Distinct antinociceptive actions mediated by different opioid receptors in the region of lamina I and laminae III–V of the dorsal horn of the rat

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1 In view of the presence of μ, δ and κ opioid receptors in the spinal dorsal horn and their apparent involvement in behavioural analgesia, the present experiments addressed the action of selective agonists ionophoresed in the vicinity of rat dorsal horn neurones which were located either in lamina I or in laminae III–V.

2 In laminae III–V, κ agonists (U50488H and dynorphin A) caused a selective inhibition of the nociceptive responses of multireceptive cells, whilst μ and δ agonists ([D-Ala2,MePhe4,Gly-ol]enkephalin and [D-Pen2,D-Pen5]enkephalin respectively) failed to alter either the spontaneous activity or the responses to noxious and innocuous cutaneous stimuli and to D,L-homocysteic acid or glutamate. Nocispecific neurones were encountered too rarely in laminae III–V to study their properties.

3 In lamina I, agonists had no effects on either nocispecific or multireceptive neurones. In contrast, the μ agonist [D-Ala2,MePhe4,Gly-ol]enkephalin consistently inhibited nociceptive responses of both multireceptive and nocispecific lamina I cells. The δ agonist [D-Pen2,D-Pen5]enkephalin consistently caused selective inhibition of the nociceptive responses of multireceptive cells but had a mixed profile of action on nocispecific cells.

4 These results suggest that μ, δ and κ opioid receptors mediate different antinociceptive actions in both laminae III–V and lamina I. The study reveals a distinct physiological role for δ receptors in modulating nociceptive inputs to lamina I neurones. In contrast to μ and κ receptor actions, δ receptors heterogeneously influence subpopulations of neurones.

Introduction

Clinical observations indicate that systemically administered morphine and other opiates selectively attenuate sensations evoked by noxious, but not innocuous stimuli (Behar et al., 1979; Wang et al., 1979). Opiates can act at both spinal and supraspinal sites to reduce neuronal responsiveness to nociceptive stimuli (see Duggan & North, 1984, for review). Intrathecal administration of opiates or opioid peptides can elicit behavioural analgesia in man and animals (Wang et al., 1979; Onofrio & Yaksh, 1983; Yaksh, 1983; Schmauss & Yaksh, 1984). Behavioural studies have suggested the efficacy of intrathecally administered opioids at both μ (Schmauss & Yaksh, 1984) and δ-sites (Hill et al., 1987). Systemic and intrathecal administration of κ-agonists have been reported as antinociceptive, particularly in visceral and mechanical nociceptive tests (Hayes et al., 1983; Schmauss & Yaksh, 1984). Other groups have observed thermal antinociception with intrathecal dynorphins and U50488H although there appears to be little margin between antinociception and motor dysfunction and paralysis (see Fleetwood-Walker, 1988; Millan, 1990, for reviews). The presence of these receptor subtypes and endogenous opioid peptides within the dorsal horn of the spinal cord, especially the superficial laminae (Hökfelt et al., 1977; Botti- celli et al., 1981; Morris & Herz, 1987; Mansour et al., 1988) suggests a complex role for opioids in influencing the spinal processing of nociceptive information at the single neurone level. Although the CNS of the rat contains relatively low levels of κ receptors (Mansour et al., 1988) their presence in dorsal horn, especially the superficial zone is now well-established (Morris & Herz, 1987).

In electrophysiological studies, single neurones of the dorsal horn in lamina I, lamina II (the substantia gelatinosa) or in the deeper dorsal horn (laminae III–V) exhibit somewhat different functional characteristics (Menetrey et al., 1977; Cervero & Igo, 1980; McMahon & Wall, 1983; Schouenborg & Sjolund, 1983) including a range of responses to noxious and/or innocuous cutaneous stimuli. The effects of microionophoretically-applied opioids (including morphine) on single dorsal horn neurones have been investigated by a number of groups (Calvillo et al., 1974; Dostrovsky & Pomeranz, 1976; Ziegglansberger & Bayerl, 1976; Duggan et al., 1977; Belcher & Ryall, 1978; Davies & Dray, 1978; Piercey et al., 1980). In general, either depression or excitation of multireceptive neurones in the deeper dorsal horn was reported. These effects were rarely reversible by naloxone. In contrast, more consistent antinociceptive and naloxone-reversible effects were observed with deeper dorsal horn neurones when opiates were administered into the substantia gelatinosa (lamina II) (Duggan et al., 1977; Sastry & Goh, 1983). These results indicated that the effects of agonists at different opioid receptors may be rather discretely mediated within distinct laminae of the dorsal horn.

