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Pharmacology of Descending Control Systems

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Phil. Trans. R. Soc. Lond. B 1985 **308**, 375-391
doi: 10.1098/rstb.1985.0038

Phil. Trans. R. Soc. Lond. B **308**, 375–391 (1985)

Printed in Great Britain

Pharmacology of descending control systems

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In the cat there is no convincing evidence that a particular compound mediates a supraspinal control of spinal transmission of nociceptive information.

There is good evidence that opioid peptides are released segmentally in response to nociceptive input to the spinal cord and that this acts to inhibit motoneurons and to reduce transmission of nociceptive information to supraspinal areas. In the cat there is no evidence that stimulation at supraspinal sites producing analgesia results in a spinal release of opioid peptides. In the rat evidence for the latter has been obtained but there are no data from other species. Tonically present supraspinal inhibition of spinal transmission of nociceptive information in the cat does not involve opioid peptides.

Indirect evidence favours a role for 5-hydroxytryptamine and noradrenaline in supraspinal control of spinal processing of nociceptive transmission. Peripheral antagonists of 5-HT have reduced spinal inhibition from stimulation at supraspinal sites but the site of action is unknown. Progress with noradrenaline involvement has been hindered by lack of a suitable antagonist.

Although the amino acids, glycine and GABA are involved in segmental inhibition of transmission of nociceptive information, no convincing evidence has indicated their involvement in supraspinal controls.

Pharmacological investigations of synaptic events in the central nervous system use a variety of preparations and methods of drug administration. First among these is the administration of substances near neurons to look for similarities between the action of an exogenous compound and that released by activity in the appropriate nerve fibres. *In vivo*, this is usually done by administration of substances electrophoretically from micropipettes, but increasingly *in vitro* experiments with tissue slices have been used since intracellular recordings are more readily obtained. A powerful tool is the use of selective antagonists of known agonists to attempt to reduce a particular synaptic event. In addition it may be possible to interfere with the synthesis, release and inactivation of a suspected synaptic transmitter. Most of these techniques have been used in investigating supraspinal control of the spinal transmission of nociceptive information, and results of some of these experiments will be discussed. It is first necessary, however, to describe briefly the descending controls which have been the subjects of pharmacological experiments.

Experimentally, analgesia has been produced by electrical stimulation at a number of brain stem sites including the periaqueductal grey, the locus coeruleus and the raphe areas of midbrain and medulla (Reynolds 1969; Liebeskind *et al.* 1973; Oliveras *et al.* 1974; Segal & Sandberg 1977). Importantly, stimulation at all of these sites has produced inhibition in the spinal cord, and in particular of the excitation of neurons by peripheral noxious stimuli (Oliveras *et al.* 1974; Fields *et al.* 1977; Guilbaud *et al.* 1977; Carstens *et al.* 1979*b*; Duggan & Griersmith 1979; Hodge *et al.* 1981; Yezierski *et al.* 1982; Morton *et al.* 1983). It is not known

if this spinal inhibition is sufficient to account for analgesia from stimulation, but it is likely to be an important component.

Electrical stimulation is, strictly speaking, an artefact and only limited conclusions can be drawn on the relevance of the effects of stimulation on animal behaviour. A system which is tonically active under particular experimental conditions or one which is invoked by the natural stimuli, is certainly not artefactual, but one has to be guarded in defining the conditions necessary for its operation. In the anaesthetized cat the spinal transmission of nociceptive information is tonically inhibited from supraspinal areas, as shown by the increased responsiveness of spinal neurons to peripheral noxious stimuli when an area of the spinal cord is cooled cephalic to the recording site (Wall 1967; Duggan *et al.* 1977*b*; Handwerker *et al.* 1975). This tonic inhibition is abolished by bilateral lesions of the lateral reticular areas of the caudal medulla (Hall *et al.* 1982), but the relevant neurons have not yet been defined with microelectrodes. It is unknown, moreover, if such inhibition is operative in conscious animals, or if it is produced by anaesthesia, or by the trauma necessary to record from neurons of the spinal cord.

Prior to discussing the involvement of particular compounds in these controls, two methodological problems require consideration. The first is that of methods of drug administration. Many of the commonly held beliefs on the pharmacology of descending controls have been derived from behavioural experiments. Typically these experiments have produced analgesia in an animal by a procedure such as stimulating in a part of the brain. Analgesia is usually defined as a prolonged latency of movement of a particular part of the body in response to a stimulus which is perceived to be painful to humans. Systemic administration of a drug may be observed to produce a change in the level of analgesia. The interpretations of this experiment are several but include the following. (*a*) The drug did indeed act in a specific manner to interfere with the action of a transmitter released through activity in particular fibres excited by the brain stimulating electrode. (*b*) The drug modified the test of analgesia (e.g. if an area of skin was heated, the drug may have altered heat transfer across the skin through alterations in cutaneous circulation). (*c*) The drug altered the excitability of neurons in the area stimulated either directly or indirectly. A different number of neurons would then be excited by the stimulating electrode. (*d*) The level of excitability of neurons involved in the test of analgesia and acted upon by the neurons excited by brain stimulation, was altered by the drug. This should result in a change in the baseline latency. (*e*) The drug did not act with the assumed specificity.

Some of these considerations equally apply when studying the firing of single neurons instead of measuring analgesia with a behavioural test. The situation can be simplified by administering drugs electrophoretically from a multibarrel micropipette which also measures cell firing, but a major problem here is that of specificity since the concentrations attained near receptors basically are unknown. This problem will be further considered subsequently with specific examples.

The second methodology consideration is that of which neurons should be studied when attempting to relate single cell studies to behavioural experiments in analgesia? In the spinal cord it would seem not unreasonable to study spinothalamic neurons excited by noxious peripheral stimuli. But these are by no means the only projecting neurons excited by such stimuli. In addition there are many other spinal neurons excited or inhibited by peripheral noxious stimuli and which are presumably concerned in the complex reflex, motor and autonomic responses to the firing of peripheral nociceptors. Even if a group of spinal neurons

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could be defined as important in transmitting impulses which are important in the perception of pain, these may not be the appropriate ones for studying a particular control. Thus a supraspinal inhibition could be exerted on a group of neurons near the first central synapse which are connected both with projecting and other neurons such that the inhibition suppresses not only the perception of pain but also the autonomic and motor responses to pain (figure 1*b*). In this case attempts to modify the inhibition by an antagonist of the transmitter released by the appropriate descending fibres may be successful with systemic administration but unsuccessful with local administration unless the neurons primarily affected are identified.

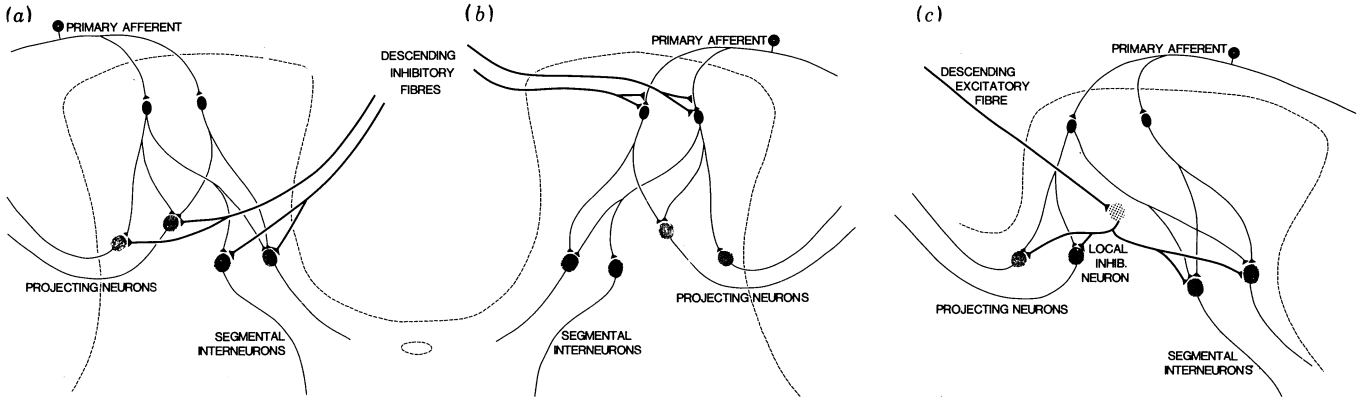

FIGURE 1. Possible mechanisms of descending inhibition. In each case a nociceptive afferent is shown making connections with a small interneuron of the upper dorsal horn, which in turn connects with a supraspinally projecting neuron and a segmental interneuron. (*a*) Descending inhibitory fibres make separate connections with projecting neurons and segmental interneurons. (*b*) Descending inhibitory fibres make axo-axonic synapses with primary afferents and axo-somatic connections with the neurons receiving primary afferents. (*c*) The descending fibres excite local inhibitory interneurons which in turn inhibit other spinal neurons.

