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STUDIES ON

SEROTONIN INVOLVEMENT IN NUCLEUS RAPHE INHIBITION AND MORPHINE DEPRESSION OF SPINAL CORD NEURONES

By

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78

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ABSTRACT

It is not clear whether 5-hydroxytryptamine (5-HT) mediates, at least partly, the nucleus raphe magnus (NRM) stimulation-produced inhibition of deep dorsal horn wide dynamic range (WDR) neuronal nociceptive activity. The role of 5-HT in the NRM phasic inhibition is suggested by the presence of 5-HT in some of the descending fibres from the NRM, the release of 5-HT into spinal perfusates upon NRM stimulation and the fact that the iontophoretic application of 5-HT in the spinal cord depresses the nociceptive activity of the deep dorsal horn WDR neurones. However, systemic administration or iontophoretic application of methysergide, a putative 5-HT antagonist in the spinal cord did not reduce the NRM phasic inhibition of the deep dorsal horn WDR neurones.

Experiments were therefore performed to determine whether 5-HT mediates the NRM phasic inhibition of WDR neurones by comparing the inhibition of the neuronal nociceptive activity to the NRM stimulation before and after administering the selective 5-HT uptake blocker, fluoxetine (6.0 mg/kg, i.v.), or the monoamine oxidase inhibitor, pargyline (30.0 mg/kg, i.v.). The NRM phasic inhibition following the drug treatment with fluoxetine or pargyline was decreased. Thus, 5-HT does not mediate but appears to reduce the NRM phasic inhibition of the deep dorsal horn WDR neurones.

Fluoxetine administration did not affect the noxious heat-evoked activity of the dorsal horn WDR neurones. This lack of effect of fluoxetine on neuronal nociceptive activity probably reflects a lack of

5-HT involvement in the tonic control of dorsal horn WDR neuronal nociceptive activity, as suggested by some authors. Pargyline treatment produced an increase in the heat-evoked activity of the dorsal horn neurones studied. The mechanism responsible for this effect is not known.

Experiments were performed to re-examine the effect of morphine on the NRM phasic inhibition of spinal cord nociceptive transmission. A controversy exists as to whether morphine elicits a supraspinal attenuation of spinal nociceptive transmission. Some investigators claim that morphine activates a NRM descending inhibition since microinjection of the drug into this nucleus produces a decrease in the nociceptive activity of the spinal cord dorsal horn WDR neurones. However, systemic morphine failed to enhance the NRM phasic inhibition of the dorsal horn WDR neurones.

To test the hypothesis that morphine activates a descending serotonergic inhibitory system from the NRM impinging on the deep dorsal horn WDR neurones, morphine and fluoxetine were given concurrently. However, with this treatment a decrease in the NRM phasic inhibition was observed which was greater than seen with fluoxetine alone. Thus, these experimental results do not favour the above hypothesis.

Morphine also suppressed the noxious heat-evoked activity of the deep dorsal horn WDR neurones. When this drug was administered concurrently with fluoxetine, the observed decrease in the nociceptive activity of the WDR neurones was not statistically different from that observed with morphine alone. This finding suggests that 5-HT does not

mediate morphine's suppressive effect on the deep dorsal horn WDR neurones and is against the hypothesis that 5-HT is intimately involved in mediating morphine inhibition of spinal cord nociceptive transmission.

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LIST OF ABBREVIATIONS

CPA	4-chloroamphetamine
DA	dopamine
DLF	dorsolateral funiculus
DLH	DL-homocysteic acid
5,7-DHT	5,7-dihydroxytryptamine
DCPS	dorsal column postsynaptic spinomedullary
ELI	enkephalin-like
EPSP	excitatory postsynaptic potential
GABA	γ-aminobutyric acid
HTM	high threshold mechanoreceptive
5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytryptamine
HRP	horseradish peroxidase
i.p.	intraperitoneal
i.v.	intravenous
kg	kilogram
K	potassium
LTM	low threshold mechanoreceptive
LSD	lysergic acid diethylamide
mg	milligram
min	minute
MAOI	monoamine oxidase inhibitor
NS	nociceptive specific

NA	noradrenaline
NRM	nucleus raphe magnus
PCPA	p-chlorophenylalanine
PAG	periaqueductal gray
PMN	polymodal nociceptor
PAD	primary afferent depolarization
sec	second
S.E.M.	standard error of mean
SG	substantia gelatinosa
SG_i	inner substantia gelatinosa
SG_o	outer substantia gelatinosa
WDR	wide dynamic range

TABLE OF CONTENTS

	Page
ABSTRACT	ii
LIST OF ABBREVIATIONS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
ACKNOWLEDGEMENT	xii
INTRODUCTION	1
REVIEW OF LITERATURE	5
Nociceptive Mechanism of Spinal Cord Dorsal Horn	5
Cutaneous Nociceptors	5
Dorsal Horn Neurones Responding to Noxious Input	9
The Nucleus Raphe Magnus-Spinal Cord Neuraxis in the Cat	20
Physiology of Descending Inhibition by Stimulation of NRM in the Cat	25
Pharmacology of the Nucleus Raphe Magnus Stimulation-Produced Inhibition of Dorsal Horn Neuronal Activity in the Cat	29
Pharmacology of Morphine on the Deep Dorsal Horn WDR Neurones	33
Tonic Descending Inhibition: Involvement of Endogenous Opioid Peptides and the Effect of Morphine	33
Intravenous Morphine on Noxious Stimulus-Evoked Responses of the Deep Dorsal Horn WDR Neurones	36
Iontophoretic Administration of Morphine	39
Supraspinal Mediated Effects of Morphine on the Deep Dorsal Horn WDR Neuronal Activity	43
Role of 5-HT in Morphine Action	45
Pharmacology of Fluoxetine and Pargyline	47

Page

METHOD AND MATERIALS..... 50

Surgical Procedures..... 50
Stimulation, Recording and Testing of Dorsal Horn Neurones.. 51
Supraspinal Stimulation..... 53
Preparation of Recording Microelectrodes..... 53
Computer Programmes..... 54
Pharmacological Studies..... 55
Calculations..... 57

RESULTS..... 59

Serotonin Involvement in NRM Stimulation-Produced Inhibition..... 59

Effect of Fluoxetine..... 59
Effect of Pargyline..... 62
Effect of Pargyline plus Fluoxetine..... 67

Effect of Morphine..... 70
Serotonin Involvement in Morphine Action..... 77
Control Experiments..... 84

DISCUSSION..... 89

Involvement of 5-HT in the NRM Phasic Inhibition of Dorsal Horn Neurones..... 89

Role of Serotonin in Morphine Depression of Nociceptive Response in the Dorsal Horn Neurones..... 102

CONCLUSION..... 108

BIBLIOGRAPHY..... 110

LIST OF TABLES

		Page
Table 1	Portion of analysis of variance for effect of morphine on noxious heat-evoked activity of individual dorsal horn neurones at different time periods.....	74
Table 2	Portion of analysis of variance and Newman-keuls test of significance for morphine effect on heat-evoked response averaged for five dorsal horn neurones.....	75
Table 3	Effect of morphine on the nucleus raphe magnus phasic inhibition of dorsal horn neuronal nociceptive activity in fluoxetine pretreated animals.....	80

LIST OF FIGURES

Figure		Page
1	Oscilloscope traces of neuronal response to noxious radiant heat and heat plus nucleus raphe magnus stimulation.....	60
2	Computer generated histograms illustrating the effect of fluoxetine and fluoxetine plus morphine, respectively, on the noxious heat-evoked and heat plus NRM stimulation-produced effect on dorsal horn neuronal nociceptive activity.....	61
3	The effect of fluoxetine on the nucleus raphe magnus stimulation-produced inhibition of the nociceptive activity of the dorsal horn neurones.....	63
4	Lack of effect of intravenous infusion of fluoxetine on the noxious heat-evoked activity of the dorsal horn neurones.....	64
5	Lack of effect of fluoxetine on the spontaneous activity of the dorsal horn neurones.....	65
6	The effect of pargyline and pargyline plus fluoxetine on the nucleus raphe magnus stimulation-produced inhibition of the dorsal horn neuronal nociceptive activity.....	66
7	The effect of pargyline and pargyline plus fluoxetine on the noxious heat-evoked activity of dorsal horn neurones.....	68
8	The effect of pargyline and pargyline plus fluoxetine on the spontaneous activity of dorsal horn neurones.....	69
9	Lack of effect of morphine on the nucleus raphe magnus stimulation-produced inhibition of nociceptive activity of dorsal horn neurones.....	71
10	The suppressive effect of cumulative doses of morphine on the noxious heat-evoked activity of dorsal horn neurones.....	73
11	Inhibition of spontaneous activity of dorsal horn neurones by cumulative doses of morphine.....	76
12	The block of nucleus raphe magnus stimulation-produced inhibition by morphine administered to fluoxetine pretreated animals. The data is expressed as percent of control.....	78

Figure		Page
13	The effect of morphine on the nucleus raphe magnus stimulation-produced inhibition in fluoxetine pretreated animal. The data is expressed as actual percent inhibition.....	79
14	The inhibitory effect of cumulative doses of morphine on noxious heat-evoked activity of dorsal horn WDR neurones in fluoxetine pretreated animals.....	82
15	The inhibition of spontaneous activity of dorsal horn neurones by morphine in fluoxetine pretreated animals....	83
16	Lack of effect of the infusion of the vehicle on the nucleus raphe magnus phasic inhibition of dorsal horn neuronal nociceptive activity.....	85
17	Lack of effect of intravenous infusion of the vehicle on the noxious heat-evoked activity of dorsal horn neurones.....	86
18	Lack of effect of intravenous infusion of the vehicle on spontaneous activity of dorsal horn neurones.....	87
19	A comparison of the percentage inhibition and noxious heat-evoked activity of dorsal horn neurones with noxious temperature applied at level of 45°C and 52°C....	88
20	A schematic neuronal arrangement illustrating some possible synaptic connections which would produce nucleus raphe magnus phasic inhibition of deep dorsal horn wide dynamic range neurone.....	92
21	A schematic neuronal arrangement illustrating the nucleus raphe magnus phasic inhibitory impingement of deep dorsal horn wide dynamic range neurone mediated via an inhibitory interneurone in the substantia gelatinosa.....	93
22	A schematic arrangement illustrating some possible synaptic connections by which the serotonergic bulbospinal neurones attenuate the nucleus raphe magnus phasic inhibition of deep dorsal horn neurone....	95
23	A schematic neuronal arrangement illustrating the serotonergic impingement on the inhibitory interneurone in the substantia gelatinosa which blocks the nucleus raphe magnus phasic inhibition of deep dorsal horn neurone.....	97

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Dedicated to my parents

INTRODUCTION

Many studies have demonstrated that electrical stimulation of the nucleus raphe magnus (NRM) in the cats can inhibit the noxious stimuli-evoked activity (Fields et al. 1977; Guilbaud et al. 1977; McCreery et al. 1979; Kajander et al. 1984; Gray and Dostrovsky 1983), and C-fibre stimulation-produced responses (Morton et al. 1983) of the deep dorsal horn wide dynamic range (WDR) neurones. This inhibition of evoked activity is by a fibre system from the NRM which descends in the dorsolateral funiculus (DLF). DLF lesions, but not ventrolateral tract lesions made rostral to the site of recording, reduced the effect in the cat (Fields et al. 1977). This is further substantiated by the anatomical study of Basbaum et al. (1978) who found that fibres from the NRM largely descend in the DLF to the spinal cord.

Some of the descending fibres from the NRM to the cat spinal cord are serotonergic (Fung et al. 1985). These serotonergic fibres can make synaptic contacts on the dendrites and soma of postsynaptic targets in the spinal cord (Ruda and Gobel 1980; Gobel et al. 1982). Stimulation of the NRM produces an increased release of 5-hydroxytryptamine (5-HT) in the spinal cord perfusates of rats (Hammond et al. 1985). When 5-HT is iontophoresed either in the vicinity of the cell body of deep dorsal horn neurones (Belcher et al. 1978) or in the region of substantia gelatinosa (SG) of the spinal cord (Headley et al. 1978), inhibition of the nociceptive activity of the deep dorsal horn neurones occurs. These lines of evidence suggest that 5-HT might

mediate, at least partly, the NRM phasic inhibition of the nociceptive activity of the dorsal horn neurones. Contrary to this speculation, Griersmith et al. (1981) reported that methysergide, a 5-HT antagonist, intravenously administered or iontophoretically applied in the SG, failed to reduce the NRM phasic inhibition of dorsal horn neuronal nociceptive activity. However, the study by Griersmith et al. (1981) is subject to criticism on the choice of antagonist used to identify the role of 5-HT. Methysergide, when microiontophoretically applied on neurones in areas of the CNS known to be innervated by 5-HT containing terminals, failed to block but instead mimicked the inhibitory effect of iontophoretic 5-HT (Haigler and Aghajanian 1977). Therefore, experiments were designed to determine whether 5-HT plays a role in the NRM phasic inhibition of dorsal horn neuronal nociceptive activity.

These experiments on NRM phasic inhibition involved the use of fluoxetine, a selective neuronal 5-HT uptake blocker, and pargyline, a monoamine oxidase inhibitor. The rationale followed was that, if 5-HT mediates the NRM stimulation-produced inhibition of dorsal horn WDR neurones, then fluoxetine and pargyline treatment should enhance 5-HT synaptic transmission and increase the extent of NRM inhibition of neuronal nociceptive activity.

There is evidence that the NRM is involved in morphine antinociception. For example, lesions of the NRM can block the increase in the rat tail-flick latency seen with systemic morphine (Proudfit and Anderson 1975; Yaksh et al. 1977). Microinjection of morphine into the NRM produces an increase in the threshold for nociception-induced

vocalization in the rats (Dickenson et al. 1979). Behavioral studies also suggest that 5-HT might be involved in morphine antinociception. For example, Taiwo et al. (1985) observed that, in the presence of intrathecal monoamine uptake blockers, subthreshold doses of parenteral morphine produced antinociception. This potentiation was prevented by depletion of 5-HT by p-chlorophenylalanine (PCPA) pretreatment or by intrathecal administration of the putative 5-HT antagonist, methysergide. Vasko et al. (1984) have reported that microinjection of morphine into the NRM of the rats produced antinociception which could be attenuated by depletion of spinal cord 5-HT using the 5-HT neurotoxin, 5,7-dihydroxytryptamine. This evidence would suggest that morphine antinociception might be mediated via the NRM through release of spinal 5-HT.

Electrophysiological studies have also attempted to identify whether morphine attenuates spinal cord nociceptive transmission through a NRM site of action. The evidence is conflicting. Le Bars et al. (1976) studied the effect of intravenous morphine (2.0 mg/kg) on the NRM phasic inhibition of deep dorsal horn neuronal nociceptive activity in decerebrate cats. Morphine was found to have no effect on this inhibition. However, when morphine (10-20 µg) was microinjected into the NRM of anaesthetized cats there was a suppression of noxious heat-evoked activity of the deeper dorsal horn WDR neurones (Du et al. 1984).

To re-examine the question of whether the NRM mediates morphine inhibition of spinal cord dorsal horn neuronal nociceptive activity, the effect of morphine on the NRM stimulation-produced inhibition of dorsal

horn neuronal activity was evaluated. The rationale was that if morphine activates a descending inhibition, from the NRM onto the deep dorsal horn neurones, then it should potentiate the NRM phasic inhibition.

Experiments were also designed to test whether morphine inhibition of neuronal nociceptive activity was affected by enhanced 5-HT synaptic transmission. If morphine inhibition of deep dorsal horn neurones is in part mediated by 5-HT, then it should be enhanced by fluoxetine treatment. Furthermore, if 5-HT is involved in NRM phasic inhibition and morphine enhances this inhibition, then morphine and fluoxetine administered concurrently should increase the inhibition more than either drug alone.

REVIEW OF LITERATURE

Nociceptive Mechanism of Spinal Cord Dorsal Horn

CUTANEOUS NOCICEPTORS: Sensory information from the skin enters the CNS via cutaneous nerves consisting of several fibre types. These include large diameter myelinated Aαβ- fibres, the small diameter Aδ- fibres and the unmyelinated C-fibres. Of these various types of cutaneous nerve fibres, it is the Aδ- and C-fibres which convey noxious information from the skin nociceptors to the spinal cord sites in the CNS (Perl 1984).

The cutaneous nociceptor units have been classified into two types, namely, high threshold mechanoreceptor units (HTM) with Aδ- and C-axons (Burgess and Perl 1967; Bessou and Perl 1969) and polymodal nociceptor units (PMN) with C-axons (Bessou and Perl 1969).

The properties of these nociceptor units have been characterized in the cat using extracellular electrodes to record unit activity from single primary afferent axons (Burgess and Perl 1967; Bessou and Perl 1969). It was observed that HTM unit axons, which generally had no background activity, responded to strong noxious pressure applied to their receptive field. Pressure thresholds required to excite these HTM axons varied from unit to unit. A linear relationship between discharge rate of units and stimulus intensity was observed. These units were resistant to activation from noxious radiant heat of their receptive fields. However, in the both cat and monkey it has been observed that

application of noxious radiant heat to the receptive field of HTM units with Aδ- axons frequently sensitized these units so that they became responsive to subsequent heat stimulation (Fitzgerald and Lynn 1977; Campbell et al. 1979). They did not respond to irritant chemicals (Burgess and Perl 1967).

The unmyelinated PMN units respond to noxious pressure, noxious heat and the application of irritant chemicals to their receptive fields (Bessou and Perl 1969). The authors observed a graded increase in response of these units to increasing skin temperature. Heat sensitization of these units also occurred after a single strong heating of the receptive field.

Beck et al. (1974) have described thermal nociceptors in the cat which respond well to noxious heat but poorly to pressure. However, doubts have been expressed as to whether these fibres are representative of a class of nociceptors distinct from PMN units. For example, Lynn (1984) has suggested that the thermal nociceptors might be PMN at the most insensitive end of the mechanical sensitivity range for the latter units. Further, these putative thermal nociceptors were not tested for their responsiveness to irritant chemicals.

Course and termination of primary afferents: Detailed analysis of the spinal cord termination of primary afferents has been done by Light and Perl (1977 and 1979). These authors combined anterograde transport of horseradish peroxidase (HRP) by cut dorsal roots with light microscopy to trace the spinal cord region of termination of these

roots. The experiments were done in three different species, namely, cat, rat and monkey. Similar pattern of termination of the dorsal root fibres in the spinal cord was observed for all three species. Fine diameter fibres were observed to enter the dorsal horn through the tract of Lissauer whereas the large diameter fibres were observed to run medially and form a bundle in the dorsal columns. Collaterals from fine fibres were seen to terminate mainly in the superficial laminae of the dorsal horn, namely, laminae I and II (also called the marginal zone and the substantia gelatinosa, respectively). Based on the diameter of the collaterals terminating in the superficial laminae the authors suggested that fibres terminating in the marginal zone were mainly of the Aδ- axon type and those terminating in the SG (substantia gelatinosa) were mainly of the C-axon type. These authors extended their experiments to monkeys in which they selectively lesioned either the medial or the lateral section of the dorsal roots and studied the pattern of termination of the intact section of the dorsal roots. It has been shown that in monkeys the lateral section of the dorsal root consists mainly of unmyelinated and thinly myelinated fibres whereas the medial portion of the dorsal root consists of myelinated fibres with medium to large diameter. When the medial division of the dorsal roots is lesioned the HRP stained fibres of the lateral division showed presumed synaptic enlargements concentrated in the marginal zone and in the SG, with some endings also appearing at the base of dorsal horn. In contrast, with the lateral division cut and the medial left intact, HRP stained terminals were found to be densely distributed in the nucleus proprius

and in the deeper layers. The termination of the two divisions of the dorsal root were found to overlap in lamina III of the primate dorsal horn.

The termination pattern of single, functionally identified primary afferents have also been characterized. For example, Light and Perl (1979a) iontophoresed HRP intracellularly into physiologically identified single Aδ- afferents and observed that the HTM units gave off collaterals which ended in terminal arbors in laminae I and V. Sometimes the terminal arbors of the HTM units also penetrated the outer lamina II (also refered to as lamina II_o). Delta hair afferent collaterals arborised and terminated mainly in ventral or inner lamina II (also referred to as lamina II_i) and laminae III- V. Attempts have also been made to identify the course and termination of C-primary afferents in the spinal cord using intracellular HRP. For example, Perl (1984a) have reported that a small sample of such units studied gave off collaterals which terminated mainly in the SG. Some collaterals were observed to pass deep and have terminals ventral to SG.

Coupling HRP histochemistry to light and electron microscopy, Rethelyi et al. (1982) observed that the collaterals of the Aδ- HTM units had <u>en passant</u> enlargements along their course. These collaterals terminated in a bouton enlargement and synaptic connections (pre or post) were found at these boutons. Such boutons either made simple axo-dendritic contact or were central terminals in glomeruli being both pre- or postsynaptic to dendrites and axons in the glomeruli. Such termination pattern for the Aδ- HTM units were observed both in laminae

I and V.

On the basis of above observations it might be concluded that the zone of termination of the small diameter myelinated and unmyelinated primary afferents, some of which are nociceptors, terminate principally in the superficial dorsal horn (laminae I and II). In contrast the large diameter primary afferents terminate mainly in lamina III and in the deeper laminae of the spinal cord dorsal horn in adult cats (see Brown 1981 for review).

DORSAL HORN NEURONES RESPONDING TO NOXIOUS INPUT: Noxious information carried by the somatic nociceptors is conveyed first to the spinal cord sites, where it is processed and relayed to supraspinal region in the CNS and also initiates segmental reflexes.

There are at least two types of spinal cord dorsal horn neurones which are excited by the noxious information entering the spinal cord. These are the wide dynamic range (WDR) or multireceptive neurones and nociceptor-specific (NS) neurones. The former class of neurones is excited by both noxious and innocuous stimuli whereas NS neurones are excited only by noxious stimuli.

Light and Durkovic (1984) have mapped the laminar organization of dorsal horn neurones which respond to the noxious stimuli of the skin. The experiments were done in decerebrate and spinalized cats. Cells which responded to the noxious pinch of their peripheral receptive field were found to be distributed throughout laminae I- VII of the spinal cord dorsal horn. The most heavy concentration of such neurones was

determined to be in the region of laminae I- II and V-VII.

WDR neurones: Light and Durkovic (1984) have reported that, in the cat, neurones of this class are concentrated in the spinal cord deep dorsal horn laminae V - VII. The electrophysiological characteristics of these deep dorsal horn WDR neurones have been described by a number of authors (Handwerker et al. 1975; Price and Browe 1973). These neurones, as mentiond earlier, were excited by both innocuous mechanical stimulation and noxious pinch of their peripheral receptive fields. A high proportion of such neurones were also found to be stimulated by noxious heating of their receptive fields and there was a graded increase in the response of these neurones to increasing skin temperature. A prominent after-discharge was seen in these neurones after the noxious thermal stimulus was removed. Some of these neurones also discharged to innocuous cooling or warming of their receptive fields.

The deep dorsal horn WDR neurones have been reported to receive inputs from both large diameter (Aαβ) and small diameter (Aδ, C-fibre) afferents (Handwerker et al. 1975; Light and Durkovic 1984). Based on the calculations of the latency of responses in these neurones to large diameter afferent stimulation, the above authors have suggested that a monosynaptic Aαβ-afferent input to the deep dorsal horn WDR neurones is possible. This is consistent with histological findings that dendrites of deep dorsal horn neurones and axonal arborization of large diameter afferents are co-terminus in these deeper laminae of dorsal horn

(Brown 1982). Polysynaptic input from Aαβ-afferents to these neurones has also been described (Handwerker et al. 1975). However, the direct activation of these deep dorsal horn neurones by nociceptive afferents is uncertain. This is especially true for the C-nociceptor afferents, most of which apparently terminate in the superficial laminae. It is possible, therefore, that many of the WDR neurones are in polysynaptic relation to nociceptors.

Various inhibitory influences on the deep dorsal horn WDR neurones in the cat have also been reported. For example, Hillman and Wall (1969) observed an inhibition of activity of these neurones by light tactile stimuli applied to areas which were adjacent to the excitatory receptive fields of these neurones. Handwerker et al. (1975) described a segmental inhibition of heat-evoked responses in these neurones by stimultion of large diameter nerve trunk afferents or the dorsal columns. Consistently, when A-fibres of nerve are polarization blocked, the C-fibre discharge of WDR neurones to stimulation of this nerve grows markedly larger (Gregor & Zimmermann 1972). Du et al. (1984a) reported an inhibition of high intensity hindlimb nerve stimulation-produced and noxious heat-evoked response in dorsal horn neurones by A-afferent stimulation of forelimb nerves. This inhibition was mediated by supraspinal structures as it could be reduced by microinjection of the local anaesthetic, lidocaine, in supraspinal sites including the NRM. Du et al. (1984a) did not classify the spinal cord dorsal horn neurones studied. However, it is possible that they were of the WDR type for Le Bars et al. (1979) had reported that in rats only the WDR

type of neurones could be inhibited by stimulation in the fore-region of the animals body. A supraspinal tonic inhibitory control on the noxious stimulus-evoked and C-fibre evoked response in WDR neurones has also been described (Handwerker et al. 1975; Soja and Sinclair 1983a).

