

ENKEPHALINS AND DORSAL HORN NEURONES OF THE CAT: EFFECTS ON RESPONSES TO NOXIOUS AND INNOCUOUS SKIN STIMULI

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1 In spinal cats anaesthetized with α -chloralose, a study was made of the effects of methionine enkephalin and methionine enkephalin amide on the responses of neurones of spinal laminae IV and V to noxious and innocuous skin stimuli. The enkephalins were ejected from micropipettes either in the region of cell bodies or in the substantia gelatinosa.

2 Administered near cell bodies the enkephalins reduced spontaneous firing and cell responses to both types of skin stimuli. These effects were antagonized by naloxone when administered near cell bodies but not when given intravenously in doses (0.3–0.6 mg/kg) more than adequate to antagonize analgesic doses of morphine.

3 Administered in the substantia gelatinosa the enkephalins were more selective in their action. The predominant effect was a reduction in nociceptive responses with little effect on non-nociceptive responses although spontaneous firing was commonly reduced. Naloxone administered either in the substantia gelatinosa or intravenously (0.1–0.3 mg/kg) reversed these effects of the enkephalins.

Introduction

The pentapeptides, methionine enkephalin and leucine enkephalin, have effects similar to those of morphine in certain pharmacological tests on peripheral tissues (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) and compete with opiates for stereospecific receptor sites in mammalian brain homogenates (Simantov, Kuhar, Pasternak & Snyder, 1976). Since their discovery there has been considerable speculation that enkephalins (or a larger molecule containing the methionine enkephalin sequence, see Goldstein, 1976) may be involved in the transmission of nociceptive information within the central nervous system. If these substances have a physiological role, then this is most likely to be revealed by applying methods used to investigate central transmitters, namely, the investigation of their presence and distribution in nervous tissue, action upon neurones, release from neural tissue under appropriate circumstances and whether antagonists of the effects of enkephalins antagonize a particular physiological event.

This paper is concerned with the effects of methionine enkephalin and methionine enkephalin amide, administered electrophoretically from micropipettes, on the responses of dorsal horn neurones of the cat to noxious and innocuous skin stimuli. Methionine enkephalin amide is similar in

action to methionine enkephalin but is more potent in opiate binding studies (Birdsall, Bradbury, Burgen, Hulme, Smyth & Snell, 1976; Chang, Fong, Pert & Pert, 1976) and in its effects on the guinea-pig ileum (Ling & Guillemin, 1976). Preliminary tests in the present experiments suggested that the amide was the more potent compound and hence it was used in the majority of experiments.

Previous work with morphine (Duggan, Hall & Headley, 1976b; 1977) showed that only when this compound was administered in the substantia gelatinosa, a region particularly high in opiate binding sites (Atweh & Kuhar, 1977), were effects observed that could be related to the analgesic properties of systemic morphine. In view of this finding the enkephalins have also been administered to both somatic and dendritic regions of dorsal horn neurones.

Methods

Experiments were performed on 28 cats anaesthetized with α -chloralose (50 mg/kg initially). Halothane was also administered throughout the period of surgery. The spinal cord was exposed by lumbar laminectomy and transected at the thoraco-lumbar junction. All animals were paralysed with gallamine triethiodide and artificially ventilated, end tidal CO₂ level being maintained at approximately 4%. Blood pressure was monitored continuously and experiments were

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terminated if systolic pressure fell below 100 mmHg.

A description of the methods used to evoke and record nociceptive and non-nociceptive responses has been published previously (Duggan *et al.*, 1976b; 1977). Briefly, nociceptive afferents were activated by radiant heat to a 3 mm diameter area of a blackened hind limb digital pad; the skin surface temperature required for threshold activation of the units studied exceeded 45°C, as monitored with a thermistor placed in contact with the skin. Non-nociceptive afferents were activated by an air jet moved by the armature of a relay and which deflected hairs close to the heated area. Infrequently, it was necessary to touch the skin of a digital pad lightly with a rod attached to the relay armature. The two types of stimuli were applied at regular intervals and reproducible responses were obtained throughout recording periods (up to 4 h per cell). The total number of action potentials occurring during nociceptive and non-nociceptive responses was measured with a gated electronic counter, and these counts were later corrected for spontaneous firing.

Drugs were ejected electrophoretically from seven barrel micropipettes, either near the cell bodies of dorsal horn neurones or in the substantia gelatinosa. In the latter case, the activity of deeper neurones of spinal lamina IV or V was recorded with an independently positioned five barrel micropipette. Micropipettes used to administer compounds in the substantia gelatinosa had larger tip diameters (6–10 µm) than those used to eject compounds near cell bodies (4–6 µm). The centre barrel of the seven barrel assembly contained 4 M NaCl, and the outer barrels contained combinations of the following compounds: methionine enkephalin (Calbiochem, 0.02 M, pH 3.2–4.0); methionine enkephalin amide (kindly supplied by Drs H. Niall and G. Tregear, the Howard Florey Institute, Melbourne, 0.02 M, pH 3.2–4.0); leucine enkephalin (Calbiochem, 0.02 M, pH 3.5); naloxone hydrochloride (a gift from Endo Laboratories, 0.1 M); Na D,L-homocysteate (0.2 M, pH 7); glycine (0.5 M, pH 3); γ -aminobutyric acid (0.5 M, pH 3); acid fast green (saturated solution in 2 M NaCl).

