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Differential antinociceptive effects of spinal opioids on foot withdrawal responses evoked by C fibre or Aδ nociceptor activation

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1 Intrathecal application of μ, δ, and κ opioids attenuate responses on several tests of animal nociception. However, the potency of these opioids differ depending on which tests were used. One factor contributing to these discrepancies is that different types of noxious stimuli activate different sets of nociceptor types, which may be differentially sensitive to opiate inhibition. To examine this hypothesis, we used a recently developed behavioural test which allows for differential assessment of nociception evoked by the activation of myelinated (Aδ) and unmyelinated C thermonociceptors.

2 Administration of a κ-selective agonist was ineffective on either type of response. δ_1 drugs were slightly more potent on C fibre-mediated responses than on Aδ-mediated responses.

3 Intrathecal μ and δ_2 drugs were antinociceptive on both Aδ and C nociceptor-mediated responses. However, unlike the δ_1 effects, the dose-response curves for μ and δ_2 drugs were significantly more steep for Aδ than for C fibre-mediated responses, potentially indicating differences in the mechanisms by which the drugs act on these 2 response types.

Keywords: Opioids; nociception; Aδ; C-fibre; nociceptor; μ-, δ- and κ-receptors

Introduction

Intrathecal injection of opioids produces antinociception in both animals and man (Wang *et al*., 1979; Yaksh, 1983; Onofrio & Yaksh, 1984). This antinociception is mediated by pre and/or post-synaptic μ, δ, and κ opiate receptors in the dorsal horn of the spinal cord which act to inhibit transmission of nociceptive information carried by primary afferents (Atweh & Kuhar, 1977; Fields *et al*., 1980; Czlonkowski *et al*., 1983; Mack *et al*., 1984; Gouarderes *et al*., 1985; Morris & Herz, 1987; Mansour *et al*., 1988; Zajac *et al*., 1989; Stewart & Hammond, 1994; Cheng *et al*., 1995; Arvidsson *et al*., 1995). Considerable evidence exists for both presynaptic (Jessel & Iversen, 1977; Yaksh *et al*., 1980; Brodin *et al*., 1983; Pan & Vasko, 1986; Go & Yaksh, 1987; Collin *et al*., 1991; Taddese *et al*., 1995; Zachariou & Goldstein, 1996) and postsynaptic (LeBars *et al*., 1976; Duggan *et al*., 1977; Belcher & Ryall, 1978; Zieglgansberger & Tulloch, 1979; Murase *et al*., 1982; Wilcockson *et al*., 1984; Jeftinija, 1988;; Grudt & Williams, 1994) antinociceptive effects of μ and δ agonists. In contrast, the preponderance of evidence indicates that the (somewhat limited) antinociceptive effects of κ opioids (Tyres, 1980; Millan, 1989; 1990) are mediated postsynaptically (Go & Yaksh, 1987; Besse *et al*., 1990; Millan, 1990; Malmberg & Yaksh, 1993; Randic *et al*., 1995).

While it is clear that opioids are, in general, more potent in attenuating nociceptive rather than non-nociceptive responses (Dickenson *et al*., 1987; Abbadie *et al*., 1994) and the specificity of some opiate receptors for different types of nociceptive modalities has been considered (e.g., Schmauss, 1987), the specifity of different classes of opioids for nociception mediated by different classes of nociceptive afferents is not well understood. One principle division in nociceptive physiology is between nociceptive afferents that have myelinated axons (Aδ) and those that are unmyelinated (C). We have previously described a rat behavioural assay which allows for the differential assessment of nociception mediated by the activation of C or Aδ nociceptors (Yeomans *et al*., 1996; Yeomans & Proudfit, 1996). Using this method, we demonstrated that systemic application of the μ opioid agonist morphine is approximately 30 times more potent in attenuating C fibre mediated nociception than for Aδ-mediated nociception (Yeomans *et al*., 1996). These data are consistent with electrophysiological experiments demonstrating a preferential effect of systemic morphine on dorsal horn neuronal responses to C vs Aδ input (LeBars *et al*., 1976). However, it has not been established whether intrathecal (i.t.) application would yield similar selectivity. The purpose of this study was to determine the selectivity of i.t. application of μ, δ, and κ opioid agonists and antagonists for nociception mediated by the activation of C or Aδ nociceptors.