Some of the deep dorsal horn WDR neurones have axons which ascend the spinal cord to supraspinal structures. For example, some of the spinocervical tract (Cervero et al. 1977), spinothalamic tract (Rucker et al. 1984), dorsal column postsynaptic spinomedullary (DCPS) tract (Brown & Fyffe 1981) and spinomesencephalic tract neurones (Yezierski & Schwartz 1984) have WDR characteristics.

Finally, a morpho-functional study on the deep dorsal horn WDR neurones have also been attempted. In such experiments physiologically indentified neurones were marked intracellularly with the HRP. This procedure allows a study of the morphology of neurones with known functional properties. For example, Ritz & Greenspan (1985) have described lamina V, WDR neurones with large soma size and dendritic spread which extended considerably in rostro-caudal and mediolateral direction. In the dorso-ventral direction the dendrites of these neurones were largely confined to Lamina III to lamina VII. In very few cases did the dendrites penetrate the superficial laminae. These authors also described a small sample of lamina VII WDR neurones. These neurones had a small soma size compared to neurones in the lamina V and their dendrites were mostly confined to the ventral horn. Bennett et al. (1984) have described dorsal DCPS neurones located in the laminae III - IV and having WDR characteristics. These neurones had

dendritic spread extending from laminae III - V, whereas those DCPS neurones which were not responsive to noxious stimuli had a dendritic spread confined to laminae III and IV.

WDR neurones have also been reported in laminae I & II of the cat dorsal horn (Bennett et al. 1981; Bennett et al. 1979; Cervero et al. 1976; Craig & Kniffki 1985; Fitzgerald 1981 and Cervero et al. 1979a). The number of WDR neurones recorded from the superficial laminae, as a percentage of the total cell population in this region, varies. For example, WDR neurones formed the majority of the neurones (64%) recorded by Fitzgerald (1981) from this region of the spinal cord in decerebrate cats, whereas, such neurones constituted a very small proportion of the sample recorded by Cervero et al. (1979a) in anaesthetized cats.

Fitzgerald (1981) has studied extensively the properties of WDR neurones of superficial laminae in cats. Most of the neurones studied had large receptive fields and responded to both brushing and noxious pinch applied to their receptive fields. Many of the neurones showed habituation to repeated brushing of the same area of their receptive field. Habituation to noxious pinch was less common. A prominent after-discharge generally developed in these neurones to pinching in receptive fields. The response to a noxious heat stimulus was not tested.

Most of the WDR neurones recorded by Fitzgerald (1981) had C-fibre input. Convergent input on these neurones of either Aδ or Aβ or both Aδ and Aβ fibres was also reported. Unlike the deeper dorsal horn neurones, the C-fibre response in the WDR neurones of the superficial

laminae was reported to occur at extremely regular latencies giving time locked spikes on stimulation of the sural nerve. The authors suggested that such time locked C-fibre response could be due to a monosynaptic C-afferent input to these neurones. No 'wind up' on C-fibre stimulation, which is typical of deeper dorsal horn WDR neurones, was seen in these neurones. The authors also calculated the latency of response in these neurones to Aβ and Aδ stimulation. Based on these calculations the author suggested that Aδ response occurred at latencies which could be monosynaptic whereas Aβ response was at latencies which might be polysynaptic.

The inhibitory responses of the superficial laminae WDR neurones also differed from that of deeper dorsal horn WDR neurones. Polarisation block of A-fibres did not enhance the C-fibre response in the former neurones (Fitzgerald 1981) which is unlike that reported for the latter group of neurones (see above). Fitzgerald (1981) also studied the effect of sural nerve stimulation at Aβ strength on the response of WDR neurones to bradykinin injected subcutaneously in their receptive fields. Whereas in lamina V WDR neurones the response to bradykinin was inhibited by a conditioning stimulus in the sural nerve at Aβ fibre intensity, no inhibition was observed in the superficial laminae neurones.

Whether these neurones project to supraspinal structures is not known. Morpho-functional studies, similar to that described before, have also been attempted for superficial laminae WDR neurones (Bennett et al. 1981; Bennett et al. 1979; Light et al. 1979; Rethelyi et al.

1983). Some of the WDR neurones thus described have characteristics of Waldeyer cells and stalked cells of the superficial laminae. Generally the dendrites of WDR neurones were confined to the superficial laminae and in some cases penetrated lamina III, with the axon also terminating in superficial laminae. The axons of some WDR Waldeyer cells could be traced to the ventral horn of the spinal cord. There might also exist a difference in the laminar arrangement of neurones in the SG such that cells in SG_0 are excited predominantly by HTM and polymodal receptors innervated by Aδ- and C-fibres, while cells in the SG_i are driven primarily by LTM (Light et al. 1979b).

NS neurones: Cells which are driven solely by noxious stimulation of their receptive fields have been described in cats, by various authors (Christensen & Perl 1970; Bennett et al. 1981; Bennett et al. 1979; Cervero et al. 1976; Craig and Kniffki 1985; Fitzgerald 1981; and Light & Durkovic 1984). Although NS neurones have also been reported in the deep dorsal horn of the spinal cord (Light and Durkovic 1984), much of the work on these type of neurones has concentrated in the region of laminae I and II of the superficial dorsal horn.

The response characteristic of these neurones to noxious stimulation of their receptive fields has been studied. Christensen & Perl (1970) described NS neurones of lamina I which either responded to noxious pinch or both noxious pinch and heat applied to their receptive fields. The former type has been called class 3a and the latter class 3b by Cervero et al. (1976). The class 3a NS neurones were desribed as

receiving input from only Aδ-cutaneous afferents whereas class 3b neurones received both Aδ-and C-fibre afferent input (Cervero et al. 1976). Fitzgerald (1981), found a number of NS neurones of the superficial laminae that developed long after-discharges lasting seconds after pinching the receptive field. On the contrary, Steedman et al. (1985) reported on superficial laminae NS neurones in which increased firing to noxious pinch of their RF was followed by total silence or decreased firing after removal of excitatory influence.

Inhibitory influences on the NS neurones of the superficial laminae of spinal cord dorsal horn have also been reported. For example, Christensen and Perl (1970), noted an inhibitory receptive field for NS cells which was generally found adjacent to their excitatory fields in a surround fashion. Only strong noxious mechanical or noxious thermal stimuli applied to the inhibitory RF of these cells was able to inhibit the activity in the NS neurones. However, recently Steedman et al. (1985) also reported an inhibitory receptive field of these neurones, which in some cases overlapped the excitatory field, and where application of innocuous mechanical stimulation produced the inhibition. Stimulation of large diameter afferents of the nerve supplying the RF of NS neurones inhibited the discharge in these neurones to noxious stimulation (Cervero et al. 1976; Steedman et al. 1985). These neurones could be inhibited by stimulation of large afferent fibres in dorsal column at intensity sufficient to excite large afferent fibres (Cervero et al. 1979d). Cervero et al. (1976) reported a supraspinal tonic inhibitory control on the activity in the

superficial laminae NS neurones. However, the tonic inhibition exercised by supraspinal structures on these neurones was reported to be weaker than the apparently similar descending tonic inhibition of deeper dorsal horn WDR cells. Cervero et al. (1979c) have also examined the extent of descending tonic inhibition on NS, SG neurones. In a small sample of six such neurones, there was no change in the background activity of four neurones and, in the remaining two, only small or transient changes were observed on application of spinal cold block.

Using intracellular recording from the superficial laminae NS neurones, Steedman et al. (1985) have calculated that the central delay before a response in these neurones could be due to the activation of Aβ or Aδ afferent fibres. Based on these calculations, the authors suggested that these neurones received a predominantly excitatory monosynaptic Aδ input and a inhibitory polysynaptic Aβ input. Like superficial laminae WDR neurones, the response to C-fibre stimulation in the superficial laminae NS neurones occurred at constant latency with no 'wind up' phenomenon.

Some of the NS lamina I neurones of the cat might be projecting to supraspinal structures. For example, Craig and Kniffki (1985) reported that a number of NS neurones from lamina I could be antidromically activated from the contralateral thalamus. However, the number of NS lamina I neurones which might project supraspinally might be small. Thus, Kumazawa and Perl (1975) could antidromically activate only one-third of these neurones from the contralateral spinal cord. Similarly Cervero et al. (1979d and 1979b) failed to backfire two-third

of the lamina I NS and SG neurones, respectively, by stimulation at a spinal cord site three spinal segments rostral to the recording site.

Morpho-functional analysis of the superficial laminae NS neurones have revealed at least three different morphological types (Bennett et al. 1981; Bennett et al. 1979; Steedman et al. 1985; Molony et al. 1981; Rethelyi et al. 1983 and Light et al. 1979). Two of these have a small perikaraya. One of these has dendrites directed in rostro-caudal fashion in lamina I and SG_0. The other type has dendrites arranged similarly as to above type plus it might have dendritic arborisation running transversely across lamina II and lamina III. The axons of these neurones are also generally directed rostro-caudally in laminae I and II. The axons branched outside the limit of the dendritic tree of this parent neurone. The third morphological type of NS neurone has a relatively larger cell body with dendrites also confined to lamina I but with some dendrite branches following the lateral curvature of lamina I into the intermediate zone of the dorsal horn. The axons of this type of neurone could be traced into lamina I and also were seen directed ventrally towards the ventral horn of the spinal cord.

In an attempt to provide a specific function for neurones in the SG in sensory transmission, Price et al. (1979) recorded from neurone pairs in the marginal zone of the monkey spinal cord. The first neurone encountered was identified as a spinothalamic tract neurone in the marginal zone while the more ventral neurone was located in the SG_0. Both neurones of each pair had similar afferent inputs with the SG_0 neurones having smaller receptive fields and shorter latencies to

appropriate nociceptive stimuli. These investigators concluded that the SG_0 cells may relay afferent nociceptive input to lamina I neurones which, in turn project to supraspinal areas.

Miscellaneous neurones: Finally, neurones in the spinal cord have been identified which respond differently from WDR or NS neurones to the application of noxious stimuli to their cutaneous receptive fields. For example, Cervero et al. (1977) have described deep dorsal horn spino-cervical tract neurones which were inhibited by noxious stimuli applied to their receptive fields (I-Class neurones). Another class of neurones described by the above authors were classified as E-I cells. These were excited vigorously by the first noxious stimulus to their receptive fields, the activity of the neurones remained high on the termination of the stimulus and when a subsequent noxious stimulus was applied the neuronal activity was inhibited. The spontaneous activity of these neurones was high and was increased slightly and transiently on cold blocking the spinal cord. Cervero et al. (1979a) have also described SG neurones which have similar characteristics to the above deep dorsal horn neurones in that a noxious stimulus applied to the receptive field inhibited the activity of the neurone. The authors called this neuronal type as class inverse-3 ($\overline{3}$). This class of SG neurone could be excited by innocuous stimuli. Another class of SG neurones described by the above authors, and called class inverse-2 ($\overline{2}$), could be inhibited by both noxious and innocuous stimulus applied to their receptive fields.

The Nucleus Raphe Magnus-Spinal Cord Neuraxis in the Cat

The nucleus raphe magnus (NRM) is a midline medullary nucleus extending from the rostral pole of the inferior olive to the level of the rostral pole of the superior olive. Detailed cytoarchitectural features of this nucleus have been described by Taber et al. (1960). These authors identified three different cell types in the NRM: big and medium sized polygonal cells present in large numbers; small, round, piriform or spindle shaped cells, and giant cells. Fibres originating within the nucleus and those crossing the nucleus were also identified as well as the presence of abundant boutons.

Dhalstrom and Fuxe (1964), using histofluorescence techniques, demonstrated that many of these cells in the NRM have a yellow fluorescence distinctive for 5-hydroxytryptamine (5-HT, serotonin). Wiklund et al. (1981) have estimated that the serotonergic neurones in the NRM constitute about 15% of the total neuronal population of this nucleus in the cat. This group identified serotonergic neurones also by using the histofluorescence technique. At least some of these neurones project to the spinal cord since a partial lesion in this nucleus results in a pronounced decrease in the level of 5-HT in the dorsal horn of the spinal cord (Oliveras et al. 1977). Employing a double labeling technique in the rat, Bowker et al. (1981) mapped the serotonin containing cells of the brainstem that project to the spinal cord. These cells were mainly in the nucleus raphe obscurus, raphe pallidus, raphe magnus and the adjacent reticular formation. Of the serotonin containing cells in the raphe nuclei at least 73.4% projected to the

spinal cord. Conversely, of spinally projecting cells in these nuclei, at least 88.6% contained serotonin. In cats, of the total number of spinally projecting neurones from the above brainstem regions 69% were serotonergic (Fung et al. 1985). West and Wolstencroft (1977) have estimated that in the cat only 10% of the NRM fibres projecting to the spinal cord are serotonergic. Their calculations were based on the assumption that the bulbospinal 5-HT units could be identified by their anticipated slow conduction velocity, due to their unmyelinated axons. However, not all bulbospinal 5-HT axons in the cat are unmyelinated (Ruda and Gobel 1980) and therefore the number of NRM bulbospinal serotonergic fibres might have been underestimated by West and Wolstencroft (1977). Indeed, using a more stringent means of identification, that of susceptibility to 5,7-dihydroxytryptamine, Wessendorf et al. (1981) found that in rats 40% of the bulbospinal units in the NRM were probably serotonergic.

Descending fibres from the NRM to the spinal cord have also been demonstrated in the cat using retrograde flow technique involving horseradish peroxidase (Basbaum et al. 1978). These fibres travel in the dorsolateral funiculus of the spinal cord and terminate in laminae I, II, and in parts of laminae V, VI, and VII. Some of these descending fibres are serotonergic since ^{3}(H)-amino acid microinjected into the NRM is anterogradely transported into morphologically identified 5-HT axonal endings in the spinal cord of cats (Ruda et al. 1981). Furthermore, thoracic transection results in a loss of spinal 5-HT below the level of section without altering that found in the cervical enlargement

(Oliveras et al. 1977). The above observations suggest that much or all of the spinal serotonin is contributed by a descending fibre system from the nucleus raphe.

A quantitative analysis of the concentration of serotonin in different regions of the spinal cord was performed by Oliveras et al. (1977). Chemical assay was used to determine the serotonin content of the spinal cord tissue obtained by punching out microdiscs from histological sections of the spinal cord. The concentration of 5-HT was at least twice as high in the gray matter as in the white matter. The regions of the spinal cord gray matter containing the most serotonin included the motor nucleus, lamina X and the dorsolateral most part of the dorsal horn (in the region of SG). Axonal terminals which take up labelled 5-HT have also been demonstrated in the superficial laminae I and II of the dorsal horn (Ruda et al. 1981).

The 5-HT axonal endings in the spinal cord synapse on dendrites and perikaya of postsynaptic targets (Ruda and Gobel 1980). Very few such axonal endings synapse on terminals of primary afferent fibres. The postsynaptic targets of 5-HT terminals include projection neurones in lamina I and stalked cells in the SG (Gobel et al. 1982). Some of these targets have enkephalin-like immunoreactivity (ELI; Glazer and Basbaum 1984). This group also found that the majority of 5-HT axonal endings did not have any definitive synaptic cleft. These anatomical observations would favour a postsynaptic action of descending 5-HT fibres and a non-synaptic action by release of 5-HT into the vicinity of target cells. Other descending projections from the NRM are

to the spinal trigeminal nucleus (pars caudalis), dorsal motor nucleus of the vagus and the solitary nucleus (Basbaum et al. 1978).

Ascending projections from this nucleus travel along the ventromedial tegmentum and the medio-longitudinal fasciculus and project to the periaqueductal gray (PAG), centralis medialis, paracentralis, centralis lateralis and dorsomedial thalamus, lateral and dorsal hypothalamic nucleus and zona incerta (Basbaum et al. 1976).

The NRM receives little or no direct input from the spinal cord. The input to the nucleus includes that from the PAG. Anatomical connections between these nuclei have been shown (Abols and Basbaum 1981) and electrophysiological evidence also exists for such a projection (Shah and Dostrovsky 1980). The input from the PAG to the NRM is predominantly excitatory for both raphe-spinal and non-spinal projecting raphe cells (Lovic et al. 1978; Maciewicz et al. 1984). There is also a large input from the nearby gigantocellular reticular formation (Rgc; Mehler 1964). Stimulation in the medullary reticular formation produces a graded depolarization of raphe cells. The NRM, in turn, projects to the medullary and pontine reticular formation (Bobillier et al. 1976) and stimulation in the nucleus raphe magnus is known to inhibit tooth pulp responses in the medullary reticular formation (Lovic and Wolstencroft 1979). Reciprocal connection between NRM and dorsal column nuclei have also been suggested (Saade et al. 1982; Jundi et al. 1982). An excitatory input to the NRM from the sensorimotor cortex has also been described (West and Wolstencroft 1978).

The raphe cells are spontaneously active in decerebrate cats and are excited by noxious and innocuous stimuli applied to their receptive field (Anderson et al. 1977). Auerbach et al. (1985) have reported that in the conscious cat, presumed serotonergic neurones in the NRM showed slow, regular rates of discharge. The authors also reported that some of these neurones could be excited by cutaneous noxious pinch and noxious heat. However, these neurones did not specifically respond to noxious stimuli as they could also be activated by non-noxious auditory and visual stimuli. Further, no difference was observed in the efficacy of low intensity and high intensity stimulation of the inferior alveolar nerve to excite these neurones. The lower intensity range used to stimulate the above nerve was adjusted to produce only Aβ-fibre activation whereas high intensity stimulation of the inferior alveolar nerve produced aversive behaviour in the animal. However, it is not clear whether the presumed 5-HT neurones studied by the authors contributed to the raphe-spinal serotonergic fibre system, though cells with similar characteristics have been shown to project to the spinal cord (Light 1981).

Serotonin is also released into the spinal cord on intense electrical stimulation of peripheral nerves (Yaksh and Tyce 1981). Some of these findings, namely (1) activation of presumed serotonergic neurones by noxious stimuli, and, (2) activation of raphe-spinal fibres by noxious stimuli (some of which might be serotonergic) and release of 5-HT into the spinal cord on intense peripheral nerve stimulation, may suggest a neuronal substrate for a negative feedback loop involving

5-HT. Noxious information may, through structures like the medullary reticular formation (a major receiving area for pain transmission pathways in cat) stimulate the NRM to produce a inhibition at the spinal level.

Co-existence of 5-HT with enkephalins in neurons of the ventral NRM has been demonstrated (Glazer et al. 1981), however their functional significance is not known.

Physiology of Descending Inhibition by Stimulation of NRM in the Cat

The NRM-spinal cord neuraxis described above is one of the descending systems by which supraspinal centres exert control over the sensory information through the spinal cord. The control by the NRM on the activity of spinal cord dorsal horn neurones to peripheral noxious stimuli has been described by Fields et al. (1977). Other supraspinal regions involved in descending control of dorsal horn neurones to noxious stimuli includes the periaqueductal gray (PAG; Liebeskind et al. 1973), the nucleus gigantocellularis (McCreery and Bloedel 1975) the red nucleus (Gray and Dostrovsky 1984), dorsolateral pons in the region of locus coerleus (Hodge et al. 1983), the lateral reticular nucleus (Morton et al. 1983), the preoptic area and basal forebrain (Carstens et al. 1983), the ventromedial septal area (Carstens et al. 1982) and the lateral tegmental field (Edeson and Ryall 1983).

The supraspinal influence on the response of dorsal horn neurones to peripheral noxious stimuli can either be tonic or phasic. The tonic control manifests as a powerful inhibition of activity in WDR dorsal horn neurones. This tonic inhibition of neuronal activity appears to be

selective for input from nociceptors vs. low threshold mechanoreceptors (LTM; Handwerker et al. 1975; Soja and Sinclair 1983a). The anatomical loci for the origin of this tonic inhibition appears to be at the level of brainstem, in the region of the lateral reticular nucleus. Lesions in this region reduces or abolishes the tonic descending inhibition on WDR dorsal horn neurones (Hall et al. 1982). Lesions in other brainstem areas including the NRM do not block this tonic inhibition (Hall et al. 1982). However, the NRM is involved in stimulation-produced (phasic) inhibition of noxious stimuli-evoked activity in the dorsal horn neurones. For example, electrical stimulation in NRM inhibited the response of dorsal horn neurones in laminae I, V and VI to high threshold mechanical stimulation (presumably in the noxious range) (Fields et al. 1977).

Guilbaud et al. (1977) reported a similar inhibition of evoked activity to pinch or intense electrical stimulation in WDR interneurones in cats. These authors and Fields et al. (1977) also reported that NRM stimulation produced a preferential inhibition of evoked activity of the dorsal horn neurones to noxious stimuli without affecting the response of these neurones to innocuous stimuli. But a wide body of evidence does not favour the specificity of inhibition on NRM stimulation. LTM and HTM cat spinothalamic neurones are both inhibited by NRM stimulation (McCreery et al. 1979). The non-noxious stimuli were more effectively inhibited when it was applied for a sustained period of time rather than repetitively or randomly. Evoked activity in NS, WDR, and LTM spinocervical tract neurones were inhibited on stimulation of NRM

(Kajander et al. 1984). Similarly, there was a lack of specificity in inhibition of evoked activity in WDR laminae IV-V spinal cord interneurones in the cat to either C-fibre stimulation of the tibial nerve or short air puffs to move hairs (Morton et al. 1983). Gray and Dostrovsky (1983) showed a nonselective inhibition of evoked activity in NS, WDR and LTM neurones having the same degree of afferent input. Thus a preponderance of evidence would suggest a nonspecific, nonselective inhibition of evoked activity in dorsal horn neurones by NRM stimulation.

Evidence also exists that electrical stimulation of the NRM decreases the spontaneous firing of cat dorsal horn neurones (Belcher et al. 1978). This inhibition of evoked and spontaneous activity is by a fibre system from the NRM which descends in the dorsolateral funiculus (DLF). DLF lesions, but not ventrolateral lesions made rostral to the site of recording, reduced the effect in the cat (Fields et al. 1977). This would be further substantiated by the anatomical study of Basbaum et al. (1978) who found that fibres from the NRM largely descend in DLF to the spinal cord.

There is evidence that this descending inhibition can occur via a presynaptic site of action. Martin et al. (1979) reported that stimulation of the NRM in cats result in primary afferent depolarization (PAD) of myelinated Aαβ- cutaneous fibres innervating rapidly and slowly adapting receptors as well as Aδ- fibres innervating hair follicle mechanoreceptors and nociceptors. They tested terminal excitability of single sural nerve fibres, innervating carefully characterized cutaneous

receptors. They concluded that stimulation of the NRM results in nonselective PAD in all types of myelinated cutaneous afferents. These effects would account for a nonselective depression of sensory inputs noted by some authors.

Paradoxically, NRM stimulation produces a hyperpolarization of C-fibres innervating polymodal nociceptors and C-mechanoreceptors and consequently the threshold for intraspinal (antidromic) excitation for these fibres is increased (Hentall and Fields 1979). This would suggest an enhancement of transmitter release to peripheral stimulation of C-primary afferents. Carstens et al. (1981), however, suggests that presynaptic inhibition of nociceptive C-afferents may occur by mechanisms other than those thought to occur for large afferents. They point to the similarity of action of opiates and NRM stimulation both increasing the threshold for intraspinal activation of primary afferents and inhibiting dorsal horn neurones (Carstens et al. 1979; Sastry 1979).

Curtis et al. (1983) questioned these findings. They found that iontophoretic 5-HT, the putative neurotransmitter involved in NRM action, increased the threshold for antidromic activation of Ia afferent terminals whereas GABA, K or tetanic stimulation of flexor muscle afferents decreased the threshold for antidromic activation. They speculated that the 5-HT effect may not be related to presynaptic control of transmitter release.

NRM stimulation may also produce postsynaptic inhibition of dorsal horn neurones. Belcher et al. (1978) reported that neuronal responses evoked by the excitant amino acid, DL- homocysteic acid (DLH), was

reduced by NRM stimulation. Using intracellular recording techniques Giesler et al. (1981) reported that in five of seven primate spinothalamic neurones tested, stimulation of the NRM produced a membrane hyperpolarization. They also found that NRM induced a block of antidromic action potentials in spinothalamic tract neurones. This evidence would support a postsynaptic inhibition of dorsal horn neurones by NRM stimulation.

Finally, not all dorsal horn neurones are inhibited by electrical stimulation of NRM. Dubisson and Wall (1980) reported excitation of laminae I and II cells upon NRM stimulation. These cells responded to pressure, touch and brushing. Based on these findings Dubisson and Wall (1980) speculated that descending inhibition produced by electrical stimulation of the NRM may occur via an excitatory input to SG cells which, in turn, inhibit deep dorsal horn neurones.