Alternatively the inhibitory fibres could be more localized in their action, directly inhibiting groups of neurons, and thus permitting differential effects on the various complex responses to nociceptive input to the spinal cord (figure 1*a*). In this case the inhibition would be reduced by the administration of the appropriate antagonist near the inhibited neurons.

The third possibility is that the descending fibres may act through activating local segmental inhibitory mechanisms (figure 1*c*). In this case an antagonist of the compound released by the relevant descending fibres may reduce the inhibition when given systemically, but be ineffective when administered near the inhibited neurons. Only an antagonist of the transmitter released by the segmental inhibitory interneurons would be effective. Thus the study of descending controls must include investigations of inhibitory mechanisms in the spinal animal and their potential interaction with descending controls. In the account which follows, considerable attention is given to these segmental inhibitory mechanisms, as experiments have indicated that they are capable of suppressing spinal transmission of nociceptive information and thus could form a suitable substrate for the action of supraspinal controls.

Despite these limitations, a considerable body of information has been acquired from neuropharmacological experiments on supraspinal control of the spinal transmission of nociceptive information. It has been found more convenient to discuss substances which could act as transmitters rather than particular descending pathways.

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1. Opioid peptides

These compounds were first isolated because of their opiate activity in pharmacological tests (Hughes 1975; Hughes *et al.* 1975) and since suppression of pain perception is a prominent effect of opiates, a role in physiological control of impulses related to pain has been intensively investigated. The evidence from anatomical and neuropharmacological techniques has supported this role, but the conditions which invoke pain reduction by opioid peptides and the mechanisms by which this is achieved are still only partly understood.

By immunocytochemical techniques, both immunoreactive enkephalins and dynorphins are present in the spinal cord (Hokfelt *et al.* 1977; Hunt *et al.* 1980; Aronin *et al.* 1981; Bennett *et al.* 1982; Ruda 1982; Przewlocki *et al.* 1983). They are relatively concentrated in the upper dorsal horn, including the substantia gelatinosa, which is the major site of termination of nociceptive primary afferent fibres (Light & Perl 1979). Most are believed to be contained within intrinsic neurons of the cord and hence little is of supraspinal origin.

(*a*) *The actions of opioid peptides on spinal neurons*

Administered iontophoretically from micropipettes, depression of firing has been the predominant effect. In experiments using extracellular recording techniques the enkephalins have depressed the spontaneous and evoked firing of neurons widely distributed in the dorsal horn (Duggan *et al.* 1977*a*; Randic & Miletic 1978; Satoh *et al.* 1979; Zieglgansberger & Tulloch 1979; Duggan *et al.* 1980). The effect is probably a postsynaptic one since synaptic excitations from both large and small diameter cutaneous afferents are reduced concomitantly (figure 2). Intracellular recordings from neurons of slice preparations of the rat dorsal horn (Murase *et al.* 1982; Yoshimura & North 1983) have observed hyperpolarization by enkephalins.

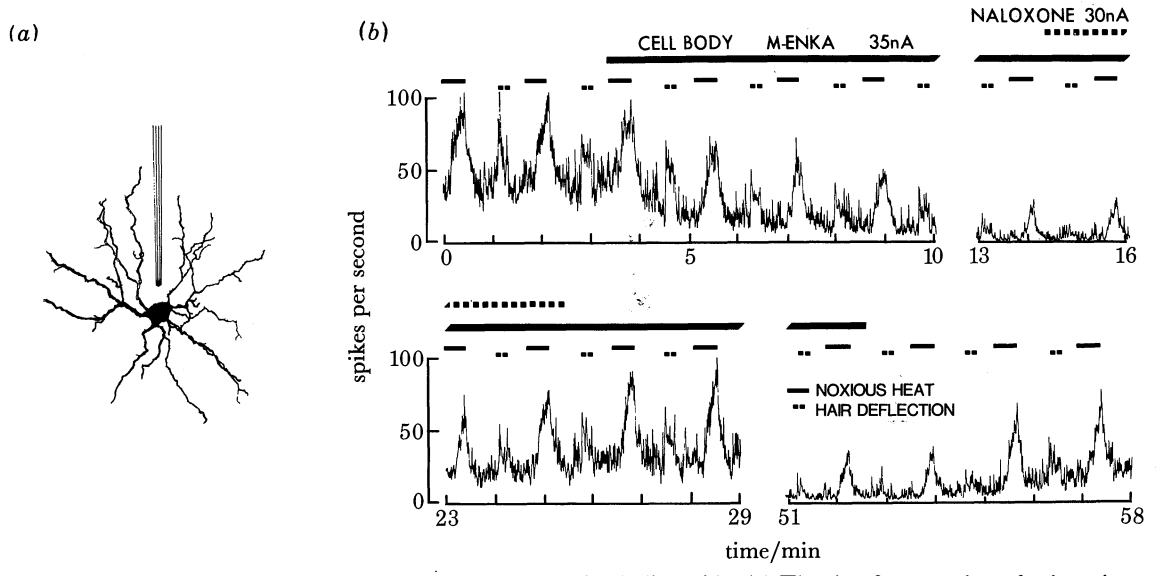

Figure 2. Depression of a spinal neuron by methionine enkephalinamide. (*a*) The tip of a seven-barrel micropipette is shown positioned adjacent to the body of a spinal neuron. (*b*) Ratemeter records of the firing rate of a cat dorsal horn neuron excited alternately by noxious heating of the skin of a digital pad (solid bars) and deflection of adjacent hairs by a moving air jet (interrupted bars). Ejection of methionine enkephalinamide (35 nA) depressed both responses and this was reversed by additional ejection of naloxone (30 nA). The action of both agonist and antagonist are shown to be reversible. (Reproduced from Duggan *et al.* (1977).)

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The substantia gelatinosa is the area of the spinal cord containing the highest levels of opioid peptides. The intrinsic neurons of this area are small and not easily recorded from with the relatively large (5–6 μm tips) multibarrel micropipette assemblies used for microelectrophoretic administration of compounds extracellularly. Duggan *et al.* (1977*a*) studied the effects of enkephalin administration in this area by using two micropipette assemblies. One had its tip positioned in the substantia gelatinosa while another recorded from a neuron in either spinal laminae IV or V and a known distance from the site of drug administration. Methionine enkephalinamide selectively reduced the excitation of these deeper neurons by noxious heating of the skin. Excitation by deflection of hairs was unaffected. This action was reversed by naloxone given either intravenously or from a micropipette.

Sastry & Goh (1983) repeated these experiments using a fine single pipette cemented to the multibarrelled assembly used to administer compounds in the substantia gelatinosa, so as to obtain recordings from neurons of this area. Another pipette recorded the firing of deeper neurons. They confirmed that methionine enkephalinamide in the substantia gelatinosa reduced the excitation of deeper neurons by noxious peripheral stimuli but found that neurons of the substantia gelatinosa were concomitantly excited. They proposed the latter to be inhibitory interneurons which acted upon the deeper neurons of laminae IV and V. In a slice preparation of rat spinal cord, however, Yoshimura & North (1983) found that neurons of the substantia gelatinosa were hyperpolarized by [D-Ala^2,D-Leu^5]enkephalin. This is a very different result from that of Sastry & Goh (1983) and suggests that opioid peptides inhibit interneurons interposed between primary afferents and deeper neurons.

(*b*) *Spinal events blocked by opioid antagonists*

In the cat the bulk of the evidence favours release of opioid peptides segmentally with little dependence on supraspinal connections. Moreover, the function of this release seems to be wider than inhibition of spinal transmission of nociceptive information alone. In the rat, there is more substantial evidence for spinal release of opioid peptides in response to supraspinal controls.

The pharmacological tool most extensively used in these experiments is naloxone. Naloxone is an opioid antagonist which has little agonist activity. With the description of multiple opioid receptors, the type of event likely to be revealed by naloxone has had to be qualified. Thus naloxone has a high affinity for the μ or morphine-preferring receptors, a lesser affinity for the δ or enkephalin-preferring receptors, and a lower affinity still for the κ receptors (Kosterlitz 1983) which may be activated physiologically by dynorphin. Thus not all of the physiology of the opioid peptides may be revealed by naloxone, and when used intravenously or iontophoretically it is not possible to say which opioid peptide is involved. The specificity of naloxone has been questioned and in particular in relation to antagonism of inhibition by GABA (Dingledine *et al.* 1978). Experiments on cultured neurons, however, have shown that millimolar concentrations of naloxone are needed for this effect (Gruol *et al.* 1980) and both the active (−) and inactive (+) isomers of naloxone show such activity. Thus before interpreting an effect of naloxone as being produced by antagonism of the action of opioid peptides, considerations of dose and stereospecificity are important.

