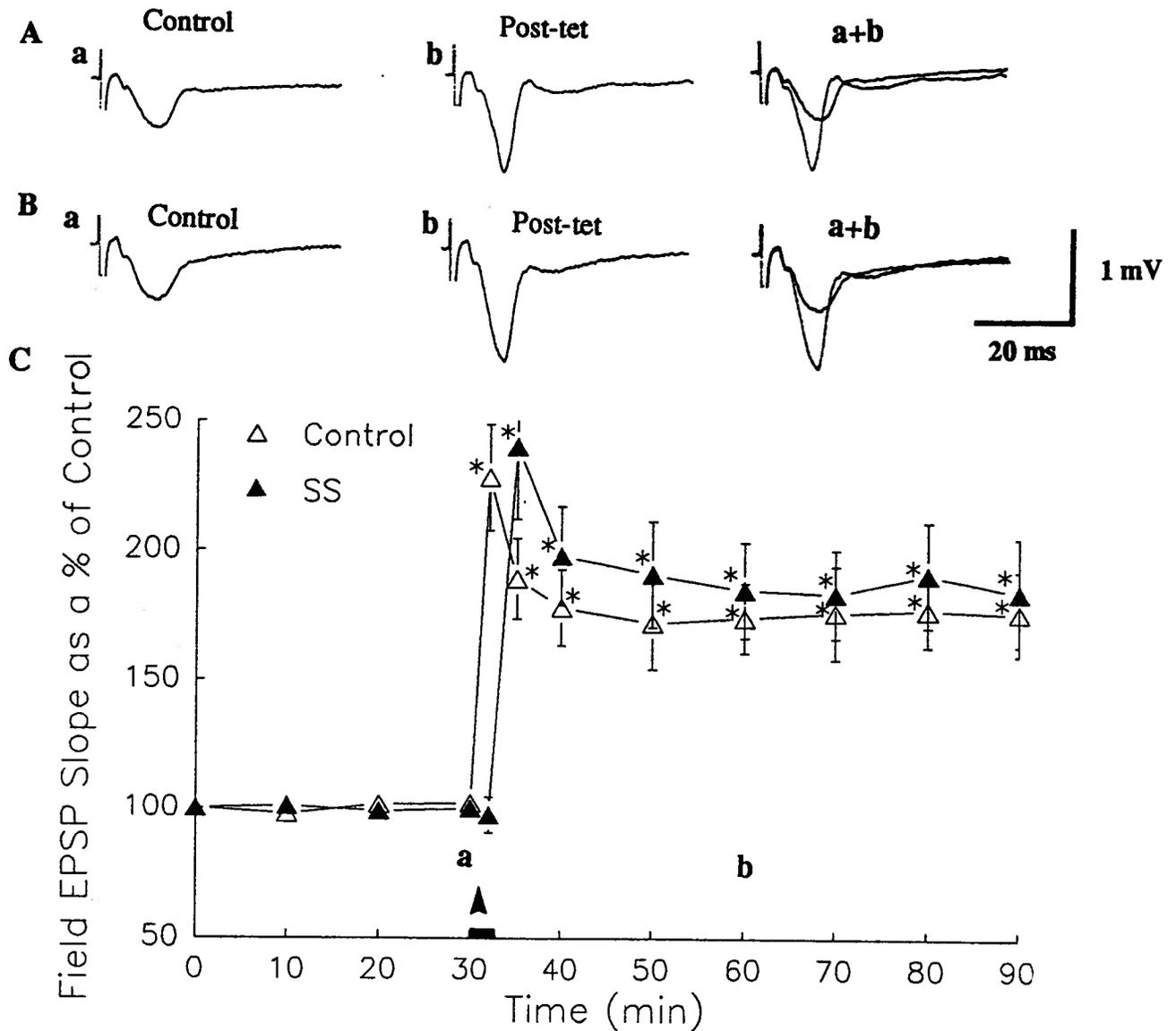


Figure 29 Effects of somatostatin on LTP of extracellular EPSPs.

In **A**, LTP of the field EPSP was caused by tetanic stimulation without the presence of somatostatin. In **B**, LTP of the field EPSP was induced by tetanic stimulation in the presence of somatostatin ($2 \mu\text{M}$, 2 min). Post-tetanic records in **A** & **B** were taken 30 min after tetanic stimulation. In **C**, LTPs of the field EPSP induced by tetanic stimulation (arrow) in the absence and presence of somatostatin ($2 \mu\text{M}$, 2 min, the short bar above abscissa) were shown. Note that tetanic stimulation in the presence of somatostatin induced a LTP of field EPSP which was not significantly different from the LTP induced by tetanic stimulation without the presence of somatostatin.

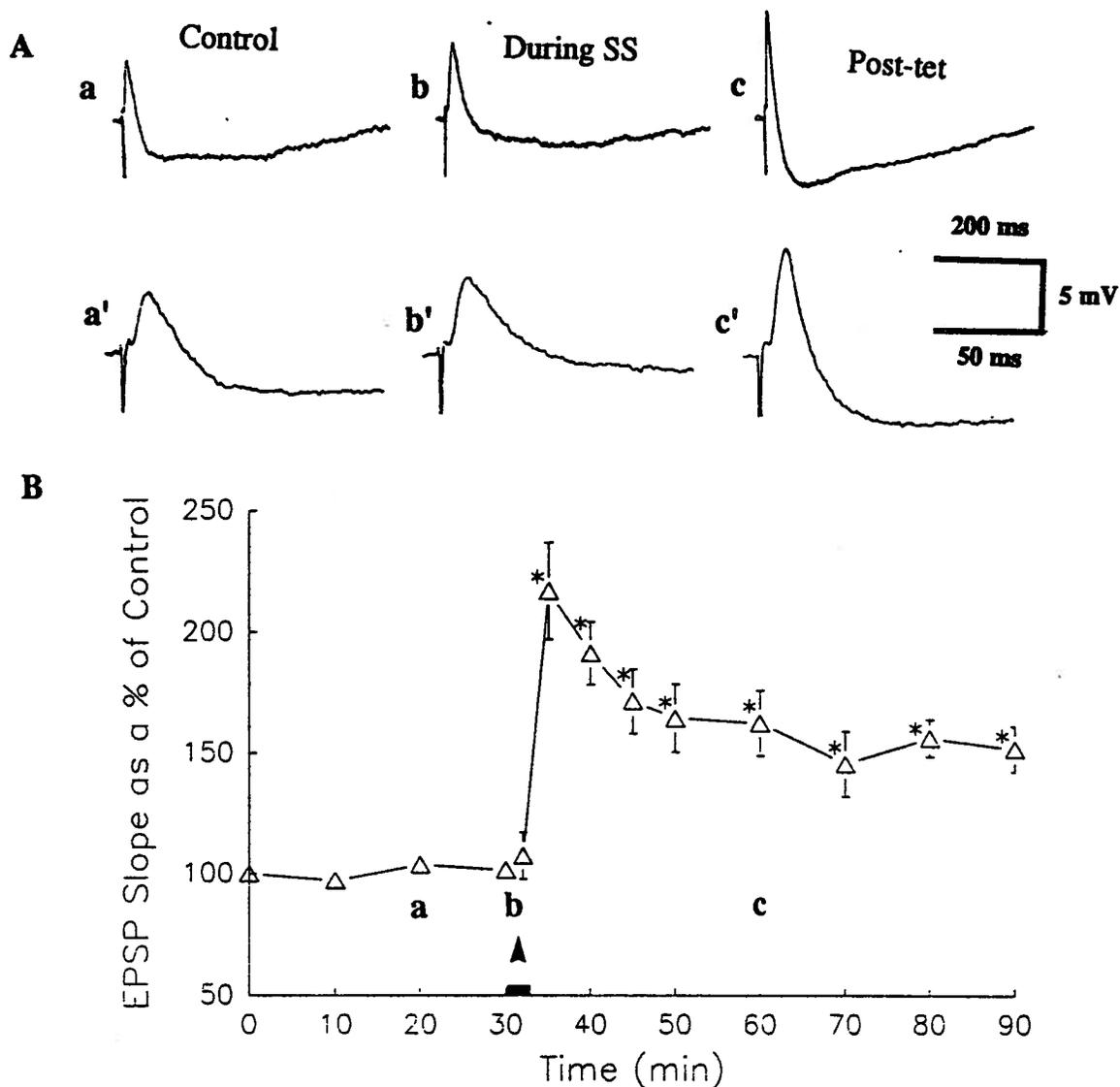


Figure 30 Induction of LTP of intracellular EPSP in the presence of somatostatin. Effects of somatostatin ($2 \mu\text{M}$) on the induction of LTP of intracellular EPSP are shown in **A** and **B**. In **A**, the records obtained from one experiment are shown in fast (**a**, **b** and **c**) and slow (**a'**, **b'** and **c'**) recording speed. Post-tetanic records were taken 30 min after tetanic stimulation. In **B**, data obtained from 8 neurons are shown in graphs. Note that somatostatin induced a hyperpolarization, a reduction in the input resistance and a suppression of the IPSPs in the CA1 neurons. Tetanic stimulation given during these actions of somatostatin induced LTP of intracellular EPSP which was not significantly different from the control LTP. In three of eight neurons, tetanic stimulation was given when the membrane potential was clamped back to the pre-application level. Since results obtained from these three cells were not different from those obtained from the other five cells, data were pooled together to plot the graph. The resting membrane potential of the neuron shown in **A** was -64 mV .

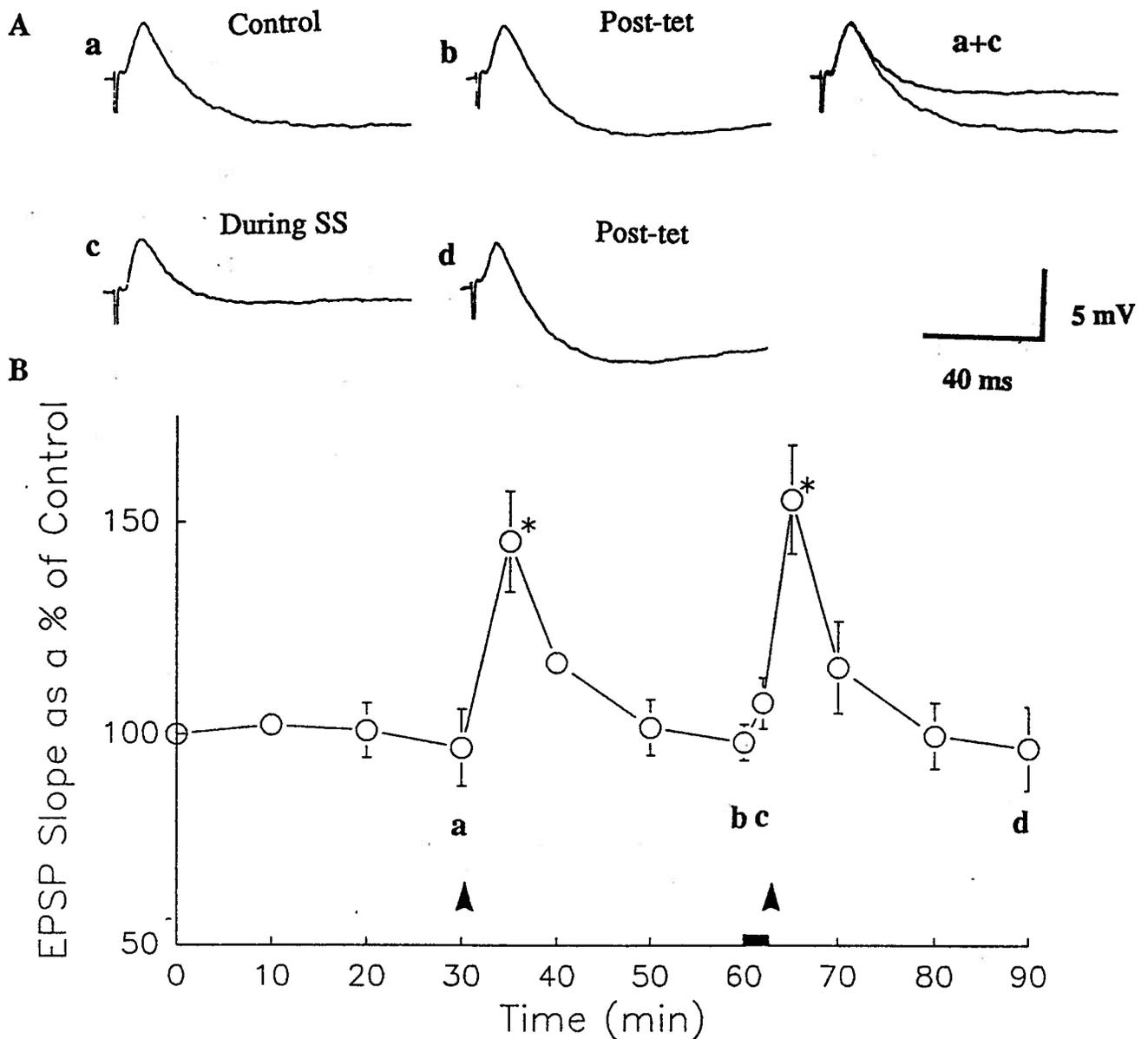


Figure 31 Effects of weak tetanic stimulation on EPSP in the presence of somatostatin

Effects of weak tetanic stimulation (arrow, 100 Hz, 0.5 s) on the EPSP in the absence and the presence of somatostatin (2 μ M, the short bar above abscissa) are shown in **A** and **B**. Weak tetanic stimulation induced STP but not LTP. After the EPSP returned to the control level, weak tetanic stimulation was given again in the presence of somatostatin. **A** shows the records of one experiment, and **B** shows data obtained from 6 neurons. Note that weak tetanic stimulation in the presence of somatostatin also induced STP only. During weak tetanic stimulation in the presence of somatostatin, the membrane potential was current-clamped back to pre-application level. The resting membrane potential of the neuron shown in **A** was -60 mV.

is primarily due to postsynaptic mechanisms. If the maintenance of LTP is presynaptic, it is possible that a release of endogenous substances from the postsynaptic cells can occur during the depolarization. The released substances may then affect the presynaptic terminals leading to changes that cause LTP. That endogenous substances released from the postsynaptic cells act as retrograde messengers in influencing the presynaptic terminals to cause LTP was suggested by Sastry et al. (1986) and Bliss et al. (1986).

Previous studies conducted in our laboratory showed that endogenous substances collected during, but not without, a tetanic stimulation in the guinea pig hippocampus or the rabbit neocortical surface caused LTP of the CA1 population spike in guinea pig hippocampal slices (Sastry et al., 1988a; Chirwa and Sastry, 1986). These substances not only induced LTP but also enhanced neurite growth in cultured PC-12 cells (Sastry et al., 1988a). The present studies were undertaken to characterize the LTP-inducing substances released during tetanic stimulation and to determine whether these substances act as retrograde messengers for LTP.

8.3.1. LTP-inducing action of the endogenous substances

The collection of endogenous substances is described in Chapter 7 (Methods and Materials). The endogenous substances were obtained from both guinea pig hippocampus and rabbit neocortex during tetanic stimulation (Chirwa and Sastry, 1986; Sastry et al., 1988a). However, it was difficult to obtain high concentrations of these endogenous substances from the guinea pig hippocampus. LTP has been shown to occur in the neocortex (Lee, 1982) and the surface of the cortex is accessible for sample collection. Therefore, the endogenous samples obtained from rabbit neocortex were used in the present study. The three types of samples collected from rabbit neocortex were as follows: (1) Tetanized neocortical sample (TNS, which was collected during a

tetanic stimulation); (2) Untetanzed neocortical sample (UNS, collected without a tetanic stimulation); (3) Untetanzed neocortical sample collected after, but not during, a tetanus (TUNS).

Application of TNS (2 ml) resulted in LTP of the stratum radiatum stimulation-induced CA1 population spike (n=16) while application of the same amount of UNS failed to cause any significant change in the population spike (n=16, Fig. 32A & B; Fig. 33). These results were consistent with the previous report from our laboratory (Sastry et al., 1988a). Studies from other laboratories showed that during LTP (some times after a tetanic stimulation), peptides or proteins were released into the extracellular fluid (Charriault-Marlangue et al., 1988; Duffy et al., 1981). Whether any LTP-inducing substances were released after a tetanic stimulation was examined in the present experiments. The samples (TUNS) were collected after a tetanic stimulation of the neocortex. Presumably, TUNS was collected during LTP that was induced in the neocortex by the previous tetanus. When TUNS (2 ml) was applied on the guinea pig hippocampal slices, no subsequent LTP could be observed (n=5, Fig. 32C). In the same slices, however, a tetanic stimulation clearly caused a subsequent potentiation of the population spike. Therefore, it appears that the release of the LTP-inducing substances from the neocortex occurs during, but not after, a tetanic stimulation of the cortical surface.

In order to check whether LTPs induced by TNS application and tetanic stimulation of hippocampal afferents are additive, TNS was applied during an established tetanus-induced LTP of the CA1 population spike. In this experiment, TNS clearly failed to further potentiate the population spike (n=6, Fig. 32D). The failure of TNS to further potentiate the population spike could not be due to a saturation of the response because a twin pulse stimulation (with a 30 ms delay) clearly showed an enhanced second population spike when tested

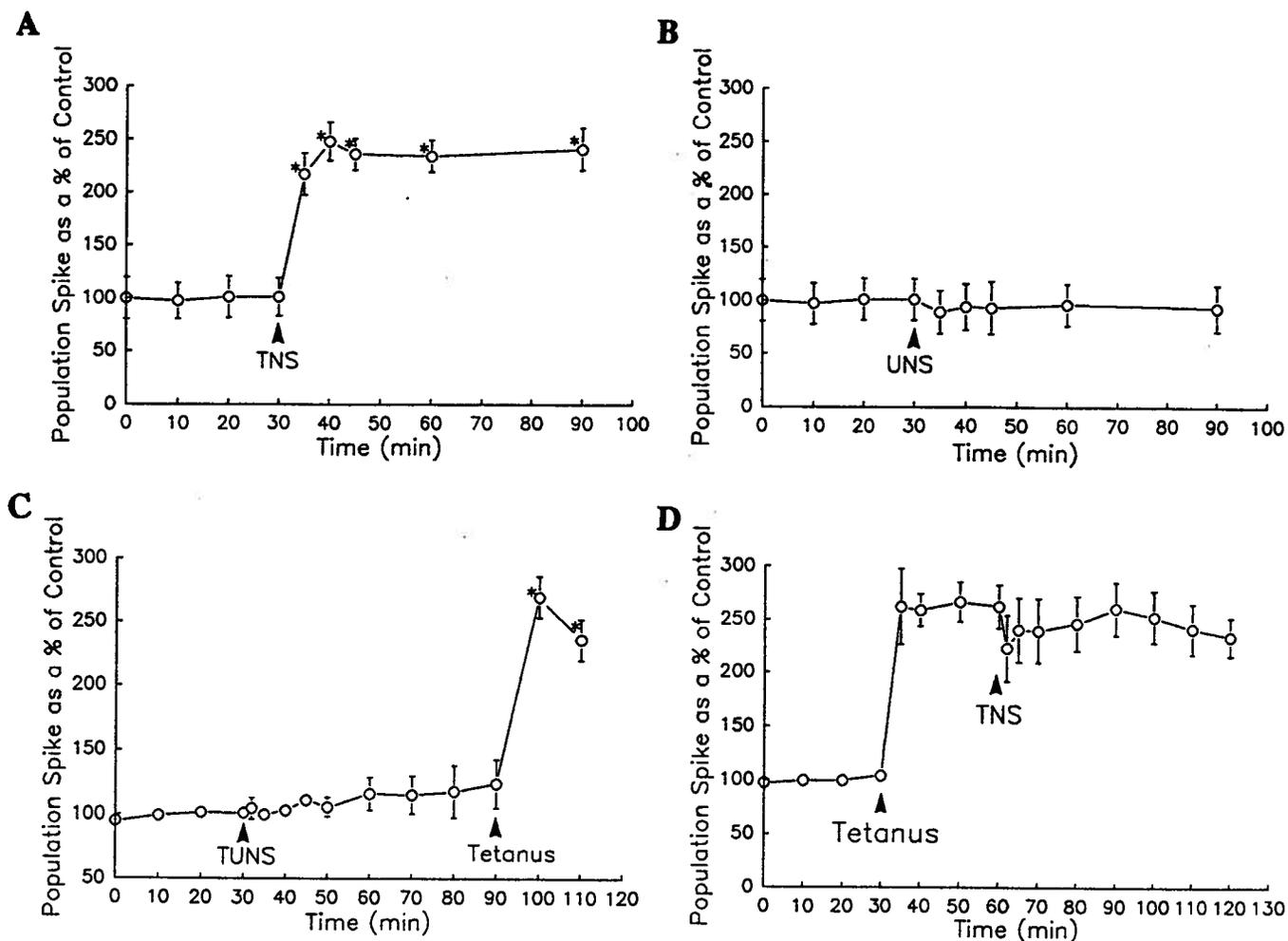


Figure 32 Effects of substances collected from rabbit neocortical surface on guinea pig hippocampal CA1 population spike

Samples were collected from the rabbit neocortex without a tetanic stimulation (untetanzed neocortical sample, UNS), during a tetanic stimulation (50 Hz, 5 s) (tetanized neocortical sample, TNS), and subsequent to (but not during) a single tetanic stimulation (untetanzed neocortical sample collected after a tetanus, TUNS). The actions of the application on the hippocampal slice of UNS (n=16), TNS (n=16) or TUNS (n=5) (2 ml each) on the population spike are illustrated in **A**, **B** and **C**, respectively. Note that TNS, but not UNS and TUNS, induced LTP. In **C**, a tetanic stimulation (tetanus) 60 min after the application of TUNS caused a post-tetanic potentiation of the population spike. In **D**, TNS was applied 30 min after the induction of LTP by tetanic stimulation (tetanus, 100 Hz, 5s). In this experiment, TNS failed to further potentiate the population spike during the established LTP. n represents the number of slices. Experiments involving TNS, UNS and TUNS in this figure and subsequent figures were conducted at 30-32 °C.

during the established LTP. Similarly, tetanic stimulation did not induce further potentiation of the population spike during the TNS-induced LTP (n=4). These results suggest that the LTP induced by tetanic stimulation of the hippocampal afferents and that induced by the application of TNS share some common mechanisms.

In order to determine whether the induction of LTP produced by TNS requires presynaptic activity, stimulation of the afferents was stopped during the application of TNS. Stimulation was resumed immediately, 2 min or 7 min after the application of TNS. When stimulation was resumed immediately or 2 min after the application of TNS, TNS could still induce LTP of the EPSP (n=4, each). However, TNS did not induce LTP when stimulation was resumed 7 min after the application (n=4). Since it is unclear as to when TNS was completely washed out, there may be two explanations for the results. One is that the LTP-inducing action of TNS is not activity-dependent because LTP was induced by TNS even when stimulation of the afferents was stopped during the application. The other is that the LTP-inducing action of TNS is activity-dependent. It is very likely that TNS requires more than 2 min to be completely washed out. Therefore, when stimulation was resumed immediately or within 2 min after the application, some TNS was still present in the perfusing chamber. Seven min after the application, TNS was completely washed out and resumption of stimulation could not induce LTP. The second explanation seems to be more reasonable because the discontinuation of stimulation for 7 min should not prevent LTP produced by TNS if this LTP involved only postsynaptic actions.

From the extracellular studies, it was not clear whether the potentiation of the population spike was, in fact, due to an enhancement in synaptic transmission and not due to some change in the CA1 neuronal excitability. It was therefore decided to examine the effects of TNS on intracellular

postsynaptic potentials. During the application of TNS, there was no significant change in the membrane potential or the input resistance of the CA1 neurons (n=6, data not shown). Following the application of TNS, however, both the slope and the height of intracellular EPSP were increased (n=10) and the increases were maintained until the recordings were terminated, suggesting that LTP occurred (Fig. 33, 34 & 35A). The effects of TNS on IPSPs were also examined. Application of TNS did not induce long-term enhancement of the fast and slow IPSPs. However, TNS induced a short-term potentiation of the fast IPSP without significantly changing the slow IPSP (Fig. 34). The TNS-induced LTP of the EPSP was not associated with any change in the membrane potential or the input resistance of the CA1 neurons (n=6) (Fig. 35 B & C). These results indicate that TNS causes LTP of the EPSP without changing the membrane potential or the input resistance of the CA1 neurons.