Pharmacology of the Nucleus Raphe Magnus Stimulation-Produced Inhibition of Dorsal Horn Neuronal Activity in the Cat

Essentially, three approaches have been used in elucidating whether 5-HT is involved in brainstem inhibition of spinal cord neuronal activity: (1) blocking synaptically released 5-HT with putative 5-HT antagonists, (2) selectively depleting 5-HT stores with p-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor or (3) increasing the concentration of synaptically released 5-HT using selective serotonergic uptake blockers.

It has already been mentioned that the NRM is thought not to be

involved in tonic inhibition of deep dorsal horn neuronal activity. Pharmacological evidence against a 5-HT involvement in tonic inhibition comes from the work of Soja and Sinclair (1980). Neither depletion of 5-HT stores by pretreatment with PCPA nor increasing the concentration of synaptically released 5-HT by fluoxetine, a serotonin uptake inhibitor, affected the response of dorsal horn cells to noxious stimuli in cats with spinal cord conduction intact or cold blocked. Since most of the spinal 5-HT is located in the descending fibre system originating in the NRM, these observations and those mentioned earlier do not support a role for the NRM in tonic descending inhibition of deep dorsal horn WDR neurones.

However, pharmacological manipulations suggest that 5-HT has a role in phasic inhibition of deep dorsal horn cells elicited by NRM or PAG stimulation. PAG inhibition of feline WDR dorsal horn neurones, which is partly relayed through the NRM, is blocked by 5-HT antagonists, methysergide (Carstens et al. 1981a; Barnes et al. 1979) and LSD (Guilbaud et al. 1973) and reduced by PCPA pretreatment (Carstens et al. 1981a). The antagonism by methysergide is reversed by intravenous 5-hydroxytryptophan (Barnes et al. 1979). However, in studies by Guilbaud et al. (1973) and Barnes et al. (1979) a substantial increase in background and noxious stimulus evoked activity was seen after administration of the 5-HT antagonists. It is, therefore, not clear whether the reduction in the degree of inhibition was due to a blockade of synaptically released 5-HT at the terminals of the bulbospinal neurones or to these changes in background or evoked activity.

However, in experiments using methysergide, no reduction of inhibition by NRM stimulation was seen. Methysergide was applied iontophoretically either in the substantia gelatinosa (SG; Griersmith et al. 1981) or on cell bodies of dorsal horn neurones (Belcher et al. 1978) or administered intravenously (Griersmith et al. 1981). Belcher et al. (1978) also observed that methysergide inhibited excitatory responses to NRM stimulation. However, methysergide per se decreased the excitability of the cells and this may have influenced the observed results.

Another technique which has been adopted to unravel the role of bulbospinal 5-HT neurones is the use of iontophoresis. Randic and Yu (1976) examined neurones in laminae I and II of the decerebrate cat which were excited by noxious cutaneous stimuli. Iontophoretic 5-HT depressed 70% of these neurones examined with the depression lasting up to 10 min. When applied in the SG, 5-HT selectively reduced responses of dorsal horn laminae IV-V neurones to peripheral noxious stimuli (Headley et al. 1978). It also preferentially inhibited evoked activity in NS neurones without affecting activity in LTM neurones when applied in the vicinity of the cell bodies (Belcher et al. 1978). These authors found, in addition, that 5-HT iontophoretically released onto cell bodies of WDR neurones nonselectively inhibited their evoked activity.

Thus, evidence would favour a selectivity of action of 5-HT depending on the region of the spinal cord to which it is applied as well as the cell type. Interestingly, the effect of 5-HT in the SG is reversed by methysergide iontophoresed into the SG prior to or

concurrently with 5-HT administration but not when applied after 5-HT iontophoresis (Griersmith and Duggan 1980). These authors suggested that 5-HT may have a modulatory role such that, once the effect of 5-HT is initiated, other changes may take place which go beyond a simple drug-receptor interaction and, therefore, methysergide can prevent but not reverse 5-HT effects. Davies and Roberts (1981) suggested that neuromodulation may occur at substance P receptors since neuronal excitation by substance P was antagonized by iontophoretic 5-HT, an effect only weakly antagonized by cinanserin, a putative 5-HT antagonist.

However, not all neuronal responses are reduced or inhibited by iontophoretic 5-HT. Iontophoretic 5-HT was found to increase the spontaneous or excitatory amino acid induced neuronal firing in the spinal cord dorsal horn (Belcher et al. 1978; Todd and Millar 1983). Todd and Millar (1983) examined the effect of iontophoretic 5-HT on interneurones located in laminae I, II and III of the cat spinal dorsal horn. The interneurones were characterized by their responsiveness to peripheral natural stimulation as LTM, HTM or WDR interneurones. Iontophoretic 5-HT excited 68% of the units examined and excitation was observed in all three classes of interneurones. The authors suggested that these interneurones were inhibitory to deep dorsal horn neurones. Thus excitation of these SG interneurones by 5-HT would inhibit activity in the deep dorsal horn cells. Indeed, Headley et al. (1978) have shown that 5-HT, iontophoresed into the SG, inhibits deep dorsal horn neurones. If 5-HT is important in mediating the

inhibition of deep dorsal horn neuronal activity upon NRM stimulation, it is possible that it does so by exciting inhibitory interneurones in the superficial laminae of the dorsal horn. This idea would be supported by the work of Dubisson and Wall (1980) who observed that stimulation of the NRM excited laminae I and II interneurones of the cat spinal cord dorsal horn. The cells studied by this group were characterized as being responsive to peripheral noxious stimuli. On the other hand, Miletic et al. (1984) reported that stimulation of the NRM markedly suppressed the response of the laminae I and II interneurones of the dorsal horn to peripheral noxious stimulation. The neurones which were inhibited also had considerable 5-HT like immunoreactive contacts onto their dendrites. However, the authors did not attempt to study whether application of 5-HT on these neurones mimicked the effect of the NRM stimulation.

Pharmacology of Morphine on the Deep Dorsal Horn WDR Neurones

TONIC DESCENDING INHIBITION: INVOLVEMENT OF ENDOGENOUS OPIOID PEPTIDES AND THE EFFECT OF MORPHINE: Whether the endogenous opioid peptides mediate the tonic supraspinal inhibition of nociceptive activity of deep doral horn WDR neurones has been investigated using opiate antagonists. The rationale used in these studies is that, if the endogenous opioid peptides mediate the tonic descending inhibition of these neurones, then administration of an opiate antagonist would be expected to reduce this inhibition. Duggan et al. (1977a) reported that

intravenous administration of the opiate antagonist, naloxone (1.3 - 3.2 mg/kg) failed to alter the tonic supraspinal inhibition impinging on these neurones. The tonic inhibition was measured as the increase in noxious stimuli-evoked response of a neurone during the cold block state of the spinal cord. A decrease in the tonic supraspinal inhibition would be expected to result in a smaller increase in the neuronal firing rate to noxious stimuli in the cold block state of the spinal cord. Furthermore, one would also expect to see an increase in the spontaneous firing of these neurones in the warm, intact state of the spinal cord. Naloxone, however, failed to produce either of these changes. Thus, these observations would suggest that endogenous opioid peptides do not mediate the tonic descending supraspinal inhibition impinging on the deep dorsal horn WDR neurones. The same conclusion was reached by Sinclair et al. (1980) based on a similar study.

The effect of morphine on the tonic descending inhibition of the dorsal horn WDR neurones has also been investigated. For example, Duggan et al. (1980) have reported that intravenous morphine (0.5 - 1.0 mg/kg), in anaesthetized cats, reduced the tonic descending inhibition of dorsal horn neurones excited by impulses in C-fibre afferents. The extent of tonic inhibition on these neurones was measured as explained in the preceding paragraph. A decrease in the difference in the evoked neuronal discharge with the spinal cord in the normal vs cold-blocked state was taken as indicative of a decrease in the tonic inhibition. However, Soja and Sinclair (1983) have questioned these findings. These authors have suggested that the decrease in the difference of the evoked

discharge, as measured by Duggan et al. 1980, was due to a reduction in the excitability of the neurones in the presence of morphine.

Jurna and Grossman (1976) have also suggested that intravenous morphine decreased the tonic inhibition on ventrolateral tract axons excited by Aδ- and C-fibre inputs. They observed that intravenous morphine reduced the Aδ- and C-fibre evoked activity in the cold block state of the spinal cord but increased the activity in these axons in the normal, warm state of the spinal cord. The authors suggested that the inhibitory effect of morphine on the axonal activity in the cold blocked state of the spinal cord was due to a spinal action of morphine, whereas, in the warm, intact state of the spinal cord morphine depressed the tonic descending inhibition on these axons which masked the spinal inhibitory actions.

The above experiments do not provide conclusive evidence that morphine has an effect on the tonic descending inhibition impinging on the deep dorsal horn neurones and the ascending axons in the anterolateral quadrant of the spinal cord. The results obtained by Duggan et al. (1980) are complicated by a change in the excitability of the neurones. The experiment of Jurna and Grossman (1976) also does not provide direct evidence of an effect of morphine on tonic descending inhibition. The excitatory effect of morphine on the ascending axons of the spinal cord might have resulted by an excitation of supraspinal facilitatory inputs to these axons or a combination of morphine's effect on the tonic descending inhibition and facilitatory inputs to these axons.

INTRAVENOUS MORPHINE ON NOXIOUS STIMULUS-EVOKED RESPONSES OF THE DEEP DORSAL HORN WDR NEURONES

Normal, intact state of the Spinal Cord: Conflicting reports have appeared on morphine's effect on dorsal horn WDR neuronal activity when using anaesthetized or decerebrated cat preparations in which the spinal cord was intact. For example, Soja and Sinclair (1983b) reported that, in chloralose anaesthetized cats, morphine (1.0 mg/kg, total dose) decreased the noxious heat evoked activity in dorsal horn WDR neurones. Similar effects have been reported by Belcher and Ryall (1978) and Davies and Dray (1978). This latter group also reported that the inhibitory effect of morphine was reversed by intravenous naloxone. Using decerebrate cats, Hanaoka et al. (1978) observed that the nociceptive response of dorsal horn lamina V NS neurones was decreased by morphine in a dose dependent manner (morphine 0.5 - 2.0 mg/kg, i.v.).

However, other authors have reported a lack of effect or variable effects of intravenous morphine on natural noxious stimuli-evoked responses of the deep dorsal horn neurones. Thus, Le Bars et al. (1976b) observed a lack of effect of morphine (2.0 mg/kg, i.v.) on noxious pinch evoked responses in WDR, lamina V neurones in the decerebrate cat. The authors suggested that this lack of effect of morphine might be due to an exaggerated descending tonic inhibition observed on these neurones which might mask the effect of morphine. However, Jurna and Grossman (1976) suggested an alternate explanation. Also using the decerebrate cat preparation, they observed that morphine

(0.5 - 2.0 mg/kg, i.v.) produced no clear effect on firing of ventrolateral tract axons to sural Aδ- and C-fibre stimulation. They postulated that morphine might reduce the tonic descending inhibition on the spinal dorsal horn neurones and thereby release the activity in their ascending axons, but at the same time it might inhibit the activity of these axons by its spinal inhibitory effect on the firing of the cell body of origin of these axons. These counter action of morphine might balance each other so that no clear response to morphine is seen.

That morphine might selectively reduce the nociceptive responses on the deep dorsal horn neurones has been suggested by Belcher and Ryall (1976). They reported that morphine, reduced responses to noxious stimuli of these cells but did not effect activity in non-nociceptive cells to peripheral innocuous stimuli. Similar observations have been made by Davies and Dray (1978). Finally, morphine (0.3 - 1.6 mg/kg, i.v.) also reduces the C-fibre stimulation-evoked responses of dorsal horn laminae IV-VII WDR neurones (Duggan et al. 1980). This effect of morphine was reversed by naloxone administered intravenously.

Transected state of the spinal cord: In anaesthetized or decerebrate cats with the spinal cord transected, the effect of intravenous morphine on the noxious stimulus-evoked activity of WDR, NS and HTM neurones have been consistently reduced by intravenous morphine (Kitahata et al. 1974; Le Bars et al. 1975; Zieglgansberger and Bayerl 1976; Hanaoka et al. 1978; Davies and Dray 1978; Calvillo et al. 1979;

Einspahr and Piercey 1980; Piercey et al. 1980; Sastry and Goh 1983). In the study by Calvillo et al. (1979), the inhibitory effect of morphine on noxious heat-evoked responses in the laminae IV-V cells was also mimicked by other opiate analgesics, namely, meperidine and fentanyl. In a few studies mentioned above, the inhibitory effect of morphine on nociceptive responses of dorsal horn WDR neurones was shown to be reversed by the intravenous administration of the narcotic antagonist, naloxone (Le Bars et al. 1975; Zieglgansberger and Bayerl 1976; Calvillo et al. 1979; Piercey et al. 1980; Einspahr and Piercey 1980). Zieglgansberger and Bayerl (1976) and Calvillo et al. (1979) also iontophoresed naloxone near the cell being recorded in the few instances. Again naloxone reversed the inhibitory effects of morphine.

The experiments quoted in the preceding paragraph would suggest the following: (1) morphine has a spinal site of action where it can act to inhibit the noxious stimulus-evoked responses in the dorsal horn WDR, NS and HTM neurones, and (2) the effect of morphine on dorsal horn neuronal firing might be mediated via an opioid receptor since it can be reversed by the opiate receptor antagonist, naloxone.

However, intravenous morphine may not selectively inhibit nociceptive responses of deep dorsal horn WDR neurones in the spinal cat preparation. For example, Einsphar and Piercey (1980) observed a reduction of both the noxious and non-noxious stimulus evoked responses. These effects of morphine were reversed by intravenous naloxone. Morphine, however, did not affect the evoked responses of the neurones which could be excited only by non-noxious stimuli.

In the spinal cat preparation intravenous morphine also reduced the late component of the Aδ- and C-fibre mediated discharge evoked in WDR neurones by electrical stimulation of peripheral nerves (Le Bars et al. 1976a; Le Bars et al. 1975; Johnson and Duggan 1981). This effect of morphine was naloxone reversible. The early component of the discharge mediated by Aβ-fibre excitation was unaffected by morphine (Le Bars et al. 1975; Le Bars et al. 1976a). Intravenous morphine in the spinal cat preparation also reduced the Aδ- and C-fibre stimulation evoked responses in the ascending ventrolateral tract axons (Jurna and Grossman 1976). Again, this effect of morphine was reversed by intravenous naloxone.

IONTOPHORETIC ADMINISTRATION OF MORPHINE

Morphine has been iontophoretically applied in the spinal cord dorsal horn in the following manner: (1) in the vicinity of the cell body of the deep dorsal horn neurone being recorded, or (2) in the SG region of the dorsal horn while recording from a deep dorsal horn neurone. When iontophoresed in the vicinity of the cell body, morphine has produced inconsistent effects on the noxious stimuli-evoked activity. For example, Calvillo et al. (1974 and 1979) reported that the narcotics morphine, meperidine and fentanyl reduced the noxious heat and pinch-evoked discharge in the majority of the neurones. Similarly, Belcher and Ryall (1978) and Zieglgansberger and Bayerl (1976) reported that morphine and levorphanol depressed the nociceptive resonses in most neurones studied. The analgesically inactive isomer of levorphanol,

dextrorphan, was found to have no or a very weak effect on the nociceptive responses of these deep dorsal horn neurones. This inhibitory effect of morphine was reversed by iontophoretic naloxone administered near the recording site (Calvillo et al. 1974; Belcher and Ryall 1978; Zieglgansberger and Bayerl 1976).

Contrary to the above reports, morphine, when iontophoresed near the region of the cell bodies, was reported to excite rather than inhibit the noxious stimuli evoked responses in the deep dorsal horn WDR neurones (Duggan et al. 1977; Davies and Dray 1978; Piercey et al. 1980). This affect of iontophoretic morphine was naloxone insensitive.

It has been suggested that this lack of uniformity of effect of morphine might result from a limitation of the iontophoretic technique (Duggan and North 1984). These authors have suggested that the receptors of importance for morphine action might be only sparsely present near the cell body.

Whether iontophoretic morphine has a site of action other than in the region of the cell body of the deep dorsal horn neurones has also been investigated. For example, Duggan et al. (1977), Davies and Dray (1978), and Sastry and Goh (1983) observed that iontophoretic application of morphine in the region of the SG of the spinal cord reduced the peripheral noxious stimuli evoked-responses in the majority of the dorsal horn WDR neurones. This effect of morphine was reversed or reduced by simultaneous or prior iontophoresis of naloxone in the SG (Duggan et al. 1977; Davies and Dray 1978). Intravenous naloxone (0.1 mg/kg) also antagonized the effects of the iontophoretic morphine (Duggan et al. 1977).

When iontophoresed in the region of the SG morphine has a selective action in that it depresses the nociceptive responses of the deep dorsal horn WDR neurones without affecting the innocuous stimuli-evoked responses of these neurones (Duggan et al. 1977; Davies and Dray 1978). Similar selectivity of morphine action has also been suggested when it is iontophoresed near the region of the cell body of deep dorsal horn neurones. For example, Calvillo et al. (1979) reported that morphine failed to affect the response of these units to innocuous peripheral stimuli, whereas, the nociceptive responses were reduced. But Zieglgansberger and Bayerl (1976) observed that morphine depressed both the innocuous and noxious stimuli-evoked responses in these neurones. The non-noxious stimulus evoked responses in the LTM units was either unaffected (Calvillo et al. 1979; Zieglgansberger and Bayerl 1976), or enhanced (Belcher and Ryall 1978).

The possible mechanism by which morphine alters the nociceptive responses of the deep dorsal horn WDR neurones has also been investigated. Both postsynaptic and presynaptic mediated effects have been suggested. Zieglgansberger and Bayerl (1976) noted that iontophoretic morphine, applied in the vicinity of the cell body, led to a reduction in the rate of rise of the EPSP recorded intracellularly. This occurred in the absence of detectable changes in the membrane potential or conductance of the cell membrane. The investigators proposed that a reduction in the rate of rise of the EPSP would reduce firing to excitatory inputs impinging on these neurones. They also noted that morphine applied in this manner reduced depolarizations by

L-glutamate and that intravenous morphine (2.0 mg/kg) produced a hyperpolarization in 50% of the cells tested. These observations would suggest a postsynaptic mechanism through which morphine exerts its suppressive effects on the deep dorsal horn neurones.

This suggested postsynaptic mechanism of action of morphine is strengthened by the observation made by other workers that morphine decreased the excitation of the deep dorsal horn neurones by L-glutamate (Calvillo et al. 1974 and 1979; Dostrovsky and Pomeranz 1976; Belcher and Ryall 1978; Piercey et al. 1980). The neurones tested by these authors were recorded extracellularly and morphine and L-glutamate were applied iontophoretically in the vicinity of the cell body of the neurone tested. However, the depressive effect of morphine in these experiments was either potentiated or unaffected by naloxone iontophoresed at the same site (Belcher and Ryall 1978; Piercey et al. 1980). Furthermore, intravenous morphine failed to reduce the excitatory amino acid induced firing of these neurones (Dostrovsky and Pomeranz 1976; Piercey et al. 1980). These latter observations do not support the suggestion that morphine exerts a suppressive effect through a postsynaptic mechanism.

A presynaptic depressive effect of morphine on the noxious stimuli evoked activity in the deep dorsal horn neurones has also been suggested. For example, in decerebrate, spinal cord sectioned cats, morphine was applied iontophoretically near the terminals of cutaneous sural nerve Aδ- and C-fibres in the superficial laminae of the dorsal horn or administered systemically. The terminal excitability of these

fibres was decreased but the PAD of these fibres produced by stimulation of small diameter afferents of the superficial peroneal nerve was enhanced (Sastry 1978 and 1979). Sastry (1979) proposed that morphine decreased the transmission of impulses from small diameter primary afferents to dorsal horn neurones by an enhancement of the presynaptic inhibition that these fibres exert on each other. The decrease in excitability during morphine application was interpreted as being due to hyperpolarization of terminals and this was considered responsible for the observed increase in PAD.

SUPRASPINAL MEDIATED EFFECTS OF MORPHINE ON THE DEEP DORSAL HORN WDR NEURONAL ACTIVITY: The effects of morphine on the tonic descending inhibition of dorsal horn WDR neurones has already been considered. It is suffice to mention here that a decrease of the tonic inhibition by intravenous morphine had been suggested (Jurna and Grossman 1976; Duggan et al. 1980; but see Soja and Sinclair 1983b). This effect of morphine was suggested to be mediated by a supraspinal site of action of morphine (Jurna and Grossman 1976). However, other authors have suggested that morphine might activate descending pathways that inhibit noxious stimuli-evoked discharges in the deep dorsal horn WDR neurones. For example, Hanaoka et al. (1978) observed that intravenous morphine produced a greater percentage decrease of the nociceptive responses of the dorsal horn neurones during normal spinal cord conduction than when the spinal cord was transected. But, Soja and Sinclair (1983b) questioned these results and suggested that the difference in the

percentage inhibition of the dorsal horn neuronal activity by intravenous morphine in the intact vs transected state of the spinal cord might have resulted from differences in the control values for neuronal response in these different state of the spinal cord. They further suggested that the inhibitory effect of intravenous morphine (1.0 mg/kg) on the dorsal horn nociceptive activity could be explained by a spinal site of action of the drug.

Sinclair (1985) has investigated the effect of perfusing morphine between the third ventricle and the cisterna magna on the nociceptive activity of the dorsal horn neurones. Concentration of 10^{-5} M and 10^{-4} M morphine were without effect on the nociceptive activity of the dorsal horn neurones. Higher concentration of perfused morphine (10^{-3} M) resulted in an increase in the spinal cord dorsal horn neuronal responses to peripheral noxious stimuli. But this effect of morphine may have been nonspecific since it was not usually reversed by naloxone. The results of this study do not support the hypothesis that morphine attenuates nociceptive activity of the dorsal horn neurones through a supraspinal mechanism.

Other approaches have been used to test whether morphine attenuates spinal cord nociceptive transmission through a supraspinal site of action. Le Bars et al. (1976b) studied the effects of intravenous morphine (2.0 mg/kg) on the NRM stimulation-evoked inhibition of deep dorsal horn neuronal nociceptive activity in decerebrated cats. Morphine was found to be ineffective. Du et al. (1984) microinjected morphine (10-20 μg) into the NRM of anaesthetized cats while recording

the activity of deep dorsal horn neurones to noxious peripheral stimuli. The result was a suppression of noxious heat-evoked activity of the dorsal horn WDR neurones. The authors suggested that morphine activates a descending inhibitory system from the NRM to suppress nociceptive transmission at the spinal cord level. But the dose of morphine microinjected in the above experiment by Du et al. (1984) might not be relevant to antinociceptive mechanism. Clark et al. (1983) have calculated that microinjection of 10 µg of morphine at a brainstem site would yield morphine concentrations at a distance within 2 mm of the injection site which would be far in excess of concentrations necessary to produce antinociception by systemic administration of the drug.

Role of 5-HT in Morphine Action

There is evidence that brainstem sites, especially NRM and PAG may mediate morphine analgesia. For example, lesion of the NRM can block the increase in the tail-flick latency seen with systemic morphine (Proudfit and Anderson 1975; Yaksh et al. 1977). Microinjection of morphine into the NRM produced an increase in the threshold for nociception-induced vocalization in rats (Dickenson et al. 1979). These experiments suggest that the NRM might be involved in mediating the analgesic effects of morphine.

Also it is known that fibres from the NRM descend to the spinal cord and some of these fibres are serotonergic. These fibres may mediate NRM stimulation induced behavioral analgesia by release of 5-HT into the spinal cord (Hammond and Yaksh 1984). This, and also the fact

that NRM has a postulated role in morphine antinociception, has resulted in considerable research in investigating the role of 5-HT in morphine antinociception. Systemically injected morphine produced an increase in spinal dorsal horn levels of 5-hydroxyindole acetic acid (5-HIAA), a metabolite of 5-HT (Shiomi et al. 1978). Spinal transection blocked the elevation of 5-HIAA elicited by morphine below the level of lesion without altering the elevation above the lesion. This suggested that the increase in levels of 5-HIAA acid was due to the supraspinal action of morphine, possibly at the NRM locus which is the major source of spinal 5-HT. The increase in the spinal dorsal horn levels of 5-HIAA might be due to increased turnover of 5-HT by morphine. It is possible that increase release of 5-HT in the spinal cord dorsal horn by morphine might mediate its antinociceptive action. Microinjection of morphine into the NRM of the rats produced antinociception which could be attenuated by depletion of spinal cord 5-HT with 5,7-dihydroxytryptamine (Vasko et al. 1984). In the presence of intrathecal monoamine uptake blockers, amitriptyline, desipramine and sertraline, subthreshold doses of parenteral morphine produced antinociception (Taiwo et al. 1985). Depletion of 5-HT by PCPA pretreatment or intrathecal administration of the putative 5-HT antagonist, methysergide, prevented the antinociceptive effect of subthreshold dose of morphine in presence of uptake blockers. When given parenterally, fluoxetine, a selective 5-HT uptake blocker, also potentiated the analgesic effect of parenteral morphine in rat tail-flick test (Larson & Takemori 1977). Depletion of 5-HT via systemic pretreatment with PCPA (Tenen 1968) or

intraventricular 5,6-DHT (Vogt 1974) antagonized the increase in tail-flick latencies produced by systemic morphine. However, intrathecal methysergide, in the absence of any 5-HT uptake blockers, failed to block the increase in tail-flick latency produced by systemic morphine (Proudfit and Hammond 1981).