In experiments employing two micropipettes, extracellular recordings of the action potentials of neurones in laminae IV and V were obtained with the 4 M NaCl filled centre barrels of five barrelled micropipettes, the outer barrels of which contained Na D,L-homocysteate (0.2 M, pH 7), glycine (0.5 M, pH 3) or γ -aminobutyric acid (GABA, 0.5 M, pH 3) and acid fast green (saturated solution in 2 M NaCl). After the seven barrel electrode had been positioned in the substantia gelatinosa, using the activity of lamina I neurones as a guide, the manipulator holding the five barrel pipette was adjusted so that this pipette, angled 18° caudally, would pass a defined distance (commonly 300 µm) vertically beneath the tip of the electrode in the substantia gelatinosa. Because it was exceptional to locate a neurone precisely at this

planned separation distance, it was decided to study neurones located from approximately 100 µm before and up to 500 µm beyond this point. If a neurone was not located within this range up to two more tracks were made with the five barrel micropipette either 50 or 100 µm medial to the first. This allows for the slight lateral shift of the dendrites of neurones of laminae IV and V as they ascend to the substantia gelatinosa.

Cells were located either by spontaneous firing, excitation by D,L-homocysteate (ejected from the advancing pipette) or by responses to one of the peripheral skin stimuli. Very few cells were found which did not respond to both stimuli, but with many it was not possible to obtain sufficiently large responses to both kinds of skin stimuli for quantitative studies. In the present study, cells were selected for reproducible responses to both noxious and innocuous skin stimuli.

In most experiments acid fast green was ejected from one or both electrodes; dye spots were subsequently located in 50 µm frozen sections. This allowed tip positions to be plotted more accurately in relation to Rexed laminae than was possible using manipulator co-ordinates alone.

Results

Ejection of methionine enkephalin and methionine enkephalin amide near cell bodies of dorsal horn neurones

Effects on spontaneous firing and on responses to noxious and innocuous skin stimuli. Methionine enkephalin (M-Enk, 20–80 nA, 8 cells) and methionine enkephalin amide (M-Enka, 3–100 nA, 23 cells) were tested for effects on the spontaneous firing and the responses to noxious and innocuous skin stimuli of 31 dorsal horn neurones. Twenty-nine of these cells were excited by both types of skin stimuli. Twenty cells were located in Rexed lamina IV and 11 in lamina V. Price & Browe (1975) and Handwerker, Iggo & Zimmerman (1975) have also found that neurones of these laminae are excited by both noxious and innocuous skin stimuli (but see Pomeranz, Wall & Weber, 1968; Guilbaud, Oliveras, Giesler & Besson, 1977).

All cells studied were spontaneously active. M-Enk depressed the spontaneous firing of only 1 of 8 cells (with 20 nA), whereas M-Enka depressed that of 21 of 23 cells tested (mean effective current 30 nA \pm 20 nA s.d.). Compared with the action of glycine and GABA, depression of spontaneous firing by both M-Enk and M-Enka was prolonged, requiring 2–4 min to become maximal and a similar time for recovery. This time course can be seen in the records of Figure 1.

These substances were tested for effects on the nociceptive and non-nociceptive responses of 28 neurones. M-Enk was tested on 8 cells and had no effect on the responses of 6. Of the latter, 2 neurones

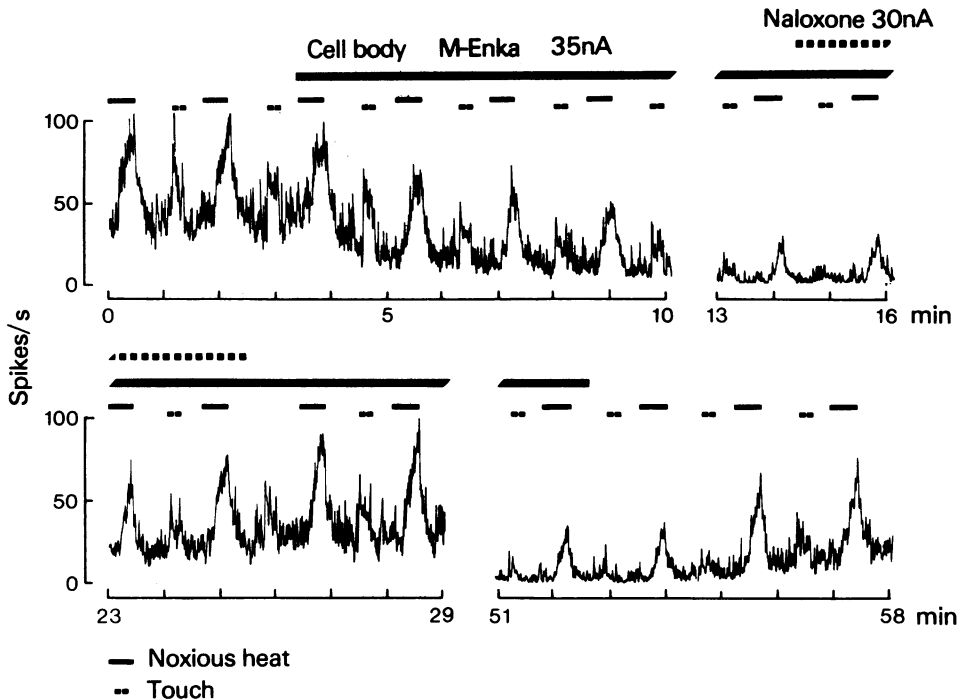


Figure 1 Depression of spontaneous firing and the responses of a dorsal horn neurone to noxious and innocuous skin stimulation by methionine enkephalin amide (M-Enka) administered in the region of the cell body and reversal of these effects by similarly administered naloxone. The neurone was located in spinal lamina V and was activated alternately by noxious heat to the third digital pad of the left hind limb and by touching the adjacent skin and hairs with a moving arm attached to the armature of a relay. Methionine enkephalin amide was ejected continuously for a total of 49 min during which naloxone was ejected continuously for 11 minutes. Some of the responses during these ejection periods have been omitted.

showed changes in action potential configuration with ejecting currents of 20 nA and 40 nA, in two experiments increases in barrel resistance limited the ejecting currents to 20 and 30 nA while with 2 other neurones no effect was seen with 50 and 80 nA. With the remaining 2 neurones M-Enk (40 nA both cells) reduced firing to noxious heat, in one case without effect on spontaneous firing or non-nociceptive responses, but in the other spontaneous firing was also reduced.

