Br. J. Pharmac. (1978), **63**, 87–96.

PHARMACOLOGICAL AND ELECTROPHYSIOLOGICAL STUDIES OF MORPHINE AND ENKEPHALIN ON RAT SUPRASPINAL NEURONES AND CAT SPINAL NEURONES

J. DAVIES & A. DRAY[1]

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

1 The actions of morphine, methionine and leucine enkephalin, administered electrophoretically, were studied on supraspinal neurones in the cortex and brainstem of the rat anaesthetized with urethane and on spinal Renshaw cells and dorsal horn interneurones in the cat anaesthetized with pentobarbitone.

2 The majority of Renshaw cells and cortical and brainstem neurones were excited by all three compounds although some supraspinal neurones were depressed.

3 Naloxone reversibly antagonized both excitatory and depressant actions of morphine and enkephalin. Acetylcholine-induced excitation but not amino acid-induced excitation was also antagonized by naloxone.

4 Neither morphine nor the enkephalins had any naloxone-reversible action on dorsal horn neurones when ejected from conventional multibarrelled electrodes. However, morphine but not enkephalin, administered into the substantia gelatinosa region of the spinal cord selectively reduced responses to noxious stimuli of neurones in deeper laminae. Naloxone administered into the same region antagonized this action of morphine.

5 Intravenous morphine also antagonized responses of dorsal horn neurones to noxious stimuli and subsequent intravenous naloxone reversed this effect.

6 It was concluded that the excitatory and inhibitory effects of morphine and enkephalin on central neurones may be mediated by actions on different opiate receptors and that depression of noxious responses of dorsal horn neurones may be relevant to the analgesic action of morphine.

Introduction

An endogenous morphine-like ligand found in mammalian brain extracts (Hughes, 1975; Terenius & Wahlström, 1974; 1975) has been identified as two pentapeptides, methionine and leucine enkephalin (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975; Simantov & Snyder, 1976). Enkephalin is unevenly distributed throughout the brain (Hughes, 1975; Simantov, Kuhar, Pasternak & Snyder, 1976a) and this distribution is generally similar to that of specific opiate binding sites (Snyder, 1975; Simantov, Kuhar, Uhl & Snyder, 1977). Additionally, in subcellular distribution studies enkephalin is found in synaptosomal fractions of brain homogenates (Pasternak, Goodman & Snyder, 1975; Simantov, Snowman & Snyder, 1976b) and immunohistochemical experiments further suggest a location in presynaptic terminals and cell bodies (Elde, Hökfelt, Johanssen & Terrenius, 1976; Elde, Hökfelt, Johansson, Ljungdahl, Nilsson & Jeffcoate, 1977).

Similarities between the actions of morphine and enkephalin have been shown on peripheral tissues (Hughes, 1975; Hughes *et al.*, 1975; North & Williams, 1976). These effects can be reversed by the opiate antagonist, naloxone. In the CNS, enkephalin, like morphine, possesses analgesic activity when administered by intracerebral injection. This action is also reversed by naloxone (Büscher, Hill, Römer, Cardinaux, Closse, Hauser & Pless, 1976; Belluzzi, Grant, Garsky, Sarantakis, Wise & Stein, 1976; Graf, Szekely, Ronai, Dunai-Kovacs & Bajusz, 1976). Such

[1] Present address: Department of Physiology and Pharmacology, Duke University Medical Centre, Durham, North Carolina., 27710, U.S.A.

observations have led to the suggestion that enkephalin may be the endogenous ligand for the central opiate receptor and furthermore may act as a neurotransmitter in the CNS (Kosterlitz & Hughes, 1975). In support of this suggestion a number of preliminary studies have shown that enkephalin may affect the activity of single central neurones when administered by electrophoresis and that these effects may be reversed by naloxone (Bradley, Briggs, Gayton & Lambert, 1976b; Davies & Dray, 1976a; Duggan, Hall & Headley, 1976b; Gent & Wolstencroft, 1976; Hill, Pepper & Mitchell, 1976; Zieglgänsberger, Fry, Herz, Maroder & Wünsch, 1976).

In the present study the effects of electrophoretically administered enkephalin were compared with those of morphine on single neurones in three different areas of the CNS which have been shown to possess stereospecific opiate receptor sites; the rat cortex (Satoh, Zieglgänsberger, Fries & Herz, 1974; Satoh, Zieglgänsberger & Herz, 1976); the rat brainstem (Bradley & Bramwell, 1977) and cat spinal cord (Duggan, Davies & Hall, 1976a; Zieglgänsberger & Bayerl, 1976).