8.3.2. *Different fractions of the endogenous substances and LTP*

Different molecular weight proteins (14-86 kDa) have been shown to be released into extracellular fluid during LTP (Duffy et al., 1981; Charriaut-Marlangue et al., 1988). It is therefore decided to determine the ability of different molecular weight fractions of TNS to induce LTP.

TNS was originally separated into three molecular weight fractions: <25 kDa, 25-50 kDa, and >50 kDa (see Chapter 7, Methods and Materials). The effects of individual fractions on the induction of LTP of the population spike were determined. The <25 kDa and >50 kDa, but not the 25-50 kDa, fractions induced LTP (n=8 for each fraction) (Fig. 36). The >50 kDa fraction lost its ability to induce LTP if the sample was kept at room temperature for 30-60 min. When the <25 kD fraction was further separated into <3 kDa, 3-10 kDa and 10-25 kDa fractions, the <3 kDa and 3-10 kDa fractions produced a potentiation that became significant only 50 min after the application (Fig. 37). It appears that the

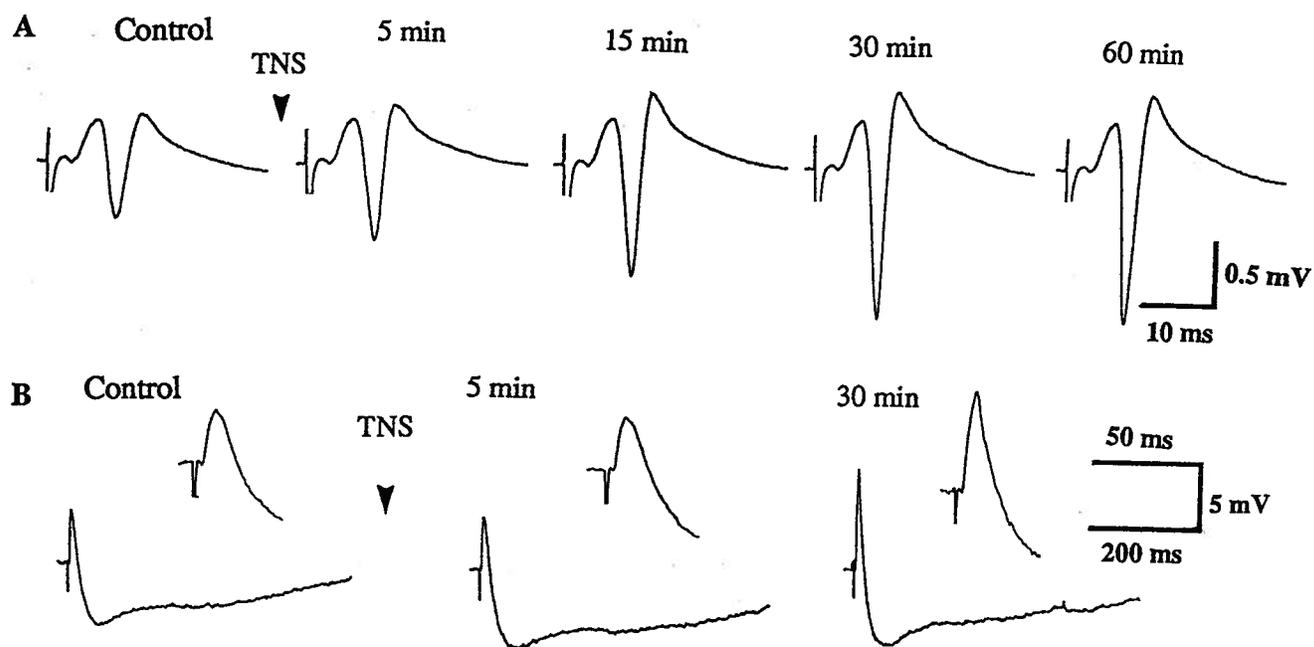


Figure 33 Representative LTP of population spike and intracellular EPSP caused by TNS.

A shows LTP of population spike induced by TNS (arrow). **B** illustrates LTP of intracellular EPSP produced by TNS. Insets show the EPSP recorded with fast recording speed. The resting membrane potential of the neuron in **B** was -62 mV.

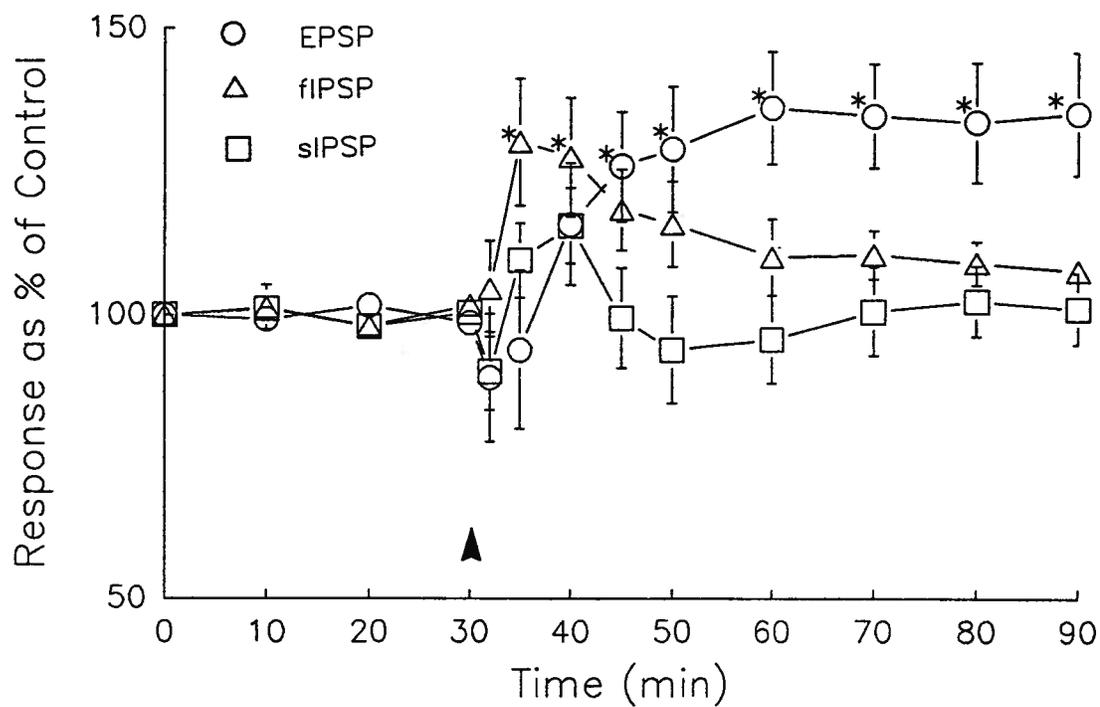


Figure 34 Changes in EPSP, fast and slow IPSP produced by TNS. Data obtained from 10 neurons are shown in graphs. Application of TNS (arrow) caused LTP of the EPSP but not the fast and slow IPSPs. TNS caused STP of the fast IPSP without significantly changing the slow IPSP in CA1 neurons.

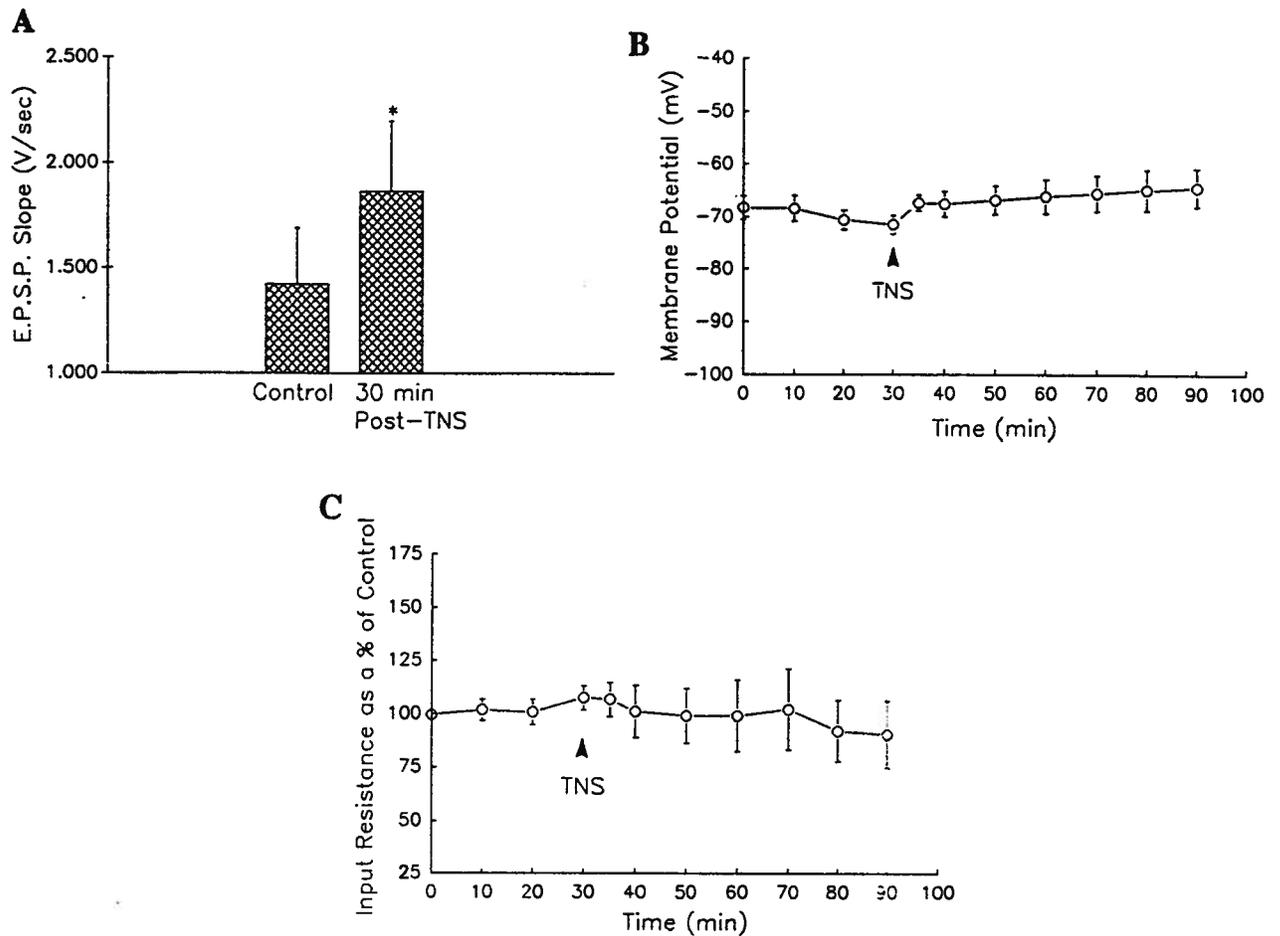


Figure 35 Effects of TNS on the membrane potential, the input resistance and the slope of the EPSP in the CA1 neurons.

The effects of TNS on the slope of the EPSP, the membrane potential and the input resistance are shown in **A**, **B** and **C**, respectively. TNS (arrow) significantly increased the slope of the EPSP without significantly changing the membrane potential and input resistance of the CA1 neurons. Data shown in **A**, **B** & **C** were obtained from 6 neurons.

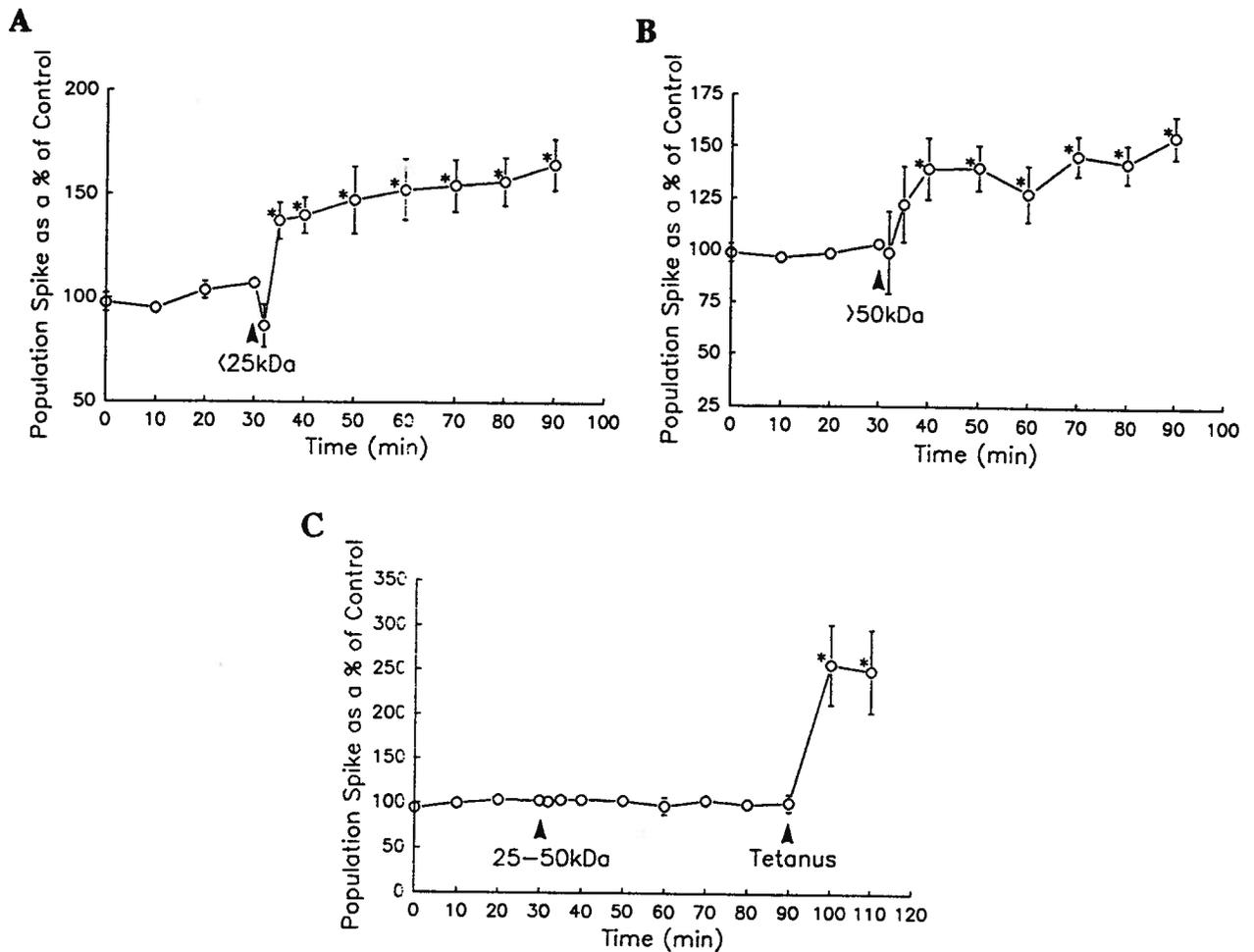


Figure 36 The ability of different fractions of TNS to induce LTP. TNS was separated into three fractions according to molecular weights (<25 kD, 25-50 kD and >50 kDa). The effects of samples containing the <25 kDa (n=8), 25-50 kDa (n=8) or >50 kDa (n=8) fractions (2 ml each) on the population spike are shown in **A**, **B** and **C**, respectively. Note that samples containing the <25 kDa and >50 kDa fractions caused LTP while the sample containing the 25-50 kDa fraction failed to induce LTP.

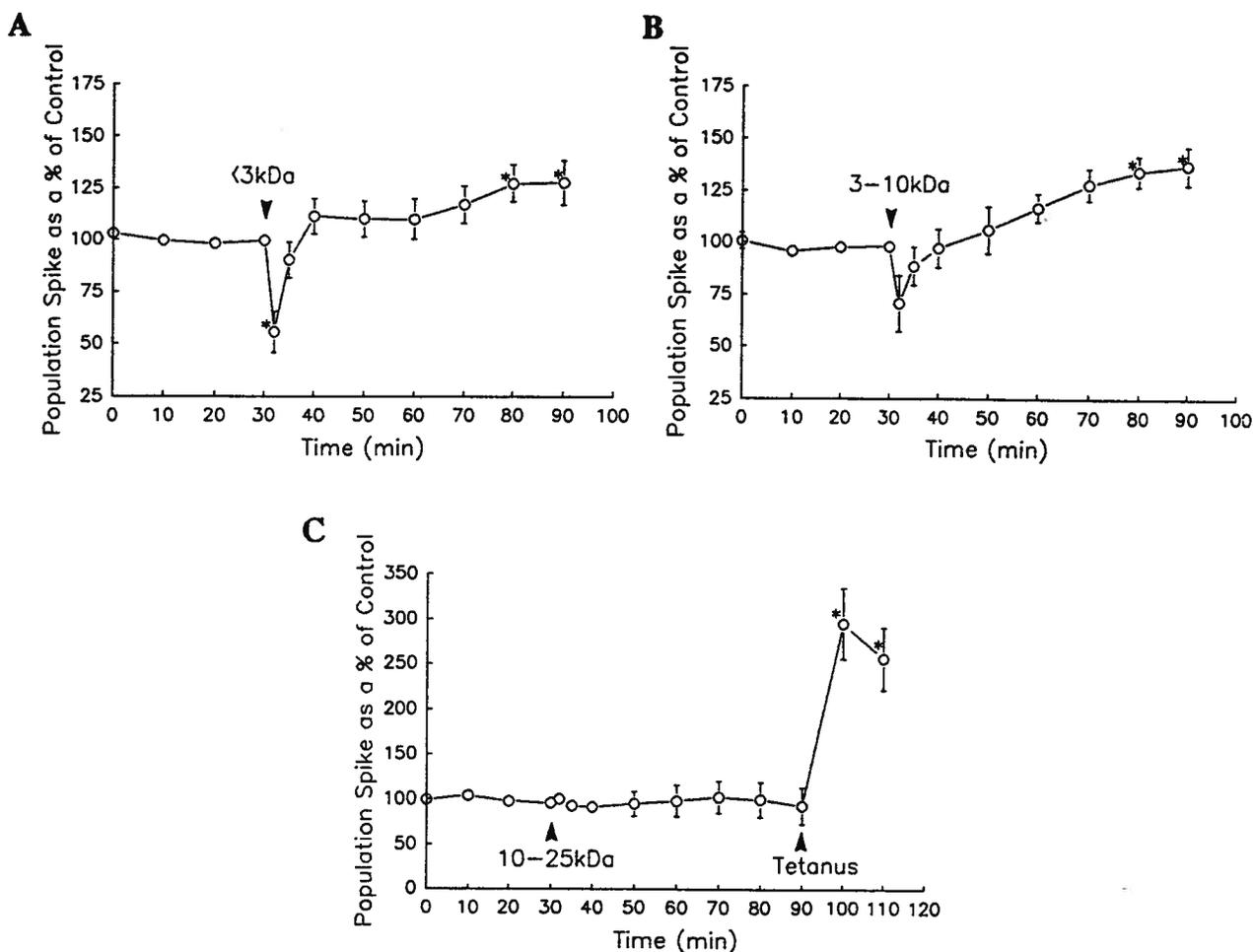


Figure 37 The effects of different fractions of TNS on the population spike. Samples containing the < 25 kDa fraction which had shown the ability to induce LTP (see previous figure) were separated into smaller molecular ranges (< 3 kDa, 3 -10 kDa and 10-25 kDa). The effects of samples containing the <3 kDa (n=10), 3-10 kDa (n=8) and 10-25 kDa (n=8) fractions on the population spike are shown in **A**, **B**, and **C**, respectively. The samples containing the <3 kDa and 3-10 kDa fractions, but not the 10-25 kDa fraction, induced LTP.

Table 6 Effects of various fractions of samples collected from the rabbit neocortical surface on the CA1 population spike in guinea pig hippocampal slices

Fraction	CA1 population spike as a % of control expressed as mean \pm SEM		
	30 min post-application	60 min post-application	No. of slices (n)
<3 kD	110.0 \pm 9.7	127.8 \pm 10.7*	10
3-10 kD	116.9 \pm 6.7	136.9 \pm 9.1*	8
10-25 kD	98.6 \pm 17.6	93.1 \pm 20.5	8
<25 kD	152.5 \pm 14.6*	164.4 \pm 12.3*	8
25-50 kD	97.3 \pm 9.7	101.4 \pm 10.0	8
>50 kD	127.8 \pm 13.4*	155.5 \pm 10.3*	8

Asterisk (*) indicates that the post-tetanus population spike is significantly different from the control population spike at a *P*-value of <0.05 using paired Student's t-test.

substances present in these two fractions when applied together (as in the <25 kDa fraction) produce a more significant potentiation (see Fig. 36). The 10-25 kDa fraction did not induce any potentiation (Fig. 37). These results indicate that the <3 kDa, 3-10 kDa <25 kDa and >50 kDa fractions of TNS induce LTP in hippocampal CA1 region. The effects of different fractions of TNS on the induction of LTP are summarized in Table 6.

8.3.3. Involvement of NMDA receptors in the release of endogenous substances

The induction of LTP in hippocampal slices by tetanic stimulation of the stratum radiatum is blocked by NMDA antagonists (Collingridge et al., 1983). Activation of NMDA receptors has been reported to cause a rapid release of specific proteins ranging from 14 to 66 kDa into the extracellular fluid (Nystrom et al., 1986). In the present study, it was therefore decided to examine whether the release of LTP-inducing substances in TNS required the activation of NMDA receptors. MK-801, a NMDA antagonist which blocks the induction of LTP in the hippocampal slices, was used because this agent can cross the blood-brain barrier (Foster et al., 1987; Gustafsson and Wigstrom, 1988). MK-801 was injected (i.p.) into the rabbit, prior to the collection of TNS (see Chapter 7, Methods and Materials). The <25 kDa and >50 kDa fractions of TNS from the MK-801 pretreated rabbits failed to cause LTP (Fig. 38). The lack of induction of LTP by these samples could not be due to the presence of significant levels of MK-801 in the collected samples because tetanic stimulation of the stratum radiatum in the hippocampal slice during the application of the TNS from the MK-801 pretreated animals still induced LTP (Fig. 38). These results indicate that the release of the LTP-inducing substances from the rabbit neocortex involves the activation of NMDA receptors.

8.3.4. Possible mechanisms of the action of LTP-inducing substances

8.3.4.1. NMDA receptors and the action of LTP-inducing substances

APV, a NMDA antagonist, is known to block the tetanus-induced LTP (Collingridge et al., 1983). It was decided to examine whether APV also affected the TNS-induced LTP. When TNS was applied in the presence of APV (40 μ M), APV failed to block the induction of LTP produced by the <3 kDa, 3-10 kDa and >50 kDa fractions (Fig. 39). In fact, the <3 kDa and 3-10 kDa fractions of TNS produced greater LTP in the presence of APV. In control experiments APV (40 μ M), applied for the same duration, clearly prevented LTP induced by tetanic stimulation (n=6). It thus appears that the LTP-inducing action of the substances in TNS does not require the activation of NMDA receptors.

8.3.4.2. ACPD receptors and the action of LTP-inducing substances

ACPD receptors have been reported to be involved in LTP (Otani and Ben-Ari, 1991; Bortolotto and Collingridge, 1992, 1993). The exact role of ACPD receptors in LTP is not very clear because of the lack of selective ACPD antagonists. L-AP3, an ACPD antagonist, has been reported to block the induction of LTP in the hippocampus (Izumi et al., 1991; Behnisch et al., 1991). Whether L-AP3 has effects on the LTP-inducing action of TNS substances was examined in the present experiments. L-AP3 (100 μ M) was applied for 20 min. When TNS was applied during the application of L-AP3, TNS could still induce LTP (n=6) (Fig. 40). This dose of AP3, however, blocked the LTP produced by tetanic stimulation (n=6). These results suggest that L-AP3 does not block the action of LTP-inducing substances in TNS.