Inhibition in the spinal cat and rabbit

Goldfarb & Hu (1976) first showed that low doses of naloxone (0.05–1.0 mg kg^{-1}) increase spinal reflexes to impulses in large diameter cutaneous and muscular afferents both in the

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anaesthetized and decerebrate cat. This has been confirmed (Duggan *et al.* 1984) and the probable involvement of opioid peptides reinforced by the stereospecificity shown with (+) and (−) isomers of furylmethylnormetazocine. The latter opioid antagonist being a benzomorphan is likely to have good affinity for both μ and κ opiate receptors but the relative importance of these in the effects of spinal reflexes is not known. Catley *et al* (1983) found that in the rabbit naloxone increased extensor reflexes of the hind limb more than flexor reflexes. This action of naloxone was stereospecific. Bladder reflexes have also been shown to be increased by low doses of naloxone (Roppolo *et al.* 1983). Further evidence for the widespread effects of the inhibition revealed by naloxone comes from intracellular recordings from motoneurons (Morton *et al.* 1982). Depolarizing potentials in motoneurons from a variety of muscular and cutaneous afferents were shown to be concomitantly increased by naloxone (figure 3).

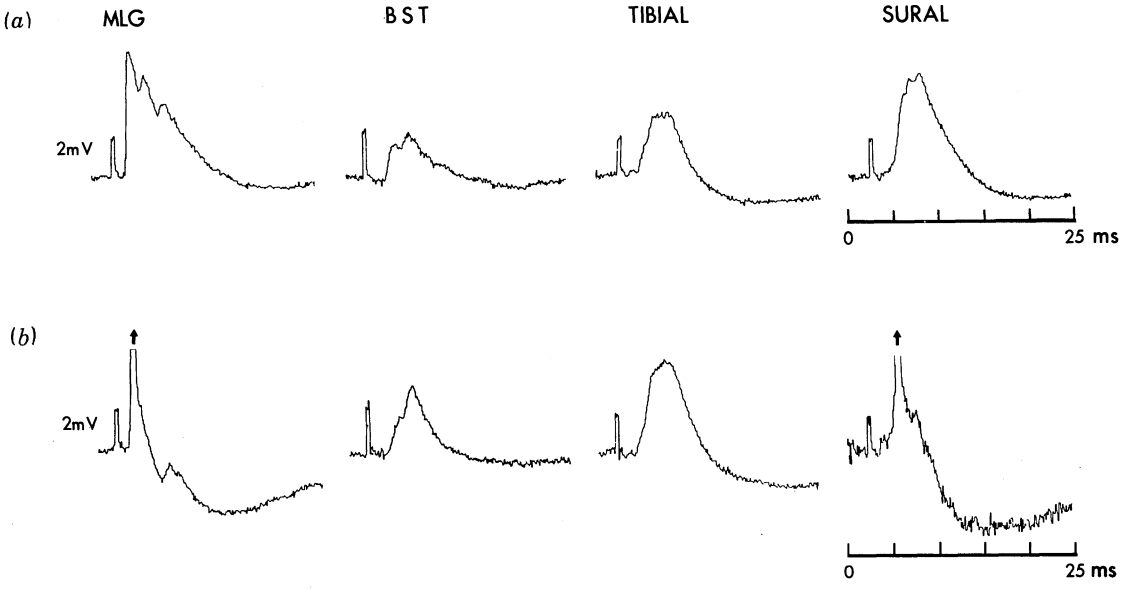

FIGURE 3. Increased depolarizing potentials evoked in a motoneuron of the cat following intravenous naloxone (*a*, *b*). The control potentials were recorded from stimulation of the combined medial and lateral nerves to the gastrocnemii (MLG) with a strength 1.6 times threshold (*T*) for the fastest conducting fibres; 1.8 *T* for the nerve to the posterior biceps semitendinosus (BST); 1.5 *T* for the tibial nerve and 1.5 *T* for the sural nerve. Each record is the average of 16 responses and the stimulus coincides with the trailing edge of the 2 mV calibration pulse. Within 4–8 min after intravenous naloxone (0.1 mg kg^{-1}) the lower potentials were recorded. Action potentials were then evoked constantly by stimulation of the m.l.g. nerve but inconstantly by stimulation of the sural nerve. Hence only one response is shown for the latter. (Reproduced from Morton *et al.* (1982).)

All of these peptidergic inhibitions in spinal animals are tonically present. In addition, two groups have demonstrated a spinally organized inhibition of reflexes from electrical stimulation of unmyelinated primary afferents. Catley *et al.* (1983, 1984) studied the reflex in the nerve to the medial gastrocnemius of the decerebrate spinal rabbit produced by stimulation of Aαβ fibres of the sural nerve. Prior stimulation of C fibres of the tibial or peroneal nerves for 10 s inhibited this reflex for 30 min, and this inhibition was reduced by naloxone 0.05 mg kg^{-1}. In the decerebrate spinal cat Chung *et al.* (1983) studied the reflex recorded in ventral roots from electrical stimulation of high threshold (Aδ and C) afferents of the sural nerve and observed inhibition for approximately 30 min following 15 and 30 min stimulation of C fibres of the tibial or common peroneal nerve. Naloxone (0.05 mg kg^{-1}) reduced this inhibition.

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Although naloxone increases the transmission of afferent impulses to the ventral roots in a relatively non-selective manner this is not seen when ascending volleys are examined by recording from various spinal funiculi. In the spinal cat large increases in the volleys recorded in the anterolateral funiculus from stimulation of unmyelinated primary afferents of the contralateral tibial nerve were produced by intravenous naloxone (0.05 mg kg^{-1}). By contrast the recordings from the ipsilateral dorsal spinocerebellar tract showed no increases despite increases in ventral root reflexes to stimulation of group I muscle afferents. With stimulation of Aαβ fibres of the sural nerves, ventral root reflexes were increased but volleys in the contralateral antero-lateral funiculus were not. This tonic inhibition of ascending volleys appears to be selective and possibly relevant to analgesia. Bernatsky *et al.* (1983) observed in the decerebrate rat that naloxone increased the firing of ascending axons of the antero-lateral funiculus to impulses to C primary afferents but had no influence on firing to Aαβ input to the spinal cord.

Since all of these effects of naloxone were observed in spinal animals, it is clear that tonic inhibition involving opioid peptides and affecting spinal reflexes and ascending information is organized in the spinal cord. The stimulus for this inhibition is uncertain but the trauma of surgery may be responsible. Studies of release of opioid peptides by perfusing the spinal cord of the cat give support to this proposal. Yaksh and Elde (1981) showed that the basal release of immunoreactive [Met]enkephalin was increased by stimulation of small, but not large, diameter afferents of the tibial nerve. A more recent study (Nyberg *et al.* 1983) has shown a complex mixture of peptides released in this way including dynorphin derivatives and the heptapeptide [Met]enkephalin–Lys^6.

The functional significance of the tonic peptidergic inhibition revealed by the study of spinal reflexes is uncertain. It seems unrelated to inhibition of the perception of pain. It is possible that it is an example of inhibition of function resulting from bodily injury and which has a role in the repair of damaged areas. The tonic inhibition revealed by the study of ascending volleys does seem appropriate to analgesia and thus could be a substrate for the action of descending controls.

The organization of either process is poorly understood. In the case of inhibition ultimately affecting motoneurons, the opioid peptides could be released near motoneurons and act pre- or postsynaptically. Equally possible is a release elsewhere in the spinal cord near interneurons on pathways to motoneurons. Morton *et al.* (1982) found no change in membrane conductance nor in resting membrane potential when recording intracellularly from motoneurons before and after naloxone administration. Thus the increase in depolarizing potentials produced by naloxone cannot be due to block of a somatic hyperpolarization. When ejected from glued parallel pipette assemblies in which the projecting pipette recorded intracellularly from motoneurons, while the multibarrel assembly administered compounds extracellularly, naloxone did not increase depolarizing potentials (Duggan & Zhao 1984). This result does not favour a release of opioid peptides near motoneurons but does not exclude it since the dendrites of these cells can extend for up to 2 mm (Brown 1981), and thus difficult to influence with iontophoretic administration near the cell body.

(*d*) *Supraspinal inhibition in the cat*

The experiments just considered have dealt with spinal animals. Attempts to implicate opioid peptides in spinal inhibition of supraspinal origin in the cat have been largely unsuccessful.