M-Enka reduced the nociceptive responses of all 20 cells tested and this was accompanied by a reduction in mechanoreceptor-induced firing in all but 2 neurones. With all cells, depression of responses to skin stimulation was associated with a reduction in spontaneous firing. It is difficult to quantify the degree of selectivity of M-Enka towards the two sensory modalities but this substance was relatively non-specific in its depressant action when ejected near cell bodies. With the responses illustrated in Figure 1, the changes in nociceptive and non-nociceptive responses

following M-Enka ejected near the cell body of this neurone were similar and this type of result was obtained with another 10 cells. With 7 neurones however, nociceptive responses were reduced to a greater extent than non-nociceptive responses.

The amino acids, glycine and GABA, are known to hyperpolarize spinal interneurons (Bruggencate & Engberg, 1968) and hence it was of interest to compare the effects of these substances with the depressant actions of the enkephalins. With 4 neurones both glycine and M-Enka were administered to the cell body region with similar effects on cell responses (3 neurones, non-selective effects; 1 neurone, nociceptive responses reduced more than non-nociceptive responses and spontaneous firing decreased). With a further 12 neurones, glycine (3 cells) and GABA (9 cells), alone, were ejected near cell bodies. With 10 neurones the depressant amino acids were non-selective reducing the three types of cell firing; on 2 neurones the predominant effect was a reduction in nociceptive responses.

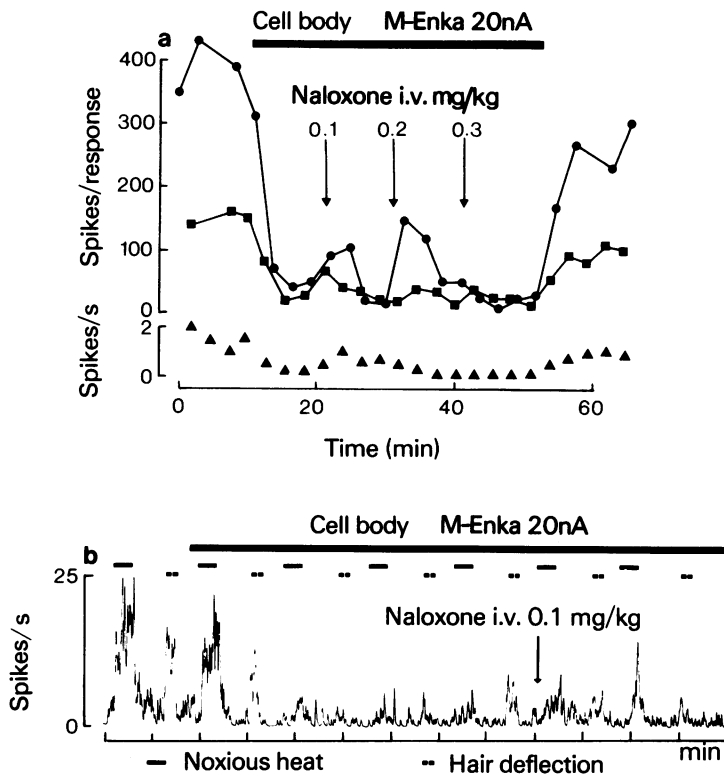


Figure 2 Failure of intravenous naloxone to reverse the depression of the spontaneous firing and nociceptive and non-nociceptive responses of a dorsal horn neurone by methionine enkephalin amide (M-Enka) administered in the region of the cell body. The neurone was located in spinal lamina IV and was activated alternately by noxious heat to the second digital pad of the left hind limb and by deflection of adjacent hairs by a moving air jet. (a) A graph of the number of action potentials, corrected for spontaneous firing, evoked by each skin stimulus, with respect to time. (●)=Noxious heat; (■)=hair deflection; (▲)=spontaneous. Spontaneous firing, in spikes per second, is also plotted. The first two doses of intravenous naloxone transiently and partially reversed the effects of M-Enka, but a third dose had no effect; (b) part of the pen recordings of cell firing rates used to prepare the graphs above. The segment illustrated has been chosen to show the effect of enkephalin on cell firing and the effect of the first dose of intravenous naloxone (0.1 mg/kg).

Antagonism of effects of methionine enkephalin amide by naloxone.

Electrophoretic naloxone. Naloxone administered electrophoretically near cell bodies produces abnormalities in action potential amplitude and configuration (Duggan, Davies & Hall, 1976a). In the present experiments, however, this precluded testing of antagonism of the effects of M-Enka on only one neurone. Of the other 12 cells tested, naloxone (10–60 nA) completely reversed the effects of M-Enka on 6, partial antagonism was observed with 4 and no effect seen with 2. In the example of Figure 1, naloxone (30 nA) after 9 min of ejection completely reversed the effects of M-Enka (35 nA) on spontaneous firing and both nociceptive and non-nociceptive responses. This similarity between the

ejecting currents of M-Enka and of naloxone was also observed with the other 9 neurones on which antagonism by naloxone occurred. Figure 1 also shows that continued ejection of M-Enka for 26 min beyond cessation of naloxone ejection was necessary to reduce responses to levels present prior to naloxone ejection. That M-Enka was depressing cell responses at this time is shown by recovery to near control levels following the termination of ejection.