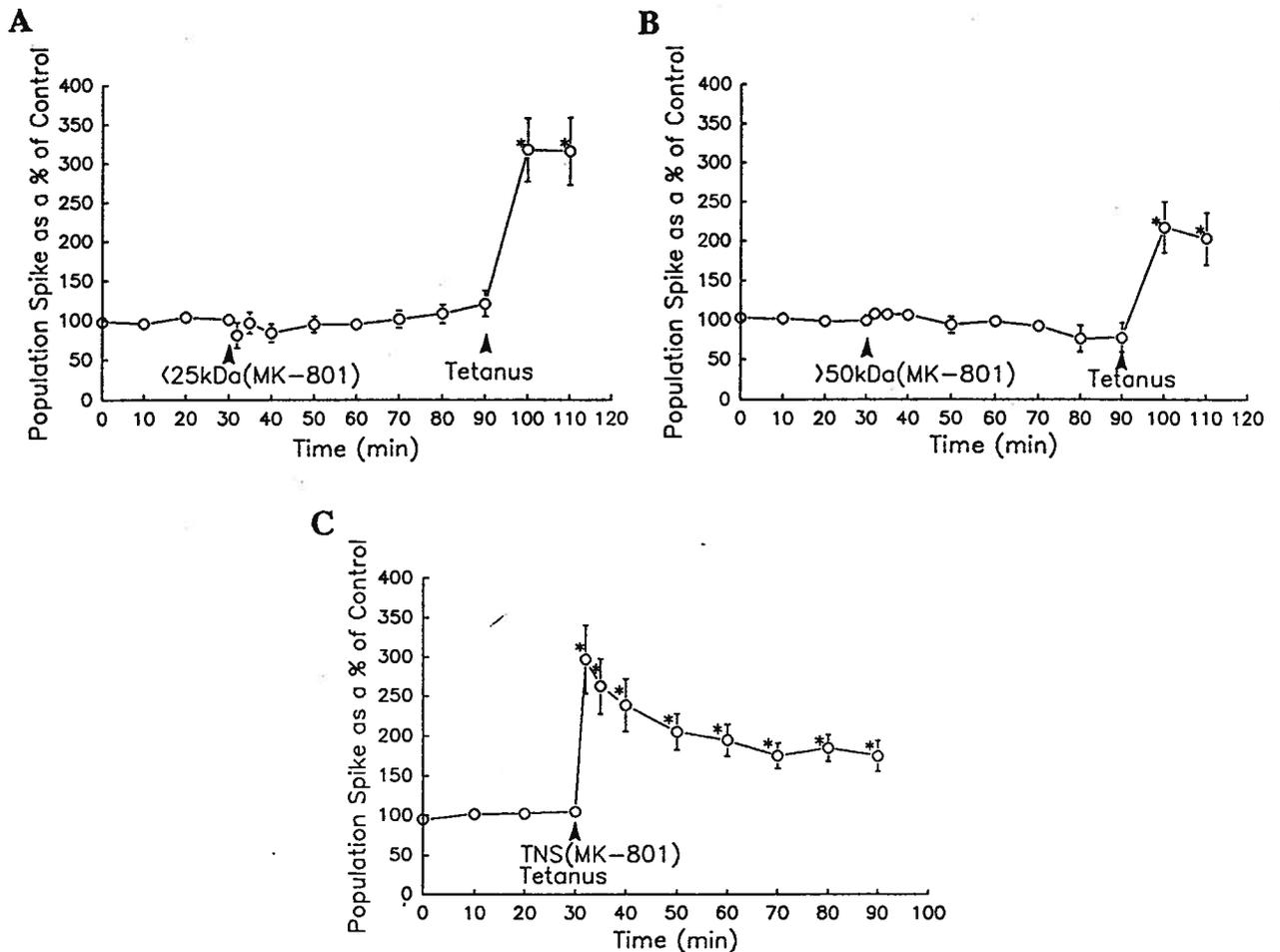


Figure 38 Effects of TNS collected from rabbit pretreated with MK-801. Samples containing the <25 kDa and the >50 kDa fractions of TNS, collected from drug-free rabbit, had shown their ability to induce LTP. The effects of the samples, collected from rabbits pre-treated with MK-801, containing the < 25 kDa (n=6) and >50 kDa (n=5) fractions are shown in **A** and **B**, respectively. These samples failed to induce LTP. In **C**, stratum radiatum in the hippocampal slice was tetanized during the last 10 sec of a 2 min application of TNS (n=6), collected from rabbits pretreated with MK-801. Note that LTP could still be induced, suggesting that the MK-801 in the TNS sample itself was inadequate to block LTP.

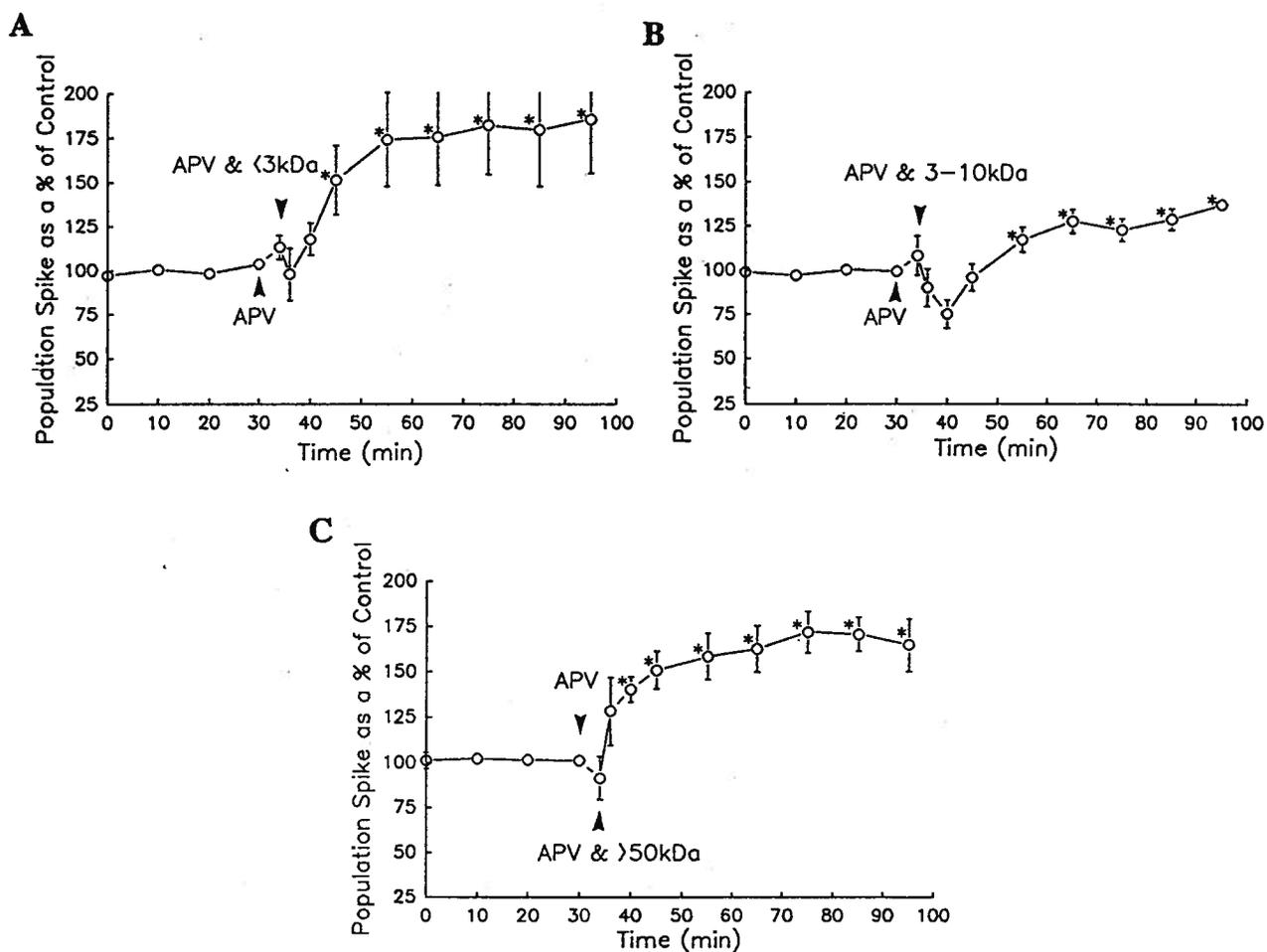


Figure 39 Effects of APV on the induction of LTP by different molecular weight fractions of TNS.

In this experiment, each of these samples contained 40 μ M APV. The hippocampal slices were superfused with APV for 4 min prior to the application of each fraction of TNS. The effects of the <3 kDa, 3-10 kDa and >50 kDa fractions in the presence of APV are shown in A, B and C, respectively. In all cases, APV did not have any significant blocking effect on the induction of LTP by these samples.

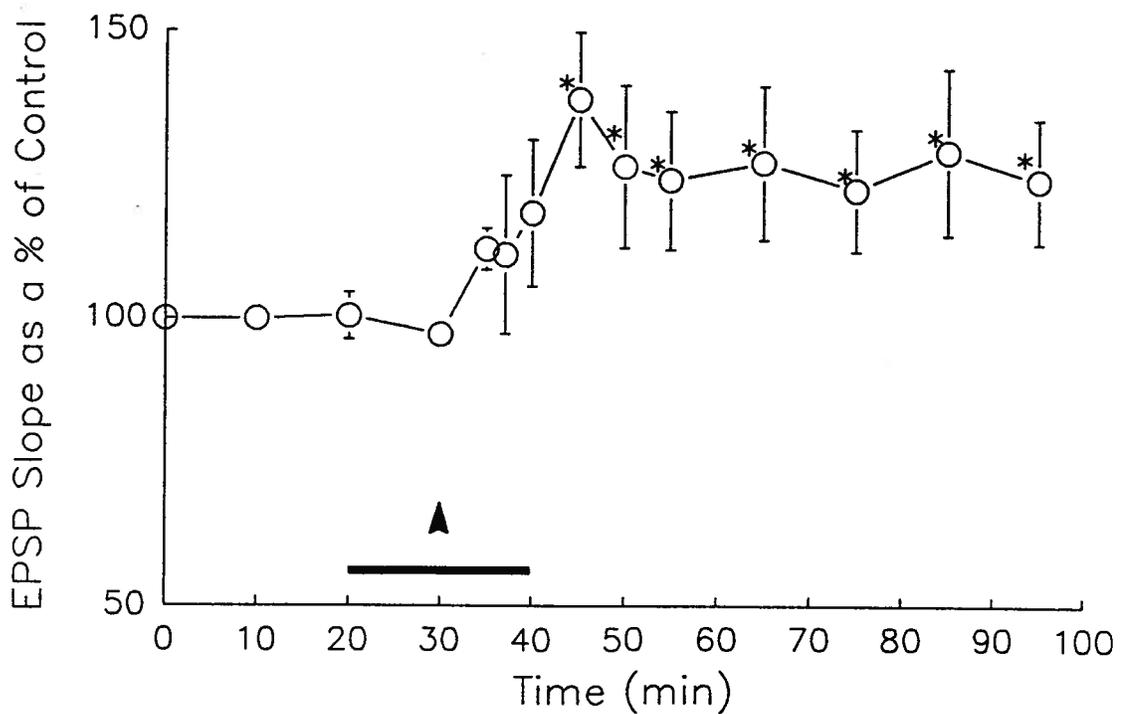


Figure 40 Effects of AP3 on the induction of LTP produced by TNS. TNS (arrow) was given during the application of L-AP3 (100 μ M, 20 min, the horizontal bar above the abscissa). Note that L-AP3 did not affect the induction of LTP of the EPSP produced by TNS. L-AP3 alone did not cause any significant change on the EPSP.

8.3.4.3. Protein kinase C and the action of LTP-inducing substances

It is believed that protein kinase C (PKC) activity increases during the induction of LTP (Akers et al., 1986). PKC inhibitors such as H-7, sphingosine, K-252b have been reported to block the induction of LTP (Lovinger et al., 1987; Malinow et al., 1988; 1989). It was therefore decided to examine whether PKC inhibitors such as sphingosine and K-252b prevent the induction of LTP produced by TNS. Sphingosine (30 μ M) was applied for 25 min. When TNS was applied during the application of sphingosine, TNS did not induce LTP (Fig. 41A). When K-252b was injected into the postsynaptic CA1 neurons, the induction of LTP produced by TNS was again prevented (Fig. 41B). These results suggest that the LTP-inducing action of the substances involve the activation of PKC.

8.3.4.4. Ca²⁺ and the action of LTP-inducing substances

Postsynaptic free Ca²⁺ seems to play a crucial role in the induction of LTP (Lynch et al., 1983). Whether the LTP-inducing action of TNS requires postsynaptic free Ca²⁺ was examined in the present experiments. BAPTA, a Ca²⁺ chelator, was injected into the CA1 neurons through the recording electrodes (see Chapter 7, Methods and Materials). Application of TNS did not induce LTP of the EPSP in BAPTA-injected neurons (n=6) (Fig. 42). In some of these neurons, TNS caused a small depression of the EPSP that lasted for 5-10 min. It therefore appears that postsynaptic intracellular free Ca²⁺ is required for the LTP-inducing action of the substances.

8.3.5. Gel electrophoresis of the endogenous substances

Proteins ranging from 14 to 86 kDa have been reported to be released into the extracellular fluid during LTP (Duffy et al., 1981; Charriault-Marlangue et al., 1988) or during depolarization of the neurons. It was therefore decided to

examine whether the endogenous substances collected from rabbit cortex during tetanic stimulation contained released proteins using gel electrophoresis. The 1-D (n=8) and 2-D (n=4) gel electrophoresis of the endogenous substances revealed that certain proteins with molecular weights >50 kDa were present in the TNS that were either absent or were present in much lower concentrations in the UNS (Fig. 43). A protein with a molecular weight of about 69 kDa was consistently seen in the TNS but not in the UNS. The 2-D gels revealed that the proteins that were increased in TNS were acidic. Although TNS contained a higher concentration of lower molecular weight peptides than UNS (Fig. 43), the resolution of the gels used in this study was not adequate to accurately estimate their molecular weights.

8.4. LTP and α -Tocopherol

α -Tocopherol (vitamin E), as an integral part of the cell membrane, is thought to be essential for the maintenance of normal structure and function of the human nervous system (Sokol, 1988; 1989; Tappel, 1962). α -Tocopherol is a major lipid-soluble antioxidant in the biological system, which can scavenge free radicals attacking from outside of the membrane and within the cell membrane (Tappel, 1962). Recently, this vitamin has been suggested to be involved in spatial learning and memory in animals (Moriyama et al., 1990). In vitamin E deficient rats, impairment of spatial learning ability is associated with an increase in lipid peroxide contents of the hippocampus. It, therefore, appears that α -tocopherol and free radicals have a role in certain types of learning and memory. Free radicals have been found to facilitate the decay of LTP (Pellmar et al., 1991). It was therefore decided to examine whether α -tocopherol induces LTP.

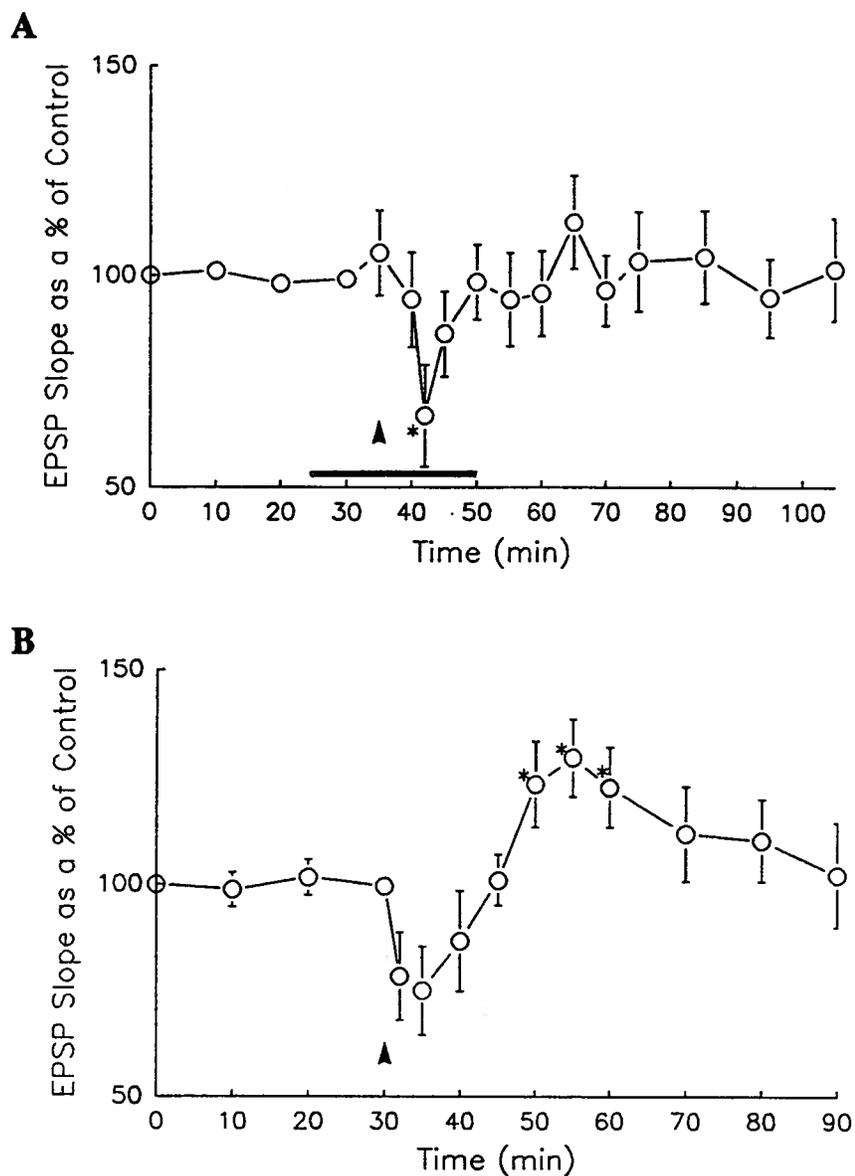


Figure 41 Involvement of protein kinase C in LTP induced by TNS. Effects of protein kinase C (PKC) inhibitors, K-252b and sphingosine, on the induction of LTP by TNS are shown in **A** and **B**. In **A**, TNS (arrow) was given in the presence of sphingosine (30 μ M, the long horizontal bar above abscissa) (n=6). Note that TNS failed to induce LTP of the EPSP in the presence of sphingosine. In **B**, K-252b was injected into the postsynaptic neurons through the recording electrodes. In K-252b-injected neurons, TNS (arrow) did not cause LTP of the EPSP (n=6).

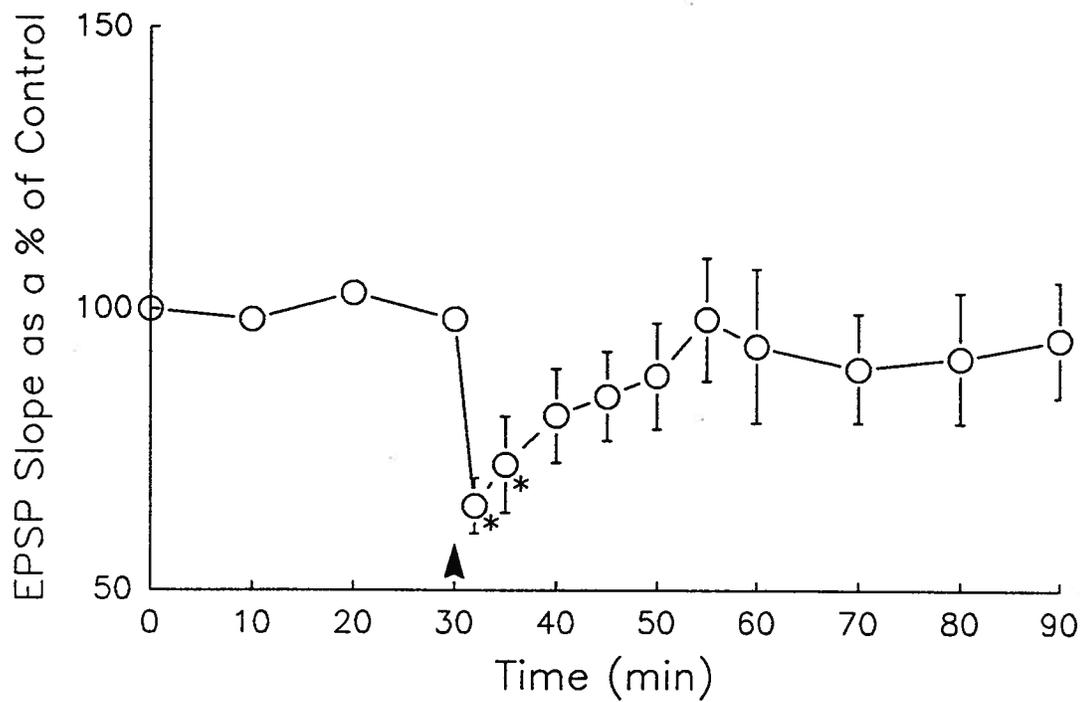


Figure 42 The role of postsynaptic Ca^{2+} in LTP produced by TNS. BAPTA, a Ca^{2+} chelator, was injected into postsynaptic cells through recording electrodes. Effects of TNS (arrow) on the EPSP in neurons injected with BAPTA are illustrated ($n=8$). Note that the application of TNS did not induce LTP of the EPSP in neurons injected with BAPTA.

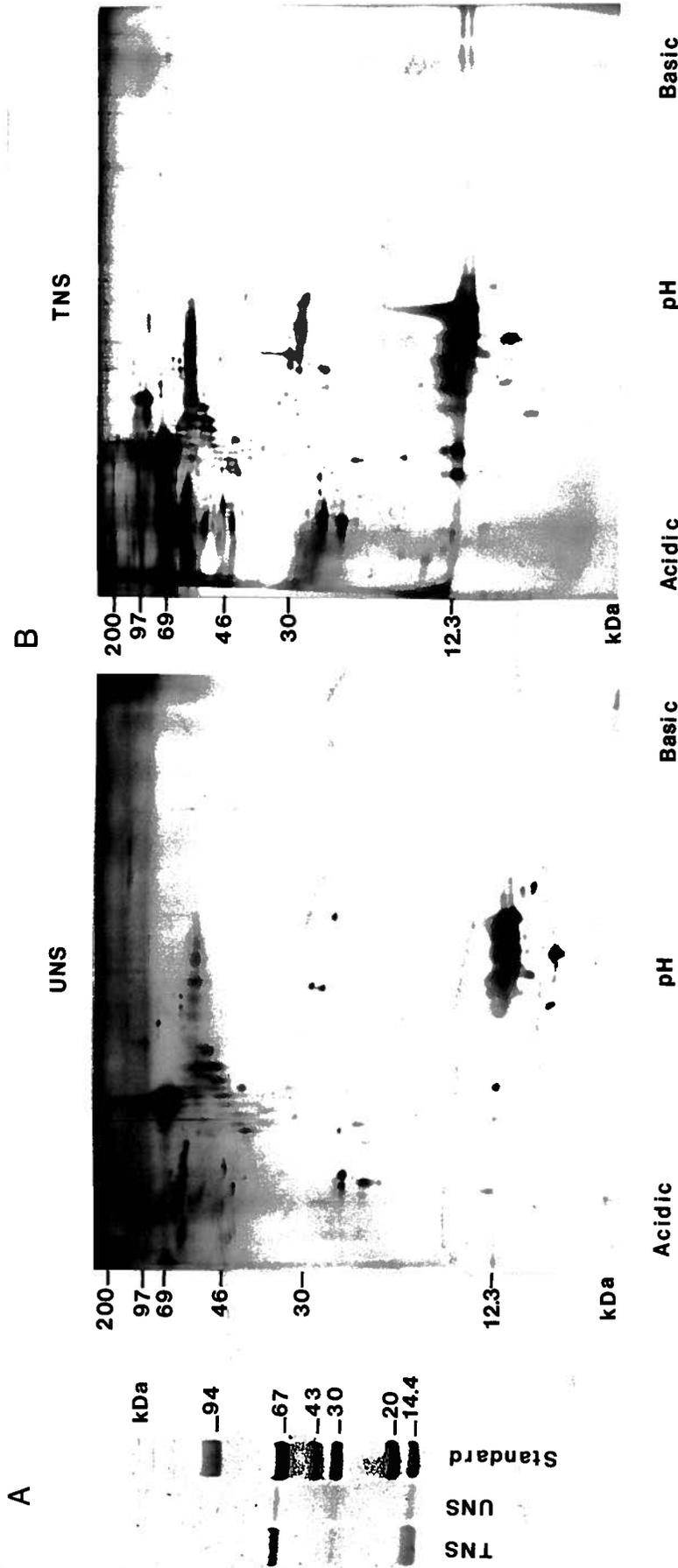


Figure 43 Electrophoretic separation of peptides from TNS and UNS respectively. In **A**, a protein of molecular weight of 69 kDa was consistently observed from TNS, but not from UNS, in the 1-D gel. In **B**, the 2-D gel showed that the 69 kDa protein was acidic. Note that more low molecular weight (<14 kDa) proteins or peptides were present more in TNS than UNS. However, the resolution of these gels would not permit identification of the molecular weights of these substances.