Pharmacology of Fluoxetine and Pargyline

Serotonin released in synaptic transmission is mainly inactivated by high affinity uptake into presynaptic terminals (Shasken and Snyder 1970). Brain synaptosomes have been effectively used for studying the uptake mechanism of 5-HT and its pharmacological manipulation. For example, Wong et al. (1974), using rat brain synaptosomes, have studied the effect of fluoxetine on 5-HT uptake in this preparation. Fluoxetine was found to produce an inhibition of 5-HT uptake in the synaptosomal preparation. The uptake inhibition was found to be selective for 5-HT. The inhibitor constant (K_i) of fluoxetine was 5×10^{-8} M for 5-HT in contrast to 1.5×10^{-5} M and 1.0×10^{-5} M for dopamine and noradrenaline, respectively. Fuller et al. (1975) compared equal doses of several 5-HT uptake blockers with fluoxetine, for their relative abilities to antagonize the depletion of 5-HT in the rat brain synaptosomes by 4-chloroamphetamine (CPA). Fluoxetine completely antagonized the action of CPA whereas chlorimipramine and imipramine had no effect. Further, fluoxetine antagonized the H75/12 induced depletion of 5-HT but not noradrenaline. In, in vivo experiments in rats,

fluoxetine decreased 5-HIAA levels in the brain which is suggestive of 5-HT uptake blockade (Fuller and Perry 1974). This effect of fluoxetine occurred in the absence of any inhibition of monoamine oxidase system. Further, fluoxetine has no affinity for serotonergic, alpha-adrenergic, muscarinic and histaminergic receptors (Wong et al. 1983) and therefore its uptake block of 5-HT might not be mediated through any receptors.

In vivo uptake block of 5-HT in the rats by fluoxetine has been suggested because of fluoxetine's ability to decrease 5-HIAA concentration in brain, antagonize serotonin depletion of the brain by CPA treatment and potentiate effects of the serotonin precursor (Fuller 1982).

Fluoxetine has been used in analgesic experiments. For example, Messing et al. (1975) studied the effect of fluoxetine on foot shock thresholds in rats. It was observed that, in rats pretreated with fluoxetine, the threshold currents required to produce a jump response was elevated compared to control animals. The analgesic effect of fluoxetine in tail-jerk test has also been reported (Hynes et al. 1985). In contrast, fluoxetine had no significant effect on hot plate reaction times in rats (Malec and Langwinski 1980). All three groups, however, demonstrated a potentiating effect of fluoxetine on morphine-induced antinociception. Fluoxetine produced this effect without altering the affinity of morphine for its receptor (Hynes et al. 1985). This is consistent with the hypothesis that an endogenous serotonergic system might play an important role in morphine

antinociception.

Pargyline is an irreversible inhibitor of monoamine oxidase (Taylor et al. 1960). It produces a rapid and long-lasting accumulation of monoamines in the brain (Spector et al. 1960; Pletscher et al. 1961). Further, it has been reported to produce a rapid accumulation of monoamine, especially 5-HT, in the cat spinal cord (Anderson et al. 1967). This effect of pargyline on spinal 5-HT levels is suggested to be responsible for the enhanced spinal cord monosynaptic reflex in cats (Sinclair and Sastry 1974). Whether pargyline has any analgesic action is not known. It is also not clear whether pargyline treatment can potentiate the antinociceptive effect of fluoxetine. However, the potentiation of fluoxetine effect on body temperature by treatment with a monoamine oxidase inhibitor (MAOI) has been demonstrated in rabbits (Sinclair and Lo 1977). The authors suggested that this was due to greatly enhanced 5-HT activity due to neuronal uptake block by fluoxetine in MAOI pre-treated animals compared to a lesser effect of fluoxetine on 5-HT activity in animals not pre-treated with MAOI.

METHODS AND MATERIALS

Surgical Procedures

These experiments were carried out in a Faraday radio frequency shielded room on adult cats of either sex. The animals were initially anaesthetized with a 3% halothane (Fluothane®)/oxygen mixture which was delivered at a rate of four L/min via a gas anaesthetic machine (Ohio). To monitor blood pressure, a carotid artery was cannulated with a polyethylene tubing (No. 160) which was prefilled with dilute sodium heparin (Upjohn Co., Canada).

A cephalic vein was cannulated with No. 90 polyethylene tubing prefilled with saline for intravenous administration of drugs. In every experiment, α-chloralose (60 mg/kg, i.v., Sigma Chemical Co.) was injected over a 10 min period while the halothane was slowly reduced to a concentration of 1.0% and maintained during surgery. Adequacy of anaesthesia was determined by a lack of sudden blood pressure or pupillary diameter change during noxious stimulation. Supplementary doses of chloralose were administered as required. The trachea was exposed and cannulated with a ribbed polyethylene Y-shaped hose connector for artificial respiration.

The animals were firmly secured to a stereotaxic animal frame (Narashigi Scientific Instruments Labs.), the hindlimbs were shaved and fastened with gypsum to an adjustable wooden platform with the toe pads up.

The lumbosacral region of the spinal cord was carefully exposed by performing a laminectomy on the L_1-S_1 segments. The dura mater was cut

and pinned back to surrounding tissue in a hammock-like fashion, gently suspending the spinal cord. In some experiments the left L_7 dorsal root was isolated and mounted intact on a silver bipolar hook electrode which was connected to one channel of an Ortec (Model 4600 Series) or a Grass (SD-9 Model) stimulator. A small hole was made in the skull above the cerebellum through which the supraspinal stimulation electrode was inserted.

The animals were paralyzed with gallamine triethiodide (Flaxedil®, Poulenc) and artificially respired using a respirator (Harvard Apparatus). End-tidal CO_2 levels were continuously monitored and maintained between 3.5 and 4.5% using a calibrated medical CO_2 gas analyzer (Beckman, Model LB-2). A bilateral pneumothorax was routinely performed so as to minimize respiration-induced spinal cord movement during recording. The entire preparation was isolated from the floor by inflating the supporting table (N_2 40 psi, Zero-G Isolation Table). This "floating" table minimized inherent floor vibrations from being transmitted through the animal frame and microcarrier to the recording electrode.

Stimulation, Recording and Testing of Dorsal Horn Neurones

Using a stereoscope (Olympus) and a pair of fine watchmaker's forceps, a small hole was carefully made in the pia mater at the left L_7 dorsal root entry zone. The recording microelectrode (glass or carbon fibre) was secured to a hydraulic microdrive holder (David Kopf Instruments), which in turn was mounted onto a fine adjusting electrode

carrier (Narashigi). The electrode was then placed perpendicular to the spinal cord surface over the pial opening and lowered by remote control in 10 µm steps.

The signal picked up by the recording microelectrode was fed into a high impedance preamplifier (M707, W.P. Instruments), amplified and subsequently displayed on a split beam storage oscilloscope (Tektronix, Model 5111). The output of the oscilloscope was fed to a window discriminator, audio monitor and spike integrator, whose output, in turn, was connected to a polygraph (Grass 79D) and microcomputers (Rockwell Aim-65, Apple II plus).

A search stimulus (1.5 V, 0.1 ms, 1.0 Hz) was applied to the L_7 dorsal root. Neurones encountered were classified according to their response to natural forms of stimuli and generally, they fell into two categories. One type responded to light mechanical stimulation, such as touch or hair movement of the receptive field. A second type responded to these stimuli and, in addition, they responded with a sustained discharge to a maintained pinch or a 10-15 sec pulse of noxious radiant heat. These neurones were term low threshold mechanoreceptive (LTM) and WDR neurones, respectively. The latter group of neurones were used in this study. Noxious heat was applied using a quartz halogen projector lamp focused on the receptive field of WDR neurones studied. The noxious temperature was set at 45-55°C and applied for 10-15 sec. A miniature thermocouple, placed on the receptive field was used to feed back control the temperature applied. In all experiments noxious radiant heat was applied automatically using a microcomputer at two

minute intervals. The temperature and duration of noxious radiant heat was carefully adjusted for each neurone so as to evoke reproducible responses.

Supraspinal Stimulation

A bipolar electrode was used to stimulate the NRM. The stereotaxic co-ordinates chosen were, AP = -7, DV = -10 and L = 0. The electrode was inserted in the sagittal plane at an angle of 14° from vertical. The nucleus was stimulated by pulse trains of 333 msec length at 100 Hz for 25 sec. The stimulation intensity was adjusted to produce about 50% inhibition of the neuronal nociceptive activity. The stimulation voltage used in the study ranged from 2.5 v to 7.0 v. In all experiments NRM stimulation was applied automatically, using a micro-computer, at four minute intervals; the onset being at the start of the heat pulse.

Preparation of Recording Microelectrodes

Microelectrodes were constructed from glass capillaries with 1.5 mm outside diameter (Glass Co. of America, Omega Dot Brand). They were pulled on a vertical electrode puller (Narashigi). Under a microscope the tip diameters were broken back to 1.0 - 1.5 µm and the electrode was then filled by capillary action with a 4 M NaCl solution. Most electrodes used had a DC resistances of 15 megohms or less.

Carbon fibre electrodes were prepared according to the method of Armstrong-James and Millar (1979). A carbon fibre of suitable length

was inserted into a glass capillary (as used above) and pulled on the vertical electrode puller. The micropipette formed had several centimeters of carbon fibre protruding from the tip. Under a light microscope, the fibre was trimmed and etched in chromic acid at 0.12 mA current so that only 15 µm of carbon fibre protruded from the micropipette. The micropipette was then filled with 4 M NaCl or saline solution (0.9%) and used for extracellular recording.

Computer Programmes

A microcomputer (Rockwell Aim-65) was programmed to receive the output from the ratemeter. In addition, outputs from the microcomputer were used to trigger the noxious radiant heat stimulator and the supraspinal stimulation apparatus. These triggering pulses were applied every two minutes to the noxious radiant heat stimulator and every four minutes to the supraspinal stimulation apparatus. The computer counted and printed the number of neuronal action potentials 25 sec before and 25 sec after the onset of each stimulus (file name = SINCL).

In addition, the Aim-65 microcomputer triggered an Apple II plus computer 25 sec before the heat pulse. This latter computer also received an input directly from the ratemeter and counted the number of action potentials for 55 sec beginning 25 sec before the onset of the heat pulse (file name = New.TS). These data were stored on discs. A program was available which allowed for the averaging of several responses (file name = New.GTS).

Pharmacological Studies

Experiments were designed to test whether 5-HT was involved in mediating the NRM stimulation produced inhibition of the deep dorsal horn WDR neurones. One WDR neurone was studied per animal. The supraspinal stimulation was adjusted so as to produce approximately 50% inhibition of evoked neuronal activity to heat. Drugs were administered after stable control responses to heat and heat plus supraspinal stimulation had been achieved. The involvement of 5-HT in the NRM phasic inhibition was investigated either by the administration of fluoxetine or pargyline. The dose of fluoxetine used was 6.0 mg/kg. This dose of fluoxetine enhances the spinal cord monosynaptic reflex in cats, an effect believed to be due to an enhanced level of synaptic 5-HT (Sastry and Sinclair 1976). The drug was administered by intravenous infusion over a period of 13 minutes to avoid any marked change in the blood pressure. The dose of pargyline used in this study was 30.0 mg/kg. This dose of pargyline is known to increase the spinal cord levels of 5-HT (Anderson et al. 1976) which is likely responsible for the enhanced monosynaptic reflex in cats (Sinclair & Sastry 1974). Again, this drug was infused slowly over a period of 30 minutes to avoid changes in the blood pressure of the cats. The effect of drug treatment on the NRM phasic inhibition was followed for a period of one hour. In the experiment with pargyline, fluoxetine was administered one hour after the start of the pargyline infusion. This protocol was followed to study whether, in presence of both fluoxetine and pargyline, the change in the NRM phasic inhibition was more than observed with administration

of fluoxetine or pargyline alone. The effect of drug treatment on the spontaneous activity and heat evoked activity of the neurones was also studied in these experiments.

Experiments were also designed to study the effect of morphine treatment on the NRM phasic inhibition and the heat-evoked activity of the deep dorsal horn WDR neurones. The experimental design was as mentioned above. Morphine was administered in doses of 0.5 mg/kg, 0.5 mg/kg and 1.0 mg/kg to a cumulative dose of 2.0 mg/kg. The first dose of 0.5 mg/kg of morphine was administered after stable controls to heat and heat plus supraspinal stimulation had been achieved. The next doses of morphine were administered after stable neuronal responses to heat and heat plus supraspinal stimulation in presence of the preceding doses were seen.

Morphine, 2.0 mg/kg cumulative dose, was also administered to fluoxetine treated cats to test whether 5-HT was involved in mediating the effect of morphine on heat-evoked activity and on NRM phasic inhibition of WDR dorsal horn neurones. Morphine was administered in these experiments 15 min after a second dose (6.0 mg/kg) of fluoxetine. The experimental protocol was as above.

Finally, control experiments were also done in which the effect of infusion of drug vehicle on the NRM phasic inhibition and heat-evoked activity of the deep dorsal horn WDR neurones was studied. In some experiments the NRM phasic inhibition was tested on different levels of excitability of the dorsal horn neurones. In these experiments the neurones were first excited by noxious heat at a temperature of 45°C

applied to their receptive fields. The NRM stimulus intensity was adjusted to produce approximately 50% inhibition of heat-evoked activity. The neurones were then excited by an application of noxious heat at a temperature of 52°C and the extent of the NRM phasic inhibition was observed without altering the NRM stimulus intensity.

On completion of the experiments the animals were killed using an intravenous injection of saturated potassium chloride solution. A lesion was then made at the supraspinal stimulation site by passing 1 mA of direct anodal current for 15 sec. The medullary region of the brain was then excised and stored in formalin for at least 48 hrs. The tissue was later sectioned using a cryostat (Damon/IEC Division) and slide-mounted for histological verification of the stimulation site.

Calculations

The data in these experiments were quantified using a microcomputer (Rockwell Aim-65) which was programmed to count cell discharges 25 sec before and after the onset of noxious radiant heat. The number of extracellular action potentials recorded 25 sec before the onset of noxious stimulus was designated as the "spontaneous activity" of the recorded cell and the cell discharge count recorded 25 sec from the onset of the noxious stimulus was designated as the "noxious heat-evoked" activity of the cell. Inhibition of noxious heat-evoked activity by the NRM stimulation was calculated as percentage of control response to noxious heat in absence of any NRM stimulation. The spontaneous activity, noxious heat-evoked activity and the inhibition of

nociceptive cell activity to NRM stimulation for each animal were calculated as percentage of control (where control was equated to 100%) for different time points and used either to plot a time course curve or averaged to plot histograms as illustrated in the Results section.

Statistical analysis of the observations during different time periods was made using two way analysis of variance followed by Newman-keuls test for comparisons of means (using "U.B.C. GENLIN" which is a general least square analysis of variance program). Absolute values of observations were used except where noted otherwise. The analysis is discussed in detail for Fig. 10 (page 72 to 75).

RESULTS

The experiments in this study were done on dorsal horn neurones characterized as WDR neurones. They could be excited by innocuous touch or brushing of the receptive field and also by noxious pinch or noxious radiant heat. The extracellular action potentials recorded were usually very large indicating that the recording was from the cell body region or a proximal dendrite of the neurone. The depth at which such recordings were made ranged from 1400 μm to 2300 μm from the surface of the spinal cord. Although actual histological verification of the spinal cord laminar corresponding to this electrode depth range was not made in this study, previous experience in our laboratory indicates that this depth range corresponded to the spinal laminae IV-VI. The results of various experiments on these neurones are detailed below.

Fig. 1 consists of oscilloscope traces illustrating the response of WDR neurone to noxious radian heat (A) and NRM phasic inhibition (B). A heat pulse of 50°C is indicated by the solid bars. Nucleus raphe magnus stimulation is indicated by the broken lines in B. It is clear that, the response of the neurone to noxious radiant heat was inhibited by NRM, stimulation.

Serotonin Involvement in NRM Stimulation-Produced Inhibition

EFFECT OF FLUOXETINE

Experiments with fluoxetine were done on seven neurones, one neurone per animal. Fig. 2 is comprised of computer generated

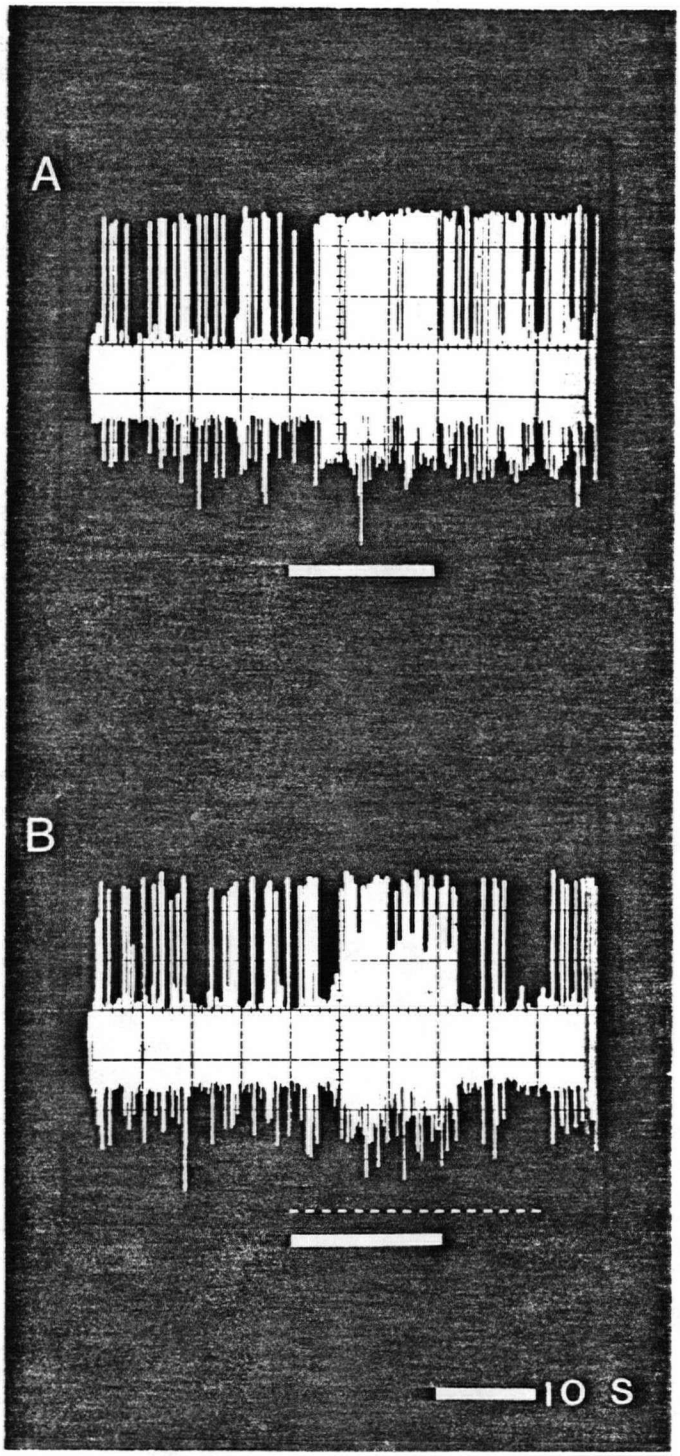

FIG: 1

Oscilloscope trace of neuronal response to noxious radiant heat and noxious heat plus nucleus raphe magnus stimulation. The noxious heat was applied during the time period indicated by the bar and the nucleus raphe was stimulated during the period indicated by broken line. The increase in the noxious heat evoked firing of the neurone (indicated in trace A) was considerably reduced by concomitant stimulation of the nucleus raphe (trace B).

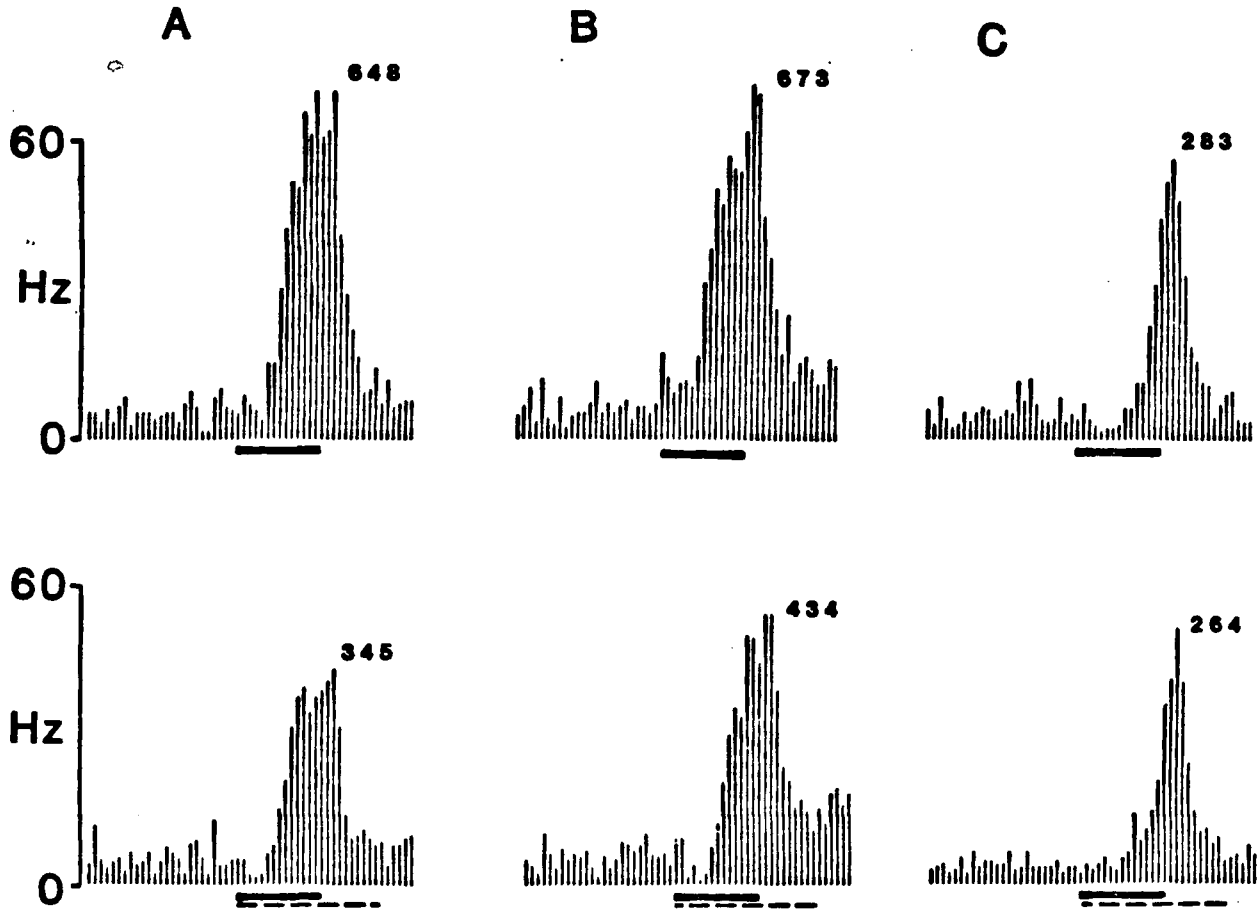

FIG: 2

Computer generated histograms, each representing the neuronal discharge over 55 sec around a noxious heat pulse. In each histogram, noxious heat was applied over the period indicated by the continuous bar and the nucleus raphe magnus was stimulated during the broken line. Each histogram was compiled from 4 consecutive noxious heat pulses. Heat was applied at a temperature of 55° C for 15 sec. The numbers at the upper right position are the number of discharges of the cell over 25 sec from the onset of the heat pulse. The nucleus raphe magnus was stimulated at 100 Hz , 2.8 v in trains of 333 msec for 25 sec. Panel A represents the control response of neurone in absence of drug treatment. Panel B represents the response of the neurone in the presence of fluoxetine (6.0 mg/kg, i.v.). Fluoxetine reduced the inhibition of neuronal discharge by nucleus raphe stimlation. Panel C represents the effect of two cumulative dose of morphine (1.0 mg/kg total dose, i.v.). Morphine decreased the discharge of the neurone to heat and to heat in presence of nucleus raphe stimulation.

histograms typically obtained from neurones used in this study. Each histogram illustrates the neuronal discharge over 55 sec around a noxious heat pulse (indicated by the bar). The NRM was stimulated during the broken lines. Each histogram is compiled of four consecutive noxious heat pulses. Panel A represents the control response of a neurone in the absence of drug treatment whereas panel B represents the response of the neurone in the presence of fluoxetine (6.0 mg/kg, i.v.). The number at the upper right position of each histogram is the average number of neuronal discharges over 25 sec from the onset of the heat pulse. It is apparent from these numbers that fluoxetine treatment had little or no effect on the response of the neurone to noxious heat whereas it attenuated the inhibition of the nociceptive activity produced by the NRM stimultion. The mean response of seven neurones to fluoxetine is illustrated in Figs. 3, 4 and 5. Fig. 3 is a graph illustrating the time course of fluoxetine on the NRM phasic inhibition. A statistically significant decrease in the NRM phasic inhibition was observed at times 4, 20 and 28 minutes after the start of fluoxetine infusion. However, these points were not different from each other. The noxious heat-evoked activity (Fig. 4) and the spontaneous activity of these neurones (Fig. 5) were not changed by fluoxetine treatment.

