MONOAMINERGIC INFLUENCES ON VARIOUS INHIBITIONS OF THE SPINAL MONOSYNAPTIC REFLEX

by

BHAGAVATULA SREE RAMA SASTRY

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Division of

Pharmacology and Toxicology

of

the Faculty of

Pharmaceutical Sciences

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

December, 1975

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmaceutical Sciences

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date January 23, 1976

ABSTRACT

The functional significance of bulbospinal 5-hydroxytryptamine (5-HT) and noradrenaline neurones is not well understood. Therefore in this study, the effects of various drugs that alter monoaminergic synaptic activity were tested on bulbospinal, presynaptic, recurrent and reciprocal Ia inhibitions of an extensor (quadriceps, QUAD) and a flexor (posterior biceps-semitendinosus, PBST) monosynaptic reflex (MSR) in unanaesthetized decerebrate cats.

The following agents were employed in the investigation: a biogenic amine neuronal uptake blocker, imipramine HCl (0.125 - 5 mg/kg); a 5-HT neuronal uptake blocker, fluoxetine HCl (Lilly 110140, 0.25 - 6 mg/kg); a 5-HT precursor, 5-hydroxytryptophan (75 mg/kg); a tryptophan hydroxylase inhibitor that depletes 5-HT, DL-p-chlorophenylalanine (300 mg/kg i.p., injected on two consecutive days before the experiment); a tyrosine hydroxylase inhibitor that depletes noradrenaline, DL-α-methyl-p-tyrosine methyl ester HCl (125 mg/kg i.p., administered 16 and 4 hours prior to the experiment); a 5-HT antagonist, cyproheptadine HCl (2.5 - 5 mg/kg); an adrenergic blocker, phenoxybenzamine HCl (2.5 - 5 mg/kg); and clonidine (2.5 - 40 μg/kg), reported to be a specific α-adrenergic agonist. A thoracic cold block, which prevents supraspinal inputs to the caudal spinal cord, was applied to test whether a drug acts through the descending systems and to determine if the inhibitions of the MSR under study are influenced by the supraspinal tonic pathways.

The physiological and the pharmacological studies have led to the following conclusions: presynaptic and recurrent inhibitions of the QUAD-

but not of the PBST-MSR are under a tonic inhibitory influence of a descending system which involves 5-HT and noradrenaline; bulbospinal inhibition of the QUAD-MSR involves both presynaptic and postsynaptic types of inhibition and both types of inhibition are antagonized by a tonically active 5-HT system; a tonically active descending system antagonizes reciprocal Ia inhibition of the extensor but not of the flexor reflex; the excitability of QUAD Ia afferents is decreased by a descending tonically active 5-HT system; and a tonically active supraspinal system has an overall excitatory influence on the extensor motoneurones.

Imipramine was more potent in antagonizing bulbospinal and recurrent inhibitions of the MSR when administered intra-arterially to the spinal cord than when injected intravenously or intra-arterially to the brain stem. Therefore, the 5-HT nerve terminals proposed to be involved in antagonizing bulbospinal and recurrent inhibitions are likely located in the spinal cord.

Clonidine antagonized all the inhibitions of the extensor and the flexor MSRs tested in this study. However, iontophoretically applied clonidine blocked the depressant effects of glycine and γ-aminobutyric acid on approximately 50% of the spinal neurones tested. This finding suggests that clonidine is not a specific α-adrenergic agonist and may have blocked the inhibitions by antagonizing glycine and γ-aminobutyric acid.

John G. Sinclair
Supervisor.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF DRUGS

Drugs	Mechanism of action	References
γ-Aminobutyric acid (GABA)	Suggested to be a synaptic transmitter involved in presynaptic inhibition.	Barker and Nicoll, 1972; Bell and Anderson, 1972; Davidoff, 1972; Eccles *et al.*, 1963.
Clonidine HCl	Suggested to be a specific α-adrenergic agonist in the central nervous system.	Anden *et al.*, 1970; Finch, 1974; Kobinger and Pichler, 1975.
DL-*p*-chlorophenylalanine (*p*-CPA)	Tryptophan hydroxylase inhibitor. Depletes 5-hydroxytryptamine in the spinal cord of the cat.	Taber and Anderson, 1973.
Cyproheptadine HCl	5-Hydroxytryptamine antagonist. Blocks 5-hydroxytryptophan effects on the monosynaptic reflex.	Banna and Anderson, 1968.
Fluoxetine HCl (Lilly 110140)	Reported to be a specific 5-hydroxytryptamine neuronal uptake blocking agent.	Fuller *et al.*, 1975; Wong *et al.*, 1975.
Glycine	Probably the inhibitory transmitter of the recurrent and reciprocal Ia inhibitory pathways in the spinal cord.	Curtis *et al.*, 1968, 1971; Werman *et al.*, 1968.
5-Hydroxytryptophan (5-HTP)	A 5-hydroxytryptamine precursor. Reported to elevate 5-hydroxytryptamine levels in the spinal cord of the cat.	Anderson and Shibuya, 1966.
Imipramine HCl	5-Hydroxytryptamine and noradrenaline neuronal uptake blocker.	Carlsson *et al.*, 1969a, b.
DL-α-methyl-*p*-tyrosine methyl ester HCl (α-MPT)	Tyrosine hydroxylase inhibitor. Reported to deplete noradrenaline in the cat's spinal cord.	King and Jewett, 1971.
Phenoxybenzamine HCl	Adrenergic blocker.	

ACKNOWLEDGEMENTS

The author is deeply indebted to Dr. John G. Sinclair for his guidance and encouragement throughout the course of this work.

He is grateful to Dr. D.M.J. Quastel for his valuable and constructive comments and criticism.

He is thankful to Dr. H. McLennan, Dr. J.H. McNeill and Dean B.E. Riedel for reviewing the thesis.

The encouragement, inspiration and help of Judith Anne are invaluable.

This study was supported by a grant from the Medical Research Council of Canada to Dr. Sinclair as well as a Medical Research Council Studentship, a Research on Drug Abuse summer Scholarship and a Warner-Lambert Research Fellowship in Pharmacy to the author.

The author wishes to thank the following for their generous contribution of drugs used in this investigation: Drs. Fuller and Hosley of Eli Lilly and Co. for fluoxetine HCl, Geigy Ltd. for imipramine HCl, Merck Sharp and Dohme Ltd. for cyproheptadine HCl and Smith Kline and French Ltd. for phenoxybenzamine HCl.

To my

parents

and

brother

INTRODUCTION

The functional significance of the bulbospinal 5-hydroxytryptamine (5-HT) and noradrenaline neurones is not well established. We have therefore been investigating the effects of various drugs, which alter monoaminergic synaptic activity, on inhibitions of the lumbosacral monosynaptic reflex (MSR) in unanaesthetized decerebrate cats.

Previous findings from our laboratory suggest that a 5-HT system antagonizes bulbospinal inhibition of the MSR and a supraspinal system involving 5-HT and noradrenaline decreases recurrent inhibition of the extensor quadriceps MSR (Sastry, 1973; Sinclair and Sastry, 1974, 1974a). In the present investigation experiments were designed to further test the above hypotheses and to determine the location of 5-HT terminals implicated in antagonizing bulbospinal and recurrent inhibitions.

Bulbospinal inhibition of the MSR is evoked by stimulation in the ventromedial bulbar reticular formation (Magoun and Rhines, 1946). This inhibition involves a postsynaptic inhibition of the MSR (Llinas and Terzuolo, 1964, 1965; Jankowska et al., 1968). Carpenter et al. (1966) suggested that activation of the dorsal but not the above ventromedial reticular formation evoked dorsal root potentials on spinal Ia afferents. However, Chan and Barnes (1972) reported that stimulation in the ventral bulbar reticular formation could evoke presynaptic inhibition of the MSR. There appears to be a discordance between the latter two studies. Therefore, experiments were conducted to test whether bulbospinal inhibition of the MSR involves presynaptic and postsynaptic types of inhibition and, if

so, to determine whether 5-HT systems antagonize both types.

Imipramine blocked spinal presynaptic inhibition of the MSR in decerebrate cats but had little effect in animals with a spinal transection (Tan and Henatsch, 1969). Moreover, this agent more effectively antagonized the inhibition of an extensor than of a flexor MSR. Imipramine is known to block the neuronal uptake of 5-HT and noradrenaline (Carlsson et al., 1969a, b). Therefore, the involvement of these biogenic amines in influencing presynaptic inhibition of the extensor and the flexor MSRs was tested. Studies were also undertaken to determine whether monoaminergic systems affect reciprocal Ia inhibition of the MSR.

LITERATURE SURVEY

Bulbospinal monoaminergic neurones:

There is strong evidence for the presence of 5-hydroxytryptamine (5-HT) and noradrenaline neurones in the spinal cord. Stimulation of the isolated rostral spinal cord of frogs or mice releases 5-HT and noradrenaline (Anden et al., 1964, 1965) while chronic transection of the rabbit spinal cord depletes these amines in the caudal spinal cord (Magnusson and Rosengren, 1963; Carlsson et al., 1963). Therefore, these monoamines in the spinal cord are associated with the descending neurones.

Histochemical fluorescence studies of Dahlstrom and Fuxe (1964, 1965) indicate that almost all the bulbospinal 5-HT neuronal somata are located in the caudal raphe nuclei of the ventromedial caudal brain stem (Raphe obscurus, pallidus and magnus) and the noradrenaline containing neurones originate mainly in the medulla oblongata between the rostral tip of the inferior olivary nucleus and the pyramidal decussation.

Some of the 5-HT and noradrenaline containing neurones in the brain stem make mutual contact while some synapse with other cells (Dahlstrom and Fuxe, 1965; Fuxe, 1965). The bulbospinal 5-HT and noradrenaline axons initially descend ventrally, lateral to the pyramidal decussation, in the brain stem. In the spinal cord, the 5-HT fibres descend in the dorsolateral and the ventromedial funiculi while the noradrenaline fibres run through the dorsolateral and the ventrolateral funiculi. Some of the monoaminergic fibres cross to the opposite side (Dahlstrom and Fuxe, 1965). Both the 5-HT and the noradrenaline nerve terminals in the lumbosacral spinal cord

of the cat were found to be dense in the substantia gelatinosa of the dorsal horn and the dorsolateral and the ventrolateral motor nuclei of the ventral horn (Dahlstrom and Fuxe, 1965; Fuxe, 1965). While some 5-HT and noradrenaline nerve terminals synapse with interneurones, some synapse with motoneurones. The monoaminergic fibres are unmyelinated and about 1 μm in diameter (Dahlstrom and Fuxe, 1965).

The spinal monosynaptic reflex:

The group Ia afferents which convey impulses from the annulospiral endings of the muscle spindles make synaptic contact with the α-motoneurones in the ventrolateral spinal motor nuclei which, in turn, innervate the extrafusal muscle fibres belonging to a synergistic muscle. This reflex is called the spinal monosynaptic reflex (MSR). Experimentally the MSR is usually evoked by stimulation of a spinal dorsal root or a peripheral afferent nerve and recorded from an appropriate ventral root. The synaptic delay across Ia afferent - α-motoneurone synapses is about 0.5 msec (Eccles, 1961). The latency between the stimulation of a dorsal root and the initiation of the compound action potential (MSR) on the ventral root is about 1 msec and includes the conduction time along the afferent fibre, the synaptic delay and the conduction along the ventral root. The MSR peaks about 0.5 msec after its initiation (Renshaw, 1940). The magnitude of the MSR is an index of the number of motoneurones that discharge simultaneously. However, the Ia afferents depolarize many motoneurones only to a subthreshold level. These motoneurones are said to be excited subliminally and do not contribute to the size of the MSR. During facilitation of the MSR, the motoneurones that were excited subliminally or those that were not activated by the test stimulus are recruited into the discharge zone. As more

motoneurones discharge, the size of the MSR enhances. The inhibition of the MSR involves elimination of some motoneurones from the discharge zone resulting in a reduction of the magnitude of the reflex.

The synaptic transmitter at the Ia afferent - α-motoneurone synapses is unknown. Substance P, which includes a group of polypeptides, is more concentrated in the dorsal than in the ventral spinal gray matter (Takahashi and Otsuka, 1975). This agent was reported to be a potent excitant of α-motoneurones. Lioresal^{R}, which blocked the synaptically induced excitation of the motoneurones, antagonized the effect of substance P (Saito et al., 1975). However, Krnjevic and Morris (1973) reported that the depolarization of cells in the cuneate nucleus produced by substance P was slow and concluded that this agent may not be the transmitter released by the primary afferents.

The levels of L-glutamate in the dorsal spinal gray matter were reported to be higher than in the ventral gray matter (Davidoff et al., 1967). The spinal neurones were excited by iontophoretically applied glutamate (Curtis et al., 1960; Haldeman and McLennan, 1972). This action of glutamate was reversed by iontophoretically administered glutamic acid diethyl ester which was suggested to be a specific glutamate antagonist (Haldeman et al., 1972; Haldeman and McLennan, 1972). However, Zieglgänsberger and Puil (1973) reported that glutamic acid diethyl ester increased the membrane conductance of spinal motoneurones and suggested that this agent is not a specific glutamate antagonist. Therefore, the possibility that glutamate is the transmitter released at the primary afferent terminals deserves further investigation.

The effects on the MSR of agents that alter monoaminergic synaptic transmission:

In unanaesthetized cats with an acute spinal transection, the 5-HT precursors, 5-hydroxytryptophan (5-HTP, 75 mg/kg i.v.) and l-tryptophan (100 mg/kg i.v.) and the monoamine oxidase inhibitor, pargyline (30 mg/kg i.v.), enhanced the MSR to about 310, 170 and 190% of control, respectively. The 5-HT levels in the spinal cord of the cat were enhanced by about 300% by 5-HTP and 70% by pargyline, at the above doses (Anderson and Shibuya, 1966; Anderson et al., 1967). Pargyline did not significantly alter the levels of noradrenaline in the spinal cord (Anderson et al., 1967). The effects of 5-HTP and l-tryptophan on the MSR were potentiated by pretreatment of the animals with pargyline (Anderson et al., 1967). Methysergide, cinanserin, d-lysergic acid diethylamide (LSD), 2-bromo-LSD and cyproheptadine reversed the facilitatory effects of 5-HTP and l-tryptophan (Banna and Anderson, 1968). The effect of pargyline was blocked by methysergide but not by the α-adrenergic blocking agent, phenoxybenzamine (Anderson et al., 1967).

In cats with a chronic spinal transection, l-tryptophan and pargyline did not enhance the MSR, however, 5-HTP still facilitated the reflex (Shibuya and Anderson, 1968). In the spinal cord caudal to a chronic transection, 25% of the control 5-HT levels (Shibuya and Anderson, 1968), 17% of the tryptophan hydroxylase activity (Clineschmidt et al., 1971a) and 20% of the dopa decarboxylase activity (Anden, 1965) remained. Therefore, Shibuya and Anderson (1968) suggested that the enhancement of the MSR by 5-HTP in the animals with a chronic spinal transection might be due to its conversion into 5-HT within the 5-HT interneurones of the spinal cord. However, there is no good evidence for the presence of 5-HT interneurones

in the spinal cord (Dahlstrom and Fuxe, 1965; Fuxe, 1965). Evidence exists to indicate that 5-HT synthesis from 5-HTP can occur extraneuronally (Kuhar et al., 1971). Moreover, 5-HTP can enter adrenergic terminals and displace catecholamines (Ng et al., 1972) or directly activate adrenergic receptors (Innes, 1962). These observations offer alternative explanations for the enhancement of the MSR by 5-HTP in animals with a chronic spinal transection as observed by Shibuya and Anderson (1968).

Taber and Anderson (1973) reported that the facilitatory effect of 5-HTP on the MSR was prevented in cats pretreated with the tryptophan hydroxylase inhibitor, DL-p-chlorophenylalanine (p-CPA, 300 mg/kg i.p. for two consecutive days). The lumbosacral 5-HT levels were depleted by about 90% in these animals. These authors suggested that in the above animals 5-HT, synthesized from 5-HTP, entered the empty synaptic vesicles but did not overflow into the synaptic clefts to stimulate the receptors.

The tricyclic antidepressants, amitriptyline, imipramine and desipramine, potentiated the facilitatory effect of 5-HTP on the MSR in cats with a spinal transection (Clineschmidt et al., 1971). Furthermore, imipramine augmented the actions of 5-HTP and pargyline on the reflex in cats with an acute but not a chronic spinal transection (Clineschmidt et al., 1971; Clineschmidt, 1972).

The above findings indicate that a descending 5-HT system in the spinal cord of the cat has a facilitatory effect on the MSR. We observed that a thoracic cold block, which eliminates supraspinal inputs to the caudal spinal cord, reduced the quadriceps but not the posterior biceps-semitendinosus MSR by about 45 per cent (Sastry, 1973).

Baker and Anderson (1970) reported that l-3,4-dihydroxyphenylalanine (l-dopa, 30 mg/kg i.v.) enhanced the MSR to about 210% of control and

pargyline pretreatment potentiated this effect of l-dopa. Phenoxybenzamine, chlorpromazine and ethobutamoxane antagonized the facilitation of the reflex by l-dopa (Baker and Anderson, 1970a). After an injection of l-dopa (20 mg/kg i.v.), dopamine levels in the spinal cord were elevated by about 30 fold, however, noradrenaline levels were not significantly increased. Therefore, Baker and Anderson (1970) concluded that the effect of l-dopa on the MSR might be mediated through dopamine. However, there is no evidence for the presence of dopaminergic pathways in the spinal cord (Dahlstrom and Fuxe, 1965; Fuxe, 1965).

The effects of iontophoretically applied 5-HT and noradrenaline on motoneurones:

Iontophoretically applied 5-HT and noradrenaline hyperpolarized many motoneurones and blocked the soma-dendritic component of the motoneurone antidromic action potentials. However, a few cells were excited by these amines (Phillis et al., 1968). Engberg and Ryall (1966) reported that noradrenaline reduced the DL-homocysteate induced firing rate of the motoneurones. Engberg and Marshall (1971) found that noradrenaline increased the motoneurone membrane resistance and the hyperpolarization produced by this agent was augmented by a conditioning hyperpolarization and reduced by a depolarization. Hence, these authors suggested that noradrenaline might reduce the permeability of the motoneurone membrane to Na^+. However, in a subsequent report Marshall and Engberg (1973) reported that H^+, when iontophoretically applied near the motoneurones, produced effects similar to those of iontophoretically applied noradrenaline. Engberg and Marshall (1971) were passing iontophoretic currents of about 100 nA to eject noradrenaline from a low pH solution. Phillis and coworkers (Phillis, 1974;

Phillis et al., 1973; Yarbrough et al., 1974) postulated that the depressant effects of 5-HT and noradrenaline on the cerebral cortical neurones might be mediated by Ca^{++}. It is unknown whether a similar situation exists for the effects of these amines on the spinal motoneurones. Krnjevic and Kisiewicz (1972) reported that injection of Ca^{++} into the motoneurones resulted in a hyperpolarization of these neurones accompanied by an increase in membrane conductance in some cells and no alteration in the membrane conductance of other cells. These authors suggested that the intracellular Ca^{++} might increase the efflux of K^{+}. From the above studies it is apparent that further investigation is necessary to understand the mechanisms of action of 5-HT and noradrenaline on the motoneurones.

Presynaptic inhibition of the MSR:

When a spinal dorsal root was stimulated an exponentially decaying positive potential, the P wave or the cord dorsum potential, was recorded from an electrode positioned on the spinal cord near the dorsal root entry zone (Gasser and Graham, 1933). Barron and Matthews (1938) reported that stimulation of a dorsal root elicited an exponentially decreasing negative potential, the negative dorsal root potential (DRP), on a rootlet of the above root and on an adjacent dorsal root. These authors postulated that the source of the P wave and the DRP was the same. The DRP represents an increase in the excitability of the primary afferents (Wall, 1958) resulting from a depolarization of these fibres (Eccles and Krnjevic, 1959; Eccles et al., 1962, 1962a, b). Although a measurement of the potential change inside the afferent terminals was not possible, it was inferred that the observed increase of the excitability originated at the terminals since the afferent fibres were depolarized (Eccles et al., 1962, 1962a, b).

When the spinal MSR was conditioned with a prior stimulation of the group I afferents belonging to a flexor or the extensor quadriceps muscle, the reflex was gradually inhibited. This inhibition of the MSR began about 5 msec after the conditioning stimulus, peaked after 20 msec and had a duration of approximately 200 msec. A similar time course was observed for the DRP (Brooks et al., 1948; Frank and Fuortes, 1957). During the above inhibition of the MSR the excitatory postsynaptic potentials (EPSPs) of a participating motoneurone were reduced. However, stimulation of the conditioning afferents did not alter the motoneurone membrane potential at its normal resting level or at a depolarized or hyperpolarized state (Frank and Fuortes, 1957; Frank, as cited by Eccles, 1964). Therefore, it is generally believed that the depression of the motoneurone EPSPs during the above inhibition of the MSR is due to a reduction in the efficacy of the presynaptic function. The inhibition is thus referred to as presynaptic inhibition (Frank and Fuortes, 1957). However, Frank (1959) suggested that if the inhibition of the motoneurones is on the dendrites, a microelectrode situated within the motoneurone soma might not be able to record the hyperpolarization and hence he called the inhibition "remote" inhibition.

Depolarization of the motor nerve terminals in the rat diaphragm resulted in a decrease in the size of the end plate potentials, however, the magnitude of the miniature end plate potentials was unaltered. Therefore, it was suggested that the reduction of the end plate potential was due to a decrease in the number of quanta of the transmitter liberated by the nerve impulse (Hubbard and Willis, 1962). Moreover, Takeuchi and Takeuchi (1962) observed that, at the giant synapses of Loligo, the magnitude of the postsynaptic potential was dependent on the size but not the level of the peak of the presynaptic action potential. Therefore, Eccles (1964) postulated

that an action potential reaching a depolarized primary afferent terminal would release less synaptic transmitter than control, thereby, producing a smaller motoneurone EPSP.

Since the latency between the conditioning stimulus and the onset of inhibition of the MSR is about 5 msec and the synaptic delay at a single central synapse is approximately 0.5 msec (Eccles, 1961), it was hypothesized that the presynaptic inhibitory pathway involves two or more serially arranged interneurones (Eccles et al., 1962). Gray (1962, 1963) discovered axo-axonal synapses in the spinal cord. Hence, Eccles (1964) suggested that the last interneurone in the above inhibitory pathway makes axo-axonal synapse with the primary afferent terminal.

A neutral amino acid, γ-aminobutyric acid (GABA), and the enzyme that decarboxylates glutamic acid to form GABA, glutamic acid decarboxylase, were found in the feline spinal cord with higher levels in the dorsal than in the ventral gray matter (Graham et al., 1967; Graham and Aprison, 1969; Albers and Brady, 1959). In the cat, loss of spinal interneurones resulted in a decrease in the levels of GABA (Miyata and Otsuka, 1972; Davidoff et al., 1967). A Ca^{++} dependent release of previously loaded ^{3}H-GABA was observed in the isolated amphibian spinal cord when the rostral spinal cord was stimulated (Collins, 1974). When GABA was topically applied on the feline spinal cord, the DRP was blocked but the afferent fibres were depolarized (Eccles et al., 1963). Similar effects were observed on the spinal cord of the frog (Schmidt, 1963; Barker and Nicoll, 1972; Davidoff, 1972). Depletion of the spinal cord GABA by pretreatment of cats with semicarbazide resulted in a blockade of the DRP (Bell and Anderson, 1972). Based on the observation that Mg^{++} blocked the DRP but did not alter the depolarization of the primary afferent filaments by GABA, Davidoff (1972) and Barker and

Nicoll (1972) suggested that the above effect of GABA was on the afferent terminals. Levy (1974) reported that intravenously injected GABA (100 mg/kg) depolarized the feline dorsal root filaments that were either cut peripherally at the dorsal root exit through the dura or sectioned centrally at the dorsal root entry zone on the spinal cord. Furthermore the GABA antagonists, bicuculline and picrotoxin, blocked the DRPs and also the action of GABA on the primary afferents (Eccles et al., 1963; Davidoff, 1972; Levy, 1974). Degroat et al. (1972) reported that GABA depolarized the dorsal root ganglia of the cat. The depolarization of amphibian sensory ganglia produced by this amino acid was reported to be dependent on the extracellular Cl^- concentration (Nishi et al., 1974). Although GABA depolarized the primary afferents when topically applied on the spinal cord or intravenously injected, this agent reduced the excitability of these fibres when iontophoretically applied at the afferent terminals (Curtis and Ryall, 1966). Therefore, although GABA appears to be involved in the production of presynaptic inhibition, its site of action is still uncertain.

During the stimulation of the primary afferents, the extracellular K^+ concentration in the spinal cord increased (Kriz et al., 1974; Singer and Lux, 1973; Krnjevic and Morris, 1972; ten Bruggencate et al., 1974). Krnjevic and Morris (1972) and Kriz et al. (1974) suggested that this increase in the K^+ levels might be responsible for the production of presynaptic inhibition. However, ten Bruggencate et al. (1974) found that picrotoxin blocked the DRPs and increased the extracellular K^+ levels whereas Nembutal^R enhanced the DRPs but reduced the extracellular K^+ concentration.

Curtis et al. (1971à) suggested that GABA, while inhibiting the spinal interneurones, may release K^+ at the inhibitory synapses. These ions may depolarize the primary afferent terminals. This suggestion is not only

consistent with the depressant effects of GABA in the cat spinal cord but also with its presumably indirect excitatory action on the afferent terminals. However, the above proposal is not supported by any experimental evidence.

Carpenter et al. (1963) reported that in unanaesthetized decerebrate cats, the DRP, evoked by stimulation of the flexor or the extensor group I afferents, was either unchanged or slightly reduced after a spinal transection. Presynaptic inhibition of the MSR was attributed to only group I afferent activation (Eccles et al., 1961). Therefore, Carpenter et al. (1963) suggested that presynaptic inhibition of the MSR is not under a tonic influence of a supraspinal system. However, group Ia and Ib afferents can produce DRPs on Ib afferents as well (Eccles et al., 1962). Furthermore, it is not known whether, in the study of Carpenter et al. (1963), the DRPs were evoked on the flexor or the extensor afferents or on both.

Stimulation of the group II and III, cutaneous and the high threshold joint afferents, collectively called the flexion reflex afferents (Eccles and Lundberg, 1959), results in inhibition of an extensor and facilitation of a flexor MSR. In decerebrated cats, the above effects of the flexion reflex afferents are suppressed but reappear after a spinal transection (Holmqvist and Lundberg, 1961). In order to evoke presynaptic inhibition of the MSR, without the interference of the effects of the flexion reflex afferents, it would be necessary to use a conditioning stimulating strength that activates only group I afferents. Eccles et al. (1962) suggested that at a conditioning interval that exceeds 25 msec the interference of postsynaptic inhibition with presynaptic inhibition of the MSR was minimal.

In decerebrate cats, the monoamine neuronal uptake blocking agent, imipramine, antagonized presynaptic inhibition of the MSR. This effect of

imipramine was not present after a spinal transection (Tan and Henatsch, 1969). Therefore, imipramine is very likely acting through a supraspinal system. Moreover, this agent had a greater blocking action on the inhibition of an extensor than on that of a flexor MSR (Tan and Henatsch, 1969).

Postsynaptic inhibition of the MSR:

Postsynaptic inhibition of the MSR involves a direct synaptic inhibition of the α-motoneurones. Examples of postsynaptic inhibition include reciprocal (direct, Ia) inhibition (Lloyd, 1941) and recurrent (antidromic) inhibition (Renshaw, 1941). Reciprocal inhibition is exerted by Ia afferents activating Ia inhibitory interneurones which impinge on α-motoneurones of antagonistic muscles (Eccles et al., 1956). Recurrent inhibition is produced by discharges in the motor axon collaterals that activate inhibitory interneurones, Renshaw cells, which in turn inhibit the motoneurones (Eccles et al., 1954a).

