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SUPPRESSION OF TRANSMISSION OF NOCICEPTIVE IMPULSES BY MORPHINE: SELECTIVE EFFECTS OF MORPHINE ADMINISTERED IN THE REGION OF THE SUBSTANTIA GELATINOSA

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1 In spinal cats anaesthetized with α-chloralose, a study was made of the effects of morphine and naloxone, administered electrophoretically from micropipettes, on the responses of dorsal horn neurones to noxious (raising of skin temperature above 45°C) and innocuous (deflection of hairs) peripheral stimuli.

2 Administered near cell bodies, morphine reduced the nociceptive responses of only 2 of 37 cells. Excitation occurred more commonly than depression and abnormalities in action potentials were commonly observed following ejection of morphine. None of these effects of morphine was antagonized by electrophoretically applied naloxone.

3 Administered in the substantia gelatinosa from one micropipette while recording responses of deeper neurones with a second micropipette, morphine reduced the nociceptive responses of 15 of 19 neurones. Firing in response to deflection of hairs was not reduced by morphine. Depression of nociceptive responses by morphine was long lasting (>20 minutes). Naloxone ejected into the substantia gelatinosa or given intravenously in doses as low as 0.1 mg/kg antagonized the effects of morphine. The effectiveness of this dose of intravenous naloxone suggests that the concentrations of morphine in the substantia gelatinosa which reduced nociceptive responses were not unlike those present after analgesic doses of systemic morphine. Naloxone alone, and excitant and depressant amino acids ejected into the substantia gelatinosa had little effect on cell firing.

4 Both the selective action of morphine on nociceptive responses and the reversal of this action by intravenous naloxone suggest that the opiate receptor present in the substantia gelatinosa is relevant to analgesia produced by opiates given systemically.

Introduction

When administered intravenously, morphine impairs spinal transmission of impulses in small, but not large, diameter primary afferents (Wikler, 1945; Koll, Haase, Block & Mühlberg, 1963; McLane & Martin, 1967b; Besson, Wyon-Maillard, Benoist, Conseiller & Hamann, 1973; Le Bars, Menétrey, Conseiller & Besson, 1975; Jurna & Grossman, 1976; Le Bars, Guilbaud, Jurna & Besson, 1976). Since nociceptive fibres are of small diameter (Iggo, 1962; Bessou & Perl, 1969) this spinal action may be a significant component of analgesia produced by morphine, particularly as in man this alkaloid has minimal effects on other sensations such as touch, vibration, vision, hearing and olfaction (Wikler, 1950; Kreuger, 1955). The recent finding that, in rats, restricted superfusion of the spinal cord with morphine produces analgesia localized to the appropriate skin segments supports this suggestion (Yaksh & Rudy, 1976).

In attempting to explain the effects of morphine on the spinal cord, several investigators have ejected morphine and the narcotic antagonist naloxone electrophoretically from micropipettes in the vicinity of spinal neurones. The results have not been uniform. Calvillo, Henry & Neuman (1974) found that, in cats anaesthetized with α-chloralose, morphine depressed the firing of neurones excited by noxious heat but not that of cells unresponsive to this stimulus. Antagonism of this effect by electrophoretic naloxone was, however, observed with only 2 of 7 cells. As the excitation of 'nociceptive' neurones by L-glutamate was also reduced, morphine probably had a post-synaptic depressant effect. In contrast, Dostrovsky & Pomeranz (1973) found that morphine depressed excitation by L-glutamate and L-aspartate, but not the spontaneous and synaptically induced firing of dorsal horn neurones of spinal cats. No distinction was made between cells on the basis of response to noxious stimuli, and these authors have subsequently reported that naloxone does not antagonize this depressant action of morphine (Dostrovsky & Pomeranz, 1976).

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Zieglgänsberger & Bayerl (1976) also found morphine to depress excitation of dorsal horn neurones by L-glutamate, but, in addition, spontaneous firing and responses to tactile stimulation were reduced. Prior ejection of naloxone blocked these actions of morphine. Noxious stimuli were not used in these experiments, and the animals were either decerebrate or anaesthetized with sodium pentobarbitone. Depression of dorsal horn neurones by morphine was not blocked by naloxone in the experiments of Duggan, Davies & Hall (1976) using cats anaesthetized with pentobarbitone. In addition dextrorphan, an inactive isomer of the opiate levorphanol, was a more potent depressant of cell firing than either morphine or levorphanol.

Most of the experiments quoted employed multibarrel micropipettes in which the orifices of recording and drug ejecting pipettes were at the same level. Drug administration would thus be predominantly in the region of cell bodies. In the micropipette assemblies of Zieglgänsberger & Bayerl (1976) the recording pipette projected 10–20 μm beyond the drug administering pipette and hence receptor sites other than those on cell bodies may have been reached.

In the present experiments, morphine and naloxone were administered electrophoretically in two ways. Firstly, from conventional multibarrel micropipettes, and thus in the vicinity of cell bodies of dorsal horn neurones activated by both noxious and non-noxious stimuli to the skin. When it became apparent that the results from this method of administration were not relevant to the effects of systemic opiates it was decided to administer both morphine and naloxone to the dendritic regions of neurones of spinal laminae IV and V. The dendrites of cells of these laminae project into the substantia gelatinosa where contacts are made with primary afferent fibres, descending fibres and axons of the intrinsic neurones of this area (Cajal, 1909; Szentágothai, 1964). Axo-axonic contacts are abundant in the substantia gelatinosa and such contacts are an important element of the 'gate control' theory of pain of Melzack & Wall (1965). The results from these experiments can be related to analgesia produced by morphine. Preliminary observations have been published (Duggan, Hall & Headley, 1976).

Methods

Experiments were performed on 36 cats (2.5–3.5 kg); 32 were anaesthetized with α-chloralose, 50 mg/kg intraperitoneally, 3 were decerebrated by midbrain coagulation under halothane anaesthesia, and the remaining animal received initially an intraperitoneal injection of sodium pentobarbitone, 35 mg/kg.

The spinal cord was exposed by lumbar laminectomy and in all experiments was sectioned at the first lumbar segment. Blood pressure was monitored continuously and experiments were terminated if mean systolic pressure fell below 100 mmHg. Animals were paralysed with gallamine triethiodide and were artificially ventilated with air, end tidal CO_2 levels being maintained at approximately 4%.

