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Opiates distinguish spinal excitation from inhibition evoked by noxious heat stimuli in the rat: relevance to theories of analgesia

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1 Experiments were performed to test the hypothesis that a significant part of the action of opiates in reducing responses to noxious stimuli is a reduction in the release of neurotransmitter from primary afferent fibres.

2 The effects of locally and systemically administered opiates were examined on the excitatory and γ-aminobutyric acid (GABA)-mediated inhibitory responses of spinal dorsal horn neurones to noxious heat stimulation in the anaesthetized rat: the inhibitions are thought to involve the same C-fibre afferents as the excitation.

3 Microionophoretically administered morphine reduced the excitatory response in a small proportion of the cells, reduced the background firing in a larger proportion but was ineffective on the inhibition.

4 Intravenously injected morphine (0.5–6 mg kg^{-1}) or etorphine (0.1–2 μg kg^{-1}) invariably attenuated the excitation of dorsal horn neurones by noxious stimuli but had no effect on the inhibition.

5 It was concluded that the data do not support the hypothesis that the production of analgesia is due mainly to a reduction in the release of transmitter from primary afferent fibres.

Introduction

This study on dorsal horn neurones was undertaken to verify the prediction that opiates would reduce the inhibitory as well as the excitatory effects evoked by stimulation of peripheral nociceptors. The prediction was based on the assumption that a major action of opiates is to reduce the release of transmitter from primary afferent fibres.

Morphine administered locally by ionophoresis into the substantia gelatinosa (Duggan *et al.*, 1977) or into deeper laminae (Calvillo *et al.*, 1974; Belcher & Ryall, 1978) reduces the ability of noxious stimulation to excite dorsal horn neurones. A similar effect is obtained when morphine or etorphine is microinjected into the brain or administered systemically (Clark & Ryall, 1983; Clark *et al.*, 1983; Gebhart *et al.*, 1984). Radiant heat was the noxious stimulus used in most of these studies. Thus it is clear that a major site of action of opiates in causing analgesia is to reduce the transmission in pain afferent pathways at the spinal level, although additional effects at supraspinal levels are also involved.

Opiates decrease the potassium-evoked release of substance P from slices of the trigeminal nucleus (Jessel & Iversen, 1977) and the stimulus-evoked release of substance P from the spinal cord *in vivo* (Yaksh *et al.*, 1980). Since substance P in primary afferent fibres is restricted to non-myelinated fibres (Hokfelt *et al.*, 1977) and such fibres mediate the response to painful stimuli, it is frequently assumed that a major action of opiates in reducing the response to pain is an interaction with opiate receptors located on the central terminals of non-myelinated primary afferent, nociceptive neurones and that the consequence of this interaction is a reduction in the release of the primary afferent transmitter, substance P.

Recently, we have shown that noxious radiant heating of the glabrous skin of the foot in the cat or the rat (Pini & Ryall 1986; Ryall & Pini, 1986) or of the hairy skin in the rat (unpublished data) produces either excitation or GABA-mediated inhibition of spontaneous or evoked neural activity of dorsal horn

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neurones, depending upon the location of the stimulus in the receptive field.

It is probable that the same type of afferent fibres mediate both the excitatory and the inhibitory responses to painful stimuli. If a major action of morphine is to reduce the release of neurotransmitter from unmyelinated primary afferent fibres, then it should reduce the inhibition as well as the excitation. The present study was therefore undertaken to test this hypothesis. A preliminary account of these findings was presented to the British Pharmacological Society (Harris & Ryall, 1986).

Methods

Experiments were performed on male Wistar rats (Bantin & Kingman, Hull) weighing 250–500 g and anaesthetized with thiobutabarbitone (Inactin, Byk; 100–118 mg kg^{-1} i.p.), supplemented with intravenous administration as required. Rectal temperature was measured and routinely maintained at 37 ± 1°C with a thermostatically controlled heating pad. The spinal cord was exposed by a thoracolumbar laminectomy and the cord transected in the lower thoracic region. The dura mater was carefully opened over the lumbar segments and the exposed cord was covered in a pool of paraffin oil.

