δ -Opioid mediated inhibitions of acute and prolonged noxious-evoked responses in rat dorsal horn neurones

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1 The effects of a selective δ -opioid agonist Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr (DSTBULET) were examined on the C- and A β -evoked responses of convergent dorsal horn neurones in the halothane anaesthetized, intact rat.

2 Intrathecal DSTBULET produced selective dose-dependent inhibitions of electrically-evoked C fibre responses of both superficial and deep neurones. A near-complete inhibition of $83 \pm 5\%$ followed $100 \mu\text{g}$ of DSTBULET and the ED₅₀ was $9 \mu\text{g}$ (13.5 nmol). Inhibitions were antagonised by intrathecal naloxone and ICI 174,864 but were not antagonised by pretreatment with intrathecal β -funaltrexamine at a dose that blocked μ -opioid effects. By contrast, DSTBULET produced excitations of electrically-evoked responses of cells recorded in a zone intermediate between the superficial and deep neurones.

3 DSTBULET ($50 \mu\text{g}$) was also tested on the more prolonged noxious neuronal response produced by subcutaneous formalin (5%, $50 \mu\text{l}$) into the receptive field. DSTBULET profoundly inhibited the response to formalin. Pretreatment with ICI 174, 864 before DSTBULET antagonised the effects of the δ -agonist on the formalin response.

4 The full peptidase inhibitor kelatorphan, known to protect endogenous enkephalins, was also tested on the formalin response. The intrathecal administration of $50 \mu\text{g}$ kelatorphan has previously been shown to inhibit electrically-evoked C fibre responses of dorsal horn neurones and to be antagonised by ICI 174,864. The same dose of kelatorphan inhibited the formalin response in the present study.

5 From this study it appears that the δ -opioid agonist DSTBULET can produce profound inhibitions of the responses of convergent neurones to nociceptive afferent inputs. Furthermore, activation of δ -opioid receptors either by DSTBULET, or by protection of endogenous enkephalins with kelatorphan, can inhibit a more prolonged chemically-evoked nociceptive input onto these dorsal horn neurones.

Introduction

The existence of the three main opiate receptor subtypes, μ , δ and κ is well established and all three appear to be present in the spinal cord of many species including man (Maurer *et al.*, 1983; Quirion *et al.*, 1983; Gourderes *et al.*, 1985; Morris & Herz, 1987; Zajac *et al.*, 1989 and refs within). However, although agonists at the μ -opioid receptor subtype clearly mediate spinal analgesia (for review see Yaksh, 1984), it has proved more difficult to establish the role of the δ -opioid subtype in spinal antinociception. δ -Opioid binding sites are located

throughout the dorsal horn, particularly in the superficial laminae where they appear to be less dense than the μ subtype (Quirion *et al.*, 1983; Gouarderes *et al.*, 1985; Waksman *et al.*, 1986; Morris & Herz, 1987; Zajac *et al.*, 1989). Despite evidence for the existence of a μ/δ -receptor complex in supraspinal sites (see Rothman & Westfall, 1982; Schoffelmeer *et al.*, 1988), in the spinal cord the δ -receptor appears distinct from the μ -receptor subtype and only a very low percentage of these receptor subtypes are colocalised on dorsal root ganglion cells (Werz *et al.*, 1987).

The peptides [Met] and [Leu]enkephalin are considered as selective but not specific endogenous