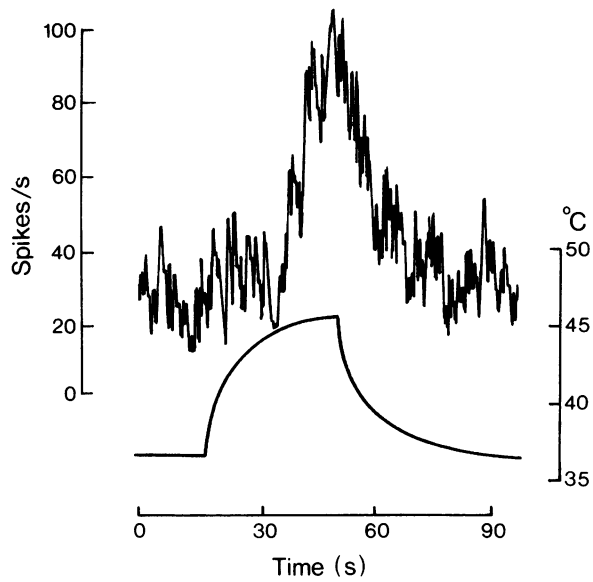

Figure 1 Firing of a lamina IV spinal neurone in response to radiant heat to the fourth left digital foot pad in the cat. Upper record; cell firing measured with a ratemeter. Lower record; skin surface temperature measured with a thermistor placed at the edge of the heated area.

These experiments sought to study quantitatively the effect of morphine on the firing of dorsal horn spinal neurones by impulses in nociceptive and non-nociceptive afferents.

Nociceptive afferents were activated by radiant heat to the left hind limb. There is evidence (Beck, Handwerker & Zimmermann, 1974) that when skin temperature is raised above 45°C the input to the spinal cord from the heated area is almost exclusively from thermal nociceptors. The posterior surface of the left hind limb was shaved, blackened with ink and held in an extended position. Radiant heat from a 45 W lamp was focused with a condenser lens to an area of approximately 3 mm diameter. Skin surface temperature was monitored by a thermistor placed at the edge of the heated area and was displayed on a pen recorder together with cell firing produced by this stimulus. Figure 1 illustrates the relationship between skin surface temperature, measured in this way, and cell firing for neurones included in Results. The firing of this neurone was not increased above the spontaneous level until a skin temperature of 45°C was reached and outlasted the stimulus by approx-

imately 20 seconds. Cells which fired with a latency shorter than that illustrated and those in which firing declined with skin temperatures above 45°C were excluded from analysis. Repeated nociceptive stimuli of this type have been reported to result in increased firing of nociceptors (Perl, Kumazawa, Lynn & Kenins, 1976). Enhanced activation of neurones was uncommon when heat stimuli of 25–30 s were repeated at intervals of 2 minutes.

Innocuous stimulation of skin was produced either by light touch to the shaved and unshaved areas using a camel hair brush, or, when quantitative responses were required, by deflection of hairs by an air jet moved by the armature of a relay activated by a Grass S8 stimulator (pulse frequency 2–4 Hz, mark: space ratio 1:1). The firing produced by these stimuli consisted of a group of action potentials commonly followed by a period of inhibition of spontaneous firing. When used for quantitative studies, the frequency of movement of the air jet was selected not only on the basis of the rapid adaptation of the peripheral receptors (Brown & Iggo, 1967) but also so that the number of evoked action potentials was not diminished by the inhibition following the preceding deflection. The time constant of the recording system (0.5 s) effectively integrated these responses (Figures 3a, 4, 6).

An automatic timer controlled both the filament current to the lamp producing noxious stimulation and the air supply to the relay device. Most results were obtained from cells activated by heat applied to a digital pad and by an air jet directed at adjacent interdigital hairs.

In the first series of experiments extracellular recordings of the firing of dorsal horn neurones were obtained with the 4 M NaCl filled centre barrels of seven barrel micropipettes. The outer barrels contained different combinations of the following compounds to be ejected electrophoretically: L-glutamate Na (0.5 M, pH 7); L-aspartate Na (0.5 M, pH 7.5); DL-homocysteate Na (0.2 M, pH 7); glycine (0.5 M, pH 3); γ-aminobutyric acid (GABA, 0.5 M, pH 3); morphine sulphate (0.07 M); naloxone hydrochloride (0.1 M in water or 0.1 M NaCl); pontamine sky blue (2% in 0.5 M Na acetate). Drugs were thus administered in the region of cell bodies.

In the later experiments, drugs were ejected in the region of the substantia gelatinosa from a seven barrel micropipette assembly, the tip of which was separated from that of a five barrel micropipette, used to record activity of deeper neurones, by a known distance.

The outer barrels of the seven barrel micropipettes contained combinations of the following solutions: morphine sulphate (0.07 M), naloxone hydrochloride (0.1 M), glycine (0.5 M, pH 3), γ-aminobutyric acid (GABA, 0.5 M, pH 3), DL-homocysteate Na (0.2 M, pH 7), L-glutamate Na (0.5 M, pH 7), pontamine sky blue (2% in 0.5 M Na acetate), acid fast green (saturated solution in 2 M NaCl).

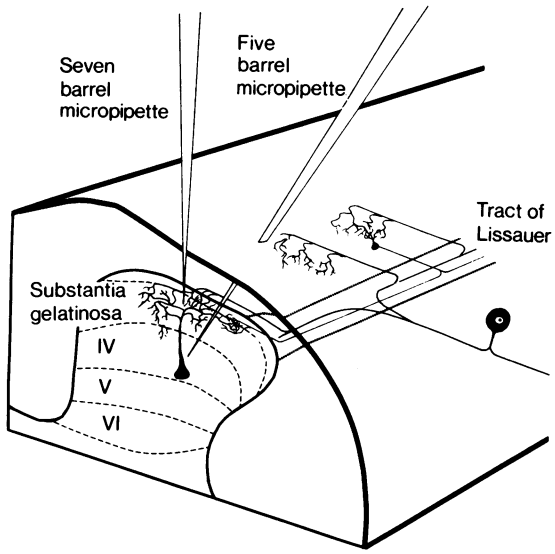

Figure 2 Arrangement of micropipettes for administering drugs in the substantia gelatinosa of the cat while recording the activity of deeper spinal neurones. The dendrites of a lamina IV neurone are shown establishing contacts with terminals of a primary afferent fibre and terminals of a substantia gelatinosa neurone of another segment. The passage of these fibres in the tract of Lissauer is also shown. The angle between the 5 and 7 barrel micropipettes was 18°.

The outer barrels of the five barrel micropipettes contained combinations of DL-homocysteate Na (0.2 M, pH 7), L-glutamate Na (0.5 M, pH 7), glycine (0.5 M, pH 3), and acid fast green (saturated solution in 2 M NaCl). The centre barrels of both types of micropipette were filled with 4 M NaCl for recording purposes.