Extracellular recordings were obtained from single dorsal horn neurones in the lower lumbar segments with single or multibarrelled microelectrodes. The recording barrels were filled with 4 M NaCl and had resistances of between 2–8 MΩ. Multibarrelled pipettes with tip diameters of 4–7 μm were used to apply morphine (100 mM as the sulphate, pH 4.5) or L-glutamate (200 mM as the sodium salt, pH 8) ionophoretically into the vicinity of single neurones. In each electrode another barrel contained 165 mM NaCl for current balancing or checking for current artefacts.

Extracellular recordings were electronically filtered and monitored on an oscilloscope. Action potentials were discriminated from the background activity, counted by a ratemeter and plotted as a continuous record of firing frequency on a chart recorder.

Cells were characterized according to their responses to noxious and non-noxious stimuli. Noxious stimuli consisted of pulses of radiant heat from a 100 W projector lamp focused by a parabolic reflector onto the plantar surface of the foot for 15 to 30 s. The skin temperature was monitored by a thermistor placed in the centre of the heated area (Clark & Ryall, 1983). The temperature was maintained between 45–52°C during heating under feedback control from the thermistor.

Morphine sulphate (Macarthy), etorphine HCl (Reckitt & Coleman Ltd.), naloxone HCl (Endo Laboratories) or sodium nitroprusside (Koch-Light) were administered through the femoral vein.

Results

Recordings were obtained from two hundred and sixteen dorsal horn neurones in Rexed's lamina III–V in thirty nine rats. The receptive fields were located on the ipsilateral hind foot. Most of the cells were excited or inhibited by noxious radiant heat and excited by non-noxious stimuli, such as light brushing or touching with a blunt instrument. They were therefore multireceptive neurones (Iggo, 1974; Menétrey *et al.*, 1977). Two hundred and three neurones responded to noxious radiant heat, thirty three of them (16.3%) being inhibited, the remainder receiving only excitation from nociceptor afferents. This proportion is similar to that found by Pini & Ryall (1986). All of the cells studied further were spontaneously active with firing rates between 1 and 25 Hz.

Effect of ionophoretic morphine

On six cells excited by the noxious stimulus, morphine (20–180 nA), administered inophoretically for 4–20 min, caused a slight reduction in the background firing frequency of one cell but had no effect on background firing of the others. There were no effects on spike amplitude. Morphine had no effect on the increase in firing evoked by noxious heat in five of the six cells tested. On the remaining cell, there was a reduction in the response to noxious heat, which was repeatable and not reproduced by a control current ejecting sodium ions from salt solution in another barrel.

Morphine, administered with currents 20–180 nA for 7–50 min to four neurones inhibited by noxious radiant heat, reduced the background firing frequency of two of these cells but had no effect on the inhibitory response to noxious heat.

Effect of intravenous morphine

The failure of ionophoretic administration of morphine to exhibit marked effects on excitatory or inhibitory effects of noxious stimulation could be due to the drug acting on primary afferent C-fibre terminals mainly at laminae more superficial than those to which it was administered in this study. Intravenous administration of opiates would obviate this difficulty and so was used in the remainder of the study.

Morphine caused transient reductions in blood pressure in these spinalized rats. To avoid large