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ligands for the δ -opioid subtype and are located in neurones intrinsic to the spinal cord and in descending systems (Hokfelt *et al.*, 1977; Hunt *et al.*, 1980; Gibson *et al.*, 1981; Lamotte *et al.*, 1983). Spontaneous release of enkephalins *in vivo* (from local and descending fibres) is increased after noxious stimulation and is altered in chronic pain states (Cesselin *et al.*, 1985; Bourgoin *et al.*, 1986). The intrathecal application of exogenous [Met] and [Leu]enkephalin and other more or less selective synthetic δ -receptor agonists produces antinociception in behavioural tests of analgesia (Tung & Yaksh, 1982; Hylden & Wilcox, 1983; Yaksh, 1983; Porreca *et al.*, 1984; Schmauss & Yaksh, 1984; Kuraishi *et al.*, 1985; Rodriguez *et al.*, 1986; Heyman *et al.*, 1987) and in electrophysiological studies on dorsal horn neurones (Duggan *et al.*, 1976; 1977; Randic & Miletic, 1978; Satoh *et al.*, 1979; Zieglgansberger & Tulloch, 1979; Sastry & Goh, 1983; Willcockson *et al.*, 1984; Fleetwood-Walker *et al.*, 1985; 1986; Dickenson *et al.*, 1987b). Antinociceptive effects also occur following the protection from degradation of endogenous enkephalins by intrathecal administration of the peptidase inhibitors bestatin, thiorphan and kelatorphan (Bourgoin *et al.*, 1986; Dickenson *et al.*, 1986; 1987a). However, in an *in vitro* study with the isolated spinal cord it has been shown that the enkephalins modulate excitatory synaptic transmission, at least in the superficial dorsal horn, by acting through the μ -opioid receptor (Jeftinija, 1989).

When examining the consequences of δ -receptor activation in the spinal cord as discussed above, an important factor to consider is the specificity of the ligands for the δ -receptor. Although the μ - and δ -receptor subtypes in the cord are thought to be separate binding sites, many δ -ligands show a lack of selectivity between the two, and similarly with μ -ligands. In addition selective δ -opioid antagonists have not been available to confirm the opioid subtype involved until relatively recently. The endogenous agonists [Leu] and [Met]enkephalin are only 10 fold more selective for δ - than for μ -binding sites (Kosterlitz & Paterson, 1985). Tyr-D-Thr-Gly-Phe-Leu-Thr (DTLET), commonly used in binding studies, has good affinity for the δ -receptor but does show residual μ -receptor cross-reactivity (Zajac *et al.*, 1983), and elicits a biphasic dose-response in electrophysiological studies (Dickenson *et al.*, 1987b). Conformational restriction of the basic peptide structure has improved receptor selectivity, as exemplified by the two cyclic enkephalin analogues Tyr-D-Pen-Gly-Phe-D-Pen (DPDPE) and Tyr-D-Pen-Gly-Phe-L-Pen (DPLPE) (Mosberg *et al.*, 1983) but this involves a significant loss of affinity and potency (Delay-Goyet *et al.*, 1985). The linear hexapeptide Tyr-D-Ser(OTbu)-Gly-Phe-Leu-Thr

(DSTBULET) has good selectivity for the δ -opioid receptor ($K_1(\delta)/K_1(\mu) = 0.012$) and better affinity than either DPDPE or DPLPE and thus is a suitable agonist for determining the role of δ -opiates in the spinal cord (Delay-Goyet *et al.*, 1987). We have already investigated the actions of DPDPE and the enkephalinase, aminopeptidase and dipeptidylaminopeptidase inhibitor, kelatorphan (Fournie-Zaluski *et al.*, 1984) on noxiously-evoked responses of dorsal horn neurones in the anaesthetized rat. Both compounds produced submaximal inhibitions which were antagonised by the selective δ -antagonist ICI 174,864 (Dickenson *et al.*, 1986; 1987b). In the present study DSTBULET was used to demonstrate the complete inhibition of C fibre-evoked responses of dorsal horn neurones in the spinal cord by a selective δ -agonist with a potency similar to the classical μ -opiate agonist morphine. The selective μ - and δ -antagonists β -funaltrexamine (Portuguese *et al.*, 1980) and ICI 174,864 (Cotton *et al.*, 1984) respectively further confirm the involvement of the δ -receptor subtype in the spinal actions of DSTBULET.