The resting membrane potential of the feline α-motoneurones is about -70 mV (Frank and Fuortes, 1955). During postsynaptic inhibition of the MSR the motoneurones are hyperpolarized producing the inhibitory postsynaptic potential (IPSP, Brock et al., 1952). During the IPSP the membrane conductance is increased. The equilibrium potential for the IPSP is about -80 mV (Coombs et al., 1955). An inward diffusion of Cl^- and an efflux of K^+ were suggested to occur during the IPSP (Coombs et al., 1955). However, Lux et al. (1970), Lux (1971) and Llinas and Baker (1972) proposed that the IPSP is generated by a selective increase in permeability to Cl^-. They also reported that K^+ permeability change is probably not involved in this process. However, in a recent lecture Eccles (1975) stated that in the hippocampal pyramidal cells the IPSP is generated by an influx of Cl^- and

probably an efflux of K^+, supporting the suggestion of Coombs et al. (1955).

a. Reciprocal Ia inhibition:

Reciprocal Ia inhibition of a MSR is produced by stimulation of group Ia afferents belonging to an antagonistic muscle. This inhibitory pathway involves an interneurone interposed between the conditioning Ia afferent terminal and the α-motoneurone (Eccles et al., 1956). The inhibition is observed when the conditioning stimulus preceeds the test stimulus by less than 1 msec, is maximal when the conditioning interval is approximately 1 msec and is present for about 2 msec (Lloyd, 1941).

Glycine, a basic amino acid, is present in the spinal cord and is more concentrated in the ventral than in the dorsal gray matter (Graham et al., 1967). Destruction of spinal interneurones resulted in a reduction in the levels of this amino acid (Davidoff et al., 1967). Iontophoretically applied glycine hyperpolarized the motoneurones and reduced the motoneurone membrane resistance. Prior hyperpolarization of the membrane reduced or reversed the glycine induced hyperpolarization. The equilibrium potentials of the inhibitory postsynaptic potential and the hyperpolarization produced by glycine were similar (Werman et al., 1968; Curtis et al., 1968). Strychnine blocks reciprocal Ia inhibition of the MSR (Eccles et al., 1954a) and is reported to be a specific glycine antagonist in the feline spinal cord (Curtis et al., 1971). Therefore, the transmitter released from the Ia inhibitory interneurones is probably glycine.

The Ia inhibitory interneurones were inhibited by the activation of the motor axon collaterals (Hultborn et al., 1971). Therefore, Hultborn et al. (1971) suggested that the enhancement of motoneuronal discharges results in a reduction in the firing of the Ia inhibitory interneurones

that innervate the antagonistic motoneurones. The disynaptic inhibition of the α-motoneurones by the descending vestibulo-, rubro-, and corticospinal tracts was found to involve the Ia inhibitory interneurones. Moreover, these interneurones were also excited by high threshold muscle afferents and cutaneous impulses (Eccles et al., 1956). Therefore, there seems to be a convergence of supraspinal and spinal afferent excitatory input to the Ia inhibitory interneurones.

b. Recurrent inhibition:

As described previously, discharges in motor axon collaterals synaptically excite Renshaw cells which in turn inhibit the motoneurones. Renshaw cells, activated in the above fashion, fire in a characteristic burst with an initial frequency of greater than 1000 spikes per sec which gradually decreases to control firing over a period of 50 msec (Eccles et al., 1954a, 1956a). A high convergence of excitatory input from the collaterals of many motor axons was suggested to be responsible for the above mentioned high frequency discharge of Renshaw cells (Eccles et al., 1956a; Ryall et al., 1972). Recurrent inhibition begins about 2 - 3 msec after the conditioning stimulation of the motor axons, peaks after approximately 5 msec and decays over a period of about 50 msec (Renshaw, 1941).

The pharmacological and physiological studies indicate that the motor axon collateral - Renshaw cell synapse is cholinergic (Curtis and Ryall, 1966a, b, c; Eccles et al., 1956a). Dihydro-β-erythroidine, which blocks cholinergic transmission at the nicotinic receptors, reduced the Renshaw cell response to synaptic activation (Eccles et al., 1956a; Curtis et al., 1966b). Eserine, an anticholinesterase drug, greatly prolonged the discharges of Renshaw cells induced by synaptic excitation (Eccles et al.,

1954a, 1956a). Intra-arterially injected acetylcholine or nicotine excite Renshaw cells (Eccles et al., 1956a; Curtis and Ryall, 1966a). The above action of acetylcholine, but not nicotine, is enhanced by eserine whereas dihydro-β-erythroidine reduced the excitatory action of both these substances (Eccles et al., 1956a). The inhibitory transmitter at the Renshaw cell - motoneurone synapses was suggested to be glycine (Curtis et al., 1968; Curtis, 1969; Curtis et al., 1971; Werman et al., 1968).

In cats anaesthetized with chloralose, antidromic volleys in the motor axons were reported to sometimes produce an inhibition of Renshaw cells instead of an excitation (Ryall, 1970). The latency observed for this inhibition suggested that the effect was brought about by a disynaptic pathway involving a Renshaw cell. Therefore, some Renshaw cells inhibit the firing of other Renshaw cells (Ryall, 1970). The Ia inhibitory interneurones were shown to be inhibited by Renshaw cells, as mentioned previously. However, there seems to be no input from the Ia inhibitory interneurones to Renshaw cells (Ryall and Piercey, 1971). Volleys in the ipsilateral flexon reflex afferents excite Renshaw cells through a polysynaptic chain, whereas, discharges in the above afferents on the contralateral side inhibit these cells (Ryall and Piercey, 1971). The collaterals of large phasic α-motoneurone axons more strongly excite Renshaw cells than those of the small tonic motoneurone axons. The small tonic motoneurones rather than the large phasic motoneurones are more effectively inhibited by Renshaw cells (Ryall et al., 1972). Wilson et al. (1960) reported that recurrent inhibition of extensor or flexor motoneurones was more effectively produced by antidromic activation of motor axons belonging to the same or synergistic muscles.

In cats anaesthetized with chloralose, stimulation of the ventromedial mesencephalic reticular formation at the level of substantia nigra (A 6.5 -

1, in the stereotaxic atlas of Snider and Niemer, 1964) or in decerebrate cats, activation of the ventrolateral bulbar reticular formation at the level of the hypoglossal nucleus (P 9.5 - 11) was found to inhibit the rate of Renshaw cell discharges evoked by antidromic volleys in the motor axons (Koizumi et al., 1959; Haase and Van der Meulen, 1961; MacLean and Leffman, 1967). The above inhibition could be produced by stimulating either side of the reticular formation but was stronger when the contralateral reticular formation was stimulated (Haase and Van der Meulen, 1961). The latency between stimulation of the mesencephalic reticular formation and the onset of the inhibition of Renshaw cell firing rate was found to be about 9 msec. This inhibition lasted at its maximum strength for approximately 25 msec. While the inhibition of Renshaw cell discharge rate by activation of the mesencephalic reticular formation was stronger, the inhibition produced by stimulation of the bulbar reticular formation had a longer duration (MacLean and Leffman, 1967). Following the stimulation of the ventral thalamus (fields of forel, zona incerta or the pericruciate cortex) the Renshaw cell discharges, evoked by activation of the motor axons, were reduced in number (MacLean and Leffman, 1967). On the other hand, stimulation of the cerebellar anterior lobe facilitated the synaptically induced Renshaw cell discharge rate (Haas and Van der Meulen, 1961). In addition, stimulation of the ventral thalamus or the pericruciate cortex could sometimes activate the Renshaw cells (MacLean and Leffman, 1967).

The above findings indicate that Renshaw cell discharges are influenced by supraspinal and spinal afferent inputs.

Recurrent inhibition of the quadriceps MSR in decerebrate cats was enhanced during a thoracic cold block, a procedure that reversibly blocks supraspinal inputs to the lumbosacral spinal cord (Sastry, 1973; Sinclair

and Sastry, 1974a). Therefore, we proposed that the above inhibition is under a tonic inhibitory influence of a descending system.

Tan and Henatsch (1969) reported that imipramine antagonized recurrent inhibition of the MSR in decerebrate cats. They also found that imipramine failed to alter the inhibition when the spinal cord was transected at the thoracic level. This agent had a greater blocking effect on recurrent inhibition of an extensor than of a flexor reflex. In a follow up study we found that in decerebrate cats, imipramine antagonized recurrent inhibition of the quadriceps but not of the posterior biceps-semitendinosus MSR. The monoamine oxidase inhibitor, pargyline, also antagonized the inhibition of the MSR. A thoracic cold block completely reversed the blockade by imipramine. Moreover, depletion of 5-HT or noradrenaline completely prevented the action of imipramine on the inhibition of the quadriceps MSR. Therefore, we proposed that a supraspinal system, which involves 5-HT and noradrenaline, antagonizes recurrent inhibition of the extensor but not of the flexor MSR (Sastry, 1973; Sinclair and Sastry, 1974a).

Iontophoretically applied 5-HT and noradrenaline either increased or decreased Renshaw cell firing but the predominant effect was inhibition (Biscoe and Curtis, 1966; Engberg and Ryall, 1966; Weight and Salmoiraghi, 1966).

Bulbospinal inhibition of the MSR:

The lumbosacral MSR is inhibited following a stimulation in the ventromedial bulbar reticular formation (Magoun and Rhines, 1946). During this bulbospinal inhibition of the MSR the motoneurones were hyperpolarized, the resistance of the motoneurone membrane was reduced and the soma-dendritic component of the action potential was blocked when the motoneurones were

activated antidromically (Llinas and Terzuolo, 1964, 1965; Jankowska et al., 1968). These findings indicate that bulbospinal inhibition of the MSR involves a postsynaptic type of inhibition. When chloride ions were iontophoretically injected into the extensor motoneurones, the hyperpolarization produced during stimulation of the bulbar reticular formation was reversed (Llinas and Terzuolo, 1964; Jankowska et al., 1968). Therefore, the ionic mechanisms responsible for bulbospinal inhibition of the extensor MSR appear to be similar to those of reciprocal Ia inhibition. Llinas and Terzuolo (1965) found that injection of Cl^- into the flexor α-motoneurones did not reverse the hyperpolarization during bulbospinal inhibition. Therefore, these authors suggested that the bulbospinal inhibitory synapses on the flexor motoneurones are on the dendrites. However, Jankowska et al. (1968) did not observe any difference between the flexor and extensor motoneurones in this regard. The discrepancy between the two above studies may be due to the difference in the experimental preparations. Jankowska et al. (1968) performed their experiments on decerebrate cats with a contralateral hemisected and an ipsilateral dorsal transected spinal cord. The spinal cord was intact in the study of Llinas and Terzuolo (1965).

The bulbospinal inhibitory pathway, which descends in the ventral quadrant of the spinal cord, likely has a disynaptic linkage involving an interneurone in the spinal cord (Jankowska et al., 1968; Clineschmidt and Anderson, 1970). The conduction velocity of this pathway was reported to be high.

Stimulation of the medial reticular formation in the caudal brain stem, 1 mm below the floor of the fourth ventricle (V about -5 to -6 in the stereotaxic atlas of Snider and Niemer, 1964), produced negative DRPs on flexor and extensor Ia afferents (Carpenter et al., 1966). These reticulospinal

fibres descend in the ventromedial spinal cord. Carpenter et al. (1966) also reported that stimulation of the ventral caudal bulbar reticular formation (about 4 mm below the floor of the fourth ventricle) did not produce DRPs on these afferents. However, Chan and Barnes (1972) found that stimulation in the ventral caudal bulbar reticular formation, 2 mm lateral from the mid-sagittal line, resulted in short and long latency DRPs on Ia afferents. Chan and Barnes also noted a time correlation between the excitability of the Ia afferents, the DRP and the inhibition of the MSR while stimulating in the bulbar area. Therefore, bulbospinal inhibition of the MSR appears to involve a presynaptic type of inhibition as well. Llinas (1964) found that strychnine (0.15 and 0.5 mg/kg i.v.) decreased the hyperpolarization of the extensor motoneurones during bulbospinal inhibition but did not antagonize the inhibition of the MSR. This failure of strychnine to reverse the inhibition of the MSR may be due to the presence of the presynaptic type of bulbospinal inhibition which may not be affected by strychnine.

Clineschmidt and Anderson (1970) reported that methysergide, cinanserin, d-lysergic acid diethylamide (LSD) and 2-bromo-LSD antagonized bulbospinal inhibition. The results of intra-arterial injection of methysergide and LSD to the spinal cord and the brain stem suggested that these agents block bulbospinal inhibition at the spinal cord level. Therefore, Clineschmidt and Anderson (1970) proposed that a 5-HT interneurone in the spinal cord is involved in the bulbospinal inhibitory pathway. However, there is no good evidence for the existence of 5-HT cell bodies in the spinal cord (Dahlstrom and Fuxe, 1965; Fuxe, 1975). In a subsequent study, Proudfit and Anderson (1973) suggested that the blockade of bulbospinal inhibition by cinanserin and methysergide could result from an enhancement of bulbo-

spinal facilitation if this facilitatory pathway is under inhibitory influence of a tonically active 5-HT system. However, we found that methysergide converted bulbospinal inhibition into a 3 - 4 fold facilitation in animals that were pretreated with p-chlorophenylalanine (Sastry, 1973; Sinclair and Sastry, 1974). Hence, methysergide does not appear to act as a 5-HT antagonist in the system. Imipramine (5 mg/kg i.v.), desipramine (4.8 mg/kg i.v.) and pargyline (30 mg/kg i.v.), drugs that would be expected to enhance 5-HT activity, antagonized bulbospinal inhibition of the MSR in unanaesthetized decerebrate cats. Moreover, the above effect of imipramine was completely prevented by a depletion of 5-HT but not noradrenaline. Therefore, we proposed that bulbospinal inhibition of the MSR is under an inhibitory influence of a 5-HT system (Sastry, 1973; Sinclair and Sastry, 1974).

Proudfit and Anderson (1974) reported that cinanserin and methysergide blocked DRPs on presumably group Ia afferents evoked by bulbar stimulation. Therefore, they proposed that a 5-HT system depolarizes these afferent terminals.

EXPERIMENTAL

Surgical procedures:

These experiments were carried out in a shielded room on a total of 119 cats of either sex weighing between 2.3 and 3.7 kg. The animals were anaesthetized with ether solvent U.S.P. and the trachea was then cannulated to artificially respire and maintain the animal under anaesthesia with the help of a Palmer respiratory pump (type AC; HP 1/4). The left carotid artery was cannulated with a No. 160 polyethylene tubing (Clay Adams) filled with diluted sodium heparin (Upjohn Company of Canada). In some experiments this tubing was connected to a P-1000-A pressure transducer which was in turn connected to a DAM-4A physiograph (Narco-Bio-Systems) for recording the blood pressure. In other experiments the tubing was attached to a Statham P23AC pressure transducer which was connected to a 79D polygraph (Grass Instruments Company) to monitor the blood pressure. The right carotid artery was ligated.

The animal's head was fixed to a Narishige stereotaxic head holder. The skull bone overlying the frontal and the parietal cerebral cortex was then removed and the animal decerebrated at the mid-collicular level. The brain tissue rostral to the transection was removed and the skull cavity packed with gauze. The cut edges of the bone were filled with bone wax to prevent air embolism and bleeding. About 5 ml of dextran (average mol. wt. 170,000, 6% W/V; Sigma Chemical Co.) were infused immediately after decerebration to compensate for blood loss. The occipital bone covering the cerebellum was removed and the dura was sectioned to expose the cerebellum.

A lumbosacral spinal laminectomy was performed and the L6, L7 and S1 ventral roots were sectioned bilaterally. In twelve animals the corresponding dorsal roots were cut bilaterally but in the rest of the animals the left dorsal roots were left intact and the peripheral nerves leading to the extensor quadriceps (QUAD) and the flexor posterior biceps-semitendinosus (PBST) muscles were isolated and cut. Bipolar platinum electrodes were attached to the central ends of the above nerves. The skin flaps on the animal's back were used to make a pool for holding mineral oil which prevents drying of the exposed spinal cord and stops the spread of current during stimulation of the spinal roots. The temperature of the mineral oil pool and the body of the animal was maintained at 36 ± 1° C with the aid of automatic D.C. temperature regulators (Richardson et al., 1965) or a heating lamp.

In most of the animals an additional spinal laminectomy was performed at the lower thoracic level (T10 - T12), the spinal cord was exposed and kept warm with mineral oil so that a functional cold block (a procedure described later in this section) could be applied.

In some animals that were used for microelectrode studies a bilateral pneumothorax was performed to minimize the respiration induced movement of the spinal cord.

To intra-arterially administer a test drug to the spinal cord, a No. 90 polyethylene tubing filled with 0.9% NaCl (W/V) was inserted in four animals through the left femoral artery into the abdominal aorta such that the tip of the cannula lay about 1 cm caudal to the exit of the renal arteries. All the major arteries below the tip of the cannula except those leading to the spinal cord were ligated as described by Holmstedt and Skoglund (1953).

In another four animals, to inject a drug intra-arterially to the brain stem, a No. 60 polyethylene tubing filled with 0.9% NaCl was inserted through the axillary artery so that the tip of the cannula lay at the branching of the vertebral artery from the left subclavian artery. The omocervical and the internal thoracic arteries were ligated (Clineschmidt and Anderson, 1970).

To inject the test drugs intravenously, a cephalic vein was cannulated with a No. 90 tubing filled with 0.9% NaCl.

Ether was discontinued following surgery and about three hours were allowed for the elimination of the anaesthetic. The animal was maintained on artificial respiration throughout the experiment.

Stimulation and recording procedures:

The central end of a cut dorsal root, usually L7, and the corresponding ventral root were placed on bipolar platinum hook electrodes. The dorsal root - ventral root monosynaptic reflex (MSR) was evoked every 5 sec by stimulation of the dorsal root (0.1 msec square wave pulse delivered from the S2 unit of a S8 Grass stimulator and passed through a S1U5 stimulation isolation unit). The stimulation strength was supramaximal for the MSR. To evoke the QUAD- or the PBST-MSR the respective peripheral nerve was stimulated using the stimulation parameters described for the dorsal root - ventral root MSR. The resulting compound action potential was recorded from the appropriate ventral root, amplified with the help of a Tektronix 2A61 differential amplifier and displayed on a Tektronix R564B storage oscilloscope.

A concentric bipolar stainless steel electrode with a tip diameter of about 0.5 mm and a separation of 0.5 mm between the two poles was positioned

in the ventromedial bulbar reticular formation (P 7.5 to 13.5; L 0 to 0.5; V -6 to -10 in the stereotaxic atlas of Snider and Niemer, 1964). Bulbospinal inhibition of the MSR was evoked by a train of square wave pulses (300 msec train, 150 Hz, 0.5 msec pulses) delivered through the above electrode. The interval between the end of the train and the stimulus to evoke the MSR was 7.5 msec. Bulbar sites and stimulus intensities were chosen which did not elicit movement of the facial, neck and the forelimb muscles or disturb the blood pressure.

Recurrent inhibition of the MSR was produced by stimulation of a ventral root (0.5 msec pulse) adjacent to the root from which the MSR was recorded. The conditioning interval was 7.5 msec.

Reciprocal Ia inhibition of the QUAD- or the PBST-MSR was induced by stimulation of the nerve belonging to the respective antagonistic muscle 1 msec prior to evoking the MSR.

The stimulation strength to evoke bulbospinal, recurrent or reciprocal Ia inhibition of the MSR was adjusted to reduce the MSR to about 40 per cent of its control value.

Presynaptic inhibition of the QUAD- or the PBST-MSR was produced by stimulation of the nerve from the respective antagonistic muscle 30 msec before evoking the MSR. The conditioning stimulus strength was adjusted so that only group I afferents were activated. This was determined by the presence of a single compound action potential recorded from the dorsal root that carried the impulses from the above conditioning nerve.

The bulbospinal, recurrent, reciprocal Ia or presynaptic conditioning stimuli were delivered from the S1 unit of the S8 stimulator and fed through a S1U5 stimulation isolation unit and a switch box. This switch box was utilized to connect or disconnect the conditioning stimuli to any

of the above stimulation sites so that all the above inhibitions of the MSR could be evoked in succession in the same animal.

Cold block:

To prevent supraspinal inputs to the lumbosacral spinal cord, about 1 cm cubes of frozen mammalian Ringer solution or artificial cerebrospinal fluid were placed on the exposed thoracic spinal cord. This procedure will be referred to as a cold block. The blockade of bulbospinal inhibition during the cold block was taken as the criterion for a functional blockade of the supraspinal inputs to the spinal cord. To reverse the cold block the cubes were removed, the cold solution was aspirated and warm mineral oil was then added and changed repeatedly until bulbospinal inhibition of the MSR returned to the pre-cold block level.

Pharmacological studies:

Imipramine HCl (5 mg/kg i.v.; Geigy Ltd.) was tested on presynaptic inhibition of the QUAD- and the PBST-MSR. The effect of a cold block was tested before and after the injection of imipramine.

Six animals were pretreated on two consecutive days with DL-p-chlorophenylalanine (p-CPA, 300 mg/kg i.p.; Sigma Chemical Co.) and the experiment was conducted on the third day when the 5-hydroxytryptamine (5-HT) levels in the spinal cord of the cat were reported to be depleted by about 90 per cent of control (Taber and Anderson, 1973).

Six other animals were pretreated with DL-α-methyl-p-tyrosine methyl ester HCl (α-MPT, 125 mg/kg i.p.; Sigma Chemical Co.) 16 and 4 hours prior to the experiment. A similar pretreatment was reported to deplete noradrenaline in the spinal cord of the cat to immeasurable levels (King and

Jewett, 1971).

The effects of a cold block, imipramine (5 mg/kg i.v.) and a second cold block were tested in sequence on presynaptic and recurrent inhibitions of the QUAD-MSR in the above p-CPA or α-MPT pretreated animals. Fluoxetine HCl (Lilly 110140; Eli Lilly and Co.), reported to be a specific 5-HT neuronal uptake blocking agent (Fuller et al., 1975; Wong et al., 1974, 1975), was administered at 10 min intervals in increasing doses. The initial dose was 0.25 mg/kg i.v. and the cumulative dose administered over 50 min was 6 mg/kg. The actions of this agent were determined on bulbospinal, recurrent, reciprocal Ia and presynaptic inhibitions of the extensor and the flexor MSRs. A cold block was applied before and after the injection of fluoxetine.

In preliminary studies 5-hydroxytryptophan (5-HTP; Sigma Chemical Co.) produced somewhat variable effects on the unconditioned MSR as reported by Clineschmidt et al. (1971). However, when the animals were pretreated with a small dose of imipramine (0.25 mg/kg i.v.) the action of 5-HTP was less variable. Therefore, 5-HTP (75 mg/kg i.v.) was administered 10 min after completing the injection of imipramine and its effects on bulbospinal, recurrent, reciprocal Ia and presynaptic inhibitions of the QUAD-MSR were determined.

The 5-HT antagonist, cyproheptadine HCl (5 mg/kg i.v.; Merck Sharp and Dohme Ltd.), was injected 40 and 60 min after completing the administration of fluoxetine or 5-HTP, respectively.

Clonidine HCl (Boehringer Ingleheim Ltd.), reported to be a specific α-adrenergic agonist in the central nervous system (Anden et al., 1970; Kobinger and Pichler, 1975), was administered at 10 min intervals in geometrically increasing cumulative doses. The initial dose was 2.5 μg/kg i.v. and the final cumulative dose over 40 min was 40 μg/kg. All the previously

mentioned inhibitions of the flexor and the extensor MSRs were tested during clonidine injections. A cold block was applied before and after the injection of clonidine. The α-adrenergic blocking agent, phenoxybenzamine HCl (5 mg/kg; Smith Kline French) was injected 30 min after the completion of the administration of clonidine and the inhibitions of the flexor MSR were tested.

Cyproheptadine (2.5 mg/kg) or phenoxybenzamine (2.5 mg/kg) was injected twice at 10 min intervals and all the inhibitions of the QUAD- and the PBST-MSR described previously were tested. In four of the twelve animals used for these experiments the inhibitions of the extensor and the flexor MSRs were evoked in the same animal.

In order to determine the site of action of imipramine in blocking bulbospinal and recurrent inhibitions of the MSR (Sastry, 1973; Sinclair and Sastry, 1974, 1974a), cumulative dose-response curves were established for the blockade of the inhibitions when the drug was injected intra-arterially to the spinal cord, intra-arterially to the brain stem or intravenously. In these experiments the initial dose of imipramine was 0.125 mg/kg when administered intra-arterially and 0.5 mg/kg when administered intravenously. Subsequent doses, increasing by geometric progression, were administered slowly at 10 min intervals. The final cumulative dose was 4 mg/kg.

Ia afferent antidromic action potentials and motoneurone field potentials:

In eight cats, a tungsten (5 μm exposed tip, about 5 MΩ) or a glass microelectrode filled with 3M NaCl (about 2 μm and 3 MΩ) was directed towards the QUAD motor nucleus while recording the orthodromic and the antidromic field potentials from the electrode tip as described by Eccles et al.

(1954). These field potentials were evoked by stimulation of the peripheral QUAD nerve (0.5 msec pulse) and the L6 ventral root (0.02 msec pulse). The tip of the electrode was assumed to be in the QUAD motor nucleus where the magnitudes of the field potentials were maximal. This position was usually 2 mm lateral to the midline and about 4.1 mm ventral from the top of the spinal cord as reported by Eccles et al. (1954).

The QUAD Ia afferents were antidromically activated by stimulating in the QUAD motor nucleus (Wall, 1958). The resulting compound action potential (QUAD-AP) was recorded from the peripheral nerve. The magnitude of the QUAD-AP was adjusted to less than 50 per cent of its maximal size. In all of the experiments the same microelectrode was used to record the maximum antidromic field potential and to evoke the QUAD-AP. The antidromic field potential and the QUAD-AP were tested during bulbospinal inhibition of the extensor MSR.

In three experiments, the time course of spinal presynaptic inhibition of the MSR and the corresponding facilitation of the QUAD-AP was followed by altering the conditioning interval. The interval where the QUAD-AP was maximally facilitated (11.5 - 13.5 msec) was chosen to study the drug and the cold block effects in all eight experiments. The conditioning interval selected for these studies on presynaptic inhibition of the QUAD-MSR was 30 msec where interference of a postsynaptic inhibition was reported to be minimal (Eccles et al., 1962).

The stimulation pulses to evoke the antidromic field potential or the QUAD-AP were delivered from the S2 unit of the S8 stimulator and passed through a S1U5 stimulation isolation unit. The QUAD-MSR, the antidromic field potential and the QUAD-AP were fed to a switch box which was used to connect one of the above inputs to a recording device. The field potential

was fed through a DAM-5 differential preamplifier or a VF-1 voltage follower (W.P. Instruments) and a Tektronix 2A61 differential amplifier and displayed on the storage oscilloscope. The QUAD-AP was recorded as described for the MSR.

The effects of imipramine (2 mg/kg i.v.; injected twice) and cyproheptadine (5 mg/kg i.v.) were tested on the unconditioned QUAD-MSR, QUAD-AP and the field potential as well as the above responses during bulbospinal and spinal presynaptic inhibitions of the MSR. In addition, the action of a cold block was tested before and after imipramine on the unconditioned and presynaptic conditioned MSR, QUAD-AP and the field potential.

Preparation of test drug solutions:

All the test drugs except cyproheptadine and phenoxybenzamine were dissolved in 0.9% NaCl just before injection. Cyproheptadine (5 mg/kg) was dissolved in 0.5 ml of propylene glycol and the resulting solution was diluted to 1 ml with 0.9% NaCl. Phenoxybenzamine (5 mg/kg) was dissolved in 1 ml of propylene glycol. The vehicle, propylene glycol (1 ml injected over 2 min), was tested on the inhibitions of the MSR in three animals.

Dextran (6% W/V) was sometimes used to maintain the blood volume and Flaxedil^{R} (Poulenc Ltd.) was routinely used to prevent movements during nerve stimulation. All agents were administered with the aid of an infusion pump.

Calculations:

In most of the experiments the signal output from the storage oscilloscope was fed through an Ortec 4623 signal averager and displayed on a Tektronix R5030 dual beam oscilloscope. Pictures of the signals were taken

on Polaroid land film, type 107, using a C-27 Tektronix oscilloscope camera.