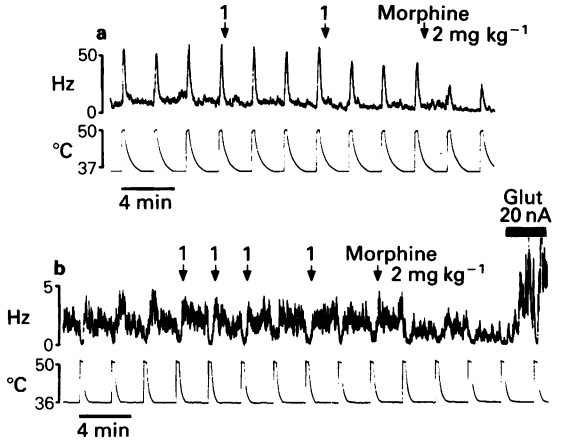

Figure 1 Effect on two dorsal horn neurones of intravenous injections of morphine sulphate on the facilitation (a) or inhibition (b) of firing produced by noxious radiant heating of the skin. The upper record in (a) and (b) shows the frequency of firing in Hz and the lower record the skin temperature, recorded with a thermistor at the heated site. The cumulative dose injected was $4\,mg\,kg^{-1}$ in (a) and $6\,mg\,kg^{-1}$ in (b). Morphine decreased the background firing in both experiments and reduced the excitatory effects of radiant heating in (a) but had no effect on the inhibition in (b). The firing frequency was elevated towards the end of the record in (b) by the ionophoresis of L-glutamate (Glut). At this time, the inhibition was still clearly marked.

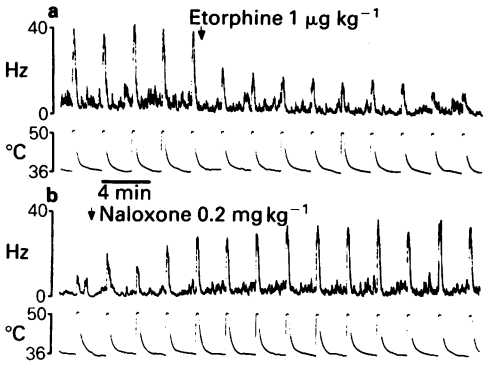

Figure 2 Depressant effect of a single intravenous injection of etorphine on the excitatory response to noxious heating of the skin and on background firing in (a) and the reversal of the depression by an injection of naloxone in (b). Records of frequency of firing and skin temperature as in Figure 1.

changes in blood pressure, it was given in small cumulative doses (Schmidt & Livingstone, 1933). In order to check that changes in blood pressure had no effects on the nociceptive responses, reductions in the blood pressure were obtained using sodium nitroprusside either as a bolus injection of $50\,\mu g$ or as an intravenous infusion at $50\,\mu g\,min^{-1}$. These injections produced falls in blood pressure similar to or greater than those produced by morphine but had no significant effects on either the background firing rate or the evoked responses to noxious stimulation. The mean ($\pm$s.e.) initial arterial blood pressure in twenty three opiate-naïve rats was 98.6 ± 2.1 mmHg. An initial dose of morphine ($0.5\,mg\,kg^{-1}$) caused a fall in blood pressure of 23.6 ± 1.8 mmHg ($n = 5$). Nitroprusside ($50\,\mu g\,min^{-1}$) caused a larger fall in pressure of 38.8 ± 6.9 mmHg ($n = 4$).

On nine cells excited by noxious radiant heat, the cumulative dose of morphine ranged from 0.5–$4\,mg\,kg^{-1}$ and reduced the background firing rate as well as reducing the evoked response to noxious heat (Figure 1a). In contrast, on seven cells inhibited by noxious radiant heat, cumulative doses of the morphine up to $6\,mg\,kg^{-1}$ in different animals also reduced the background firing rate of these cells but had no effect on the inhibitory responses to noxious heat (Figure 1b). Since the inhibitory responses following the reductions of background firing rate by morphine were sometimes difficult to evaluate, the background firing rate of such cells was elevated by the ionophoretic administration of L-glutamate. During the period when the firing rate was elevated, the inhibitory response to noxious heat pulses to the receptive field of these neurones was still present: there was a marked decrease in the firing frequency during the noxious stimulus to a level similar to that produced by noxious heat before drug administration.

Effect of intravenous etorphine

Etorphine is a potent agonist on μ-type opiate receptors but is not selective. It has a shorter duration of action than morphine which makes it easier to follow the time course of the effects of an intravenous administration.