Some preliminary results of this study have been communicated to the British Pharmacological Society (Davies & Dray, 1976b).

Methods

Experiments were performed on adult male albino rats lightly anaesthetized with urethane (1.4 g/kg i.p.) and on adult female cats anaesthetized with pentobarbitone sodium (35 mg/kg, i.p. initially and supplemented i.v.). Rats were prepared for brainstem recording by the method previously described (Bradley & Dray, 1973). Other animals were used for recording from the sensorimotor cortex following removal of the skull and incising the overlying dura.

In cats the spinal cord was exposed by lumbar laminectomy between L1 and S1. In some experiments the spinal cord was sectioned at L1. The L7 and S1 ventral roots were located, sectioned close to their entry through the dura and the central ends were mounted on bipolar silver stimulating electrodes. The exposed cord and adjacent tissue was covered by paraffin oil (maintained at 37°C) contained in elevated skin flaps. The left saphenous vein was cannulated for intravenous administration of drugs and blood pressure was continuously monitored via a carotid cannula. The animal's temperature was maintained at 37–38°C by means of a thermostatically controlled, heating blanket.

In all brain areas examined the activity of single neurones was recorded extracellularly with the centre barrel of a 7-barrelled glass micropipette. The recording barrel contained 3 M sodium chloride while another barrel contained 1 M sodium chloride and was used for current balancing or for the ejection of Na^+ or Cl^- to test for electrophoretic current effects. The other barrels contained the following compounds for electrophoretic administration: morphine sulphate 0.05 M or 0.07 M; naloxone hydrochloride 0.1 M; L-glutamate sodium 0.5 M, pH 7.2; acetylcholine chloride 0.5 M; glycine 0.2 M, pH 3.5; γ-aminobutyric acid (GABA) 0.2 M, pH 3.5; D,L-homocysteic acid 0.2 M, pH 7.2; leucine-enkephalin 0.018 M, pH 4; methionine-enkephalin 0.018 M, pH 4; pontamine sky blue 2% in 0.5 M sodium acetate. In most experiments a conventional configuration was used but in other experiments a separate recording pipette was glued to the multibarrelled electrode such that its tip protruded by 15–600 μm beyond the multibarrel electrode. This latter arrangement allowed microelectrophoretic drug administration to sites distant from the cell body. Conventional techniques were used for microelectrophoresis and for the recording and displaying of action potentials (see e.g. Davies, 1976).

Neurones in the rat brainstem and cortex were randomly sampled preference being given to spontaneously active cells. Renshaw cells in cats were identified by their response to stimulation of L7 and S1 ventral roots at strengths supramaximal for antidromic activation of α motoneurones (0.1 ms pulse width, 4 Hz). Dorsal horn neurones were located in segments L6–S1 by their response to stimulation of the ipsilateral hind foot. This stimulation consisted of: (a) non-noxious stimulation—application of light pressure to the foot with glass rods, displacement of hairs with jets of air or (b) noxious stimulation—application of radiant heat to a small area of a toe pad by means of an electric filament (3 mm diameter) placed 0.5–1 cm from the pad such that the surface of the pad attained a temperature of 45–55°C (measured with a thermocouple) within 4–10 seconds. The position of the cells recorded in the dorsal horn was determined by locating a dye mark (pontamine sky blue) in frozen sections (80 μm) cut within 24 h of completion of the experiment.

Results

Rat brainstem

Micropipette penetrations were made from 0.5–2.0 mm rostral to the obex and 1.5 mm on either side of the midline. Morphine was tested on 65 brainstem neurones, all of which were excited by acetylcholine (ACh). Brief expulsions of morphine (30–100 nA, mean 57.2 nA; 10–60 s) excited 55 cells (Figure 1) and depressed 3. Excitation by morphine was of somewhat longer latency than that of ACh and outlasted the period of ejection by 1–30 s (Figure 1).