The magnitudes of the unconditioned and the conditioned responses (MSR, antidromic field potential or the QUAD-AP) were determined at 10 min intervals by averaging 8 consecutive unconditioned and the following 4 conditioned responses. The average unconditioned response before and after a drug administration was expressed as a per cent of the final control response. The actual per cent inhibition or facilitation of the response on the final control test was calculated and equated to 100 per cent inhibition or facilitation, respectively. The actual per cent inhibition or facilitation of the previous control tests and tests subsequent to a drug injection were adjusted on the basis of this final control figure. Therefore, a procedure which enhanced the inhibition or facilitation of a response would give a value of greater than 100 per cent whereas a blockade of the above would result in a reduction in the per cent figure.

Iontophoretic study:

In six animals a five barrel micropipette (3 - 7 μm tip diameter), mounted on a micromanipulator, was inserted into the spinal gray matter. The central barrel of the micropipette was filled with 3M NaCl (3 - 10 MΩ) and each of the outer barrels was filled with one of the following solutions: glycine (0.5M, pH 3.5 HCl; Sigma Chemical Co.), γ-aminobutyric acid (GABA, 0.5M, pH 3.5 HCl; Sigma Chemical Co.), clonidine HCl (0.01M in 0.1M NaCl, pH 4 HCl) and Na glutamate (0.2M, pH 7.5 NaOH; Sigma Chemical Co.). The NaCl barrel was connected in series to a VF-1 Voltage follower, a Tektronix 2A61 differential amplifier and an oscilloscope. The extracellular action potentials of 34 unidentified single spinal neurones were recorded through the NaCl barrel. The signals were fed through a Ferch

rate meter and recorded on a polygraph as spikes per second.

The drug ions were held in the pipette by a backing current of about 10 nA. The effect of clonidine on the firing rate of the cells was tested by passing a positive current (usually from 0 backing current to about 30 nA). The maximum clonidine current that did not significantly alter the neurone firing frequency was then determined. Glycine or GABA was ejected by passing 2 - 20 nA positive current and the effect on the cell firing rate was determined before, during and after an ejection of clonidine that did not significantly alter the control firing rate. A negative current (5 - 40 nA) was passed through the glutamate barrel to increase and maintain the firing rate of most of the cells at a reasonably constant level. Current effects were checked by passing a negative current through the clonidine barrel and a positive current through the glutamate barrel.

Preparation of the micropipettes:

The micropipettes (Vancouver Scientific Glass Blowers) were pulled using a Narishige vertical micropipette puller. The micropipette tip was then broken during observation through a microscope so that the tip diameter was 3 - 7 μm. All solutions were passed through a millipore filter before use. A few of the micropipettes were filled with the drug solutions by centrifuging at 9000 revolutions per min for about 10 min. Other pipettes were immersed in gently boiling distilled water for about an hour. The water was then cooled and the micropipettes were found to be filled. The distilled water from the stems of the pipettes was removed using a 31 gauge hypodermic needle and syringe. Then the pipettes were filled with the drug solutions and left overnight in a cold room with the tips immersed in distilled water. The next day these pipettes were centrifuged at 6000 revolutions per min for 5 min before use.

RESULTS

Presynaptic inhibition:

The thoracic cold block enhanced presynaptic inhibition of the QUAD-MSR but did not alter the inhibition of the PBST-MSR (Fig. 1,2,5,7 and 8). Imipramine HCl (5 mg/kg i.v.; Fig. 1 and 8) and fluoxetine HCl (Lilly 110140, 0.25 - 6 mg/kg i.v.; Fig. 2) antagonized the inhibition of the extensor but not of the flexor MSR. The blockade of the inhibition by imipramine or fluoxetine was completely eliminated by a cold block. The 5-HT antagonist, cyproheptadine HCl (5 mg/kg i.v.), also reversed the blocking action of fluoxetine.

In animals that were pretreated with p-CPA or α-MPT, neither a cold block nor imipramine had an effect on the inhibition of the QUAD-MSR (Fig. 3).

The small dose of imipramine (0.25 mg/kg i.v.) had no significant effect on presynaptic inhibition of the extensor MSR. However, when 5-HTP was administered 10 min after the completion of the injection of imipramine, the inhibition was gradually antagonized. This blocking effect reached maximum in about 60 min. Cyproheptadine (5 mg/kg i.v.) partially reversed the above action of 5-HTP (Fig. 4).

Clonidine HCl (2.5 - 40 μg/kg i.v.) antagonized presynaptic inhibition of the extensor and the flexor MSRs. A cold block did not alter the above effects of clonidine. Phenoxybenzamine HCl (5 mg/kg i.v.) also had no effect on the above blockade of the PBST-MSR (Fig. 5).

Fig. 6 depicts a parallelism between the time course of the facili-

tation of the QUAD-AP and that of the inhibition of the QUAD-MSR, suggesting a cause - effect relationship between the two events.

The cold block enhanced presynaptic inhibition of the QUAD-MSR as well as the unconditioned and the conditioned QUAD-AP (Fig. 8). This effect on the conditioned QUAD-AP was not apparent from Fig. 7 but was observed in six of eight experiments. Imipramine (2 mg/kg i.v., administered twice) reduced the unconditioned QUAD-AP, antagonized presynaptic inhibition of the QUAD-MSR and blocked the facilitation of the QUAD-AP during the conditioning (Fig. 7 and 8). A cold block and cyproheptadine (5 mg/kg i.v.) partly reversed the above effects of imipramine.

Cyproheptadine (2.5 mg/kg i.v.) or phenoxybenzamine (2.5 mg/kg i.v.), injected twice, enhanced the inhibition of the extensor but partially antagonized the inhibition of the flexor MSR in the experiments in which the inhibitions of the above MSRs were evoked in the same animal and in those in which the inhibition of either MSR was produced (Fig. 9).

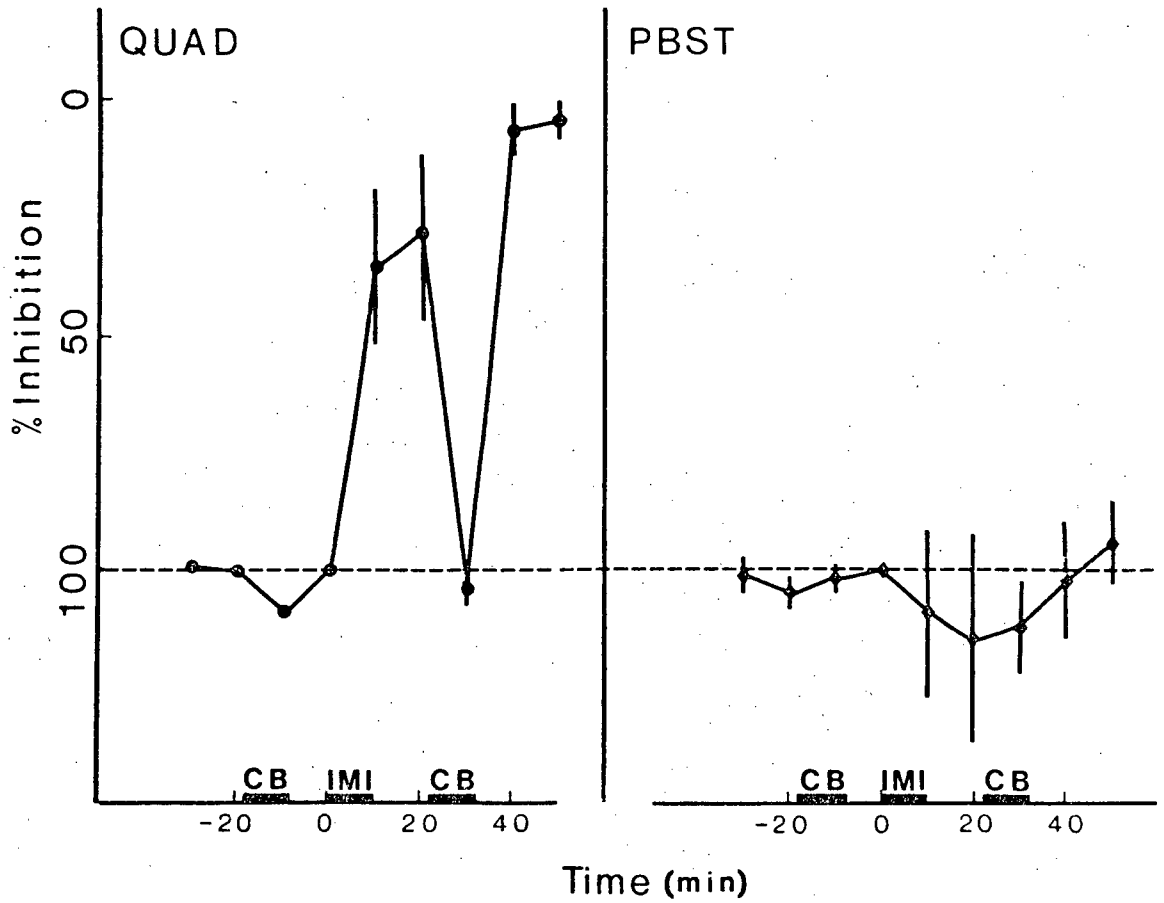

Fig. 1. The effects of imipramine HCl (IMI, 5 mg/kg) on presynaptic inhibition of the QUAD- (n=6) and the PBST- (n=6) MSRs. A cold block (CB) was applied before and after the administration of imipramine as indicated on the abscissa. In this and the subsequent graphs: 1. each point equals the mean ± S.E.M. and 2. the duration of a drug injection or a cold block application is indicated on the abscissa.

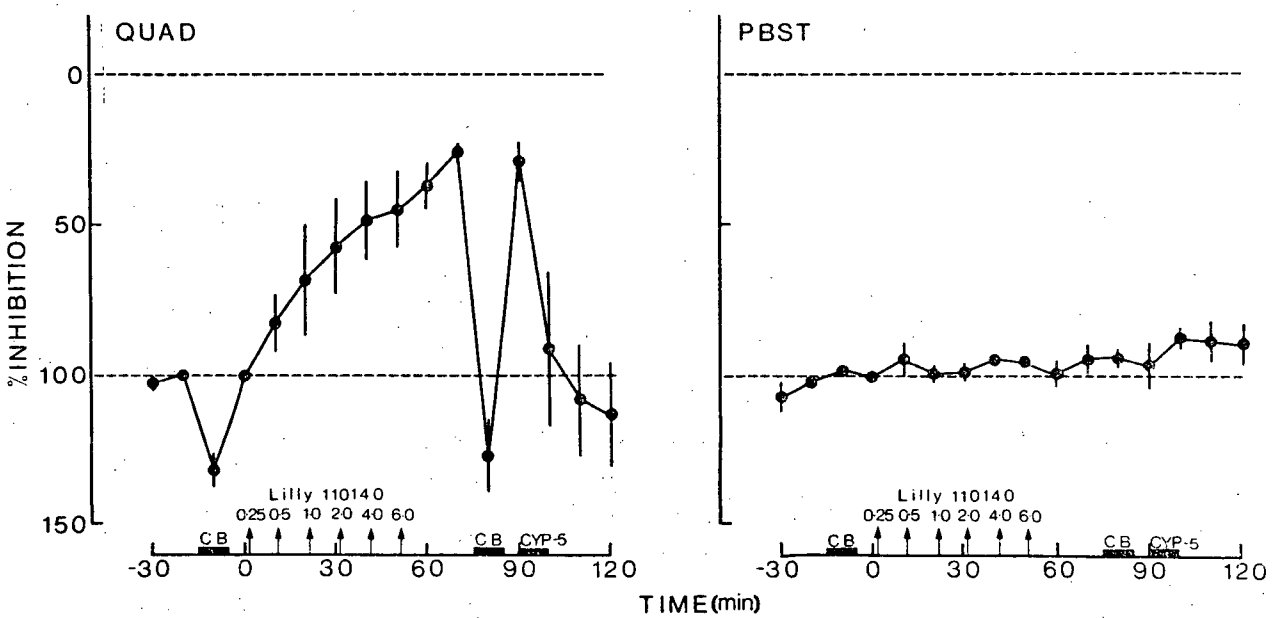

Fig. 2. The actions of fluoxetine HCl (Lilly 110140, increasing cumulative doses in mg/kg as indicated on the abscissa) on presynaptic inhibition of the QUAD- (n=5) and the PBST- (n=5) MSRs. A cold block (CB) was applied prior and subsequent to the injection of fluoxetine. Cyproheptadine HCl (CYP, 5 mg/kg) was administered 40 min after the completion of the injection of fluoxetine.

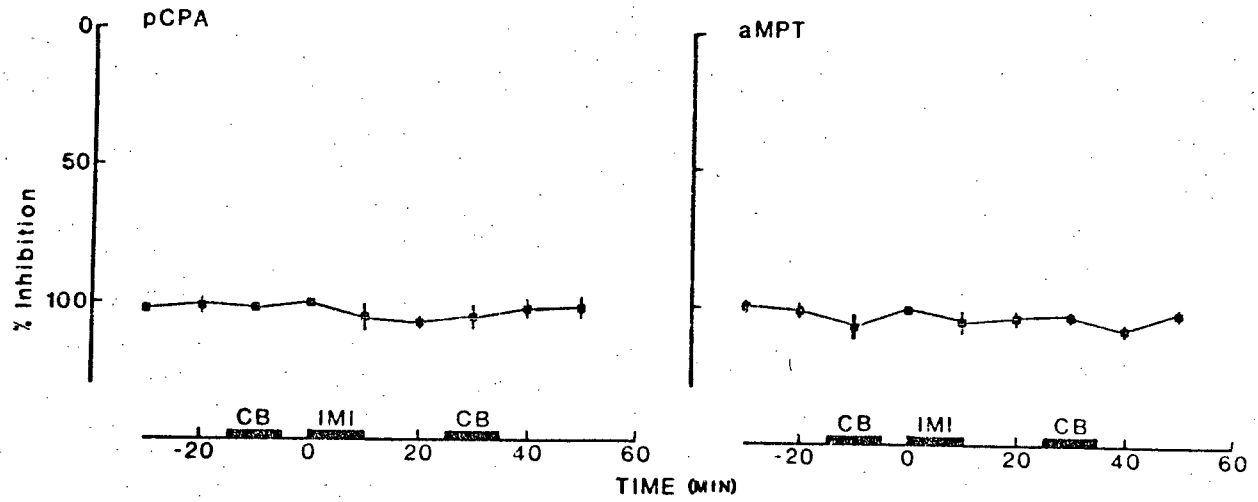

Fig. 3. The effects of a cold block (CB), imipramine HCl (IMI, 5 mg/kg) and a second cold block, tested in sequence, on presynaptic inhibition of the QUAD-MSR in cats pretreated with p-CPA (n=6) or α-MPT (n=6).

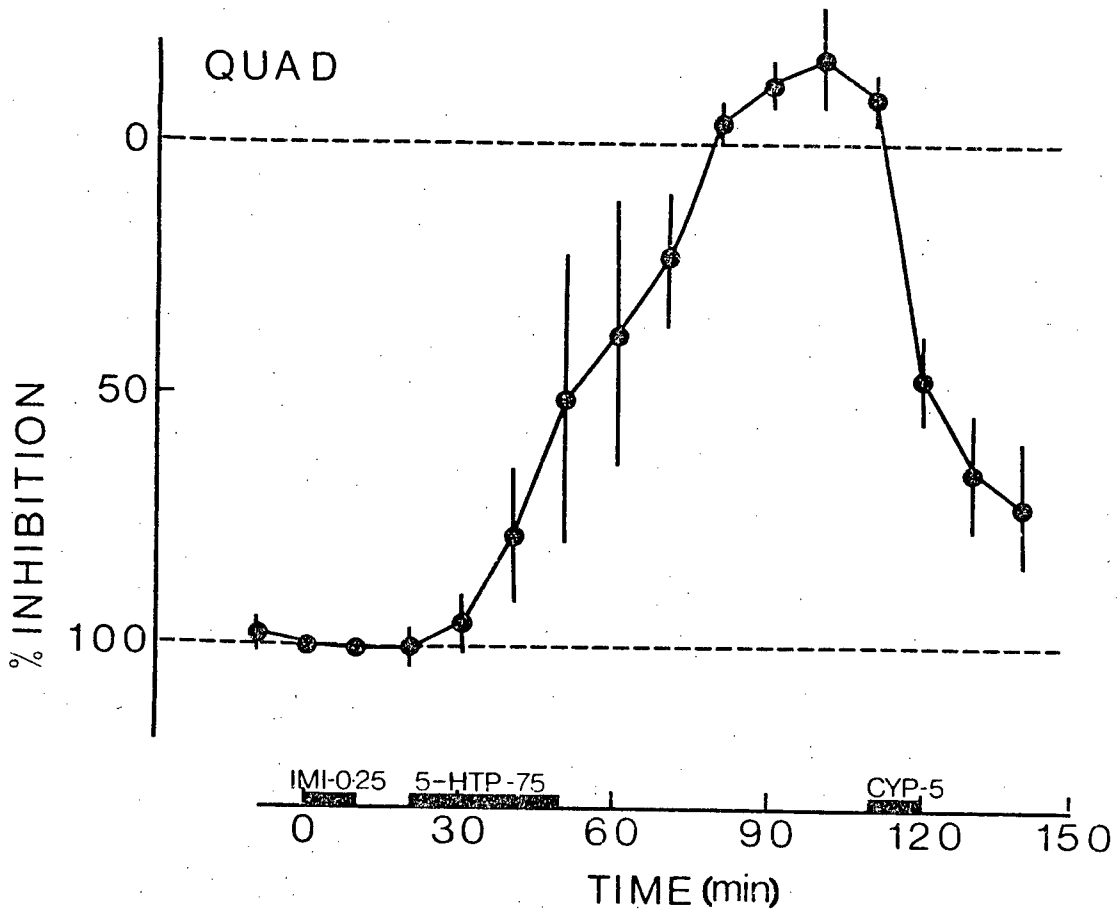

Fig. 4. The effects of a small dose of imipramine HCl (IMI, 0.25 mg/kg), 5-HTP (75 mg/kg) and cyproheptadine HCl (CYP, 5 mg/kg) on presynaptic inhibition of the QUAD-MSR (n=6).

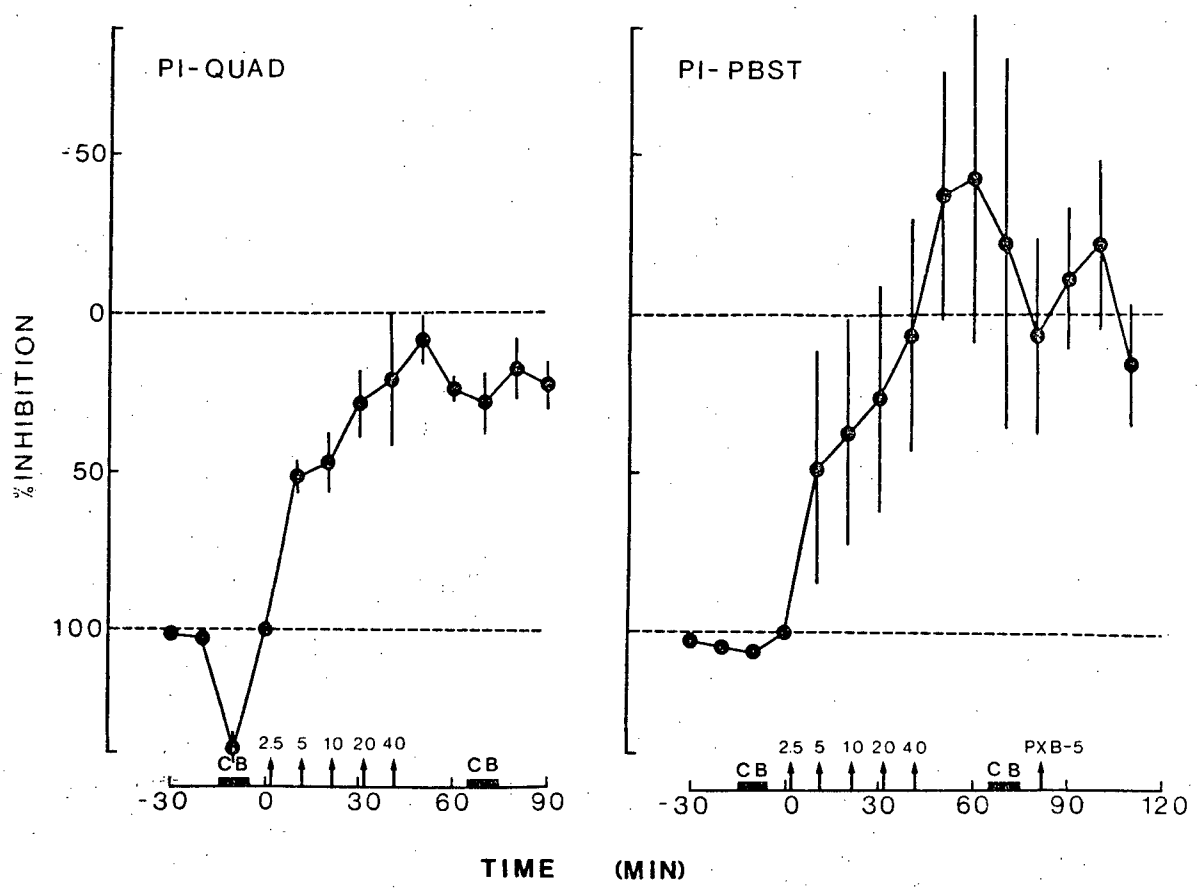

Fig. 5. The blockade of presynaptic inhibition of the QUAD- (PI-QUAD, n=6) and the PBST- (PI-PBST, n=4) MSRs by clonidine HCl administered in geometrically increasing cumulative doses in μg/kg as indicated on the abscissa. A cold block (CB) was applied before and after the injection of clonidine. The action of phenoxybenzamine HCl (PXB, 5 mg/kg) was tested on the inhibition of the flexor reflex after the second cold block.

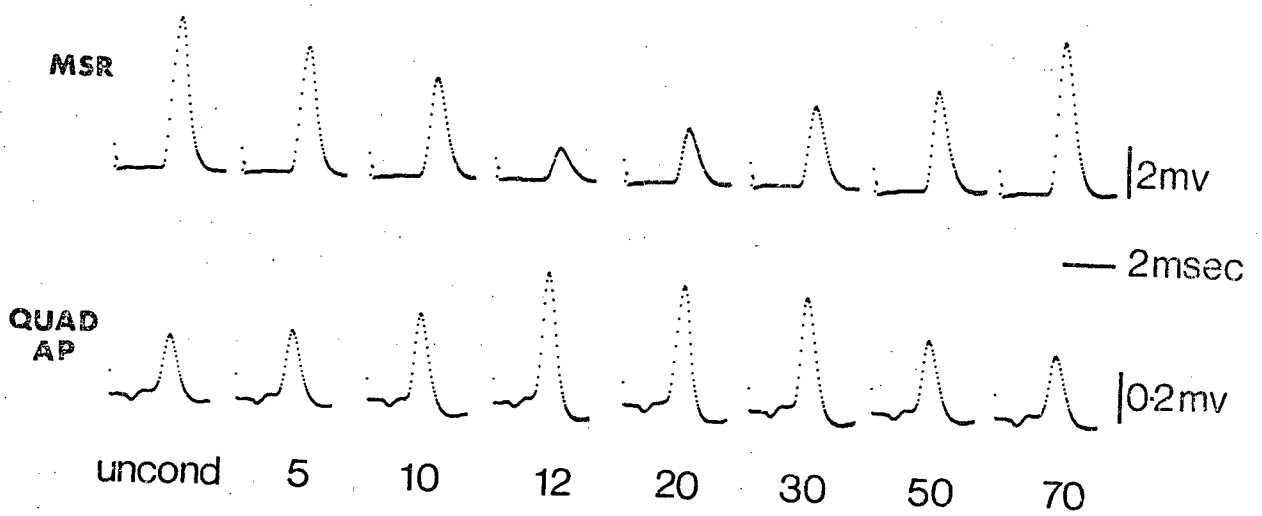

Fig. 6. The time course of presynaptic inhibition of the QUAD-MSR (MSR) and facilitation of the QUAD-AP during the conditioning by PBST group I afferents. The extreme left frames illustrate the unconditioned (uncond) responses. The conditioning intervals in msec are indicated at the bottom of each frame. Each signal represents the averaged response of four sweeps.

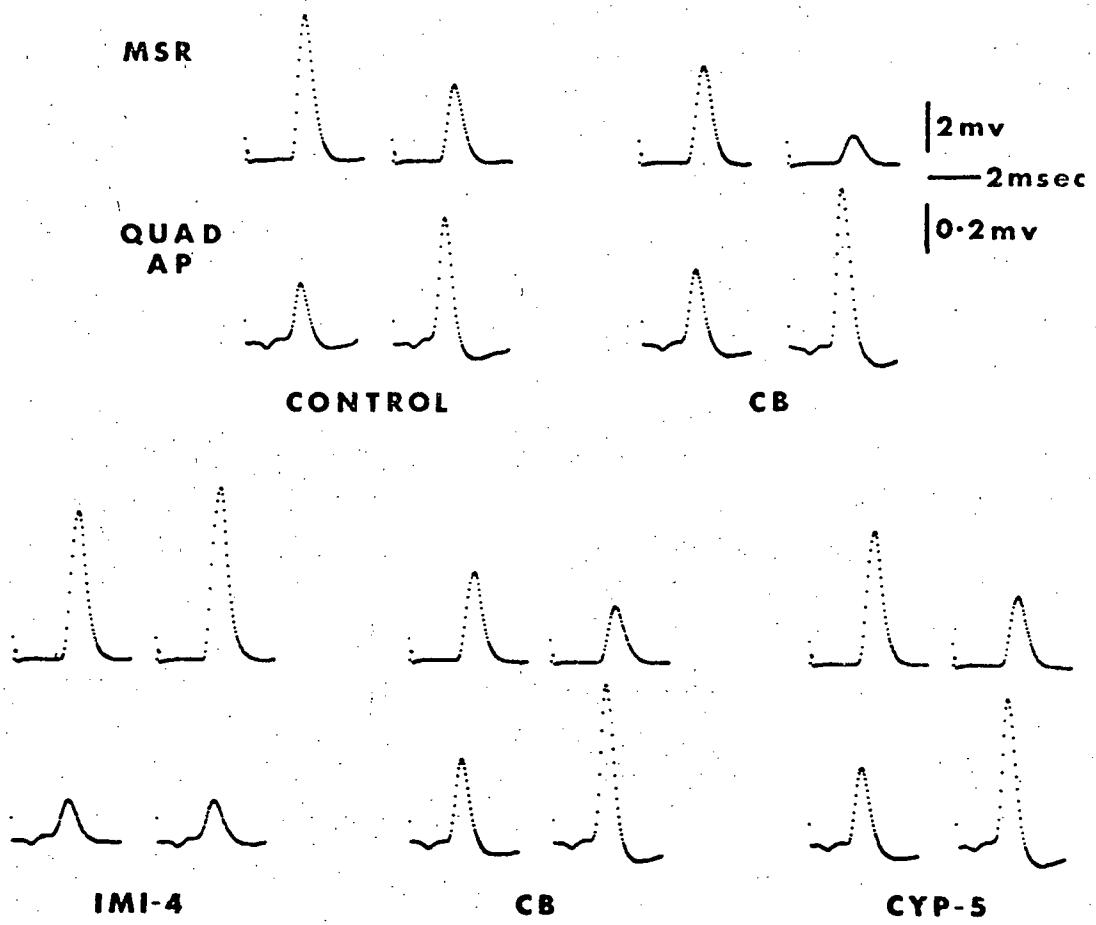

Fig. 7. The blockade of presynaptic inhibition of the QUAD-MSR and the corresponding facilitation of the QUAD-AP by imipramine HCl (IMI, 2 mg/kg injected twice over 20 min). In each panel the top frames represent the QUAD-MSR (MSR) and the bottom frames represent the QUAD-AP. In each panel the left signals illustrate the unconditioned responses and the right ones portray the responses conditioned by PBST group I afferents. A cold block (CB) was applied 10 min before and 10 min after the injection of 4 mg/kg of imipramine. Cyproheptadine HCl (CYP, 5 mg/kg) was administered 20 min after the injection of imipramine. Each signal represents the averaged response of four sweeps.