A recent study from our laboratory supported this hypothesis. Fleetwood-Walker et al. (1988) reported that when administered near to spinocervical tract (SCT) neurones in laminae III–V of cat spinal cord, only κ agonists exerted a selective antinociceptive action whilst μ and δ agonists were ineffective. In contrast, μ, but not δ or κ agonists ionophoretically applied in the region of the substantia gelatinosa had a selective antinociceptive effect on SCT neurones. In these experiments there was little evidence for any significant antinociceptive role of δ agonists at either of these two loci. However, results of intrathecal studies showed that δ, as well as μ and κ, agonists have distinct analgesic actions (Onofrio & Yaksh, 1983; Hill et al., 1987). Whilst the effects of receptor-selective opioid agonists administered in lamina II and laminae III–V were investigated in detail by Fleetwood-Walker et al. (1988), effects on cells in lamina I remained to be studied. Furthermore, the majority of neurones tested in previous studies were multireceptive. The present study investigated the actions of μ, δ and κ agonists on nocispecific and multireceptive neurones in lamina I and compared these effects with actions on multireceptive laminae III–V cells, in the rat. Preliminary reports of this work have

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appeared in abstract form (Fleetwood-Walker et al., 1986; Hope et al., 1987).

Methods

Experiments were carried out on rats (270–330 g) anaesthetized with intravenous α-chloralose (35 mg kg⁻¹) and urethane (700 mg kg⁻¹), after induction with halothane. Supplemental doses of anaesthetic were given as required. Core temperature was maintained at 37–38°C and carotid blood pressure was monitored throughout the experiment. If blood pressure fell below 90 mmHg, the experiment was terminated. Humidified oxygen (0.11 min⁻¹) was passed through one arm of a patient Y-shaped cannula to enrich the inspired air of spontaneously respiring rats. A cranotomy was performed to allow stereotaxic placement of electrodes at supraspinal sites and a laminectomy (thoracic 12–lumbar 4) allowed access for electrophysiological recordings from dorsal horn neurones.

Electrophysiological methods

Extracellular recordings were made via the central barrel (4 M NaCl, pH 4.0–4.5) of a 7 barrelled glass microelectrode. Electrode tip sizes were 4.0–4.5 μm and d.c. resistances were 5–8 MΩ. The band-width of the recording amplifier was 1 Hz–7 kHz.

One side barrel contained 1 M NaCl (pH 4.0–4.5) for automatic current balancing and current controls (Neurophore Ionophoresis System, Medical Systems Corporation). Another side barrel contained Pontamine Sky Blue (2% in 0.5 M sodium acetate for marking recording sites by ejection for 50 μA min). Other barrels contained various opioid receptor-selective agonists or antagonists (Corbett et al., 1984; Fleetwood-Walker et al., 1988): 2.5 mM dynorphin A₁₋₁₃ (DYN0), 5 mM [d-Ala₂,MePhe₄,Gly-ol]enkephalin (DAMG), 5 mM [d-Pen₂,d-Pen₄]enkephalin (DPPDE), 100 mM naloxy hydrochloride, 30 mM ICI 174864 (N,N-diallyl-Tyr-Alb-Alb-Phe-Leu) (all in aqueous solution at pH 4.0–4.5), 5 mM US50488H (trans-3,4-dichloro-N-methyl-N-(2-pyrrolidiny]kyl)clohexyl benzene acetamide, US50) in 100 mM NaCl at pH 4.0–4.5 and 100 mM d$_{1}$-homocysteic acid (DLH) and 100 mM sodium glutamate in aqueous solution at pH 8.0–8.5. Synthetic peptides were obtained from Sigma or Cambridge Research Biochemicals, except for ICI 174864 which was a gift from IC Pharmaceuticals plc.