8.4.1. Effects of α -Tocopherol on LTP

In preliminary studies, it was found that α -tocopherol dissolved in 0.5 % dimethyl sulfoxide (DMSO) and 0.3% ethanol could induce long-term enhancement of the EPSP. Since it was not possible to dissolve α -tocopherol in aqueous solution, and since DMSO and ethanol could have effects of their own, subsequent experiments were performed with the disodium salt of α -tocopherol phosphate (referred to as α -tocopherol in subsequent text), which dissolves in aqueous solutions (see Chapter 7, Methods and Materials). After obtaining stable control responses, different concentrations (0.05-0.5 mM) of α -tocopherol phosphate were applied to determine the concentration which produced consistent effects on the CA1 neurons of the hippocampus. Concentrations of 0.1 mM (n=6) and 0.2 mM (n=16), applied for 5 min, were found to induce a significant increase in the EPSP, without changing the membrane potential and the input resistance of the neurons. Since the facilitation of the EPSP was larger with 0.2 mM, this concentration was used for subsequent quantitative studies. The potentiation of EPSP produced by α -tocopherol phosphate could not be due to an increase in phosphate because the action of α -tocopherol phosphate in the normal medium was not significantly different from that in the phosphate-free medium. The EPSP was not significantly changed during the application of α -tocopherol. The EPSP, however, started to increase about 10 min after the drug application and reached a plateau in another 10 to 20 min (EPSP slope 30 min after the drug application as a percentage of control: 158.36 ± 12.28 , n=16) (Fig. 44A & 45A). The potentiation of the EPSP lasted until the recording was terminated 60 min after the drug application. This result suggests that α -tocopherol can induce a slowly developing LTP of the EPSP. Results from the input-output (I/O) curves generated by applying different stimulation strengths to the stratum radiatum and recording the resultant CA1 neuronal EPSPs (Fig. 46)

further indicate that α -tocopherol enhances the efficacy of the excitatory synaptic transmission in these neurons.

In order to examine whether LTPs of the EPSP induced by α -tocopherol and by tetanic stimulation of hippocampal afferents are additive, the stratum radiatum was tetanized (two trains of 100 Hz for 1 s, 20 s interval) during the established α -tocopherol-induced LTP of the EPSP. In this experiment, tetanic stimulation of the hippocampal afferents did not produce any further LTP of the EPSP ($n=10$, Fig. 44B). During an established LTP of the EPSP caused by tetanic stimulation of the stratum radiatum, there was no further potentiation of the EPSP when α -tocopherol was applied. These results suggest that the LTP of the EPSP induced by α -tocopherol and that produced by tetanic stimulation of hippocampal afferents might share similar mechanisms.

8.4.2. Effects of α -Tocopherol on IPSPs and electrical properties of CA1 neurons

α -Tocopherol induced LTP of the EPSP in the CA1 neurons, without producing significant effects on the fast and slow IPSPs (response 30 min after the drug application as a percentage of control: fast IPSP 107.75 ± 7.97 ; slow IPSP 105.25 ± 9.35) ($n=8$, Fig. 45A). The number of action potentials in the CA1 neurons elicited with depolarizing rectangular current pulses (0.2-0.4 nA, 200 ms) was not changed during LTP of the EPSP induced by the drug ($n=4$, Fig. 45B). The membrane potential and the input resistance of the neurons were also unaffected by the drug (pre-drug control: resting membrane potential 62.63 ± 2.15 mV, input resistance 40.5 ± 2.48 M Ω ; during the last minute of α -tocopherol application: resting membrane potential 62.13 ± 2.06 mV, input resistance 41.15 ± 2.42 M Ω ; 30 min post-drug application: resting membrane potential 60.13 ± 2.06 mV, input resistance 43.88 ± 2.62 M Ω ; $n=16$) (Fig. 45C). These results indicate that α -tocopherol induces LTP of the EPSP, without

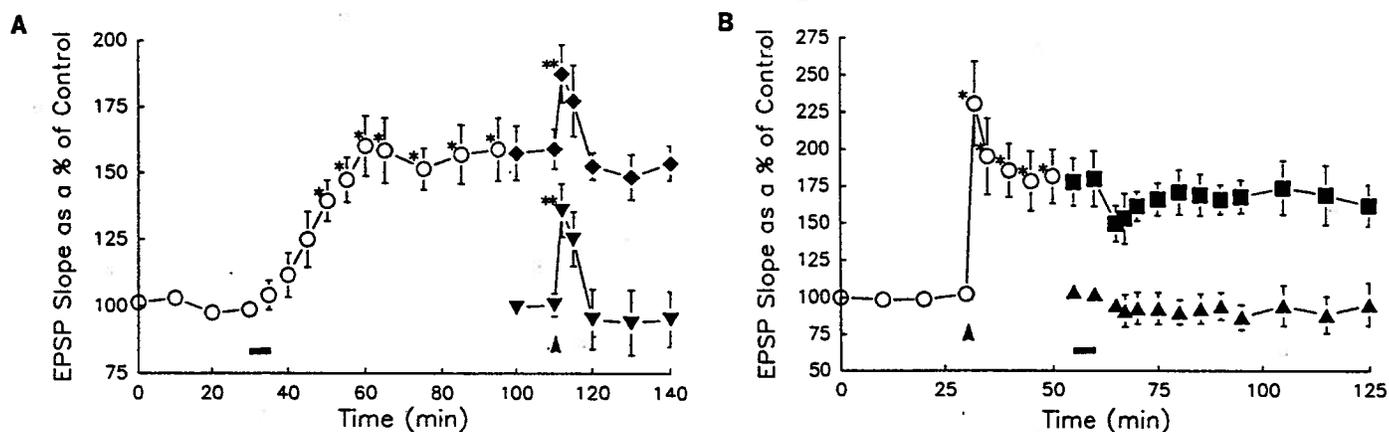


Figure 44 Effects of α -tocopherol phosphate on the EPSP of guinea pig hippocampal CA1 neurons.

α -Tocopherol phosphate (0.2 mM; n=16 neurons; O) was applied for 5 min (solid bar above abscissa). In A, tetanic stimulation (arrowhead) was given to the stratum radiatum of the hippocampus after the established α -tocopherol phosphate-induced LTP, in two sub-groups of these 16 experiments 60 min after α -tocopherol phosphate application, as described below. In 6 neurons (▼), the EPSP was adjusted to the pre-drug control level by reducing the stimulation strength 60 min after the drug application (LTP was well established by this time). The tetanic stimulation was given 10 min after decreasing the stimulation strength. During the tetanic stimulation, the stimulation strength was, however, increased to the pre-drug level. In 4 neurons (◆), in which EPSP still had more room to potentiate even though LTP had occurred, the tetanic stimulation was given without decreasing the stimulation strength. In either group, only short term potentiation, but not LTP, was observed after the tetanic stimulation. In B, the stratum radiatum was tetanized after obtaining adequate control (O, n=10). During an established LTP of the EPSP, α -tocopherol phosphate was applied 30 min post-tetanus. In some experiments, the stratum radiatum stimulation strength was not altered (■, n=4) while in the others, it was decreased so that the EPSP was comparable to the pre-tetanic level (▲, n=6). Note that, in either case, the vitamin did not cause any further increase in the EPSP. Asterisks (*) indicate that the post-drug EPSPs are significantly different from the control EPSPs. Asterisks (**) indicate that the post-tetanic EPSPs are significantly different from the pre-tetanic EPSPs.

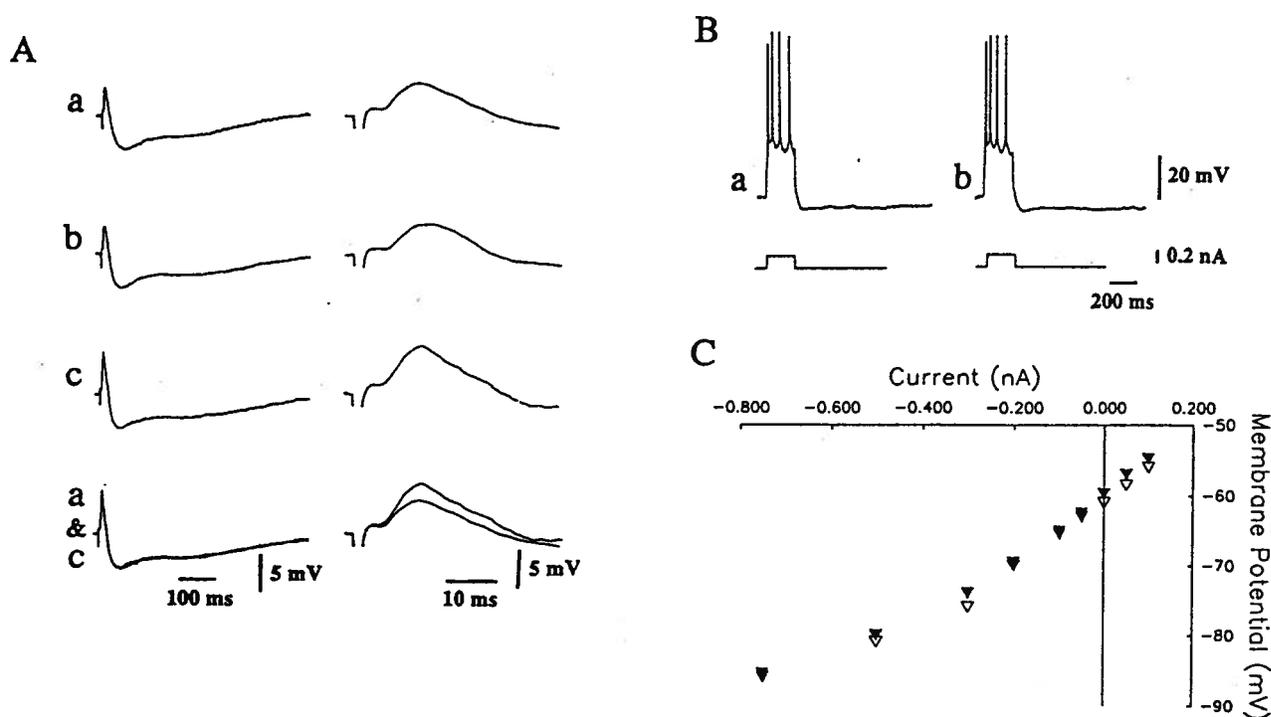


Figure 45 Actions of α -tocopherol phosphate on the evoked synaptic responses, input resistance and action potential generation in CA1 neurons.

The EPSPs (shown in slow and fast recording speed) and the IPSPs, recorded before (Aa), during (Ab) and 30 min after (Ac) the application of α -tocopherol phosphate (0.2 mM, applied for 5 min), are shown in A. Note that the agent did not change the EPSP or the fast and slow IPSPs during the application. However, the drug significantly potentiated the EPSP without changing the fast and slow IPSPs 30 min after its application (see the superimposed records, a & c in A). In B, the ability to discharge action potentials in response to an intracellularly injected depolarizing rectangular current pulse before (Ba) and 30 min after (Bb) drug application, is shown. The I-V curves before (∇) and 30 min after (\blacktriangledown) the drug application, are illustrated in C (The + and - signs on the abscissa [current] indicate de- and hyper-polarizing currents, respectively). The responses in A, B and C were obtained from the same neuron with the resting membrane potential of -62 mV.

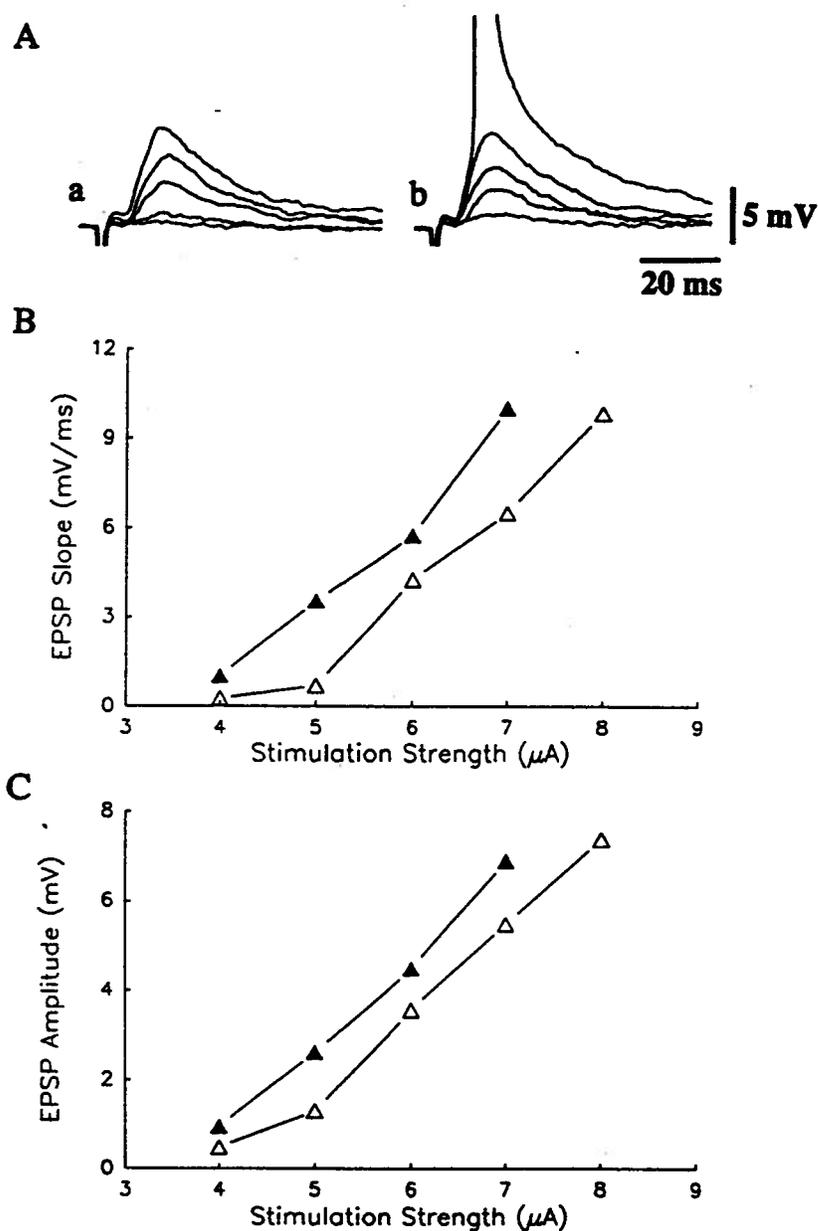


Figure 46 Effects of α -tocopherol phosphate on the input-stimulus/output-response (I/O) relationship.

The EPSPs evoked by 5 different intensities of stratum radiatum stimulation before (**Aa**) and 30 min after the drug application (**Ab**) are illustrated in **A**. The plots of the EPSP slopes vs. stimulus strengths (4-8 μA , 0.2 ms) and EPSP amplitudes vs. stimulus prior to the application of α -tocopherol phosphate (Δ) and 30 min post-drug application (\blacktriangle) are shown in **B** and **C**, respectively. In this neuron, the action potential was evoked by the 8 μA stimulation in the stratum radiatum 30 min after, but not prior to, the drug application. The spike shown in **Ab** was an averaged record and its shape was distorted because of the variability in the latency of onset; data from this record were not included in the graphs in **B** and **C**. **A**, **B** and **C** were from the same neuron with a resting membrane potential of -58 mV.

changing the membrane potential, the input resistance and the inhibitory synaptic transmission in the CA1 neurons.

8.4.3. Possible mechanisms of LTP-inducing action of α -tocopherol

8.4.3.1. Glutamate receptors and LTP-inducing action of α -tocopherol

A recent report suggests that oxygen free radicals can modulate the NMDA receptor function (Aizenman et al., 1990). Therefore, I have examined whether α -tocopherol induced LTP of the EPSP by affecting the NMDA receptor function. Co-application of α -tocopherol and APV (40 μ M) did not result in a significantly reduced LTP (EPSP slope 30 min after the application of α -tocopherol and APV as a percentage of control: 138.35 ± 9.36 , $n=6$) (Fig. 47). The possible involvement of ACPD receptors in the LTP-inducing action of α -tocopherol was also examined. In the presence of L-AP3 (100 μ M), α -tocopherol did not induce LTP of the EPSP (EPSP slope 30 min after the application of drugs as a 86.89 ± 9.65 , $n=8$) (Fig. 48).

Since the LTP-induced by α -tocopherol is developed slowly, NMDA- and non-NMDA receptor sensitivity can be changed after the application of α -tocopherol. The present experiments were conducted to determine whether the LTP-inducing action of α -tocopherol was due to changes in NMDA and non-NMDA receptor-mediated depolarizations by glutamate. In Mg^{2+} free medium, application of NMDA (10 μ M) induced a depolarization of the CA1 neurons. The NMDA-induced depolarization obtained during or 30 min after the application of α -tocopherol was not significantly different from the depolarization obtained before the vitamin application ($n=6$) (Fig. 49A). The non-NMDA receptor-gated response was examined in the standard medium containing APV (40 μ M). In this medium, application of glutamate (1 mM) produced a depolarization of the CA1 neurons. The glutamate-induced depolarization was primarily mediated by non-

NMDA receptors because the NMDA response was blocked by APV and Mg^{2+} in the standard medium. α -Tocopherol did not change the glutamate-induced depolarization in this medium ($n=4$) (Fig. 49B).

These results suggest that α -tocopherol does not alter the NMDA- and non-NMDA-receptor mediated depolarization. The α -tocopherol-induced LTP is blocked by AP3, but not by APV. It is possible that ACPD receptors are involved in the LTP-inducing action of α -tocopherol. However, a more selective antagonist is required to confirm the involvement of ACPD receptors.

8.4.3.2. Protein kinase C and LTP-inducing action of α -tocopherol

It is thought that PKC is involved in LTP. The LTP-inducing action of α -tocopherol is blocked by L-AP3, which is known to inhibit the ACPD-stimulated phosphoinositide hydrolysis in the hippocampal slices (Schoepp et al., 1991). Increases in phosphoinositide hydrolysis can lead to an activation of PKC and an increase in intracellular Ca^{2+} concentration. It was therefore decided to examine whether protein kinase C activation is involved in the LTP-inducing action of α -tocopherol. α -Tocopherol when applied in the presence of sphingosine (30 μ M) did not induce a potentiation of the EPSP ($n=6$, Fig. 50A). In K-252b injected neurons also, α -tocopherol failed to induce LTP of the EPSP ($n=6$, Fig. 50B). It therefore appears that PKC activation is required for the LTP-inducing action of α -tocopherol.

8.4.3.3. Ca^{2+} and LTP-inducing action of α -tocopherol

Postsynaptic free Ca^{2+} plays a crucial role in the induction of LTP (Lynch et al. 1983). Whether postsynaptic free Ca^{2+} has a role in the LTP-inducing action of α -tocopherol was examined in the present study. Intracellular injection of BAPTA into the CA1 neurons blocked the induction of LTP produced by α -tocopherol ($n=6$, Fig. 51). It appears that chelation of postsynaptic Ca^{2+} blocks

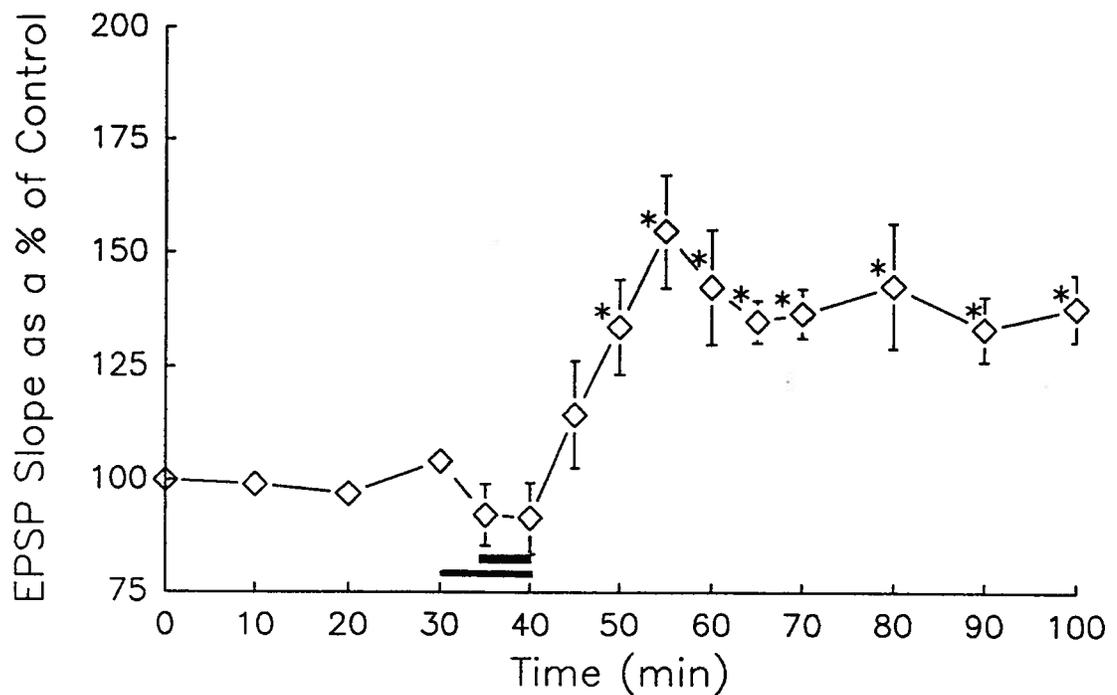


Figure 47 Effects of APV on the induction of α -tocopherol phosphate-induced LTP.

APV (40 μ M; the long horizontal bar above the abscissa) application was initiated 5 min prior to the application of α -tocopherol phosphate (the short bar above abscissa) and terminated when the superfusion of the latter was terminated (n=6). Note that APV did not block the LTP induced by α -tocopherol phosphate.

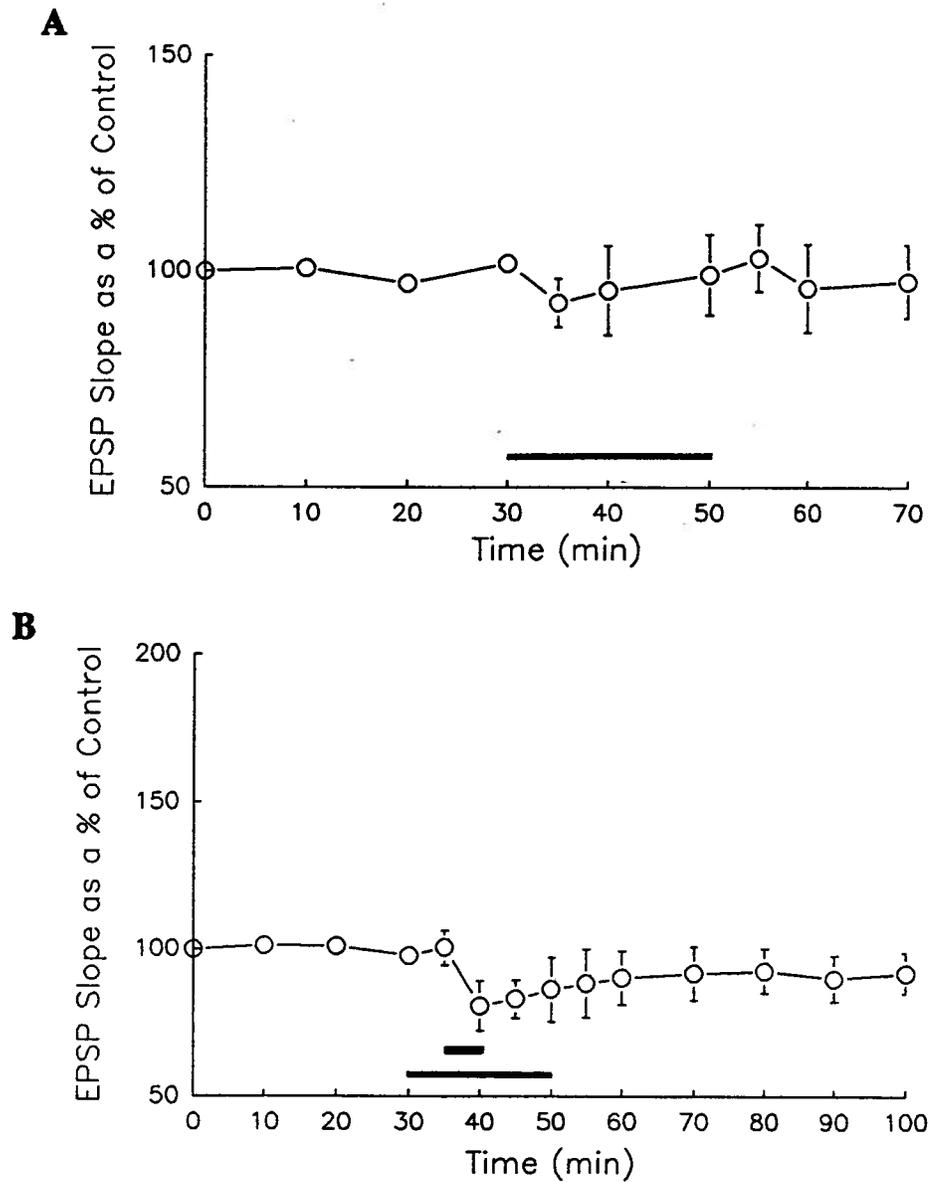


Figure 48 Effects of L-AP3 on the α -tocopherol-induced LTP.