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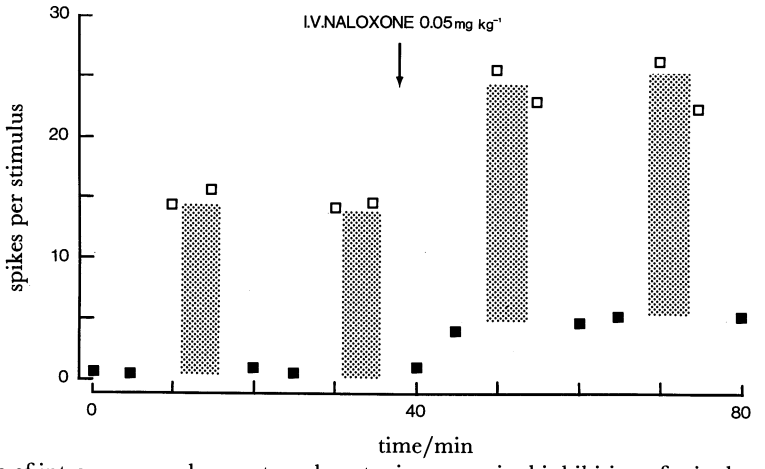

FIGURE 4. Failure of intravenous naloxone to reduce tonic supraspinal inhibition of spinal transmission of impulses in unmyelinated primary afferents of the cat. The right tibial nerve was stimulated with a strength adequate to excite unmyelinated primary afferents. Recordings were obtained with a pair of ball electrodes placed on the left antero-lateral spinal funiculus and an electronic counter gated so that with each nerve stimulus, only action potentials produced by impulses in unmyelinated primary afferents were counted. The means of 16 responses are plotted with respect to time (■). The standard errors of the means are not plotted but in all cases were less than two spikes. Cooling the spinal cord with a metal chamber placed on the cord dorsum cephalic to the recording site and which was perfused with a water ethylene glycol mixture at −2 °C, increased these responses (□). The hatched areas thus indicate the extent of tonic inhibition. Administration of naloxone (0.05 mg kg^{-1}) increased the responses observed with normal spinal conduction but did not reduce the increase in these responses by blocking spinal conduction by cooling.

The powerful supraspinal tonic inhibition revealed by cooling the spinal cord of the cat cephalic to the recording site, was uninfluenced by systemic naloxone (Duggan *et al.* 1977*b*). These experiments studied neurons of spinal laminae IV and V excited by noxious cutaneous stimuli and possible projections were unknown. Effects on projecting neurons have been examined by recording ascending volleys in the antero-lateral spinal funiculus of the first lumbar segment produced by peripheral noxious stimulation of areas innervated by distal segments. The tonic supraspinal inhibition of the transmission of such volleys revealed by the cold block technique was uninfluenced by naloxone (as illustrated in figure 4). Thus although there is a tonic opioid peptidergic system inhibiting supraspinal transmission of nociceptive information in surgically prepared cats, it is wholly of spinal origin.

In the conscious cat, Oliveras *et al.* (1977*b*) found that i.m. naloxone (0.3 mg kg^{-1}) reduced the analgesia produced by electrical stimulation near the medullary raphe (N centralis inferior). When neurons of the dorsal horn have been studied in anaesthetized cats, powerful inhibition of nociceptive responses has been produced by stimulation of the medullary raphe but this was unaltered by systemic naloxone (Duggan & Griersmith 1979). Electrical stimulation in the region of the midbrain periaqueductal grey (p.a.g.) of the anaesthetized cat produces selective inhibition of the excitation of dorsal horn neurons by peripheral noxious stimulus and this inhibition is unchanged by systemic naloxone (Duggan & Griersmith 1979; Carstens *et al.* 1979*a*).

Thus, these studies of supraspinal-derived inhibition in the cat stand in contrast to the abundance of inhibitions involving opioid peptides which are organized segmentally in the spinal cord. It may be that anaesthesia and surgery have a depressant effect on supraspinal controls involving opioid peptides but even under these conditions there is no difficulty in producing inhibition from supraspinal sites.

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(*e*) *Inhibition in the rat*

In the rat, spinal inhibition from supraspinal sites has been reduced by naloxone. Rivot *et al.* (1979) inhibited the excitation of lumbar dorsal horn neurons by unmyelinated primary afferents by stimulation in the nucleus raphe magnus (n.r.m.) of the medulla. Naloxone (0.2 mg mg^{-1}) elevated the basal responses of these neurons and reduced brain stem induced inhibition by 30%. Supporting this is the finding of Zorman *et al.* (1982), that the inhibition of tail flick from stimulation in the n.r.m. of lightly anaesthetized rats was reduced by intrathecal administration of naloxone. Jurna (1980), however, found no effect by systemic naloxone on the inhibition, by a stimulation of the p.a.g., of the firing of ascending axons recorded in the antero-lateral spinal funiculus of the rat to electrical stimulation of unmyelinated primary afferents.

The influence of naloxone on reflexes in the spinal rat does not appear to have been investigated. The experiments of Fitzgerald & Woolf (1980) in finding effects by naloxone on the spontaneous firing of neurons of the dorsal horn of spinal rats suggest that such segmental inhibition involving opioid peptides does occur.

(*f*) *Man*

Naloxone has been studied for effects on reflexes in man. In normal volunteers, naloxone (0.8 mg total dose) produced a small increase in the H reflex (a monosynaptic extensor reflex) but no change in a presumed nociceptive reflex (Boureau *et al.* 1978). In paraplegic man (long recovered from the injury) no effects were observed (Willer & Bussell 1980). There are no reports of the effect of naloxone on reflexes in humans soon after a spinal lesion.

2. Amino acids

The amino acids glycine and γ-aminobutyric acid (GABA) are the best established inhibitory transmitters in the spinal cord. There is good evidence that glycine is released from the terminals of Renshaw cells (Curtis *et al.* 1976) and Ia inhibitory interneurons (Lodge *et al.* 1977) when inhibiting motoneurons. GABA is the transmitter mediating presynaptic transmission of impulses in group I muscle afferents (Curtis *et al.* 1977). The synthesizing enzyme of GABA, glutamate decarboxylase, is present in high quantities in the substantia gelatinosa (McLaughlin *et al.* 1975). The spinal actions of glycine can be blocked by the appropriate concentrations of strychnine (Curtis *et al.* 1979) while those of GABA are selectively reduced by bicuculline (Curtis *et al.* 1971).

Recent experiments have shown that the inhibition of nociceptive transmission produced by dorsal column stimulation is probably mediated by GABA. The procedure is used clinically and was originally proposed as a means of stimulating large diameter, primary afferents without activating small fibres. By the gate control theory of Melzack & Wall (1965) this was predicted to inhibit the spinal transmission of impulses in C fibres presynaptically and thus reduce the perception of pain.

When studying multireceptive neurons in laminae I and IV, the excitation of these cells by cutaneous noxious heat was readily reduced by continuous stimulation of the dorsal columns. Dorsal column stimulation always fired the neurons producing a short burst of spikes followed by a prolonged period of inhibition. At a frequency of stimulation of 10–50 Hz the neurons

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were effectively inhibited between dorsal column evoked bursts, and excitation by noxious heating of the skin was greatly reduced. Administering bicuculline near such neurons reduced the inhibition produced by dorsal column stimulation. Strychnine was without effect. It is not possible to say whether the process reduced by bicuculline was pre- or postsynaptic inhibition or both.

These results were obtained in spinal animals and thus were not dependent on supraspinal controls. It is possible, however, that supraspinal sites could activate segmental mechanisms in producing inhibition and thus GABA could be involved. This proposal has not been intensively investigated.

Lovick & Wolstencroft (1983) found that electrical stimulation in the medullary raphe of the cat reduced the excitation of medial reticular neurons by impulses in tooth pulp afferents. Administering bicuculline near the reticular neurons reduced this inhibition.

Attempts to implicate amino acids in other descending controls of spinal transmission of nociceptive information have been largely unsuccessful. Tonic supraspinal inhibition which is measured by cooling an area of the spinal cord between the brain and the recording site was not reduced by bicuculline nor by strychnine (Duggan *et al.* 1981). Both strychnine and bicuculline were administered near the bodies of the neurons inhibited, and at more dorsal sites up to, and including, the substantia gelatinosa, and while both reduced a segmental inhibition, tonic descending inhibition was unchanged (figure 5).