All drugs were applied with a cathodal current except DLH and glutamate (anodal). Retention currents of 10–15 nA were applied to minimize drug leakage between tests.

The location of each neurone was examined histologically, having been marked by Pontamine Sky Blue dye ejection, at the end of each test. Many neurones located in lamina I were also identified as belonging to the spinomensephalic tract (SMT) by use of standard antidromic criteria (Lipski, 1981). Concentric bipolar electrodes (Rhodes Medical SNE-100) were stereotaxically placed in the region of the contralateral parabrachial nucleus (posterior 9.5–10.0 mm, ventral 6.5–7.0 mm and lateral 0.5–1.0 mm, with respect to bregma (Hylden et al., 1986; Paxinos & Watson, 1986). The positions of all stimulating electrodes were marked by iron deposition from the electrode (30–60 s, 30 μA d.c.) and verified histologically, following ferricyanide staining.

Test protocols

Action protocols of the recorded neurones were of uniform size and shape and could be clearly discriminated from other field potentials. All dorsal horn neurones had cutaneous excitatory receptive fields on the ipsilateral hindlimb. The field properties were carefully examined by use of both noxious and innocuous cutaneous stimuli before and after testing with drugs. Neurones were selected that could be classified as either multireceptive or nocispecific. Multireceptive neurones responded to both innocuous stimuli (light brushing, gentle squeeze and/or tapping) and noxious stimuli (pinch and/or radiant heat, 46–49°C). In contrast, nocispecific neurones responded only to noxious stimuli and gave no response to any innocuous stimuli tested. Having thus classified the receptive field of the neurone, controlled stimuli were applied (usually for 10 s periods) to adjacent cutaneous areas, within the receptive field. The innocuous stimulus used was a motorized rotating brush. Noxious stimuli were provided by a calibrated pinch (serrated forceps with a graduated controlled displacement) or a thermocouple-controlled radiant heat lamp (giving a surface temperature ramp between 30–49°C). Responses to these stimuli and DLH- or glutamate-evoked activity (in response to ionophoresis at 5–60 nA for 10–15 s) were regularly repeated over 3 or 4 min cycles. These responses were always submaximal and approximately matched in terms of neuronal firing rates. Duplicate or triplicate controls varied by less than 15%. Neuronal firing was recorded on FM tape and firing rates (400 ms bins) were plotted on-line by computer (Cromemco System III), together with analogue signals from the stimuli and the ionophoresis unit. Responses to pinch, heat, brush, DLH or glutamate and epochs of spontaneous activity were integrated over appropriate time scales and normalized against control values measured from the duplicate or triplicate pre-drug control responses. These normalized values were expressed graphically (see Figure 1, for example) to facilitate comparisons of drug effects on different classes of evoked activity. All drugs were continuously ionophoresed for 1 min before and for the duration of the test cycle. Ionophoretic ejection was started at currents of 5 nA and was increased between each subsequent cycle of tests by 5, 10 or 20 nA intervals until a distinct action of an opioid agonist was observed. Ionophoresis of agonists was then either terminated and testing continued until responses recovered to control levels or was maintained for testing of reversal by an opioid antagonist.

Results

Characteristics of cells

The excitatory receptive fields of all neurones tested were carefully mapped and were always located on the ipsilateral hind paw or limb. Inhibitory or contralateral receptive fields were rarely seen and were not tested. Twenty-seven neurones were subsequently located in the region of lamina I. Histological examination of transverse sections of the spinal cord revealed that 27 Pontamine Sky Blue dye spots located immediately dorsal to the substantia gelatinosa (lamina II) demonstrating the loci of the lamina I neurones. Six dye spots were located ventral to the substantia gelatinosa, revealing the loci of neurones in the deeper dorsal horn laminae III–V.