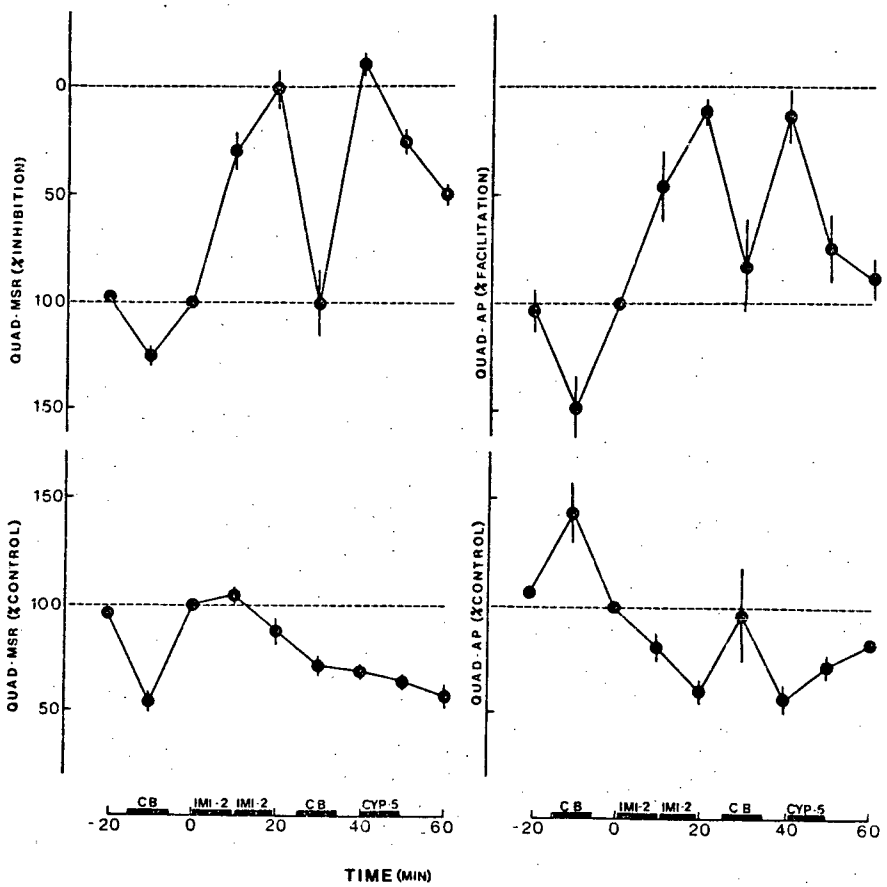

Fig. 8. The effects of imipramine HCl (IMI, 2 mg/kg administered twice) on the unconditioned QUAD-MSR (bottom left graph), presynaptic inhibition of the QUAD-MSR (top left), the unconditioned QUAD-AP (bottom right) and the conditioned QUAD-AP (top right). A cold block (CB) was applied before and after imipramine. Cyproheptadine HCl (CYP, 5 mg/kg) was administered after the second cold block as indicated on the abscissa. (n=8).

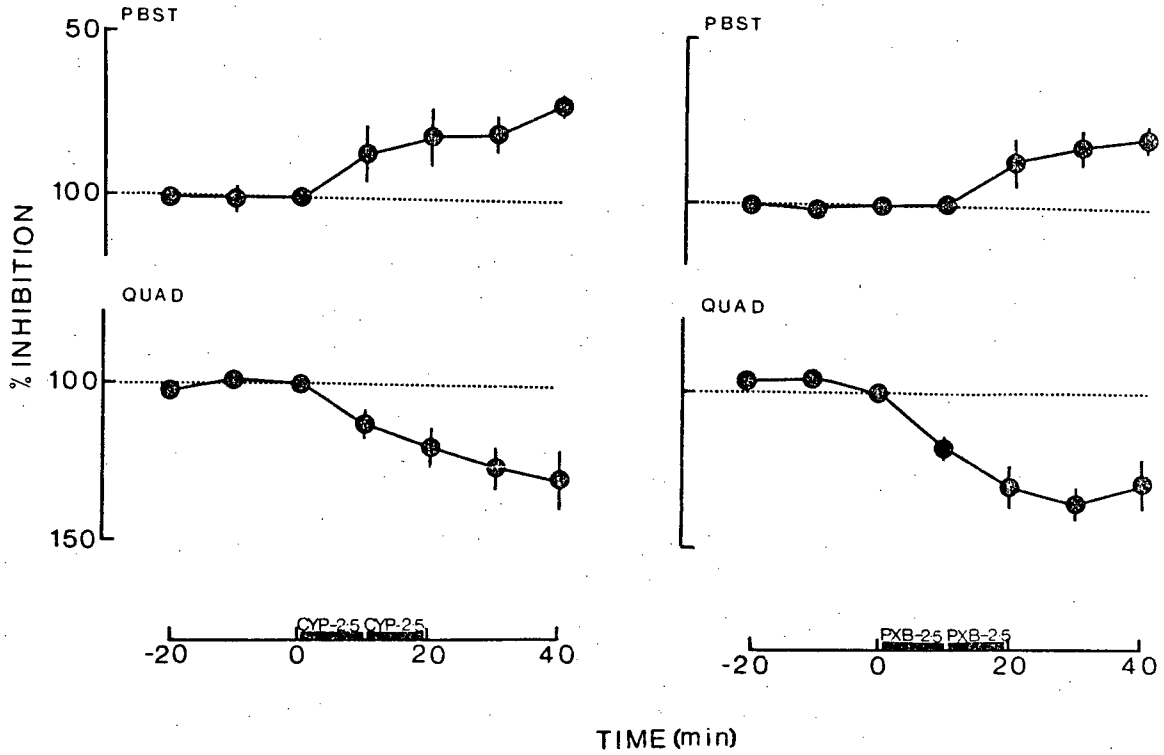

Fig. 9. The enhancement of presynaptic inhibition of the QUAD-MSR by cyproheptadine HCl (CYP, 2.5 mg/kg administered twice; bottom left graph) and phenoxybenzamine HCl (PXB, 2.5 mg/kg injected twice; bottom right). The inhibition of the PBST-MSR was partially blocked by cyproheptadine (top left) and phenoxybenzamine (top right). For each graph n=4.

Bulbospinal inhibition:

Fluoxetine (0.25 - 6 mg/kg i.v.) antagonized bulbospinal inhibition of both the extensor and the flexor MSRs, although the effect was greater on the inhibition of the extensor reflex. This blockade by fluoxetine was partially reversed by cyproheptadine (5 mg/kg i.v.; Fig. 10 and 11).

Imipramine (0.25 mg/kg i.v.) did not significantly alter the inhibition of the QUAD-MSR but the subsequent 5-HTP (75 mg/kg i.v.) administration converted the inhibition into a facilitation in four of six animals tested (Fig. 12). The antagonism gradually reached maximum about 60 min after the start of injection. Cyproheptadine (5 mg/kg i.v.) partially reversed the blockade by 5-HTP (Fig. 12).

Clonidine (2.5 - 40 μg/kg i.v.) converted the inhibition of the QUAD-MSR into a facilitation in four of six experiments (Fig. 13, left panel). Bulbospinal inhibition of the PBST-MSR was similarly blocked by this agent. Phenoxybenzamine (5 mg/kg i.v.) did not alter the blockade of the flexor MSR.

Fig. 14 illustrates that cyproheptadine (2.5 - 5 mg/kg i.v.) enhanced and phenoxybenzamine (2.5 - 5 mg/kg i.v.) antagonized the inhibition of both the QUAD- and the PBST-MSRs. The magnitude of cyproheptadine's action was greater on the inhibition of the extensor MSR than on that of the flexor MSR.

The dose-response curve for intra-arterial injection of imipramine to the brain stem was not significantly different from the curve for intravenous administration of this agent (Fig. 15). However, the dose-response curve for imipramine was shifted to the left of the other two curves when the drug was administered intra-arterially to the spinal cord. The points after the doses 0.25 - 2 mg/kg on the dose-response curve for intra-arterial

injection to the spinal cord were significantly different ($P < 0.05$) than the corresponding points on the other two curves (Fig. 15). Therefore, imipramine was more potent when injected intra-arterially to the spinal cord than when administered by the other two routes.

During bulbospinal inhibition of the QUAD-MSR, the antidromic field potential was reduced and the QUAD-AP facilitated (Fig. 16). Imipramine (2 mg/kg i.v., administered twice) antagonized the above inhibition of the QUAD-AP while blocking bulbospinal inhibition of the QUAD-MSR (Fig. 16 and 17). Cyproheptadine (5 mg/kg i.v.) partially reversed this blockade.

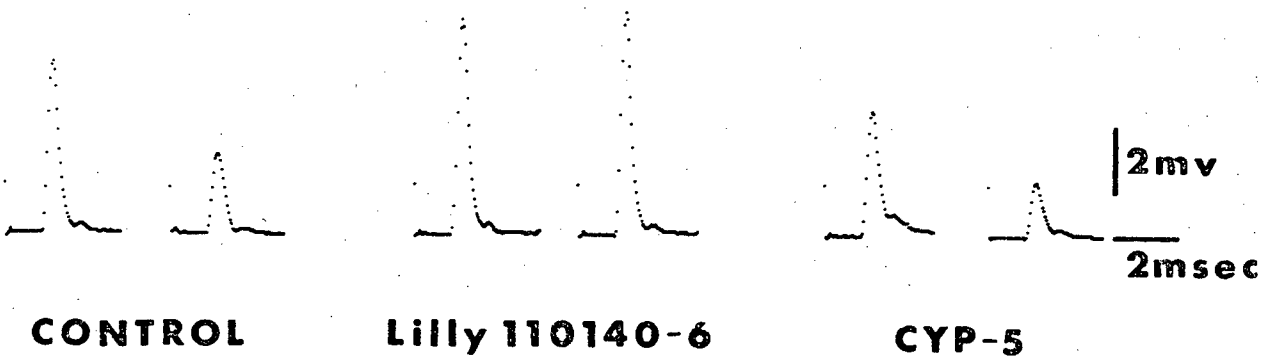

Fig. 10. The blockade of bulbospinal inhibition of the QUAD-MSR by fluoxetine HCl (Lilly 110140, total dose of 6 mg/kg injected over 50 min) and a reversal of the blockade by cyproheptadine HCl (CYP, 5 mg/kg) administered 50 min after the completion of fluoxetine injection. The left frame in each panel represents the unconditioned and the right the conditioned MSR. Each signal represents the averaged response of four sweeps.

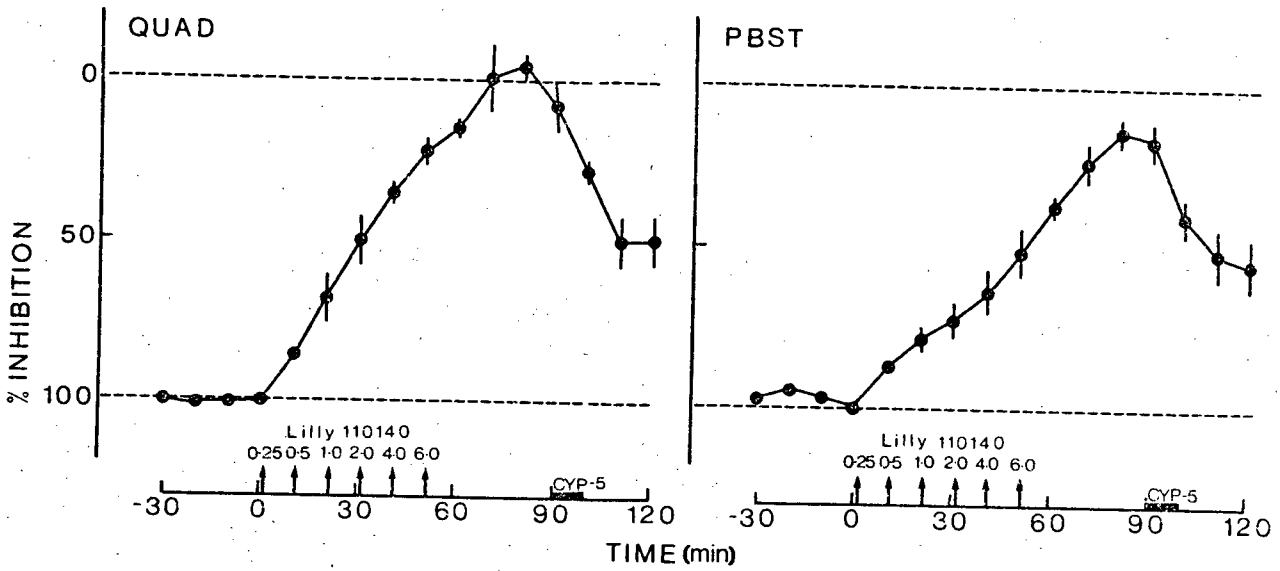

Fig. 11. The effects of fluoxetine HCl (Lilly 110140, administered in increasing cumulative doses in mg/kg as indicated on the abscissa) and cyproheptadine HCl (CYP, 5 mg/kg) on bulbospinal inhibition of the QUAD- (n=5) and the PBST- (n=5) MSRs.

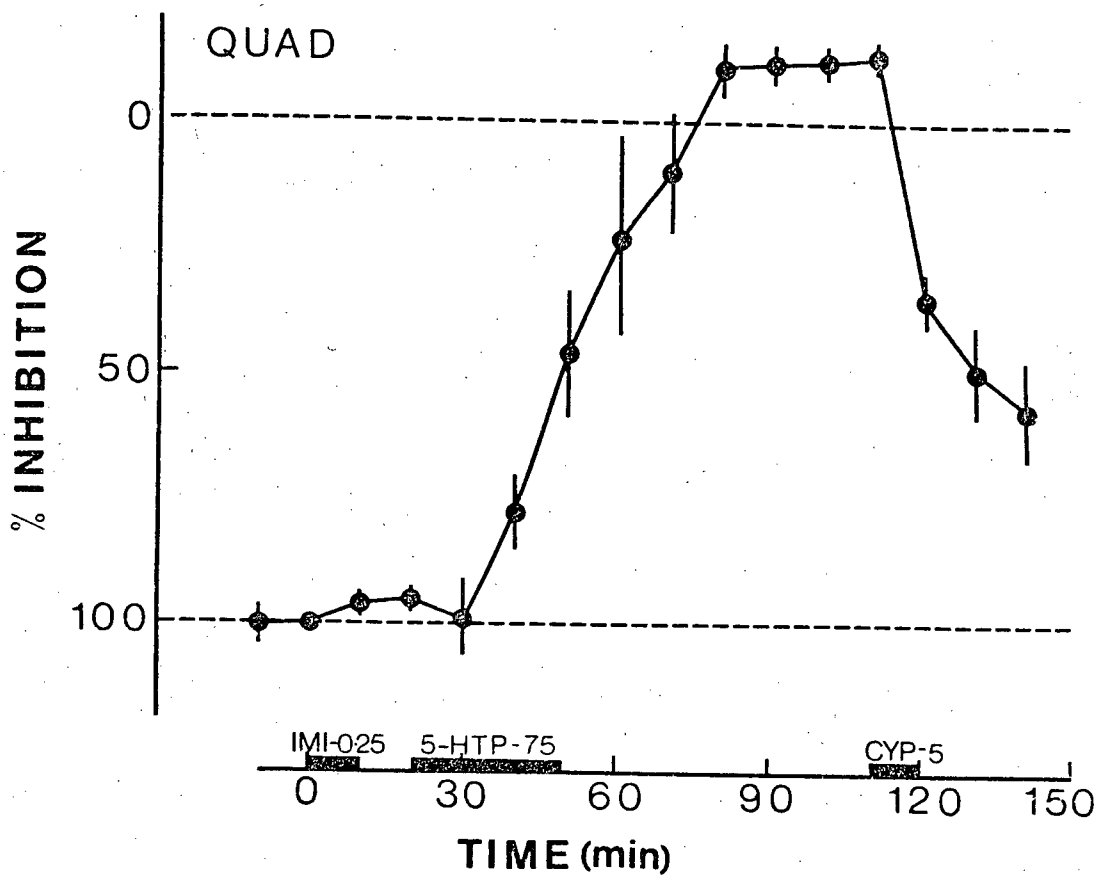

Fig. 12. The effects of imipramine HCl (IMI, 0.25 mg/kg), 5-HTP (75 mg/kg) and cyproheptadine HCl (CYP, 5 mg/kg), tested in sequence, on bulbospinal inhibition of the QUAD-MSR (n=6).

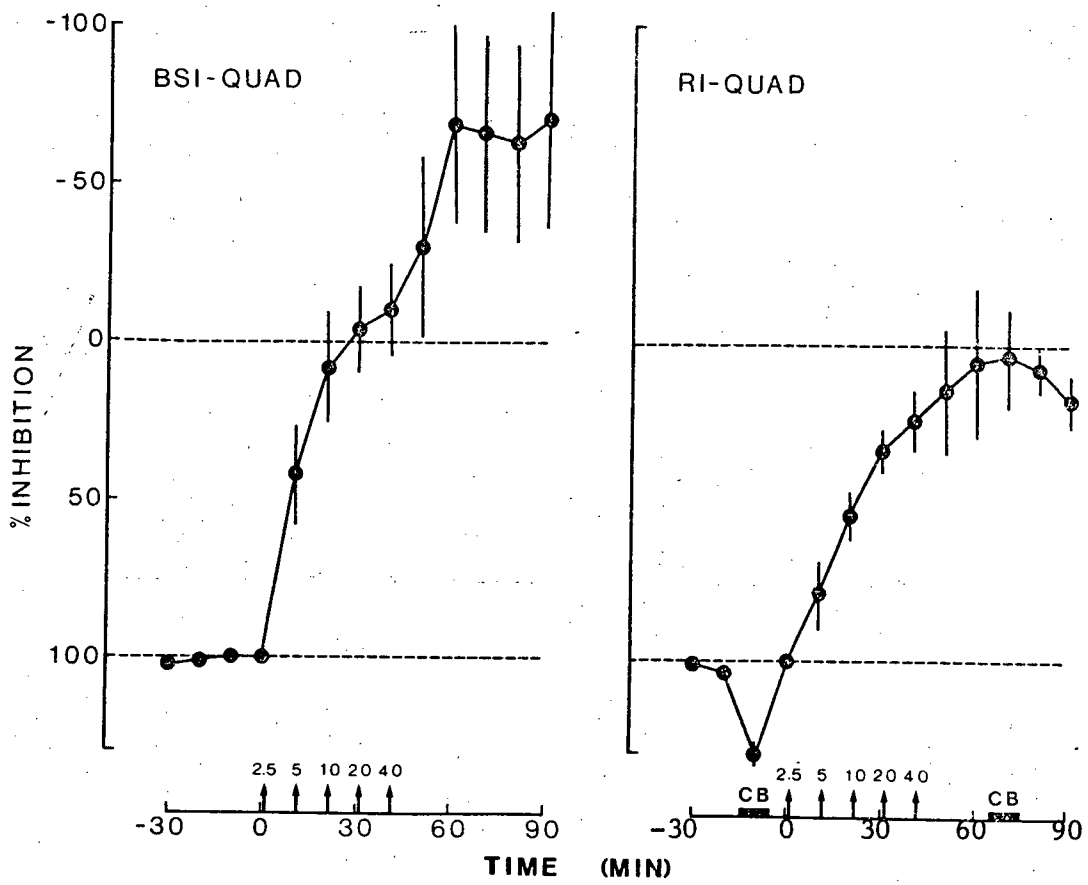

Fig. 13. The blockade of bulbospinal (BSI-QUAD, n=6) and recurrent (RI-QUAD, n=6) inhibitions of the QUAD-MSR by clonidine HCl (administered in geometrically increasing cumulative doses in μg/kg as indicated on the abscissa). The effects of a cold block (CB) application was tested on recurrent inhibition before and after the injection of clonidine.

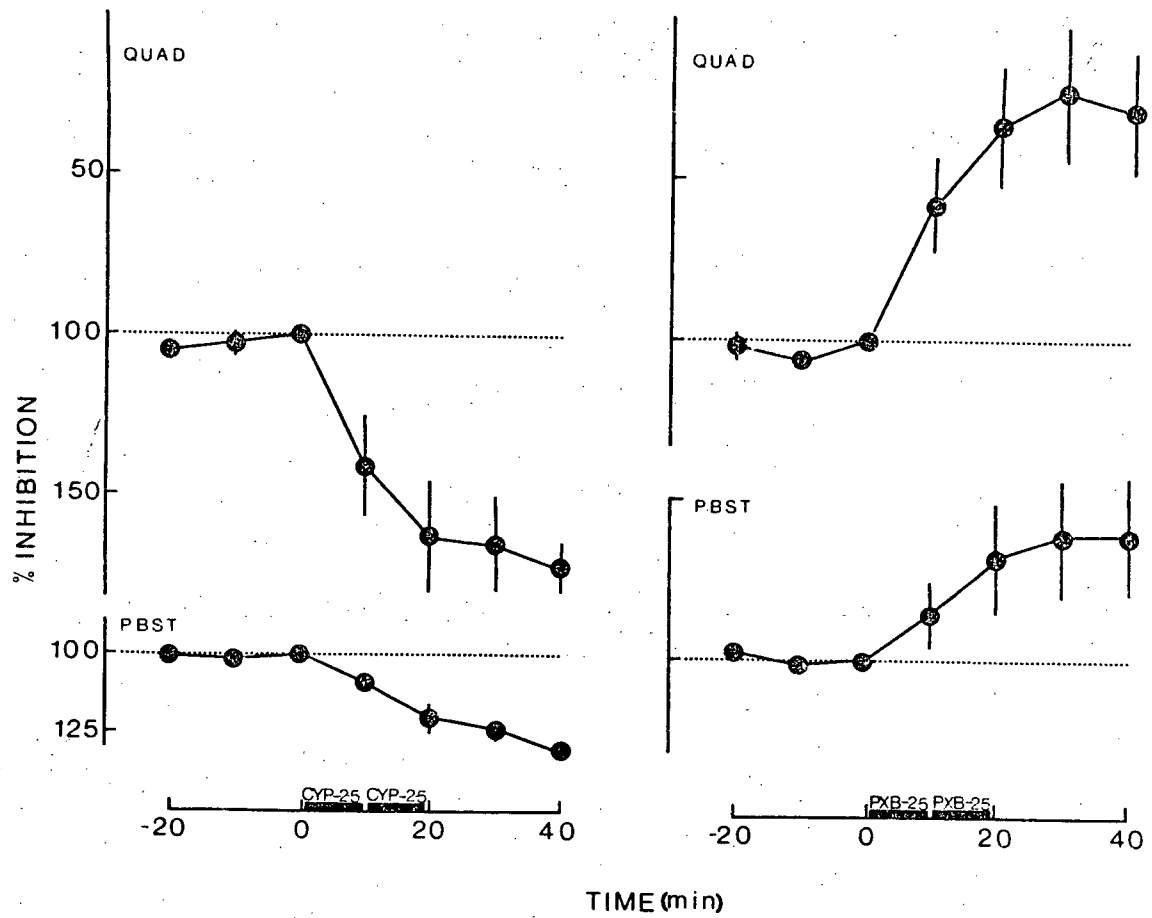

Fig. 14. The enhancement of bulbospinal inhibition of the QUAD- (top left graph) and the PBST- (bottom left) MSRs by cyproheptadine HCl (CYP, 2.5 mg/kg administered twice). The antagonism of the inhibition of the QUAD- (top right) and the PBST- (bottom right) MSRs by phenoxybenzamine HCl (PXB, 2.5 mg/kg injected twice) is also illustrated. For each graph n=4.

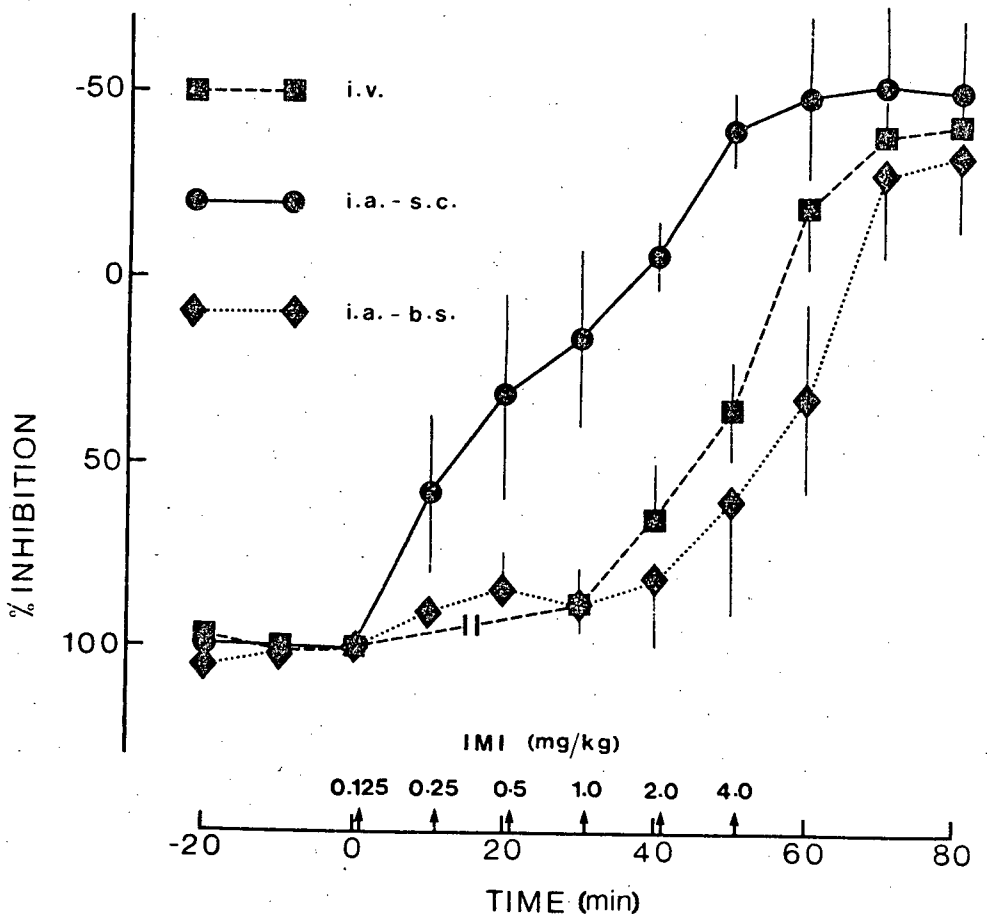

Fig. 15. The blockade of bulbospinal inhibition of the dorsal root - ventral root MSR by imipramine HCl (IMI). The drug was administered intravenously (i.v., 0.5 - 4 mg/kg), intra-arterially to the spinal cord (i.a. - s.c., 0.125 - 4 mg/kg) and intra-arterially to the brain stem (i.a. - b.s., 0.125 - 4 mg/kg) in geometrically increasing cumulative doses. For each curve n=4.