In **A**, L-AP3 (100 μ M, the long horizontal bar above the abscissa) was applied to the hippocampal slices after stable control EPSP was obtained ($n=6$). Note that application of L-AP3 did not affect the basal responses. In **B**, α -tocopherol phosphate (the short bar above the abscissa) was applied in the presence of L-AP3 (the long bar above the abscissa) ($n=8$). Note that α -tocopherol phosphate failed to induce LTP in the presence of L-AP3.

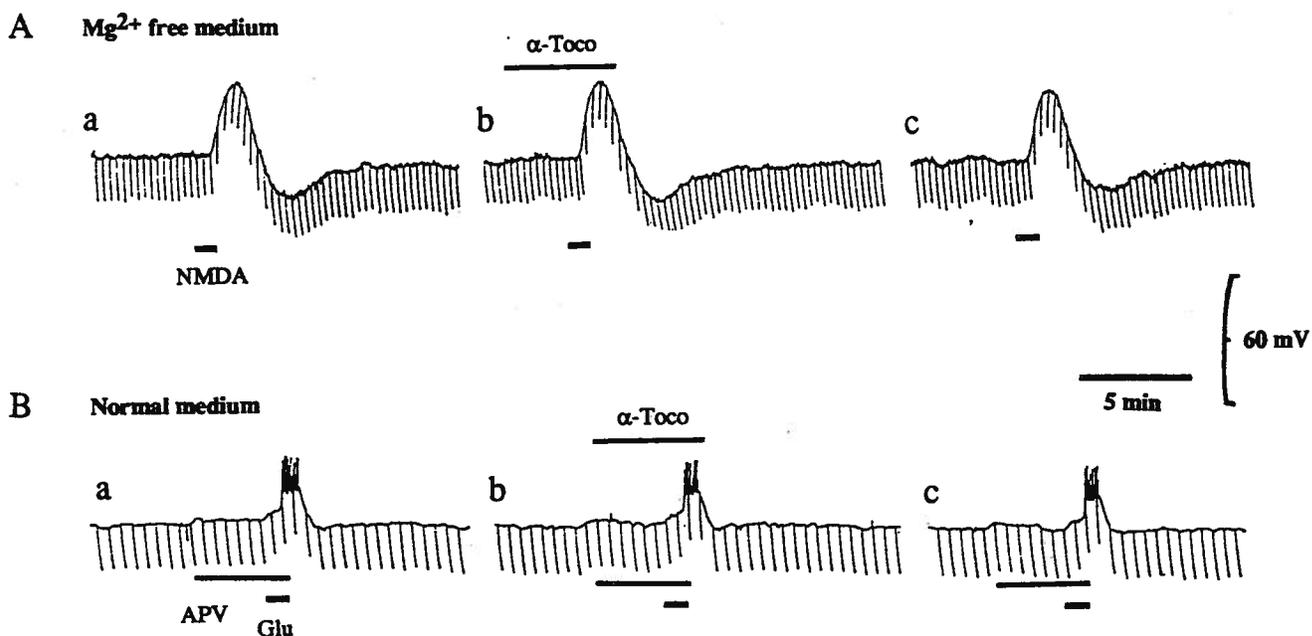


Figure 49 Effects of α -tocopherol phosphate on the NMDA and non-NMDA responses.

In **A**, the hippocampal slice was superfused with Mg^{2+} free medium throughout the experiment. NMDA ($10 \mu M$, applied for 40 sec, the short bar below the trace) was applied to the slices before (**Aa**), during (**Ab**) and 30 min after (**Ac**) the application of α -tocopherol phosphate (0.2 mM , applied for 5 min, the horizontal bar above the trace). Note that the depolarization induced by NMDA application was not affected by the application of α -tocopherol phosphate. In **B**, the hippocampal slices were superfused with normal medium throughout the experiment. APV ($40 \mu M$, the long horizontal bar below the trace) application was initiated 3 min prior to the application of glutamate (1 mM , applied for 50 sec, the short horizontal bar below the trace) and terminated when the superfusion of the latter was terminated. Glutamate was applied before (**Ba**), during (**Bb**) and 30 min after (**Bc**) the application of α -tocopherol phosphate (0.2 mM , applied for 5 min, the horizontal bar above the trace). Note that the glutamate-induced depolarization in the normal medium containing APV was not changed by the application of α -tocopherol phosphate. **A** and **B** were from different neurons. The resting membrane potential of the neurons in **A** and **B** were -64 mV and -59 mV , respectively.

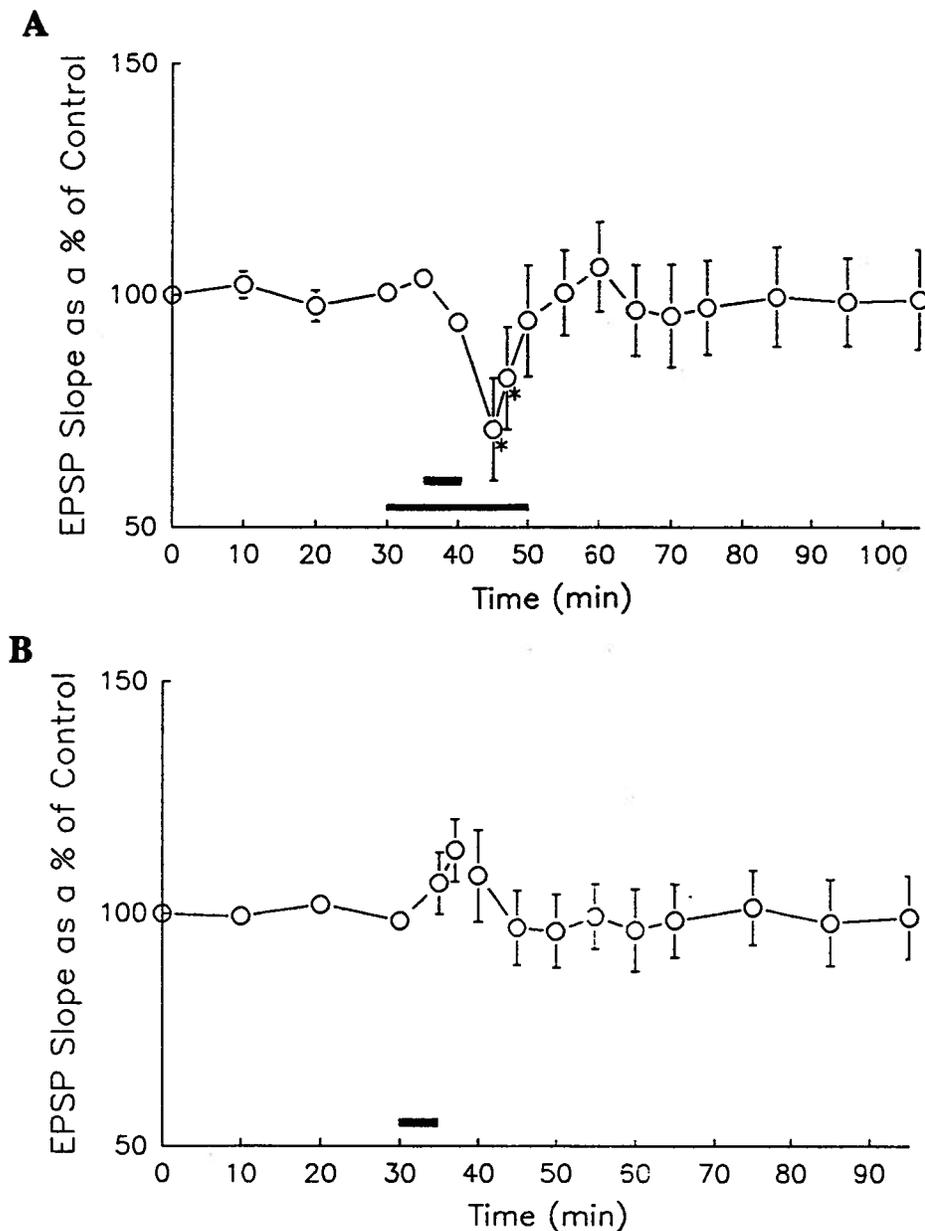


Figure 50 Effects of protein kinase C inhibitors on the LTP-inducing action of α -tocopherol phosphate

In **A**, α -Tocopherol phosphate (0.2 mM, the short bar above abscissa) was applied in the presence of sphingosine (30 μ M, the long bar above abscissa) (n=6). Note that the LTP-inducing actions of α -tocopherol phosphate were blocked by sphingosine. In **B**, α -tocopherol phosphate (the solid bar above abscissa) was applied following the injection of K-252b into the CA1 neurons (n=6). Note that α -tocopherol phosphate failed to cause LTP of the EPSP in the K-252b injected neurons.

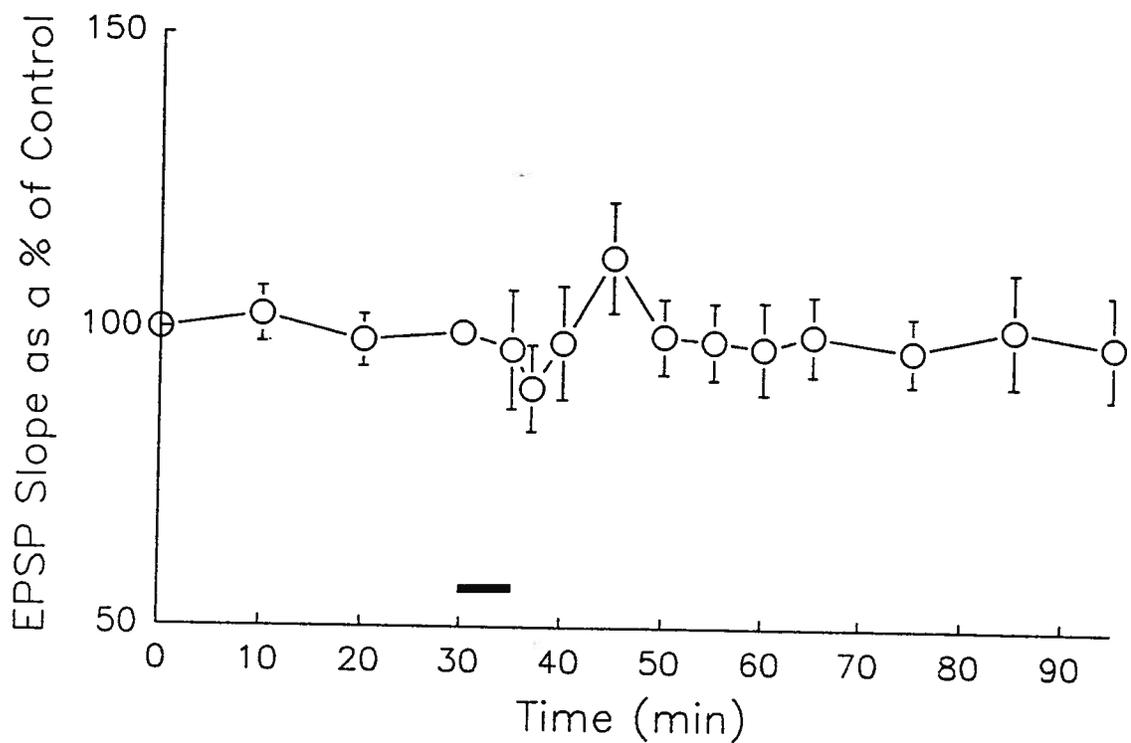


Figure 51 Effects of chelation of postsynaptic Ca^{2+} on the LTP-inducing action of α -tocopherol phosphate

BAPTA, a Ca^{2+} chelator, was injected to the CA1 neurons through recording electrodes. α -Tocopherol phosphate (0.2 mM, the solid bar above abscissa) was applied to the BAPTA-injected neurons ($n=6$). Note that α -tocopherol phosphate did not induce LTP of the EPSP in the BAPTA-injected neurons.

the LTP-inducing action of α -tocopherol.

8.4.3.4. Antioxidant and LTP-inducing action of α -tocopherol

α -Tocopherol is a lipophilic antioxidant, which can scavenge free radicals within the cell membrane (Tappel, 1962). Whether α -tocopherol induced LTP of EPSP through its antioxidative effect is, however, unclear. It was therefore decided to examine whether other antioxidants can induce LTP. Ascorbic acid, a water-soluble antioxidant, can remove free radicals in the aqueous phase. DMSO is a lipid-soluble antioxidant, which can readily cross the membrane of neurons. The actions of ascorbic acid (Na salt) and DMSO on the CA1 neuronal EPSP were, therefore, examined to compare these effects with those of α -tocopherol.

Ascorbic acid (0.1-5 mM, applied for 10 min) did not produce a significant potentiation of the EPSP (EPSP slope 30 min after the application of 3 mM ascorbic acid as a percentage of control: 98.83 ± 11.029 , $n=6$) (Fig. 52). The membrane potential and the input resistance were not significantly altered by these concentrations of ascorbic acid. However, at a concentration of 10 mM, ascorbic acid induced an increase in the EPSP in 2 of 6 neurons examined. This increase was associated with a reduction in the input conductance of the cells. Therefore, the increase in the EPSP induced by 10 mM ascorbic acid in the two neurons might be due to the reduction of the input conductance of the cells. It appears that ascorbic acid does not induce LTP of the EPSP. It is possible that this lack of effect by ascorbic acid is due to its inability to remove free radicals within the membrane. Therefore, DMSO, a lipid-soluble antioxidant, was examined in some experiments. Application of DMSO (3%) potentiated the EPSP of the CA1 neurons ($n=12$, Fig. 53). The potentiation of the EPSP reached a plateau within 5-10 min after starting the application and lasted as long as the application of the agent continued. However, the EPSP

quickly returned to the pre-application level once the application of DMSO was stopped. Perhaps DMSO failed to induce LTP because the drug was washed out very quickly. It is possible that the potentiation of the EPSP produced by DMSO is due to the antioxidant effect of the drug. However, unlike α -tocopherol, DMSO also caused an increase in the IPSPs in some CA1 neurons when the agent was present (Fig. 53B). It is apparent that the actions of DMSO on the EPSP and the IPSPs are different from those of α -tocopherol.

8.4.4. LTP in vitamin E deficient rats

Since impairment of spatial learning ability was reported in vitamin E deficient rats (Moriyama et al., 1990), experiments were conducted to determine whether LTP is affected in vitamin E deficient rats. Rats (two weeks old) were divided into two groups. One group was designated as control group which was fed with vitamin E control diet. The other group, designated as the vitamin deficient group, was fed with vitamin E deficient diet, which had all components of the control diet except vitamin E. If rats were fed with these diets for one month, LTP induced by tetanic stimulation occurred in slices obtained from both groups of rats ($n=6$ for each group). Similar results were observed in rats which were given these diets for 2 months ($n=6$ for each group). However, when rats were fed with these diets for 3 months, the tetanus-induced LTP occurred only in the slices obtained from vitamin E control group ($n=8$). Tetanic stimulation failed to induce LTP in slices obtained from a paired vitamin E deficient group ($n=12$, Fig. 54 & 55). The appearance, weight and behaviour of the rats were not significantly different between control and the deficient group. The membrane potential, input resistance and evoked synaptic responses of CA1 neurons recorded from the hippocampal slices of the vitamin E deficient rats were not significantly different from those obtained from the hippocampal slices of control rats ($n=8$, data not shown). However, it appears that LTP is affected in vitamin E

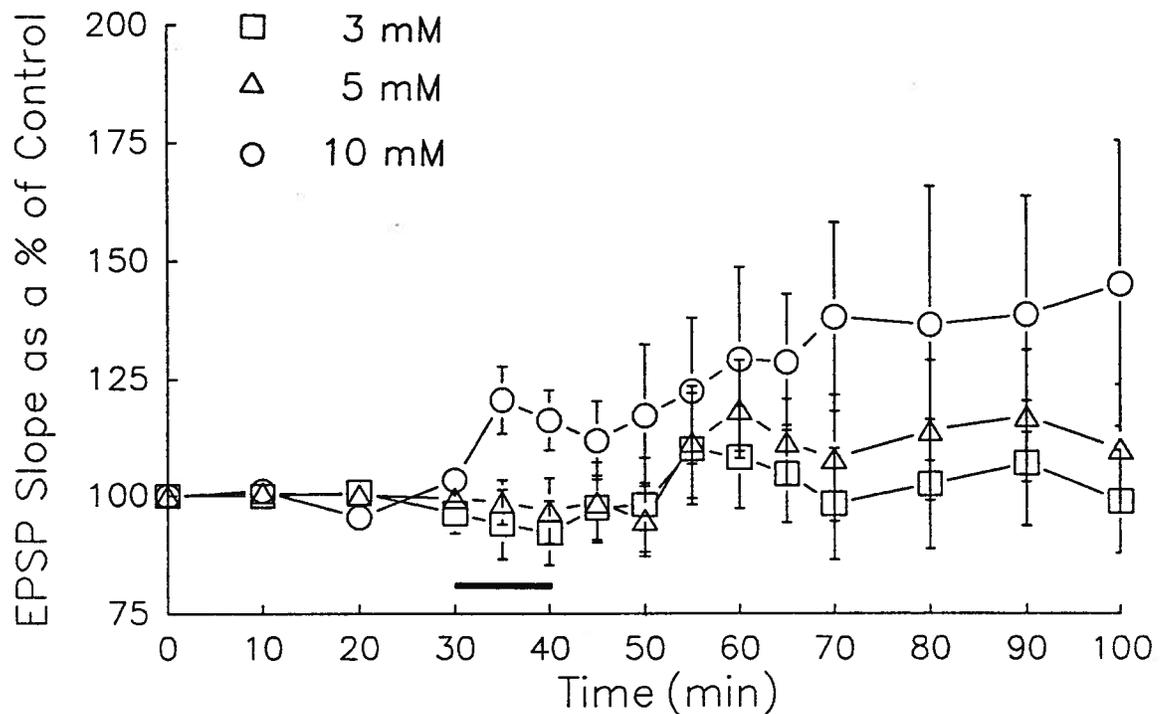


Figure 52 The inability of sodium ascorbate to induce LTP in the CA1 neurons. The actions of sodium ascorbate (applied for 10 min, the horizontal bar above the abscissa), a water soluble antioxidant, on the EPSP of the CA1 neurons are illustrated (3 mM, n=6; 5 mM, n=4; 10 mM, n=6). Note that sodium ascorbate did not induce LTP of the EPSP in the CA1 neurons. At a concentration of 10 mM, sodium ascorbate induced an increase in the EPSP in 2 of 6 neurons examined. However, the increase was associated with a reduction in the input conductance of the cells.

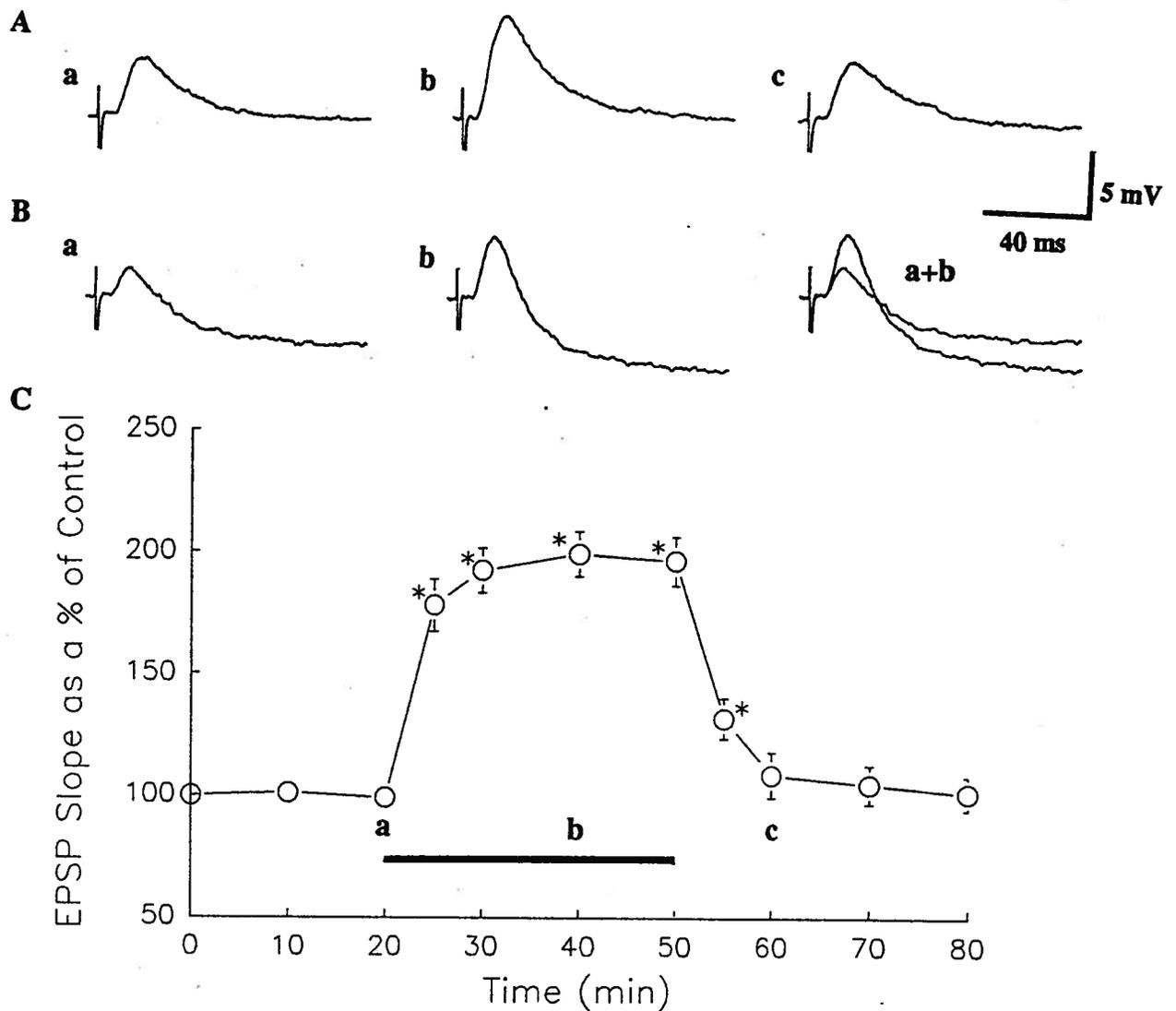


Figure 53 Effects of DMSO on the EPSP of the CA1 neurons

Effects of DMSO (3%, the long bar above the abscissa) on the EPSP were shown in A, B and C. The EPSP of the CA1 neurons were potentiated during the application of DMSO (C, $n=16$). The potentiated EPSP returned to the pre-application level quickly once the application of DMSO was terminated. Note that DMSO did not induce LTP of the EPSP. A and B show the records of control (a), during (b) and after (c) application of DMSO in two individual experiments. DMSO also increased the IPSPs in some neurons (B). The resting membrane potentials of the neurons shown in A and B were -68 mV and -61 mV, respectively.