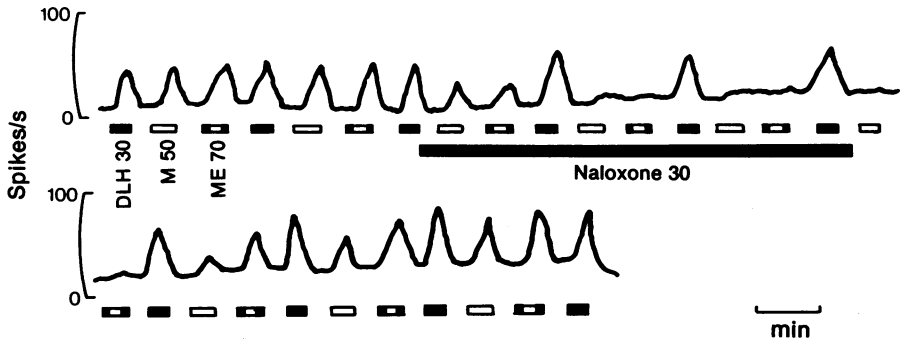

Figure 1 Continuous ratemeter records, spikes/s against time (min) of a spontaneously firing brainstem neurone. These records show reproducible excitation by D,L-homocysteic acid (DLH), morphine (M) or methionine enkephalin (ME). During the continuous administration of naloxone (30 nA), morphine and ME-induced excitations were reversibly reduced. The upper and lower records are continuous.

Depression by morphine was prolonged, lasting 15–60 s after the termination of the ejecting current.

Methionine enkephalin (ME) expelled with cationic current (30–150 nA, mean 63.8 nA; 10–60 s) excited 22 and depressed 10 of 44 brainstem neurones tested. The responses to ME were of a similar time course and magnitude to those of morphine (Figure 1). Leucine-enkephalin (LE) (25–150 nA, mean 59 nA) expelled in a similar manner to ME excited 7 of 14 cells tested. No depressant responses were seen, but the excitatory effects of LE were of similar time course to ME. There was a close correlation in the direction of response of brainstem neurones to morphine and ME, 24 of 42 cells tested with both agents responding in the same direction. However, on 7 cells morphine was excitatory, whereas ME produced depression. On 13 cells where morphine, ME and LE were tested, 7 cells were excited by all three substances. Excitatory or inhibitory responses were found within the same penetration using the same micropipette and such responses were not a particular feature of any individual micropipette.

Naloxone (30–50 nA, mean 38 nA; 2.5–9 min, mean 5.4 min) reversibly reduced both morphine (9 of 11 cells), ME (6 of 8 cells) and LE (1 cell) excitation without affecting the excitatory responses to DLH on the same neurones (Figure 1). Similar administration of naloxone reduced ACh excitation (8/10 cells) but not depression by GABA (0/3 cells). A prolonged ejection of naloxone occasionally produced an increase in background firing which was sometimes accompanied by high frequency bursts of action potentials.

Rat cortex

Morphine (40–80 nA, mean 55.3 nA), administered with similar currents and for similar periods as in the brainstem experiments described above, excited 20 and inhibited 9 of 37 cortical cells tested. Both the excitatory (Figure 2) and inhibitory (Figure 2b) responses were of similar time course to those observed in the brainstem. ME (40–80 nA, 53.3 nA) excited 12 and inhibited 7 of 28 cortical neurones tested. In two cells the excitation was preceded by a period of inhibition. In tests on 4 cells, LE excited 3 and had no effect on the other. As in the brainstem, the direction of responses to morphine and ME on the same neurones was similar. Thus, of 28 cells tested, 12 were excited by morphine and ME while 3 were depressed and 4 were unaffected by both substances. However, on two cells morphine was excitatory whereas ME produced a depression of firing (Figure 2a).

Both the depressant and excitatory responses to morphine or ME (Figure 2) were reversibly antagonized by *similar* amounts of naloxone (15–40 nA mean 23.0 nA; for 2.5–6.5 min, mean 5.0 minutes). Naloxone reduced morphine inhibition on all 5 cells and excitation in all 3 cells tested. ME-induced inhibition (Figure 2) was reduced in all 6 cells tested and excitation on 3 of 4 cells tested. Excitation by LE (one cell) or DLH (3 cells) was unaffected.

Experiments with protruding electrodes

In 2 brainstem and 3 cortex experiments, morphine, ME and naloxone were ejected some 15–30 μm from the recording sites by means of microelectrodes with a protruding recording pipette (protrusion 15–30 μm), Satoh *et al.* (1976) suggest that ejection from such electrode assemblies is in the region of the dendritic tree rather than the cell soma. In tests on 6 cholinoceptive brainstem neurones, all were excited by morphine but 4 were excited and 2 depressed by ME.