In addition to the effects of DSTBULET on electrically-evoked neuronal activation we also studied the action of this δ -agonist on more prolonged noxiously-evoked neuronal responses produced by subcutaneous formalin. The lower analgesic potency of δ -agonists against mechanical stimuli and in the acetic acid writhing test, in behavioural studies of antinociception, has led to the suggestion that spinal opiate receptor subtypes differentiate between thermal, mechanical and chemical noxious stimuli (Schmauss & Yaksh, 1984). The potency of μ -opioid agonists in inhibiting the formalin-evoked response of dorsal horn neurones has been shown previously (Dickenson & Sullivan, 1987). In the present study DSTBULET and the peptidase inhibitor kelatorphan were also found to be potent against the formalin response and do not therefore appear to differentiate between electrically-evoked and chemically-evoked cutaneous afferent input.

Methods

Male Sprague-Dawley rats (200–250 g) were initially anaesthetized with 3% halothane in a 66% N₂O, 33% O₂ gaseous mix and then maintained on 1.5–2% halothane for subsequent surgery. Following cannulation of the trachea the rat was mounted in a stereotaxic frame and a laminectomy performed, exposing the L1–L3 region of the spinal cord. The vertebrae caudal and rostral to the exposed cord were supported by clamps to hold the cord rigid and a glass-coated tungsten electrode lowered into the dorsal horn. Throughout the experiment the halothane concentration was held at between 0.8 and

1.5% depending on the individual animal. This level of anaesthesia was sufficient to maintain complete areflexia whilst maintaining the animal in good physiological condition with no apparent depression of respiration or neuronal activity. Recording of a single neurone could be continued for several hours.

Single unit extracellular recordings were made of dorsal horn neurones responding to both innocuous (brush) and noxious (pinch, heat) stimuli applied to the ipsilateral receptive fields in the plantar region of the hind paw. The depth from the surface of the cord of the neuronal recording site was established using a SCAT microdrive (Digitimer). Once the neurone had been characterized by means of natural stimuli, 16 electrical pulses (0.5 Hz, 2 ms wide) were applied via needles inserted into the centre of the receptive field. This provided a constant reproducible test stimulus for the experiment. Stimulation was applied at 3 times the threshold current for C fibre activation and a post-stimulus time histogram (PSTH) was built and displayed by means of a C.E.D. interface and MRATE software from which the C fibre-evoked neuronal response could be separated by latency and threshold from the A β fibre-evoked response and quantified. A β fibre-evoked responses were measured separately again at 3 times the threshold for A β fibre activation. For the acute studies, after at least 3 control tests at 5 min intervals which did not differ by more than 10%, drugs were applied directly onto the cord in a volume of 50 μ l. Tests were carried out 5 and 10 min after each application and subsequently every 10 min.

In the experiments where formalin was used as the noxious stimulus, neurones were initially characterized as already described. C and A β fibre-evoked responses to electrical stimulation were measured by PSTH recordings. Then the spontaneous activity of the neurone was recorded for 10 min. Typically the convergent neurones studied in this preparation had no or very low levels of spontaneous activity and any cell showing a rate higher than 20 spikes min⁻¹ was not used for these experiments. Formalin (5%, 50 μ l) was then injected into the receptive field and the neuronal response monitored for 60 min. Intrathecal drugs were applied as described above 20 min before the formalin injection.

All results are expressed as a percentage of the mean control response and analysed by Student's *t* test.

Drugs

Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr (DSTBULET), kelatorphan and Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGOL), were synthesised by B.P. Roques (Delay-Goyet *et al.*, 1985). Allyl₂-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864) was obtained from Cambridge

Research Biochemicals and β -funaltrexamine was a gift from Prof. Portuguese.