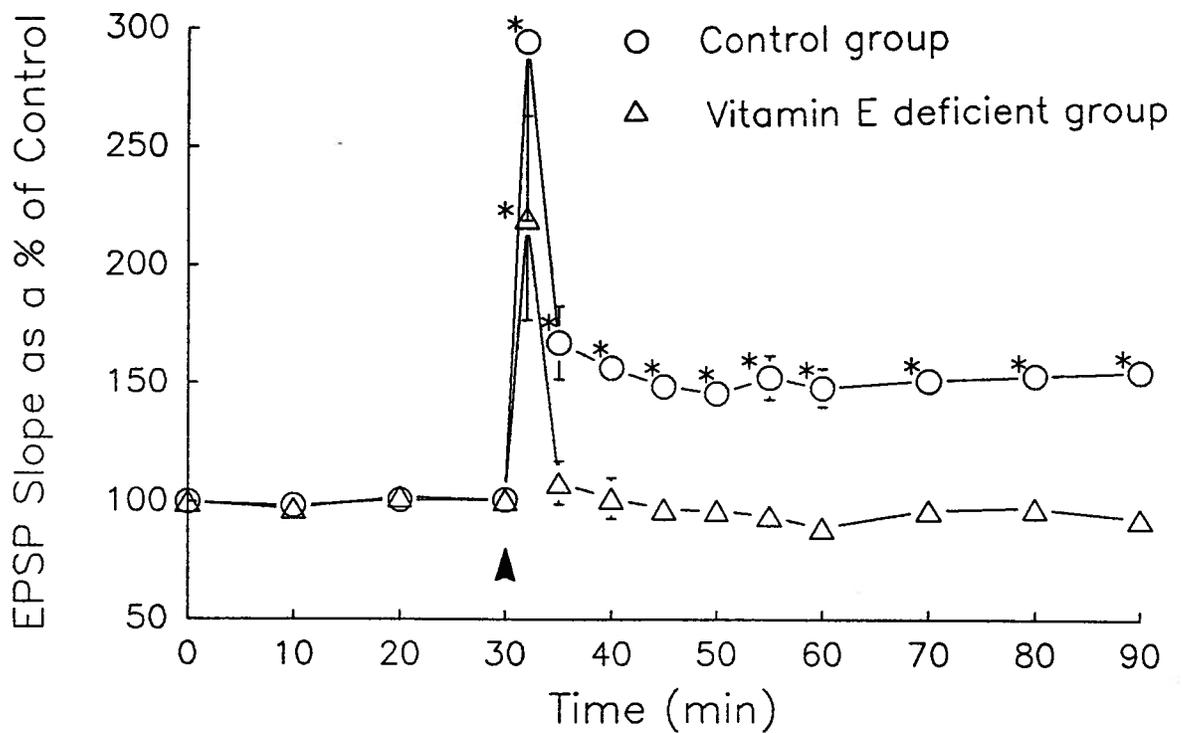


Figure 54 The inability of tetanic stimulation to produce LTP in vitamin E deficient rat hippocampal CA1 neurons.

Tetanic stimulation (2 trains of 100 Hz for 1 sec, 20 sec interval) was given to the stratum radiatum. In hippocampal slices obtained from the vitamin E deficient rats, tetanic stimulation failed to produce LTP of the EPSP in the CA1 neurons (n=12). Similar tetanic stimulation was able to induce LTP of the EPSP in the CA1 neurons of hippocampal slices obtained from the matching control rats (n=8). Note that other than vitamin E, the components of the vitamin deficient diet were the same as those of the control diet.

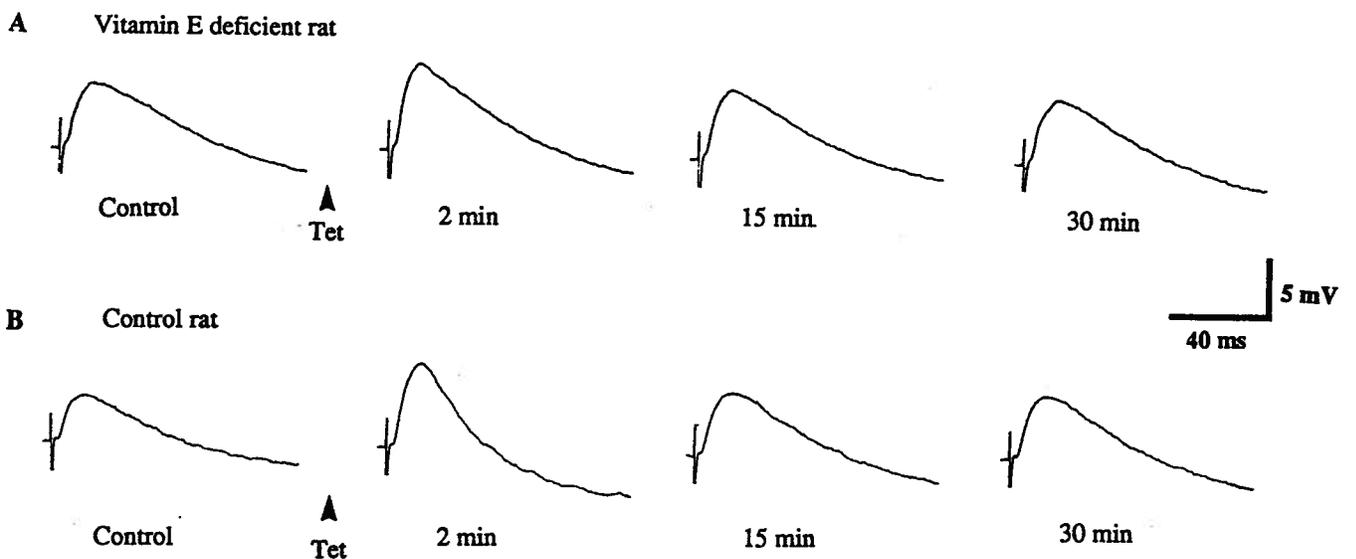


Figure 55 Tetanic stimulation of the stratum radiatum in vitamin E deficient and control rat hippocampal slices.

A shows the records obtained from vitamin E deficient rat hippocampal slice. **B** illustrates the records obtained from matching control rat hippocampal slice. Note that tetanic stimulation induced LTP of the EPSP in the hippocampal slices obtained from matching control rats but not vitamin E deficient rats. The resting membrane potentials of the neurons in **A** and **B** were -67 mV and -61 mV, respectively.

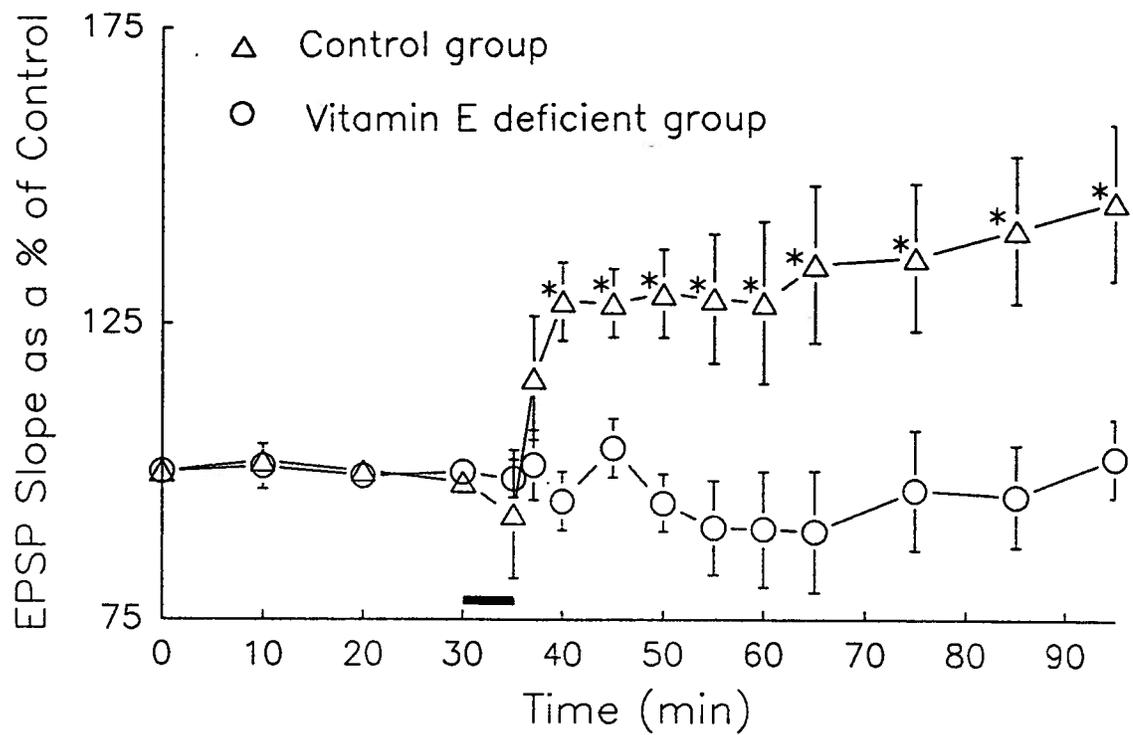


Figure 56 The failure of α -tocopherol phosphate to induce LTP in hippocampal CA1 neurons of vitamin E deficient rats. α -Tocopherol phosphate (0.2 mM, the solid bar above abscissa) was applied to the hippocampal slices. LTP was observed after the application of α -tocopherol phosphate in hippocampal slices obtained from matching control rat slices (n=6), but not from vitamin E deficient rats (n=6).

deficient rats. Application of α -tocopherol induced LTP in the slices obtained from control rats, but not in the slices obtained from vitamin E deficient rats (n=6, Fig. 56). These results indicate that application of α -tocopherol was not able to overcome the effects caused by prolonged vitamin E deficiency on LTP.

9. DISCUSSION

9.1. LTP and endogenous substances

Previous studies conducted in our laboratory first showed that substances collected during, but not without, a tetanic stimulation in guinea pig hippocampus (Chirwa and Sastry, 1986) or rabbit neocortical surface (Sastry et al., 1988a) caused LTP of the CA1 population spike in guinea pig hippocampal slices. However, the mechanisms underlying the release and action of the LTP-inducing endogenous substances were unknown. What role the released substances play in the LTP process was also unclear. In the present study, the substances collected from rabbit neocortical surface during tetanic stimulation (TNS) produced LTP of both the population spike and the EPSP in guinea pig hippocampal slices. In contrast, the fluid collected from rabbit neocortex without tetanic stimulation failed to cause LTP. The present results confirm the previous findings of Sastry and his colleagues (1988a). The application of TNS not only potentiated the population spike but also increased the slope and height of the intracellular EPSP in the CA1 neurons. TNS did not significantly change the membrane potential and the input resistance of the CA1 neurons. Therefore, it is apparent that the potentiation induced by TNS is due to an increase in synaptic transmission rather than to a general change in the excitability of the CA1 neurons. If TNS induces a general enhancement of synaptic transmission, it should increase both excitatory and inhibitory synaptic transmissions in the CA1 area of the hippocampus. TNS caused only a short-term potentiation of the GABA_A receptor-mediated fast IPSP which lasted for 10 min. TNS did not

significantly change the GABA_B receptor-mediated slow IPSP. It is apparent that the application of TNS selectively induces a long-term enhancement of the excitatory synaptic transmission without inducing a long-term effect on the inhibitory synaptic transmission in the CA1 area. The result rules out the probability of general effects of TNS on synaptic transmission in the CA1 area. Since LTP induced by a tetanic stimulation of the hippocampal afferents prior to the application of TNS occluded any further LTP induced by TNS application, and *vice versa*, the tetanus- and TNS-induced LTP may share some common mechanisms in the chain of events leading to the potentiation. The LTP-inducing action of TNS seems to be activity-dependent because LTP cannot be induced if the stimulation of afferents is stopped during the application of TNS. Tetanus induced LTP is input specific in that only the tetanized synapses, and not the quiescent synapses on the same postsynaptic cell, are potentiated.

9.1.1. Characterization of the LTP-inducing substances

Proteins with different molecular weights ranging from 14 to 86 kDa have been reported to be released during LTP *in vitro* (Duffy et al., 1981; Otani et al., 1992) and *in vivo* (Charriaut-Marlangue et al., 1988; Fazeli et al., 1988; Fazeli et al., 1990). Depolarization induced by application of NMDA into the hippocampus causes a release of proteins with molecular weights of 14 to 66 kDa (Nystrom et al., 1986). Whether the proteins released during LTP are directly involved in the LTP process is not clear. Inhibitors of protein synthesis such as cycloheximide and anisomycin have been shown to interfere with the LTP process (Stanton and Sarvey, 1984; Deadwyler et al., 1987; Frey et al., 1988). Certain peptides such as mast cell degranulating peptides (MCD) have been reported to induce LTP (Cherubini et al., 1987; Aniksztejn et al., 1990; Kondo et al., 1992). It is possible that the endogenous substances collected from rabbit neocortex during tetanic stimulation contain some released peptides which are responsible for the

induction of LTP. The notion is supported by the evidence that the LTP-inducing action of TNS is greatly diminished if TNS is heated at 60 °C for 30 min and cooled prior to the application (Sastry et al., 1988a). It is unlikely that the LTP-inducing action of TNS is due to an increase in the release of endogenous glutamate or acetylcholine during tetanic stimulation because preheating of glutamate does not abolish the effects of glutamate in the hippocampus. Furthermore, cholinergic antagonists such as atropine do not block the LTP-inducing action of TNS (Sastry et al., 1988a; Chirwa, 1988).

In the present study, the results of gel electrophoresis revealed that some proteins with molecular weights greater than 50 kDa were present in TNS and these proteins were either absent or present in much lower concentrations in UNS. TNS also contained higher concentrations of some lower molecular weight peptides than UNS. However, the resolution of the gels used in the present study was not able to identify their molecular weights. It is apparent that tetanic stimulation caused an increase in the release of proteins with different molecular weights from the neocortical surface into the extracellular fluid. It is, however, not clear whether proteins with different molecular weights in TNS play a different roles in the LTP process. It was, therefore, decided to fractionate TNS to determine the approximate molecular weight of the substances which were responsible for LTP in guinea pig hippocampal slices (Table 6). The fractions containing substances with molecular weights of <3, 3-10 and >50 kD were clearly shown to produce LTP. The <3 and 3-10 kD fractions of TNS, when applied separately, could only produce marginal and slowly developing LTP whereas a combination of the two fractions (<25 kDa fraction of TNS) clearly produced a significant LTP that developed much more rapidly. If the >50 kDa fraction is compared with the <3 kDa and 3-10 kDa fractions of TNS, it is clear that the LTP induced by the higher molecular weight fraction reached a

maximum much faster than the lower molecular weight fractions. The LTP induced by TNS was relatively larger and developed faster than the LTPs produced by different fractions of TNS. Perhaps the three fractions are needed to work in concert with each other to establish LTP. The different fractions of TNS may be involved in LTP at different stages, the >50 kDa fraction in the early and the other fractions in the late stages of LTP. The 69 kDa protein present in TNS was probably involved in the LTP-inducing action of the >50 kDa fraction of TNS. The LTP-inducing substances present in <25 kDa fractions, including the <3 and 3-10 kDa fractions, have not been identified in the present study. It has been reported that arachidonic acid released during tetanic stimulation of hippocampal afferents is involved in LTP (Williams et al., 1989). Application of arachidonic acid can induce a slowly developing LTP which has a time course similar to that of LTP induced by <3 kDa. If arachidonic acid is indeed released during tetanic stimulation as Williams et al. proposed, then it could be present in the low molecular weight fractions of TNS.

9.1.2. Mechanisms of the release of the endogenous substances

The substances collected from rabbit neocortical surface during, but not subsequent to, tetanic stimulation, could induce LTP. It is apparent that the LTP-inducing substances are released only during tetanic stimulation that can induce a depolarization of the cells and activate NMDA receptors. The depolarization caused by tetanic stimulation may cause the release of substances such as proteins and peptides from the cells. APV, a NMDA antagonist, has been reported to prevent tetanus-induced protein release (Otani et al., 1992). Application of NMDA has been shown to cause a release of proteins from cells into extracellular fluid (Nystrom et al., 1986). It was therefore decided to examine whether the release of the LTP-inducing substances in TNS was dependent on the activation of NMDA receptors. In the present study, TNS

collected from rabbits pretreated with MK-801 failed to induce LTP. The result suggests that NMDA receptor activation is involved in the release of the LTP-inducing substances. The failure of the TNS from pretreated rabbits to induce LTP could not be due to the presence of adequate quantities of MK-801 in TNS because a tetanic stimulation of the afferents in the hippocampal slice during the application of the above sample could still induce LTP. The exact sites of the release of the LTP-inducing substances are not clear. The LTP-inducing substances can be released postsynaptically and/or presynaptically. The substances can also be released from glia. Since the release of LTP-inducing substances involves NMDA receptor activation, the release sites very likely contain NMDA receptors. The presence of NMDA receptors on postsynaptic sites is well known (Collingridge and Singer, 1990, Nicoll et al., 1990; see the review section of 4.1.1). It is not very clear whether NMDA receptors are also present on the presynaptic terminals. NMDA and NMDA antagonists such as CPP and APV have been shown to affect the release of glutamate, aspartate or GABA from the CA1 area of the hippocampal slices evoked by exposing the hippocampal slices to high K^+ medium (Martin et al., 1991). Similar results have been reported in rat striatum *in vivo* (Bustos et al., 1992). The authors suggested that the release of amino acids could be mediated through presynaptic NMDA receptors. However, the exposure of the hippocampal slices to high K^+ medium and the application of NMDA can activate postsynaptic NMDA receptors which may mediate the release of amino acids from the presynaptic terminals that impinge on these cells through a retrograde mechanism. On the other hand, the activation of the neurons by NMDA can release transmitters from the terminals of the same neurons. Therefore, the results cannot confirm the presence of NMDA receptors on the presynaptic terminals of the CA1 area. Smirnova et al. (1993) have recently shown that a

33-kDa protein (GR33) obtained from a rat striatal cDNA library forms glutamate-activated ion channels when expressed in *Xenopus* oocytes. The channels possess some pharmacological properties, such as being APV-sensitive, in a manner similar to the postsynaptic NMDA receptor-gated channels. Since the sequence of GR33 was reported to be identical to that of the presynaptic protein syntaxin (Bennett et al., 1992), Smirnova et al. (1993) suggested that this protein may be a presynaptic glutamate receptor. Despite the evidence discussed above, clear evidence proving the existence of presynaptic NMDA receptors is still lacking. However, the presence of such receptors cannot be ruled out until further evidence is obtained from studies carried out directly from presynaptic terminals. Glial cells are another source for the release of the LTP-inducing substances. It has been shown that cultured astrocytes have glutamate receptors, primarily kainate and AMPA (quisqualate) receptors (Bowman and Kimelberg, 1984; Kettenmann et al., 1984; Kettenmann and Schachner, 1985). It has been suggested that NMDA receptors do not exist on glia because the application of NMDA does not induce any response in these cells even in the presence of glycine or in Mg^{2+} -free solution (Backus et al., 1989; Usowicz et al., 1989; Kettenmann and Schachner, 1985). However, a recent report has shown that agonists for non-NMDA and NMDA receptors induce Ca^{2+} flux in cultured astrocytes (Holopainen and Akerman, 1990). The Ca^{2+} flux-induced by NMDA is increased in Mg^{2+} -free medium and blocked by APV. More recently, an NMDA-evoked current has been recorded in Bergmann glial cells in cerebellar slices (Muller et al., 1993). The NMDA-activated current is mimicked by homocysteate, a NMDA receptor agonist. The current is inhibited by NMDA antagonists such as ketamine but not by CNQX, a non-NMDA antagonist. However, a high dose of NMDA is required to induce the current in these glial cells. The reversal potential of the NMDA-evoked current in Bergmann glial

cells is approximately -50 mV which is different from that of the neuronal NMDA current. Glycine and Mg^{2+} do not affect the NMDA current in these glial cells. Although the properties of NMDA-evoked current in glial cells may be different from those in neurons, the finding provides evidence for the existence of NMDA-like receptors in at least some types of glial cells in cerebellum. Whether similar NMDA receptors are also present in some glial cells in hippocampus and neocortex remain to be seen. Although the present study cannot directly confirm the release sites for the LTP-inducing substances, it is not opposed to the postsynaptic cells to be the most likely primary site for the release of the substances. NMDA receptors are dense in postsynaptic cells, particularly in the dendritic area, and depolarization-induced by tetanic stimulation can facilitate the opening of NMDA channels. The depolarization of the neurons and the activation of NMDA may allow the release of certain substances which are involved in the induction of LTP. The release may also occur in glial cells. In fact, depolarization of glia through current injection, when paired with stimulations of afferents, can cause LTP of the EPSP in the nearby CA1 neurons (Sastry et al., 1988c). The mechanism for this LTP is not clear. It is possible that some substances, which are involved in the induction of LTP in the CA1 neurons, are released from glial cells during the depolarization. Giulian et al. (1993) recently found that certain proteins with molecular weights greater than 10 kDa are released from cultured astroglia during CNS injury. These proteins released from astroglia act as trophic factors to promote neuronal growth. The LTP-inducing substances in the present study have also been shown to have trophic effects such as enhancing neurite growth in cultured PC-12 cells (Sastry et al., 1988a). It is therefore logical to speculate the involvement of glial cells in the release of the LTP-inducing substances. It is less likely that the LTP-inducing substances are released from the presynaptic terminal because there is

no direct evidence for the existence of NMDA receptors at present. However, it is possible that some of the substances collected from rabbit neocortex during tetanic stimulation are released from the presynaptic terminals. One possibility is that the activation of NMDA receptors in postsynaptic sites and/or glial cells may indirectly control the release of substances from the presynaptic terminals through a retrograde mechanism. Another possibility may be that NMDA receptors are, indeed, present in presynaptic terminals and have not been detected yet.

9.1.3. Mechanisms of the LTP-inducing action of the endogenous substances

Tetanic stimulation can induce LTP of the EPSP in hippocampus (Schwartzkroin and Wester, 1975). Under normal conditions, the tetanus-induced LTP requires the activation of NMDA receptors (Collingridge et al., 1983). Recently, ACPD receptors have also been reported to be involved in LTP (Otani and Ben-Ari, 1991; Bortolotto and Collingridge, 1992, 1993). Furthermore, protein kinase C (Lovinger et al., 1987; Malinow et al., 1989; Malenka et al., 1989) and postsynaptic free Ca^{2+} play a critical role in the induction of LTP (Lynch et al., 1983; Malenka et al., 1988; Morishita and Sastry, 1991). Since the tetanus- and TNS-induced LTPs occlude each other, these two LTPs may share some common mechanisms. It is of interest to determine whether the TNS-induced LTP involve the activation of NMDA and ACPD receptors.

In the presence of APV, an NMDA antagonist, the LTP-inducing action of TNS is not affected. This result indicates that the activation of NMDA receptors is not required for the induction of TNS-induced LTP. It is likely that TNS induces LTP by acting on the steps following the activation of NMDA receptors. Previous findings reported by our laboratory (Morishita et al., 1992) showed that

saccharin blocks the tetanus- as well as the TNS-induced LTP in hippocampal slices. Since saccharin does not interfere with NMDA receptors, the blockade of the tetanus-induced LTP by saccharin appears not to involve NMDA receptors. The result supports the notion that TNS may induce LTP by acting on some elements other than NMDA receptors. TNS has been reported to induce neurite growth in PC-12 cells, which is blocked by saccharin (Sastry et al., 1988a; Morishita et al., 1992). Some growth-inducing substances present in TNS may be responsible for the induction of LTP.

Activation of ACPD receptors has been reported to be involved in LTP in hippocampus (Otani and Ben-Ari, 1991). ACPD receptor antagonists such as L-AP3 and MCPG have been shown to block the induction of LTP produced by tetanus (Izumi et al., 1991; Behnisch et al., 1991; Bortolotto and Collingridge, 1992, 1993). However, the selectivity of ACPD receptor antagonists such as L-AP3 and MCPG (a new ACPD receptor antagonist) has been questioned (Schoepp and Conn, 1993). One of the reasons for the non-selectivity of ACPD receptor antagonists may be due to the existence of multiple subtypes of ACPD receptors. L-AP3 is a ACPD receptor antagonist which has been reported to block the ACPD-induced PI turnover (Schoepp et al., 1990). TNS still induced LTP of the EPSPs in the CA1 neurons in the presence of L-AP3. It therefore appears that the LTP-inducing action of TNS does not require the activation of the ACPD receptor subtype which is involved in PI turnover in hippocampus slices.