Intravenous naloxone. In previous experiments (Duggan *et al.*, 1976b; 1977) doses of intravenous naloxone adequate to antagonize the analgesic action of systemic morphine also antagonized the effects of morphine administered electrophoretically in the substantia gelatinosa. When drugs are administered electrophoretically, concentrations in the vicinity of

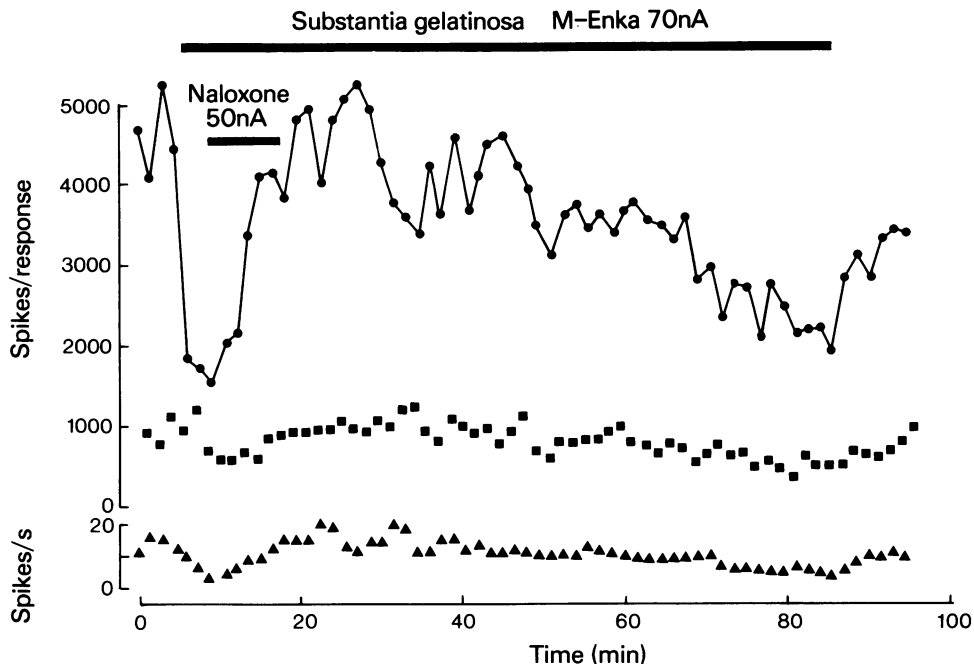


Figure 3 Depression of the spontaneous firing and nociceptive responses of a dorsal horn neurone by methionine enkephalin amide (M-Enka) administered in the substantia gelatinosa and reversal of these effects by similarly administered naloxone. The neurone was located in spinal lamina IV and was activated by noxious heat (●) to the fourth digital pad of the left hind limb and by deflection of lateral hairs (■); (▲)=spontaneous. The separation between recording and drug administering micropipettes was 430 μ m. The total number of action potentials evoked by each stimulus has been corrected for spontaneous firing and plotted with respect to time. Spontaneous firing, in spikes per second, is also plotted.

neurones are unknown, but reversal of the effects of electrophoretic morphine by intravenous naloxone suggests that the concentrations of morphine attained at opiate receptors in the substantia gelatinosa were not inappropriate to those necessary to produce analgesia. Hence it was of interest to determine if the effects of enkephalin on cell bodies were reduced by similar doses of intravenous naloxone.

This was tested in six experiments. In two, intravenous naloxone (up to 0.5 mg/kg in divided doses) had no effect on the depression of cell firing by M-Enka. With 3 neurones, partial (10–20%) and transient (lasting less than 6 min) reversal was observed with low doses of naloxone (0.1–0.2 mg/kg), but this effect was not repeated with subsequent higher doses. Results from one such experiment are illustrated in Figure 2. With this neurone, the first two doses of intravenous naloxone (0.1 and 0.2 mg/kg) both transiently increased nociceptive responses but the third dose (0.3 mg/kg) had no effect. Longer lasting (up to 15 min), but nonetheless partial antagonism was observed with 1 neurone after naloxone (0.1 mg/kg), but this was not repeated with subsequent doses of 0.1 and 0.3 mg/kg. Lack of

antagonism can be further demonstrated if recovery from the action of an agonist occurs in the presence of the potential antagonist. In the present experiments spontaneous firing and the responses to skin stimulation recovered rapidly following the cessation of M-Enka ejection, despite the relatively high circulatory levels of naloxone (Figure 2a).

Ejection of methionine enkephalin and methionine enkephalin amide in the substantia gelatinosa

Effects on spontaneous firing and responses to noxious and innocuous skin stimulation. M-Enk (90–200 nA, 8 cells), and M-Enka (15–250 nA, 29 cells) ejected in the substantia gelatinosa, were tested for effects on the nociceptive and non-nociceptive responses of 8 lamina IV and 29 lamina V spinal neurones. The separation between drug administering and recording micropipettes ranged from 150 to 830 μ m.