Excitatory neuronal responses were evoked from 12 out of 27 neurones located in lamina I and 6 out of 6 neurones in laminae III–V, by both noxious and innocuous cutaneous stimuli. These multireceptive lamina I neurones, including 4 SMT neurones, all responded to an unequivocally noxious pinch and 4 out of 6 cells tested also responded to noxious radiant heat (44–48°C). In all 6 deeper dorsal horn cells tested and 7 out of 12 lamina I cells the initial vigorous response evoked by innocuous stimulation could be subsequently maintained throughout the experiment. However, 5 out of 12 lamina I cells demonstrated an adaptation to testing with the first two or three innocuous brush stimuli applied. Thereafter repeated brush-evoked responses were consistently maintained at a lower level without alteration of the stimulus, for the duration of the testing period. This characteristic adaptation to non-nociceptive stimuli was seen both in SMT neurones and in lamina I neurones unidentified in terms of their ascending projections. However, neither the nociceptive responses of any lamina I neurones nor any responses of neurones located in laminae III–V demonstrated this feature of adaptation to controlled cutaneous stimuli. In such cases,
stimuli could be repeatedly evoked at 3 min intervals for over 2 h.

A variety of innocuous stimuli (including brushing, gentle squeeze or tap) completely failed to evoke any response from 15 out of 27 lamina I neurones, including 10 SMT neurones. These neurones, however, consistently responded to noxious cutaneous stimuli and were thus classified as nocispecific.

Both nocispecific and multireceptive neurones in lamina I showed a characteristic lack or minimal level (<2 Hz) of spontaneous background activity. These neuronal characteristics were seen in both SMT and other lamina I neurones, unidentified in terms of their ascending projections. In contrast, neurones in the deeper dorsal horn consistently demonstrated background activity ranging from 2 to 10 Hz.

**Effects of opioids applied in the vicinity of multireceptive neurones located in lamina I**

Twelve multireceptive neurones in lamina I (of which 4 were SMT cells) were tested. In all cases both the μ (DAMGO) and the δ (DPDPE) agonists had profound selective antinociceptive effects, inhibiting both mechanical and thermal nociceptive responses whilst responses to innocuous and DLH- or glutamate-evoked activity were not altered from control levels (Table 1 and Figure 1). In the 4 cases where recovery from DAMGO (20 nA) was investigated, the substantial inhibition of nociceptive responses had recovered to near control values (98 ± 5% for pinch) within 5 ± 2 min (means ± s.e.mean, n = 4). Corresponding recovery from 15–20 nA DPDPE was similar, with pinch responses being restored to 93 ± 5% of controls in 7 ± 2 min (n = 4). The action of DAMGO was reversed by naloxone (20–60 nA) but not by a selective δ-antagonist, ICI 174864 (60–75 nA). In contrast, ICI 174864 (20–50 nA) consistently reversed typical DPDPE-mediated effects, as shown in Figure 2. In the 4 cases of DAMGO action in which reversal was investigated, the substantial inhibition of nociceptive responses caused by 15–20 nA DAMGO was reversed to 96 ± 12% of controls (pinch) by 4 ± 2 min of naloxone at 20–60 nA. Intravenous naloxone was not tested. The δ antagonist, ICI 178864 was ineffective, with DAMGO-suppressed pinch responses remaining at 26 ± 4% of controls despite ionophoresis of ICI 174864 at 60–75 nA for 4 ± 1 min (n = 3). In contrast, the substantial inhibition of nociceptive pinch responses caused by 15–20 nA DPDPE was reversed to 84 ± 6% of control responses by 20–50 nA of ICI 174864 for 5 ± 1 min (n = 4). In no case was there any effect of antagonists alone. In contrast to the selective antinociceptive effects evoked by ionophoresis of μ and δ agonists at low ejection currents (5–25 nA) on both SMT and unidentified revealed Pontamine Sky Blue dye spots located immediately dorsal to the substantia gelatinsa (lamina II). Six dye spots were located ventral to the substantia gelatinsa, revealing the loci of neurones in the deeper dorsal horn laminae III–V.