Inhibition of PKC activity (Lovinger et al., 1987; Malinow et al., 1989) and chelation of postsynaptic free Ca^{2+} (Lynch et al., 1983; Morishita and Sastry, 1991) have been shown to block the induction of LTP produced by tetanus. Both presynaptic and postsynaptic PKC play a crucial role in the induction and/or maintenance of LTP. In the presence of sphingosine, a PKC inhibitor,

TNS failed to induce LTP of the EPSP. Injection of K-252b, a potent and hydrophilic PKC inhibitor, into the hippocampal CA1 neurons prevented the TNS-induced LTP in these neurons. It is possible that an increase in PKC activity is required for the LTP-inducing action of TNS. It is not certain whether both presynaptic and postsynaptic PKC activations are needed for the TNS-induced LTP. In K-252b injected neurons in which only postsynaptic PKC was inhibited, short-term potentiation (STP) was observed following the application of TNS. Application of sphingosine to the slices presumably blocked both presynaptic and postsynaptic PKC activities. Neither STP nor LTP of the EPSP was produced by TNS in the presence of sphingosine. It is likely that both presynaptic and postsynaptic PKC are involved in the LTP-inducing action of TNS and mediates different phases of LTP produced by TNS. An increase in the postsynaptic free Ca^{2+} occurs following tetanic stimulation. The rise of Ca^{2+} may activate protein kinases such as PKC, which are responsible for LTP. Chelation of postsynaptic free Ca^{2+} blocks the induction of LTP (Lynch et al. 1983). In BAPTA-injected neurons, TNS failed to induce LTP of the EPSP. In these neurons, TNS in fact produced a transient depression of the EPSP. However, the significance of the transient depression is not understood at present. It appears that postsynaptic free Ca^{2+} is required for the LTP-inducing action of TNS.

Overall, the LTP-inducing substances in TNS may act on both postsynaptic and presynaptic sites because both postsynaptic PKC activity and free Ca^{2+} are required for the LTP-inducing action of TNS, and because some presynaptic elements such as presynaptic activity may be involved in the LTP-inducing action of TNS. The LTP-inducing action of TNS does not involve the activation of NMDA receptors and the ACPD receptor subtype that mediates the PI turnover in the hippocampus.

9.1.4. *The possible feedback mechanisms*

It is generally believed that the induction of LTP, under normal conditions, is primarily mediated through postsynaptic mechanisms, mainly via the activation of NMDA receptors (Collingridge et al., 1983). NMDA antagonists such as APV and MK-801 prevent the induction of LTP (Collingridge et al., 1983). However, the processes that follow the activation of NMDA receptors are not very clear. An increase in postsynaptic free Ca^{2+} using photolysis of nitr-5 leads to a potentiation of the EPSP (Malenka et al., 1988). Ca^{2+} influx through the open NMDA channels may trigger the activation of a series of protein kinases such as PKC. The activation of protein kinases may modify certain receptors and channels. An increase in sensitivity of AMPA receptors during LTP has been reported (Davies et al., 1989). The increase is prevented by K-252b, a PKC inhibitor. While postsynaptic modification seems to occur during LTP, presynaptic modification is thought to play a major role in the maintenance of LTP. An increase in glutamate release has been suggested to be primarily responsible for the maintenance of LTP (Dolphin et al., 1982; Bliss et al., 1986; also see Aniksztejn et al., 1989; Roisin et al., 1990). Since it is believed that the induction of LTP is primarily mediated through postsynaptic mechanisms and the maintenance of LTP is mainly due to presynaptic mechanisms, interactions between postsynaptic cells and presynaptic terminals must occur during the LTP process. Furthermore, depolarization of the postsynaptic cell, when paired with the stimulation of afferents, induces LTP of the EPSP (Sastry et al., 1986; Kelso et al., 1986; Wigstrom et al., 1986). The depolarization may cause a release of some substances from the postsynaptic cell and the released substances can then act in a retrograde manner to modify the presynaptic terminals leading to LTP (Sastry et al., 1986). Therefore, retrograde messengers have been proposed to play a role in the communication between postsynaptic cells and

presynaptic terminals (Sastry et al., 1986; Bliss et al., 1986). The hypothesis of retrograde messengers involves three basic steps: (1) some substances are released from postsynaptic cells during the induction of LTP; (2) the released substances quickly diffuse to presynaptic terminals through the extracellular fluid; (3) the substances interact with presynaptic terminals causing an increase in transmitter release that is responsible for the maintenance of LTP. Several agents such as arachidonic acid, NO and CO have been suggested to be retrograde messengers for LTP. However, there is lack of clear evidence to prove that these agents are retrograde messengers at present (see review section in this thesis). Therefore, the search for retrograde messengers continues. In the present study, samples collected from the extracellular fluid during, but not without or after, tetanic stimulation of rabbit neocortex induced LTP in the guinea pig hippocampal slices. This result indicates that the LTP-inducing substances are released into the extracellular fluid during tetanic stimulation (presumably during the induction of LTP in the tetanized neocortex). Do these substances act as retrograde messengers for LTP? To answer this question, one has to decide whether the substances are released from postsynaptic cells and whether they cause LTP by interacting with presynaptic terminals. The release, but not the action, of the LTP-inducing substances collected from rabbit neocortex during tetanic stimulation was blocked by NMDA antagonists. It is apparent that the substances are released during the activation of NMDA receptors and then act on the steps after the activation of NMDA receptors. Since the release of the LTP-inducing substances is NMDA receptor-dependent and NMDA receptors are primarily localized on postsynaptic cells, it is logical to suggest that the release of the LTP-inducing substances mainly occurs in postsynaptic cells during the activation of NMDA receptors produced by tetanus. Some presynaptic elements are likely to be involved in the

LTP-inducing actions of the substances because of the requirement of presynaptic activity. It is believed that stimulation of the afferents is needed for activating NMDA and/or ACPD receptors during the induction of LTP. This is however not the case for the LTP-inducing action of the substances since LTP induced by TNS is independent of the activation of NMDA and ACPD receptors. It is possible that interactions between TNS and the presynaptic terminals occur in an activity-dependent manner. Therefore, the LTP-inducing substances seem to follow the three basic steps (from release to action) to act as retrograde messengers for LTP. In fact, fractions with different molecular weights of TNS can induce LTPs which develop with different time courses. The >50 kDa fraction of TNS induced LTP much faster than the <3 kDa and 3-10 kDa fractions of TNS. It is possible that substances with different molecular weights are responsible for different stages of LTP. The findings discussed above support the notion that some of the substances in TNS may play a role as retrograde messengers for LTP. However, some of the present results cannot be explained simply by a retrograde interaction mechanism alone. Intracellular injection of K-252b (a PKC inhibitor) or BAPTA (a Ca^{2+} chelator) into postsynaptic CA1 neurons prevented LTP produced by TNS in these neurons. This result indicates that postsynaptic free Ca^{2+} and PKC activity are involved in the TNS-induced LTP. Some substances in TNS must cause postsynaptic modifications which are crucial for LTP. There are at least two explanations for the involvement of postsynaptic modifications in the LTP-inducing action of TNS. One is that postsynaptic PKC and free Ca^{2+} are needed for the TNS-induced LTP even though the postsynaptically released TNS directly interacts with only the presynaptic terminals. The other is that TNS is released from more than one source during tetanic stimulation and then acts on both postsynaptic and presynaptic sites to cause LTP. Chelation of postsynaptic Ca^{2+} and inhibition of

PKC do not affect normal postsynaptic responses such as EPSP and IPSPs (Lynch et al., 1983; Morishita and Sastry, 1991; Xie and Sastry, 1991). If the TNS-induced LTP is mediated by a persistent increase in transmitter release alone, chelation of postsynaptic free Ca^{2+} and inhibition of PKC should not block the LTP-inducing action of TNS. Therefore, the first explanation seems to be difficult to understand. Recent evidence from both quantal analysis and biochemical studies indicates that the process of the tetanus-induced LTP may involve both presynaptic and postsynaptic changes (Kullmann and Nicoll, 1992; Liao et al., 1992; Larkman et al., 1992; Otani and Ben-Ari, 1993). Following the activation of NMDA receptors, both postsynaptic free Ca^{2+} and PKC activity are needed for postsynaptic modifications such as phosphorylation of receptors and channels which may change the sensitivity of postsynaptic receptors irreversibly. Even though the activation of NMDA receptors is not required for the TNS-induced LTP, postsynaptic free Ca^{2+} and PKC activity are needed for TNS to cause postsynaptic modifications. Therefore, the second explanation is more reasonable. In addition to postsynaptic cells, other sources such as glia and presynaptic terminals, particularly glial cells, can also release some substances contained in TNS which are responsible for LTP. It is known that certain proteins which have growth-inducing effect can be released from glial cells (Lindsay, 1979). In goldfish, some of the proteins released from glia are related to learning (Shashoua, 1979). It has been reported that LTP can be induced in the CA1 neurons when stimulations of afferents are paired with depolarization of nearby glial cells (Sastry et al., 1988c). Therefore, it will not be surprising if some of the LTP-inducing substances are released from glial cells. It is debatable whether NMDA receptors exist in glial cells. However, the release of certain LTP-inducing substances from glial cells can be regulated by NMDA receptors in glial cells and/or postsynaptic neurons. APV has been reported to

reduce the depolarization of glial cells induced by tetanic stimulation (Sastry, et al., 1988c). If NMDA receptors are indeed present in glial cells, they can directly mediate the release of the substances. On the other hand, activation of postsynaptic NMDA receptors during tetanic stimulation can release K^+ into extracellular space and subsequently facilitate the glial depolarization leading to the release of the substances. The released substances can act on both presynaptic terminals and postsynaptic cells and cause LTP in CA1 neurons. Postsynaptic NMDA receptors may control the release of substances from the presynaptic terminals in a similar manner. Therefore, the release of the LTP-inducing substances from the terminals should not be completely ruled out although there is no conclusive and direct evidence for the existence of NMDA receptors in presynaptic terminals. Finally, it is necessary to determine whether the interaction between the LTP-inducing substances and the presynaptic terminals causes an increase in glutamate release from the presynaptic terminals in order to make a conclusion on the exact role of these substances. Further investigation is required to resolve this matter.

In summary, the present studies provide further evidence to support the previous finding from our laboratory that some substances are released from rabbit neocortex and guinea pig hippocampus during tetanic stimulation and these substances can induce LTP when collected and re-applied to the hippocampal slices (Chirwa and Sastry, 1986; Sastry et al., 1988a). The release, but not the action, of the LTP-inducing substances is NMDA receptor-dependent. The LTP-inducing substances are probably released from multiple sources, primarily postsynaptic cells and glial cells. The exact sources for the release of the LTP-inducing substances require further investigation. The released substances act downstream from the NMDA receptors. It is apparent that postsynaptic free Ca^{2+} and PKC activity, and activation of presynaptic

terminals are required for the LTP produced by the endogenous substances. Whether the LTP-inducing action involves an increase in glutamate release from presynaptic terminals remains to be determined. Substances with different molecular weights may mediate certain stages of LTP. Some substances in TNS may act as retrograde messengers for LTP. However, the present findings cannot be explained by the hypothesis of retrograde messengers alone, but supports the idea that both presynaptic and postsynaptic modifications occur during the LTP process after the activation of NMDA receptors.

Although results in the present study show the ability of different substances released from the neocortical surface to induce LTP in hippocampal slices, whether the substances released from the hippocampus during LTP-inducing tetanic stimulation would behave in a similar manner needs further examination. The reasons for analyzing the actions of the endogenous substances released from the neocortex relate to the ease with which the samples could be collected in sufficient concentration and to the well-known similarities between LTP in the neocortex and the hippocampus. Furthermore, it is known from previous studies from our laboratory that TNS collected from the guinea pig hippocampus can cause LTP in hippocampus slices.

9.2. Involvement of α -tocopherol in LTP

α -Tocopherol (vitamin E), as a major antioxidant in the biological system, can scavenge free radicals attacking from outside of the membrane and within the membrane and is essential for the maintenance of normal structure and function of the human nervous system. There were several reasons leading to our present investigation of involvement of α -tocopherol in LTP. First, vitamin E has been suggested to be involved in spatial learning and memory. The impairment of spatial learning ability is correlated with an increase in lipid peroxide content of the vitamin E deficient rat hippocampus (Moriyama et al.,

1990). LTP is thought to be the cellular mechanism underlying learning and memory. Second, free radicals such as hydrogen peroxide have recently been shown to facilitate the decay of LTP (Pellmar et al., 1991). Third, in cultured central neurons, α -tocopherol has been shown to act as a growth-inducing factor which supports the survival of the neurons and enhances neurite growth (Nakajima et al., 1991). Neurotrophic factors have been reported to facilitate LTP (Sastry et al., 1988b; Patterson et al., 1992). Fourth, changes in the levels of free radicals and antioxidants such as vitamins E and C have been observed in patients with Alzheimer' disease, which is associated with memory impairment (Wartanowicz et al., 1984; Jeandel et al., 1989; Carney et al., 1991; Smith et al., 1991). These lines of evidence suggest that vitamin E and free radicals are probably associated with certain forms of learning and memory. Therefore, it is worthwhile to examine the effects of α -tocopherol on LTP.

Although increasing evidence shows that α -tocopherol plays a significant role in the normal functioning of the brain (Tappel, 1962; Sokol, 1988, 1989), the electrophysiological effect of the agent is not very well-known. One of the main reasons is that the neutral form of α -tocopherol cannot be readily dissolved without using relatively high concentration of solvent such as DMSO or ethanol. DMSO and ethanol themselves have electrophysiological effects on the hippocampal neurons. Therefore, α -tocopherol phosphate (disodium salt) which dissolves in aqueous solutions and has the properties of α -tocopherol was chosen in the present study.

The observation that α -tocopherol phosphate has the ability to induce a slowly developing long-lasting potentiation of the EPSP in hippocampal CA1 neurons suggests that this agent can induce LTP. Since this action of α -tocopherol occurred without either a significant change in the membrane potential and the input resistance or an alteration in the fast and the slow IPSPs

in the CA1 neurons, it is possible that the potentiation of the EPSP is due to a selective action of the agent. The finding that the LTP caused by α -tocopherol occluded any further LTP by a tetanic stimulation of the stratum radiatum, and vice versa, suggests that the drug- and the tetanus-induced LTP may share some common mechanisms.

To further confirm the involvement of α -tocopherol in LTP, the ability of tetanic stimulation and application of α -tocopherol to generate LTP in vitamin E deficient rats was examined. It is known that vitamin E deficiency in animals takes months to develop using a vitamin E deficient diet (Horwitt, 1962). In rats fed with vitamin E deficient diet for 1 or 2 months, the LTP produced by tetanic stimulation did not show any significant difference from the LTP obtained from the hippocampal slices of control rats. However, the ability of tetanic stimulation to generate LTP was significantly impaired in the rats fed with vitamin E deficient diet for 3 months. This finding indicates that vitamin E plays a significant role in the LTP process although the exact mechanisms for the impairment in LTP generation in the vitamin E deficient rats are not clear at present. Application of α -tocopherol also failed to induce LTP in the vitamin E deficient rats. It is possible that the vitamin E deficiency causes severe malfunctioning of certain receptors or channels, and the acute application of α -tocopherol cannot ameliorate the effects of chronic vitamin E deficiency.

9.2.1. Possible mechanisms for the actions of α -tocopherol

Free radicals have been shown to modulate NMDA receptor function (Aizenman et al., 1990). However, since APV did not significantly affect the LTP produced by the vitamin, and since the vitamin did not change the NMDA-induced depolarization of the CA1 neurons, it is unlikely that NMDA receptor activation is required for the LTP-inducing action of α -tocopherol. The LTP-inducing action of α -tocopherol is not due to a change in non-NMDA receptor

sensitivity because α -tocopherol did not change the non-NMDA receptor-mediated depolarization. Since the LTP-produced by α -tocopherol is blocked by L-AP3, it is possible that the LTP-inducing action of the agent involves the activation of certain subtype of ACPD receptors. In the light of the selectivity of L-AP3 on ACPD receptors being controversial, a conclusion of the involvement of ACPD receptors in the LTP-inducing action of α -tocopherol cannot be made until more selective ACPD receptor antagonists are available. L-AP3 has been reported to block the ACPD-induced PI turnover in hippocampal slices which can cause PKC activation and release of internal Ca^{2+} (Schoepp et al., 1990). Both inhibition of PKC activity and chelation of postsynaptic free Ca^{2+} could prevent the α -tocopherol-induced LTP. It is not certain whether the blockade of the LTP-inducing action of α -tocopherol produced by PKC inhibitors and Ca^{2+} chelators are directly related to the activation of the ACPD receptor subtype which is sensitive to L-AP3. Further investigation is required to answer this question. The requirement of PKC activity and postsynaptic free Ca^{2+} for the LTP-inducing action of α -tocopherol are similar to that for the tetanus-induced LTP. The result further supports the idea that the tetanus- and α -tocopherol-induced LTP share some common mechanisms even though the mechanism for the activation of PKC and the rise of postsynaptic free Ca^{2+} may not be the same in both cases. While the tetanus-induced LTP has a more rapid onset, the vitamin E-induced LTP takes several minutes to develop. It is believed that the tetanus-induced LTP is composed of several components (Racine et al., 1983). LTPs induced by different fractions of the endogenous substances (in TNS) discussed above develop with different time courses. It is possible that α -tocopherol is involved in the development of the late stages of LTP. Previous results show that the LTP-inducing action of the endogenous substances occurs in the steps following NMDA receptor activation. If α -tocopherol is one of the substances released by

tetanic stimulation, then it is not surprising that its LTP-inducing action does not require the activation of NMDA receptors. Further investigation is required to examine this possibility.

The LTP-inducing action of the vitamin may be due to its antioxidant property, since α -tocopherol is a major lipid-soluble antioxidant in the biological system. L-Ascorbic acid (vitamin C), a water-soluble antioxidant, was used to compare the effects of α -tocopherol. Application of sodium ascorbate did not cause a significant LTP of the EPSP in the hippocampal CA1 neurons. It is not clear whether the lack of effect by ascorbic acid is due to its inability to remove free radicals within the membrane, or whether the scavenging of free radicals is not a mechanism for the LTP-inducing action. If the latter is true, the mechanisms of the LTP-inducing action may be unrelated to its ability to combat the detrimental actions of free radicals, but be due to a specific interaction between α -tocopherol and certain receptors. Whether α -tocopherol directly bind to certain receptors to induce LTP remains to be determined. On the other hand, it is possible that the removal of damaging effects of the free radicals in the membrane, rather than on the outer membrane, is involved in LTP. Perhaps, ascorbic acid has effects only on the outer membrane whereas α -tocopherol, being lipid soluble, produce its effects within the cell membrane. Trolox-C, a structural analog of α -tocopherol, and DMSO are antioxidants which can cross the biological membranes. It has been reported that Trolox-C and DMSO do not change the population EPSP or the population spike in the CA1 area during application (Pellmar et al., 1989). The long-term effects of these agents have not been reported. In fact, DMSO was used in the present study. The EPSP was potentiated by the application of DMSO, but returned to the baseline once the drug was withdrawn. It is apparent that DMSO does not have the LTP-inducing action. However, both Trolox-C and DMSO are very weak antioxidants.

The negative results of these drugs cannot rule out the probability that the LTP-inducing action of α -tocopherol is due to its antioxidant property. Further examination using more comparable antioxidants will help to resolve this matter. One interesting possibility is that free radicals are continually being generated in the tissue and that they dampen the expression of LTP. Hydrogen peroxide has been shown to facilitate the decay of LTP (Pellmar et al., 1991). If tetanic stimulations that induce LTP also generate significant levels of free radicals, the LTP process may be affected. On the other hand, vitamin E may also be released by tetanic stimulation as a protective measure against the damaging effects of free radicals so that the process of LTP consolidation may take place.

It has been suggested that arachidonic acid and/or its metabolites can act as second messengers for the induction of LTP (Williams et al., 1989; Williams and Bliss, 1989). An increase in arachidonic acid concentration has recently been found in a postsynaptic membrane fraction after the induction of LTP in the dentate gyrus of the hippocampus (Clements et al., 1991). α -Tocopherol has been shown to modulate the metabolism of the arachidonic acid cascade (Reddy et al., 1988). There is also evidence that α -tocopherol can stimulate arachidonate release from human endothelial tissues (Tran and Chan, 1988). However, the agent induces the opposite effect in some other tissues (Reddy et al., 1988). The precise effect of vitamin E on arachidonic acid in the CA1 area of the hippocampus is, however, unknown. Therefore, further investigation is required to examine whether the LTP-inducing action of α -tocopherol occurs via arachidonic acid.

α -Tocopherol has been reported to have a growth-inducing action in cultured neurons (Nakajima et al., 1991; Sato et al., 1993). It is not clear whether the growth-inducing action of α -tocopherol is due to its antioxidant activity or to its direct interactions with certain receptors. Neurotrophic factors

have been reported to be involved in the induction of LTP (Sastry et al., 1988b). The endogenous substances collected from rabbit neocortex during tetanic stimulation not only induce LTP in the hippocampal slices but also enhance neurite growth in cultured PC-12 neurons (Sastry et al., 1988a). The possibility that the LTP-inducing action and the growth-inducing effect of α -tocopherol share some common mechanisms cannot be ruled out. Further investigations are required to examine this possibility.

9.2.2. *The significance of the LTP-inducing action of α -tocopherol*

Evidence is available in literature which implicates an increase in the damage caused by free radicals in the process of aging and in the development of degenerative diseases (Wartanowicz, 1984). A reduction of α -tocopherol levels have also been reported in the serum of Alzheimer's patients (Jeandel et al., 1989; Smith et al., 1991). It has been suggested that the memory process, which is adversely affected in Alzheimer's patients, may be a target for free radicals, although further evidence is required to accept this as a mechanism involved in the onset of the disease. In both human and animal studies, α -tocopherol was shown to prevent or reduce the damaging effects causing free radicals by slowing the cellular aging process (Meydani et al., 1985). Since LTP is thought to be involved in learning and memory (Teyler and DiScenna, 1984; Thompson, 1986), presumably the LTP-inducing action of the vitamin is involved in some of the actions mentioned above. The revelation of the mechanisms underlying the involvement of vitamin E in LTP will not only help us understand how the LTP process can be modulated by a variety of factors, but also aid the discovery of agents which can improve learning and memory.

9.3. Modulation of LTP by GABAergic inhibition

9.3.1. LTP and IPSPs

Stimulation of the afferents which evokes EPSPs in CA1 neurons, also induces fast and slow IPSPs in these neurons, mediated through GABA_A and GABA_B receptors, respectively (Dutar and Nicoll, 1988). Under normal conditions, tetanic stimulation not only induces LTP of the EPSP but also causes long-term changes in the fast and slow IPSPs. It is however a controversial issue as to whether tetanic stimulation of the afferents causes long-term potentiation or depression of the IPSPs (Abraham et al., 1987). Changes in the IPSPs significantly affect the LTP process because the modification of GABAergic inhibition by GABA_A and GABA_B agonists and antagonists can modify the LTP process (Wigstrom and Gustafsson, 1983; Davies et al., 1991; Mott and Lewis, 1991; Ople and Karlsson, 1990). If tetanic stimulation induces a long-term potentiation or depression of the IPSPs in the CA1 neurons, the expression of LTP of the EPSP will be affected. Postsynaptic GABA_B receptors are primarily localized in the apical dendrites close to the sites where most of the NMDA receptors are located. Activation of GABA_B receptors causes an increase in the membrane conductance which can shunt the currents generated by NMDA channel activation. Postsynaptic GABA_A receptors are localized in both the soma and dendrites of the CA1 neurons. Activation of GABA_A receptors greatly affects the expression and the shape of the NMDA receptor-mediated EPSP. Therefore, it is important to determine as to what kind of changes occur following tetanic stimulation and how the changes modify LTP of the EPSP.