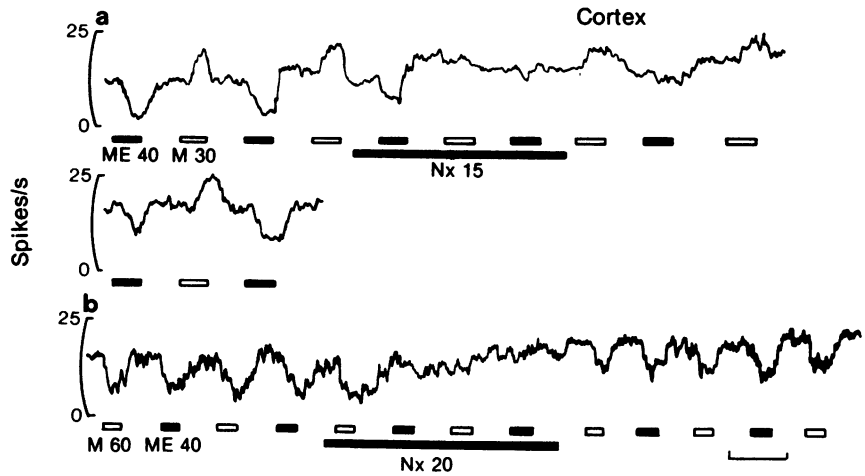

Figure 2 Ratemeter records showing the effects of electrophoretic naloxone on the responses of cortical neurones to morphine (M 30 nA) and methionine-enkephalin (ME 40 nA). Record (a) shows that this cell was reproducibly inhibited by ME but excited by M. Naloxone (Nx 15 nA) reversibly reduced the responses to both compounds. The trace (b) from another cortical neurone shows reproducible depression by M and ME. As before, naloxone (20 nA) reversibly reduced both responses. The time bar = 1 minute.

Naloxone reversibly reduced morphine excitation and ME inhibition of the same two cells.

In the cortex, morphine excited 4, inhibited 4 and had no effect on a further 4 cells tested. Of 10 cells tested, ME depressed 6 and in 2 others the excitation was preceded by a period of inhibition. Naloxone reversibly reduced the inhibition by both morphine and ME on 3 cells tested and the excitation by morphine and inhibition by ME on 3 others. In these experiments the amounts of naloxone required to antagonize morphine or ME were similar to those used in the experiments employing conventional microelectrodes.

Cat spinal cord

Renshaw cells Methionine enkephalin and morphine were tested on 44 Renshaw cells. When ME was ejected with a cationic current (30–100 nA, mean 79 nA) it excited 26 cells; morphine (30–80 nA, mean 70 nA) excited 39 cells. Excitation by ME was rapid in onset and recovery, and was similar to that observed with morphine (Figure 3). The spontaneous activity, observed in some Renshaw cells, was unaffected by morphine and ME. The excitation by ME was not due to ejection of H^+ from acidified solutions (pH 4) since electrophoresis of ME from solutions at pH 9 also excited Renshaw cells in 4 tests. Leucine-enkephalin (60–90 nA, mean 80 nA) excited 6 of 12 Renshaw cells tested. There were no clear differences in the potency or duration of action of LE compared with ME.

Naloxone (30–80 nA, mean 49 nA for 2–8 min) reversibly antagonized the excitation induced by morphine and ME but not by DLH in tests on 7 of 8 cells (Figure 3). In 5 of these cells also tested with ACh, naloxone reduced ACh excitation concomitantly with that of morphine and ME. In one cell the excitation by LE was reversibly antagonized by naloxone. Depression by glycine or GABA was unaffected by similar amounts of naloxone (4 cells). During these tests with naloxone, spontaneous (2 of 12 cells) and DLH-induced excitation (3 of 8 cells) was enhanced. More prolonged ejections (3 min or more) produced high frequency bursts of action potentials. Similar effects of naloxone on Renshaw cells have been reported previously (Duggan, *et al.*, 1976a).

Dorsal horn cells Morphine and ME were tested on 46 dorsal horn neurones. These were classified into three groups: (a) non-nociceptive units, activated by light pressure or jets of air to the ipsilateral hindfoot but not by noxious radiant heat, (b) nociceptive units, activated when the surface temperature of a toe pad exceeded 45°C following the discrete application of radiant heat; these units also responded to other sensory modalities, (c) unidentified units whose sensory modalities were not determined.