Etorphine was injected intravenously as the hydrochloride whilst recording from eleven cells excited by noxious radiant heat and seven cells inhibited by noxious heat. The evoked excitation was regularly reduced by $0.5–1.0\,\mu g\,min^{-1}$ etorphine in different animals (Figure 2). In one instance, $0.2\,\mu g\,kg^{-1}$ increased the background firing frequency and the evoked response to heating the skin and this effect recovered after 30 min.

Seven dorsal horn neurones inhibited by noxious radiant heat were challenged with etorphine at doses of $0.1–2\,\mu g\,kg^{-1}$. These doses reduced the background firing rates of the cells but had no effect on the inhibitory responses to noxious heat (Figure 3).

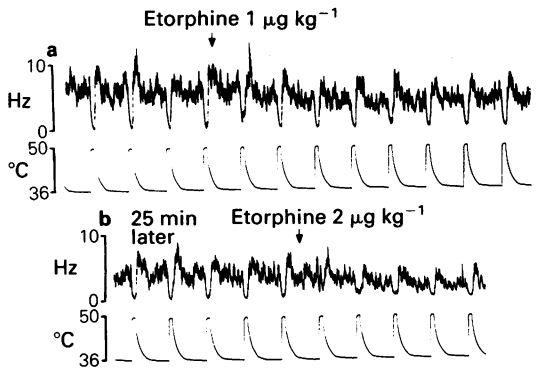

Figure 3 Depression of background firing by etorphine, with no effect on the inhibition evoked by noxious radiant heat. Records of frequency of firing and skin temperature as in Figure 1.

In five rats, an initial dose of $1\,\mu g\,kg^{-1}$ of etorphine reduced the blood pressure by 29.2 ± 6.6 mmHg. This effect was smaller than that produced by sodium nitroprusside which was without effect on the firing of the dorsal horn neurones.

Effect of naloxone

Naloxone hydrochloride, given in doses of 0.1–$0.5\,mg\,kg^{-1}$, reversed the effects of intravenous morphine on the background firing and partially reversed inhibition by morphine of excitatory responses to noxious heat on all four cells tested.

On two cells the effects of etorphine were reversed by naloxone at doses of 0.2 and $1\,mg\,kg^{-1}$ (Figure 2).

Two further cells inhibited by noxious radiant heat were challenged with intravenously administered naloxone. Naloxone ($1–20\,mg\,kg^{-1}$) had no effect on the background firing rate or on the inhibitory response to noxious heat.

Discussion

We have previously demonstrated that the ionophoretic administration of morphine to cells in the deeper laminae of the dorsal horn in the cat reduces their responses to noxious stimulation by heat or bradykinin in about half of the cells tested (Belcher & Ryall, 1978), although the excitation by DL-homocysteic acid was attenuated on the majority of the cells. In the present experiments in rats, there was a reduction in the response to noxious heat on only one of six cells tested, although the background firing frequency was reduced in three of ten cells. The apparently lower incidence of attenuation of the response to noxious heat in the rat may have been due either to a lower sensitivity of the dorsal horn cells in the rat compared to the cat or to the small size of the sample in rats. Regardless of the explanation, we are unable to attribute much significance to the failure to reduce the inhibitory response to noxious heat on any of the four cells tested.

The advantage of systemic administration of opiates in spinal animals is that no assumptions are made regarding the locus of action in the spinal cord. Accordingly, the intravenous administration of morphine reduced the background firing frequency and the excitatory response to noxious heat stimuli on all cells from which recordings were obtained. The amounts injected ranged from 0.5 to $4\,mg\,kg^{-1}$, which is similar to the range of doses employed in the cat (see Clark *et al.*, 1983 for references). Etorphine, which is effective in the microgram range in the cat (Clark & Ryall, 1983), is similarly effective on the excitatory response to noxious heat in the rat.

These data on excitatory response to noxious heat are, of course, compatible with any site of morphine action on opiate receptors on the pathway from nociceptor afferent C-fibres to the dorsal horn neurones, from which the recordings are obtained, and are consistent with either presynaptic sites of action, or with postsynaptic sites in the substantia gelatinosa or in deeper laminae. In particular, they may be reconciled with the view that a major action of opiates in the production of analgesia is a reduction in the release of a primary afferent neurotransmitter, possibly substance P, mediated through opiate receptors on the terminals of the primary afferent C-fibres.