Excitatory neuronal responses were evoked from 12 of the neurones located in lamina I and 6 of the neurones in laminae III–V, by both noxious and innocuous cutaneous stimuli. These multireceptive lamina I neurones, including 4 SMT neurones, all responded to an unequivocally noxious pinch and 4 out of 6 cells tested also responded to noxious radiant heat (44–48°C). In all 6 deeper dorsal horn cells tested and 7 out of 12 lamina I cells the initial vigorous response evoked by innocuous stimulation could be subsequently maintained throughout the experiment. However, 5 out of 12 lamina I cells demonstrated a gradual adaptation to testing with the first two or three innocuous brush stimuli applied. Thereafter repeated brush-evoked responses were consistently maintained at a lower level without alteration of the stimulus, for the duration of the experiment. This characteristic adaptation to non-nociceptive stimuli was seen both in SMT neurones and in lamina I neurones unidentified in terms of their ascending projections. However, neither the nociceptive responses of any lamina I neurones nor any responses of neurones located in laminae III–V demonstrated this feature of adaptation to controlled cutaneous stimuli. In such cases, stimuli could be repeatedly evoked at 3 min intervals for over 2 h.

A variety of innocuous stimuli (including brushing, gentle squeeze or tap) completely failed to evoke any response from 15 out of 27 lamina I neurones, including 10 SMT neurones. These neurones, however, consistently responded to noxious cutaneous stimuli and were thus classified as nocispecific.

Both nocispecific and multireceptive neurones in lamina I showed a characteristic lack or minimal level (<2 Hz) of spontaneous background activity. These neuronal characteristics were seen in both SMT and other cells, κ agonists (U50488H or DYNO) had no effect at either the same (5–25 nA) or much higher (up to 100 nA) ejection currents, in 4 out of 5 neurones tested (Figure 1). In a single example, DYNO inhibited all evoked activity at ejection currents around 60 nA. In 8 neurones, multiple testing of different agonists was carried out, including 3 where μ, δ and κ agonists were all studied. All

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**Table 1** Opioid effects on lamina I neurones

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<thead>
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<th>Selective antinociceptive effect</th>
<th>No effect</th>
<th>Non-selective effect</th>
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<tbody>
<tr>
<td>multiresponse</td>
<td>nocispecific</td>
<td>multiresponse</td>
</tr>
<tr>
<td>DAMGO</td>
<td>9/9</td>
<td>0/9</td>
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<tr>
<td>DPDPE</td>
<td>8/8</td>
<td>5/15</td>
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<tr>
<td>U50488H or DYNO</td>
<td>0/5</td>
<td>0/4</td>
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DAMGO = [D-Ala2,MePhe4,Gly-ol]enkephalin; DPDPE = [D-Pen2,D-Pen5]enkephalin; U50488H = trans-3,4-dichloro-N-methyl-N-(2-pyrrolidinyl)cyclohexyl-benzene acetamide.
such results were consistent with the overall conclusion that \( \mu \) and/or \( \delta \) agonists would suppress nociceptive inputs to any individual neurone, whereas \( \kappa \) agonists were ineffective.

Effects of opioids applied in the vicinity of nocispecific neurones located in lamina I

A total of 15 cells in lamina I were classified as nocispecific, responding only to noxious cutaneous stimuli and ionophoresed DLH, including 10 neurones antidromically identified as SMT cells. In contrast to the consistent selective antinociceptive effects on multireceptive lamina I neurones described above, the \( \delta \) agonist DPDPE had 3 different actions on nocispecific cells (Table 1 and Figure 3). DPDPE selectively inhibited the nociceptive responses of 5 out of 15 neurones, whilst the DLH-evoked responses were unaffected, with ejection currents of up to 25 nA (Figure 3a). In one neurone of this type for which recovery was closely examined, the substantial inhibition of nociceptive responses due to DPDPE was reversed to more than 90% of control levels 5 min after stopping ejection of DPDPE. With the same test protocol both the noxious and DLH-evoked responses were inhibited to a similar degree in 7 out of 15 cells (Table 1 and Figure 3c). In the 3 of these neurones for which recovery was examined, the DPDPE-induced suppression of evoked responses had recovered to near control values [for example 102 ± 12% (DLH) and 87 ± 9% (pinch)] by 6 ± 1 min after stopping ejection of DPDPE. Both of these actions of DPDPE were reversed by ionophoresis of the \( \delta \) antagonist, ICI 174868 (20-70 nA). In all 3 of the selectively affected neurones that were tested with the \( \delta \) antagonist (including 2 SMT cells), the effects of DPDPE were also readily reversed by ICI 174864. Pinch responses, for example, were restored to 92 ± 9% of controls by 20-60 nA ICI 174864 applied for 4 ± 1 min (n = 3). In all 4 of the non-selectively affected neurones that were tested with the \( \delta \) antagonist (including 2 SMT cells), the effects of DPDPE were also readily reversed by ICI 174864. For example, the suppressed responses to DLH and to noxious pinch were reversed to 88 ± 12% and 85 ± 3% of controls by 30-70 nA ICI 174864 applied for 5 ± 1 min (n = 4). No effect of ICI 174864 alone was ever apparent.