Results

A total of 72 convergent cells was recorded from 34 rats. All cells had very little or no spontaneous activity and were separated into 3 distinct populations in terms of depth. Superficial cells (recorded between 0 and 250 μ m from the surface of the dorsal horn, mean depth 125.8 ± 24 μ m, *n* = 25) and deep cells (greater than 500 μ m from the surface, mean depth 762.1 ± 48 μ m, *n* = 38), were separated by an intermediate group recorded between 250 μ m and 500 μ m (mean depth 381.1 ± 27 μ m, *n* = 9). Superficial and deep cells responded similarly to opiates and could not be distinguished from each other by their neuronal responses. The threshold for activation of C fibre evoked responses in superficial and deep cells was 0.82 ± 0.14 and 0.64 ± 0.1 mA respectively. These intensities evoked a mean C fibre response of 201 ± 32 and 252 ± 36 spikes per 16 stimuli. The threshold for A β fibre-evoked responses were approximately 10 times less in most cells, a mean 0.058 ± 0.017 mA in superficial cells and 0.043 ± 0.001 mA in deep cells. Since cells occurring in either the superficial or deep layers of the dorsal horn responded similarly to opiates, dose-response curves were constructed from the results of all these cells. Neurones recorded between 250 and 500 μ m in an intermediate zone showed similar thresholds to the other cells for A β and C fibre activation (0.041 ± 0.11 , 0.68 ± 0.11 mA respectively) and a similar C fibre-evoked response (230 ± 19 spikes). However, the response of these neurones to intrathecal opiates were generally opposite to that of the superficial and deep cells and they have therefore been considered separately in the results.

Acute neuronal responses to electrically-evoked C and A β fibre stimulation

DSTBULET produced dose-dependent selective inhibitions of C fibre-evoked responses in all superficial and deep cells (Figure 1a). An almost complete mean inhibition of $83 \pm 5\%$ (*n* = 14) was produced by 100 μ g of the peptide and the ED₅₀ was estimated from the dose-response curve to be 9 μ g (13.5 nmol). The peak effect occurred 31 ± 3 min (mean $\pm$ s.e.mean) after drug administration and was then maintained for over 30 min. There was no difference in response of the superficial and the deep cells to the effects of the peptide, either in terms of degree of inhibition or time-course. A β fibre-evoked activity was only significantly inhibited by the higher doses of the peptide and to a much lesser degree than the

Figure 1 Dose-response curve of the effects of (a) DSTBULET and (b) L-DSTBULET on dorsal horn neuronal responses evoked by electrical stimulation at C fibre (●) and A fibre (○) intensities. Each point represents the mean and vertical lines show s.e.mean; $n = 4$ to 14. * $P < 0.05$; ** $P < 0.005$ (tested for significant difference against controls).

corresponding C fibre response (Figure 1a). A maximal inhibition of only $33 \pm 6\%$ of A β fibre-evoked responses occurred with $100 \mu\text{g}$ DSTBULET compared to the almost complete inhibition of C fibre responses by this dose.

In a group of 9 cells recorded in the intermediate zone only 2 cells were inhibited by DSTBULET. For the other 7 cells enhancement of the C fibre-evoked responses above control levels occurred after intrathecal application of the peptide. The sample was too small to determine any dose-response relationship overall and individual cells varied considerably

Figure 2 The antagonism of the inhibition by DSTBULET (open columns) on C fibre evoked responses of deep and superficial cells by $25 \mu\text{g}$ naloxone (N), $125 \mu\text{g}$ ICI 174,864 (I) but not by pretreatment with $0.05 \mu\text{g}$ β -funaltrexamine (F) (hatched columns).

in the degree of excitation. However, a mean excitation of $48 \pm 12\%$ was produced by $5 \mu\text{g}$ DSTBULET in 4 cells compared with a mean inhibition of $30 \pm 6\%$ produced by the same dose of DSTBULET in the superficial/deep cells. The subsequent application of a higher dose of DSTBULET reversed the excitation produced by the agonist in 2 cells and an inhibition of C fibre-evoked responses occurred.