The majority of neurones (28) responding to noxious heat stimulation were located in lamina IV and V (Rexed, 1952), although a few were found in lamina I. These cells had small receptive fields, they discharged for several seconds after termination of the radiant heat stimulus and also responded to low in-

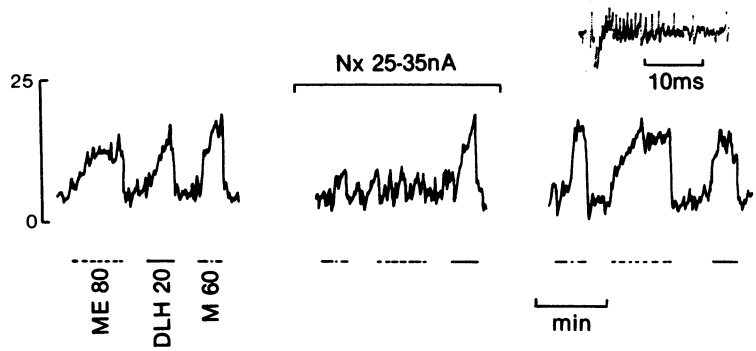

Figure 3 Ratemeter record shows, from left to right, the excitatory effects of methionine enkephalin (ME 80 nA), DL-homocysteic acid (DLH 20 nA) and morphine (M 60 nA) on a Renshaw cell; the depression of ME and M responses by the ejection of naloxone (Nx 25–35 nA for 6 min) from an adjacent barrel of the micro-electrode and subsequent recovery of the excitatory effect of M and ME 2 min after terminating the Nx expelling current. The inset above the ratemeter record indicates a single oscilloscope sweep of the synaptic response of this Renshaw cell to a ventral root stimulus.

tensity mechanical skin stimulation and clearly fall into the category of class 2 neurones described by Iggo (1974). Neurones responding only to non-noxious stimuli (10) were mainly located in lamina IV, V and VI, and probably correspond to the class 1 cells described by Iggo (1974).

Irrespective of the cell classification, morphine and ME were without effect on the spontaneous or DLH-induced firing of 34 of 41 dorsal horn neurones. Only three cells were inhibited by morphine and ME and on these naloxone was either ineffective in reversing these actions or itself produced a depression of firing. Morphine (mean 140 nA, 8 cells), ME (mean 120 nA, 8 cells) and LE (mean 90 nA, 2 cells) were all without effect on noxious or non-noxious responses. It was unlikely that the lack of effect of morphine and ME and LE on dorsal horn neurones was due to uncertain electrophoretic release since the effectiveness of these substances had been and could be subsequently demonstrated with the same electrodes on Renshaw cells in the same experiment.

It was recently reported that administration of morphine into the substantia gelatinosa region of the spinal cord selectively reduced responses of dorsal horn neurones to noxious stimuli (Duggan, Hall & Headley, 1976b). To try and confirm these observations the effects of morphine and ME on noxious responses of 20 lamina IV or V neurones were determined when these substances were ejected 30–600 μm (mean 50 μm) from the recording site with protruding microelectrode assemblies. Subsequent histological verification of the location of the tip of the multibarrel electrode indicated that in the majority of cases drug ejection was in the vicinity of lamina II and III (substantia gelatinosa). Morphine (50–200 nA mean 162 nA) ejected for 1–19 min (mean 11 min) reduced the response to noxious heat stimulation by 60–100% in 13 of these cells. Two cells were directly excited by morphine and the others were unaffected. Moreover, in 12 of the 13 cells where morphine depressed responses induced by noxious heat, submaximal responses induced by movement of interdigital hairs (air jet) (8 cells) or electrophoretic DLH (4 cells) were unchanged (Figure 4).

The depression of noxious heat responses by morphine was prolonged. Partial recovery was observed in 2 cells, 10 min after cessation of the morphine ejection. In 9 other cells recovery occurred only after the electrophoretic administration of naloxone (30–100 nA, mean 80 nA) for 2–14 min (mean 5 minutes). On 3 cells the responses to noxious heat were markedly increased above control levels following naloxone reversal of the effect of morphine. Naloxone itself did not modify the responses to non-noxious stimuli on the same neurones. In contrast to the effects of morphine, ME (100–150 nA) ejected for 8–14 min had no discernible effects on responses of 4 neurones to a noxious or non-noxious stimuli.