DPDPE failed to alter the responses of the remaining 3 neurones at either similar (5-20 nA) (Figure 3b) or in 2 of these cases, even at much higher ejection currents (up to 100 nA) (not shown). The \( \kappa \) agonists US50488H and DYNO failed to cause any change in responses tested (5-15 nA) (Figure 4) or even at up to 40 nA (not shown). However, in all 9 cells tested, including 5 SMT cells, the selective \( \mu \) agonist, DAMGO inhib-
The effects of \( \mu \) and \( \kappa \)-selective agonists ionophoresed in the vicinity of nocispecific lamina I neurones. Responses were integrated and expressed as a percentage of control levels. Mean values from samples of neurones (n indicated) are shown; s.e.mean shown by vertical bars. Responses were evoked by noxious heat (○), noxious pinch (□), or (DLH) (●). (a) The \( \mu \) agonist, [\( \mu \)-Ala\(_2\),MePhe\(_4\),Gly-ol]enkephalin (DAMGO), inhibited only nociceptive responses, leaving DLH-evoked activity unaffected (n = 9). (b) In contrast the \( \kappa \) agonist, U50488H (U50), failed to alter the levels of any of the evoked activity, in all neurones tested (n = 3).

**Effects of opioids applied in the vicinity of multireceptive neurones located in laminae III–V**

On six multireceptive neurones located in the deeper dorsal horn (laminae III–V) neither the \( \mu \) (DAMGO) nor the \( \delta \) (DPDPE) agonists caused selective antinociceptive effects (Figure 5), which were not apparent for lamina I neurones. However, the \( \kappa \) agonists (DYNO or U50488H) typically caused a selective antinociceptive effect on the pinch response of all neurones tested, as shown in Figure 5. On 2 neurones tested, U50488H also selectively inhibited the noxious heat response. Three of the 4 neurones tested with U50488H were examined for recovery. The substantial inhibition of pinch responses caused by 15 nA U50488H had recovered to 83 ± 10% of control values in 12 ± 3 min after stopping ionophoresis of the drug. No further studies were carried out on these neurones, but when examining the same phenomenon in cat laminae III–V neurones, reversal by naloxone and inactivity of [des-Tyr\(_1\)]\( \mu \)-dynorphin \( \Delta \)1–13 were seen (Fleetwood-Walker et al., 1988). Ionophoresis of either naloxone or ICI 174864 alone had no apparent effect on the evoked responses tested in lamina I or laminae III–V. No clear differences in the profile of drug actions could be discerned between antidromically-identified SMT neurones and unidentified nocispecific neurones, with examples of SMT cells showing each of the profiles of DPDPE action and the typical responses to \( \mu \) and \( \kappa \) agonists. The administration of antagonists by other routes was not tested.

**Discussion**

A variety of antinociceptive actions are mediated by \( \mu \), \( \delta \) and \( \kappa \) opioid agonists. As in the cat (Fleetwood-Walker et al., 1988), the antinociceptive action of \( \kappa \) agonists was exerted here on both the thermal and mechanical nociceptive responses of laminae III–V neurones to an apparently similar degree. Systemic or intrathecal administration of selective \( \kappa \) agonists are most effective against visceral and mechanical nociception (Hayes et al., 1983; Schaus & Yaksh, 1984). Then there is little margin between doses causing antinociception and motor dysfunction or paralysis (see Millan, 1990 for review). In the present study, the range over which opioids could act was considerably restricted by virtue of the experimental design, although some diffusion of ionophoresed drugs must occur. The excitatory effects observed by Knox & Dickinson (1987) may have involved actions of intrathecal administration \( \kappa \) agonists at various sites remote to the dorsal horn neurone being recorded. In our studies, the sites mediating such excitation may have been beyond the reach of ionophoresed \( \kappa \) agonists.