EFFECT OF PARYGLINE

The effect of pargyline (30.0 mg/kg, i.v.) infused over 30 minutes on the NRM stimulation is illustrated in Fig. 6. Pargyline produced a

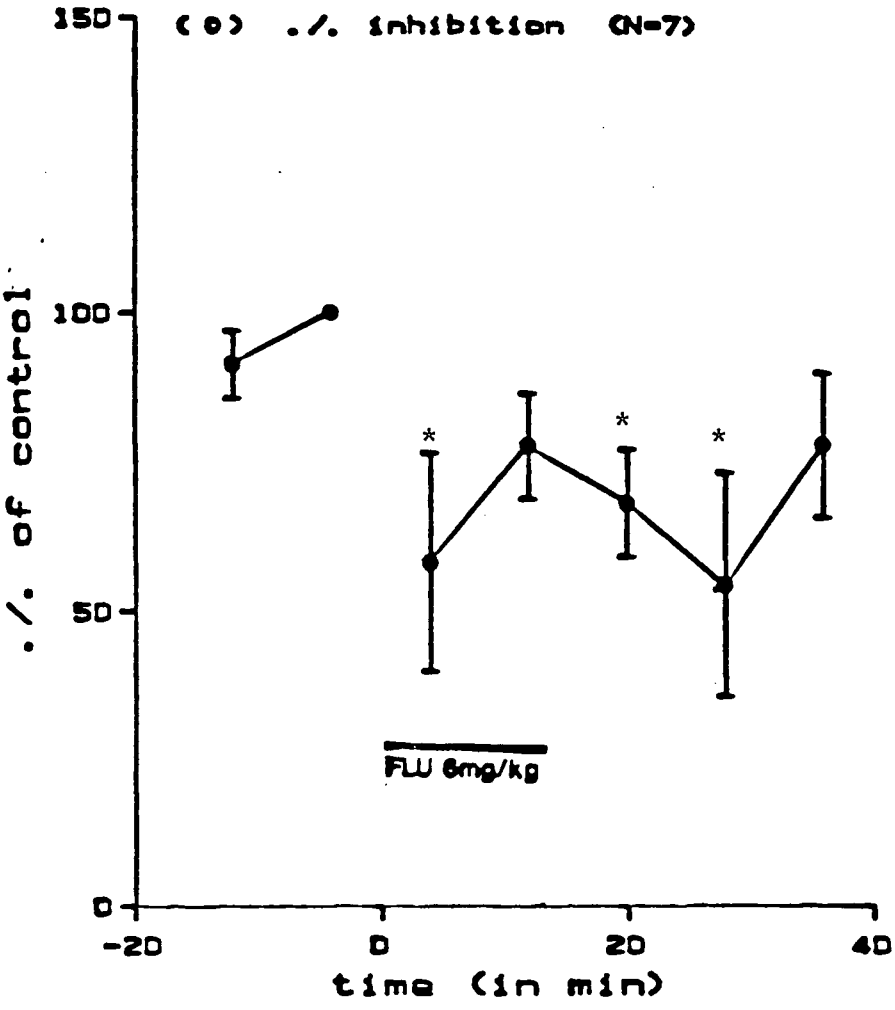

FIG: 3

The effect of the fluoxetine (6.0 mg/kg, i.v.) on the nucleus raphe magnus stimulation-produced inhibition of the nociceptive activity of wide dynamic neurones. The drug was infused over the time period indicated by the bar. The value at time four minutes before the start of the infusion (or -4 min) was equated to 100%. Values before and after time -4 min were calculated based on this figure. Each point represents the mean ± S.E.M., n=7. The inhibition at time + 4, + 20 and + 28 min was found to be significantly decreased from the 100% control (* $P < 0.05$)

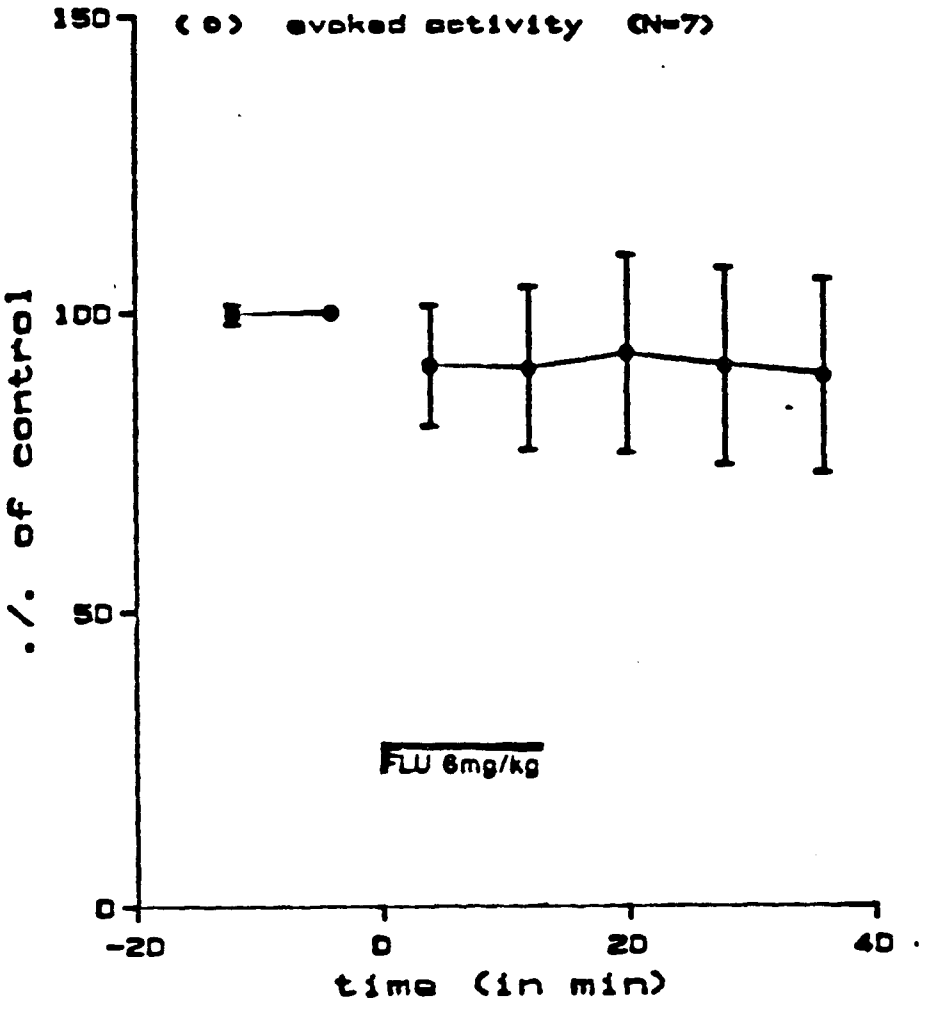

FIG: 4

Lack of effect of intravenous infusion of fluoxetine (6.0 mg/kg, i.v.) on the noxious heat evoked-activity of wide dynamic range neurones. The drug was infused over the time period indicated by the bar. Data are expressed as in Fig.3. Each point represents the mean ± S.E.M., n=7.

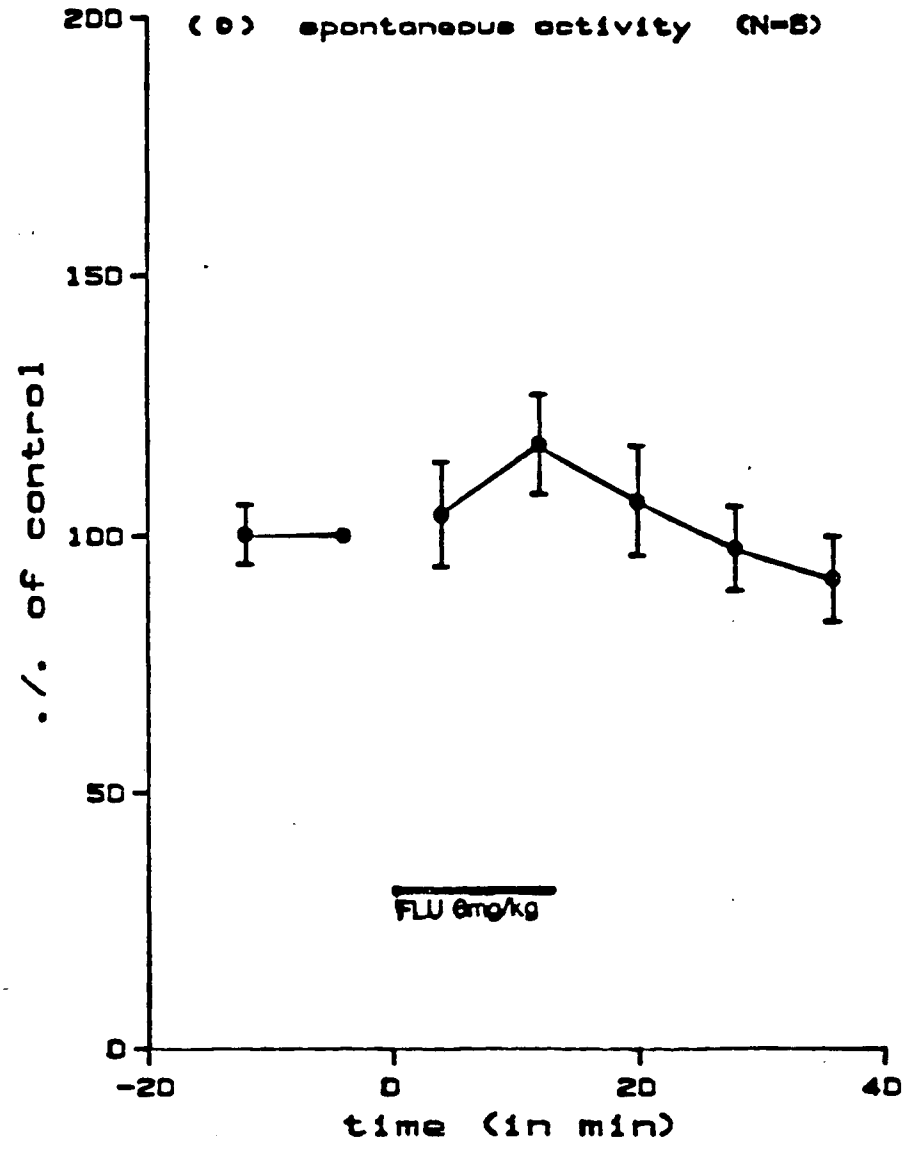

FIG: 5

Lack of effect of intravenous infusion of fluoxetine (6.0 mg/kg, i.v.) on the spontaneous activity of wide dynamic range neurones. The drug was infused over the time period indicated by the bar. Data are expressed as in Fig.3. Each point represents the mean ± S.E.M., n=6.

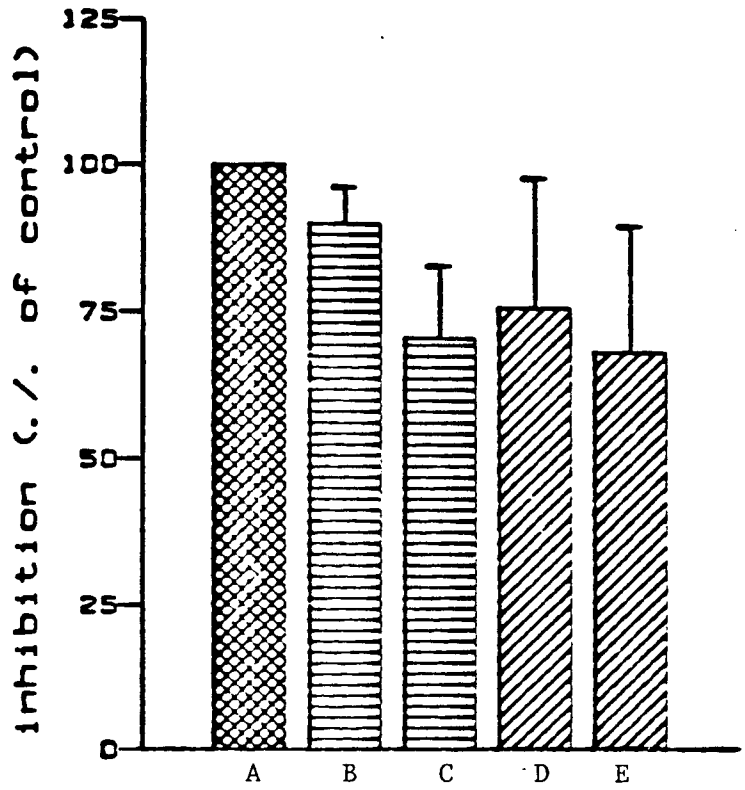

FIG: 6

The effect of pargyline (30.0 mg/kg, i.v.) and pargyline plus fluoxetine (6.0 mg/kg, i.v.) on the nucleus raphe magnus stimulation-produced inhibition of the dorsal horn neuronal activity. Pargyline was infused over a time period of 30 min. Thirty minutes later fluoxetine was infused over a time period of 13 min. Bar A represents the mean inhibition between the time period 12 and 4 min before the start of pargyline infusion. This value was equated to 100% and the values of subsequent bars were calculated based on this figure. Bar B represents the mean inhibition during the pargyline infusion whereas bar C represents the mean inhibition up to 26 min following pargyline infusion. Bar D represents the mean inhibition during the fluoxetine infusion and the bar E represents the mean inhibition in the next 24 min. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B *, A-C *, A-D *, A-E *, B-C *, C-D, and C-E. N=4.

time-dependent decrease in the NRM phasic inhibition. The decrease in the NRM phasic inhibition with pargyline in the time period of up to 20 minutes post-infusion was more than the decrease observed for the NRM phasic inhibition during pargyline infusion. Pargyline also produced a time-dependent increase in the noxious heat-evoked activity of these neurones such that the increase in the post-infusion period was more than observed during pargyline infusion (Fig. 7). Little or no effect of pargyline was seen on the spontaneous activity of these neurones (Fig. 8).

EFFECT OF PARGYLINE PLUS FLUOXETINE

In these experiments, animals treated with pargyline were then administered fluoxetine. Fluoxetine was administered about one hour after pargyline administration. Administration of fluoxetine did not produce any further change in the NRM phasic inhibition compared to the inhibition with pargyline alone in the post infusion period although it was significantly less compared to the 100% control value (Fig. 6). However, fluoxetine increased the noxious radiant heat-evoked activity of the neurones (Fig. 7). The increase observed with fluoxetine was statistically significantly different from the mean of the evoked activity observed with pargyline during the infusion and the post-infusion time period as well as from pre-drug control value. The change in evoked activity with fluoxetine during the infusion and post-infusion time period was no different from each other.

Fluoxetine administered to pargyline treated animals produced a

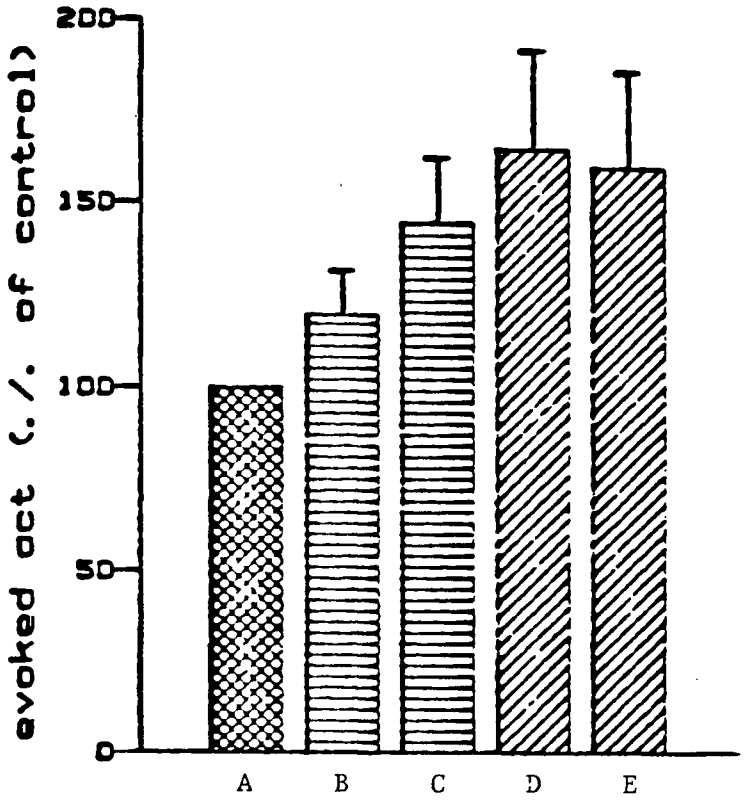

FIG: 7

The effect of pargyline (30.0 mg/kg, i.v.) and pargyline plus fluoxetine (6.0 mg/kg, i.v.) on the noxious heat-evoked activity of dorsal horn wide dynamic range neurones. Data are expressed as indicated in Fig. 6. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B *, A-C *, A-D *, A-E *, B-C *, C-D * and D-E. N=4.

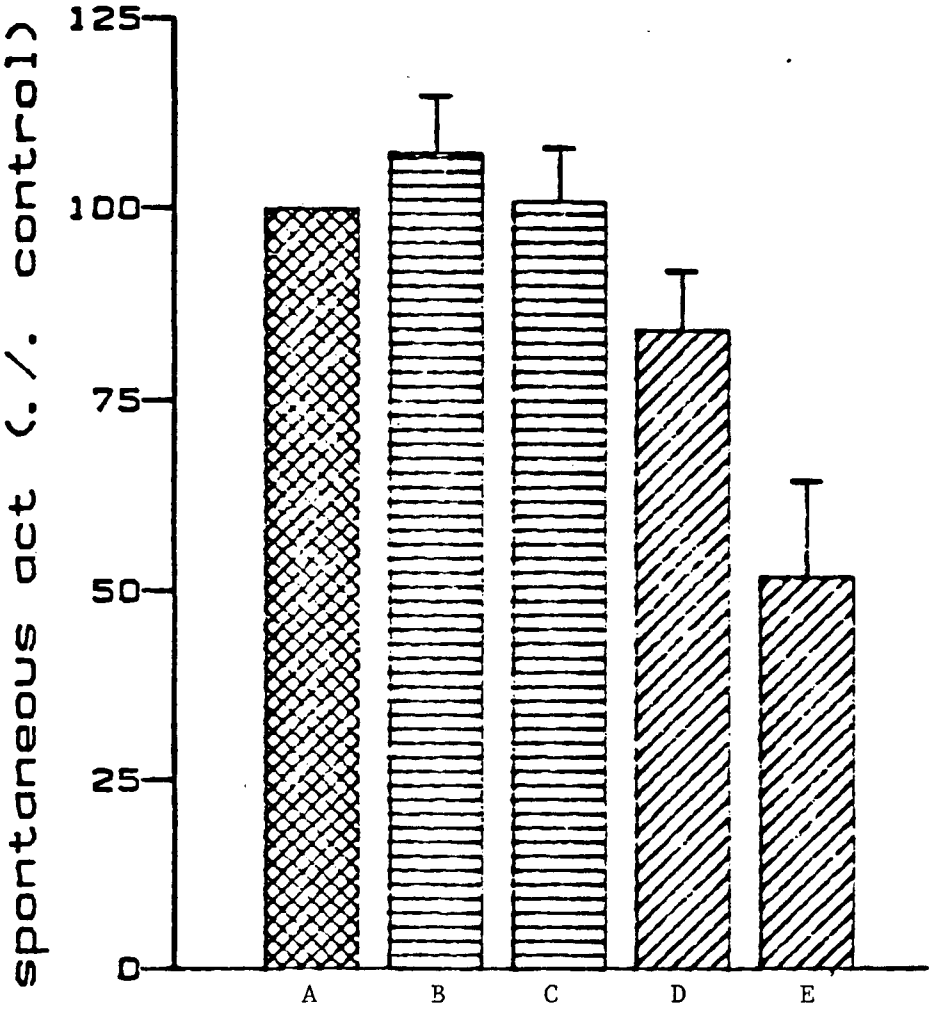

FIG: 8

The effect of pargyline (30.0 mg/kg, i.v.) and pargyline plus fluoxetine (6.0 mg/kg, i.v.) on the spontanous activity of dorsal horn neurones. Data are expressed as indicated as in Fig. 6. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B, A-C, A-D *, A-E *, D-E *, C-D *, and C-E *. N=4.

decrease in the spontaneous activity of the neurones studied (Fig. 8). The decrease was statistically significant from the pre-drug control values and from pargyline treated values. This effect of fluoxetine in pargyline treated animals was also time-dependent in that the mean spontaneous activity in the fluoxetine post-infusion time period was significantly less than the mean spontaneous activity during the infusion of fluoxetine.

Effect of Morphine

Morphine was given in three divided doses of 0.5 mg/kg, 0.5 mg/kg and 1.0 mg/kg, respectively to a cumulative dose of 2.0 mg/kg. The results of the experiments are illustrated in Figs. 9, 10 and 11.

The effect of morphine on the inhibition of nociceptive activity by the NRM stimulation was inconsistent in that it either increased or decreased the NRM phasic inhibition of the cell and no statistically significant change in either direction was found. The increase in inhibition was observed in 3 neurones and the inhibition was decreased in 2 neurones. The maximum increase in inhibition was observed with a morphine cumulative dose of 2.0 mg/kg. This increase ranged from 113 to 145 percent of the pre-drug control value. The maximum decrease in inhibition was also seen with a morphine cumulative dose of 2.0 mg/kg. The decreases observed in these 2 neurones were -35 (i.e., rather than observing an inhibition, a facilitation of noxious heat-evoked activity was observed upon NRM stimulation) and 50 percent of the pre-drug control value. The grouped data on these five neurones is illustrated in Fig. 9.

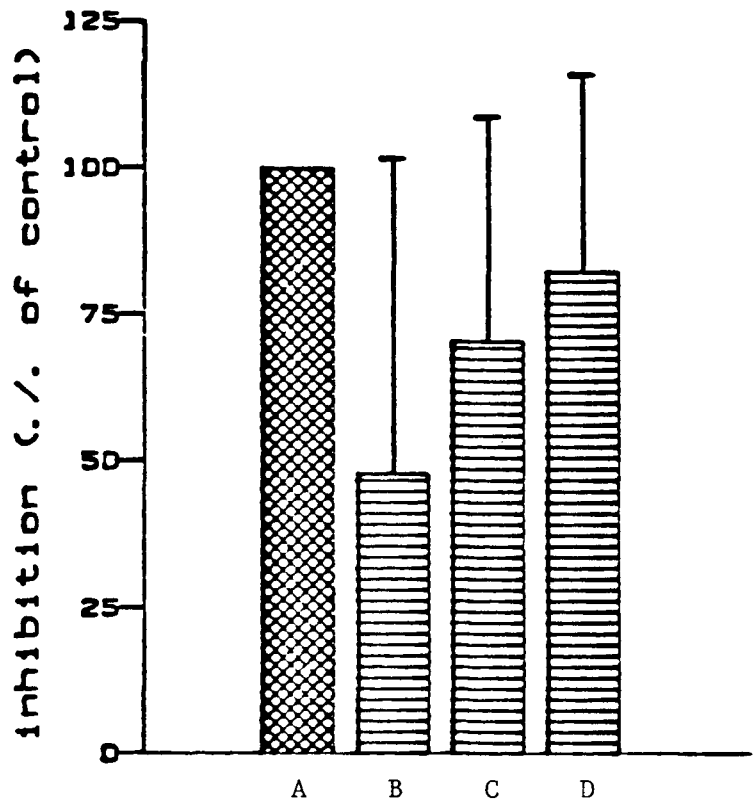

FIG: 9

Lack of effect of morphine (2.0 mg/kg total dose, i.v.) on the nucleus raphe magnus stimulation-produced inhibition of nociceptive activity of dorsal horn neurones. Morphine was infused in three cumulative doses of 0.5, 0.5, and 1.0 mg/kg respectively . Bar A represents the mean inhibition between the time period 12 and 4 min. before start of morphine infusion. This value was equated to 100./.. Values represented by bars following this were calculated based on this figure. Bars B, C and D represents the mean inhibition, between the time period 14 to 22 min, 10 to 22 min and 6 to 14 min post-infusion, to the three cumulative doses of morphine respectively. N=5.

Morphine produced a decrease in the heat-evoked activity of the neurones (Fig. 10). The decrease was observed to be dose-dependent with a decrease observed with a dose of 0.5 mg/kg of morphine and a maximal decrease of evoked activity with morphine cumulative dose of 2.0 mg/kg.