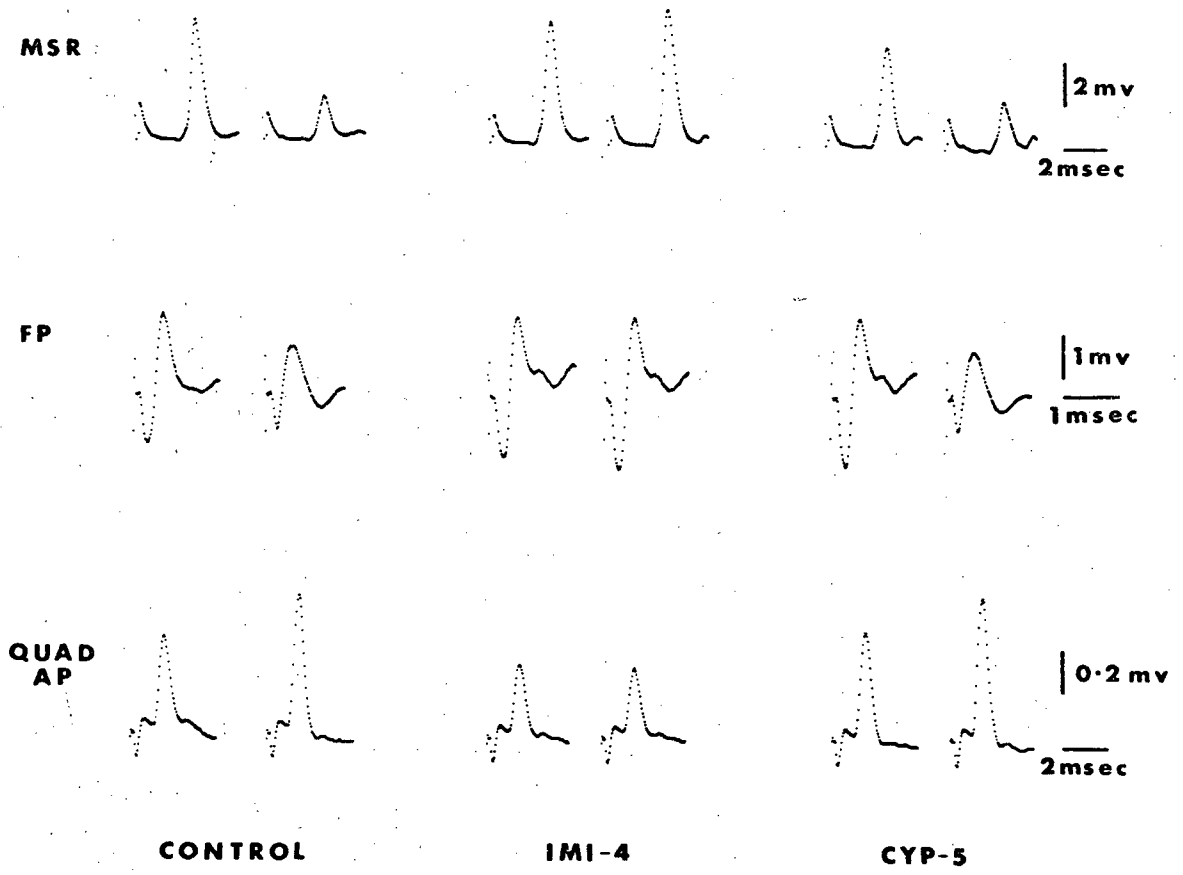

Fig. 16. The blockade of bulbospinal inhibition of the QUAD-MSR (MSR), and the associated reduction in the antidromic motoneurone field potential (FP) and the facilitation of QUAD-AP by imipramine HCl (IMI, 2 mg/kg administered twice over 20 min). A reversal of the blockade by cyproheptadine HCl (CYP, 5 mg/kg injected 20 min after the completion of imipramine administration). The left signals in each pair represent the unconditioned responses and the right signals illustrate the conditioned responses. Each signal represents the averaged response of 4 sweeps.

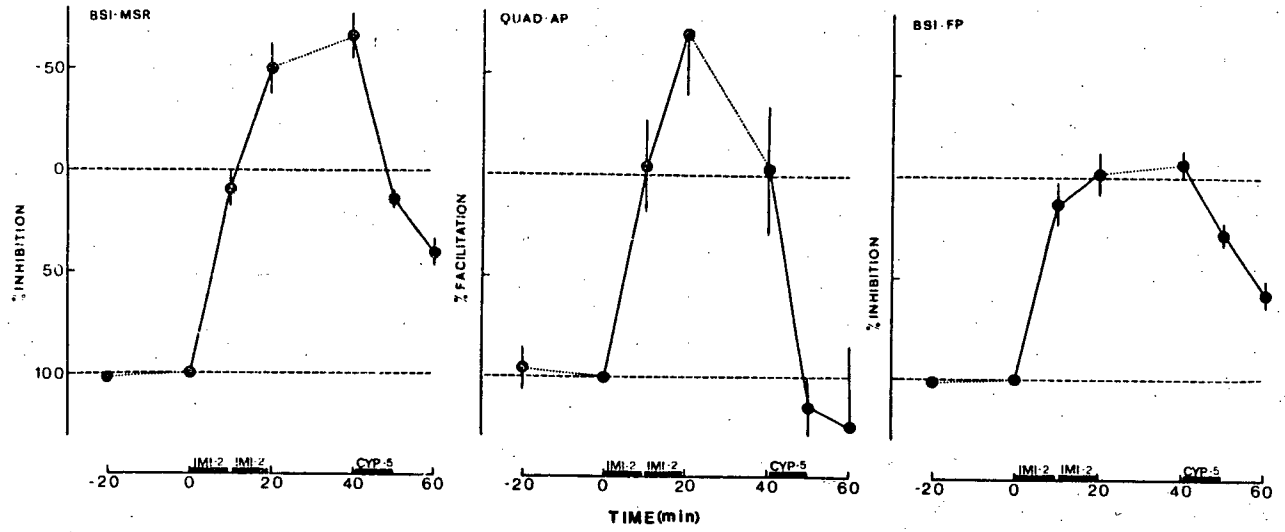

Fig. 17. The blockade of bulbospinal inhibition of the QUAD-MSR (BSI-MSR), and the associated facilitation of the QUAD-AP and inhibition of the antidromic motoneurone field potential (BSI-FP) by imipramine HCl (IMI, 2 mg/kg administered twice). The effects of cyproheptadine HCl (CYP, 5 mg/kg) are also shown (n=8).

Recurrent inhibition:

Clonidine (2.5 - 40 μg/kg i.v.) blocked the inhibition of the QUAD- (Fig. 13, right panel) and the PBST- (not shown) MSRs. A cold block did not alter the above action of clonidine. Also, phenoxybenzamine (5 mg/kg i.v.) failed to reverse clonidine's effect on the inhibition of the flexor MSR.

When supraspinal inputs to the spinal cord were eliminated by the application of a thoracic cold block, recurrent inhibition of the QUAD-MSR was facilitated (Fig. 13, right panel and Fig. 18, left panel). The inhibition of the PBST-MSR was, however, unaffected (Fig. 18). Fluoxetine (0.25 - 6 mg/kg i.v.), given following the recovery of the cold block, antagonized the inhibition of the QUAD- but not of the PBST-MSR. A second cold block, applied after the injection of fluoxetine, completely reversed the above blockade. Cyproheptadine (5 mg/kg i.v.) also partially reversed the action of fluoxetine on the inhibition of the extensor MSR (Fig. 18).

In the cats pretreated with p-CPA or α-MPT neither a cold block nor imipramine (5 mg/kg i.v.) altered recurrent inhibition of the QUAD-MSR (Fig. 19).

The inhibition of the extensor MSR was unaltered by the small dose of imipramine (0.25 mg/kg i.v.), however, 5-HTP (75 mg/kg i.v.) gradually blocked the inhibition. This blockade reached maximum in about 60 min. Cyproheptadine (5 mg/kg i.v.) reversed the blockade by 5-HTP (Fig. 20).

Cyproheptadine (2.5 - 5 mg/kg i.v.) and phenoxybenzamine (2.5 - 5 mg/kg i.v.) enhanced the inhibition of the extensor but partially reversed the inhibition of the flexor MSR in the experiments in which recurrent inhibition of the QUAD- and the PBST-MSRs was evoked in the same animal and in those in which the inhibition of either MSR was produced (Fig. 21).

The dose-response curve for intra-arterial administration of imipramine to the spinal cord was shifted to the left of the curves for intra-arterial injection to the brain stem or intravenous administration (Fig. 22). The points after the doses 0.25 - 2 mg/kg on the curve for intra-arterial injection to the spinal cord were significantly different ($P < 0.05$) from the corresponding points on the other two curves. These results indicate that imipramine antagonized recurrent inhibition by a spinal site of action.

Reciprocal Ia inhibition:

Imipramine (5 mg/kg i.v., n=6), fluoxetine (0.25 - 6 mg/kg i.v., n=5), cyproheptadine (2.5 - 5 mg/kg i.v., n=4) and phenoxybenzamine (2.5 - 5 mg/kg i.v., n=4) had no significant effect on reciprocal Ia inhibition of the extensor and the flexor MSRs. Moreover, 5-HTP (75 mg/kg i.v., n=6) also failed to alter the inhibition of the extensor MSR.

A cold block enhanced the inhibition of the extensor but not of the flexor MSR. Clonidine (2.5 - 40 μg/kg i.v.) antagonized reciprocal Ia inhibition of both the QUAD- and the PBST-MSRs (Fig. 23). A subsequent cold block did not alter clonidine's effect. Furthermore, phenoxybenzamine (5 mg/kg i.v.) also failed to alter the blockade of the PBST reflex by clonidine.

Control experiments:

To test whether the inhibitions were stable throughout the time course of experiments, bulbospinal and recurrent inhibitions of the dorsal root - ventral root MSR were recorded for 2½ hours in two experiments. The maximum mean deviation from the control values was 7.8% for bulbospinal and

9.3% for recurrent inhibition.

In five experiments the size of the QUAD-MSR was reduced to about 50 per cent of its control value by decreasing the stimulation strength. In these animals recurrent and presynaptic inhibitions were tested on the control and the reduced MSR without altering the conditioning stimulation strength. The inhibitions of the reduced MSR were not significantly altered from those of the control (recurrent: 103 ± 4.2% S.E.M.; presynaptic: 105 ± 6.7% S.E.M.). However, in these animals a cold block reduced the MSR by 46 ± 5.9% S.E.M. and increased recurrent inhibition to 122 ± 5.2% S.E.M. and presynaptic inhibition to 128 ± 7.1% S.E.M. of control. These results suggest that the enhancement of the inhibitions during a cold block is not due to a reduction in the size of the unconditioned MSR.

Propylene glycol (1 ml) enhanced bulbospinal, recurrent and presynaptic inhibitions of the QUAD-MSR. However, the inhibitions returned to control values about 1 min after the injection, indicating that the actions of cyproheptadine and phenoxybenzamine on the above inhibitions were independent of the effects of the vehicle.

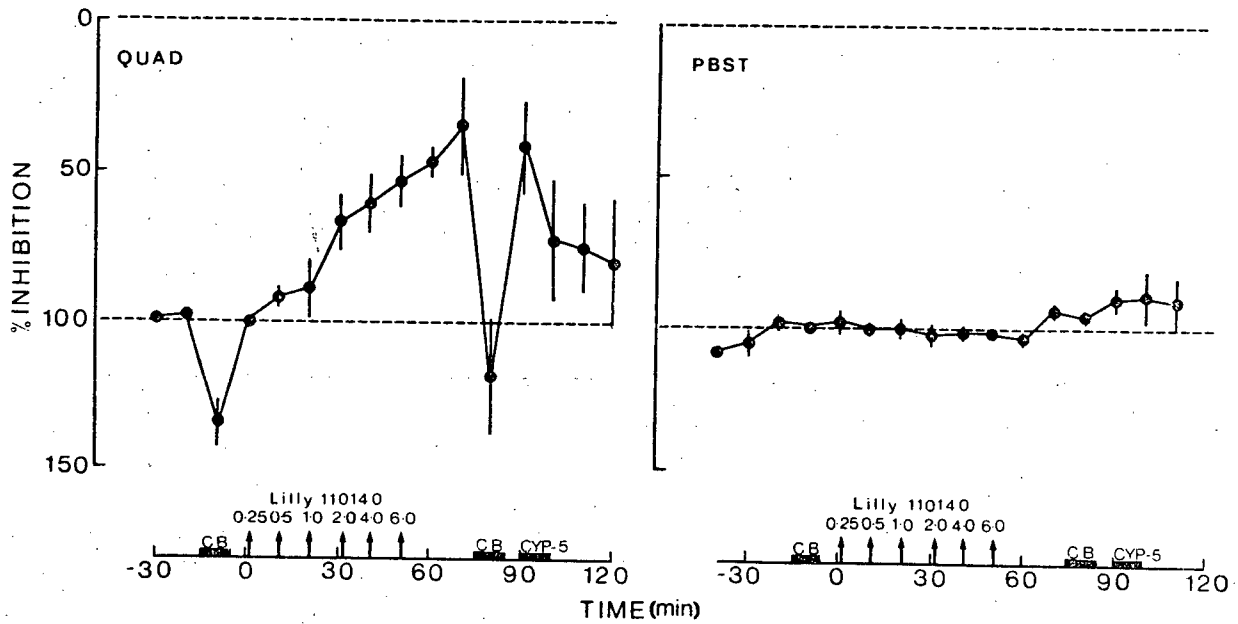

Fig. 18. The actions of fluoxetine HCl (Lilly 110140, administered in increasing cumulative doses in mg/kg as indicated on the abscissa) on recurrent inhibition of the QUAD- (n=5) and the PBST- (n=5) MSRs. A cold block (CB) was applied before and after the injection of fluoxetine. The effects of cyproheptadine HCl (CYP, 5 mg/kg) were also tested.

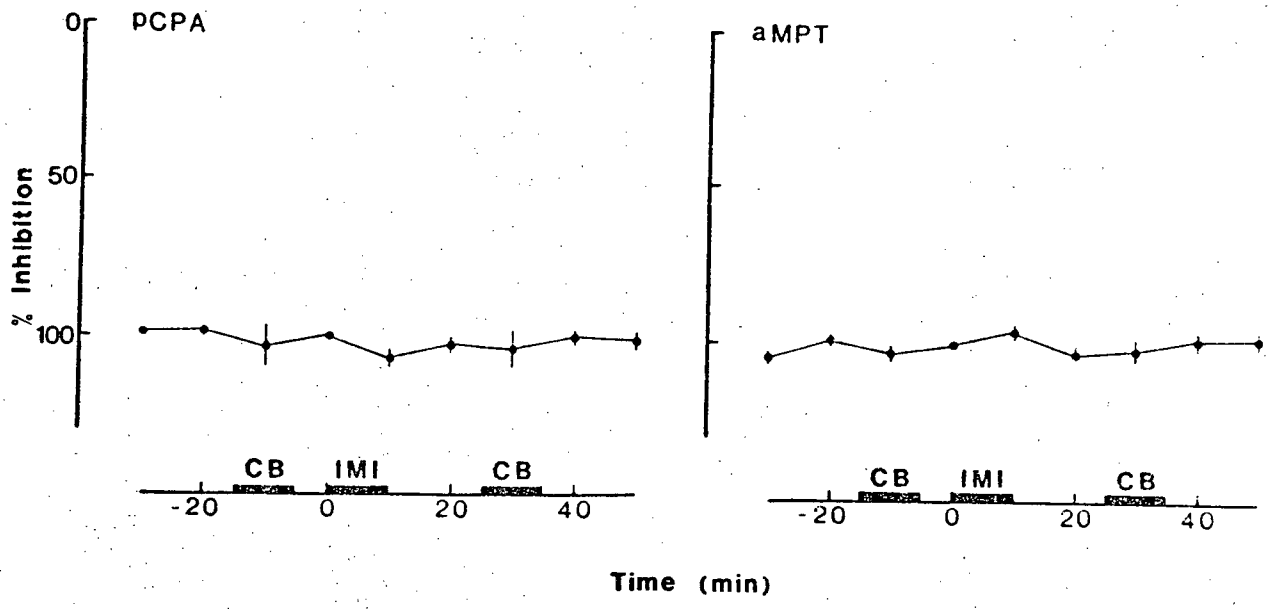

Fig. 19. The effects of a cold block (CB), imipramine HCl (IMI, 5 mg/kg) and a second cold block, tested in sequence, on recurrent inhibition of the QUAD-MSR in cats pretreated with p-CPA (n=6) or α-MPT (n=6).

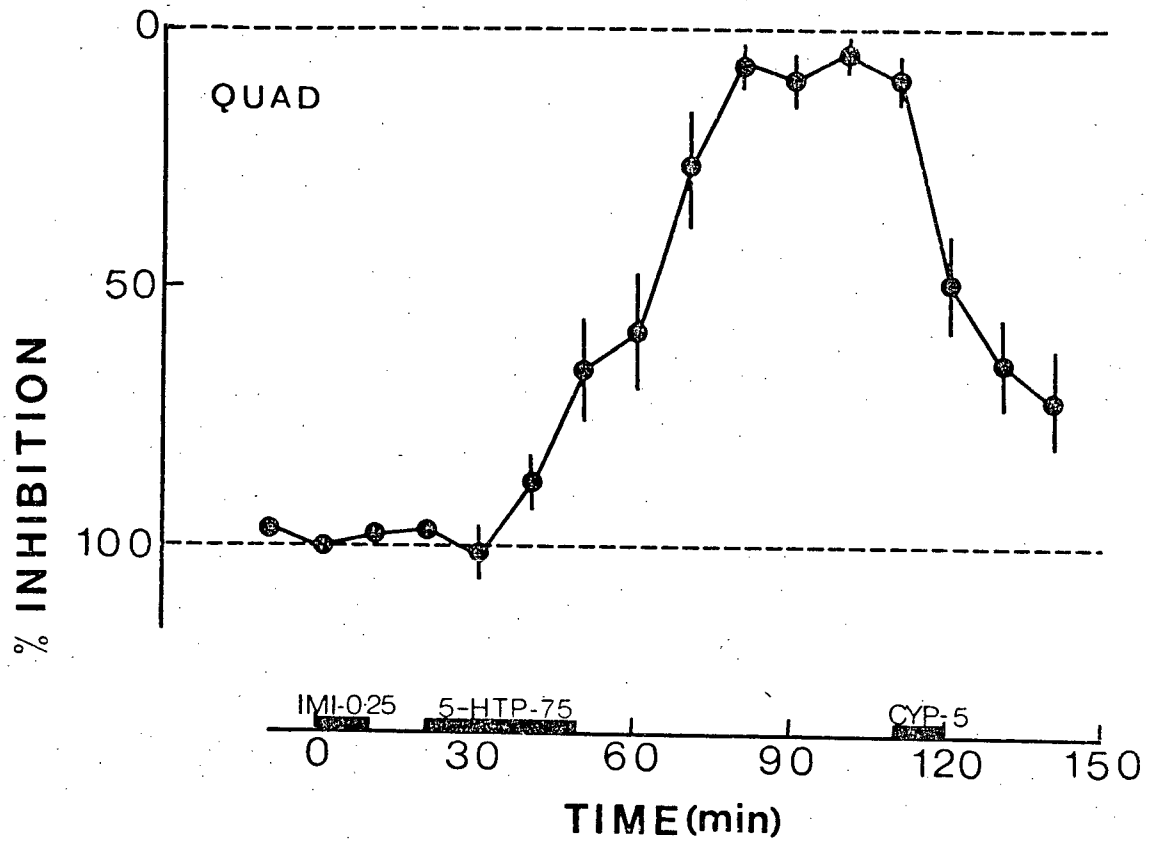

Fig. 20. The effects of a small dose of imipramine HCl (IMI, 0.25 mg/kg), 5-HTP (75 mg/kg) and cyproheptadine HCl (CYP, 5 mg/kg) on recurrent inhibition of the QUAD-MSR (n=6).

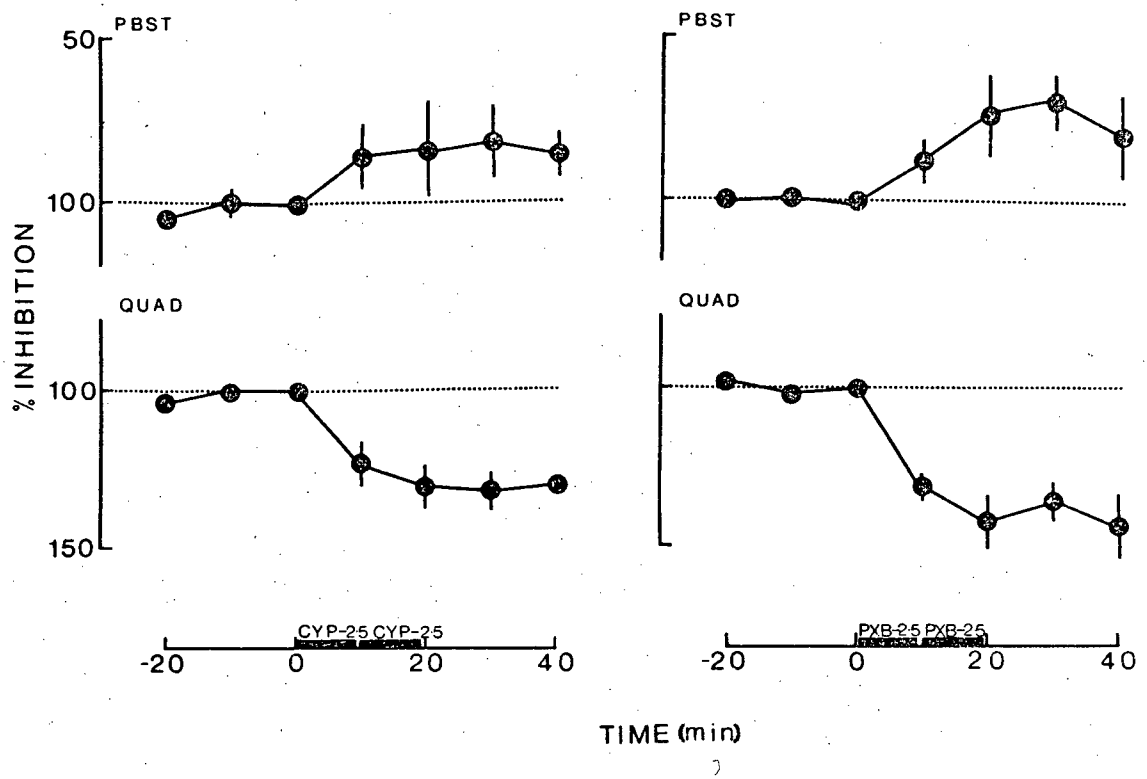

Fig. 21. The enhancement of recurrent inhibition of the QUAD-MSR by cyproheptadine HCl (CYP, 2.5 mg/kg administered twice; bottom left graph) and phenoxybenzamine HCl (PXB, 2.5 mg/kg injected twice; bottom right). The inhibition of the PBST-MSR was partially blocked by cyproheptadine (top left) and phenoxybenzamine (top right). For each graph n=4.

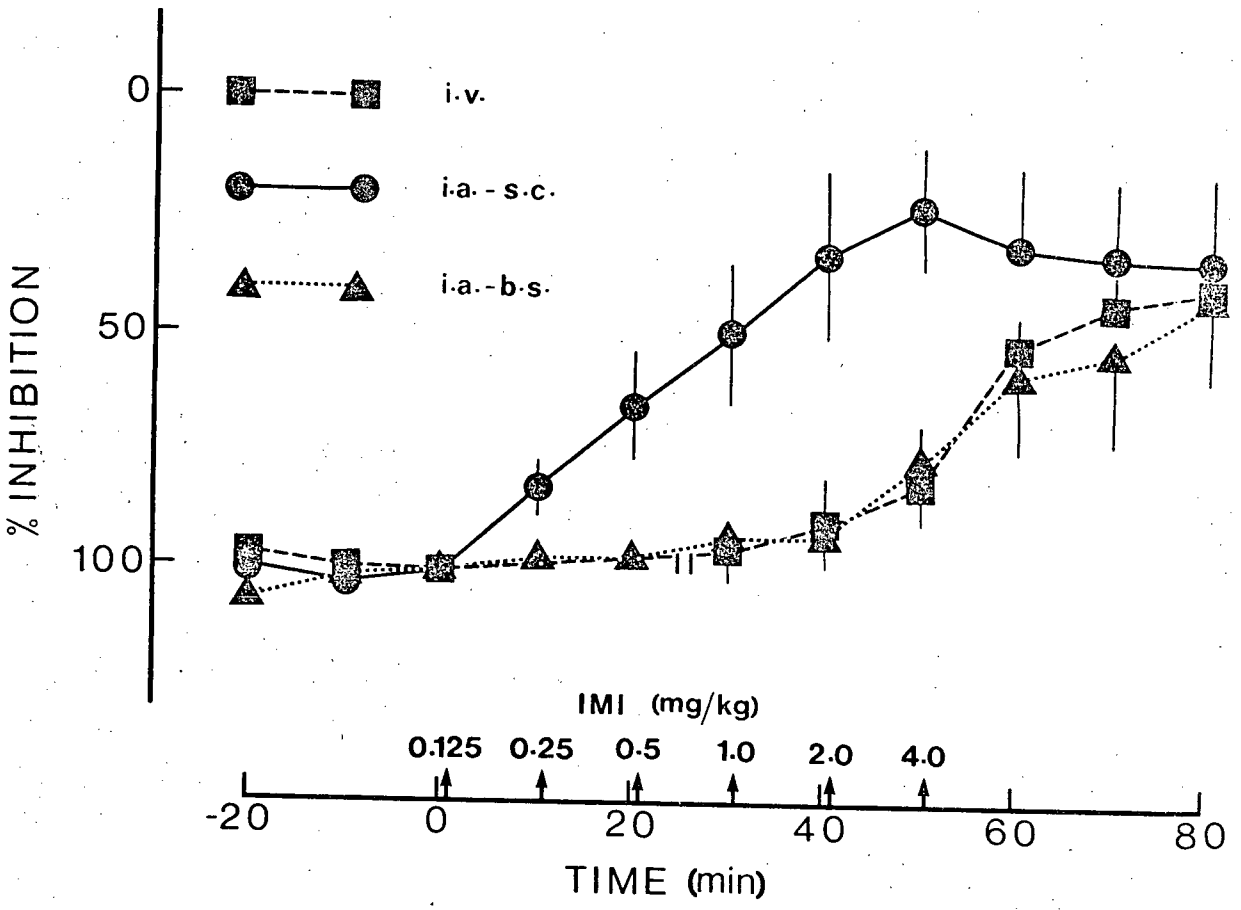

Fig. 22. The antagonism of recurrent inhibition of the dorsal root - ventral root MSR by imipramine HCl (IMI). The drug was injected intravenously (i.v., 0.5 - 4 mg/kg), intra-arterially to the spinal cord (i.a. - s.c., 0.125 - 4 mg/kg) and intra-arterially to the brain stem (i.a. - b.s., 0.125 - 4 mg/kg) in geometrically increasing cumulative doses. For each curve n=4.