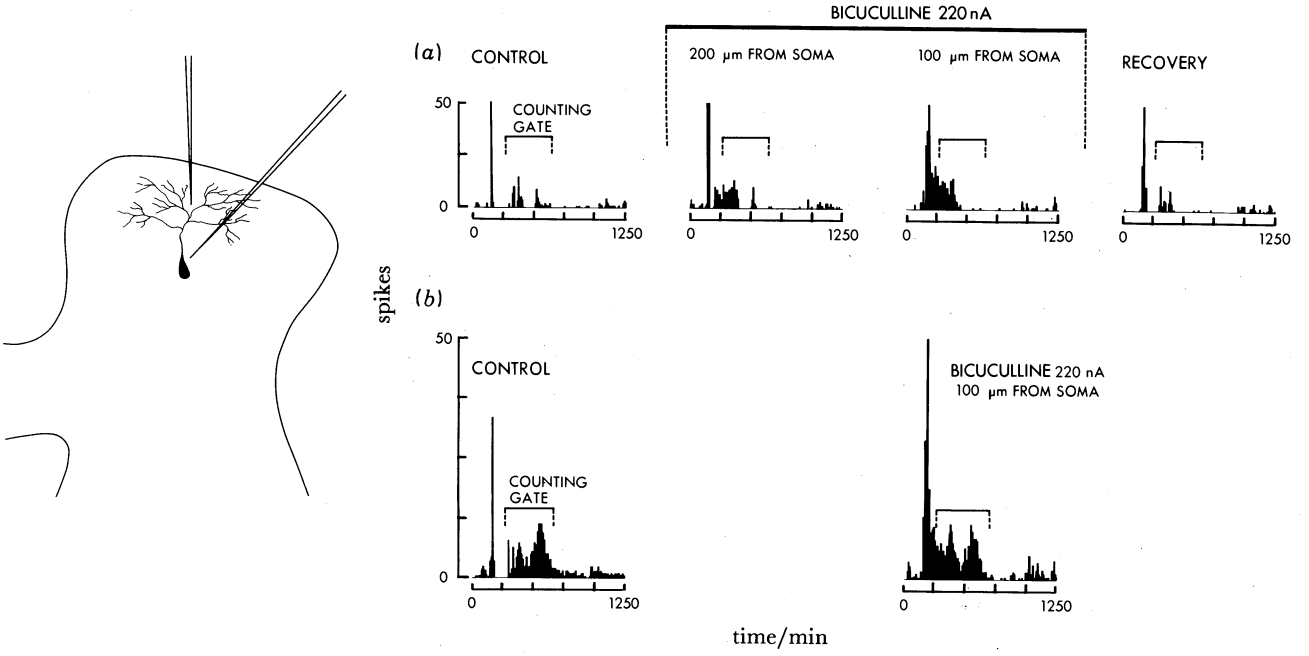

FIGURE 5. Reduction of a segmental but not supraspinal inhibition by bicuculline. (*a*) The records are peristimulus histograms of the excitation of a lamina IV neuron by electrical stimulation of the tibial nerve, with a strength adequate to excite both myelinated and unmyelinated primary afferents. Each histogram is the sum of 16 responses with a memory address dwell time of 10 ms. Because of the slow time base the stimulus artefact is included in the first memory address of the early peak of firing. (*b*) Histograms obtained with block of spinal conduction cephalic to the recording site. Bicuculline was ejected electrophoretically from a seven barrel micropipette which was independent of the recording electrode and the tip was positioned 200 and 100 μm dorsal to the tip of the recording electrode. Note that bicuculline abolished the inhibition of firing between the early and late peaks but did not reduce the increase in the latter produced by spinal cold block. (Reproduced from Duggan *et al.* (1981).)

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Belcher *et al.* (1978) studied inhibition of spontaneous firing of neurons of the dorsal horn produced by electrical stimulation near the raphe of the cat. Bicuculline had no effect on this inhibition but strychnine did reduce inhibition in some neurons, not all of which were excited by noxious cutaneous stimuli.

The involvement of inhibitory amino acids in the transmission of nociceptive information warrants further investigation with the finding that 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol (THIP) is an analgesic in both rodents and man (Hill *et al.* 1981).

3. Monoamines

The monoamines 5-hydroxytryptamine (5-HT) and noradrenaline have been favoured candidates for mediating the effects of supraspinal areas in controlling spinal transmission of nociceptive information. Much of the evidence is indirect. Thus many of the brain stem sites producing analgesia on electrical stimulation have 5-HT containing neurons. These include the dorsal raphe of the midbrain and the nucleus raphe magnus and its lateral extension the nucleus alatus (Watkins *et al.* 1983) in the medulla. Stimulation in the region of the locus coeruleus, which contains catecholamine-containing cells, also produces analgesia (Segal & Sandberg 1977). The problem with these experiments is that it is not possible to activate selectively one group of neurons by electrical stimulation in the brain. Indeed in the case of the medullary raphe the studies of West & Wolstencroft (1977) indicate a wide range of conduction velocities for raphe spinal cells with the implication that the 5-HT containing neurons would be the least excitable to electrical stimulation.

Starting with the studies of Dahlstrom & Fuxe (1964) to more recent work using antisera to 5-HT (Ruda *et al.* 1982; Light *et al.* 1983) the distribution of 5-HT has been interpreted as suggesting a role in the control of nociceptive transmission in the spinal cord. There is a dense plexus of 5-HT containing endings in the upper dorsal horn (Carlsson *et al.* 1964; Oliveras 1977*a*) and recent studies have shown 5-HT within endings making mainly dendritic connections with neurons in this area (Ruda *et al.* 1982; Light *et al.* 1983). By light microscopy, noradrenaline-containing endings are also concentrated in the upper dorsal horn (Dahlstrom & Fuxe 1964; Westlund *et al.* 1982).

(*a*) *Spinal actions of 5-HT and noradrenaline*

Perfusion of the surface of the spinal cord with 5-HT-containing solutions produces analgesia in rodents (Yaksh & Wilson 1979). Noradrenaline is particularly potent in producing analgesia by this route (Reddy & Yaksh 1980). Administering these compounds from micropipettes near single neurons has in general given supportive evidence that these amines may be important in dorsal horn function. The results are particularly interesting with administration in the upper laminae.

Randić & Yu (1976) examined neurons in laminae I and II of the decerebrate cat which were excited by noxious cutaneous stimuli. Iontophoretic 5-HT depressed 70% of these neurons with depression lasting up to 10 min. A prolonged action of 5-HT was also observed by Headley *et al.* (1978) who administered 5-HT in the substantia gelatinosa and observed a selective inhibition of excitation of deeper neurons by noxious cutaneous stimuli. The recent experiments of Todd & Millar (1983) with carbon fibre microelectrodes to record from the small neurons of laminae II found 5-HT mainly excited neurons, again with a prolonged action. They

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suggested that these neurons were inhibitory to deeper cells and were responsible for the inhibition observed by Headley *et al.* (1978). 5-HT was found to depress the spontaneous firing of the majority of spinothalamic neurons of the monkey (Jordan *et al.* 1978). If 5-HT is important in descending controls these experiments suggest that it could do so by directly inhibiting neurons, or indirectly by exciting inhibitory cells.

When administered iontophoretically near neurons of the dorsal horn and trigeminal nucleus, noradrenaline has depressed spontaneous firing and excitation by peripheral stimuli. With neurons excited by both noxious and non-noxious stimuli, noradrenaline has been relatively selective, producing a greater reduction in responses to noxious than to non-noxious stimuli (Headley *et al.* 1978; Belcher *et al.* 1978) and in this respect differs from methionine enkephalin, 5-HT and the amino acids, glycine and GABA (Headley *et al.* 1978). Satoh *et al.* (1979), however, found noradrenaline to be a non-selective depressant. In the substantia gelatinosa, noradrenaline has been the most potent compound tested in selectively reducing the nociceptive responses of deeper neurons of laminae IV and V (Headley *et al.* 1978). As with 5-HT, Todd & Millar (1983) have obtained evidence that this may be produced through excitation of intrinsic neurons of the substantia gelatinosa.

These selective effects of noradrenaline can be explained by a presynaptic action on the terminals of nociceptive afferents and Carstens *et al.* (1981*b*) have obtained evidence for this. Noradrenaline has been shown to hyperpolarize both motoneurons (Engberg & Marshall 1971), cerebellar Purkinje cells (Hoffer *et al.* 1973) and neurons of a slice preparation of the locus coeruleus (Egan *et al.* 1983). Thus it is possible that noradrenaline affects both postsynaptic membrane and fibre terminals when administered from micropipettes.

(*b*) *Antagonists of* 5-*HT and noradrenaline*

When superfused over the spinal cord the analgesia produced by 5-HT is reduced by methysergide and cyproheptadine (Yaksh & Wilson 1979). With iontophoretic administration peripheral antagonists of 5-HT have been of limited use (Haigler & Aghajanian 1974). Griersmith & Duggan (1980), however, showed that the prolonged depressant action of 5-HT in the substantia gelatinosa was blocked by prior administration of methysergide.