The procedure used for positioning the two micropipettes independently was as follows. By the use of a stereoscopic microscope, the two micropipettes were first aligned tip to tip, 50–200 μm above the surface of the cord, and the appropriate micromanipulator readings were noted. The seven barrel pipette, with which extracellular activity was also recorded, was then lowered vertically as far as spinal lamina I. Ejection of dye showed that this lamina could be reliably identified by the appearance of cells responding to electrophoretic DL-homocysteate or L-glutamate and to cutaneous stimulation. The electrode was then advanced a further 50–200 μm. Drugs were thus ejected in spinal laminae II and III, the laminae usually accepted as comprising the substantia gelatinosa (Szentágothai, 1964; Scheibel & Scheibel, 1968). The distance of this final site from the convergence point above the cord was then measured. The second pipette, of 5 barrels, angled 18° caudally, was then inserted in the spinal

cord at an appropriate distance rostrally so that it would pass a defined distance vertically below the tip of the substantia gelatinosa pipette. The planned vertical separation of tips ranged from 150 to 400 μm (300 μm was most commonly used) but, because it was exceptional to locate a neurone precisely when this planned separation had been reached, it was decided to study neurones located approximately 100 μm before and up to 500 μm beyond this point. Actual tip separations varied from 150–680 μm with two thirds lying in the 300–500 μm range.

The relationship of the two micropipettes is shown diagramatically in Figure 2.

The relative positions of the two electrode tips were verified for one pair of positions in each experiment by deposition of dye, either acid fast green or pontamine sky blue. Dyespots were subsequently located in 50 μm frozen sections.

Neuronal firing rates, measured with a ratemeter, were displayed continuously on a pen recorder. In later experiments the total number of action potentials evoked by noxious heat and hair deflection was determined with a gated electronic counter. With nociceptive responses the gate duration needed to outlast the stimulus by several seconds whereas with hair deflection the gate and the stimulus were usually of the same duration. Integrals determined in this way are affected by changes in spontaneous firing and since the gate durations were different for the nociceptive and non-nociceptive responses the two will be unequally affected. This was allowed for by subtracting from each response a number determined by the gate duration and the mean spontaneous firing just prior to each response (see Figure 6). In earlier experiments where response integrals were not determined response amplitude, in spikes per s, was plotted with respect to time.

Results

A. Morphine and naloxone administered in the region of cell bodies

An attempt was made to sample neurones in several areas of the dorsal horn. Table 1 summarizes the responses of neurones of the various layers of Rexed to noxious and innocuous skin stimulation.

In agreement with others (Handwerker, Iggo & Zimmermann, 1975; Price & Browe, 1975) the majority of cells were excited by both types of stimulus and hence the terms 'nociceptive' and 'non-nociceptive' have not been used to categorize neurones.

(i) Effects of electrophoretic morphine.

(a) Spontaneous firing. Ejected with currents of up to 80 nA, morphine had no effect on the spontaneous firing of the five neurones of lamina I, excited 2 of 14 neurones of lamina IV and excited 5 of 13 neurones of lamina V. Inhibition of spontaneous firing was not seen with cells of these superficial laminae. In laminae VI and VII, 4 neurones were inhibited, 4 excited and 5 unaffected. No correlation was found between the responses of these neurones to skin stimulation and the effect of morphine on spontaneous firing.

During these experiments, the ejecting current of morphine was increased in a stepwise manner till abnormalities in action potential amplitude and configuration appeared (Duggan & Curtis, 1972). This occurred with currents of the order of 60 to 80 nA. Excitation by morphine, which occurred with the higher ejecting currents, took the form of firing in groups of action potentials (bursts) and thus differed from that previously observed with Renshaw cells (Davies & Duggan, 1974).

(b) Firing by noxious and innocuous skin stimulation. Morphine, ejected with increasing currents of up to 70 nA, depressed the nociceptive responses of only 2 of 37 cells tested. With 9 cells the response was enhanced together with an increase in spontaneous firing. To test for possible selectivity in the effects of morphine towards nociceptive responses, 6 cells were activated alternately by noxious heat to an area of the left hind limb and by a moving air jet directed at adjacent hairs. In no case was the response to either sensory modality significantly reduced during morphine ejection. With 3 neurones responses to both

Table 1 Responses to skin stimulation of dorsal horn neurones classified by laminae of Rexed

		Responses to skin stimulus					
		Excited by noxious heat only	*Excited by innocuous mechanical only*	*Excited by noxious and innocuous*	*Inhibited by noxious heat*	*Not affected*	*Totals*
I	I			5			5
Rexed	IV		1	11	1	1	14
Lamina	V			12		1	13
	VI & VII	1	2	4	4	2	13
Totals		1	3	32	5	4	45

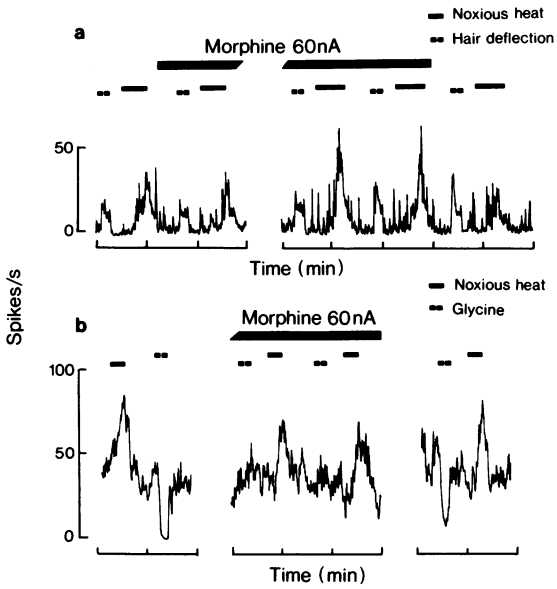

Figure 3 (a) Effect of electrophoretic morphine on the excitation of a lamina V spinal neurone of the cat by noxious heat to the fourth digital pad of the left hind limb and by deflection of adjacent hairs by a moving air jet. Morphine was ejected for a total of 7 minutes. (b) Failure of concentrations of morphine, adequate to antagonize depression of cell firing by glycine, to reduce activation of a lamina VI spinal neurone by noxious heat. The three sets of records are, from left to right, before, during and 2 min after ejection of morphine. Noxious heat was applied to the fourth digital pad of the left hind limb. Morphine was ejected for 8 min before the period illustrated.

stimuli were enhanced and this was associated with irregular firing in bursts (Figure 3a).

Morphine blocks the inhibitory action of glycine on spinal neurones (Curtis & Duggan, 1969). As a further safeguard that the essentially negative results presented above were not due to failure to eject an adequate amount of morphine, on five cells depression of firing by glycine was alternated with excitation by noxious heat. In all cases currents of morphine more than twice those adequate to block the action of glycine had no significant effect on activation by noxious heat (Fig. 3b).