Of the 37 cells tested, the enkephalins had no effect on 16 and reduced the nociceptive responses of 21, of which 2 were with M-Enk (110 and 120 nA) and 19 with M-Enka (mean 115 nA \pm 52 nA s.d.). In contrast

to the effects of these substances ejected near cell bodies, relative selectivity was shown by M-Enk and M-Enka when ejected into the substantia gelatinosa. Thus, of these 21 neurones, selectivity (reduced nociceptive responses with no significant effect on spontaneous firing or on non-nociceptive responses), was seen with 6 whereas lack of specificity (similar reduction of all three firing patterns) was seen with only 1. With the remaining 14 neurones the reduction in nociceptive responses was accompanied by decreased spontaneous firing (as illustrated in Figure 4). With 9 of these the non-nociceptive responses were also reduced, but such effects on non-nociceptive responses were usually slight in relation to the changes in nociceptive responses. With the results illustrated in Figure 3 the total number of action potentials evoked by noxious and innocuous skin stimuli shows that, when corrected for changes in spontaneous firing, the responses of this neurone to noxious heat were reduced by approximately 70% following ejection of M-Enka whereas those to innocuous skin stimulation were reduced by only 20%. Both Figure 3 and Figure 4 show a significant depression of spontaneous firing accompanying the reduction in nociceptive responses.

Table 1 has been prepared to contrast the differing effects of M-Enka administered near cell bodies and in the substantia gelatinosa. It shows that whilst partial selectivity was observed at both sites of administration, complete selectivity was seen only with ejection in the substantia gelatinosa, whereas, non-selectivity occurred almost exclusively following administration near cell bodies.

The time course of the onset of reduction of nociceptive responses by the enkephalins was not determined accurately since heat stimuli were delivered at intervals of 1.5 to 2 minutes. Frequently, however, there was a clear effect within one cycle time (Figures 3 and 4), a situation quite different from that found with morphine (see Figures 4-6 in Duggan *et al.*, 1977). The depression by enkephalin of spontaneous firing, when apparent, was similar in time of onset with ejection into the substantia gelatinosa as with ejection near cell bodies, although the effect with substantia gelatinosa ejection was smaller in amplitude.

After the termination of M-Enk or M-Enka ejection, recovery occurred progressively over 4 to 10 (Figures 3 and 4), or occasionally up to 15, minutes. This time course of recovery was thus considerably faster than that following the ejection of morphine, since the effects of the latter compound could remain unchanged for at least 30 min after the cessation of ejection (Duggan *et al.*, 1977).

Antagonism of the effects of methionine enkephalin and methionine enkephalin amide

Electrophoretic naloxone. Ejected electrophoretically from the same micropipettes from which

Table 1 Method of administration and the effects of methionine enkephalin amide on the spontaneous firing and nociceptive and non-nociceptive responses of dorsal horn neurones; results with methionine enkephalin have not been included

Site of administration	Effects on cell firing: numbers of neurones				
	No effect	Nociceptive responses alone reduced	Nociceptive responses and spontaneous firing reduced. Non-nociceptive responses unaffected	Greater reduction in nociceptive than non-nociceptive responses. Spontaneous firing decreased	Similar reduction in nociceptive and non-nociceptive responses. Spontaneous firing decreased
Substantia gelatinosa	10	5	4	9	1
Cell bodies	0	0	2	7	11

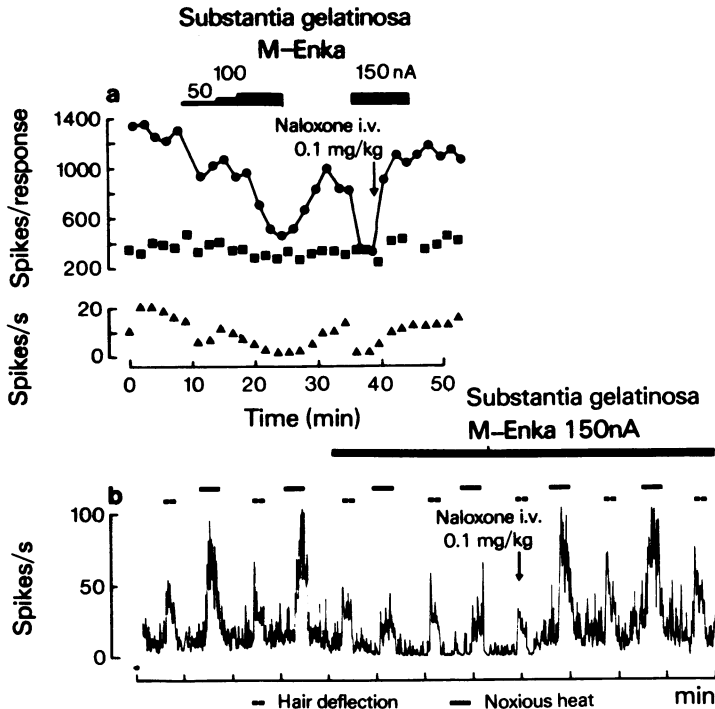


Figure 4 Reversal by intravenous naloxone of the depression of spontaneous firing and nociceptive responses of a dorsal horn neurone by methionine enkephalin amide (M-Enka) administered in the substantia gelatinosa. The neurone was in spinal lamina IV and was activated by noxious heat (●) to the fourth digital pad of the left hind limb and by deflection of adjacent hairs (■); (▲)=spontaneous. Separation between drug administering and recording micropipettes, 220 μ m. The graphs (a) and pen recordings (b) of cell responses are as in Figure 2.