Several antagonists were used to determine whether the inhibitions produced by the higher doses of DSTBULET in the superficial and deep cells were mediated via the δ -receptor subtype. Intrathecal naloxone ($25 \mu\text{g}$) antagonised the effects of DSTBULET back to a mean of $83 \pm 4\%$ of control (Figure 2). However, this dose would not be expected to be selective for the μ -opioid receptor type. Intrathecal ICI 174,864 ($125 \mu\text{g}$), a selective δ -opioid antagonist was tested against both μ - and δ -agonist mediated effects. In 3 cells the inhibition ($15 \pm 4\%$ of control) of C fibre-evoked responses by $5 \mu\text{g}$ DAGOL was not antagonised by application of ICI 174,864, the inhibition remaining unchanged over 1 h ($14 \pm 5\%$ of control). Subsequent application of $5 \mu\text{g}$ naloxone rapidly antagonised the DAGOL-mediated inhibitions back to $73 \pm 18\%$ of control. In contrast to the lack of antagonism of μ -mediated effects, $125 \mu\text{g}$ ICI 174,864 completely antagonised the inhibitory effects of DSTBULET in 3 out of 11 cells and partially antagonised the agonist in the remaining 8 cells. Overall the responses of all 11 cells were antagonised to $72 \pm 5\%$ of control (Figure 2). The action of the selective μ -antagonist β -funaltrexamine (β FNA) was

also investigated. An intrathecal dose of 0.05 µg β-FNA was given 2 h before the administration of 5 µg DAGOL and subsequently 100 µg DSTBULET. An example of the antagonism of DAGOL by βFNA and subsequent inhibition of a single neurone by DSTBULET is shown in Figure 3. In 6 cells in which the action of DAGOL was completely blocked by βFNA the highest dose of DSTBULET still produced maximal inhibitions (mean $89 \pm 7\%$), which were not significantly different from the effects of that dose of DSTBULET alone (Figure 2). Thus the effects of DSTBULET are likely to be mediated solely through the δ -receptor, although the incomplete antagonism of DSTBULET by ICI 174,864 must also be considered.

The inactive isomer of DSTBULET was tested on A β and C fibre-evoked responses in 6 cells. No inhibition or excitation of electrically evoked neuronal activity was seen to occur after intrathecal application of 1 to 100 µg Tyr-L-Ser(Otbu)-Gly-Phe-Leu-Thr (Figure 1b).

Prolonged neuronal responses to subcutaneous formalin

DSTBULET was also tested on the response of convergent dorsal horn neurones to a more prolonged chemical noxious stimulus. In the control experiments a subcutaneous injection of 5% formalin produced a biphasic pattern of activity in these neurones over a period of 1 h, as shown in Figure 4a. The activity within the first 10 min following the injection of formalin was denoted as the 1st peak and the more prolonged response over the next 50 min the 2nd peak. A dose of 50 µg DSTBULET 20 min before the injection of formalin completely abolished the 2nd peak of the response and profoundly inhibited the first peak (Figure 4b). The co-administration of ICI 174,864 with DSTBULET completely antagonised the effect of the agonist on the 1st peak (Figure 4c). ICI 174,864 also partially but significantly ($P < 0.005$) antagonised the action of DSTBULET on the 2nd peak. The peptidase inhibitor kelatorphan was also tested on the formalin

Figure 3 An example of the selective antagonism of DAGOL but not DSTBULET by pretreatment with β -funaltrexamine (βFNA). (a) The post-stimulus-time histogram of the responses of a single deep dorsal horn neurone to electrical stimulation after 2 h of pretreatment with 0.05 µg βFNA. There was no change in the response 30 min after the administration of 5 µg DAGOL (b). This dose would normally produce a complete inhibition of the C fibre-evoked response. Subsequently 100 µg of DSTBULET abolished the C fibre-evoked response within 30 min (c) and the inhibition was reversed by 25 µg naloxone (d).

Figure 4 Rate recordings (1 s bins) of the neuronal response evoked in individual dorsal horn neurones by subcutaneous formalin. In a neurone with saline pretreatment 2 peaks of activity occurred over the 1 h of recording (a). Pretreatment with 50 μ g DSTBULET 20 min before the injection of formalin inhibited both phases of the response in another neurone (b). When 125 μ g ICI 174,864 was coadministered with DSTBULET there was a partial recovery of the formalin response (c).

response. In previous studies we have found that the intrathecal application of 50 μ g kelatorphan inhibited the C fibre-evoked response by 51% and this effect was antagonised by ICI 174,864 (Dickenson *et al.*, 1986). In the present study a similar dose of the peptidase inhibitor completely abolished the 2nd peak of the neuronal response in 4 cells. Figure 5 shows the overall results for the effects of DSTBULET and kelatorphan on both phases of the formalin-evoked response.