Selective inhibition of nociceptive responses, without affecting innocuous, DLH-evoked or spontaneous activity, indicates that inhibitory influences may be exerted specifically on the nociceptive pathway, rather than generally on any polysynaptic input or directly on the neurones being tested, either in laminae III–V and lamina I. For example, \( \kappa \) agonists may inhibit nociceptive transmission to the deeper dorsal horn neurone being tested, via a closely antecedent interneurone, rather than by action on the nociceptive afferent fibres which predominantly terminate in the superficial dorsal horn (Cervero & Igo, 1980). In the case of \( \kappa \) agonists, these proposed interneurones are unlikely to be located as far dorsal as the region of the substantia gelatinosa, because Fleetwood-Walker et al. (1988) reported that ionophoresis of \( \kappa \) agonists there failed to influence neurones located in laminae III–V. Similarly, the quite different profiles of drug action when applied in superficial laminae rather than laminae III–V suggest that drugs did not readily diffuse between the two. The majority (but not all) of the \( \kappa \) receptors are present in superficial laminae (Slater & Patel, 1983; Morris & Herz, 1987) as are the endogenous \( \kappa \) agonists (Botticelli et al., 1981). At first sight there appears to be a mismatch between structure and function. However, the superficial dynorphin-related peptides may have other roles than the modulation of acute cutaneous nociceptive inputs surveyed in the present study. In contrast to the lack of action in the vicinity of deeper dorsal horn neurones, \( \mu \) and \( \delta \) receptor selective agonists often had profound influences on both nocispecific and multireceptive lamina I neurones. Autoradiographic studies have revealed \( \mu \) opioid binding sites correspondingly concentrated in the superficial dorsal horn (laminae I and II) (Atweh & Kuhar, 1977; Traynor et al., 1982; Mack et al., 1984; Dash-
wood et al., 1985; Morris & Herz, 1987) and µ agonists can exert an antinoceptive influence on deeper dorsal horn neurones, via the substantia gelatinosa, in the cat (Fleetwood-Walker et al., 1988). It is possible that the actions of µ agonists in the substantia gelatinosa are mediated by the vicinity of lamina I neurones in both the nociceptive and non-nociceptive pathways, or directly on the neurone being tested. 

A recent autoradiographic study by Morris & Herz (1987) found δ binding sites restricted to lamina I of the dorsal horn of the rat. This evidence is in accordance with our findings that δ agonists failed to influence somatosensory inputs to deeper dorsal horn neurones (laminae III–V) when either applied locally to these cells (in the rat, this study; in the cat, Fleetwood-Walker et al., 1988) or in the substantia gelatinosa dorsal to the cells being recorded (Fleetwood-Walker et al., 1988). The use of the selective δ antagonist, ICI 174864, confirmed the involvement of δ receptors in the modulation of somatosensory inputs to both multi- and nociceptive neurones in lamina I. Although the terminal excitability of primary afferent Aδ- and C-fibres is reduced by δ-agonist derivatives active both at µ and δ sites (Carstens et al., 1979; Hentall & Fields, 1983) there must be some differential action of µ and δ agonists to account for the different effects caused by DAMGO and DPDPDE on nocispecific lamina I neurones. Whilst DAMGO consistently inhibited only nociceptive responses, leaving non-nociceptive activity unaffected in multi- and nociceptive neurones, DPDPDE also regularly caused inhibition of both noxious and DLH-evoked responses to nocispecific cells. It therefore seems that there is not an ubiquitous inhibitory mechanism mediated by δ-receptors. The variety of both selective and non-selective actions of δ agonists indicates a complex and perhaps important role for δ-receptor sites, restricted to lamina I (Morris & Herz, 1987) in the modulation of nociceptive transmission through the superficial dorsal horn and on to supraspinal sites.

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