M-Enk and M-Enka were ejected in the substantia gelatinosa, naloxone (mean 60 nA \pm 14 nA s.d.) antagonized the actions of the enkephalins on all 5 of the neurones tested. With all of these neurones nociceptive responses were reduced by the enkephalins, but with 1 spontaneous firing was also reduced and with 3 both spontaneous firing and non-nociceptive responses were also reduced. Naloxone reversed all of these effects. Such a result is illustrated in Figure 3. With this neurone, reversal of the effects of M-Enka occurred within 4 min of the start of ejection of naloxone. In previous experiments (Duggan *et al.*, 1977), naloxone did not merely antagonize the depression of nociceptive responses by morphine but enhanced these responses well above control values. In the present study this effect was observed with 2 of 5 cells when nociceptive responses had been depressed by the enkephalins, although the effect appeared to be less than that observed with morphine.

The antagonism by naloxone of the action of the enkephalins, like that of morphine, could be long-lasting. With 3 neurones the ejection of M-Enka was

continued after the termination of the ejection of naloxone until nociceptive responses had once more been reduced; the times for this to occur were 8, 20, and in the example of Figure 3, 60 minutes.

Intravenous naloxone. In eight experiments naloxone was given intravenously when the nociceptive responses of a neurone had been significantly depressed by the ejection of an enkephalin in the substantia gelatinosa (M-Enk, 1 neurone; M-Enka, 7 neurones). The ejection of enkephalin was continuous throughout the period of administration of naloxone. With 6 of these 8 neurones a single dose of naloxone of 0.1 mg/kg reversed the effects of M-Enk (1 cell) or M-Enka (5 cells). Results from one experiment are illustrated in Figure 4. The records of the rate of cell firing show that reversal of the effects of M-Enka occurred within 1 min of injecting naloxone intravenously, a time course observed with the other 5 neurones. The adequacy of this antagonism contrasts strongly with the limited effect of intravenous naloxone when

enkephalins were ejected near cell bodies (see Figure 2). Further doses of naloxone were given to 4 of these 6 cells, in 2 cases without further effect, in one increasing spontaneous firing and in another further increasing the nociceptive response. Naloxone increased the spontaneous firing and nociceptive responses of two cells above control levels and also the non-nociceptive response of one of these two.

Of the remaining 2 cells, naloxone 0.1 mg/kg, partially reversed the effects of M-Enka (200 nA) on one, but additional doses up to a total of 0.8 mg/kg had no further effect. With the remaining cell, divided doses to a total of 0.8 mg/kg had no effect though electrophoretic naloxone had previously fully antagonized the action of M-Enka (50 nA).

Effects of excitant and depressant amino acids ejected into the substantia gelatinosa. In an attempt to determine whether the effects of the enkephalins could be explained by simple excitation or depression or neuronal firing, amino acids were also ejected in the substantia gelatinosa.

The excitant D,L-homocysteate (DLH, ejected with currents as high as 250 nA) was tested on 14 cells, the nociceptive responses of 9 of which were depressed by an enkephalin. In these tests relatively low ejecting currents of DLH (30–50 nA) were used initially and were only increased if no effect was observed. Seven cells, 5 of which were affected by an enkephalin, were excited: responses to both types of skin stimulation were increased together with spontaneous firing, but with 2 cells nociceptive responses were enhanced more than non-nociceptive responses or spontaneous firing. The latency to this excitation was long in comparison to the usual latencies when amino acids are ejected near cell bodies: with these 7 cells, the latency to excitation was between 30 and 60 seconds. Recovery from the effects of DLH, however, always occurred within a few seconds of terminating the ejection.

With 5 other cells, 4 of which were influenced by an enkephalin, DLH (50–150 nA) depressed spontaneous firing. This depression was in all cases transient, having a short latency (<10 s) and lasting no more than 3 min despite the continued administration of DLH. In one case depression was succeeded by excitation.

The inhibitory amino acid GABA was tested on 6 cells of which 4 were not affected by ejecting currents of up to 100–150 nA; glycine (100 nA) had no effect on the one cell tested. With 2 neurones, GABA (60 and 80 nA) depressed cell firing, in one case decreasing all three types of firing (latency 30 s) and in the other transiently decreasing (latency 1 min) spontaneous firing (the only parameter studied).

Thus the effects of amino acids ejected in the substantia gelatinosa were different from those of the enkephalins, just as in previous experiments (Duggan *et al.*, 1977) they differed from those of morphine.

Discussion

Since the enkephalins were discovered there have been various hypotheses centred upon a regulatory role for these, or related peptides, in the transmission of nociceptive information in the central nervous system. The present experiments have some relevance to such a proposed role.

When ejected electrophoretically in the vicinity of cell bodies, enkephalins depressed the spontaneous activity of dorsal horn neurones. A similar result has been obtained with neurones in several areas of the central nervous system including brain stem (Bradley, Briggs, Gayton & Lambert, 1976), thalamus (Hill, Pepper & Mitchell, 1976), cerebral cortex (Zieglgänsberger, Fry, Herz, Moroder & Wunsch, 1976) and periaqueductal grey matter (Frederickson & Norris, 1976) of the rat, and the brain stem of the cat (Gent & Wolstencroft, 1976). This depressant action does not occur with all neurones since cells of the posterior cerebral cortex of the rat were not affected by enkephalin (Frederickson & Norris, 1976) and Renshaw cells of cat and rat were excited by this substance (Davies & Dray, 1976). Of these investigators only Hill *et al.* (1976) tested enkephalin for effects on excitation of neurones by noxious stimuli. Although nociceptive responses were depressed, modality selectivity was not tested and the depressant amino acid GABA had similar effects to those of enkephalin. Hence this action of enkephalin may not be related to specific control of the transmission of nociceptive information. In the present study, dorsal horn neurones were activated by innocuous as well as by noxious skin stimuli and enkephalins administered to cell bodies reduced the responses to both sensory modalities. Such an action does not seem suited for regulation of nociceptive information alone.