Discussion

In previous studies on the same preparation the intrathecal application of the selective δ -opioid agonist DPDPE or the enkephalinase inhibitor kelatorphan produced a maximal 50–60% inhibition of C fibre-evoked responses (Dickenson *et al.*, 1986; 1987a,b). DTLET, a mixed δ/μ -agonist could produce 100% inhibitions of C fibre-evoked activity but the biphasic dose-response curve suggested that

Figure 5 The overall inhibitory actions of 50 μ g DSTBULET (D) and 50 μ g kelatorphan (K) on the 1st acute peak (0–10 min) and the 2nd more prolonged phase (11–60 min) of the formalin response (C, control). Note the complete antagonism of DSTBULET effects on the 1st peak and partial antagonism in the 2nd peak by ICI 174,864 (I) coadministered with DSTBULET. $n = 3$ to 5. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$ (tested for significant difference against the control response).

μ -receptor activation was necessary to inhibit completely C fibre-evoked responses (Dickenson *et al.*, 1987b). It therefore appeared that δ -receptor activation either via an exogenous agonist, or through the protection of endogenous enkephalins, was unable to inhibit completely noxiously-evoked responses of convergent dorsal horn neurones in the spinal cord. This was in contrast to the profound maximal inhibitions of these responses of convergent dorsal horn neurones by a large range of μ -agonists, including DAGOL, morphine and methadone (Dickenson & Sullivan, 1986; Dickenson *et al.*, 1987b; 1988; McQuay *et al.*, 1989). However, the present study clearly demonstrates that a selective δ -opioid agonist DSTBULET can almost completely abolish C fibre responses in superficial and deep dorsal horn neurones with a potency similar to morphine. Although antagonism of the peptide by ICI 174,864 was often incomplete, pretreatment with β FNA, an irreversible selective μ -antagonist, at doses which blocked μ -mediated effects on the same neurone, did not reduce the inhibitory action of DSTBULET. Thus, it can be concluded that even at the highest doses tested DSTBULET is likely to be acting solely on the δ -receptor opioid subtype. The marked but incomplete antagonism of DSTBULET by ICI 174,864 may result from the relatively lower affinity of this antagonist for the δ -opioid receptor (Kosterlitz & Paterson, 1985).

The dose-response curve for the inhibition of C fibre-evoked responses by DSTBULET is similar to that of μ -opioid agonists with respect to the maximum drug effect attained. However, no excitations of neuronal activity were seen after the lowest doses of DSTBULET, unlike the pronounced facilitations which occur after low doses of the μ -agonists morphine and DAGOL in this preparation (Dickenson & Sullivan, 1986; 1987b). In addition other δ -opioid agonists studied in this preparation did not produce facilitations of responses at low doses (Dickenson *et al.*, 1987b). μ -Opioid mediated biphasic actions have also been observed on the nociceptive flexion reflex in the rat (Wiesenfeld-Hallin & Duranti, 1987), on glutamate-evoked responses of primate spinothalamic tract neurones (Willcockson *et al.*, 1986) and on action potentials in primary afferent neurones *in vitro* (Higashi *et al.*, 1982; Crain & Shen, 1988). The enhanced potassium- or capsaicin-evoked release of substance P-like immunoreactivity in the rat spinal cord slice by μ -opioid receptor activation (Mauborgne *et al.*, 1987; Pohl *et al.*, 1989) could be one mechanism by which the facilitations occur. Reduction rather than enhancement of substance P-like immunoreactivity occurs with DPDPE, DTLET, DADLE, [Met]- and δ -enkephalin (Hirota *et al.*, 1985; Go & Yaksh, 1987; Mauborgne *et al.*, 1987). Another possible explanation for the facilitations is presynaptic autoinhibition of [Met]enkephalin release (Ueda *et al.*, 1986). The lack of low-dose excitation by DSTBULET implies that this putative autoreceptor might be the μ -opioid receptor subtype. However, in *in vitro* studies low doses of both μ - and δ -opioid agonists prolonged the duration of the calcium component of the action potential (APD) in dorsal root ganglion cells in culture, before higher doses of the agonists shortened the APD leading to depression of neurotransmitter release (Crain & Shen, 1988).