On this basis, it was predicted that opiates would attenuate any effect consequent on the activation of nociceptive C-fibres. However, there was no effect of morphine or etorphine on the inhibitory response to noxious heating of the skin, which we believe to be mediated by the same sensory fibres that mediate the excitatory response, the difference being due to the location of the stimulus in the receptive field on glabrous or hairy skin in the cat and the rat (Pini & Ryall, 1986; Ryall & Pini, 1986; Harris & Ryall, unpublished observations).

Figure 4 illustrates two possible explanations for the failure of opiates to attenuate the C-fibre mediated inhibition from nociceptive afferents. In (a) there is only one set of primary afferent fibres whereas in (b) there are two. It is considered that the more likely hypothesis is that shown in Figure 4a in which inhibition or excitation is dependent only on the location of the stimulus in the receptive field: there is physiological and pharmacological evidence for the intervention of a γ-aminobutyric acid (GABA)ergic interneurone in the inhibitory pathway

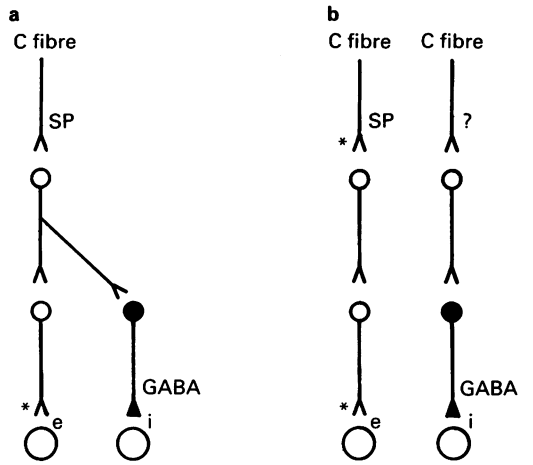

Figure 4 Two alternative proposals to account for the inability of opiates to attenuate inhibition of dorsal horn neurones evoked by noxious heating of the skin while reducing background firing and the excitation evoked by the same stimulus. The asterisks indicate the proposed major site of action of opiates. The preferred mechanism is that shown in (a) (see Discussion). In (a), the same afferent fibres mediate both the inhibition (i) and the excitation (e) but there is a divergence of the pathway for inhibition of neurones which are excited from an adjacent part of the receptive field. The primary afferent transmitter may be substance P (SP) but this is not essential to the argument. The reduction in transmitter release at the first order synapse does not account for the opiate effects. An alternative mechanism is shown in (b) in which two sets of primary afferent C-fibres are postulated. These may or may not contain different transmitters but it is necessary to specify that the opiate receptors are located only on the pathway which excites the dorsal horn neurones and that the other set of C-fibres, which have no opiate receptors on their terminals, mediates only inhibition.

(Pini & Ryall, 1986; Ryall & Pini, 1986; Harris & Ryall, unpublished observations). Nevertheless, the alternative hypothesis shown in Figure 4b must be considered. This shows two independent pathways for excitation and inhibition. Fibres in one set, those mediating excitation, have opiate receptors on them whereas fibres in the other set, which mediates the inhibition, do not. Arguments related to the second hypothesis (Figure 4b) include the following:

(i) It is possible that the peripheral sensory fibres mediating the inhibition utilize another neurotransmitter at their central terminals and that opiates do not reduce the release of this transmitter. Substance P is shown as the transmitter for the excitatory pathway in Figure 4b but this is not essential to the argument. It has been suggested (Hokfelt *et al.*, 1976; Kuraishi *et al.*, 1985; Yaksh, 1986; Weisenfeld-Hallin, 1986) and refuted (Leah *et al.*, 1985; Duggan *et al.*, 1987) that noxious stimulation releases both substance P and somatostatin which are contained in distinct sets of afferent fibres. If the suggestion is correct it is possible that the opiate receptors are restricted to the substance P-containing fibres.