(c) Excitation by L-glutamate. The effects of morphine on excitation by L-glutamate were variable. Of 13 cells excited both by noxious and innocuous stimulation of skin, morphine (ejecting currents of up to 60 nA), enhanced the response to glutamate of 5 cells and this was accompanied by increased spontaneous firing. A further increase in excitation by L-glutamate occurred immediately after the termination of morphine ejection. Morphine depressed the excitation by L-glutamate of 7 cells but this was associated with abnormalities in action potential amplitude and configuration.

(ii) Effects of electrophoretic naloxone. Ejected with increasing currents of up to 60 nA, naloxone had no significant effect on spontaneous firing and responses to skin stimulation. When morphine had an effect on cell firing, either excitation or depression, no antagonism by naloxone was observed (10 neurones). Attention needs, however, to be drawn to an apparent antagonism of morphine which occurred in these tests.

Naloxone reduced the excitation by L-glutamate of 4 of 7 cells, but, following the termination of ejection, with 5 neurones the responses to L-glutamate were enhanced by up to 100% above control values for as long as 10 minutes. When responses to L-glutamate had been depressed by morphine, simultaneously administered naloxone either had no effect or further reduced the action of the amino acid. When the ejection of naloxone was terminated, however, (that of morphine continuing) excitation by L-glutamate was greater than that prior to ejection of naloxone. Such an action cannot be interpreted in terms of antagonism between morphine and naloxone as it occurred in the absence of morphine.

B. Morphine and naloxone ejected in the region of the substantia gelatinosa

(i) Distribution of cells studied. Cells were chosen on the basis of distance from the drug-administering micropipette and reproducibility of responses to both nociceptive and non-nociceptive stimuli. Twenty one were in Rexed lamina IV, and nine in lamina V.

(ii) Morphine and responses to noxious heat and deflection of hairs. Morphine, ejected in the region of the substantia gelatinosa with currents of 30–250 nA, reduced the response to noxious heat of 12 of 15 cells activated alternately by deflection of hairs and noxious heat to the appropriate part of the hind limb. With 3 neurones neither response was reduced. Of the 12 neurones with which nociceptive activation was reduced by morphine, the non-nociceptive response was unchanged with 7 cells and increased above controls with 5. With a further 4 cells nociceptive responses alone were studied and morphine reduced these in 3.

Unlike the experiments on cell bodies, where ejecting currents of morphine of the order of 60–80 nA caused abnormalities in spike configuration, when morphine was administered in the substantia gelatinosa, the relatively large currents (mean 146 nA $\pm$ 62 nA s.d.) needed to reduce nociceptive responses did not affect action potentials.

Depression of nociceptive activation by morphine

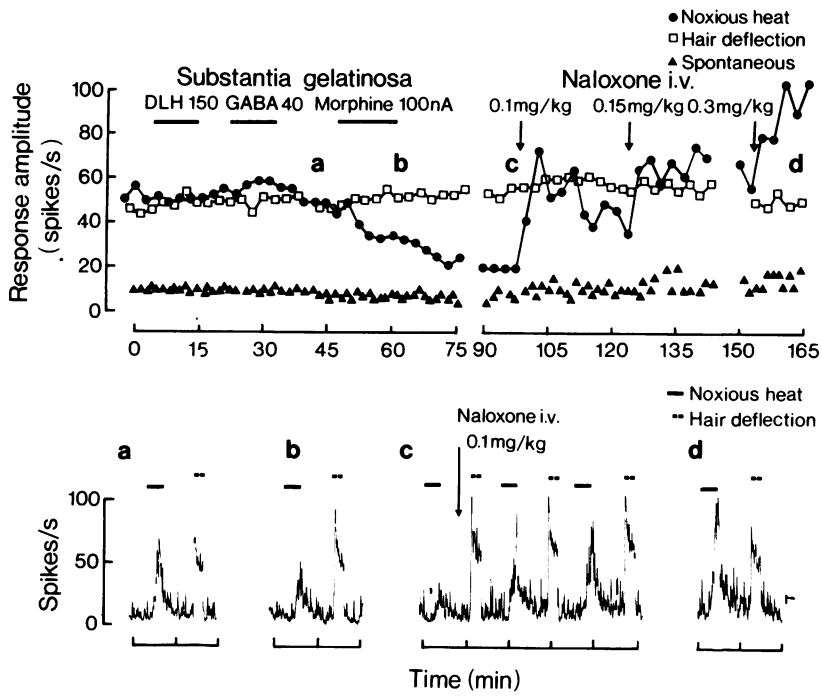

Figure 4 Depression by morphine, ejected in the substantia gelatinosa, of nociceptor but not mechanoreceptor activation of a dorsal horn neurone and reversal of this effect by intravenous naloxone in the cat. This neurone was located in spinal lamina IV and was activated by noxious heat to the third digital pad of the left hind limb and by deflection of adjacent hairs; separation between drug administering and recording micropipettes, 230 μm. The lower tracings are pen recordings of cell firing; the upper record is a graph of response amplitude in spikes per second with respect to time for the noxious and innocuous stimuli. As the response to deflection of hairs consisted of an initial burst followed by a slow decline in cell firing, the firing frequency present mid-way through the stimulus period has been plotted. Spontaneous firing is also plotted. The bars above the graph indicate the times of ejection of DL-homocysteate (DLH), γ-aminobutyric acid (GABA) and morphine in the substantia gelatinosa. Ejection currents are in nanoamperes (nA). The letters a, b, c and d indicate the times at which the corresponding lower traces were recorded.

without effect on mechanoreceptor-induced firing is shown in Figure 4. Ejection of DL-homocysteate and GABA in the substantia gelatinosa had no effect on the firing of this neurone. Compared with control observations (Figure 4a), those recorded after 15 min of morphine ejection into the substantia gelatinosa (Figure 4b) show approximately a 40% reduction in firing to noxious heat but no change in the response to hair deflection. With this and other neurones, further depression of nociceptive responses occurred following cessation of morphine ejection (Figure 4c).

Figure 4 also shows that, on this neurone, spontaneous firing was not depressed concomitantly with nociceptor-induced firing, an observation also made with 9 other neurones (see Figure 6). With 4 cells, however, both spontaneous firing and nociceptive responses were reduced in parallel, a result illustrated in Figure 5. With the remaining neurone, the nociceptive responses of which were reduced by morphine, spontaneous firing was increased.

The time course of the effects of morphine ejected in the substantia gelatinosa showed differences from those usually observed when drugs are administered electrophoretically near cell bodies. Firstly, there was considerable variation in the time to onset of the reduction of nociceptive activation (4–35 minutes). Even when these times were corrected to effective doses of morphine (total charge in microcoulombs passed through the morphine containing barrel up to the time of reduction of nociceptive responses) the scatter was considerable: the mean effective dose was 102 μC ± 79 s.d. This scatter probably represents in part the variation in the positioning of the drug administering pipette with respect to the receptors affecting each neurone.