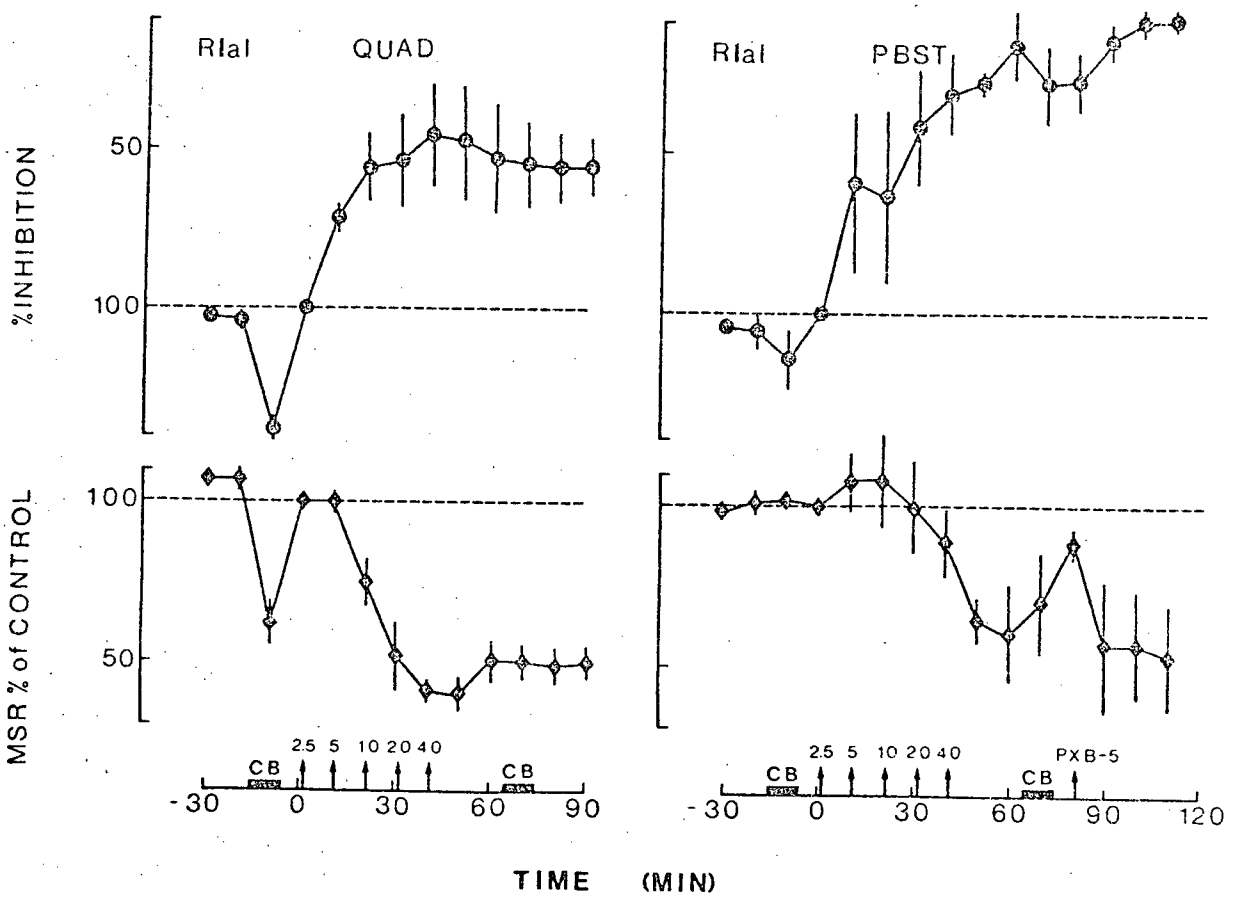

Fig. 23. The blockade of reciprocal Ia inhibition of the QUAD- (top left, n=6) and the PBST- (top right, n=4) MSRs by clonidine HCl administered in geometrically increasing cumulative doses in μg/kg as indicated on the abscissa. The depression of the QUAD- (bottom left, n=6) and the PBST- (bottom right, n=4) MSRs by clonidine is also illustrated. A cold block (CB) was tested before and after the clonidine injection. The action of phenoxybenzamine HCl (PXB, 5 mg/kg) was tested on the inhibition of the flexor reflex after the second cold block.

The unconditioned MSR:

Imipramine (5 mg/kg i.v.) decreased the unconditioned QUAD- and the PBST-MSRs by about 45 per cent. This observation is consistent with previous reports (Sastry, 1973; Sinclair and Sastry, 1974). Imipramine also reduced the extensor MSR in p-CPA pretreated animals.

A cold block decreased the extensor MSR in unpretreated animals (Fig. 23 and 24) but failed to alter this MSR in animals pretreated with p-CPA. The flexor reflex was unaltered by the cold block application (Fig. 23).

A cold block enhanced the QUAD-AP but reduced the field potential (Fig. 24). Imipramine (2 - 4 mg/kg i.v.) reduced the QUAD-MSR, the QUAD-AP and the field potential. A cold block partly reversed the above action of imipramine on the QUAD-AP but not on the MSR and the field potential (Fig. 24). In 4 of 5 experiments the submaximal field potential was augmented by imipramine (2 mg/kg i.v.) but was depressed by a second dose.

Fluoxetine (total dose of 6 mg/kg i.v.) enhanced the extensor and the flexor MSR to 139 ± 10.5% S.E.M. and 130 ± 4% S.E.M. of control value, respectively (n=5).

In six animals, imipramine (0.25 mg/kg i.v.) had no significant effect on the QUAD-MSR but in three of the above animals 5-HTP (75 mg/kg i.v.), administered subsequent to imipramine, enhanced this extensor reflex (190.6, 306.7 and 238.1% of control; 90 min after injection). In two animals, 5-HTP initially reduced (71.4 and 50.1%; 20 min after the start of injection) but later enhanced (207.1 and 248%; 90 min after injection) the MSR. In the remaining animal the MSR was depressed throughout the time tested (61.9%; 90 min after 5-HTP).

Cyproheptadine blocked the above facilitation of the QUAD-MSR by 5-HTP (91.9 ± 11.2 S.E.M., MSR % of control) and converted the enhancement of the

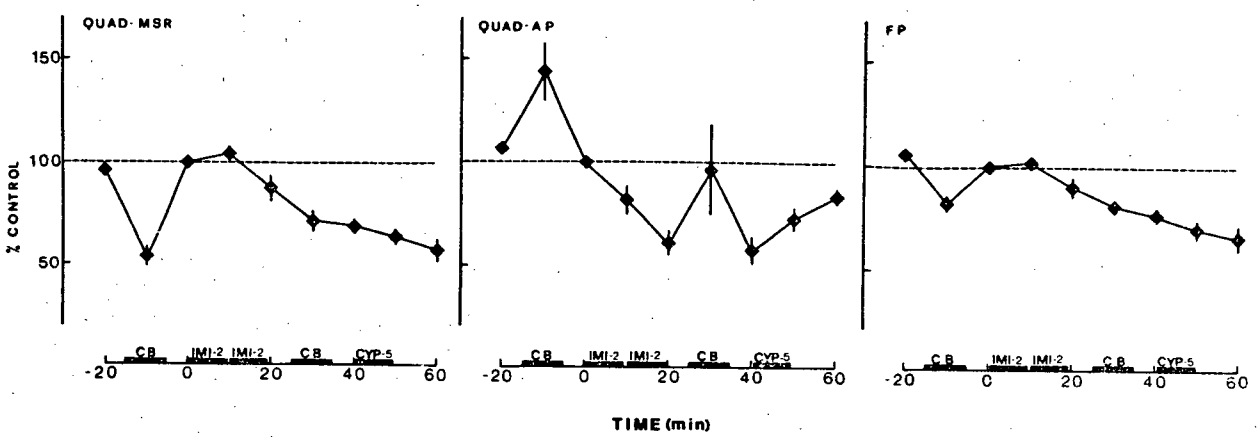

Fig. 24. The effects of a cold block (CB), imipramine HCl (IMI, 2 mg/kg administered twice), a second cold block and cyproheptadine HCl (CYP, 5 mg/kg), tested in sequence, on the QUAD-MSR, the QUAD-AP and the antidromic field potential (FP). For each graph n=8.

QUAD- and the PBST reflexes produced by fluoxetine into a depression (QUAD: 48.4 ± 8.7% S.E.M.; PBST: 73.5 ± 9.5% S.E.M.). When administered alone, cyproheptadine (2.5 - 5 mg/kg i.v.) reduced the extensor and the flexor MSR to 62.8 ± 13.7 S.E.M. and 70 ± 12.7 S.E.M., % of control), respectively (n=4). Phenoxybenzamine also, at the same dose, depressed these reflexes (QUAD: 61 ± 8.9% S.E.M.; PBST: 45 ± 3.7% S.E.M.; n=4).

Blood pressure:

Fluoxetine (0.25 - 6 mg/kg i.v.) did not alter the blood pressure of the animals. 5-HTP usually had a biphasic action, an initial enhancement of the systolic pressure by about 23 per cent that lasted over 30 min followed by a fall in the diastolic pressure by about 12.5 per cent that usually lasted throughout the experiment. A similar observation was made by Anderson and Shibuya (1966). However, there was no correlation between these blood pressure changes and the blocking action of this agent on the inhibitions of the extensor MSR.

Imipramine did not significantly alter the blood pressure when given in geometrically increasing doses at 10 min intervals (the total dose over 50 min was 4 mg/kg). This agent, however, reduced the mean blood pressure to about 75 and 85 per cent of control when administered at a dose of 5 mg/kg over 10 min and at a total dose of 4 mg/kg over 20 min, respectively. These effects of imipramine on the blood pressure very likely did not contribute to this agent's action on presynaptic inhibition of the MSR since imipramine blocked the inhibition of the QUAD- but not the PBST-MSR and a cold block which had no effect on the blood pressure completely eliminated the above effect of imipramine.

Clonidine (2.5 - 40 μg/kg i.v.) had an initial transient hypertensive

effect followed by a reduction of about 15% of the mean blood pressure which persisted throughout the experiment.

Cyproheptadine (5 mg/kg i.v.) and phenoxybenzamine (5 mg/kg i.v.) reduced the mean blood pressure by about 18.5 and 25%, respectively. This effect in both cases lasted throughout the course of the experiment. The depressant effects of these agents on blood pressure would not appear to be related to their action on bulbospinal, recurrent and presynaptic inhibitions of the MSR since cyproheptadine enhanced whereas phenoxybenzamine antagonized bulbospinal inhibition. Moreover, both the agents enhanced presynaptic and recurrent inhibitions of the QUAD- but not of the PBST-MSR. Noradrenaline and 5-HT had been implicated in antagonizing these inhibitions of the extensor reflex (see discussion and Sinclair and Sastry, 1974a).

Iontophoretic study:

Iontophoretically applied clonidine had varied effects on the discharge rate of spinal neurones that were either spontaneously firing or whose discharge rate was increased by glutamate. Clonidine currents of less than 10 nA usually had no significant effect; higher currents increased, decreased or did not alter the firing rate.

The discharge rate of 33 cells tested was decreased by γ-aminobutyric acid (GABA; Fig. 25 and 26). Clonidine, at currents that did not significantly change the control firing rate, antagonized the effect of GABA on 49 per cent of the neurones (Fig. 25 and 26). However, in 12 per cent of the cases clonidine potentiated the action of GABA. Clonidine failed to alter the amino acid's effect on 39 per cent of the neurones.

Glycine also reduced the discharge rate of 31 neurones tested (Fig. 26). The effect of this amino acid on 42 per cent of the above cells was

antagonized by clonidine (Fig. 26). Clonidine augmented the effect of glycine on 18 per cent of the cells but did not alter the amino acid's action on 40 per cent of the neurones.

On 4 cells clonidine antagonized the effect of GABA but had no effect on the action of glycine when these amino acids were tested on the same neurone. Similarly, in three cases the action of glycine but not of GABA was blocked by clonidine.

Fig. 25. The blockade by clonidine (10 nA) of the depression of a spontaneously firing spinal neurone discharge rate by γ-aminobutyric acid (GABA, 5 nA). Both agents were applied iontophoretically. A: control. G: illustrates the effect of positive current (15 nA).

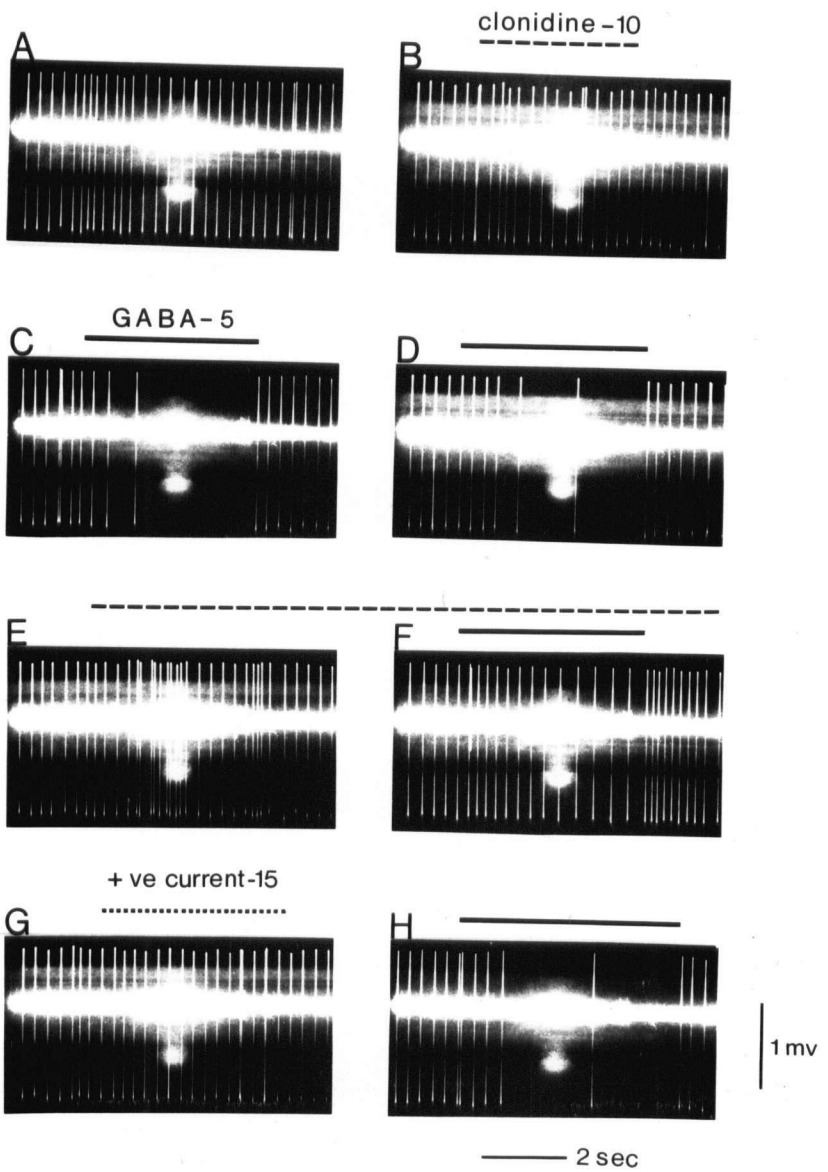

Fig. 25

Fig. 26. The blockade of the effects of A: γ-aminobutyric acid (GABA, iontophoretically applied, 5 - 25 nA) and B: glycine (5 - 15 nA) on unidentified spinal neurones by clonidine (5 or 10 nA as indicated). The cell in A was spontaneously firing; the discharge rate of the neurone in B was increased and maintained at an approximately constant level by glutamate (15 nA).

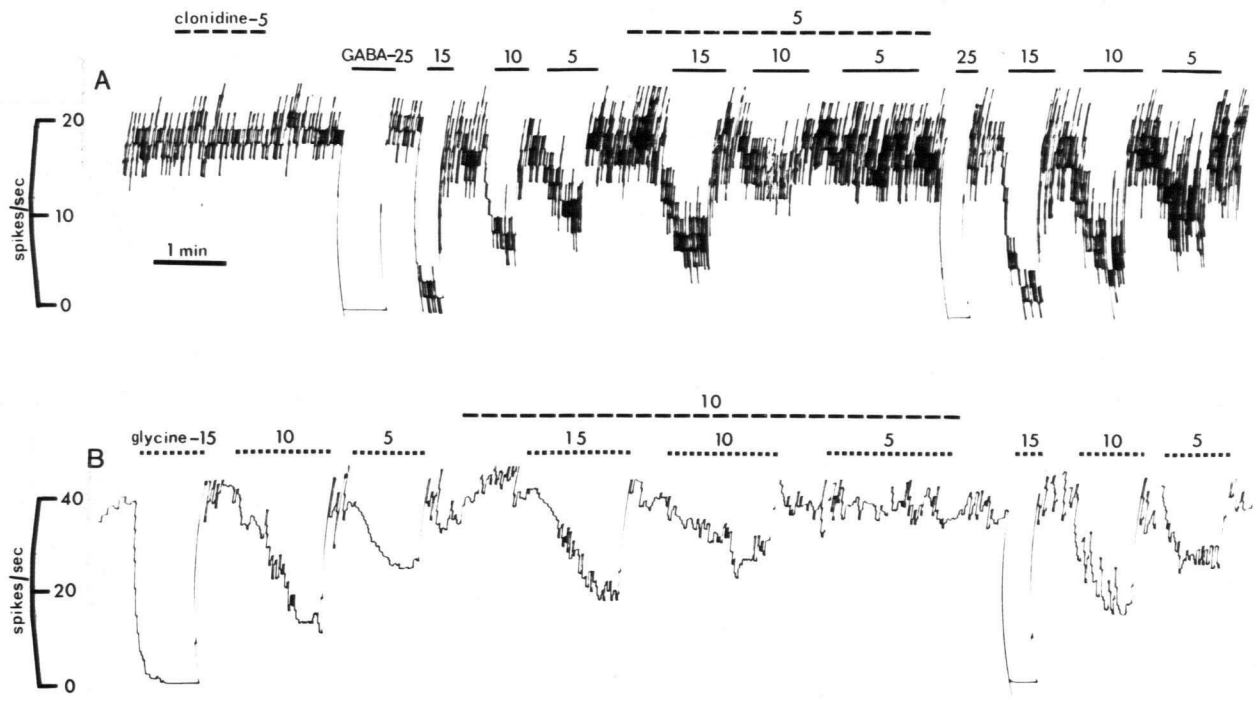
clonidine-5
5
GABA-25
15
10
5
15
10
5
25
15
10
5
A
20
10
0
spikes/sec
1 min
10
glycine-15
10
5
15
10
5
15
10
5
B
40
20
0
spikes/sec

Fig. 26

DISCUSSION

The neuronal circuitry upon which drugs used in this study act is not entirely known. Diagrammatic neuronal arrangements have been proposed which illustrate the monoaminergic influences on presynaptic, bulbospinal and recurrent inhibitions of the quadriceps (QUAD) monosynaptic reflex (MSR), as shown in Fig. 27, 28 and 29, respectively. A possible neuronal scheme for 5-HT influences on the unconditioned reflex appears in Fig. 30. The following discussion may be considered with reference to these figures.

Presynaptic inhibition of the MSR:

The blockade of presynaptic inhibition of the extensor QUAD but not of the flexor posterior biceps-semitendinosus (PBST) MSR by imipramine is consistent with a similar finding by Tan and Henatsch (1969). The complete elimination of imipramine's blocking action on presynaptic inhibition by a thoracic cold block, which prevents supraspinal inputs to the caudal spinal cord, indicates that imipramine exerts this effect through a descending system.

Since imipramine failed to antagonize presynaptic inhibition in cats pretreated with either the tryptophan hydroxylase inhibitor, DL-p-chlorophenylalanine (p-CPA) or the tyrosine hydroxylase inhibitor, DL-α-methyl-p-tyrosine methyl ester HCl (α-MPT), the blockade of this inhibition by imipramine is likely mediated through 5-hydroxytryptamine (5-HT) and noradrenaline. Moreover, the complete elimination of imipramine's blocking action by either of the above pretreatments suggests that 5-HT and nor-

adrenaline neurones are serially arranged in the same descending system through which imipramine exerts this effect (Fig. 27). At present we have no information concerning the order of the arrangement in this system.

Fluoxetine was reported to be a specific 5-HT neuronal uptake blocking agent (Fuller et al., 1975; Wong et al., 1974, 1975). 5-Hydroxytryptophan (5-HTP) elevated 5-HT levels in the spinal cord of the cat (Anderson and Shibuya, 1966). In the present study, fluoxetine and 5-HTP antagonized presynaptic inhibition of the extensor MSR presumably by enhancing 5-HT synaptic activity. The finding that the blockade of the inhibition by fluoxetine was reversed by a cold block indicates that this agent produces its effect through a supraspinal system. Fluoxetine did not alter the inhibition of the flexor MSR supporting the notion that 5-HT does not influence this inhibition.

The enhancement of presynaptic inhibition of the QUAD- but not of the PBST-MSR by a thoracic cold block suggests that the inhibition of the extensor MSR is under a tonic inhibitory influence of a descending system. Furthermore, the finding that a cold block failed to enhance the inhibition of the extensor MSR in p-CPA or α-MPT pretreated cats, suggests that the tonic inhibitory system involves 5-HT and noradrenaline. This latter notion is strengthened by the enhancement of presynaptic inhibition of the QUAD-MSR by the 5-HT antagonist, cyproheptadine, and the adrenergic antagonist, phenoxybenzamine.

Carpenter et al. (1963) suggested that, in decerebrate cats, presynaptic inhibition of the MSR is not under a tonic inhibitory influence of a supraspinal system. This proposal is at variance with the present hypothesis that presynaptic inhibition of the QUAD-MSR is tonically inhibited by a descending monoaminergic system. The discrepancy may be due to different

experimental procedures adopted in these two studies. In the present investigation the effect of a cold block was tested on the inhibition of the MSR, whereas Carpenter et al. (1963) observed the effect of a spinal transection on the negative dorsal root potentials (DRPs) evoked by stimulation of group I afferents. Eccles et al. (1962) reported that group I afferents can produce DRPs on both Ia and Ib afferents. Moreover, in the study of Carpenter et al. (1963) it is not known whether DRPs were generated on extensor or flexor afferents or on both. The present study clearly involves inhibition of an extensor or a flexor MSR. The reflex was conditioned by group I afferents and a conditioning interval of 30 msec was maintained to eliminate the interference of postsynaptic inhibition (Eccles et al., 1962). Moreover in the present experiments, utilizing the primary afferent excitability testing procedure, a cold block augmented the facilitation of the antidromic compound action potentials on QUAD Ia afferents (QUAD-APs) evoked by a conditioning stimulus from the flexor group I afferents. In the above experiments a cold block also enhanced presynaptic inhibition of the QUAD-MSR. Imipramine antagonized the facilitation of the QUAD-APs and blocked presynaptic inhibition. Both a cold block and cyproheptadine reversed the effects of imipramine. These observations strengthen our proposal implicating a supraspinal monoaminergic system in antagonizing presynaptic inhibition of the QUAD-MSR.

A cold block enhanced and imipramine decreased the unconditioned QUAD-AP. This action of imipramine was reversed by a cold block and cyproheptadine. These results suggest that a tonically active descending 5-HT system decreases the excitability of QUAD Ia afferents (Fig. 30).

It is unknown how the above monoaminergic system exerts its blocking action on presynaptic inhibition of the extensor MSR. The spinal inter-

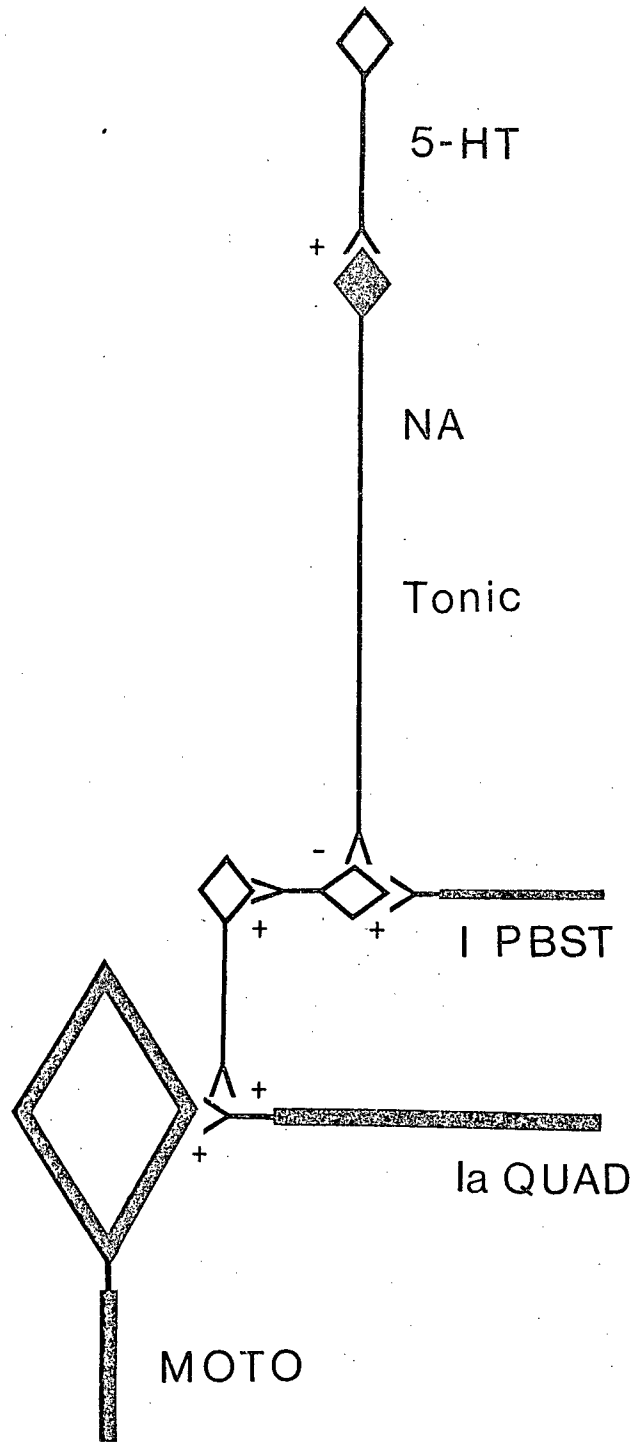

Fig. 27. A diagrammatic neuronal arrangement illustrating the supraspinal system, involving 5-HT and noradrenaline (NA), implicated in tonically antagonizing presynaptic inhibition of the QUAD-MSR. In this and the subsequent figures the symbols + and - indicate excitatory and inhibitory synapses, respectively.

neurones activated by flexion reflex afferents or group Ib afferents were proposed to be tonically inhibited by certain reticulospinal pathways (Engberg et al., 1968a, b: Holmqvist and Lundberg, 1959, 1961). In the present study, a cold block did not alter the magnitude of the PBST-MSR suggesting that these flexor Ia afferents are not under a tonic inhibitory influence of a descending system. Therefore the monoaminergic terminals, proposed to tonically inhibit the presynaptic inhibitory pathway from the PBST group I afferents, likely synapse with interneurones in the inhibitory pathway.

We do not know why cyproheptadine and phenoxybenzamine antagonized the inhibition of the flexor MSR. It is unlikely that 5-HT and noradrenaline facilitate the above inhibition since imipramine and fluoxetine did not enhance this inhibition.

The effects of clonidine on presynaptic inhibition of the MSR are discussed at the end of this section.

In conclusion, the findings in this study indicate that: 1. a descending 5-HT system tonically decreases the excitability of the QUAD Ia afferents (Fig. 30) and 2. a supraspinal system, involving both 5-HT and noradrenaline, tonically antagonizes presynaptic inhibition of the QUAD-MSR (Fig. 27).

Bulbospinal inhibition of the MSR:

The blockade of bulbospinal inhibition by the 5-HT precursor, 5-HTP, and the 5-HT neuronal uptake blocking agent, fluoxetine, as well as a partial reversal of the above blockade by the 5-HT antagonist, cyproheptadine, are consistent with our proposal implicating 5-HT in antagonizing bulbospinal inhibition (Sastry, 1973: Sinclair and Sastry, 1974).

Bulbospinal inhibition of the QUAD-MSR was blocked by imipramine

(Sastry, 1973; Sinclair and Sastry, 1974) and fluoxetine and enhanced by cyproheptadine to a greater extent than the inhibition of the PBST-MSR. This would suggest that the bulbospinal inhibitory pathway to the above extensor MSR receives a greater inhibitory influence of the 5-HT system than that of the flexor MSR.

Assuming that the 5-HT system that is proposed to antagonize bulbospinal inhibition is tonically active, where 5-HT is steadily released into the synaptic clefts, imipramine and fluoxetine likely enhance the 5-HT synaptic activity by blocking the uptake of this amine into the nerve terminals. This assumption is strengthened by the finding that cyproheptadine enhances bulbospinal inhibition. Moreover, in the p-CPA pretreated animals, in which the 5-HT nerve terminals are likely functionally inoperative, imipramine failed to alter the inhibition (Sastry, 1973; Sinclair and Sastry, 1974).

Since imipramine was more potent in antagonizing bulbospinal inhibition of the MSR when administered intra-arterially to the spinal cord than when injected intra-arterially to the brain stem or intravenously, the spinal cord is very likely the site of its blocking action. This implies that the 5-HT nerve terminals that are involved in antagonizing bulbospinal inhibition are located in the spinal cord since imipramine very likely blocks this inhibition by blocking the 5-HT neuronal uptake (Sinclair and Sastry, 1974).