In 4 experiments morphine (0.25–2 mg/kg) was administered intravenously in gradually increasing doses to minimize changes in blood pressure, while recording from a cell activated alternatively by noxious heat stimulation and non-noxious air jet stimulation. Morphine selectively reduced the response to noxious heat and this effect was subsequently reversed by intravenous naloxone (0.05–0.1 mg/kg) in each case (Figure 4).

Discussion

In the present series of experiments the effects of enkephalin have been compared with those of mor-

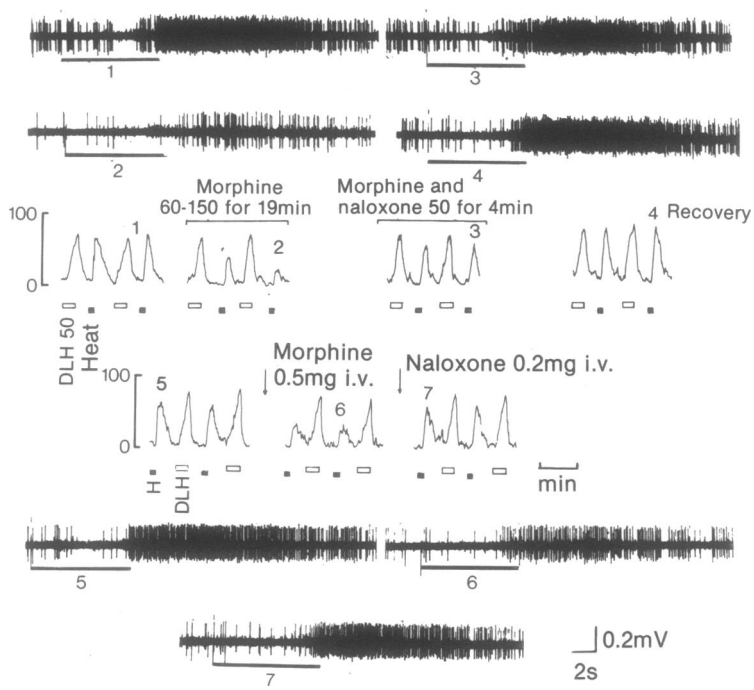

Figure 4 Ratemeter and spike record showing effects of morphine and naloxone on responses of a lamina IV neurone to alternate noxious heat stimulus and electrophoretic DL-homocysteic acid (DLH). Recordings were made from a single electrode protruding 50 μm beyond the multibarrel microelectrode. The latter was situated in the ventral border of lamina III. The upper ratemeter record illustrates selective antagonism of the noxious heat response by morphine 60–150 nA ejected for 19 min and the subsequent reversal of this effect when naloxone 50 nA was ejected for 4 min during the continued ejection of morphine. The lower ratemeter record illustrates that intravenous administration of morphine and naloxone has a similar effect to that of electrophoretic administration of these substances on this neurone. Examples of spike records of noxious heat responses corresponding to the numbered ratemeter responses are also illustrated. The middle and last part of the lower ratemeter record are examples of recordings made 3 and 2 min respectively after the injection of morphine and naloxone.

phine on single neurone activity and on synaptic responses of central neurones. The enkephalins had similar actions to morphine in most respects.

In the rat brainstem, morphine had both excitatory and inhibitory actions. The most common response observed was excitation, in agreement with previous studies in this area (Bradley & Dray, 1974; Bramwell & Bradley, 1974; Bradley, Bramwell & Dray, 1976a; Bradley & Bramwell, 1977). However, other electrophoretic studies with morphine and other opiates suggest that inhibition is the only response in the rat locus coeruleus (Baird & Kuhar, 1977) and that this response predominates in the cat brain stem (Gent & Wolstencroft, 1976). ME also had both excitatory and inhibitory actions whereas LE, tested on a smaller sample of neurones, was only excitatory. Moreover there was a good correlation between the response of the same neurones to morphine and to ME or LE. As with morphine, excitatory responses were observed more frequently than inhibitions. On the other hand previous studies suggest that the most common effect of the enkephalins in both rat (Bradley *et al.*, 1976a; Hill *et al.*, 1976; Bradley *et al.*, 1977) and cat brainstem (Gent & Wolstencroft, 1976) is inhibition. The reason for these differences in the effectiveness of morphine or enkephalin in this brain area is unclear. They may represent sampling differences from heterogeneous areas (e.g. see Bird & Kuhar, 1977) or species differences (e.g. see Gent & Wolstencroft, 1976; Anderson, Lobataz & Proudfit, 1977).