Enkephalins have been shown to hyperpolarize neurones of the myenteric plexus of the guinea-pig (North, 1977). Such a hyperpolarizing action could explain the effects of these substances administered near cell bodies in the present experiments since there was a similarity between the effects of M-Enka and those of glycine and GABA, both of which are known to hyperpolarize spinal interneurones (Bruggencate & Engberg, 1968).

Antagonism of the effects of electrophoretically administered enkephalins on cell bodies by similarly administered naloxone indicates that these effects were mediated by 'opiate' receptors. These receptors were not revealed in previous experiments with morphine on dorsal horn neurones (Duggan *et al.*, 1976a; Dostrovsky & Pomeranz, 1976; Duggan *et al.*, 1977; but see also Calvillo, Henry & Neuman, 1974; Zieglgänsberger & Bayerl, 1976). This may have resulted from the interference by both morphine and naloxone with spike generation. Two findings, however, suggest that even the naloxone-sensitive

receptors on the bodies of the neurones studied in the present experiments are not of significance to opiate-induced analgesia. Firstly, responses to innocuous as well as noxious stimuli were reduced by enkephalin at this site. Secondly, doses of intravenous naloxone which are adequate to antagonize analgesic doses of morphine failed to antagonize the action of enkephalin on cell bodies. This latter finding suggests that the activation of these receptors by the concentrations of enkephalins attained in the present experiments was greater than the activation produced by analgesic doses of morphine.

When ejected in the substantia gelatinosa, the enkephalins were more selective in that responses to noxious skin stimuli were reduced with a lesser change in responses to innocuous skin stimuli and in spontaneous firing. However, such effects were only seen with ejecting currents considerably higher than those used for ejection near cell bodies. No definitive explanation can be offered for this difference, but it is probable that to depress the firing of neurones of laminae IV and V, it was necessary for the enkephalins administered in the substantia gelatinosa to affect structures distributed through a greater volume of tissue surrounding the micropipette tip than was the case with ejection near the cell bodies.

With substantia gelatinosa administration the enkephalins were thus basically morphine-like (see Duggan *et al.*, 1977). However, there were two differences from the action of morphine ejected in this region. Firstly, the duration of action of the enkephalins was relatively brief when compared with that of morphine. Nociceptive responses recovered in 2–15 min after the termination of enkephalin ejection whereas the effects of morphine remained unchanged for periods in excess of 30 min (Duggan *et al.*, 1976b, 1977). This may reflect a relatively rapid degradation of the polypeptides as has been suggested as an explanation for the transient effects of M-Enk injected intracranially (Belluzzi, Grant, Garsky, Sarantakis, Wise & Stein, 1976; Büscher, Hill, Römer, Cardinaux, Closse, Hauser & Pless, 1976). Secondly, the selectivity of the enkephalins towards the three types of firing studied, that is spontaneous firing and nociceptive and non-nociceptive responses, was less than that of morphine administered in the substantia gelatinosa. With morphine, nociceptive responses were depressed without a change in spontaneous firing or non-nociceptive responses in approximately two-thirds of cells studied whereas this selectivity was observed with only about one-third of neurones affected by enkephalin.

No adequate explanation can be offered for this difference. Although it was clear that the enkephalins

could reduce nociceptive responses independently of changes in spontaneous firing, with many neurones it is still possible that the observed reductions in responses to noxious and innocuous skin stimuli were merely the consequence of a reduction in spontaneous firing. Nociceptive responses may be reduced in this manner to a greater extent than non-nociceptive responses because of a difference in the pattern of firing induced by the two types of stimuli; thus the regular firing induced by nociceptive activation may be more susceptible to inhibition than the burst-like firing induced by activation of rapidly adapting mechanoreceptors.

The results with glycine and GABA ejected near cell bodies make it unlikely that mere depression of spontaneous firing can explain the effects of enkephalins administered in the substantia gelatinosa. With these amino acids, nociceptive and non-nociceptive responses were usually reduced in parallel with spontaneous firing. These findings, together with the differing effects of the enkephalins at the two sites of administration, argue in favour of a specific action by the polypeptides on some structure on the nociceptive pathway in the region of the substantia gelatinosa. The lack of activity of amino acids ejected in the substantia gelatinosa suggests that a presynaptic action may be more likely, a view favoured by the strict localization of opiate binding within the substantia gelatinosa (Pert, Kuhar & Snyder, 1976; Atweh & Kuhar, 1977) and also by the decrease of binding following dorsal root section (Lamotte, Pert & Snyder, 1976).

The present experiments have shown that the responses of dorsal horn neurones to skin stimuli can be altered by enkephalins acting at receptors situated both on the bodies of these cells and on an unknown structure in the substantia gelatinosa. While the results with intravenous naloxone both in these and previous studies (Duggan *et al.*, 1977) suggest that the 'opiate' receptors on cell bodies are not of significance to analgesia produced by systemic morphine, these receptors may still be of importance to a substance released at axosomatic synapses in relatively high concentrations. Activation of these cell body receptors would then reduce the transmission of several sensory modalities; on the other hand, activation of receptors in the substantia gelatinosa would result in a more selective reduction in the transmission of nociceptive impulses. Thus the enkephalins may have a wider physiological role than effects on nociception alone.