Phenoxybenzamine did not enhance bulbospinal inhibition. Furthermore, pretreatment of cats with α-MPT did not alter the blocking action of imipramine on this inhibition (Sastry, 1973; Sinclair and Sastry, 1974). Therefore, it is unlikely that a noradrenaline system antagonizes bulbospinal inhibition. Phenoxybenzamine enhanced recurrent (see discussion on

recurrent inhibition) and presynaptic inhibitions of the QUAD-MSR where noradrenaline was implicated in tonically antagonizing these inhibitions (see presynaptic inhibition - this section; Sinclair and Sastry, 1974a). At present we have no explanation for the blocking action of phenoxybenzamine on bulbospinal inhibition. It is improbable that a noradrenaline system mediates bulbospinal inhibition since, in p-CPA pretreated cats in which noradrenaline would be expected to be present, imipramine did not enhance this inhibition. Moreover desipramine, an effective noradrenaline neuronal uptake blocking agent, antagonized rather than enhanced the inhibition (Sastry, 1973; Sinclair and Sastry, 1974).

During bulbospinal inhibition of the QUAD-MSR, the QUAD-AP was facilitated and the antidromic motoneurone field potential was reduced, indicating that bulbospinal inhibition of this extensor MSR involves both presynaptic and postsynaptic types of inhibition. This finding would confirm similar observations made by Chan and Barnes (1972). Imipramine antagonized both the above facilitation of the QUAD-AP and the reduction of the field potential, while blocking bulbospinal inhibition of this MSR. Moreover, cyproheptadine partially reversed the above effects of imipramine. These results suggest that 5-HT is involved in antagonizing both the presynaptic and the postsynaptic types of bulbospinal inhibition of the QUAD-MSR (Fig. 28).

In the majority of the experiments, minimal stimulation strength utilized to evoke bulbospinal inhibition reduced the antidromic field potential without altering the magnitude of the QUAD-AP. Therefore, bulbospinal inhibition of the MSR may be predominantly a postsynaptic type of inhibition. This postsynaptic inhibitory pathway likely contains a disynaptic link involving a spinal interneurone (Jankowska et al., 1968). Hultborn and Udo (1972) showed that the Ia inhibitory interneurones, associated with recip-

rocal Ia inhibition of the MSR, receive excitatory inputs from the descending cortico-, rubro- and vestibulospinal tracts. It is unknown whether the above interneurones are involved in bulbospinal inhibition of the MSR. The hyperpolarization of motoneurones evoked by bulbospinal inhibition is reduced by strychnine (Llinas, 1964). This agent also blocks reciprocal Ia inhibition by antagonizing the effect of the neurotransmitter, probably glycine, that is released from the Ia inhibitory interneurones (Eccles et al., 1954a; Curtis and Duggan, 1969; Curtis et al., 1971). Therefore, it is possible that these interneurones are connected with the bulbospinal inhibitory pathway. However, reciprocal Ia inhibition does not appear to be inhibited by a 5-HT system (see discussion on this inhibition). If the Ia inhibitory interneurones are involved in the bulbospinal inhibitory pathway, the 5-HT terminal in the spinal cord must end on the axon terminals of the bulbospinal neurones.

In the present investigation, since a train duration of 300 msec was used to evoke bulbospinal inhibition, we are unable to predict the conduction velocity and the neuronal arrangement of the presynaptic bulbospinal inhibitory pathway. The tonic inhibition on various pathways activated by flexion reflex afferents or group Ib afferents, in decerebrate cats, were suggested to be mediated at the spinal interneurone level (Engberg et al., 1968a, b; Holmqvist and Lundberg, 1959, 1961). Similarly, presynaptic type of bulbospinal inhibition of the MSR might also be tonically inhibited by the 5-HT system at an interneurone level. However, we have no evidence to support this idea.

Clineschmidt and Anderson (1970) found that methysergide, cinanserin, d-lysergic acid diethylamide (LSD) and 2-bromo-LSD, but not cyproheptadine, antagonized bulbospinal inhibition of the MSR. These authors also found

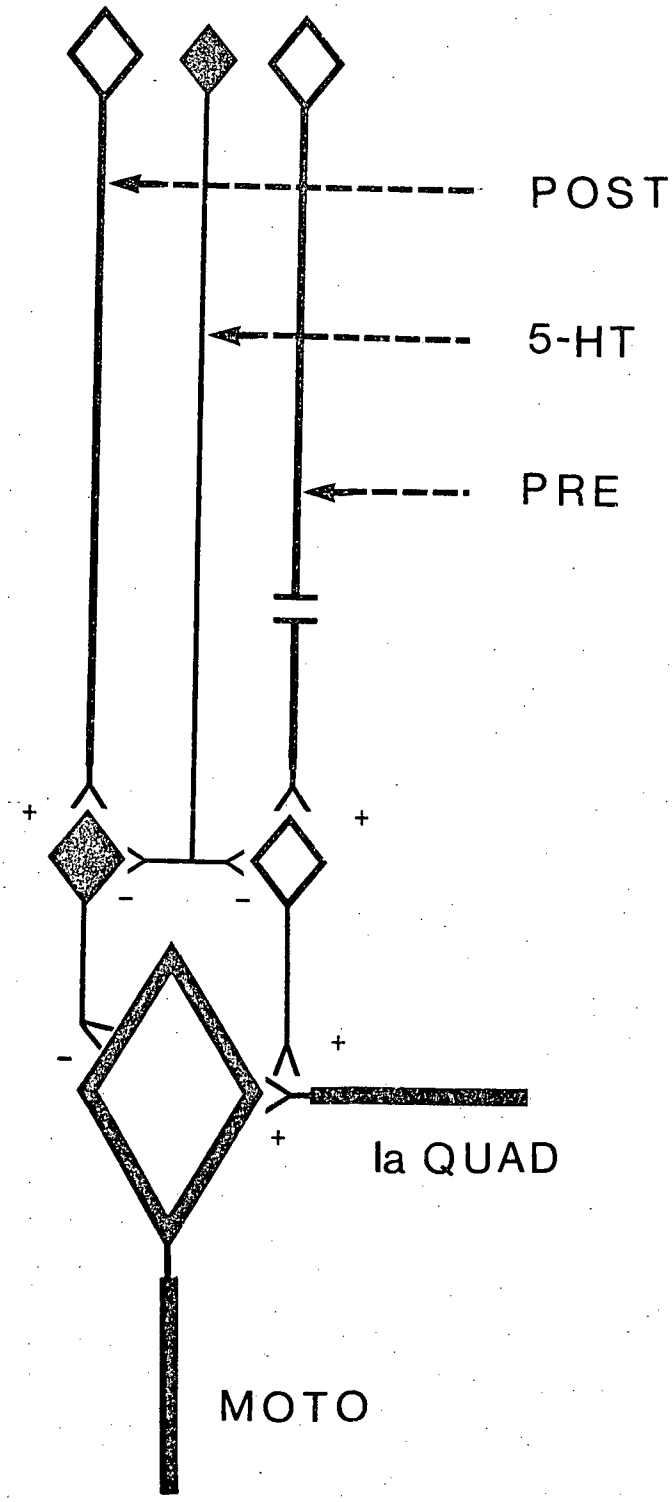

Fig. 28. A diagrammatic neuronal circuitry representing the tonically active 5-HT system that is proposed to antagonize pre- and postsynaptic types of bulbospinal inhibition of the QUAD-MSR.

that methysergide and LSD had a spinal site of action. Therefore, they proposed that a 5-HT interneurone in the spinal cord is involved in this inhibitory pathway. However, there is no convincing evidence for the existence of 5-HT cell bodies in the spinal cord (Dahlstrom and Fuxe, 1965; Fuxe, 1965; Proudfit and Anderson, 1973). Proudfit and Anderson (1973) suggested that the blockade of bulbospinal inhibition by cinanserin and methysergide could result from an enhancement of bulbospinal facilitation if this facilitatory pathway is under an inhibitory influence of a tonically active 5-HT system. However, we observed that methysergide converted the inhibition into a facilitation in animals that were pretreated with p-CPA (Sastry, 1973; Sinclair and Sastry, 1974). Hence, methysergide is probably not a 5-HT antagonist in this system. In addition, our data do not fit the proposals presented by Clineschmidt and Anderson (1970) or Proudfit and Anderson (1973).

The action of clonidine on this inhibition is discussed at the end of this section.

In conclusion, the findings in the present study along with our earlier observation (Sastry, 1973; Sinclair and Sastry, 1974) indicate that a descending 5-HT system in the spinal cord of the cat has a tonic inhibitory influence on presynaptic and postsynaptic types of bulbospinal inhibition of the MSR (Fig. 28). Although in Fig. 28 the 5-HT nerve terminals are shown to impinge on the interneurones in the spinal cord, we do not know where in the spinal cord these terminals exert their blocking action.

Recurrent inhibition of the MSR:

The antagonism of recurrent inhibition of the QUAD-MSR by 5-HTP and fluoxetine as well as the partial reversal of the above effects by cyproheptadine are consistent with our proposal that a monoaminergic system which

involves serially arranged 5-HT and noradrenaline neurones inhibits this inhibition of the extensor MSR (Sastry, 1973; Sinclair and Sastry, 1974a).

The finding that the blocking effect of fluoxetine was prevented by a cold block suggests that this agent, like imipramine (Sastry, 1973; Sinclair and Sastry, 1974a), is exerting its effect through a descending system.

Fluoxetine did not alter recurrent inhibition of the PBST-MSR. It was previously shown that imipramine also failed to alter this inhibition (Sinclair and Sastry, 1974a). These findings suggest that recurrent inhibition of this flexor MSR is not influenced by a 5-HT or a noradrenaline system.

The dose-response curves for imipramine's blocking action on recurrent inhibition of the MSR, when the agent was administered intravenously or intra-arterially to the spinal cord or the brain stem, indicate that imipramine very likely exerts its effect in the spinal cord. This agent has been shown to preferentially block the uptake of 5-HT over that of noradrenaline (Carlsson et al., 1969a, b; Ross and Renyi, 1969; Shaskan and Snyder, 1970). Therefore, in the proposed monoaminergic system that inhibits recurrent inhibition, the 5-HT terminals are likely located in the spinal cord.

Presuming that imipramine antagonized recurrent inhibition by blocking the neuronal uptake of the biogenic amines, the descending monoaminergic system must be tonically active. This idea is strengthened by the observation that imipramine acts through a supraspinal system but has a spinal site of action. Moreover, a cold block, which enhanced the inhibition of the QUAD-MSR in control animals (Sastry, 1973; Sinclair and Sastry, 1974a), failed to alter the inhibition in animals pretreated with p-CPA or α-MPT. The finding that cyproheptadine and phenoxybenzamine enhanced the inhibition of the extensor reflex further indicates that the monoaminergic system is tonically active.

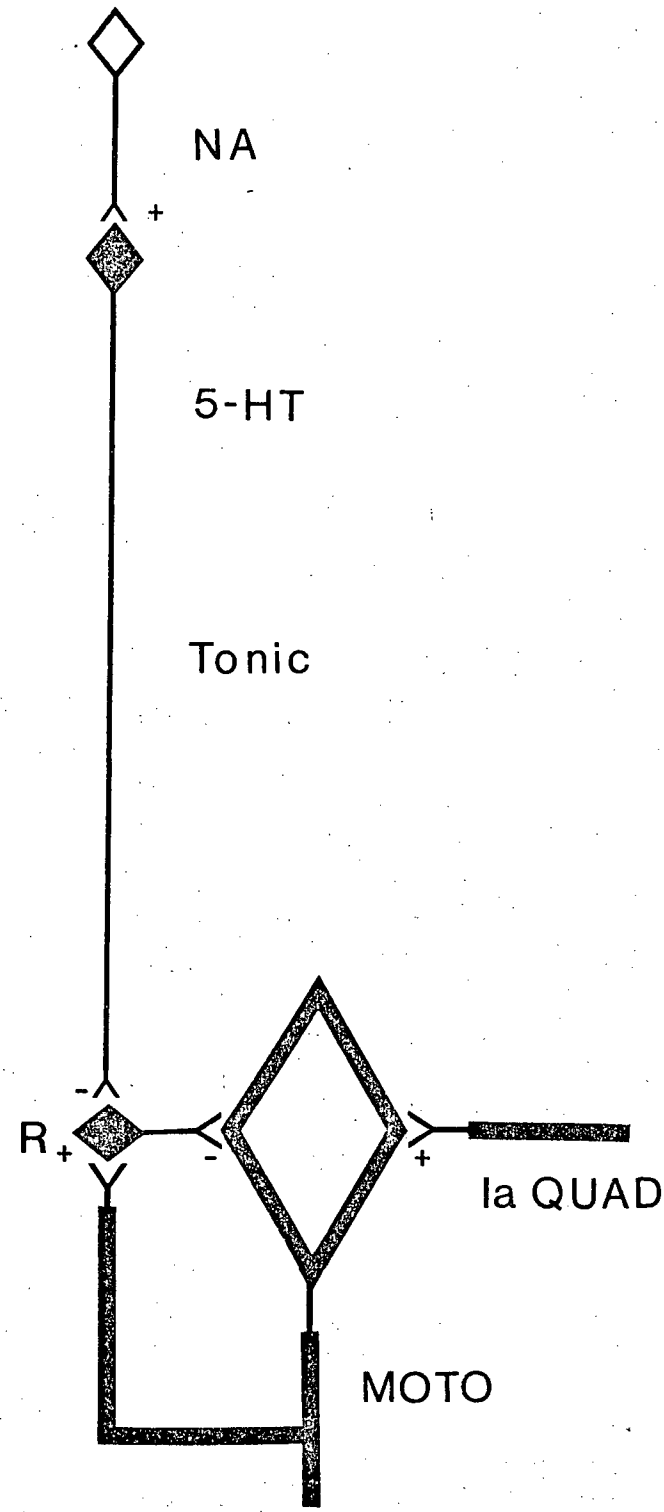

Fig. 29. A diagrammatic neuronal scheme portraying the tonically active supraspinal system, involving noradrenaline (NA) and 5-HT, implicated in antagonizing recurrent inhibition of the QUAD-MSR.

The present study does not indicate where in the spinal cord the monoaminergic system antagonizes the recurrent inhibitory pathway. Since the major effect of iontophoretically applied 5-HT and noradrenaline on Renshaw cells is inhibition (Biscoe and Curtis, 1966; Engberg and Ryall, 1966; Weight and Salmoiraghi, 1966), perhaps the above system terminates on Renshaw cell bodies.

The effects of clonidine on recurrent inhibition are discussed elsewhere in this section.

In conclusion these results, along with our earlier findings (Sastry, 1973; Sinclair and Sastry, 1974a), indicate that recurrent inhibition of the extensor QUAD- but not of the flexor PBST-MSR is under a tonic inhibitory influence of a descending system which involves 5-HT and noradrenaline. We also propose that the 5-HT terminals in this monoaminergic system are located in the spinal cord (Fig. 29).

Reciprocal Ia inhibition of the MSR:

Since imipramine, fluoxetine, 5-HTP, cyproheptadine and phenoxybenzamine did not significantly alter reciprocal Ia inhibition of the MSR, 5-HT and noradrenaline do not likely affect this inhibition. A cold block enhanced the inhibition of the QUAD-MSR but not of the PBST-MSR suggesting that the inhibition of the above extensor MSR is under a tonic inhibitory influence of a descending system.

The unconditioned MSR:

The enhancement of the MSR by fluoxetine and 5-HTP as well as a reversal of this augmentation by cyproheptadine are consistent with the reports indicating that a 5-HT system has an overall facilitatory effect on the MSR

(Anderson, 1972; Clineschmidt et al., 1971). In cats with an acute but not a chronic spinal transection, pargyline and l-tryptophan enhanced the MSR whereas imipramine potentiated the facilitation of the MSR by 5-HTP (Clineschmidt et al., 1971; Shibuya and Anderson, 1968). These results indicate that imipramine, pargyline and l-tryptophan act through a descending pathway. In the present investigation, a cold block decreased the QUAD-MSR by about 45 per cent but did not alter the PBST-MSR. Therefore, a supraspinal system has an overall tonic facilitatory effect on this extensor MSR. Since a cold block failed to alter the QUAD-MSR in p-CPA pretreated animals, the above supraspinal system likely involves 5-HT. A cold block enhanced and imipramine decreased the QUAD-AP. This effect of imipramine was reversed by a cold block or cyproheptadine. These results suggest that a descending tonically active 5-HT system decreases the excitability of QUAD Ia afferents (Fig. 30).

The size of the QUAD-AP is inversely related to the magnitude of the QUAD-MSR (Fig. 6). Therefore, the decrease in the QUAD-AP by imipramine suggests that this agent enhances the MSR. However, the reflex was depressed rather than augmented by imipramine in most of the experiments. The decrease in the size of the antidromic field potential by this agent may be responsible for the above reduction of the MSR.

Barnes et al. (1962) reported that, in cats anaesthetized with pentobarbital, a functional blockade of supraspinal inputs to the spinal cord resulted in a hyperpolarization of the motoneurones. In the present study, the antidromic motoneurone field potential was reduced by application of a cold block. The above observations indicate that the supraspinal systems have an overall tonic excitatory effect on the motoneurones. It is unknown whether the motoneurones in the study of Barnes et al. (1962) innervate the

flexor or the extensor muscles. In the present study, although the field potential might involve both flexor and extensor motoneurones, since a cold block decreased the extensor but not the flexor MSR, the descending tonic facilitatory effect is very likely on the extensor rather than the flexor motoneurones (Fig. 30).

Imipramine augmented the submaximal field potential at a dose of 2 mg/kg but reduced this potential when a second dose was administered. It is unknown whether the above facilitatory effect is mediated through 5-HT. The maximal field potential was decreased by imipramine. The MSR in control and in p-CPA pretreated animals was also depressed by this agent. Moreover, cyproheptadine did not reverse imipramine's action on the MSR and the field potential. Therefore, the depressant effects of imipramine on the MSR and the field potential do not appear to be mediated through 5-HT. The decrease in the MSR and the field potential produced by imipramine appears to be dependent on the descending inputs since little depression, by this agent, occurred during a cold block (Fig. 24).

The depressant effect of 5-HTP on the MSR, observed in some experiments, is probably not due to a 5-HT involvement as imipramine potentiated the facilitatory action (Clineschmidt et al., 1971) but reduced the depressant effect of this agent. Furthermore, the reduction in the MSR by 5-HTP was rapid in onset and was not usually present 90 min after the start of injection, when 5-HT levels in the spinal cord were reported to be maximal (Anderson and Shibuya, 1966).

The blockade of various inhibitions by fluoxetine and 5-HTP is not due to their excitatory effect on the MSR since these compounds did not alter reciprocal Ia inhibition. Fluoxetine enhanced the flexor and the extensor MSR. However, this agent antagonized recurrent and presynaptic inhibitions

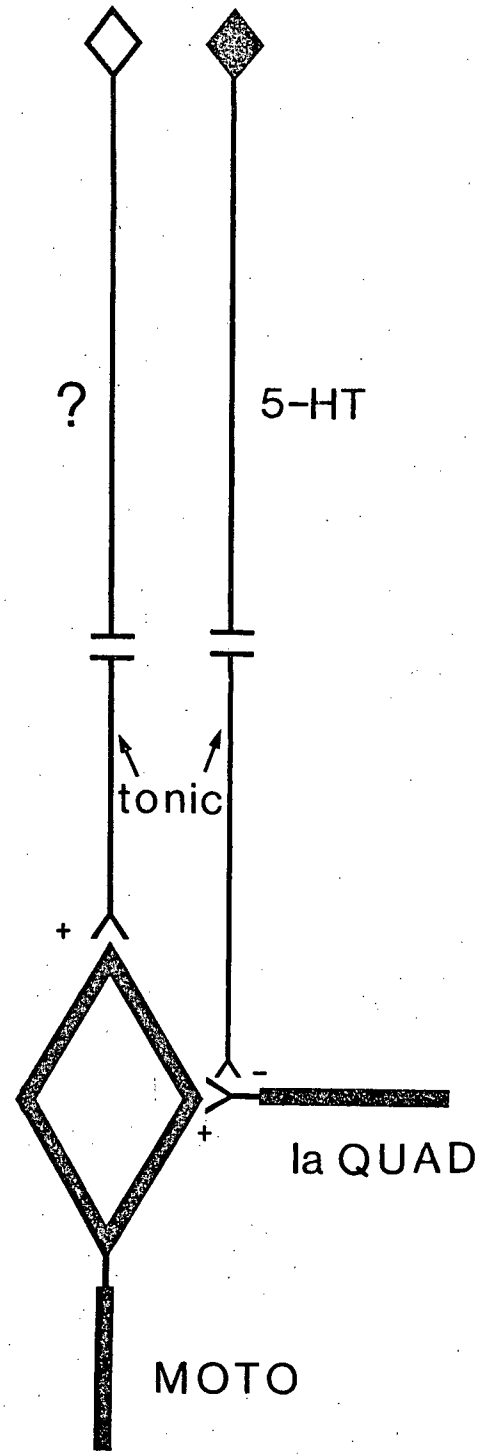

Fig. 30. A schematic neuronal arrangement illustrating: 1. the proposed descending tonically active 5-HT system that decreases the excitability of QUAD Ia afferents and 2. the supraspinal tonic facilitatory system that impinges on the extensor motoneurones.

of the extensor but not of the flexor reflex. 5-HTP increased or decreased the MSR but always antagonized the inhibitions.

In conclusion, the results in the present investigation suggest that: 1. a tonically active 5-HT system decreases the excitability of QUAD Ia afferents and 2. a tonic supraspinal system has an excitatory effect on QUAD motoneurones (Fig. 30).

Clonidine effects:

Clonidine was reported to be a specific α-adrenergic receptor stimulant in the central nervous system (Anden et al., 1970; Finch, 1974; Kobinger and Pichler, 1975). In support of these reports, Anderson and Stone (1974) found that this agent mimicked the depressant effects of noradrenaline on the majority of cerebral cortical and medullary neurones tested. Moreover, bulbocapnine reversed the effects on some cells of both noradrenaline and clonidine, but not of 5-HT. Therefore, clonidine was included in the present study to test the proposals implicating noradrenaline in antagonizing presynaptic and recurrent inhibitions of the extensor MSR.

All the inhibitions of the QUAD- and the PBST-MSRs examined in this investigation were antagonized by clonidine. Other observations in the present study do not implicate noradrenaline in antagonizing bulbospinal and reciprocal Ia inhibitions of the extensor or the flexor MSRs or presynaptic and recurrent inhibitions of the flexor reflex. It is noteworthy that the adrenergic blocking agent, phenoxybenzamine, enhanced the inhibitions which were proposed to be under a tonic inhibitory influence of noradrenaline but failed to increase other inhibitions. Moreover, this agent did not alter the blocking action of clonidine on bulbospinal, reciprocal Ia, presynaptic and recurrent inhibitions of the PBST-MSR. Therefore, the

blockade by clonidine of various inhibitions, where noradrenaline is not suggested to be involved, is puzzling. The finding that a cold block had no effect on clonidine's action on reciprocal Ia, presynaptic and recurrent inhibitions of the QUAD and the PBST reflexes indicates that this agent is acting in the spinal cord.

Although clonidine antagonizes the inhibitions of the MSR, it does not induce convulsions in the animals. The flexor and the extensor MSRs were reduced by clonidine. The blockade of the inhibitions, the depression of the unconditioned MSR and the lack of convulsant effect by clonidine could be explained if this agent decreases the release of synaptic transmitters. A local anaesthetic type of action by clonidine is improbable because this agent did not alter the size of the neurone action potentials.

The amino acid, γ-aminobutyric acid (GABA), has been implicated in presynaptic inhibition of the MSR (Barker and Nicoll, 1972; Bell and Anderson, 1974; Davidoff, 1972; Eccles et al., 1963; Levy, 1974). Glycine is probably the inhibitory transmitter involved in reciprocal Ia and recurrent inhibitions of the MSR (Curtis et al., 1968; Curtis, 1969; Curtis et al., 1971; Werman et al., 1968). The finding that strychnine, reported to be a specific glycine antagonist (Curtis et al., 1971), reduced the hyperpolarization of motoneurones during bulbospinal inhibition (Llinas, 1964) suggests that glycine may be involved in this inhibition. Huffman and McFadin (1972) found that bicuculline, reported to be a specific GABA antagonist (Curtis et al., 1971a), blocked bulbospinal inhibition of the PBST-MSR but not of the QUAD-MSR. This observation suggests that GABA may be involved in bulbospinal inhibition of the above flexor MSR. Hence, the possibility that clonidine antagonizes the effects of GABA and glycine was tested. Clonidine did antagonize the reduction of discharge rate produced by these

amino acids in about 50 per cent of the spinal neurones. Thus, clonidine may have blocked the inhibitions by antagonizing GABA and glycine.

The reason for the finding that in some cells clonidine blocked the effects of either glycine or GABA but not of both is not clear. It should be mentioned that only clonidine currents that had no significant effect on the control cell firing rate were used to test this agent's antagonism towards the actions of the amino acids. Higher clonidine currents might have blocked the effects of both glycine and GABA on the above cells but were not tested.

Clonidine did not alter the effects of glycine and GABA on about 40 per cent of the cells. Even when very high currents were passed to eject clonidine, through one of the pipettes used in this study, the firing rate of 4 cells tested was unaltered. It may be possible that, in the above case, the micropipette barrel containing clonidine was obstructed and this agent could not be ejected. However in the rest of the cases, although clonidine failed to alter the effects of the amino acids, this agent increased or decreased the discharge rate of the neurones when higher currents were passed, indicating that clonidine was released. The present experiments were performed on unidentified spinal neurones. A better approach would have been to test the actions of clonidine, glycine and GABA on neurones that were identified to receive endogenous glycine and GABA neuronal inputs.

At present, the reason for the potentiation of the amino acid effects by clonidine, observed in a few cases, is not clear.

The population of neurones tested in this study is small. Further investigation is necessary to make more conclusive remarks.

In view of the present findings that clonidine antagonized glycine

and GABA effects on some spinal neurones, this agent does not appear to be a specific α-adrenergic agonist. This idea is supported by several reports in the literature. Csongrady and Kobinger (1974) reported that clonidine activates histamine$_2$ receptors on the guinea pig atria. Bloch et al. (1974) suggested that clonidine exerts its hypotensive effect through a dopaminergic, but not a noradrenergic, system in the brain stem. Clonidine was proposed to antagonize the release of noradrenaline (Starke et al., 1972). Anderson and Stone (1974) reported that clonidine did not alter the firing rate of 32 out of 185 cortical and 5 out of 62 medullary cells whose discharge rate was reduced by noradrenaline. Furthermore, clonidine enhanced the firing rate of 15 cortical and 4 medullary cells which were depressed by noradrenaline.

Therefore, the results that clonidine antagonized bulbospinal and reciprocal Ia inhibitions of the flexor and the extensor MSRs, as well as presynaptic and recurrent inhibitions of the flexor MSR, need not negate the hypotheses that the above inhibitions are not under a tonic influence of noradrenaline.

CONCLUSIONS

In this investigation, the influences of bulbospinal 5-hydroxytryptamine (5-HT) and noradrenaline neurones on presynaptic, bulbospinal, recurrent and reciprocal Ia inhibitions of the extensor quadriceps (QUAD) and the flexor posterior biceps-semitendinosus (PBST) monosynaptic reflexes (MSRs) were examined in cats decerebrated at the mid-collicular level. The observations led to the following proposals:

1. Presynaptic inhibition of the QUAD- but not of the PBST-MSR is under a tonic inhibitory influence of a descending system which involves 5-HT and noradrenaline (Fig. 27).

2. Stimulation in the ventromedial bulbar reticular formation evokes both presynaptic and postsynaptic inhibitions of the extensor reflex (Fig. 28). This finding supports a similar observation made by Chan and Barnes (1972).

3. A tonically active 5-HT system, in the spinal cord, antagonizes presynaptic and postsynaptic types of bulbospinal inhibition of the QUAD reflex (Fig. 28).

4. A descending system involving 5-HT and noradrenaline has a tonic inhibitory influence on recurrent inhibition of the extensor MSR (Fig. 29).

5. The excitability of QUAD Ia afferents is decreased by a descending tonically active 5-HT system (Fig. 30).

6. A tonically active supraspinal system has an overall excitatory influence on the extensor motoneurones (Fig. 30).

7. A tonically active supraspinal system, which does not involve 5-HT or noradrenaline, antagonizes reciprocal Ia inhibition of the extensor MSR.