In the cerebral cortex, morphine and enkephalin also produced excitation or inhibition. Often responses to both substances were in the same direction, though on some neurones the responses to morphine were opposite to those of enkephalin. Previously both substances have been shown to depress the activity of a high proportion of cortical neurones in rats (Satoh *et al.*, 1974; 1976; Zieglgänsberger *et*

al., 1976) although in the cat, cortical neurones appear to be insensitive to morphine (Duggan, *et al.*, 1976a). These differences may be explained by the observation that during repeated administration, tachyphylaxis occurred both to morphine and enkephalin-induced depression (Zieglgänsberger *et al.*, 1976) with the consequence that excitation may thus be revealed. While no obvious tachyphylaxis to morphine or enkephalin effects were observed in the present study, its occurrence cannot necessarily be excluded. Thus the final proportion of neurones affected by opiates reported here include only those cells where the first and subsequent electrophoretic administration gave a reproducible effect. Also Satoh and others (1974; 1976) using protruding electrodes, reported that higher amounts of morphine were usually required to produce excitation. Therefore it is possible that opiate receptors mediating excitation or inhibition might be situated on different sites e.g. soma, dendrites or terminals. The present experiments with similar micropipettes gave no clear insight into these possibilities. Both excitation and depression was observed in the brainstem and the cortex. Thus, any apparent differences in opiate responses in this and other studies cannot be readily attributed to technical differences. Interestingly, differences in the responses to morphine and enkephalin have also been observed in other areas. Thus, Segal (1977) reported that neither morphine nor enkephalin had significant effects on hippocampal cell firing although some neurones were excited and some depressed. On the other hand, others report that hippocampal neurones are predominantly excited by morphine and enkephalin (Nicoll, Siggins, Ling, Bloom & Guillemin, 1977) and that the direction of the responses appears to be determined by the level of background activity (Hill, Pepper & Mitchell, 1977). In the present experiments there appeared to be no obvious relationship between the direction of the responses and background activity either in brainstem or cortex. In agreement with previous studies however (Davies & Duggan, 1974; Duggan *et al.*, 1976a; Davies, 1976; Davies & Dray, 1976a) morphine and enkephalin consistently excited spinal Renshaw cells.

It is likely that excitation by morphine or enkephalin was mediated by activation of specific opiate receptors since this action was selectively and reversibly antagonised by naloxone in the brain stem and cortex. Our observation of naloxone-reversible excitation of Renshaw cells confirms previous reports (Duggan *et al.*, 1969; Davies & Duggan, 1974; Davies, 1976; Davies & Dray, 1976a). Recently others have also shown naloxone-sensitive excitations by opiates in several other areas of the brain and spinal cord after electrophoretic and systemic administration (Nicoll *et al.*, 1977; Hill *et al.*, 1977; Belcher & Ryall, 1977; Chou & Wang, 1977; Anderson, Basbaum & Fields, 1977). Though it is arguable that the effects of systemic drugs may be mediated indirectly, these latter observations support the notion for excitatory opiate receptors. However since others have more commonly reported that morphine and enkephalin-induced inhibition is readily antagonized by naloxone it has been suggested that inhibition is of greater physiological significance (see Bradley *et al.*, 1977). Indeed naloxone readily antagonized morphine and enkephalin inhibition in the present experiments in the cortex though there appeared to be little difference in the amounts which also antagonised excitatory responses in this area. These collective observations would therefore support the hypothesis (Lord, Waterfield, Hughes & Kosterlitz, 1977) that multiple receptors for opiates and enkephalins exist in the CNS as in peripheral tissues.