In the present study, tetanic stimulation not only induced LTP of the EPSP, but also caused a small LTP of the fast IPSP without changing the slow IPSP in the CA1 neurons. The potentiation of the fast IPSP had a time course similar to LTP of the EPSP. The slow IPSP basically remained unchanged after

tetanic stimulation. In the picrotoxinin-treated slices, tetanic stimulation caused a small depression of the slow IPSP. How did these changes in IPSPs take place? It is known that tetanic stimulation of the afferents causes Ca^{2+} influx and PKC activation which probably trigger LTP of the EPSP in the CA1 neurons. Intracellular free Ca^{2+} and PKC, acting as second messengers, affect not only the EPSP but also the IPSPs. An elevation of intracellular free Ca^{2+} has been reported to decrease the fast IPSP (Chen et al., 1990), and an increase in PKC activity has been shown to depress the fast and slow IPSPs (Baraban et al., 1985; Stelzer, 1992). It is expected that chelation of postsynaptic free Ca^{2+} and inhibition of PKC activity will prevent some of the changes in the EPSP and the IPSPs produced by tetanic stimulation. Our observation that LTP of the EPSP did not occur in BAPTA- or K-252b- contained CA1 neurons is in agreement with the reports by others (Lynch et al., 1983; Morishita and Sastry, 1991; Malinow et al., 1989). Interestingly, unlike the EPSP, both the fast and the slow IPSPs in the BAPTA- or K-252b-injected neurons increased significantly after tetanic stimulation. LTP of the fast IPSP in the BAPTA- and K-252b-injected neurons were much larger than that of the control neurons. The potentiation of the slow IPSP occurred only in the neurons injected with BAPTA or K-252b. It is apparent that postsynaptic free Ca^{2+} and K-252b play a significant role in modulating the IPSPs. Increases in postsynaptic free Ca^{2+} and PKC activity by tetanic stimulation cause not only the potentiation of the EPSP but also the suppression of the increase in the fast and slow IPSP in the CA1 neurons. Since either the blockade of the fast IPSP by GABA_A antagonists or the suppression of the slow IPSP by GABA_B antagonists can facilitate the induction of LTP at excitatory synapses, the suppression of the potentiation of both the fast and the slow IPSPs, caused by the rise of intracellular Ca^{2+} and the increase in PKC activity during tetanic stimulation, may minimize a dampening of the expression of LTP

of the EPSP by the potentiated IPSPs. It is known that the blockade of the fast IPSP enhances the amplitude and the duration of the EPSP because the late phase of EPSP and the early phase of fast IPSP largely overlap (Davies et al., 1990). Enhancement of the fast IPSP can reduce the NMDA receptor-mediated EPSP. Before the induction of LTP of the slow IPSP, application of phaclofen blocked the slow IPSP but had little effect on the EPSP. It is probably due to the time courses of the EPSP and slow IPSP are quite far apart so that the influence of the two responses on each other is minimal. However, after the induction of LTP of the slow IPSP, phaclofen not only blocked the slow IPSP but also enhanced the duration and the amplitude of the EPSP in most cells tested. It appears that the potentiated slow IPSP has more influence on the EPSP. This evidence supports the idea that LTP of the IPSPs produced by tetanic stimulation would interfere with the expression of LTP of the EPSP. However, under normal conditions, LTP of the IPSPs is suppressed by postsynaptic free Ca^{2+} and PKC so that LTP of the EPSP can be better expressed. The findings in the present study indicates that LTP of the IPSPs occurs in CA1 pyramidal neurons in addition to LTP of the EPSP. The fast and slow IPSPs may not directly control the induction of LTP of the EPSP, but they certainly can modulate the expression of LTP of the EPSP to a great extent under normal conditions as the roles of postsynaptic Ca^{2+} and PKC are not only to potentiate the EPSP but also to depress the IPSPs.

9.3.2. Possible mechanisms for LTP of the IPSPs

Since both fast and slow IPSPs recorded in CA1 neurons comprise monosynaptic and polysynaptic components, LTP of the IPSPs cannot be simply explained by the change of free Ca^{2+} concentration and PKC activity in CA1 pyramidal neurons. The expression of the IPSPs in pyramidal CA1 cells can be controlled by both postsynaptic and presynaptic sites. Postsynaptic

modifications probably include changes in receptor sensitivity and reversal potentials of certain ion channels. Since reversal potentials of the fast and slow IPSPs do not change during LTP of the IPSPs, they are not responsible for the potentiation of the IPSPs. It has been reported that tetanic stimulation causes a reduction of GABA receptor sensitivity which is blocked by APV (Stelzer, 1992; Rai and Stelzer, 1993). It has been suggested that the decrease in GABA receptor sensitivity is due to Ca^{2+} influx through NMDA channels. The idea is supported by the present finding that the IPSPs were potentiated but not depressed after tetanic stimulation in the neurons injected with BAPTA or K-252b. Presynaptic modification is more complicated because it involves changes in CA1 interneurons which give off axons to innervate CA1 pyramidal cells. Alterations in interneurons can directly affect the IPSPs in CA1 pyramidal cells. It has been reported that tetanic stimulation of the stratum radiatum not only induces LTP of the EPSP in CA1 pyramidal cells, but also causes an increase in excitability of CA1 interneurons (Rai and Stelzer, 1993). Furthermore, LTP of the EPSP in CA1 interneurons has been observed (Taube and Schwartzkroin, 1987; Reece and Redman, 1992; Rai and Stelzer, 1993). The mechanisms involved in interneuron LTP are not clear yet. It is apparent that the induction of interneuron LTP of the EPSP and the increase in excitability of interneurons may contribute to LTP of the IPSPs in CA1 pyramidal cells.

The observation that tetanic stimulation failed to induce LTP of the slow IPSP in the control, BAPTA- and K-252b-injected neurons in the presence of APV suggests that NMDA receptors play a role in the induction of LTP of the slow IPSP. At the same time, tetanic stimulation still caused a small LTP of the fast IPSP in the presence of APV. However, this LTP of the fast IPSP has no significant difference among the control, BAPTA- and K-252b-injected neurons, and it was smaller than the LTP of the fast IPSP obtained in the neurons injected

with BAPTA or K-252b in the absence of APV. It is apparent that APV reduces LTP of the fast IPSP and prevents the induction of LTP of the slow IPSP. Presumably, APV blocked the Ca^{2+} influx through NMDA receptors during tetanic stimulation. If an increase in intracellular free Ca^{2+} and PKC activity decreases GABA receptor sensitivity in CA1 pyramidal cells, the blockade of Ca^{2+} influx by APV should promote LTP of the IPSPs. This was not the case in the present study. These results might not be due to Ca^{2+} influx through voltage-gated Ca^{2+} currents because no difference had been observed between data obtained from the control and BAPTA-injected neurons. It is apparent that there are other factors involved in altering the IPSPs. LTP of the EPSP in CA1 interneurons should have direct influence on the change of the IPSPs. If interneuron LTP is blocked by APV, LTP of the IPSPs in CA1 pyramidal cells will be depressed. Although evidence in the literature shows that LTP of the EPSP in CA1 interneurons occurs after tetanic stimulation, it is not known whether interneuron LTP is sensitive to APV (Rai and Stelzer, 1993). In order to further examine the possible involvement of interneuron LTP of the EPSP in LTP of the IPSPs in CA1 pyramidal cells, monosynaptic IPSPs were examined. In the presence of APV and CNQX, glutamatergic transmission in hippocampal slices was presumably abolished. In this case, the IPSPs in CA1 pyramidal cells were evoked by direct activation of the axons of interneurons. Recurrent IPSP in CA1 pyramidal cells was also blocked. Tetanic stimulation did not cause LTP of the slow IPSP and induced only a small LTP of the fast IPSP in the control, BAPTA- and K-252b-injected CA1 pyramidal cells. It is not unexpected that the changes in the IPSP showed no difference among control, BAPTA- and K-252b injected neurons because APV and CNQX blocked the depolarization caused by tetanic stimulation, and subsequently prevented Ca^{2+} influx through both NMDA channels and voltage-gated channels. The findings suggest that LTP of the

IPSPs may be greatly affected by changes in interneuron EPSP. The small LTP of the fast IPSP observed in the presence of APV and CNQX was similar to the one obtained in the presence of APV alone. The mechanism of this small LTP is not clear (it is, however, possible that a residual glutamatergic transmission is present during a tetanic stimulation in the presence of the antagonists). Perhaps, the fast IPSP can be potentiated by direct stimulation of the axons of interneurons, and the IPSP in pyramidal cells can increase further if LTP of the EPSP in the CA1 interneurons occurs after tetanic stimulation. The slow IPSP did not increase at all after tetanic stimulation in the presence of APV and CNQX or APV alone. In fact, the slow IPSP following tetanic stimulation was slightly decreased in the presence of these antagonists. The difference between the post-tetanic changes in the fast and the slow IPSPs is not very clear. It is possible that the function of GABA_A and GABA_B receptors is mediated through different mechanisms. It has also been reported that GABA_A and GABA_B receptor-mediated responses are innervated by different groups of interneurons (Lacaille et al., 1989). Furthermore, stronger stimulation strength is usually required to evoke the slow IPSP (Dutar and Nicoll, 1988). Further investigation is needed to clarify these matters.

In summary, the IPSPs in pyramidal neurons are controlled by the changes in CA1 pyramidal neurons and interneurons. In CA1 pyramidal cells, an increase in intracellular free Ca²⁺ and PKC activity can depress the fast and slow IPSPs which allow a better expression of the EPSP in the pyramidal cells. The increases in intracellular free Ca²⁺ and PKC activity may be mediated through the activation of NMDA channels at the excitatory synapses. It may be one of the examples in which excitatory and inhibitory synapses interact with each other through second messengers in postsynaptic cells. In the presynaptic sites, excitability of interneurons may have direct influence on the release of

inhibitory transmitters such as GABA from the axons which make synaptic contacts with the pyramidal cells. The idea is supported by the finding that the blockade of glutamatergic transmission completely blocked LTP of the slow IPSP and reduced LTP of the fast IPSP. The small LTP of the fast IPSP observed in the presence of APV and CNQX may represent the real change of the fast IPSP produced by direct stimulation of the axons of interneurons. The finding also suggests that the fast and slow IPSPs are either mediated through different mechanisms or innervated by different groups of interneurons in CA1 area. Under normal conditions, the balance between the postsynaptic and presynaptic forces will determine the expression of the IPSPs in CA1 pyramidal cells. During the LTP process, both the fast and the slow IPSPs are suppressed by tetanic stimulation through free Ca^{2+} and PKC activity, which in turn allows a better expression of LTP of the EPSP in CA1 pyramidal cells.

9.3.3. Somatostatin and GABA-ergic inhibition

As previously discussed, GABA-ergic inhibition can modulate LTP of the EPSP in CA1 pyramidal cells. It is interesting to examine whether somatostatin (SS), an endogenous neuropeptide which is localized in some GABA-ergic interneurons in CA1 area (Kunkel and Schwartzkroin, 1988; Somogyi et al., 1984), can modulate the induction of LTP of the EPSP. The interactions between the actions of somatostatin and GABA-ergic IPSPs at synapse may occur since it is possible that the peptide and the amino acid are co-released from the same terminal. SS has been reported to hyperpolarize the CA1 pyramidal cells (Pittman and Siggins, 1981). SS has also been shown to suppress the GABA receptor-mediated fast and slow IPSPs in the CA1 neurons (Scharfman and Schwartzkroin, 1989). The mechanisms underlying the actions of SS on hippocampal neurons have been under debate. However, since it is apparent that SS has interactions with GABA-ergic inhibition, the peptide is

probably able to modulate the induction of LTP of the EPSP in CA1 pyramidal neurons. Before examining the effects of SS on LTP of the EPSP, it is important to decide how SS interacts with GABA-ergic inhibition in CA1 pyramidal cells.

As reported in the literature (Pittman and Siggins, 1981), SS hyperpolarized the CA1 neurons. This effect was associated with a reduction in the input resistance of the cells. The reversal potential for this action of the peptide was close to -90 mV, supporting the idea that it activated a K⁺ conductance (Twery et al., 1991). The type of K⁺ channel activated by SS was not investigated in this study, although it has been suggested that it might activate different types of K⁺ currents, including the fast transient (I_A) (Chen et al., 1990), the delayed rectifier (I_K) (Chen et al., 1990) and the M (I_M) (Moor et al., 1988) currents.

SS suppressed the GABA_A receptor-mediated fast IPSP and the GABA_B receptor-mediated slow IPSP in CA1 pyramidal cells, supporting the results from earlier studies (Scharfman and Schwartzkroin, 1989). The action of the peptide appears not to be due to a hyperpolarization (Twery and Gallagher, 1990), since clamping the membrane potential back to the pre-somatostatin level maintained the suppression. It is possible that the decrease in the input resistance caused by the peptide can account for the depressant action on the IPSPs. Since the EPSP is not significantly affected, the somewhat selective depression of the IPSPs by the peptide may be related to the distribution of SS receptors on the neurons.

If SS and GABA are co-released by the activation of GABA-ergic neurons, the peptide can access the subsynaptic GABA receptors. It is possible that SS may depress the IPSPs by directly interacting with GABA receptors. However, this possibility is ruled out by the finding that the actions of the peptide were not blocked by either picrotoxinin, a GABA_A antagonist, or phaclofen, a postsynaptic

GABA_B antagonist, at concentrations that blocked the fast and the slow IPSPs, respectively. It is unlikely that the peptide caused a hyperpolarization of the neurons through a presynaptic action since the action was observed on slices exposed to a Ca²⁺-free and high Mg²⁺-TTX-containing medium.

It has been suggested that SS can suppress the IPSPs by acting presynaptically (Scharfman and Schwartzkroin, 1989). Since the suppression of the fast IPSP caused by the peptide was not different in the presence or the absence of 2-OH-saclofen, which had been suggested to block the presynaptic GABA_B receptors involved in suppressing GABA release (Davies, et al., 1990), it is unlikely that SS acts on the presynaptic GABA_B receptors.

It appears that SS does not induce the actions on GABA-ergic inhibition by acting directly on GABA receptors. Since the hyperpolarization caused by baclofen or SS is mediated through K⁺ channels (Alger, 1984; Andrade et al., 1986) and certain types of G protein (Andrade, 1986), it is possible that both GABA_B and SS receptors are coupled to the same type of G-protein or channels. The observation that the activation of GABA_B receptors by baclofen occluded the hyperpolarizing action of SS in the CA1 neurons supports this idea. QX-314 suppresses the GABA_B receptor-mediated slow IPSPs (Nathan et al., 1990), as well as the hyperpolarization caused by baclofen or SS. QX-314 could depress the slow IPSP and the hyperpolarizing actions of SS or baclofen by acting on the agonist receptor-gated channels or intracellular messengers that modulate these channels. The suppressive effect of SS, but not of baclofen, on the fast IPSP was blocked in neurons injected with QX-314. It is, therefore, apparent that SS-induced suppression of the IPSP is due to a QX-314-sensitive postsynaptic effect, while the action of baclofen on this IPSP is not dependent on such a postsynaptic mechanism. The action of baclofen on the fast IPSP is probably mediated through presynaptic mechanisms such as the activation of presynaptic

GABA_B receptors (Dutar and Nicoll, 1988; Harrison et al., 1988; Lanthorn and Cotman, 1981; Misgeld et al., 1984; Ople et al., 1982).

Changes in intracellular free Ca²⁺ and PKC activity can alter the GABA receptor-mediated responses. However, neither chelation of postsynaptic free Ca²⁺ nor inhibition of PKC activity could interfere with the actions of SS in CA1 pyramidal neurons.

The present findings provide evidence that interactions between SS and GABA-ergic inhibition occur in CA1 pyramidal neurons. The interactions probably occur at the level of the channels or intracellular second messengers but not at the receptor level.

9.3.4. LTP and somatostatin

The functional significance of the SS-induced hyperpolarization and suppression of the GABA-ergic IPSPs is not known. SS has been reported to be involved in learning and memory (Haroutunian et al., 1987). Changes in the number of SS binding sites have been reported in patients with epilepsy (Riekkinen and Pitkanen, 1990) or Alzheimer's disease (Chan-Palay, 1987). Recently, SS has been shown to facilitate LTP of the population spike in the mossy fibre-CA3 pathway (Matsuoka et al., 1991). The mechanisms for this facilitatory action of SS are not known. It has not been reported whether this action involves the modulation of GABA receptors by SS. It is known that the mechanisms underlying LTP in Schaffer-collateral/commissural-CA1 and in mossy fibre-CA3 pathways are different. For example, unlike LTP in Schaffer collateral/commissural-CA1 pathways which is NMDA receptor-dependent, LTP in mossy fibre-CA3 pathways is not sensitive to NMDA antagonists (Zalutsky and Nicoll, 1990). Therefore, the action of SS on LTP in CA1 neurons may not be the same as that in CA3 neurons. Here actions of SS on LTP in CA1 neurons are discussed. The modulation of GABA-ergic inhibition has been known to

interfere with the induction of LTP of the EPSP. The depression of the fast and slow IPSP can facilitate the opening of NMDA channels by enhancing the depolarization caused by tetanic stimulation. On the other hand, hyperpolarization of the CA1 neurons during tetanic stimulation by injecting current can prevent the induction of LTP of the EPSP (Malinow and Miller, 1986). The effects of SS on CA1 neurons include a hyperpolarization, a reduction of input resistance and a depression of the IPSPs. The SS-induced depression of the IPSPs in CA1 neurons should facilitate the induction of LTP. On the other hand, the SS-induced hyperpolarization would prevent the induction of LTP. Therefore, the net effect of facilitatory and inhibitory forces may determine whether SS enhances or reduces LTP of the EPSP in CA1 neurons. The LTP induced by tetanic stimulation during the application of SS was not significantly different from the LTP produced by tetanic stimulation in the absence of SS. It is apparent that SS does not block the induction of LTP under normal conditions. Under these conditions, it is somewhat difficult to determine whether the peptide has facilitatory effects on LTP. Weak tetanus which can induce only short-term potentiation (STP) under normal conditions can induce LTP in the presence of picrotoxinin, a GABA_A antagonist, because picrotoxinin blocks the fast IPSP and facilitates the opening of NMDA channels during tetanic stimulation (Wigstrom and Gustafsson, 1983). Since SS, like picrotoxinin, suppressed the IPSPs, it was decided to determine whether this weak tetanus could induce LTP in the presence of SS. The result was that the peptide could not convert the STP induced by weak tetanus to a LTP in the CA1 neurons. The finding indicates that application of SS does not affect the induction of LTP in hippocampal CA1 neurons under normal conditions although the peptide can interact with GABA-ergic inhibition. It is possible that the effects of SS-induced depression of the IPSPs on LTP of the EPSP are minimized by

the effects of the hyperpolarization and the reduction in the input resistance of CA1 neurons caused by SS so that the LTP process is not disrupted by SS. However, the present finding cannot completely rule out the role of endogenous SS in LTP of CA1 neurons since endogenous SS released from presynaptic terminals may act more specifically than SS applied through the extracellular medium. The lack of selective SS receptor antagonists limits the studies of endogenous SS on synaptic transmission at present. The availability of selective SS receptor antagonists will provide a useful tool for exploring the possibility of involvement of endogenously released SS in LTP.

10. SUMMARY AND CONCLUSIONS

The findings reported in this thesis provide evidence that the LTP process can be modulated by a variety of factors. The studies focus on three major issues: (1) possible roles of endogenous substances collected during tetanic stimulation on the LTP process; (2) role of vitamin E in the LTP process; (3) interactions between LTP in excitatory and inhibitory synapses. The major findings can be summarized as follows:

1. Tetanic stimulation of rabbit neocortex releases substances which, when collected during tetanic stimulation and applied to guinea pig hippocampal slices, can induce LTP of the EPSP and population spike in those slices. The finding indicates that certain endogenous substances, which can induce LTP, transiently diffuse into extracellular fluid during tetanic stimulation.
2. The release process for the LTP-inducing substances requires NMDA receptor activation. The exact locus of the release remains to be determined. However, postsynaptic cells and/or glial cells may be the primary candidates for the sites of the release.

3. The LTP-inducing action of the substances does not require the activation of NMDA receptors and the subtype of ACPD receptors which are sensitive to L-AP3. This suggests that the endogenous substances act on the steps following the activation of NMDA.
4. Protein kinase C activity and postsynaptic free Ca^{2+} are required for the LTP inducing action of the endogenous substances. The LTP inducing action of the endogenous substances also requires the activation of presynaptic fibres. Since LTP induced by the endogenous substances involves both presynaptic and postsynaptic actions, the LTP-inducing action of these substances cannot simply be explained by the hypothesis of retrograde messengers alone.
5. Different molecular weight fractions of the sample collected from neocortex during tetanic stimulation can induce LTPs with different time courses when the fractions are applied separately. The finding suggests that multiple LTP-inducing substances are released during tetanic stimulation and these different substances may be responsible for different stages of LTP.
6. Gel electrophoresis of samples reveals the presence of a protein with a molecular weight of about 69 kDa in the samples collected from rabbit neocortex during, but not without, tetanic stimulation. The samples collected during tetanic stimulation may also contain more low molecular weight substances than those collected without tetanic stimulation; but the gel used in the present studies cannot accurately identify the low molecular weight (<10 kDa) substances. These findings further support the idea that different substances are involved in different stages of LTP of the EPSP.
7. Application of α -tocopherol phosphate can induce a slowly developing LTP of the EPSP without affecting the IPSPs and the excitability of the CA1 neurons in hippocampal slices. LTPs induced by tetanic stimulation and

α -tocopherol occlude each other. The finding suggests that the two LTP may share some common mechanisms.

8. The LTP caused by α -tocopherol does not require the activation of NMDA receptors. However, this LTP may be mediated at least in part by the activation of the subtype of ACPD receptors which is sensitive to L-AP3. α -Tocopherol does not change the NMDA and non-NMDA receptor-mediated responses.
9. Protein kinase C activity and postsynaptic free Ca^{2+} in CA1 neurons are needed for the induction of α -tocopherol-induced LTP.
10. Ascorbic acid (water soluble antioxidant) and DMSO (weak lipid soluble antioxidant) do not induce LTP of the EPSP in hippocampal slices.
11. The inability of tetanic stimulation to induce LTP in vitamin E deficient rats further supports the involvement of α -tocopherol in the LTP process. The failure of α -tocopherol to cause LTP in vitamin E deficient animal may be due to the long-term effects of the vitamin deficiency on the CA1 neurons.
12. Tetanic stimulation can induce LTP not only at excitatory synapses but also at inhibitory synapses in hippocampal CA1 pyramidal neurons under certain circumstances. LTPs of the EPSP and the IPSPs can interfere with each other.
13. LTP of the fast and slow IPSPs is affected by the postsynaptic protein kinase C activity and $[\text{Ca}^{2+}]_i$ concentration as well as the conditions of interneurons which make synaptic contacts with the CA1 neurons.
14. Under normal conditions, LTP of the IPSPs is suppressed by the activation of postsynaptic protein kinase C and the rise of intracellular free Ca^{2+} caused by tetanic stimulation of the afferents so that LTP of the EPSP can be better exhibited.

15. The finding that the potentiation of the IPSPs, especially the slow IPSP, is dependent on the activation of glutamate receptors demonstrates the possible involvement of interneurons in LTP of the IPSPs in CA1 pyramidal cells or the interactions between excitatory and inhibitory synapses in the CA1 cells.
16. Somatostatin, an endogenous peptide which is co-localized with GABA in the inhibitory presynaptic terminals of CA1 pyramidal cells, can interact with GABA-ergic inhibition. Application of SS causes a depression of both fast and slow IPSPs, a hyperpolarization and a reduction of the input resistance of the CA1 neurons.
17. Application of SS does not have effects on the induction of LTP in the CA1 neurons. However, the study on the role of somatostatin on LTP is limited by the lack of selective somatostatin receptor antagonists.
18. In conclusion, the findings presented in this thesis provide evidence that certain substances are released into the extracellular fluid during tetanic stimulation; these substances are responsible for the interactions among postsynaptic, presynaptic and/or glial cells, and subsequently initiate the LTP process through both postsynaptic and presynaptic modifications. Under normal conditions, changes in inhibitory synapses after tetanic stimulation also contribute to a better expression of LTP of the EPSP. The involvement of vitamin E in the LTP process suggests that certain levels of vitamin E in brain tissues are required for the maintenance of proper functions of the cell membrane and receptors which are critical for the LTP process.

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