Secondly, the reduction of nociceptive activation by

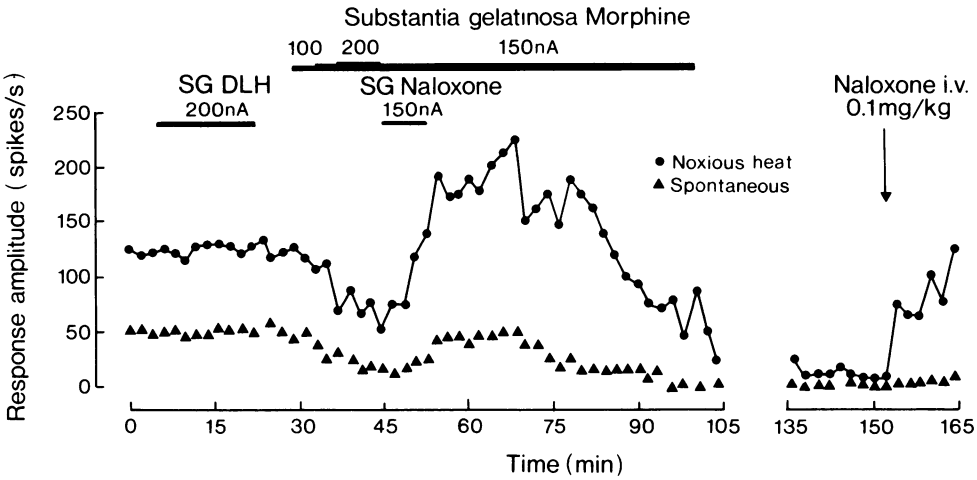

Figure 5 Prolonged effect of morphine, ejected in the substantia gelatinosa, in reducing nociceptive activation of a dorsal horn neurone and reversal of this effect by naloxone administered both electrophoretically and intravenously in the cat. Lack of effect by DL-homocysteate is also shown. The neurone was located in spinal lamina IV and activated by noxious heat to the fourth digital pad of the left hind limb; separation of tips of drug administering and recording micropipettes 200 μm. Response amplitude and spontaneous firing are plotted with respect to time. The times of ejection of DL-homocysteate (DLH), morphine and naloxone are indicated by bars above the graph.

morphine was long lasting but, because its abrupt reversal by intravenous naloxone was considered necessary evidence of specificity, the duration of action was not determined with any accuracy. In five experiments depression by morphine was still unchanged at 12, 17, 21, 50 and 56 min after cessation of morphine ejection into the substantia gelatinosa.

Figures 4 and 5 illustrate results from two such experiments. In each case both the prolonged control observations and the abrupt increase in firing by noxious heat which followed intravenous naloxone suggest strongly that morphine was still active in reducing nociceptive responses 40–50 min after cessation of ejection in the substantia gelatinosa.

(iii) Reversal by naloxone

(a) Electrophoretic naloxone. Naloxone, ejected electrophoretically with currents of 30–200 nA in the region of the substantia gelatinosa from the same pipettes used to administer morphine, reversed the depression of nociceptive responses by morphine on all 13 cells tested. In these experiments naloxone ejection was added to that of morphine, after the latter drug had reduced nociceptive responses, and morphine ejection was continued beyond that of naloxone until nociceptive activation had once more been reduced (Figure 5). There were two types of result. In the first (4 neurones) naloxone restored nociceptive responses to control levels with no significant effect on non-nociceptive responses or spontaneous firing. In the second group (9 neurones) naloxone not only antagonized the effect of morphine but enhanced nociceptive responses to values well above those of controls (Figures 5 and 6). This was accompanied by an increase in cell excitability as shown by increased spontaneous firing and non-nociceptive responses when the latter were tested (7 neurones). As Figure 6 shows, however, the increases in nociceptive responses were considerably greater than the changes in the other firing parameters.

On each neurone the ejecting current of naloxone necessary to antagonize the action of morphine was approximately equal to the current of morphine necessary to reduce nociceptive responses (see Figures 5 and 6). As Figure 5 illustrates, large ejecting currents of morphine for prolonged periods were required to reduce responses to levels similar to those present prior to naloxone ejection.

(b) Intravenous naloxone. When administered intravenously after morphine ejection was terminated, naloxone abruptly reversed the depression of nociceptive activation by morphine in 4 of 5 experiments. In three experiments the first dose of naloxone was effective (0.1, 0.3 and 0.3 mg/kg) and in the remaining one, two doses of 0.3 mg/kg were necessary. In 3 of these 4 experiments, subsequent doses of intravenous naloxone increased firing by

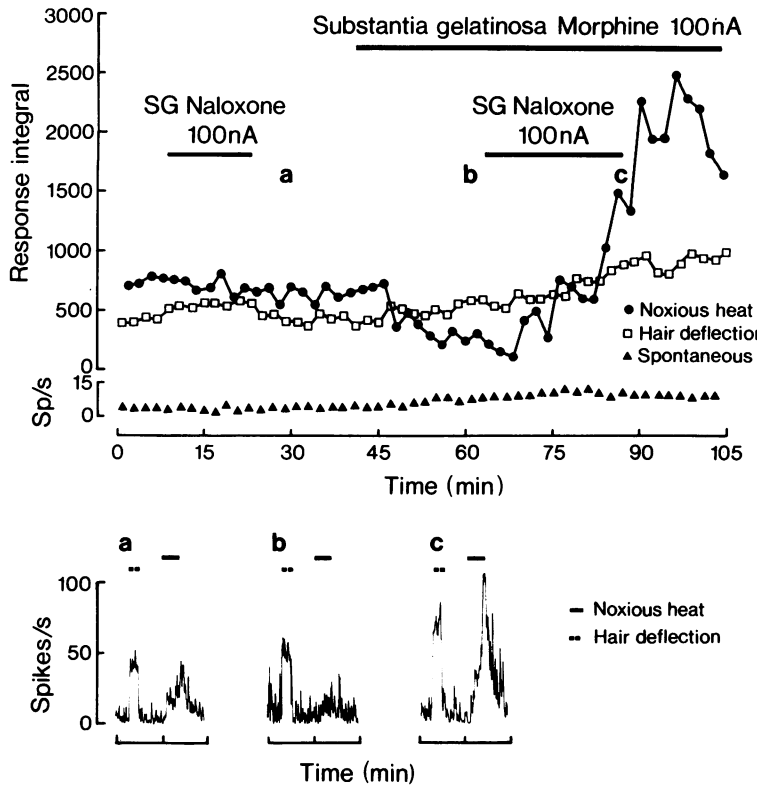

Figure 6 Contrasting effects of naloxone ejected alone in the substantia gelatinosa and naloxone ejected after morphine, on the firing of a dorsal horn neurone in the cat. This neurone was located in lamina IV and activated by noxious heat to the fifth digital pad of the left hind limb and deflection of adjacent hairs; separation between drug administering and recording micropipettes, 240 μm. Upper record – a graph of the total number of action potentials evoked by each stimulus (response integral) with respect to time. These counts have been corrected for spontaneous firing (gate duration for the noxious stimulus, 40 s; for the innocuous stimulus, 15 s). Spontaneous firing rate is also plotted. Lower tracings – cell firing measured with a rate meter. The letters a, b and c refer to the times on the graph at which the records illustrated were observed.

noxious heat to levels above those of control observations and this was accompanied by a lesser increase in spontaneous firing (Figure 4d).