In the convergent neurones examined in this study C fibre-evoked responses were selectively inhibited with little effect on A β fibre responses, except at the highest doses. The loss of only 50% of δ -binding sites in laminae 1 and 2 after dorsal rhizotomy (Zajac *et al.*, 1989) indicates the presence of pre-synaptic receptors on the primary afferent terminals but also postsynaptic receptors, presumably located on intrinsic neurones and on the dendrites and soma of convergent output cells (see Besson & Chaouch, 1987). Both pre- and post-synaptic δ -receptor activation could result in the inhibition of C fibre-evoked responses in convergent neurones (Sastri, 1979; Zieglansberger & Tulloch, 1979; Miletic & Randic, 1982; Hentall & Fields, 1983). The selectivity of opioid action on C fibre-evoked responses has also been demonstrated in iontophoretic studies where opiates, ejected into the substantia gelatinosa, inhibit

the noxiously evoked responses of deep dorsal horn neurones without affecting the responses to non-noxious stimuli (Duggan *et al.*, 1976; 1977; 1981; Davies & Dray, 1978; Fleetwood-Walker *et al.*, 1988). However, when ejected close to the cell bodies opiates generally either had no effect or mediated a non-selective inhibition of both noxious and non-noxious evoked responses (Duggan *et al.*, 1976; 1977; Zieglansberger *et al.*, 1970; Fleetwood-Walker *et al.*, 1988). Although in several studies enkephalins inhibit noxiously-evoked responses of deep cells whilst sparing non-noxious responses when iontophoretically applied into the SG (Duggan *et al.*, 1976; 1977; 1981; Davies & Dray, 1978), it has also been found that specific δ -opioid agonists were only selective in inhibiting the response of multireceptive neurones located in lamina I and not those of laminae III-V spinocervical tract neurones (Fleetwood-Walker *et al.*, 1986). We have not characterised the cells in the present study in terms of output but, nevertheless, we could not distinguish between the effects of DSTBULET on superficial compared with deep convergent neurones.

We have not investigated the extent to which intrathecal opiates penetrate into the dorsal horn. However, from the electrophysiological studies mentioned above, and the evidence from binding studies indicating a superficial location of δ -opiate receptors, it is likely that a selective C fibre inhibition results from anatomical differences in A β and C fibre termination in the dorsal horn. It is interesting to note that inhibition of A β fibre-evoked responses did occur at higher doses of DSTBULET and the maximum degree of inhibition (40%) was similar to that observed previously for other opiates, including DAGOL and morphine (Dickenson & Sullivan, 1986; Dickenson *et al.*, 1987b). Possibly this represents the penetration of the drug to deeper opiate receptors postsynaptic to both A β and C fibre terminals and therefore results in the inhibition of all types of response of the cell. The thick myelinated A β afferent fibres terminate much deeper in the dorsal horn in laminae III-VI, whereas the smaller unmyelinated C fibres terminate in the superficial laminae I and II (Brown, 1982). It is also possible that the inhibition of A δ fibre-evoked activity contributes to effects of higher doses of DSTBULET on the short latency response. It is often difficult to separate A β - and A δ -evoked responses in terms of latency. Certainly it appears that a certain degree of inhibition of the short latency response, whether evoked by A β or A δ fibres, always occurs whenever C fibre-evoked responses are completely inhibited.