On visual inspection of the Fig. 10 the dose-dependent effect of cumulative doses of morphine is not apparent. This is because Fig. 10 is based on the average of noxious heat-evoked activity and its S.E.M. of 5 neurones, from different animals, which exhibit considerable animal to animal variation. However, use of analysis of variance effectively allows removal of animal to animal variation and permits examination of treatment effects on a given animal for which measured activity is quite stable for a given treatment. For example, Table 1, animal 1, treatment 1 (control) the mean activity is 1124.5 and standard deviation of 87.64. In the same animal for treatment 2 (morphine 0.5 mg/kg), the value was 699.33 with a standard deviation of 57.49. Thus, these standard deviations are reasonable and the same is seen for all treatments within this animal and with different animals. However, when a given treatment is compared for animals 1 to 5 considerable animal to animal variation in the mean of heat-evoked activity is observed. Table 2 shows that when observations are averaged for all 5 animals, there is a dose-dependent effect of morphine treatment. This is shown by the four subsets of the Newman-keuls test.

Morphine also decreased the spontaneous activity of the neurones (Fig. 11). The decrease in spontaneous activity by morphine was

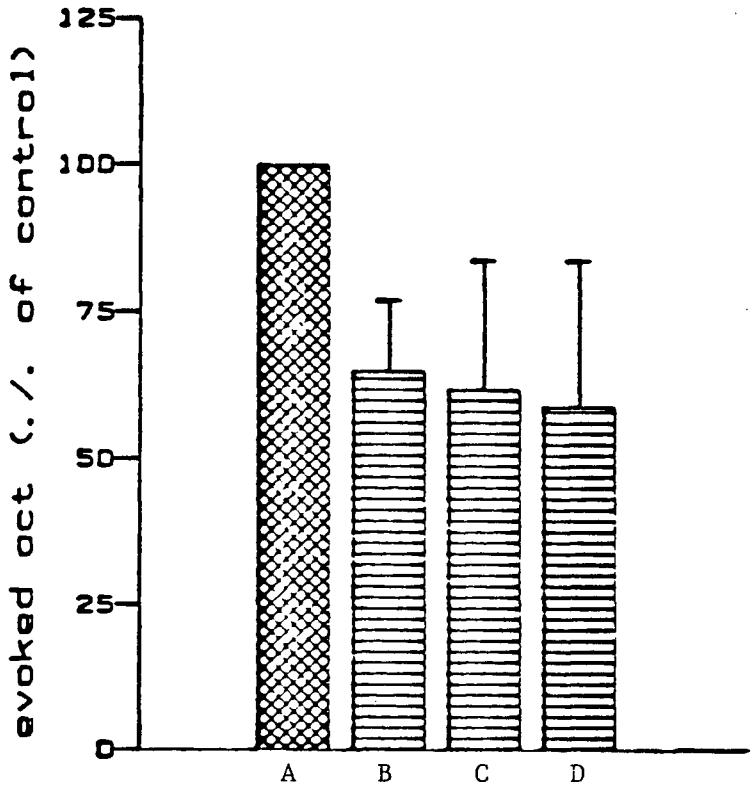

FIG: 10

The effect of morphine (2.0 mg/kg total dose, i.v.) on the noxious heat-evoked activity of dorsal horn neurones. Morphine was administered in three cumulative doses as in Fig. 9. Data are expressed as indicated in Fig. 9. Morphine administration decreased the heat-evoked activity of the neurones. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B *, A-C *, A-D *, B-C *, B-D *, and C-D *. N=5.

Table 1

Portion of analysis of variance for effect of morphine on noxious heat-evoked activity of individual dorsal horn neurones at different time periods

Frequencies, means, standard deviations for A*DDT

	1[a]	2[a]	3[a]	4[a]	5[a]
.1[b]	6[n]	6[n]	6[n]	6[n]	6[n]
0 MEAN	1124.5	480.50	1269.5	249.50	1384.0
0 STDV	87.644	40.004	59.288	62.788	9.2952
.2[b]	6[n]	6[n]	6[n]	6[n]	6[n]
0 MEAN	699.33	501.00	503.67	102.67	1067.7
0 STDV	57.497	76.407	40.849	34.448	75.197
.3[b]	6[n]	8[n]	8[n]	6[n]	8[n]
0 MEAN	388.83	715.12	508.50	92.167	676.00
0 STDV	18.519	70.516	32.196	36.706	73.644
.4[b]	6[n]	6[n]	6[n]	6[n]	12[n]
0 MEAN	272.33	760.17	459.50	89.000	551.50
0 STDV	65.534	66.189	47.627	31.279	78.151

Multiple range tests

Newman-Keuls test at 5% probability level
There are 10 homogeneous subsets which are listed as follows:

(4 4, 4 3, 4 2)

(4 1, 1 4)

(1 3)

(3 4, 2 1, 2 2, 3 2, 3 3)

(2 1, 2 2, 3 2, 3 3, 5 4)

(5 3, 1 2, 2 3)

(1 2, 2 3, 2 4)

(5 2, 1 1)

(3 1)

(5 1)

N.B.: a signifies animals such that 1^a indicates 1st animal, 2^a means 2nd animal and so on.

b signifies treatment such that 1^b is control, 2^b is response to morphine 0.5 mg/kg, 3^b is response to morphine cumulative dose of 1.0 mg/kg and 4^b is response to 2.0 mg/kg cumulative dose of morphine.

n signifies number of observations made.

Table 2

Portion of analysis of variance and Newman-keuls test of significance for morphine effect on heat-evoked response averaged for five dorsal horn neurones

	30^n	30^n	36^n	36^n
0 MEAN	901.60	574.86	502.30	447.33
0 STDV	462.60	323.73	225.45	226.73

Multiple range tests

Newman-Keuls test at 5% probability level

There are 4 homogeneous subsets which are listed as follows:

(.4)

(.3)

(.2)

(.1)

Time for multiple range test was 0.64974E-02 seconds. Cumulative time is 0.17889 seconds.

N.B.: n signifies number of observations made.

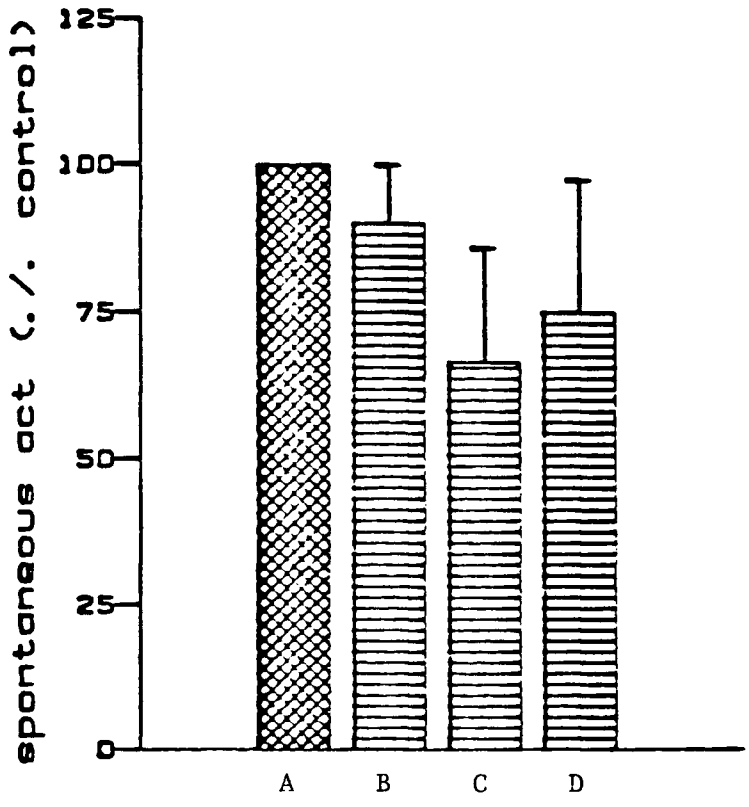

FIG: 11

The effect of morphine (2.0 mg/kg total dose, i.v.) on the spontaneous activity of dorsal horn neurones. Morphine was administered as in Fig. 9. Data are expressed as indicated in Fig. 9. Morphine decreased the spontaneous activity of the neurones. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B *, A-C *, A-D *, B-C *, B-D *, and C-D. N=5.

observed with a dose of 0.5 mg/kg and was maximal with the cumulative dose of 1.0 mg/kg.

Serotonin Involvement in Morphine Action

In these experiments, after obtaining stable neuronal responses to fluoxetine, the animals were administered morphine in three cumulative doses as described in the Material and Methods section. The rationale was to study whether enhanced serotonergic synaptic transmission in the presence of fluoxetine affected the neuronal response to morphine. A result typical of this study is illustrated in Fig. 2 panel C. Morphine, when administered in a cumulative dose of 1.0 mg/kg to fluoxetine treated animals, reduced the NRM phasic inhibition of the neurones nociceptive activity. The grouped data for five neurones tested in this manner is illustrated in Fig. 12. Morphine, in fluoxetine treated animals, produced a statistically significant reduction of the NRM phasic inhibition at all dose levels when compared to the NRM phasic inhibition in the presence of fluoxetine alone or with the pre-drug control. A decrease in inhibition was observed with the morphine dose of 0.5 mg/kg which was the same as observed with morphine cumulative dose of 1.0 mg/kg, and the maximal decrease was with the cumulative dose of 2.0 mg/kg.

The variation, particularly with the morphine cumulative dose of 2.0 mg/kg, is due in part to the method of expressing the data as percentage of control. The same data plotted as the actual percent inhibition is shown in Fig. 13. The values for the above figures of the individual neurones are reported in Table 3. Normally, converting the

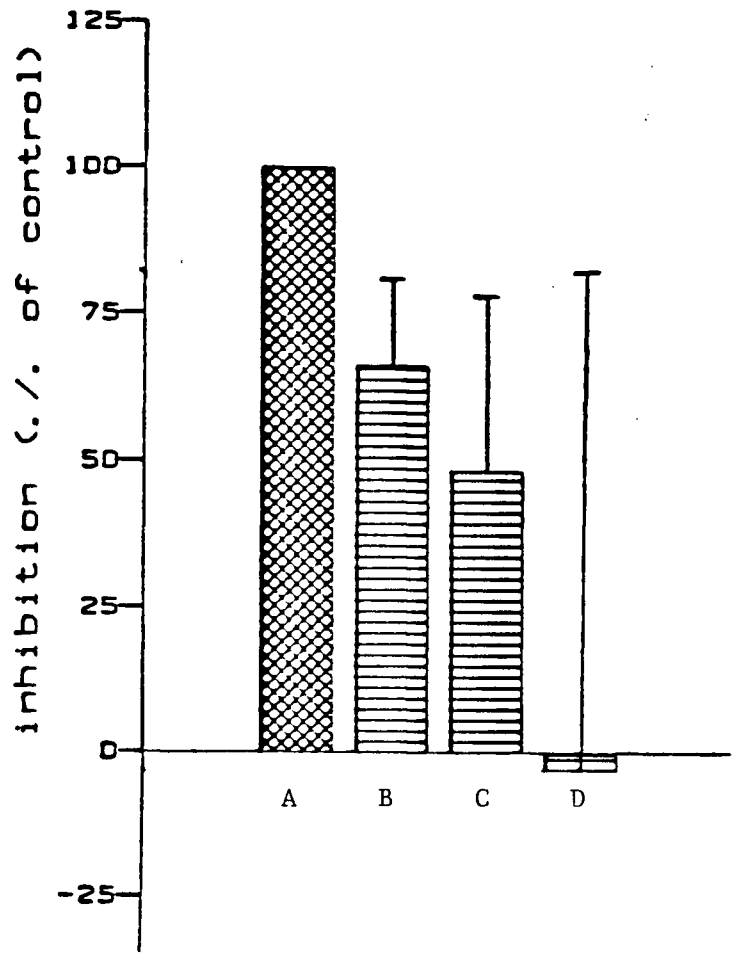

FIG: 12

The block of nucleus raphe magnus stimulation-produced inhibition by morphine (2.0 mg/kg total dose, i.v.). Morphine was administered as in Fig. 9. At the time of infusion of the first dose of morphine the animals had already been treated with fluoxetine (6.0 mg/kg. i.v.). Bar A represents the mean inhibition to fluoxetine between time 10 and 4 min before the start of morphine infusion. This value was equated to 100 %. Bars B, C and D represent the mean inhibition, between the time periods indicated in Fig. 9, to the three cumulative doses of morphine respectively. Each of the three bars is represented as % of the fluoxetine control. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B *, A-C *, A-D *, B-C, B-D *, and C-D *. N=5.

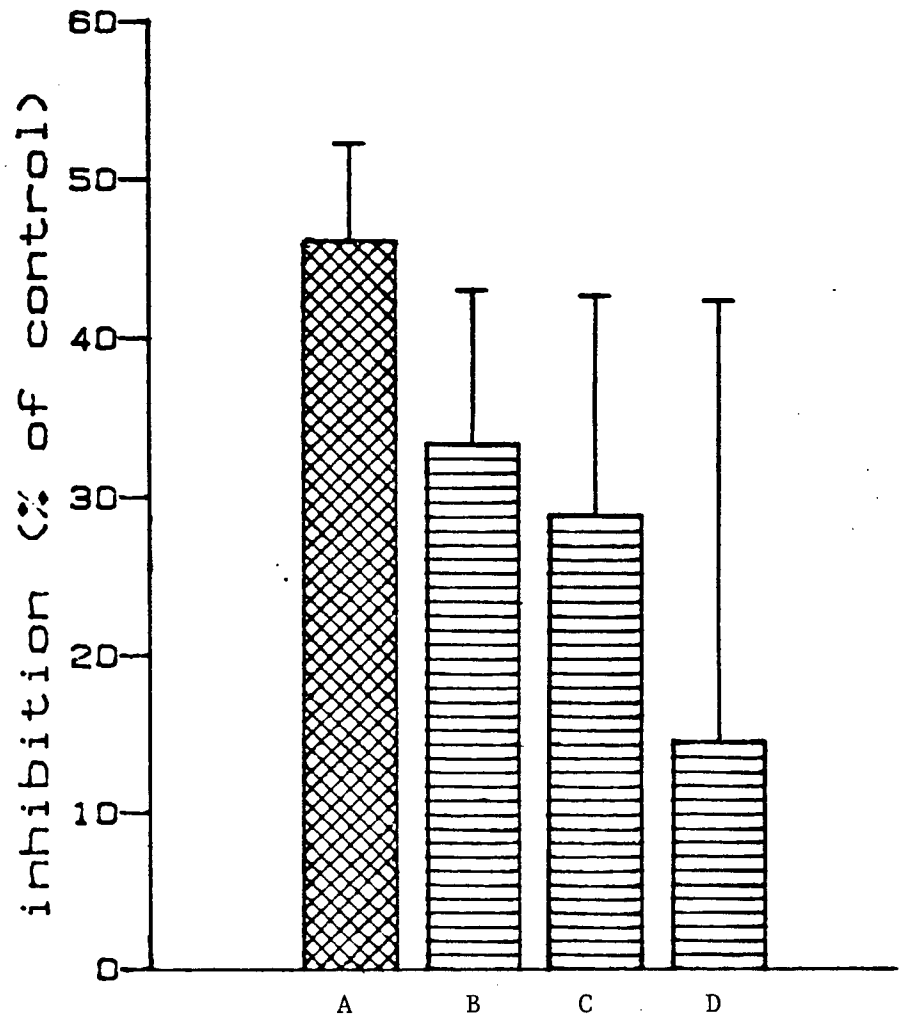

FIG: 13

The effect of morphine (2.0 mg/kg total dose, i.v.) on the nucleus raphe magnus stimulation-produced inhibition of dorsal horn neuronal nociceptive activity. Morphine was administered as in Fig. 9 to fluoxetine pretreated animals. The data plotted is same as in Fig. 12, except in this Fig. actual percentage inhibition are plotted. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B *, A-C *, A-D *, B-C, B-D *, and C-D *. N=5.

Table 3

Effect of morphine on the NRM phasic inhibition of dorsal horn neuronal nociceptive activity in fluoxetine pretreated animals

Neurone	% Inhibition to NRM Stimulation of a Noxious Heat Evoked Activity							
	Control		Morphine Cumulative Doses (0.5 mg/kg		(1.0 mg/kg		(2.0 mg/kg	
	-10 to -2 min		+14 to +22 min		+10 to +22 min		+6 to +14 min	
1	35.4	(100)	14.6	(41.4)	11.5	(32.5)	16.2	(45.7)
2	61.4	(100)	48.7	(79.3)	52.9	(86.2)	50.2	(81.7)
3	27.8	(100)	7.6	(27.2)	-17.1	(-61.3)	-107.0	(-332.4)
4	51.7	(100)	57.5	(111.3)	51.6	(99.9)	39.5	(76.4)
5	53.8	(100)	38.4	(71.3)	45.4	(84.4)	59.4	(110.3)
Mean ± SEM	46.0 ± 6.2	(100 ± 0.0)	33.9* ± 9.6	(66.1 ± 14.2)	28.9* ± 13.7	(48.3 ± 29.7)	14.5* ± 27.7	(-3.6 ± 82.8)

N.B.: Control values represent the response of neurones to fluoxetine prior to infusion of morphine and is averaged for time period on the table. The averaged response of neurones to different cumulative doses of morphine for the time period mentioned following drug infusion are also given above. The values in brackets represent the inhibition expressed as percent of control (with pre-morphine response equated to 100%) whereas non-bracketed values are actual percent inhibitions. Asterisk indicates a significant difference of the morphine response from the control. When the response to morphine was compared with each other, the change with morphine cumulative dose of 0.5 mg/kg and 1.0 mg/kg was identical but different from change seen with dose of 2.0 mg/kg.

data to percent of control decreased the apparent variation. However, in this case it was increased. Converting the actual percent inhibition to percent of control caused an amplification of changes observed due to drug treatment, the amplification being greatest for the neurone having the smallest control percent inhibition (i.e., neurone 3). This neurone also showed the greatest change in the inhibition following morphine treatment. Thus, the variation shown in Fig. 12 is exceedingly large.

Morphine, in fluoxetine treated animals produced a dose-dependent decrease in the noxious heat-evoked activity compared to pre-drug control values or with the fluoxetine control (Fig. 14). This group was also compared with the group where the effect of morphine alone was determined on the noxious heat-evoked activity. This was done to determine whether enhanced 5-HT synaptic transmission, in the presence of fluoxetine, affected the inhibitory action of morphine on the neuronal nociceptive activity. Since this was an unpaired comparison it was necessary to normalize morphine effects in both the groups. This was done by equating the control pre-drug response in both the groups to 100%. The effect of the three cumulative doses of morphine in both the test groups was normalized to their respective 100% control and then compared statistically.

When thus compared, the effect of morphine at all three dose levels on neuronal nociceptive activity in fluoxetine treated groups was not statistically different from the effect of the three cumulative doses of morphine alone. Morphine in fluoxetine treated animals also produced a dose-dependent decrease in spontaneous activity of the neurones when compared to their paired controls (Fig. 15).

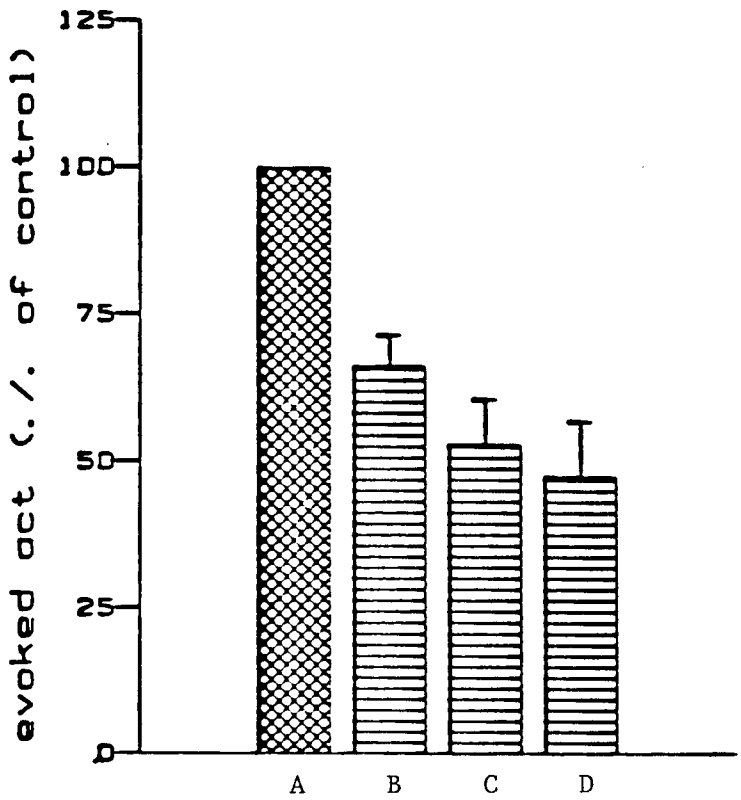

FIG: 14

The inhibitory effect of morphine (2.0 mg/kg, i.v.) on noxious heat-evoked activity of dorsal horn neurones. Morphine was administered as in Fig. 9. At the time of infusion of the first dose of morphine the animals had already been treated with fluoxetine (6.0 mg/kg, i.v.). Data are expressed as indicated in Fig. 12. The inhibition of evoked activity by morphine was dose-dependant. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B *, A-C *, A-D *, B-C *, B-D *, and C-D *. N=5.

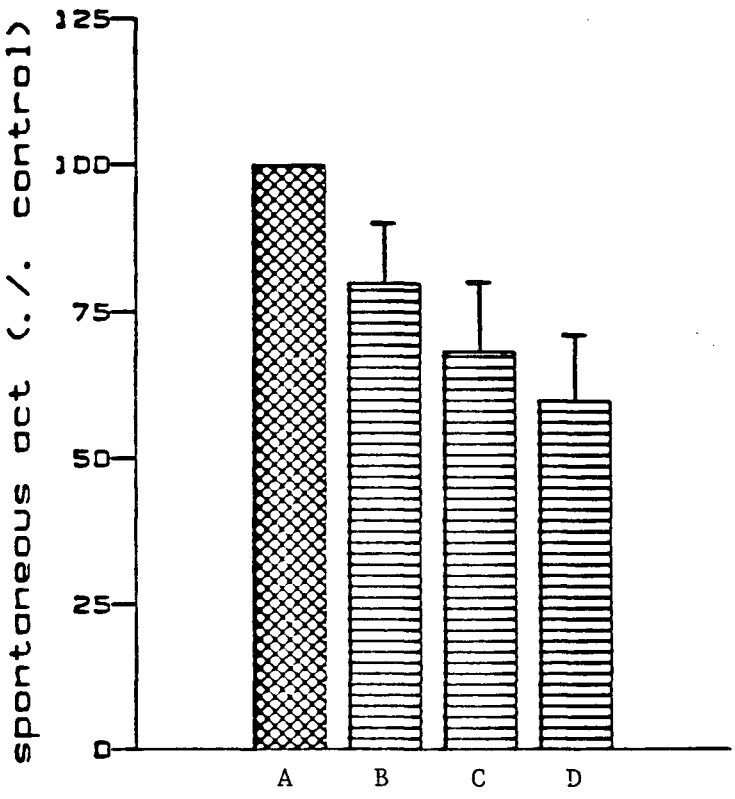

FIG: 15

The inhibition of spontaneous activity of dorsal horn neurones by morphine (2.0 mg/kg total dose, i.v.). Morphine was administered as in Fig. 9. At the time of infusion of the first dose of morphine the animals had already been treated with fluoxetine (6.0 mg/kg, i.v.). Data are expressed as indicated in Fig. 12. The decrease of spontaneous activity by morphine from the fluoxetine control was dose-dependent. The following comparisons were made and * indicates a significant difference ($P < 0.05$): A-B *, A-C *, A-D *, B-C *, B-D *, and C-D *. N=5.

Control Experiments

In these experiments the response of WDR neurones to heat and heat plus NRM stimulation was studied with the infusion of the drug vehicle (distilled water). The results of these experiments are illustrated in Figs. 16, 17 and 18. The NRM phasic inhibition, evoked activity and the spontaneous activity of the neurones was not affected by infusion of the vehicle.

Control experiments were also designed to test whether changing the excitability of neurones affected the NRM phasic inhibition of the cell activity. The results are illustrated in Fig. 19. The heat-evoked response of the neurones at 52°C (986.12 ± 179.37) was more than the response at 45°C (634.01 ± 141.96). This increase in cell activity at 52°C vs 45°C was statistically significant. The NRM phasic inhibition showed a decline with the increasing cell activity. The mean % inhibition at 52° was 46.79 ± 16.04 compared to 56.79 ± 16.04 at 45°C. This decrease in the NRM phasic inhibition was also statistically significant.