(ii) An excitatory amino acid is another candidate for the role of primary afferent excitatory transmitter in C-fibres (Schouenborg & Sjolund, 1986). Schouenborg & Sjolund showed that an antagonist (D-glutamyl glycine) reduced the excitation of lamina I–IV neurones in the rat. There is no evidence to indicate that opiates differentially reduce the release of one neurotransmitter such as substance P but not another such as an excitatory amino acid from primary afferent nociceptive fibres. Even if there was a differential reduction in neurotransmitter release, the observation by Schouenborg & Sjolund that the excitation of dorsal horn neurones was reduced by an amino acid antagonist shows that the excitatory amino acid is involved in the excitatory pathway rather than, or in addition to involvement in the inhibitory pathway to the dorsal horn cells.

(iii) The peripheral fibres mediating the inhibition may be excited by the same stimuli and utilize the same neurotransmitter as those mediating excitation to the dorsal horn neurones, but the former may lack opiate receptors upon their terminals. If this postulate is correct then it should be possible to demonstrate two functionally distinct sets of primary afferent C-fibres, both sets being activated by noxious heat stimuli but only one set having opiate receptors upon their central terminals: such fibres have yet to be demonstrated.

(iv) The postulate in Figure 4b requires some improbable assumptions without physiological precedent in the central nervous system. These are that some peripheral afferent fibres mediate only excitation at central nociceptive neurones whereas other fibres, identical in their responses to peripheral stimuli in all other respects save location in the receptive field but differing in the absence of opiate receptors on their terminals, mediate only inhibition.

These considerations lead us to conclude that our data are best explained by postulating a mechanism which does not require a reduction in the release of primary afferent neurotransmitter. This mechanism must be located on that part of the pathway to the nociceptive dorsal horn neurone which is not shared by the inhibitory, GABAergic pathway. Such a site is indicated by the asterisk in Figure 4a: it could be either presynaptic to the final neurone or post-synaptic on the soma region of the penultimate neurone. The GABAergic neurone must be devoid of opiate receptors or such receptors may not be vital

to its function. This hypothesis accounts for the reduction of excitation of the nociceptive neurone by opiates while leaving the inhibition unaffected and requires no new assumptions regarding the organization of function in peripheral fibres.

An extrapolation from this reasoning is that the indubitable reduction in release of substance P by opiates may be unimportant for the analgesic effect. Such a conclusion is not new. Firstly, Wall *et al.* (1981) and Wall & Fitzgerald (1982) questioned the role of substance P as a transmitter from the primary afferent fibres by depleting the peptide from the terminals in the substantia gelatinosa with capsaicin. They were unable to show a reduction in spinal transmission of impulses from C-fibres in the cut nerve.

Secondly, there is evidence (Mauborgne *et al.*, 1987) from *in vitro* studies for the involvement of both δ- and μ-receptors in the release of substance P from the spinal cord: μ-receptor activation increases release whereas action on δ-receptors leads to a reduction in release. It was concluded that opiate analgesia, which seems to be mainly a μ-receptor-mediated effect, cannot be due to the reduced release of substance P. However, the reduced release of primary afferent neurotransmitter could be more important for other analgesic substances which have a relatively greater effect on the δ-receptors than on the μ-receptors.

Woolf & Fitzgerald (1982) showed that the inhibition of lamina IV and V neurones in the rat by impulses in large diameter primary afferent fibres is reduced by naloxone. Inhibitory responses recorded in the trigeminal nucleus to noxious stimulation are blocked by naloxone in the rat (Mayer & Hill, 1978), but it is unknown whether these responses are mediated by A-fibres. We believe that the inhibition in the spinal cord evoked by noxious heating of the skin is mediated by small, non-myelinated afferents. In two animals this inhibition was unaffected by systemically administered naloxone. This strengthens the view that the inhibition is not mediated by large diameter afferents and indicates that the inhibitory GABAergic system is not controlled by an intraspinal enkephalinergic system.

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