The analgesia produced by perfusion of the spinal cord with noradrenalin is reversed by phentolamine (Reddy *et al.* 1980; Kuraishi *et al.* (1979). In the experiments of Egan *et al.* (1983) hyperpolarization of neurons of a slice of the rat locus coeruleus was reduced by both yohimbine and phentolamine added to the superfusate. Administered from micropipettes near neurons of the dorsal horn, however, the effects of noradrenaline have not been consistently reduced by any of the peripheral antagonists of noradrenaline. This creates difficulties in experiments aimed at assigning physiological significance to these effects of noradrenaline and is a puzzling result in view of the findings from intrathecal administration.

(*c*) 5-*HT Antagonists and supraspinal controls*

If descending systems are tonically active then administration of antagonists alone should produce changes consistent with enhanced spinal transmission of nociceptive information. Intrathecal administration of methysergide in amounts adequate to reduce the action of similarly administered 5-HT has produced hyperalgesia in rats (Proudfit & Hammond 1981).

In single neuron studies, however, there are few reports that an antagonist of 5-HT has increased the firing of spinal neurons to peripheral noxious stimuli (Belcher *et al.* 1978; Yezierski

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et al. 1982; Carstens *et al.* 1981*a*; Griersmith *et al.* 1981). The one exception is the study of Guilbaud *et al.* (1973) who observed that LSD 0.01 mg mg^{-1} i.v. increased the spontaneous firing of lamina V neurons of the chloralose anaesthetized cat. In the study of Griersmith *et al.* (1981) tonic inhibition was measured by the cold block technique. Iontophoretic administration of methysergide near, and dorsal to, the neurons studied failed to reduce this inhibition. Intravenous methylsergide did reduce tonic descending inhibition but far from increasing responses they were depressed by the drug because of a powerful direct spinal action.

When studying inhibition from electrical stimulation near 5-HT containing neurons the results have been variable. Stimulation near the medullary raphe has not been consistently antagonized by purported 5-HT antagonists (Belcher *et al.* 1978; Griersmith *et al.* 1981; Yezierski *et al.* 1982). Inhibition from stimulation near the dorsal raphe of the midbrain has been reduced by systemic LSD (Guilbaud *et al.* 1973) and from stimulation in the periventricular grey by methysergide (Carstens *et al.* 1981*a*; Yezierski *et al.* 1982). It was suggested that stimulation in the periventricular grey selectively excited 5-HT containing and spinally projecting neurons of the medullary raphe and, since this is not possible with electrical stimulation in the medullary raphe itself, 5-HT antagonists would be more successful in reducing inhibition from electrical stimulation of the p.a.g. than of the medullary raphe (Yezierski *et al.* 1982). Perhaps supporting this is the report of Chitour *et al.* (1982) that i.v. cinanserin and metergoline reduced inhibition of dorsal horn neurons of the rat by remote noxious stimuli (diffuse noxious inhibitory control, d.n.i.c.). This suggests that d.n.i.c. is mediated by 5-HT containing neurons of the medullary raphe. It was proposed that 5-HT antagonists were more successful in reducing this inhibition than that from stimulation of the raphe because the former process selectively excited 5-HT containing neurons.

A major problem in interpreting these results is that the site of action of the drugs reducing an inhibition is not known with systemic administration. For example, systemic LSD has been shown to slow the spontaneous firing of some 5-HT containing neurons (Aghajanian *et al.* 1972) and this changed excitability could result in a reduced number of neurons being excited by a stimulus (electrical or natural) thus resulting in a reduction in an associated inhibition. Griersmith *et al.* (1981) found that methysergide in doses of 1–2 mg kg^{-1} was such a depressant at the spinal level that it was not possible to interpret effects on supraspinal inhibition as necessarily resulting from antagonism of the effects of 5-HT released from spinal terminals of descending fibres.

(*d*) *Noradrenaline and descending controls*

A major difficulty in assigning a physiological role to the actions of noradrenaline is that no synaptic event relevant to control of nociception has been shown to be reduced by a selective noradrenergic antagonist. There are reports of inhibition at other sites reduced by noradrenergic antagonists. These include inhibition of cerebellar Purkinje cells by stimulation near the locus coeruleus (Hoffer *et al.* 1973), and presumed recurrent inhibition of locus coeruleus neurons (Egan *et al.* 1983). A distributing report is that of Hodge *et al.* (1983). Thus while inhibition of the responses of dorsal horn neurons of the cat to both noxious and non-noxious cutaneous stimuli was produced by stimulation near the locus coeruleus, this was unchanged by prior treatment with reserpine or intrathecal administration of 6-hydroxydopamine sufficient to deplete noradrenaline levels to less than 10% of normal.

While lesions which reduce tonic-descending inhibition are near catecholamine containing

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neurons of the caudal medulla, the few experiments which have examined the possibility that noradrenaline is released by the relevant descending fibres have not supported this hypothesis. Depletion of catecholamines in the cat by reserpine administration did not reduce tonic inhibition of the nociceptive responses of dorsal horn neurons in the experiments of Jurna & Grossman (1976) and Soja & Sinclair (1983).

References

Aghajanian, G. K., Haigler, H. J. & Bloom, F. E. 1972 Lysergic acid diethylamide and serotonin: direct actions on serotonin-containing neurons in rat brain. *Life Sci.* **11**, 615–622.

Aronin, N., Di Figlia, M., Liotta, A. S. & Martin, J. B. 1981 Ultra-structural localization and biochemical features of immunoreactive leu-enkephalin in monkey dorsal horn. *J. Neurosci.* **1**, 561–577.

Belcher, G., Ryall, R. W. & Schaffner, R. 1978 The differential effects of 5-hydroxytryptamine, noradrenaline and raphé stimulation on nociceptive and non-nociceptive dorsal horn interneurones in the cat. *Brain Res.* **151**, 307–321.

Bennett, G. J., Ruda, M. A., Gobel, S. & Dubner, R. 1982 Enkephalin immunoreactive stalked cells and lamina IIb islet cells in cat substantia gelatinosa. *Brain Res.* **240**, 162–166.

Bernatzky, G., Doi, T. & Jurna, I. 1983 Effects of intrathecally administered pentobarbital and naloxone on the activity evoked in ascending axons of the rat spinal cord by stimulation of afferent A and C fibres. Further evidence for a tonic endorphinergic inhibition in nociception. *Arch. Pharmac.* **323**, 211–216.

Boureau, F., Willer, J. C. & Danthier, C. 1978 Study of naloxone in normal awake man: effects on spinal reflexes. *Neuropharmacology* **17**, 565–568.

Brown, A. G. 1981 *Organization in the spinal cord.* Berlin: Springer-Verlag.

Carlsson, A. B., Falck, B., Fuxe, K. & Hillarp, N. A. 1964 Cellular localization of monoamines in the spinal cord. *Acta physiol. scand.* **60**, 112–119.

Carstens, E., Fraunhoffer, M. & Zimmermann, M. 1981*a* Serotonergic mediation of descending inhibition from midbrain periaqueductal gray, but not reticular formation of spinal nociceptive transmission in the cat. *Pain* **10**, 149–167.

Carstens, E., Klumpp, D., Randić, M. & Zimmermann, M. 1981*b* Effects of iontophoretically applied 5-hydroxytryptamine on the excitability of single primary afferent C and A fibers in the cat spinal cord. *Brain Res.* **220**, 151–158.

Carstens, E., Klumpp, D. & Zimmermann, M. 1979*a* The opiate antagonist, naloxone, does not affect descending inhibition from midbrain of nociceptive spinal neuronal discharges in the cat. *Neurosci. Lett.* **11**, 323–327.

Carstens, E., Yokota, T. & Zimmermann, M. 1979*b* Inhibition of spinal neuronal responses to noxious skin heating by stimulation of mesencephalic periaqueductal gray in the cat. *J. Neurophysiol.* **42**, 558–568.

Catley, D. M., Clarke, R. W. & Pascoe, J. E. 1983 Naloxone enhancement of spinal reflexes in the rabbit. *J. Physiol., Lond.* **339**, 61–73.

Catley, D. M., Clarke, R. W. & Pascoe, J. E. 1984 Post-tetanic depression of spinal reflexes in the rabbit and the possible involvement of opioid peptides. *J. Physiol., Lond.* **352**, 483–493.

Chitour, D., Dickenson, A. H. & Le Bars, D. 1982 Pharmacological evidence for the involvement of serotonergic mechanisms in diffuse noxious inhibitory controls. *Brain Res.* **236**, 329–338.

Chung, J. M., Fang, Z. R., Cargill, C. L. & Willis, W. D. 1983 Prolonged, naloxone-reversible inhibition of the flexion reflex in the cat. *Pain* **15**, 35–53.

Curtis, D. R., Duggan, A. W., Felix, D. & Johnstone, G. A. R. 1971 Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord. *Brain Res.* **32**, 69–96.