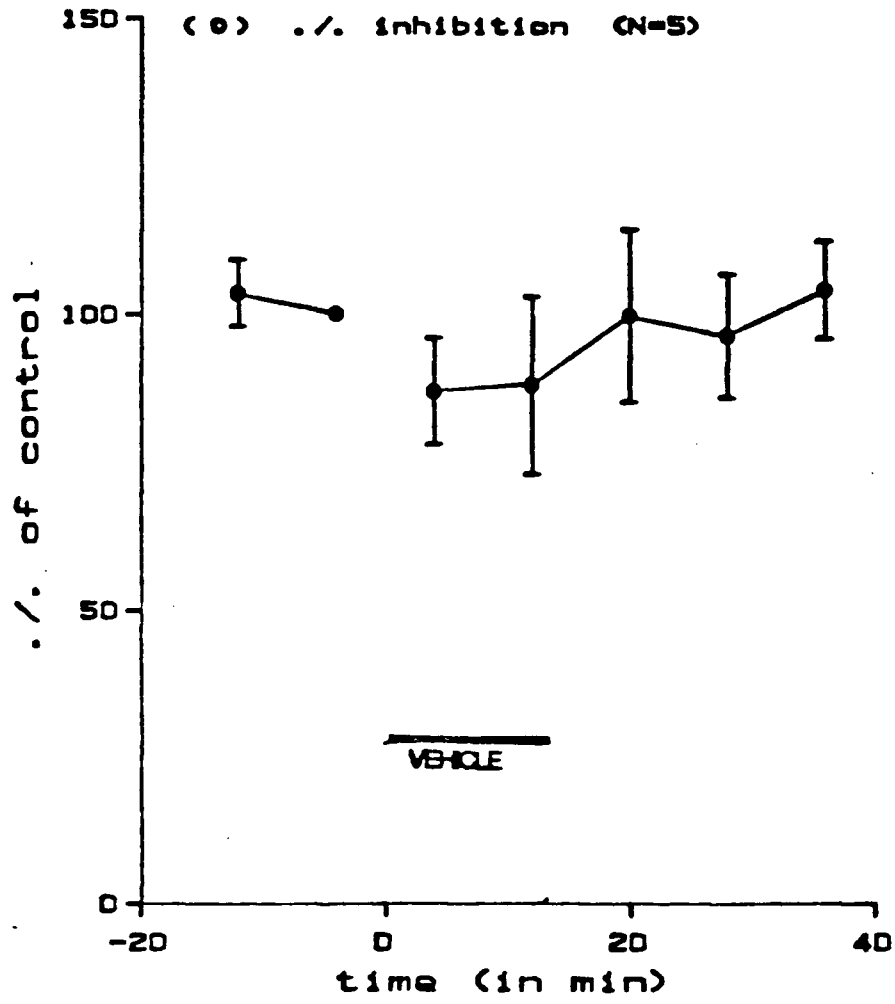

FIG: 16

The effect of the vehicle (distilled water, 1.5 ml/kg, i.v) on the nucleus raphe magnus stimulation-produced inhibition of the nociceptive activity of the dorsal horn neurones. The vehicle was infused over the time period indicated by the bar. The value at time four minutes before the start of the infusion (or -4 min) was equated to 100%. Values before and after time -4 min were calculated based on this figure. Each point represents the mean ± S.E.M.,n=5. The inhibition at each point was found not to be different from the 100% control.

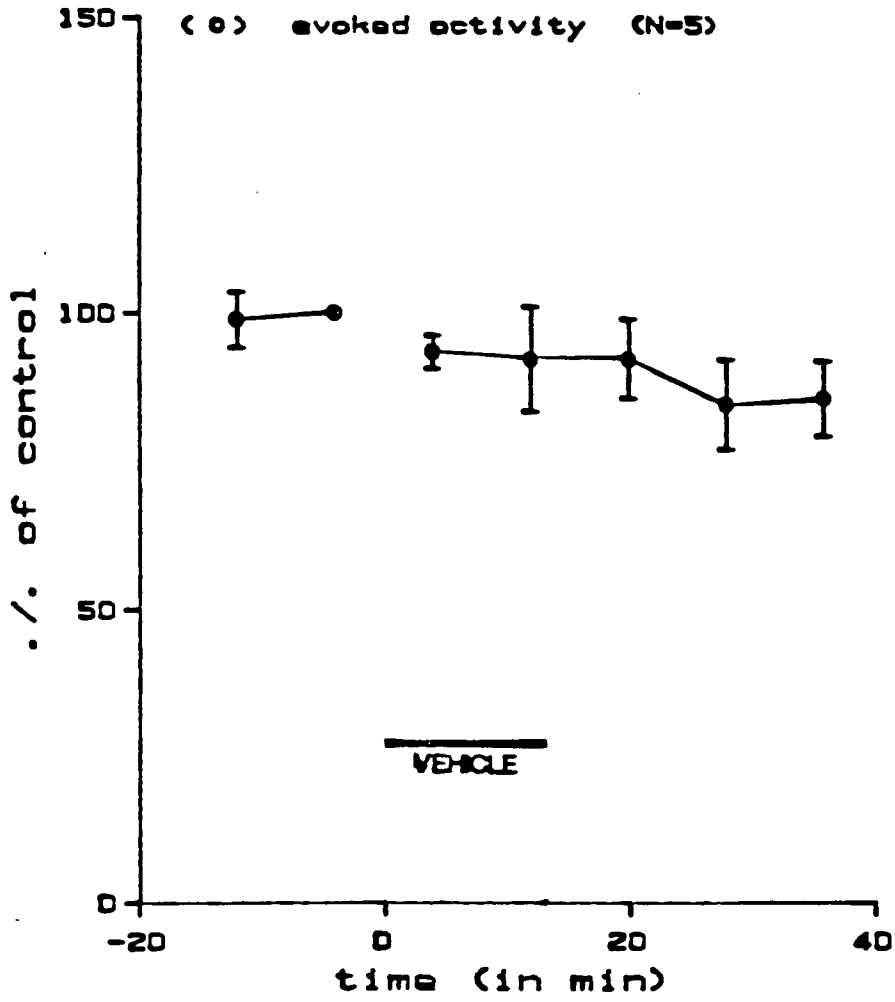

FIG: 17

Lack of effect of intravenous infusion of the vehicle on the noxious heat-evoked activity of the dorsal horn neurones. The vehicle was infused over the time period indicated by the bar. Data are expressed as in Fig. 15. Each point represents the mean ± S.E.M., n=5.

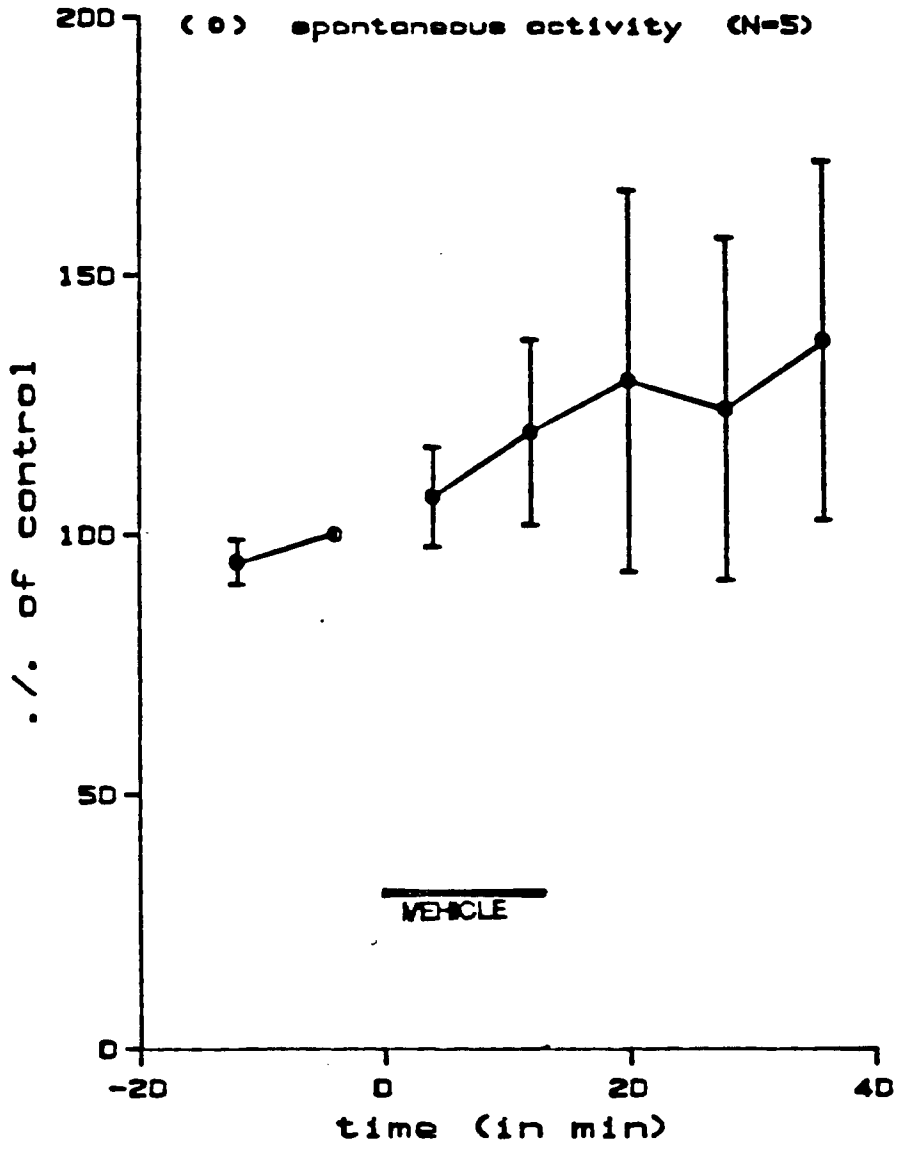

FIG: 18.

Lack of effect of intravenous infusion of vehicle on the spontaneous activity of dorsal horn neurones. The vehicle was infused over the time period indicated by the bar. Data are expressed as in Fig. 15. Each point represents the mean ± S.E.M., n=5.

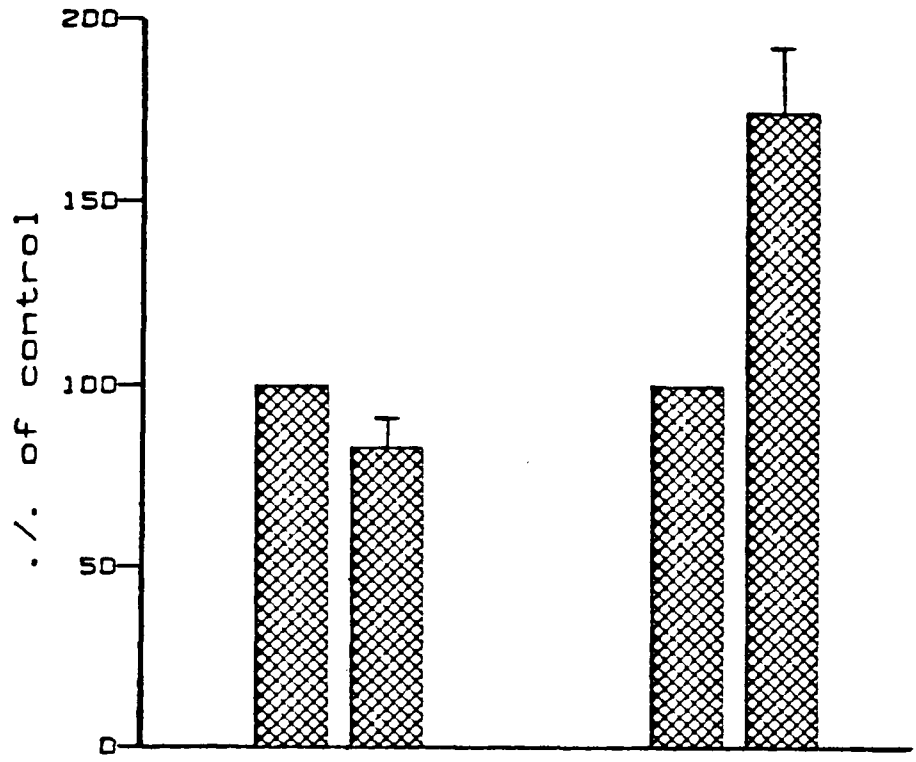

FIG: 19

A comparison of the percentage inhibition of noxious heat-evoked activity of dorsal horn neurones with noxious heat applied at 45° C and 52° C. The pair of histograms on the left compare the percentage inhibition at the same stimulus intensity of the nucleus raphe magnus whereas the pair on the right compares the heat-evoked activity. The mean inhibition at 52° C was significantly decreased compared to the inhibition at 45° C and the heat-evoked response at 52° C was significantly increased compared to the reponse at 45° C ($P < 0.05$). N=6.

DISCUSSION

Involvement of 5-HT in the NRM Phasic Inhibition of Dorsal Horn Neurones

It has been speculated that 5-HT might mediate, at least partly, the NRM phasic inhibition of deep dorsal horn WDR neurones. To test this hypothesis, experiments were designed to enhance synaptic transmission of 5-HT in the cat spinal cord using fluoxetine, a 5-HT uptake blocker, and pargyline, a monoamine oxidase inhibitor. The rationale of such a design was that, under conditions of enhanced spinal 5-HT synaptic transmission, the NRM phasic inhibition of dorsal horn neurones, if mediated by 5-HT, should be increased. However, treatment with fluoxetine and pargyline, contrary to the expressed speculation produced a decrease in the NRM phasic inhibition of deep dorsal horn WDR neurones. The involvement of serotonin in this drug effect and the possible implication for the role of 5-HT in the NRM stimulation-produced inhibition of the deep dorsal horn WDR neurones is discussed below.

There is both biochemical and functional evidence to suggest that fluoxetine and pargyline can enhance 5-HT synaptic transmission. Relevant biochemical evidence has been mentioned earlier in the text. Briefly, fluoxetine is reported to be a very selective uptake blocker of 5-HT in rat synaptosomes and only in very high doses does it block the uptake of noradrenaline (NA) and dopamine (DA; Wong et al. 1974; Fuller et al. 1975). Similarly, pargyline is reported to markedly increase 5-HT, but not NA levels, in the cat spinal cord (Anderson et al. 1967).

This evidence would suggest that the drugs, fluoxetine and pargyline, would have a greater effect on serotonergic systems as compared to the other monoaminergic systems. Sinclair and Sastry (1974) have provided evidence that the bulbospinal inhibition of the cat spinal monosynaptic reflex (MSR) is under an inhibitory influence of a 5-HT system. In this study, imipramine, which preferentially blocks the uptake of 5-HT, antagonized the bulbospinal inhibition of the MSR and the effect of imipramine was blocked in 5-HT depleted animals. Pargyline and fluoxetine, administered systemically and in same doses used in the present study, also reduced the bulbospinal inhibition of the MSR in cats (Sinclair and Sastry 1974; Sastry and Sinclair 1976). By extrapolating from the above evidence it can be postulated that administration of either fluoxetine or pargyline to the experimental animals in this study would have resulted in enhanced 5-HT synaptic transmission. Since both drug treatments resulted in an antagonism of the NRM phasic inhibition of the deep dorsal horn WDR neurones, this drug effect might be due to the increased 5-HT synaptic activity.

There is other supporting evidence which does not suggest the idea that 5-HT mediates the NRM phasic inhibition of the deep dorsal horn WDR neurones in the cat. For example, Griersmith et al. (1981) have reported that the putative 5-HT antagonist, methysergide, administered either intravenously or by iontophoresis in the region of SG, failed to reduce the NRM stimulation-produced inhibition of nociceptive activity of dorsal horn neurones. Similarly, Belcher et al. (1978) failed to observe a reversal of the NRM phasic inhibition of dorsal horn neuronal

nociceptive activity by methysergide iontophoresed in the vicinity of the neuronal cell body.

The neuronal circuitry upon which fluoxetine and pargyline act to exert the effects reported in this study is not known. Hypothetical neuronal circuits are proposed in Figs. 20 and 21 to explain the mechanism of the NRM phasic inhibition.

The NRM stimulation-produced inhibition of nociceptive activity of the deep dorsal horn WDR neurones may occur by several mechanisms: (1) It may operate via a direct inhibitory input onto the deep dorsal horn neurones. Descending fibres from the NRM have been observed to make soma-dendritic contacts in the deep dorsal horn laminae V-VII (Light and Kavookjian 1985). (2) The nociceptive afferent drive to deep dorsal horn WDR neurones may also be reduced by an inhibition of an interneurone in the superficial laminae which may be part of the excitatory polysynaptic afferent input onto these deep dorsal horn neurones. Consistent with this idea, soma-dendritic synaptic contacts made by descending NRM fibres have also been observed in the superficial laminae of spinal cord dorsal horn in cats (Light and Kavookjian 1985; Ruda et al. 1981). (3) Presynaptic inhibition of nociceptor primary afferent terminals or interneurones in the afferent pathway produced by the NRM stimulation would also reduce the nociceptive drive to the deep dorsal horn WDR neurones. Martin et al. (1979) reported that stimulation of the NRM in cats resulted in primary afferent depolarization (PAD) of afferent terminals of Aδ-nociceptors. Finally, (4) NRM stimulation may excite interneurones in the superficial laminae

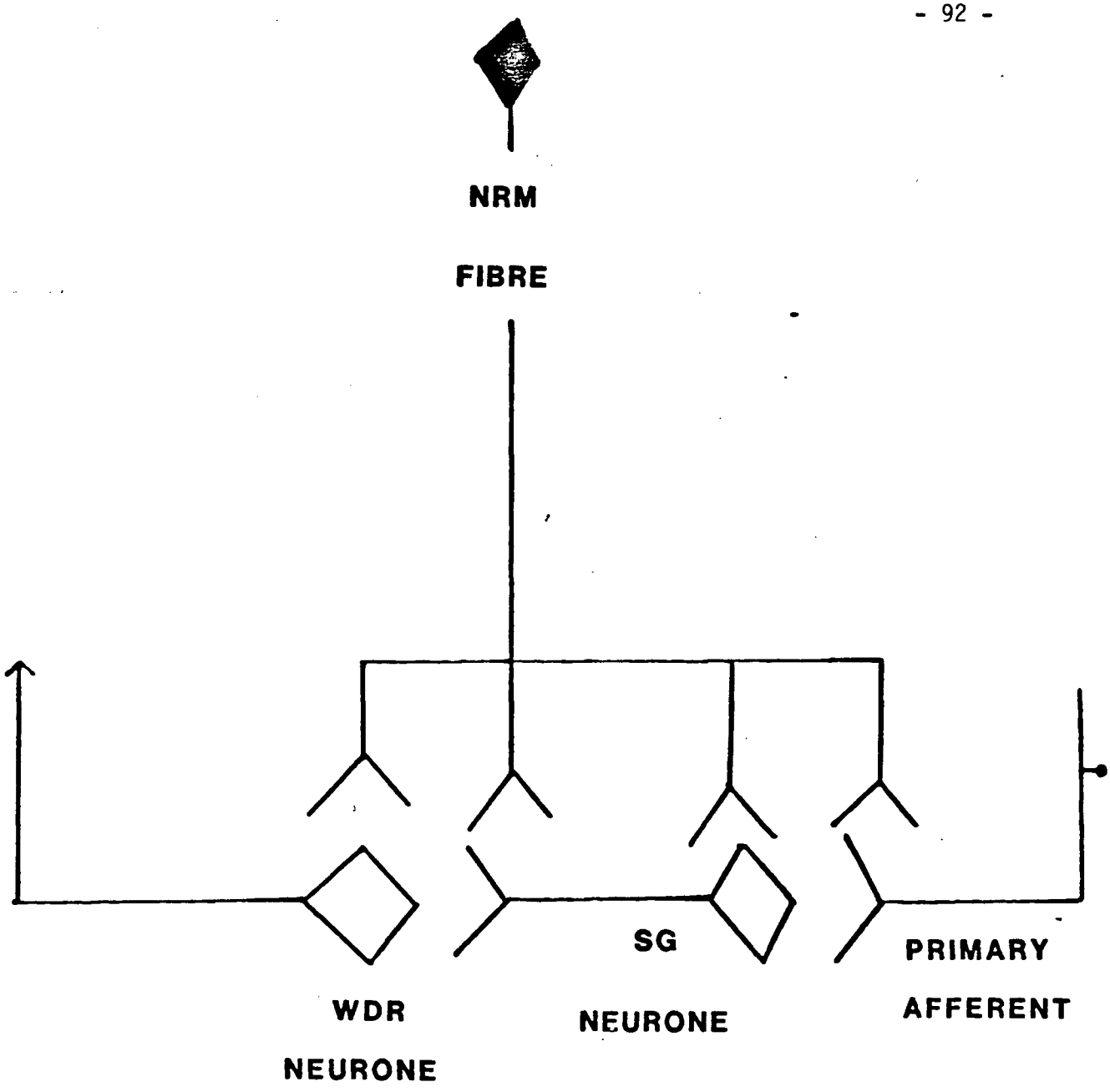

FIG: 20

A schematic neuronal arrangement illustrating some possible synaptic connections which would produce nucleus raphe magnus phasic inhibition of deep dorsal horn wide dynamic range neurones. In this and the following figures, filled cell bodies indicate that the neurones are inhibitory whereas open cell bodies indicate that the neurones have excitatory effect on the postsynaptic targets (see text for details).

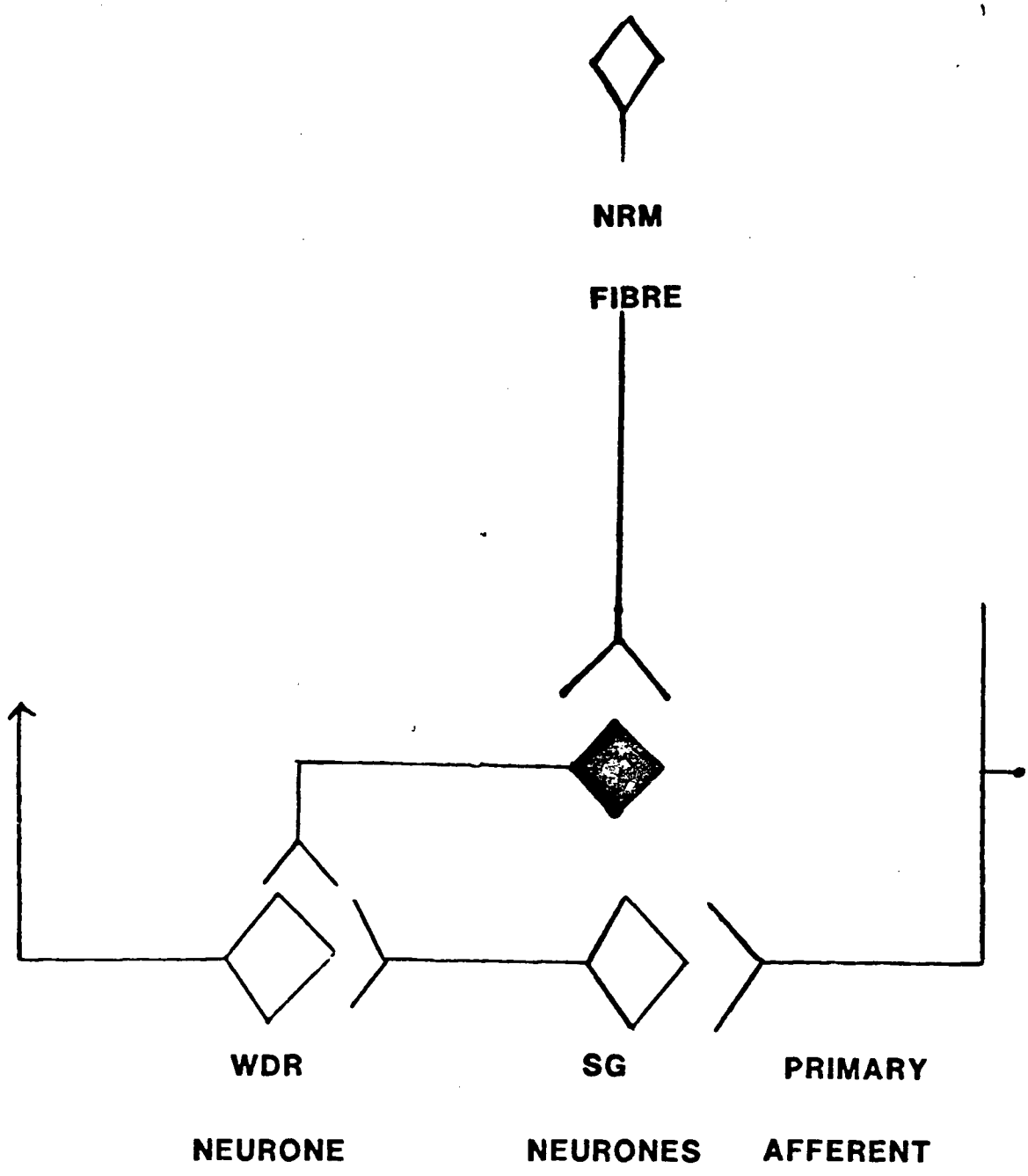

FIG: 21

A schematic neuronal arrangement illustrating the nucleus raphe magnus phasic inhibitory impingement of deep dorsal horn neurones mediated via an inhibitory interneurone in the substantia gelatinosa.

which are inhibitory to the WDR neurones of the deep dorsal horn (Dubisson and Wall 1980).