Administered intravenously during the ejection of morphine, naloxone (0.5–1.2 mg/kg) antagonized the reduction of nociceptive responses by morphine in 3 of 6 experiments. All of these results were obtained from cells on which the effects of morphine had previously been antagonized by electrophoretic naloxone and on which prolonged ejection of morphine was necessary to reduce responses below control levels. In the experiments in which intravenous naloxone had no effect, the approximate amounts of morphine previously ejected were 150 nA for 90 min, 200 nA for 105 min and 200 nA for 150 minutes. In those in which intravenous naloxone reversed the effects of morphine the ejecting currents of morphine were 200 nA for 120 min, 30 nA for 70 min and 60 nA for 90 minutes.

(iv) Effects of naloxone alone. Naloxone, amongst the opiate antagonists, is said to exhibit the least agonist activity (Blumberg & Dayton, 1972). In two thirds of the present experiments, naloxone, administered electrophoretically after morphine, did not merely antagonize the effects of morphine but raised the three types of firing studied, spontaneous, nociceptive and non-nociceptive, above control levels (Figure 6). Hence it became necessary to test this substance for possible excitant effects *per se* when administered in the region of the substantia gelatinosa.

Twelve cells were studied in this way, 8 in lamina IV and 4 in lamina V.

Ejected with currents and times similar to those used to reverse the effects of morphine, naloxone had no effect on the firing of 9 neurones. With 2 cells the three parameters (spontaneous firing and the responses to both noxious and innocuous skin stimuli) were elevated just above control values during

naloxone ejection and with one cell a similar effect was observed on spontaneous and nociceptive-induced firing, the only two firing patterns studied.

In no instance were the effects of naloxone, administered alone, similar to those observed when this substance was given after morphine. Figure 6 illustrates these differing actions of naloxone on one neurone. In this experiment naloxone 100 nA for 14 min had no effect on cell firing. Ejected after morphine had reduced nociceptive responses, this dose of naloxone restored nociceptive responses to control values. Continued ejection increased nociceptive responses to levels well above controls.

(v) Effects of amino acids. In an attempt to gain some insight into the possble site and mode of action of morphine, excitant (L-glutamate, DL-homocysteate) and depressant (glycine, GABA) amino acids were also ejected from micropipettes positioned in the substantia gelatinosa. In contrast to morphine there was considerable variation in the effects on spontaneous firing and on the responses to noxious and innocuous stimulation.

Of the 15 cells on which morphine was subsequently shown to be effective in reducing nociceptive responses, an excitant amino acid was also ejected from the morphine administering pipette with 11; with 4 no effect on any firing pattern was observed even after ejections of up to 200 nA for 13 min (Figures 4, 5); with 2 the spontaneous firing was variably increased but the responses to peripheral stimulation were unaffected; with 3 spontaneous firing and nociceptive, but not non-nociceptive, responses were increased; with one all three types of firing were increased and with one both spontaneous firing and the response to nociceptive afferents were reduced. Three cells, the nociceptive responses of which were unaffected by morphine, were all excited by an excitant amino acid but there was no consistent effect on the responses to skin stimulation.

Glycine and GABA seldom had any effect. Of 4 cells on which morphine subsequently reduced the nociceptive response, these amino acids had no effect on 3 (maximum current 200 nA for 5 min) and depressed the spontaneous firing and both responses (the non-nociceptive response being reduced before the nociceptive) with one, after ejection at 30–100 nA for a total of 15 minutes. Of 11 other cells on which glycine or GABA was tested, effects were seen on only 2 cells, both with GABA: with one spontaneous firing alone was reduced, and with the other the spontaneous and the nociceptive response were reduced.

At no time was the specific reduction of nociceptive responses produced by ejection of morphine observed following the ejection of amino acids.

A further difference from morphine lies in the time course of action of the amino acids. Although the time to onset of any detectable action of amino acids was frequently very prolonged (in relation to the usual latencies when amino acids are ejected near cell bodies), the reversal from such actions following the termination of ejection invariably occurred within 2 minutes.

Discussion

As the most common effect of morphine, administered near cell bodies, was to excite cells and produce abnormalities in action potential configuration, and these effects were not antagonized by naloxone, it is probable that the results obtained from this method of administration have no relevance to the depression by systemic opiates of the responses of dorsal horn neurones to noxious skin stimuli (Besson *et al.*, 1973; Le Bars *et al.*, 1975, Jurna & Grossman, 1976). This is in disagreement with the results of Calvillo *et al.* (1974) and Zieglgänsberger & Bayerl (1976) but supports the findings of Dostrovsky & Pomeranz (1976).

By contrast, when ejected in the region of the substantia gelatinosa, the effect of morphine was characterized by selectivity in the sensory modality affected, long duration and reversal by naloxone administered either electrophoretically or intravenously. Since the spinal cord was transected in all experiments these actions of morphine are independent of supraspinal influences. That these effects are relevant to the action of morphine administered systemically is supported by the following considerations:

Sensory modality selectivity

In humans, analgesic doses of morphine are without significant effect on olfaction, vision, hearing, touch, vibration and two-point discrimination (Wikler, 1950; Kreuger, 1955).

At the level of the spinal cord, several studies in cats indicate that intravenous morphine-like compounds have a preferential effect in reducing responses to impulses in small diameter primary afferent fibres. Using a combination of noxious natural stimulation and electrical stimulation of large diameter cutaneous afferents, Le Bars *et al.* (1975) found that morphine, 2 mg/kg, depressed the responses of lamina V neurones to the noxious stimulus alone. A similar result has been observed with phenoperidine (Besson *et al.*, 1973). Fentanyl (20–40 mg/kg) suppresses the discharge of spinal interneurones by impulses in Aδ but not in larger diameter fibres (Iwata & Sakai, 1971). Experiments on spinal reflexes have indicated that intravenous morphine preferentially reduces those reflexes produced by impulses in small diameter afferents (Wikler, 1945; Takagi, Matsumura, Yanai & Ogiu, 1955; Koll *et al.*, 1963; Krivoy, Kroeger & Zimmermann, 1973).