Unlike the profound inhibitions of superficial and deep cells DSTBULET produced excitations of neuronal responses recorded in the intermediate zone. An excitatory effect on substantia gelatinosa

(SG) neurones by systemic morphine and iontophoretic [Met] enkephamide has been previously demonstrated in electrophysiological studies *in vivo* (Woolf & Fitzgerald, 1981; Sastry & Goh, 1983). We have also observed a selective excitation of neurones recorded in an intermediate zone by other intrathecal opiates including morphine in this *in vivo* preparation (unpublished). We have not demonstrated that cells recorded in this intermediate zone are in fact located in the SG. However, excitations of neuronal responses by opiates including DSTBULET have also been recorded in the isolated spinal cord slice (Dickenson & Magnuson, 1989). In this preparation the cells are clearly located in the translucent zone of the dorsal horn which is generally accepted to be the SG (Brown, 1982). It is likely that the neurones recorded *in vivo* are also located in the same zone i.e. the SG, since they exhibit a similar excitatory response to opiates. The SG has been implicated in segmental control of nociceptive transmission via inhibitory interneurons releasing enkephalins and γ -aminobutyric acid (GABA) (see Besson & Chaouch, 1987). It seems probable that the mechanism of excitation of the intermediate cells by DSTBULET in this study is indirect and is in fact the consequence of the blockade by DSTBULET of a tonic interneuronal inhibition of these cells. A similar mechanism occurs in the hippocampus where opioid-induced excitation is mediated by inhibition of inhibitory interneurons (Zieglansberger *et al.*, 1970; Neumaier *et al.*, 1988).

Differential inhibition of thermal, pressure and chemical noxious inputs have been observed for agonists acting at different opiate receptor subtypes in the spinal cord (Schmauss & Yaksh, 1984). It has been suggested that whereas μ -opioid agonists inhibit all three noxious stimuli, δ -agonists are selective for thermal stimuli and are ineffective against chemically-evoked noxious input. In behavioural studies DPDPE and [Met]enkephalin appear more potent against thermal compared with mechanically-evoked noxious stimuli (Kuraishi *et al.*, 1985; Rodriguez *et al.*, 1986) and both DADLE and [Met]enkephalin are ineffective in the acetic acid writhing test (Schmauss & Yaksh, 1984). However, in the present study DSTBULET was equally effective in inhibiting neuronal responses evoked by both electrical and chemical noxious stimulation. Furthermore, the enhancement of endogenous enkephalins in the spinal cord by kelatorphan was also sufficient to abolish the neuronal excitation by formalin. It is possible that the δ -agonists distinguish between cutaneous and visceral inputs rather than the modality of input. Both the noxious activity evoked by electrical stimulation and by the chemical irritant formalin were cutaneous in origin, whereas the acetic writhing test involves visceral inputs. An alternative explanation

tion arises from the differences in analgesic potency of μ - and δ -agonists in behavioural tests. Generally, δ -agonists are less effective than μ against noxious stimuli (Hylden & Wilcox, 1983; Porreca *et al.*, 1984; Heyman *et al.*, 1987; Russell *et al.*, 1987). If the intensity of the noxious stimuli is much greater in the writhing test, the lower potency of DPDPE or [Met]enkephalin might be insufficient to produce any visible analgesia. However, in a comparison of the writhing test and the hot-plate test, Shaw *et al.* (1988) concluded that the former is a less intense stimulus than the latter. It would be interesting to study DSTBULET against a visceral stimulus to see if the ligand, test or receptor type determines these differences.

To summarise, the selective δ -opioid DSTBULET

is as potent as morphine in inhibiting noxiously-evoked input to the rat spinal cord. C fibre-evoked responses of dorsal horn neurones due to either electrical stimulation of the receptive field or subcutaneous injection of formalin could be abolished by this agonist. Kelatorphan was equally effective in inhibiting the formalin response, confirming the involvement of endogenous enkephalins in spinal antinociception. We conclude that the activation of δ -opioid receptors can play an important role in the mediation of antinociception in the spinal cord.

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