The chemical nature of the transmitter mediating the NRM phasic inhibition of deep dorsal horn WDR neurones is not known. Electrical stimulation of the NRM is likely to excite both serotonergic and non-serotonergic bulbospinal fibres. The balance of such activation results in inhibition of WDR neurones. However, since fluoxetine and pargyline antagonized rather than enhance the inhibition, it appears that 5-HT is not the principle mediator of the inhibition. Furthermore, these results suggest that 5-HT may be involved in antagonzing this bulbospinal inhibition. A possible mechanism of action of 5-HT in the spinal cord to block the NRM phasic inhibition is discussed below.

One of the most obvious sites of 5-HT action is in the substantia gelatinosa of the spinal cord dorsal horn since the concentration of 5-HT in the spinal cord of the cat is highest in this region (Oliveras et al. 1977). Iontophoretic application of 5-HT in the vicinity of the cell bodies of laminae I and II neurones is known to excite these neurones (Todd and Millar 1983), some of which were characterized to be of the WDR type. Therefore, it is possible that these superficial laminae interneurones are a link in the polysynaptic nociceptive afferent input to deep dorsal horn WDR neurones. NRM stimulation, through 5-HT release, may block the concurrent NRM phasic inhibition by activating these superficial laminae excitatory interneurones (Fig. 22). Alternatively, serotonin might also block the NRM phasic inhibition of deep dorsal horn neurones by inhibiting the superficial laminae

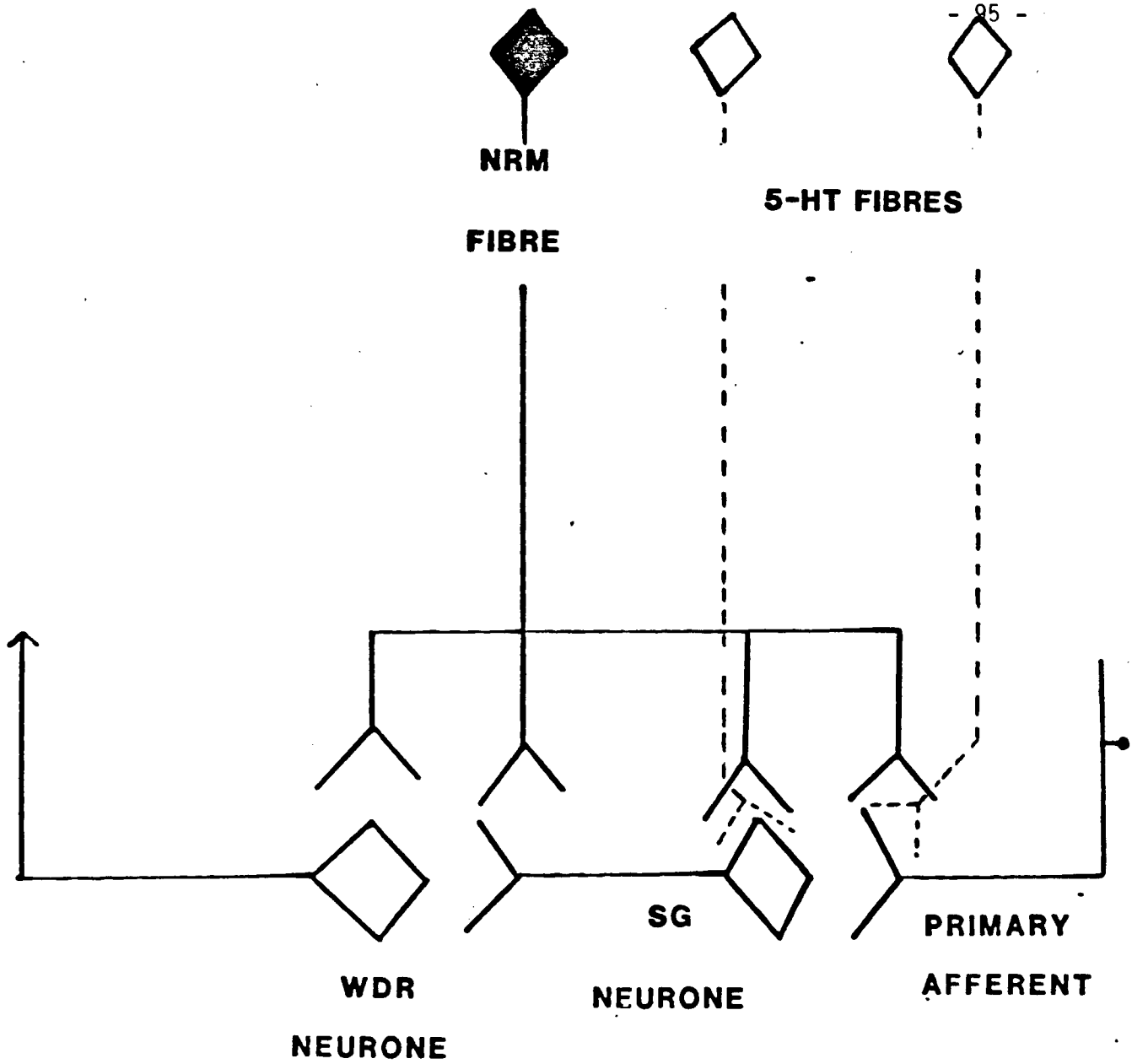

FIG: 22

A schematic neuronal arrangement illustrating some possible synaptic connections by which the serotonergic bulbospinal neurones attenuate the nucleus raphe magnus phasic inhibition of deep dorsal horn neurones.

interneurones, which are inhibitory to deep dorsal horn neurones (Fig. 23). For example, Randic and Yu (1976) have reported that iontophoretic application of 5-HT inhibited the majority of superficial laminae neurones in their study.

Serotonin released in the SG might also act presynaptically to decrease the NRM phasic inhibition of the deep dorsal horn neurones. There is some evidence in favour of this postulate. For example, iontophoretic 5-HT increases the threshold for antidromic activation of Aδ- and C-fibres in the cat spinal cord (Carstens et al. 1981b). This effect of 5-HT on the primary afferent terminal excitability is opposite to that which occurs during PAD which is thought to result in a decreased transmitter release during afferent activation. Therefore, the effect of 5-HT on the and Aδ- and C-afferent fibre terminal excitability might lead to an enhancement of transmitter release by peripheral activation of these afferents. Since some of the Aδ- and C-fibres are nociceptors (Burgess and Perl 1967; Bessou and Perl 1969) one can speculate that enhancement of transmitter release by nociceptor afferents in the presence of 5-HT would result in greater activation of dorsal horn neurones to peripheral noxious stimuli applied to their receptive fields. This increased nociceptive afferent drive on dorsal horn neurones might then decrease the inhibition of neurones by the NRM stimulation.

In the text above it has been suggested that the decrease in the NRM phasic inhibition of nociceptive activity of the deep dorsal horn neurones by fluoxetine and pargyline, respectively, might be due to the enhancement of 5-HT synaptic transmission in the spinal cord.

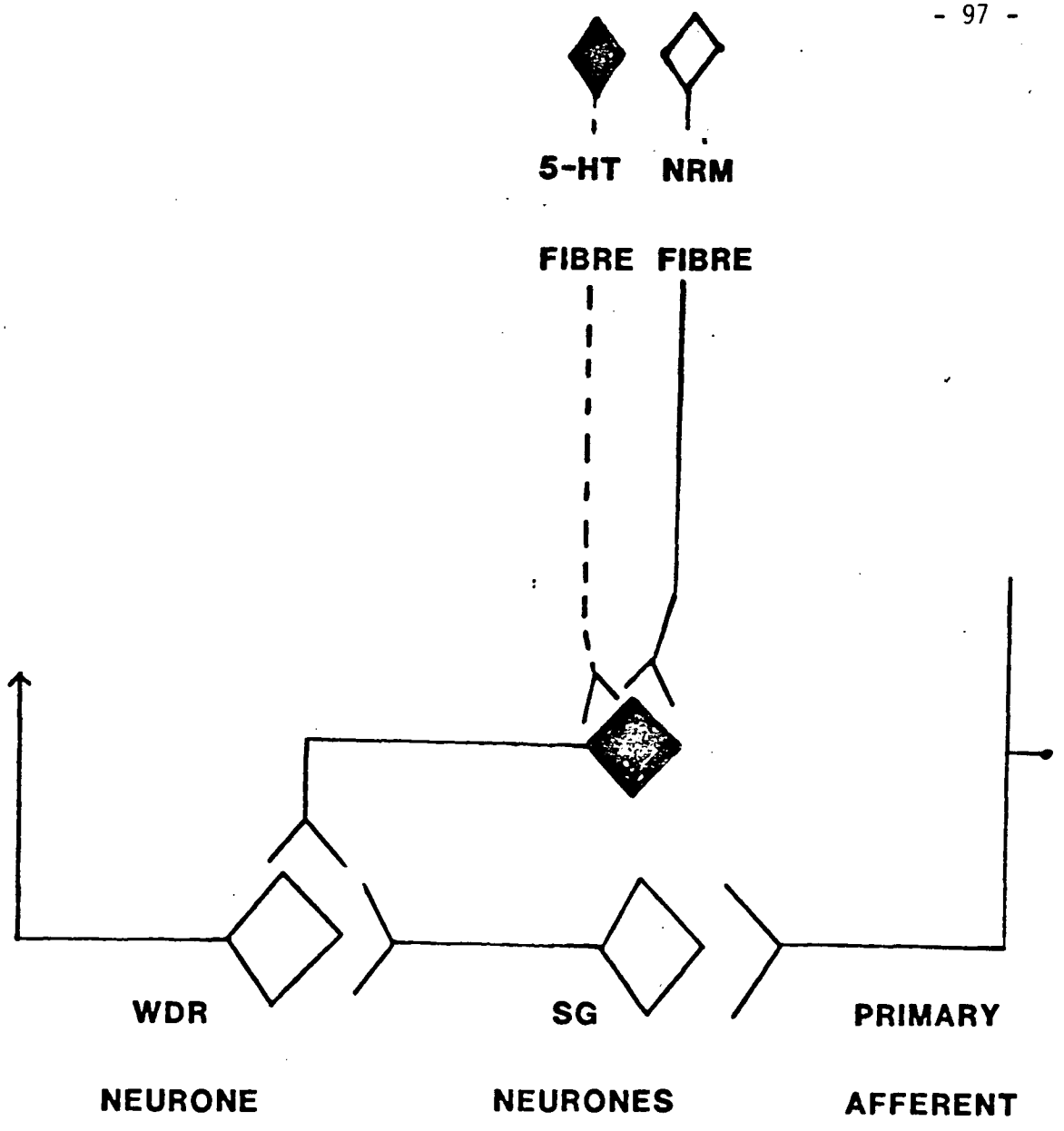

FIG: 23

A schematic neuronal arrangement illustrating the serotonergic synaptic impingement on the inhibitory interneurone in the substantia gelatinosa which attenuates the nucleus raphe magnus inhibition of deep dorsal horn neurones.

It is also possible that the effect seen with these drug treatment might not only be mediated through a spinal site of action but also through a supraspinal component. The supraspinal site(s) of drug action is not known but it is possible that fluoxetine and pargyline alter the excitability of the NRM serotonergic neurones. Aghajanian (1978) postulated that monoaminergic neurones of the central nervous system are under feedback regulation whereby an increase in the availability of monoamine transmitter would be compensated by a decrease in the monoamine neuronal firing rate. In keeping with this hypothesis, it has been observed that parenteral administration of drugs which enhance 5-HT availability, such as monoamine oxidase inhibitors like pargyline (Aghajanian et al. 1970), and 5-HT uptake blockers like chlorimipramine (Gallagher and Aghajanian 1975) and zimelidine (de Montigny et al. 1981), depressed the firing rate of dorsal raphe serotonergic neurones. It is possible that treatment with parenteral pargyline and fluoxetine similarly decreased the excitability of serotonergic neurones of the NRM in the present study. Consistent with this view is the finding of Hammond et al. (1985) that, in rats, parenteral administration of fluoxetine did not produce a quantitative increase of basal or NRM stimulation-produced 5-HT release into the spinal perfusate. This can be explained by a scenario in which an increased release of spinal 5-HT by fluoxetine, as a result of its serotonin uptake blocking activity, is obscured by a reduction in the release of 5-HT due to inhibition of the serotonergic raphe neurones firing rate. In this hypothetical model, if fluoxetine and pargyline decreased the excitability of the NRM

serotonergic neurones, then the number or the extent of excitation of such neurones by the NRM stimulation might also decrease. This, in turn, would decrease the synaptic 5-HT input to the spinal cord. If 5-HT is indeed the transmitter responsible for the NRM phasic inhibition of the nociceptive activity of the deep dorsal horn WDR neurones then, in accordance with the above scenario, the NRM phasic inhibition of neuronal activity would decrease.

In the above model the speculated decreased excitability of the NRM serotonergic neurones by fluoxetine and/or pargyline might be due to an enhanced supraspinal 5-HT synaptic transmission. Consistent with this notion are the observations that iontophoretic application of 5-HT in the NRM inhibited the activity of NRM-spinal neurones in primates (Willcockson et al. 1983), identified NRM bulbospinal serotonergic neurones in rats (Wessendorf and Anderson 1983) and systemic administration of 5-HT agonist, 5-methoxy-N-N-dimethyltryptamine, reduced the firing of presumed serotonergic neurones of the cat NRM (Fornal et al. 1985).

From the above discussion it follows that the decrease in the NRM phasic inhibition of dorsal horn neuronal nociceptive activity by administration of fluoxetine and pargyline might be due to the enhancement of 5-HT transmission at spinal and supraspinal sites. The contribution of spinal vs supraspinal sites of drug action cannot be resolved from this study.

Fluoxetine treatment did not effect the spontaneous and the heat-evoked activity of the neurones in this study. A similar effect of

fluoxetine have been earlier reported by Soja and Sinclair (1980). However, treatment with pargyline produced an increase in the responsiveness of the deep dorsal horn WDR neurones to noxious heat applied to their receptive fields. This effect of pargyline cannot be satisfactorily explained. The increase is possibly due to an action of the drug on the descending tonic inhibition impinging on these neurones, however, we did not examine this possibility in this study. Fluoxetine, in the presence of pargyline, produced a small but significant increase in heat-evoked activity of neurones and a significant decrease in the spontaneous activity of the neurones studied. The mechanism of action of fluoxetine in the presence of pargyline is not known.

Duggan and Griersmith (1979) have suggested that changes in the blood pressure with a drug treatment might affect the neuronal responsiveness to noxious heat. For example, these authors have reported that infusion of the vasodepressor agent, isobutyl methylxanthine, produced a marked increase in cell firing to noxious heat. The authors suggested that the fall in B.P. might have decreased the dissipation of heat from the receptive field resulting in greater activation of nociceptors and an increase in the noxious drive to the spinal cord neurones. However, it is believed that the increase in the neuronal responsiveness to noxious heat with pargyline treatment in this study is not a reflection of blood pressure changes because (i) the increase in the noxious heat-evoked activity by pargyline was accompanied by a small and gradual increase in blood pressure, whereas,

according to Duggan & Griersmith (1979), an increase in neuronal cell firing to heat is accompanied with a marked fall in blood pressure, (ii) Clark and Ryall (1983), in cats, did not observe any change in cell firing to noxious heat applied to the receptive field when the blood pressure was made to suddenly fall or rise with infusion of hexamethonium or adrenaline, respectively, and (iii) Lynn (1979), in rabbits, reported that noxious heat-evoked responses in polymodal nociceptors was independant of the local blood flow.

It is possible that the increase in the excitability of neurones to noxious heat with pargyline treatment influenced the NRM phasic inhibition of these neurones. To test this possibility control experiments were done in which it was observed that increasing the activity of neurones by increasing the temperature of noxious heat applied to the receptive field decreased the NRM phasic inhibition. In these control experiments, the average increase in the response of neurones to noxious heat was about 55% which was accompanied by an average decrease of 10% in the NRM phasic inhibition. In the pargyline treated group of neurones, the average increase in the neuronal response to noxious heat in the pargyline post-infusion period was about 44% with an average decrease of 14% in the NRM phasic inhibition. The decrease in the NRM phasic inhibition with pargyline treatment, if it had been solely due to change in the excitability of the neurones, should have been less than 10%. Because it was otherwise it suggests that the drug, pargyline, decreased the NRM phasic inhibition, partly, as the result of its pharmacological effect, with the increase in cell excitability also

contributing to its action.

Finally, control experiments were run in which the NRM stimulation-produced inhibition of dorsal horn neuronal nociceptive activity was studied with the infusion of the vehicle. The inhibition was followed for period of up to one hour which is the same time period for which most of the drug effects were studied. In these control experiments the NRM phasic inhibition did not change significantly with the infusion of the vehicle nor with time. The noxious heat-evoked and the spontaneous activity of the neurones in this control experiment were also unaffected.

Role of Serotonin in Morphine Depression of Nociceptive Response in the Dorsal Horn Neurones

There is some evidence to suggest that the NRM serotonin might play an important role in morphine antinociception in the spinal cord (see text). It has been suggested that the systemic administration of morphine might activate descending serotonergic fibres originating in the NRM to produce an increase in the release of spinal cord serotonin. This spinally released serotonin might partly mediate, morphine antinociception. To investigate this idea, experiments were designed wherein morphine was administered to animals already treated with fluoxetine. It was reasoned that if 5-HT did mediate, at least partly, morphines inhibition of dorsal horn neuronal nociceptive activity, then the effect of morphine would be enhanced by fluoxetine. Clearly, this was not the case. Morphine, administered systemically in three

cumulative doses, produced a dose-dependent inhibition of nociceptive activity of the deep dorsal horn WDR neurones which was no different from that observed when morphine and fluoxetine were administered concurrently.

Recent electrophysiological findings also support the observation made in this study that 5-HT does not mediate morphine inhibition of dorsal horn neuronal nociceptive activity. For example, Auerbach et al. (1985) have observed that analgesic doses of morphine (2.0 mg/kg, i.p) did not increase the activity of presumed serotonergic neurones in the conscious cat. Similarly, Chiang and Pan (1985) have reported that, in the anaesthetized rats, administration of morphine (5.0 mg/kg, i.p.) did not affect the activity in the majority of presumed NRM-spinal projecting serotonergic neurones. Clearly, if morphine had increased the activity of this type of neurone, it would have been strong evidence that the attenuation of dorsal horn neuronal nociceptive activity by morphine was mediated by 5-HT released synaptically in the spinal cord dorsal horn. A lack of excitation of serotonergic neurones by morphine strengthens the observation made in this study.

Furthermore, there is no conclusive evidence that analgesic relevant doses of morphine attenuates the nociceptive activity of dorsal horn WDR neurones by activating a descending inhibitory system. Systemic morphine can inhibit dorsal horn neuronal nociceptive activity in both spinal cord intact (Soja and Sinclair 1983b) and transected cats (Hanaoka et al. 1978). The latter authors also observed that systemic morphine produced a greater percentage decrease of the dorsal horn

neuronal nociceptive responses during normal spinal cord conduction than when the spinal cord was transected. Based on this observation the authors suggested that morphine activates descending pathways that inhibit noxious stimuli-evoked discharges in deep dorsal horn WDR neurones. But, Soja and Sinclair (1983b) have suggested that the difference in the percentage inhibition of the dorsal horn neuronal activity by systemic morphine in the intact vs transected state of the spinal cord might have resulted from differences in the control values for neuronal responses in these different state of the spinal cord. Du et al. (1984) observed that microinjection of 10-20 μg of morphine into the NRM produced an inhibition of noxious heat-evoked activity of the dorsal horn WDR neurones. The authors also suggested that morphine activates a descending inhibitory system from the NRM to suppress nociceptive transmission at the spinal cord level. However, this effect of microinjected morphine might not reflect the action of systemic morphine because, when given systemically, the drug effect might be a reflection of its action on a number of neuronal pools. Thus, when a larger supraspinal area was exposed to morphine by infusing the drug between the third ventricle and the cisterna magna, no inhibition of dorsal horn neuronal nociceptive activity was observed (Sinclair 1985). Further, the doses of morphine microinjected by Du et al. (1984) in the NRM would produce concentrations of the drug in the region around the nucleus which were calculated to be far in excess of concentrations necessary to produce antinociception by systemic administration of the drug (Clark et al. 1983).

The hypothesis that morphine activates a descending inhibition to the spinal cord from the NRM was investigated in this study by considering the effects of the drug on the NRM phasic inhibition of the deep dorsal horn WDR neurones. The rationale of this experiment was that if morphine does activate a descending inhibition from the NRM, then it should potentiate the NRM phasic inhibition of the dorsal horn neurones. However, the results of this study were ambiguous. Morphine either increased or decreased the NRM stimulation-produced inhibition. Further experiments were carried out to investigate whether 5-HT might mediate or influence the morphine effect on the NRM phasic inhibition, by administering morphine to fluoxetine treated animals. Here morphine consistently produced a decrease in the NRM phasic inhibition which was greater than that observed with fluoxetine. Thus, under conditions of enhanced 5-HT synaptic transmission, morphine produced a decrease in the NRM phasic inhibition which is not consistent with the hypothesis that morphine activates a descending serotonergic system from the NRM to inhibit spinal cord nociceptive transmission.

There are a number of possibilities as to how morphine might interact with 5-HT systems, especially of the NRM, to decrease the NRM stimulation-produced inhibition of the dorsal horn neurones. For example, morphine might enhance the 5-HT synaptic transmission of the NRM serotonergic neurones and therefore potentiate the effect of fluoxetine in decreasing the NRM phasic inhibition. However, recent electrophysiological evidence does not support this possibility. That is, morphine given systemically does not affect the activity of the

presumed 5-HT interneurones in the NRM of conscious cats (Auerbach et al. 1985) or of the NRM-spinal serotonergic neurones in anaesthetized rats (Chiang and Pan 1985).

It is also possible that morphine can interact with 5-HT in the spinal cord to block the NRM phasic inhibition. The possible mechanism of interaction of 5-HT and morphine in the spinal cord is not known but it might occur at one or more of the sites described previously for spinal serotonin action on the descending inhibition.

The decrease in the NRM phasic inhibition by treatment with morphine in fluoxetine treated animals is unlikely due to the change in the excitability of dorsal horn WDR neurones to noxious heat. This conclusion was reached based on control experiments which showed that a decrease in excitability of neurones produced an enhancement of the NRM phasic inhibition of nociceptive activity. The decrease in the excitability of these neurones was accompanied by a decrease in the phasic inhibition.

The electrophysiological results obtained in this study are not consistent with those obtained in certain behavioural studies which mainly use the rat tail-flick test. Some of these studies provide evidence that morphine antinociception is mediated in part via 5-HT systems. The following factors could be important in these conflicting results. (i) The present experiments were performed on cats whereas most of the behavioural experiments were done in rats. Therefore, a species difference might explain the results obtained. (ii) This study was done in anaesthetized animals. Anaesthesia may have altered the

results obtained. For example, pentobarbital anaesthesia was found to diminish the antinociceptive potency of intracranial morphine (Ossipov and Gebhart 1984). Thus, it is possible that anaesthesia blocked the supraspinal component of morphine's action in this study. (iii) Morphine antinociception in behaving animals might occur by its action on different spinal or supraspinal neuronal pools than the one studied here. Some of these possibilities might also explain the results obtained by LeBars et al. (1980). These authors reported that microinjection of 5 µg of morphine into the NRM of rats was antinociceptive in behavioural experiments but excited or had no effect on the C-fibre response of dorsal horn WDR neurones of the same anaesthetized animals.

CONCLUSION

1. The magnitude of the nucleus raphe magnus (NRM) phasic inhibition of the deep dorsal horn wide dynamic range (WDR) neurones was not increased but rather decreased by the selective 5-HT reuptake inhibitor, fluoxetine, and by treatment with the monoamine oxidase inhibitor, pargyline. These results indicate that enhancement of 5-HT transmission does not result in increased NRM phasic inhibition of dorsal horn neuronal nociceptive activity.

2. Morphine did not produce a consistent effect on the NRM phasic inhibition. It either produced an increase or a decrease of this inhibition. However, in the presence of fluoxetine, morphine reduced the NRM phasic inhibition. These observations are not consistent with the hypothesis that morphine activates a descending serotonergic system from the NRM to inhibit spinal cord nociceptive transmission.

3. Fluoxetine treatment did not increase the noxious heat-evoked activity of dorsal horn WDR neurones. This observation is similar to the earlier reported effect of fluoxetine by Soja and Sinclair (1980) who suggested, based on this and other evidence, that 5-HT is not involved in the tonic control of nociceptive activity of these neurones.

4. Morphine administration produced an inhibition of dorsal horn neuronal nociceptive activity. This inhibitory effect of morphine was no different than that seen when morphine was administered to fluoxetine pretreated animals. These results suggest that 5-HT does not mediate the morphine inhibitory effect on the spinal cord deep dorsal horn wide dynamic range neurones.

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