The present experiments, by demonstrating that

morphine administered in the region of the substantia gelatinosa was selective in reducing firing by nociceptive but not by non-nociceptive afferents, thus show good correlation with the effects of systemic morphine. This selectivity is unlikely to be due to failure of morphine to reach terminals of mechanoreceptor afferents for although these fibres establish contacts with dorsal horn neurones outside the substantia gelatinosa, the terminals of both large diameter and small diameter primary afferent fibres are widely distributed within the substantia gelatinosa (Cajal, 1909; Ranson & Billingsley, 1916; Szentágothai, 1964; Scheibel & Scheibel, 1968).

Antagonism by intravenous naloxone

When substances are administered electrophoretically, tissue concentrations are unknown and hence antagonism of the effects of morphine by electrophoretically administered naloxone may not be relevant to the action of systemic morphine. However, reversal of the effects of electrophoretic morphine by doses of intravenous naloxone as low as 0.1 mg/kg suggests that the concentrations of morphine in the substantia gelatinosa were not inappropriate to those present after analgesic doses of morphine administered intravenously to cat (Jurna & Grossman, 1976) and dog (McLane & Martin, 1967a).

When administered into the substantia gelatinosa after morphine, naloxone did not behave simply as an antagonist; rather, with most neurones, nociceptive responses were enhanced well above control levels and excitability was increased as shown by increased spontaneous firing and slightly enhanced responses to mechanoreceptor stimulation. As naloxone alone did not show such activity the effects of this compound were dependent on whether or not cells had previously been exposed to morphine. These findings parallel results obtained when morphine and naloxone are administered systemically. Given alone in doses of 0.05–0.1 mg/kg naloxone has a negligible effect on the firing of lamina V neurones and ventrolateral tract axons in response to stimulation of Aδ and C fibres, but, when given after morphine, responses depressed by morphine are enhanced above control values (Le Bars *et al.*, 1975; 1976; Jurna & Grossman, 1975).

The long duration of the effect of morphine administered in the region of the substantia gelatinosa differs from any other report of an effect of this substance given electrophoretically (Dostrovsky & Pomeranz, 1973; Bramwell & Bradley, 1974; Calvillo *et al.*, 1974; Satoh, Zieglgänsberger, Fries & Herz, 1974; Davies & Duggan, 1974; Gent & Wolstencroft, 1976). When neurophysiological parameters are measured, intravenous morphine is not a short acting drug (McLane & Martin, 1967b; Herz & Teschemacher, 1971; Le Bars *et al.*, 1975). A prolonged analgesic action of morphine is observed after intraventricular administration (Herz & Teschemacher, 1971) and has been attributed to the difficulty with which the hydrophilic morphine molecule penetrates nervous tissue. In the present experiments other processes such as a slow rate of removal from the substantia gelatinosa or a slow dissociation from receptors may have been responsible for the prolonged action of morphine.

The anatomical localization of the opiate receptor studied in the present experiments is not known. Autoradiographic studies in the rat (Pert, Kuhar & Snyder, 1975) have found the substantia gelatinosa to be an area rich in opiate receptors. Binding studies in monkey (Lamotte, Pert & Snyder, 1976) have found a similarly high level of opiate receptors in the upper dorsal horn (which includes the substantia gelatinosa). Both the distribution of silver grains in the former study and the results of dorsal root section in the latter have suggested a location of opiate receptors on structures other than cell bodies, a finding consistent with the results of the present experiments.

Because of the complexity of organization of the substantia gelatinosa any of the following mechanisms could explain the effects of morphine in the present experiments: (1) Inhibition of transmitter release from the terminals of nociceptive afferents. This could be produced by block of conduction in fine terminals or by activity at axo-axonic synapses known to be abundant in the substantia gelatinosa (Réthelyi & Szentágothai, 1969). (2) Antagonism of the postsynaptic action of the transmitter released by nociceptive afferents. The relevant receptors could be located on the dendrites of neurones of lower spinal laminae or on neurones of the substantia gelatinosa. (3) Depression of excitatory interneurones or excitation of inhibitory interneurones of the substantia gelatinosa, although the inactivity of amino acids when ejected in this region may exclude this explanation.

The importance of the present experiments lies in the demonstration that morphine, ejected in the region of the substantia gelatinosa, has effects that can be related to analgesia induced by systemic opiates. Because of this, the technique offers a means of investigating the physiological role, if any, of the endogenous polypeptides (Hughes, 1975) which compete with morphine for stereospecific receptor sites in brain homogenates (Simantov, Kuhar, Pasternak & Snyder, 1976).

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References

BECK, P.W., HANDWERKER, H.O. & ZIMMERMANN, M. (1974). Nervous outflow from the cat's foot during noxious radiant heat stimulation. *Brain Res.*, **67**, 373–386.

BESSON, J.M., WYON-MAILLARD, M.C., BENOIST, J.M., CONSEILLER, C. & HAMANN, K.F. (1973). Effects of phenoperidine on lamina V cells in the cat dorsal horn. *J. Pharmac. exp. Ther.*, **187**, 239–245.

BESSOU, P. & PERL, E.R. (1969). Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. *J. Neurophysiol.*, **32**, 1025–1043.

BROWN, A.G. & IGGO, A. (1967). A quantitative study of cutaneous receptors and afferent fibres in cat and rabbit. *J. Physiol. Lond.*, **193**, 707–733.

BLUMBERG, H. & DAYTON, H.B. (1972). Naloxone and related compounds. In *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*, ed. Kosterlitz, H.W., Collier, H.O.J. & Villarreal, J.E. pp. 110–119. London: Macmillan.

BRAMWELL, G.J. & BRADLEY, P.B. (1974). Actions and interactions of narcotic agonists and antagonists on brain stem neurones. *Brain Res.*, **73**, 167–170.

CAJAL, S. RAMÓN Y. (1909). *Histologie du Système Nerveux de l'Homme et des Vertébrés*, Vol. 1. Paris: Maloine.

CALVILLO, O., HENRY, J.L. & NEUMAN, R.S. (1974). Effects of morphine and naloxone on dorsal horn neurones in the cat. *Can. J. Physiol. Pharmac.*, **52**, 1207–1211.

CURTIS, D.R. & DUGGAN, A.W. (1969). Depression of spinal inhibition by morphine. *Agents and Actions*, **1**, 14–19.

DAVIES, J. & DUGGAN, A.W. (1974). Opiate agonist-antagonist effects on Renshaw cells and spinal neurones. *Nature, Lond.*, **250**, 70–71.

DOSTROVSKY, J. & POMERANZ, B. (1973). Morphine blockade of amino acid putative transmitters on cat spinal cord sensory interneurones. *Nature, New Biol.*, **246**, 222–224.