Curtis, D. R., Game, C. J. A., Lodge, D. & McCulloch, R. M. 1976 A pharmacological study of Renshaw cell inhibition. *J. Physiol., Lond.* **258**, 227–242.

Curtis, D. R., Hösli, L., Johnston, G. A. R. & Johnston, I. H. 1968 The hyperpolarization of spinal motoneurones by glycine and related amino acids. *Expl Brain Res.* **5**, 235–258.

Curtis, D. R., Lodge, D. & Brand, S. J. 1977 GABA and spinal afferent terminal excitability in the cat. *Brain Res.* **130**, 360–363.

Dahlstrom, A. & Fuxe, K. 1964 Evidence for the existence of monoamine neurones in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neurone system. *Acta physiol. scand.* **64** (Suppl. 247).

Dingledine, R., Iversen, L. L. & Breuker, E. 1978 Naloxone as a GABA antagonist: evidence from iontophoretic receptor, binding and convulsant studies. *Eur. J. Pharmacol.* **47**, 19–28.

Duggan, A. W. & Griersmith, B. T. 1979 Inhibition of the spinal transmission of nociceptive information by supraspinal stimulation in the cat. *Pain* **6**, 149–161.

Duggan, A. W., Griersmith, B. T. & Johnson, S. M. 1981 Supraspinal inhibition of the excitation of dorsal horn neurones by impulses in unmyelinated primary afferents: lack of effect by strychnine and bicuculline. *Brain Res.* **210**, 231–241.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Duggan, A. W., Hall, J. G. & Headley, P. M. 1977*a* Enkephalins and dorsal horn neurones of the cat: effects on responses to noxious and innocuous skin stimuli. *Br. J. Pharmac.* **61**, 399–408.
Duggan, A. W., Hall, J. G., Headley, P. M. & Griersmith, B. T. 1977*b* The effect of naloxone on the excitation of dorsal horn neurones of the cat by noxious and non-noxious cutaneous stimuli. *Brain Res.* **138**, 185–189.
Duggan, A. W., Johnson, S. M. & Morton, C. R. 1981 Differing distributions of receptors for morphine and met^5-enkephalinamide in the dorsal horn of the cat. *Brain Res.* **229**, 379–387.
Duggan, A. W., Morton, C. R., Johnson, S. M. & Zhao, Z. Q. 1984 Opioid antagonists and spinal reflexes in the anaesthetized cat. *Brain Res.* **297**, 33–40.
Duggan, A. W. & Zhao, Z. Q. 1984 Microelectrophoretic administration of naloxone near motoneurones fails to reproduce the effects of systemic naloxone in anaesthetized cats. *Neurosci. Lett.* **45**, 305–310.
Egan, T. M., Henderson, G., North, R. A. & Williams, J. T. 1983 Noradrenaline-mediated synaptic inhibition in rat locus coeruleus neurones. *J. Physiol., Lond.* **345**, 477–488.
Engberg, I. & Marshall, K. C. 1971 Mechanism of noradrenaline hyperpolarization in spinal cord motoneurones of the cat. *Acta physiol. scand.* **83**, 142–144.
Fields, H. L., Basbaum, A. I., Clanton, C. H. & Anderson, S. D. 1977 Nucleus raphé magnus inhibition of spinal cord dorsal horn neurones. *Brain Res.* **126**, 441–453.
Fitzgerald, M. & Woolf, C. J. 1980 The stereospecific effect of naloxone on rat dorsal horn neurones: inhibition in superficial laminae and excitation in deeper laminae. *Pain* **9**, 293–306.
Goldfarb, J. & Hu, J. W. 1976 Enhancement of reflexes by naloxone in spinal cats. *Neuropharmacology* **15**, 785–792.
Griersmith, B. T. & Duggan, A. W. 1980 Prolonged depression of spinal transmission of nociceptive information by 5HT administered in the substantia gelatinosa: antagonism by methysergide. *Brain Res.* **187**, 231–236.
Griersmith, B. T., Duggan, A. W. & North, R. A. 1981 Methysergide and supraspinal inhibition of the transmission of nociceptive information in the anaesthetized cat. *Brain Res.* **204**, 147–158.
Gruol, D. L., Barker, J. L. & Smith, T. G. 1980 Naloxone antagonism of GABA-evoked membrane polarizations in cultured mouse spinal cord neurones. *Brain Res.* **198**, 323–332.
Guilbaud, G., Besson, J.-M., Oliveras, J. L. & Liebeskind, J. C. 1973 Suppression by LSD of the inhibitory effect exerted by dorsal raphé stimulation on certain spinal cord interneurones in the cat. *Brain Res.* **61**, 417–422.
Guilbaud, G., Oliveras, J. L., Giesler, G. & Besson, J.-M. 1977 Effects induced by stimulation of the centralis inferior nucleus of the raphé on dorsal horn interneurones in cat's spinal cord. *Brain Res.* **126**, 355–360.
Haigler, H. J. & Aghajanian, G. K. 1974 Peripheral serotonin antagonists: failure to antagonize serotonin in brain areas receiving a prominent serotonin input. *J. Neural Trans.* **35**, 257–273.
Hall, J. G., Duggan, A. W., Morton, C. R. & Johnson, S. M. 1982 The location of neurones tonically inhibiting dorsal horn neurones of the cat. *Brain Res.* **244**, 215–222.
Handwerker, H. O., Iggo, A. & Zimmermann, M. 1975 Segmental and supraspinal actions on dorsal horn neurones responding to noxious and non-noxious skin stimuli. *Pain* **1**, 147–166.
Headley, P. M., Duggan, A. W. & Griersmith, B. T. 1978 Selective reduction by noradrenaline and 5-hydroxytryptamine of nociceptive responses of cat dorsal horn neurones. *Brain Res.* **145**, 185–189.
Hill, R. C., Maurer, H., Buescher, H. & Roemer, D. 1981 Analgesic properties of the GABA-minetic THIP. *Eur. J. Pharmac.* **69**, 221–225.
Hodge, C. J., Apkarian, A. V., Stevens, R., Vogelsang, G. & Wisnicki, H. J. 1981 Locus coeruleus modulation of dorsal horn unit responses to cutaneous stimulation. *Brain Res.* **204**, 415–420.
Hodge, C. J., Apkarian, A. V., Stevens, R. T., Vogelsang, G. D., Brown, O. & Franck, J. I. 1983 Dorsolateral pontine inhibition of dorsal horn cell responses to cutaneous stimulation: lack of dependence on catecholaminergic systems in cat. *J. Neurophysiol.* **50**, 1220–1235.
Hoffer, B. J., Siggins, G. R., Oliver, A. P. & Bloom, F. E. 1973 Activation of the pathway from locus coeruleus to rat cerebellar Purkinje neurones: pharmacological evidence of noradrenergic central inhibition. *J. Pharmac. exp. Ther.* **184**, 553–569.
Hökfelt, T., Ljungdahl, A., Terenius, L., Elde, R. & Nilsson, G. 1977 Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: enkephalin and substance P. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3081–3085.
Hughes, J. W. 1975 Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.* **88**, 295–308.
Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, G. A. & Morris, H. R. 1975 Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature, Lond.* **258**, 577–579.
Hunt, S. P., Kelly, J. S. & Emson, P. C. 1980 The electron microscopic localization of methionine-enkephalin within the superficial layers (I and II) of the spinal cord. *Neuroscience* **5**, 1871–1890.
Jordan, L. M., Kenshalo, D. R., Martin, R. F., Haber, L. H. & Willis, W. D. 1978 Depression of primate spino-thalamic tract neurons by iontophoretic application of 5-hydroxytryptamine. *Pain* **5**, 135–142.
Jurna, I. 1980 Effect of stimulation in the periaqueductal grey matter on activity in ascending axons of the rat spinal cord: selective inhibition of activity evoked by Aδ and C fibre stimulation and failure of naloxone to reduce inhibition. *Brain Res.* **196**, 33–42.
Jurna, I. & Grossman, W. 1976 The effect of morphine on the activity evoked in ventrolateral tract axons of the cat spinal cord. *Expl Brain Res.* **24**, 473–484.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Kosterlitz, H. 1983 Opioid peptides and pain – an update. In *Advances in pain research and therapy* (ed. J. J. Bonica, U. Lindblom & I. Iggo), Vol. 5, pp. 199–208. New York: Plenum Press.

Kuraishi, Y., Harada, Y. & Takagi, H. 1979 Noradrenaline regulation of pain-transmission in the spinal cord mediated by α-adrenoceptors. *Brain Res.* **174**, 333–336.