8. Iontophoretically applied clonidine, reported to be a specific α-adrenergic agonist, antagonized the depressant effects of γ-aminobutyric acid and glycine on about 50 per cent of the spinal neurones tested.

REFERENCES

ALBERS, R.W. and BRADY, R.O. (1959). Distribution of glutamic decarboxylase in the nervous system of the Rhesus monkey. J. Biol. Chem. 234: 926-928.

ANDEN, N.E. (1965). Distribution of monoamines and dihydroxyphenylalanine decarboxylase activity in the spinal cord. Acta Physiol. Scand. 64: 197-203.

ANDEN, N.E., CARLSSON, A., HILLARP, N.A. and MAGNUSSON, T. (1964). 5-hydroxytryptamine release by nerve stimulation of the spinal cord. Life Sci. 3: 473-478.

ANDEN, N.E., CARLSSON, A., HILLARP, N.A. and MAGNUSSON, T. (1965). Noradrenaline release by nerve stimulation of the spinal cord. Life Sci. 4: 129-132.

ANDEN, N.E., CORRODI, H., FUXE, K., HOKFELT, B., HOKFELT, T., RYDIN, C. and SVENSSON, T. (1970). Evidence for a central noradrenaline receptor stimulation by clonidine. Life Sci. 9: 513-523.

ANDERSON, E.G., BAKER, R.G. and BANNA, N.R. (1967). The effects of monoamine oxidase inhibitors on spinal synaptic activity. J. Pharmacol. Exp. Ther. 158: 405-415.

ANDERSON, E.G. and SHIBUYA, T. (1966). The effect of 5-hydroxytryptophan and l-tryptophan on spinal synaptic activity. J. Pharmacol. Exp. Ther. 153: 352-360.

ANDERSON, E.G. (1972). Bulbospinal serotonin - containing neurones and motor control. Fed. Proc. 31: 107-112.

ANDERSON, C. and STONE, T.W. (1974). On the mechanism of action of clonidine: effects on single central neurones. Br. J. Pharmac. 51: 359-365.

BAKER, R.G. and ANDERSON, E.G. (1970). The effects of L-3,4-dihydroxyphenylalanine on spinal reflex activity. J. Pharmacol. Exp. Ther. 173: 212-223.

BAKER, R.G. and ANDERSON, E.G. (1970a). The antagonism of the effects of L-3,4,-dihydroxyphenylalanine on spinal reflexes by adrenergic blocking agents. J. Pharmacol. Exp. Ther. 173: 224-231.

BARKER, J.L. and NICOLL, R.A. (1972). Gamma-aminobutyric acid: role in primary afferent depolarization. Science 176: 1043-1045.

BANNA, N.R. and ANDERSON, E.G. (1968). The effects of 5-hydroxytryptamine antagonists on spinal neuronal activity. J. Pharmacol. Exp. Ther. 162: 319-325.

BARNES, C.D., JOYNT, R.J. and SCHOTTELIUS, B.A. (1962). Motoneuron resting potentials in spinal shock. Amer. J. Physiol. 203: 1113-1116.

BARRON, D.H. and MATTHEWS, B.H.C. (1938). The interpretation of potential changes in the spinal cord. J. Physiol. (Lond.) 92: 276-321.

BELL, J.A. and ANDERSON, E.G. (1972). Semicarbazide induced depletion of γ-aminobutyric acid and blockade of presynaptic inhibition. Brain Res. 43: 161-169.

BISCOE, T.J. and CURTIS, D.R. (1966). Noradrenaline and inhibition of Renshaw cells. Science 151: 1230-1231.

BLOCH, R., BOUSQUET, P., FELDMAN, J. and SCHWARTZ, J. (1974). Action hypotensive de la clonidine appliquee sur la surface ventrale du bulbe rachidren: analogie avec la dopamine. Therapie 29: 251-259.

BROCK, L.G., COOMBS, J.S. and ECCLES, J.C. (1952). The recording of potentials from motoneurones with an intracellular electrode. J. Physiol. (Lond.) 117: 431-460.

BROOKS, C. McC., ECCLES, J.C. and MALCOLM, J.L. (1948). Synaptic potentials of inhibited motoneurones. J. Neurophysiol. 11: 417-430.

CARLSSON, A., CORRODI, H., FUXE, K. and HOKFELT, T. (1969a). Effect of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-α-dimethyl-meta-tyramine. Eur. J. Pharmacol. 5: 357-366.

CARLSSON, A., CORRODI, H., FUXE, K. and HOKFELT, T. (1969b). Effects of some antidepressant drugs on the depletion of intraneuronal brain catecholamine stores caused by 4-α-dimethyl-meta-tyramine. Eur. J. Pharmacol. 5: 367-373.

CARLSSON, A., MAGNUSSON, T. and ROSENGREN, E. (1963). 5-hydroxytryptamine in spinal cord, normally and after transection. Experientia 19: 359.

CARPENTER, D., ENGBERG, I., FUNKENSTEIN, H. and LUNDBERG, A. (1963). Decerebrate control of reflexes to primary afferents. Acta Physiol. Scand. 59: 424-437.

CARPENTER, D., ENGBERG, I. and LUNDBERG, A. (1966). Primary afferent depolarization evoked from the brain stem and the cerebellum. Arch. ital. Biol. 104: 73-85.

CHAN, S.H.H. and BARNES, C.D. (1972). A presynaptic mechanism evoked from brain stem reticular formation in the lumbar cord and its temporal significance. Brain Res. 45: 101-114.

CLINESCHMIDT, B.V. (1972). Spinal monoamines and the toxic interaction between monoamine oxidase inhibitors and tricyclic antidepressants. Eur. J. Pharmacol. 19: 126-129.

CLINESCHMIDT, B.V. and ANDERSON, E.G. (1970). The blockade of bulbospinal inhibition by 5-hydroxytryptamine antagonists. Exp. Brain Res. 11: 175-186.

CLINESCHMIDT, B.V., PIERCE, J.E. and SJOERDSMA, A. (1971). Interactions of tricyclic antidepressants and 5-hydroxyindole alkylamine precursors on spinal monosynaptic transmission. J. Pharmacol. Exp. Ther. 179: 312-323.

CLINESCHMIDT, B.V., PIERCE, J.E. and LOVENBERG, W. (1971a). Tryptophan hydroxylase and serotonin in spinal cord and brain stem before and after chronic transection. J. Neurochem. 18: 1593.

COLLINS, G.G.S (1974). The spontaneous and electrically evoked release of ^{3}H-GABA from the isolated hemisected frog spinal cord. Brain Res. 66: 121-137.

COOMBS, J.S., CURTIS, D.R. and ECCLES, J.C. (1955). The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. J. Physiol. 130: 326-373.

CSONGRADY, A. and KOBINGER, W. (1974). Investigations into the positive inotropic effect of clonidine in isolated hearts. Naunyn - Schmiedeberg's Arch. Pharmacol. 282: 123-128.

CURTIS, D.R. (1969). The pharmacology of spinal postsynaptic inhibition. Mechanisms of synaptic transmission. Progress in Brain Research. Vol. 31. Akert, K and Waser, P.G., Editors. Amsterdam, Elsevier. p. 171-189.

CURTIS, D.R. and DUGGAN, A.W. (1969). The depression of spinal inhibition by morphine. Agents and Action 1: 14-19.

CURTIS, D.R., DUGGAN, A.W. and JOHNSTON, G.A.R. (1971). The specificity of strychnine as a glycine antagonist in the mammalian spinal cord. Exp. Brain Res. 12: 547-565.

CURTIS, D.R., DUGGAN, A.W., FELIX, D. and JOHNSTON, G.A.R. (1971a). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. Brain Res. 32: 69-96.

CURTIS, D.R., HOSLI, L. JOHNSTON, G.A.R. and JOHNSTON, I.H. (1968). The hyperpolarization of spinal motoneurones by glycine and related amino acids. Exp. Brain Res. 5: 235-258.

CURTIS, D.R., PHILLIS, J.W. and WATKINS, J.C. (1960). The chemical excitation of spinal neurones by acidic amino acids. J. Physiol. (Lond.) 150: 656-682.

CURTIS, D.R. and RYALL, R.W. (1966). Pharmacological studies upon spinal presynaptic fibres. Exp. Brain Res. 1: 195-204.

CURTIS, D.R. and RYALL, R.W. (1966a). The excitation of Renshaw cells by cholinomimetics. Exp. Brain Res. 2: 49-65.

CURTIS, D.R. and RYALL, R.W. (1966b). Acetylcholine receptors of Renshaw cells. Exp. Brain Res. 2: 66-80.

CURTIS, D.R. and RYALL, R.W. (1966c). The synaptic excitation of Renshaw cells. Exp. Brain Res. 2: 81-96.

DAHLSTROM, A. and FUXE, K. (1964). Evidence for the existence of monoamine containing neurones in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurones. Acta Physiol. Scand. 62:

Suppl. 232: 1-55.

DAHLSTROM, A. and FUXE, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neurone systems. Acta Physiol Scand. 64: Suppl. 247: 1-36.

DAVIDOFF, R.A. (1972). Gamma-aminobutyric acid antagonism and presynaptic inhibition in the frog spinal cord. Science 175: 331-333.

DAVIDOFF, R.A., GRAHAM, L.T., SHANK, R.P., WERMAN, R. and APRISON, M.H. (1967). Changes in amino acid concentrations associated with loss of spinal interneurones. J. Neurochem. 14: 1025-1031.

DEGROAT, W.C., LALLEY, P.M. and SAUM, W.R. (1972). Depolarization of dorsal root ganglia in the cat by GABA and related amino acids: antagonism by picrotoxin and bicuculline. Brain Res. 44: 273-277.

ECCLES, J.C. (1961). The mechanisms of synaptic transmission. Ergebn. Physiol. 51: 299-430.

ECCLES, J.C. (1964). Presynaptic inhibition in the spinal cord. Physiology of spinal neurones. Progress in Brain research vol. 12. Eccles, J.C. and Schade, J.P., Editors. Amsterdam, Elsevier. p. 65-91.

ECCLES, J.C. (1975). Speach to the Society for Neurosciences, British Columbia Chapter.

ECCLES, J.C., ECCLES, R.M. and FATT, P. (1956a). Pharmacological investigations on a central synapse operated by acetylcholine. J. Physiol. (Lond.) 131: 154-169.

ECCLES, J.C., ECCLES, R.M. and MAGNI, F. (1961). Central inhibitory action attributable to presynaptic depolarization produced by muscle afferent volleys. J. Physiol. (Lond.) 159: 147-166.

ECCLES, J.C., FATT, P. LANDGREN, S and WINSBURY, G.J. (1954). Spinal cord potentials generated by volleys in the large muscle afferents. J. Physiol. (Lond.) 125: 590-606.

ECCLES, J.C., FATT, P. and KOKETSU, K. (1954a). Cholinergic and inhibitory synapses in a pathway from motor - axon collaterals to motoneurones. J. Physiol. (Lond.) 126: 524-562.

ECCLES, J.C., FATT, P. and LANDGREN, S. (1956). The central pathway for the direct inhibitory action of impulses in the largest afferent nerve fibres to muscles. J. Neurophysiol. 19: 75-98.

ECCLES, J.C., KOSTYUK, P.G. and SCHMIDT, R.F. (1962a). Central pathways responsible for depolarization of primary afferent fibres. J. Physiol. (Lond.) 161: 237-257.

ECCLES, J.C., KOSTYUK, P.G. and SCHMIDT, R.F. (1962b). The effect of electric polarization of the spinal cord on central afferent fibres and on their

excitatory action. J. Physiol. (Lond.) 162: 138-150.

ECCLES, J.C. and KRNJEVIC, K. (1959). Potential changes recorded inside primary afferent fibres within the spinal cord. J. Physiol. (Lond.) 149: 250-273.

ECCLES, R.M. and LUNDBERG, A. (1959). Supraspinal control of interneurones mediating spinal reflexes. J. Physiol. (Lond.) 147: 565-584.

ECCLES, J.C., MAGNI, F. and WILLIS, W.D. (1962). Depolarization of central terminals of group I afferent fibres from muscle. J. Physiol. (Lond.) 160: 62-92.

ECCLES, J.C., SCHMIDT, R.F. and WILLIS, W.D. (1963). Pharmacological studies on presynaptic inhibition. J. Physiol. (Lond.) 168: 500-530.

ENGBERG, I., LUNDBERG, A. and RYALL. R.W. (1968). Reticulospinal inhibition of transmission in reflex pathways. J. Physiol. (Lond.) 194: 201-223.

ENGBERG, I., LUNDBERG, A. and RYALL, R.W. (1968a). Reticulospinal inhibition of interneurones. J. Physiol. (Lond.) 194: 225-236.

ENGBERG, I. and MARSHALL, K.C. (1971). Mechanism of noradrenaline hyperpolarization in spinal cord motoneurones of the cat. Acta Physiol. Scand. 83: 142-144.

ENGBERG, I. and RYALL, R.W. (1966). The inhibitory action of noradrenaline and other monoamines on spinal neurones. J. Physiol. (Lond.) 185: 298-322.

FINCH, L. (1974). The cardiovascular effects of intraventricular clonidine and Bay 1470 in conscious hypertensive cats. Br. J. Pharmac. 52: 333-338.

FRANK, K. (1959). Basic mechanisms of synaptic transmission in the central nervous system. I.R.E. Trans. Med. Electron. ME-6: 85-88.

FRANK, K. and FUORTES, M.G.F. (1957). Presynaptic and postsynaptic inhibition of monosynaptic reflex. Fed. Proc. 16: 39-40.

FRANK, K. and FUORTES, M.G.F. (1955). Potentials recorded from the spinal cord with microelectrodes. J. Physiol. (Lond.) 130: 625-654.

FULLER, R.W., PERRY, K.W. and MOLLOY, B.B. (1975). Effect of 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. J. Pharmacol. Exp. Ther. 193: 796-803.

FUXE, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand. 64: Suppl. 247: 37-84.

GASSER, H.S. and GRAHAM, H.T. (1933). Potentials produced in the spinal cord by stimulation of dorsal roots. Amer. J. Physiol. 103: 303-320.

GRAHAM, L.T. and APRISON, M.H. (1969). Distribution of some enzymes associated with the metabolism of glutamate, aspartate, γ-aminobutyrate and

glutamine in cat spinal cord. J. Neurochem. 16: 559-566.

GRAHAM, L.T., SHANK, R.P, WERMAN, R. and APRISON, M.H. (1967). Distribution of some synaptic transmitter suspects in cat spinal cord: glutamic acid, aspartic acid, γ-aminobutyric acid, glycine and glutamine. J. Neurochem. 14: 465-472.

GRAY, E.G. (1962). A morphological basis for presynaptic inhibition? Nature (Lond.) 193: 82-83.

GRAY, E.G. (1963). Electron microscopy of presynaptic organelles of the spinal cord. J. Anat. (Lond.) 97: 101-106.

HAASE,J. and VAN DER MEULEN, J.P. (1961). Effects of supraspinal stimulation on Renshaw cells belonging to extensor motoneurones. J. Neurophysiol. 24: 510-520.

HALDEMAN, S., HUFFMAN, R.D., MARSHALL, K.C. and MCLENNAN, H. (1972). The antagonism of the glutamate - induced and synaptic excitations of thalamic neurones. Brain Res. 39: 419-425.

HALDEMAN, S. and MCLENNAN, H. (1972). The antagonistic action of glutamic acid diethylester towards amino acid - induced and synaptic excitations of central neurones. Brain Res. 45: 393-400.

HOLMQVIST, B. and LUNDBERG, A. (1961). Differential supraspinal control of synaptic actions evoked by volleys in the flexon reflex afferents in alpha motoneurones. Acta Physiol. Scand. 54: Suppl. 186: 1-51.

HOLMQVIST, B. and LUNDBERG, A. (1959). On the organization of the supraspinal inhibitory control of interneurones of various spinal reflex arcs. Arch. ital. Biol. 97: 340-356.

HOLMSTEDT, B. and SKOGLUND, C.R. (1953). The action on spinal reflexes of dimethyl-amido-ethoxy-phosphoryl cyanide, "tabun", a cholinesterage inhibitor. Acta Physiol. Scand. 29: Suppl. 106: 410-427.

HUBBARD, J.I. and WILLIS, W.D. (1962). Reduction of transmitter output by depolarization. Nature (Lond.) 193: 1294-1295.

HUFFMAN, R.D. and MCFADIN, L.S. (1972). Effects of bicuculline on central inhibition. Neuropharmacology 11: 789-799.

HULTBORN, H., JANKOWSKA, E. and LINDSTROM, S. (1971). Relative contribution from different nerves to recurrent depression of Ia IPSPs in motoneurones. J. Physiol. 215: 637-664.

HULTBORN, H. and UDO, M. (1972). Recurrent depression from motor axon collaterals of supraspinal inhibition in motoneurones. Acta Physiol. Scand. 85: 44-57.

INNES, I.R. (1962). An action of 5-hydroxytryptamine on adrenaline receptors. Br. J. Pharmac. 19: 427-441.

JANKOWSKA, E., LUND, S., LUNDBERG, A. and POMPEIANO, O. (1968). Inhibitory effects evoked through ventral reticulospinal pathways. Arch. ital. Biol. 106: 124-140.

KING, D. and JEWETT, R. (1971). The effects of α-methyltyrosine on sleep and brain norepinephrine in cats. J. Pharmacol. Exp. Ther. 177: 188-195.

KOBINGER, W. and PICHLER, L. (1975). The central modulatory effect of clonidine on the cardiodepressor reflex after suppression of synthesis and storage of noradrenaline. Eur. J. Pharmacol. 30: 56-62.

KOIZUMI, K., USHIYAMA, J. and BROOKS, C.M. (1959). A study of reticular formation action on spinal interneurones and motoneurones. Jap. J. Physiol. 9: 282-303.

KRIZ, N., SYKOVA, E., UJIC, E. and VYKLICKY, L. (1974). Changes of extracellular potassium concentration induced by neuronal activity in the spinal cord of the cat. J. Physiol. (Lond.) 238: 1-15.

KRNJEVIC, K. and LISIEWICZ, J.M. (1972). Injections of calcium ions into spinal motoneurones. J. Physiol. (Lond.) 225: 363-390.

KRNJEVIC. K. and MORRIS, M.E. (1972). Extracellular K^+ activity and slow potential changes in spinal cord and medulla. Can. J. Physiol. Pharmacol. 50: 1214-1217.

KRNJEVIC, K. and MORRIS, M.E. (1973). Depolarizing action of substance P in the cuneate nucleus of the cat. In: Abstr. Ann. Meeting Soc. Neurosci. 3rd., San Diego. p. 349.

KUHAR, M.J., ROTH, R.H. and AGHAJANIAN, G.K. (1971). Selective reduction of tryptophan hydroxylase activity in rat forebrain after midbrain raphe lesions. Brain Res. 35: 167-176.

LEVY, R.A. (1974). GABA: a direct depolarizing action at the mammalian primary afferent terminal. Brain Res. 76: 155-160.

LLINAS, R. (1964). Mechanisms of supraspinal actions upon spinal cord activities. Pharmacological studies on reticular inhibition of alpha-extensor motoneurones. J. Neurophysiol. 27: 1127-1137.

LLINAS, R. and BAKER, R. (1972). A chloride - dependent inhibitory postsynaptic potential in cat trochlear motoneurones. J. Neurophysiol. 35: 484-492.

LLINAS, R. and TERZUOLO, C.A. (1964). Mechanisms of supraspinal actions upon spinal cord activities. Reticular inhibitory mechanisms on alpha-extensor motoneurones. J. Neurophysiol. 27: 579-591.

LLINAS, R. and TERZUOLO, C.A. (1965). Mechanisms of supraspinal actions upon spinal cord activities. Reticular inhibitory mechanisms upon flexor motoneurones. J. Neurophysiol. 28; 413-421.

LLOYD, D.P.C. (1941). A direct central inhibitory action of dromically

conducted impulses. J. Neurophysiol. 4: 184-190.

LUX, H.D. (1971). Ammonium and chloride extrusion: hyperpolarizing synaptic inhibition in spinal motoneurones. Science 173: 555-557.

LUX, H.D. and LIEBL, L. (1974). Possible relationships between extracellular potassium activity and presynaptic inhibition in the spinal cord of the cat. Pflugers Arch. ges. Physiol. 349: 301-317.

LUX, H.D., LORACHER, C. and NEHER, E. (1970). The action of ammonium on postsynaptic inhibition of cat spinal motoneurones. Exp. Brain Res. 11: 431-447.

MACLEAN, J.B. and LEFFMAN, H. (1967). Supraspinal control of Renshaw cells. Expl. Neurol. 18: 94-104.

MAGNUSSON, T. and Rosengren, E. (1963). Catecholamines of the spinal cord normally and after transection. Experientia. 19: 229.

MAGOUN, H.W. and RHINES, R. (1946). An inhibitory mechanism in the bulbar reticular formation. J. Neurophysiol. 9: 165-171.

MARSHALL, K.C. and ENGBERG, I. (1973). Action of iontophoretically applied hydrogen ion on spinal neurones. Can. Fed. Biol. Soc. 16: 33.

MIYATA, Y. and OTSUKA, M. (1972). Distribution of γ-aminobutyric acid in cat spinal cord and the alteration produced by local ischemia. J. Neurochem. 19: 1833-1834.

NG, L.K.Y., CHASE, T.N., COLBURN, R.W. and KOPIN, I.J. (1972). Release of ^{3}(H) dopamine by L-5-hydroxytryptophan. Brain Res. 45: 499-505.

NISHI, S., MINOTA, S. and KARCZMAR, A.G. (1974). Primary afferent neurones: the ionic mechanism of GABA - mediated depolarization. Neuropharmacology 13: 215-219.

PHILLIS, J.W. (1974). The role of calcium in the central effects of biogenic amines. Life Sci. 14: 1189-1201.

PHILLIS, J.W., LAKE, N. and YARBROUGH, G.G. (1973). Calcium mediation of inhibitory effects of biogenic amines on cerebral cortical neurones. Brain Res. 53: 465-469.

PHILLIS, J.W., TEBECIS, A.K. and YORK, D.H. (1968). Depression of spinal motoneurones by noradrenaline, 5-hydroxytryptamine and histamine. Eur. J. Pharmacol. 4: 471-475.

PROUDFIT, H.K. and ANDERSON, E.G. (1973). Influence of serotonin antagonists on bulbospinal systems. Brain Res. 61: 331-341.

PROUDFIT, H.K. and ANDERSON, E.G. (1974). New long latency bulbospinal evoked potentials blocked by serotonin antagonists. Brain Res. 65: 542-546.

RENSHAW, B. (1940). Activity in the simplest spinal reflex pathways.

J. Neurophysiol. 3: 373-387.

RENSHAW, B. (1941). Influence of discharge of motoneurones upon excitation of neighbouring motoneurones. J. Neurophysiol. 4: 167-183.

RICHARDSON, T.W., APRISON, M.H. and WERMAN, R. (1965). An automatic direct-current operating temperature - control device. J. Appl. Physiol. 20: 1355-1356.

ROSS, S.B. and RENYI, A.L. (1969). Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. Eur. J. Pharmacol. 7: 270-277.

RYALL, R.W. (1970). Renshaw cell mediated inhibition of Renshaw cells. Patterns of excitation and inhibition from inputs in motor axon collaterals. J. Neurophysiol. 33: 257-270.

RYALL, R.W. and PIERCEY, M.F. (1971). Excitation and inhibition of Renshaw cells by impulses in peripheral afferent nerve fibres. J. Neurophysiol. 34: 242-251.

RYALL, R.W., PIERCEY, M.F., POLOSA, C. and GOLDFARB (1972). Excitation of Renshaw cells in relation to orthodromic and antidromic excitation of motoneurones. J. Neurophysiol. 35: 137-148.

SAITO, K. KONISHI, S. and OTSUKA, M. (1975). Antagonism between Lioresal and substance P in rat spinal cord. Brain Res. 97: 177-180.

SASTRY, B.S.R. (1973). Serotonin involvement in the blockade of bulbospinal and recurrent inhibition of the monosynaptic reflex. M.Sc. thesis. The University of British Columbia.

SCHMIDT, R.F. (1963). Pharmacological studies on the primary afferent depolarization of the toad spinal cord. Pflugers Arch. ges. Physiol. 277: 325-346.

SHASKAN, E.G. and SNYDER, S.H. (1970). Kinetics of serotonin uptake into slices of rat brain: Relationship to catecholamine uptake. J. Pharmacol. Exp. Ther. 175: 404-418.

SHIBUYA, T. and ANDERSON, E.G. (1968). The influence of chronic cord transection on the effects of 5-hydroxytryptophan, l-tryptophan and pargyline on spinal neuronal activity. J. Pharmacol. Exp. Ther. 164: 185-190.

SINCLAIR, J.G. and SASTRY, B.S.R. (1974). The blockade of bulbospinal inhibition by imipramine, desipramine and pargyline. Neuropharmacology 13: 643-650.

SINCLAIR, J.G. and SASTRY, B.S.R. (1974a). Supraspinal monoaminergic involvement in the blockade of recurrent inhibition of the monosynaptic reflex. Neuropharmacology 13: 741-747.

SINGER, W. and LUX, H.D. (1973). Presynaptic depolarization and extracellular potassium in the cat lateral geniculate nucleus. Brain Res. 64: 1-17.

SNIDER, S. and NIEMER, T.W. (1964). A stereotaxix atlas of the cat brain. The University of Chicago press. Second edition.

STARKE. K., WAGNER, J. and SCHUMANN, H.J. (1972). Adrenergic neuron blockade by clonidine: comparison with guanethidine and local anesthetics. Arch. int. Pharmacodyn. 195: 291-308.

TABER, C.A. and ANDERSON, E.G. (1973). Paradoxical blockade by p-chlorophenylalanine of 5-hydroxytryptophan facilitatory actions on spinal reflexes. J. Pharmacol. Exp. Ther. 187: 229-238.

TAKAHASHI. T. and OTSUKA, M. (1975). Regional distribution of substance P in the spinal cord and nerve - roots of the cat and the effect of dorsal root section. Brain Res. 87: 1-11.

TAKEUCHI, A. and TAKEUCHI, N. (1962). Electrical changes in pre- and postsynaptic axons of the giant synapse of Loligo. J. gen. Physiol. 45: 1181-1193.

TAN, U. and HENATSCH, H.D. (1969). Wirkungen von Imipramin auf die spinalmotorischen Extensor- und Flexor-Systeme der Katze. Naunyn-Schmiedeberg's Arch. Pharmacol. 262: 337-357.

WALL, P.D. (1958). Excitability changes in afferent fibre terminations and their relation to slow potentials. J. Physiol. (Lond.) 142: 1-21.

WEIGHT, F.F. and SALMOIRAGHI, G.C. (1966). Adrenergic responses of Renshaw cells. J. Pharmacol. Exp. Ther. 154: 391-397.

WERMAN, R., DAVIDOFF, R.A. and APRISON, M.H. (1968). Inhibitory action of glycine on spinal neurones of the cat. J. Neurophysiol. 31: 81-95.

WILSON, V.J., DICKE, F.P.J. and TALBOT, W.H. (1960). Distribution of recurrent facilitation and inhibition in cat spinal cord. J. Neurophysiol. 23: 144-153.

WONG, D.T., HORNG, J.S., BYMASTER, F.P., HAUSER, K.L. and MOLLOY, B.B. (1974). A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. Life Sci. 15: 471-479.

WONG, D.T., BYMASTER, F.P., HORNG, J.S. and MOLLOY, B.B. (1975). A new selective inhibitor for uptake of serotonin into synaptosomes of rat brain: 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. J. Pharmacol. Exp. Ther. 193: 804-811.

YARBROUGH, G.G., LAKE, N. and PHILLIS, J.W. (1974). Calcium antagonism and its effect on the inhibitory actions of biogenic amines on cerebral cortical neurones. Brain Res. 67: 77-88.

ZIEGLGÄNSBERGER, W. and PUIL, E.A. (1973). Intracellular investigations on the effect of microelectrophoretically applied glutamate antagonists upon spinal neurones of the cat. Naunyn-Schmiedeberg's Arch. Pharmacol. Suppl. R89.