DOSTROVSKY, J.O. & POMERANZ, B. (1976). Interaction of iontophoretically applied morphine with responses of interneurones in cat spinal cord. *Expl. Neurol.*, **52**, 325–338.

DUGGAN, A.W. & CURTIS, D.R. (1972). Morphine and the synaptic activation of Renshaw cells. *Neuropharmac.*, **11**, 189–196.

DUGGAN, A.W., DAVIES, J. & HALL, J.G. (1976). Effects of opiate agonists and antagonists on central neurones of the cat. *J. Pharmac. exp. Ther.*, **196**, 107–120.

DUGGAN, A.W., HALL, J.G. & HEADLEY, P.M. (1976). Morphine, enkephalin and the substantia gelatinosa. *Nature, Lond.*, **264**, 456–458.

GENT, J.P. & WOLSTENCROFT, J.H. (1976). Effects of methionine-enkephalin and leucine-enkephalin compared wth those of morphine on brainstem neurones in cat. *Nature, Lond.*, **261**, 426–427.

HANDWERKER, H.O., IGGO, A. & ZIMMERMANN, M. (1975). Segmental and supraspinal actions on dorsal horn neurons responding to noxious and non-noxious skin stimuli. *Pain*, **1**, 147–165.

HERZ, A. & TESCHEMACHER, H.J. (1971). Activities and sites of antinociceptive action of morphine-like analgesics, and kinetics of distribution following intravenous, intracerebral and intraventricular application. *Adv. Drug. Res.*, **6**, 79–119.

HUGHES, J. (1975). Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.*, **88**, 295–308.

IGGO, A. (1962). Non-myelinated visceral, muscular and cutaneous afferent fibers and pain. In *International Symposium on Assessment of Pain in Man and Animals*, ed. Keele, C.A. & Smith, R., pp. 74–87. Edinburgh: Livingston.

IWATA, M. & SAKAI, Y. (1971). Effects of fentanyl upon the spinal interneurones activated by Aδ afferent fibres of the cutaneous nerve of the cat. *Jap. J. Pharmac.*, **21**, 413–416.

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.

KOLL, W., HAASE, J., BLOCK, G. & MÜHLBERG, B. (1963). The predilective action of small doses of morphine on nociceptive spinal reflexes of low spinal cats. *J. Neuropharmac.*, **2**, 57–66.

KREUGER, H. (1955). Narcotics and analgesics. In *The Alkaloids Chemistry and Physiology*, Vol. V., ed. Manske, R.H.F., pp. 2–78. New York: Academic Press.

KRIVOY, W., KROEGER, D. & ZIMMERMANN, E. (1973). Actions of morphine on the segmental reflex of the decerebrate-spinal cat. *Br. J. Pharmac.*, **47**, 457–464.

LAMOTTE, C., PERT, C.B. & SNYDER, S.H. (1976). Opiate receptor binding in primate spinal cord: distribution and changes after dorsal root section. *Brain Res.*, **112**, 407–412.

LE BARS, D., GUILBAUD, G., JURNA, I. & BESSON, J.M. (1976). Differential effects of morphine on responses of dorsal horn lamina V type cells elicited by A and C fibre stimulation in the spinal cat. *Brain Res.*, **115**, 518–524.

LE BARS, D., MENÉTREY, D., CONSEILLER, C. & BESSON, J.M. (1975). Depressive effects of morphine upon lamina V cells activities in the dorsal horn of the spinal cat. *Brain Res.*, **98**, 261–277.

McLANE, T.K. & MARTIN, W.R. (1967a). Antagonism of the spinal cord effects of morphine and cyclazocine by naloxone and thebaine. *Int. J. Neuropharmac.*, **6**, 325–327.

McLANE, T.K. & MARTIN, W.R. (1967b). Effects of morphine, nalorphine, cyclazocine and naloxone on the flexor reflex. *Int. J. Neuropharmac.*, **6**, 89–98.

MELZACK, M. & WALL, P.D. (1965). Pain mechanisms: a new theory. *Science*, **150**, 971–979.

PERL, E.R., KUMAZAWA, T., LYNN, B. & KENINS, P. (1976). Sensitization of high threshold receptors with unmyelinated (C) afferent fibers. *Prog. Brain Res.*, **43**, 263–277.

PERT, C.B., KUHAR, M.J. & SNYDER, S.H. (1975). Autoradiographic localization of the opiate receptor in rat brain. *Life Sci.*, **16**, 1849–1854.

PRICE, D.D. & BROWE, A.C. (1975). Spinal cord coding of graded non-noxious and noxious temperature increases. *Expl. Neurol.*, **48**, 201–221.

RANSON, S.W. & BILLINGSLEY, P.B. (1916). The conduction of painful afferent impulses in the spinal nerves. *Am. J. Physiol.*, **40**, 571–584.

RÉTHELYI, M. & SZENTÁGOTHAI, J. (1969). The large synaptic complexes of the substantia gelatinosa. *Expl. Brain Res.*, **7**, 258–274.

SATOH, M., ZIEGLGÄNSBERGER, W., FRIES, W. & HERZ, A. (1974). Opiate agonist-antagonist interaction at

cortical neurones of naive and tolerant/dependent rats. *Brain Res.*, **82**, 378–382.

SCHEIBEL, M.E. & SCHEIBEL, A.B. (1968). Terminal axonal patterns in cat spinal cord. II. The dorsal horn. *Brain Res.*, **9**, 32–58.

SIMANTOV, R., KUHAR, M.J., PASTERNAK, G.W. & SNYDER, S.H. (1976). The regional distribution of a morphine-like factor, enkephalin in monkey brain. *Brain Res.*, **106**, 189–197.

SZENTÁGOTHAI, J. (1964). Neuronal and synaptic arrangement in the substantia gelatinosa Rolandi. *J. comp. Neurol.*, **122**, 219–240.

TAKAGI, H., MATSUMURA, M., YANAI, A. & OGIU, K. (1955). The effect of analgesics on the spinal reflex activity of the cat. *Jap. J. Pharmac.*, **4**, 176–187.

WIKLER, A. (1945). Effects of morphine, nembutal, ether and eserine on two-neurone and multineurone reflexes in the cat. *Proc. Soc. exp. Biol. Med.*, **58**, 193–196.

WIKLER, A. (1950). Sites and mechanisms of action of morphine and related drugs in the central nervous system. *Pharmac. Rev.*, **2**, 435–461.

YAKSH, T.L. & RUDY, J.A. (1976). Analgesia mediated by a direct spinal action of narcotics. *Science*, **192**, 1357–1358.

ZIEGLGÄNSBERGER, W. & BAYERL, H. (1976). The mechanism of inhibition of neuronal activity by opiates in the spinal cord of the cat. *Brain Res.*, **115**, 111–128.

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