Liebeskind, J. C., Guilbaud, G., Besson, J. M. & Oliveras, J. L. 1973 Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioural observations and inhibitory effects on spinal cord interneurones. *Brain Res.* **50**, 441–446.

Light, A. R., Kavookjian, A. M. & Petrusz, P. 1983 The ultrastructure and synaptic connections of serotonin – immuno reactive terminals in spinal laminae I and II. *Somatosens. Res.* **1**, 33–50.

Light, A. R. & Perl, E. R. 1979 Re-examination of the dorsal root projection to the spinal dorsal horn including observations on the differential termination of coarse and fine fibres. *J. comp. Neurol.* **186**, 117–132.

Lodge, D., Curtis, D. R. & Brand, S. J. 1977 A pharmacological study of the inhibition of ventral group I a-excited spinal interneurones. *Expl Brain Res.* **29**, 97–105.

Lovick, T. A. & Wolstencroft, J. H. 1983 Actions of GABA, glycine, methionine-enkephalin and β-endorphin compared with electrical stimulation of nucleus raphé magnus on responses evoked by tooth pulp stimulation in the medial reticular formation in the cat. *Pain* **15**, 131–144.

McLaughlin, B. J., Barber, R., Saito, K., Roberts, E. & Wu, J. Y. 1975 Immunocytochemical localisation of glutamate decarboxylase in rat spinal cord. *J. comp. Neurol.* **164**, 305–322.

Melzack, R. & Wall, P. D. 1965 Pain mechanisms – a new theory. *Science, Wash.* **150**, 971–979.

Morton, C. R., Johnson, S. M. & Duggan, A. W. 1983 Lateral reticular regions and the descending control of dorsal horn neurones of the cat: selective inhibition by electrical stimulation. *Brain Res.* **275**, 13–21.

Morton, C. R., Zhao, Z. Q. & Duggan, A. W. 1982 A function of opioid peptides in the spinal cord of the cat: intracellular studies of motoneurones during naloxone administration. *Neuropeptides* **3**, 83–91.

Murase, K., Nedeljkov, V. & Randić, M. 1982 The actions of neuropeptides on dorsal horn neurons in the rat spinal cord slice preparation: an intracellular study. *Brain Res.* **234**, 170–176.

Nyberg, G., Yaksh, T. L. & Terenius, L. 1983 Opioid activity released from cat spinal cord by sciatic nerve stimulation. *Life Sci.* **33**, 17–20 (Suppl. 1).

Oliveras, J. L., Besson, J. M., Guilbaud, G. & Liebeskind, J. C. 1974 Behavioural and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. *Expl Brain Res.* **20**, 32–44.

Oliveras, J. L., Bourgoin, S., Hery, F., Besson, J. M. & Hamon, M. 1977*a* The topographical distribution of serotonergic terminals in the spinal cord of the cat: biochemical mapping by the combined use of microdissection and microassay procedures. *Brain Res.* **138**, 393–406.

Oliveras, J. L., Hosobuchi, Y., Redjemi, F., Guilbaud, G. & Besson, J. M. 1977*b* Opiate antagonist, naloxone, strongly reduces analgesia induced by stimulation of a raphé nucleus (centralis inferior). *Brain Res.* **120**, 221–229.

Proudfit, H. K. & Hammond, D. L. 1981 Alterations in nociception thresholds and morphine-induced analgesia produced by intrathecally administered amine antagonists. *Brain Res.* **218**, 393–399.

Przewlocki, P., Gramsch, C., Pasi, A. & Herz, A. 1983 Characterization and localization of immunoreactive dynorphin, α-neo-endorphin met-enkephalin and substance P in human spinal cord. *Brain Res.* **280**, 95–104.

Randić, M. & Miletic, V. 1978 Depressant actions of methionine-enkephalin and somatostatin on cat dorsal horn neurones activated by noxious stimuli. *Brain Res.* **152**, 196–202.

Randić, M. & Yu, H. H. 1976 Effects of 5-hydroxytryptamine and bradykinin in cat dorsal horn neurones activated by noxious stimuli. *Brain Res.* **111**, 197–203.

Reddy, S. V. R., Maderdrut, J. L. & Yaksh, T. L. 1980 Spinal cord pharmacology of adrenergic agonist-mediated antinociception. *J. Pharmac. exp. Ther.* **213**, 525–533.

Reddy, S. V. R. & Yaksh, T. L. 1980 Spinal noradrenergic terminal system mediates antinociception. *Brain Res.* **189**, 391–402.

Reynolds, D. V. 1969 Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science, Wash.* **164**, 444–445.

Rivot, J. P., Chaouch, A. & Besson, J. M. 1979 The influence of naloxone on the C-fiber response of dorsal horn neurons and their inhibitory control by raphé magnus stimulation. *Brain Res.* **176**, 355–364.

Roppolo, J. T., Booth, A. M. & de Groat, W. C. 1983 The effects of naloxone on the neural control of the urinary bladder of the cat. *Brain Res.* **264**, 355–358.

Ruda, M. A. 1982 Opiates and pain pathways: demonstration of enkephalin synapses on dorsal horn projection neurons. *Science, Wash.* **215**, 1523–1525.

Ruda, M. A., Coffield, J. & Steinbusch, H. W. M. 1982 Immunocytochemical analysis of serotonergic axons in laminae I and II of the lumbar spinal cord of the cat. *J. Neurosci.* **2**, 1660–1671.

Sastry, B. R. & Goh, J. W. 1983 Actions of morphine and met-enkephalinamide on nociceptor driven neurones in substantia gelatinosa and deeper dorsal horn. *Neuropharmacology* **22**, 119–122.

Satoh, M., Kawajiri, S.-T., Ukai, Y. & Yamamoto, M. 1979 Selective and non-selective inhibition by enkephalin and noradrenaline of nociceptive responses of lamina V type neurons in the spinal dorsal horn of the rabbit. *Brain Res.* **177**, 384–387.

Segal, M. & Sandberg, D. 1977 Analgesia produced by electrical stimulation of catecholamine nuclei in the rat brain. *Brain Res.* **123**, 369–372.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Soja, P. & Sinclair, J. G. 1983 Evidence that noradrenaline reduces tonic descending inhibition of cat spinal cord nociceptor-driven neurones. *Pain* **15**, 71–81.

Todd, A. J. & Millar, J. 1983 Receptive fields and responses of iontophoretically applied noradrenaline and 5-hydroxytryptamine of units recorded in laminae I–III of cat dorsal horn. *Brain Res.* **288**, 159–168.

Wall, P. D. 1967 The laminar organization of dorsal horn and effects of descending impulses. *J. Physiol., Lond.* **188**, 403–423.

Watkins, L. R., Young, E. G., Kinscheck, I. B. & Mayer, D. J. 1983 The neural basis of footshock analgesia: the role of specific ventral medullary nuclei. *Brain Res.* **276**, 305–316.

West, D. C. & Wolstencroft, J. H. 1977 Location and conduction velocity of raphé spinal neurons in nucleus raphé magnus and raphé pallidus in the cat. *Neurosci. Lett.* **5**, 147–151.

Westlund, K. N., Bowker, R. M., Ziegler, M. G. & Coulter, J. D. 1982 Descending noradrenergic projections and their spinal terminations. *Prog. Brain Res.* **57**, 219–238.

Willer, J. C. & Bussell, B. 1980 Evidence for a direct spinal mechanism in morphine-induced inhibition of nociceptive reflexes in humans. *Brain Res.* **165**, 57–65.

Yaksh, T. L. & Elde, R. P. 1981 Factors governing release of methionine enkephalin-like immunoreactivity from mesencephalon and spinal cord of the cat *in vivo*. *J. Neurophysiol.* **46**, 1056–1075.

Yaksh, T. L. & Wilson, P. R. 1979 Spinal serotonin terminal system mediates antinociception. *J. Pharmac. exp. Ther.* **208**, 446–453.

Yezierski, R. P., Wilcox, T. K. & Willis, W. D. 1982 The effects of serotonin antagonists on the inhibition of primate spinothalamic tract cells produced by stimulation in nucleus raphé magnus or periaqueductal gray. *J. Pharmac. exp. Ther.* **220**, 266–277.

Yoshimura, M. & North, R. A. 1983 Substantia gelatinosa neurones *in vitro* hyperpolarized by enkephalin. *Nature, Lond.* **305**, 529–530.

Zieglgansberger, W. & Tulloch, I. F. 1979 The effects of methionine and leucine enkephalin on spinal neurones of the cat. *Brain Res.* **167**, 53–64.

Zorman, G., Belcher, G., Adams, J. F. & Fields, H. L. 1982 Lumbar intrathecal naloxone blocks analgesia produced by microstimulation of the ventro-medial medulla in the rat. *Brain Res.* **236**, 77–84.

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