It is difficult to extrapolate from the observations made on single brainstem, cortical or Renshaw cells to explain the analgesic properties of morphine or enkephalin. However, opiate receptor binding studies (for reference see Atweh & Kuhar, 1977) and measurements of enkephalin distribution in various brain regions (Simantov *et al.* 1976a, b; Elde *et al.*, 1976; Simantov *et al.*, 1977) suggest that both the cortex and brainstem exhibit activity in both these respects. The brainstem in particular has several important loci for analgesic mechanisms and the actions of opiates (for reference see Bradley *et al.*, 1977; Atweh & Kuhar, 1977). On the other hand while Renshaw cells clearly possess opiate receptors and may be involved in mediating some spinal actions of opiates, it is unlikely that they are involved in pain processes (Davies, 1976). However, recent data suggest opiates exert analgesic actions at the spinal cord level (Le Bars, Menétrey, Conseiller & Besson, 1975; Yaksh & Rudy, 1977). Moreover, high opiate binding (Pert, Kuhar & Snyder, 1975; Lamotte, Pert & Snyder, 1976; Atweh & Kuhar, 1977) and high enkephalin levels are associated particularly with dorsal horn structures which receive primary afferent fibres (Simantov *et al.*, 1977; Elde *et al.*, 1977). Enkephalin containing cell bodies and terminals have been found in laminae II-V (Elde *et al.*, 1977) and these cells may represent a system of interneurones at all levels of the spinal cord. Thus, effects on dorsal horn structures may have more relevance to the analgesic properties of opiates.

Previous reports on the effects of electrophoretic morphine on dorsal horn neurones are conflicting. Using conventional microelectrodes or electrodes where the recording barrel only protruded by 10–20 μm (Zieglgänsberger & Bayerl, 1976), morphine depressed spontaneous firing, glutamate-induced excitation and responses induced by noxious skin stimulation (Dostrovsky & Pomeranz, 1973; Calvillo, Henry & Newman, 1974; Zieglgänsberger & Bayerl, 1976). Moreover, the depression of glutamate-induced

firing and responses to noxious skin stimulation were antagonized by naloxone (Calvillo *et al.*, 1974; Zieglgänsberger & Bayerl, 1976). The data presented in this paper support the previous findings of other laboratories that both morphine and ME ejected from conventional microelectrodes generally have little effect on dorsal horn neurones whether these are responsive to noxious stimuli or not (Davies, 1976; Duggan *et al.*, 1976a; Duggan, Hall & Headley 1977). In addition, electrophoretic naloxone invariably had similar effects to those of morphine or failed to antagonize the action of morphine in the present experiments and those reported by others (Dostrovsky & Pomeranz, 1976; Duggan *et al.*, 1976a).

There is no clear explanation for these differences. It is unlikely that failure to affect dorsal horn cells in the present study was due to inadequate electrophoretic release of morphine since positive effects were demonstrated with the same electrodes on Renshaw cells in the same experiments. Duggan *et al.* (1976b; 1977) presented evidence suggesting that morphine and enkephalin exert selective naloxone reversible effects on dorsal horn neurones if administered into the substantia gelatinosa region of the spinal cord. The present findings that morphine ejected in the vicinity of laminae II and III (substantia gelatinosa region) depressed responses of cells recorded in deeper laminae (laminae IV and V) to noxious stimuli support such observations. The absence of effects of ME ejected from similar electrodes with protruding recording barrels is not necessarily at variance with an action of opiates in the substantia gelatinosa but may be related to rapid inactivation of ME (Hambrook, Morgan, Rance & Smith, 1976). Duggan *et al.* (1976b) reported that ME only affected 2 of 6 neurones tested whereas the more stable analogue, methionine enkephalin amide, affected 10 of 11 cells tested. Indeed, we have also found that electrophoretic ejection of a less labile penta-peptide [D-Ala^2]-leucine-enkephalin (Baxter, Geoff, Miller & Saunders, 1977) into the substantia gelatinosa selectively reduces responses of laminae IV and V neurones to noxious stimuli (Davies & Dray, unpublished observations). Whether morphine affected only the activity of substantia gelatinosa neurones which may synapse with neurones in deeper laminae or had additional actions on the dendrites of laminae IV or V neurones which project into this region (Webster, 1977) is uncertain. It is also unclear whether the reduction of nociceptive responses involved the activation of excitatory or inhibitory receptors or a modification of the release of primary sensory transmitters such as substance P (Jessell & Iversen, 1977). However, irrespective of the mechanisms involved the present results are consistent with the hypothesis that the enkephalins may be endogenous ligands for central opiate receptors and may function as neurotransmitters.

This work was supported by an MRC Programme Grant to Professor D.W. Straugham. We thank Endo Laboratories Inc., for gifts of naloxone, Dr B.A. Morgan of Reckitt & Colman Ltd., Hull, and Dr M.J. Turnbull of I.C.I. Alderly Park for gifts of enkephalins.

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(Received August 15, 1977.
Revised November 14, 1977.)