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References

- ATWEH, S.F. & KUCHAR, M.J. (1977). Autoradiographic localization of opiate receptors in rat brain. I. Spinal cord and lower medulla. *Brain Res.*, **124**, 53–67.
- BELLUZZI, J.D., GRANT, N., GARSKY, V., SARANTAKIS, D., WISE, C.D. & STEIN, L. (1976). Analgesia induced *in vivo* by central administration of enkephalin in rat. *Nature, Lond.*, **260**, 625–626.
- BIRDSALL, N.J.M., BRADBURY, A.F., BURGESS, A.S.V., HULME, E.C., SMYTH, D.G. & SNELL, C.R. (1976). Interactions of peptides derived from the C-fragment of β -lipotropin with brain opiate receptors. *Br. J. Pharmac.*, **58**, 460–461P.
- BRADLEY, P.B., BRIGGS, I., GAYTON, R.J. & LAMBERT, L.A. (1976). Effects of microiontophoretically applied methionine-enkephalin on single neurones in rat brain stem. *Nature, Lond.*, **261**, 425–426.
- BRUGGENCATE, G. TEN. & ENGBERG, I. (1968). Analysis of glycine actions on spinal interneurons by intracellular recording. *Brain Res.*, **11**, 446–450.
- BÜSCHER, H.H., HILL, R.C., RÖMER, D., CARDINAUX, F., CLOSSE, A., HAUSER, D. & PLESS, J. (1976). Evidence for analgesic activity of enkephalin in the mouse. *Nature, Lond.*, **261**, 423–425.
- 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.
- CHANG, J.-K., FONG, B.T., PERT, A. & PERT, C.B. (1976). Opiate receptor affinities and behavioural effects of enkephalin: structure-activity relationship of ten synthetic peptide analogues. *Life Sci.*, **18**, 1473–1381.
- DAVIES, J. & DRAY, A. (1976). Effects of enkephalin and morphine on Renshaw cells in feline spinal cord. *Nature, Lond.*, **262**, 603–604.
- DOSTROVSKY, J.O. & POMERANZ, B. (1976). Interaction of iontophoretically applied morphine with responses of interneurons in cat spinal cord. *Expl Neurol.*, **52**, 325–338.
- DUGGAN, A.W., DAVIES, J. & HALL, J.G. (1976a). Effects of opiate agonists and antagonists on central neurons of the cat. *J. Pharmac. exp. Ther.*, **196**, 107–120.
- DUGGAN, A.W., HALL, J.G. & HEADLEY, P.M. (1976b). Morphine, enkephalin and the substantia gelatinosa. *Nature, Lond.*, **264**, 456–458.
- DUGGAN, A.W., HALL, J.G. & HEADLEY, P.M. (1977). Suppression of transmission of nociceptive impulses by morphine: selective effects of morphine administered in the region of the substantia gelatinosa. *Br. J. Pharmac.*, **61**, 65–76.
- FREDERICKSON, R.C.A. & NORRIS, F.H. (1976). Enkephalin-induced depression of single neurones in brain areas with opiate receptors—antagonism by naloxone. *Science*, **194**, 440–442.
- GENT, J.P. & WOLSTENCROFT, J.H. (1976). Effects of methionine-enkephalin and leucine-enkephalin compared with those of morphine on brainstem neurones in cat. *Nature, Lond.*, **261**, 426–427.
- GOLDSTEIN, A. (1976). Opioid peptides (endorphins) in pituitary and brain. *Science*, **193**, 1081–1086.
- GUILBAUD, G., OLIVERAS, J.L., GIESLER, G., Jr. & BESSON, J.M. (1977). Effects induced by stimulation of the central inferior nucleus of the raphe on dorsal horn interneurons in cat's spinal cord. *Brain Res.*, **126**, 355–360.
- 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**, 147–165.
- HILL, R.G., PEPPER, C.M. & MITCHELL, J.F. (1976). Depression of nociceptive and other neurones in the brain by iontophoretically applied met-enkephalin. *Nature, Lond.*, **262**, 604–606.
- HUGHES, J.W., SMITH, T.W., KOSTERLITZ, H.W., FOTHERGILL, L.A., MORGAN, B.A. & MORRIS, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature, Lond.*, **258**, 577–579.
- 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.
- LING, N. & GUILLEMIN, R. (1976). Morphinomimetic activity of synthetic fragments of β -lipotropin and analogs. *Proc. natn Acad. Sci. U.S.A.*, **73**, 3308–3310.
- NORTH, R.A. (1977). Hyperpolarization of myenteric neurones by enkephalin. *Br. J. Pharmac.*, **59**, 504–505P.
- PERT, C.B., KUCHAR, M.J. & SNYDER, S.H. (1976). Autoradiographic localization of the opiate receptor in rat brain. *Life Sci.*, **16**, 1849–1954.
- POMERANZ, B., WALL, P.D. & WEBER, W.V. (1968). Cord cells responding to fine myelinated afferents from viscera, muscle and skin. *J. Physiol., Lond.*, **199**, 511–532.
- PRICE, D.D. & BROWNE, A.C. (1975). Spinal cord coding of graded nonnoxious and noxious temperature increases. *Expl Neurol.*, **48**, 201–221.
- SIMANTOV, R., KUCHAR, 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.
- 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.
- ZIEGLGÄNSBERGER, W., FRY, J.P., HERZ, A., MORODER, L. & WÜNSCH, E. (1976). Enkephalin-induced inhibition of cortical neurones and the lack of this effect in morphine tolerant/dependent rats. *Brain Res.*, **115**, 160–164.

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