Methods

Animal preparation

Female Sprague-Dawley rats (300–350 g; Sasco, Omaha, NE) were lightly anaesthetized with urethane (Sigma; 1000 mg kg^{-1}, i.p.) to impede avoidance learning and to prevent non-specific movements during long latency response periods. The dose of urethane used has been shown not to alter reflexive responses compared to unanaesthetized rats (Maggi & Meli, 1986; Yeomans *et al*., 1996). Thirty minutes later, deep anaesthesia was induced with ether so that an incision could be made in the atlanto-occipital membrane. An intrathecal catheter (PE10) was then inserted through the incision and advanced to the lumbar enlargement. Both feet were blackened with India ink to provide uniform skin heating during the application of radiant heat.

Assessment of nociception

Responses evoked by thermal activation of Aδ and C nociceptors were separately assessed as previously described (Yeomans *et al*., 1996). Briefly, latencies were measured to foot withdrawals elicited by the output of a projection bulb focused on the dorsolateral or dorsomedial surfaces of either hindpaw. The bulb intensity was set to as as to increase the surface skin temperature either at a high (6.5°C s^{-1}) or low (0.9 °C s^{-1}) average rate. The low rate evokes responses mediated by C fibre activation, whereas the high rate evokes responses

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mediated by the activation of Aδ nociceptors (Yeomans & Proudfit, 1996). The response latencies of the 4 skin surfaces were averaged and expressed as the foot withdrawal latency (FWL). To minimize tissue damage by prolonged heating in the absence of foot responses, trials were terminated after cut-off latencies of 20 s for the low and 6 s for the high heating rate trials.

Effects of intrathecal injections of opiate agonists and antagonists on nociception

Baseline foot-withdrawal latencies were determined. Thereafter, rats were given an i.t. injection of one of the agonists, antagonists or vehicles as listed below (Table 1). Six animals were used for each dose. Drug effects on response latencies were determined immediately after the drug injection, and at 5 or 10 min intervals for 2 h. In 1 group of animals per agonist (except U50488), approximately 20 min after giving the highest dose of agonist, the antagonist selective for the same receptor was administered. Testing then proceeded for an additional 20 min. U50488 did not produce antinociception at a dose of 500 nmol, this drug was not tested further.

Analysis

To determine whether the drugs administered significantly attenuated nociceptive responsiveness, separate analyses of variance were performed for each agonist, antagonist and vehicle with response latency as the dependent variable. Follow up analyses (Dunnett's) were performed to determine which individual doses produced significant antinociceptive effects. Data derived from sessions in which these doses were administered were used for determination of dose-response functions (see below). To determine whether the antagonists significantly attenuated the antinociceptive effect of the agonists, paired *t* tests were performed comparing peak responses after administration of the antagonist to responses that occurred at the same time points in animals that received the same dose of agonists, but did not receive the antagonist.

Dose-response functions with latency shifts could not be calculated directly. This is because the latency values and maximal latency shifts are unequal for high and low rate skin heating responses. However, the subsurface (tissue) skin temperatures of response for the two heating rates were approximately equal, and so, for direct comparison of effects on high vs low rate responses, latencies were converted to subsurface response temperatures by use of formulae based on skin temperature data gathered previously (Yeomans & Proudfit, 1994).

For conversion of response latencies to response temperatures (subsurface temperature at response):

$$T_r = (FWL \times HR) + T_b$$

Where T_r is response temperature in °C, FWL is foot withdrawal latency in seconds, HR is the rate of increase in subsurface skin temperature in °C s^{-1} for a particular bulb intensity, and T_b is the estimated subsurface baseline temperature in °C. The heating rate constants are from published measurements taken approximately 0.5 mm below the surface of the skin (Yeomans & Proudfit, 1994). For the high rate this number is 2.5°C s^{-1}. For the low rate the number is 0.6°C s^{-1}. The subsurface baseline temperature varies little and is usually approximately 35°C. Separate dose-response lines were constructed for the high and low heating rates by use of peak response latency values after i.t. injection of the opioid agonists. The dose-response relationships for the antinociceptive effects of the agonists were assessed by one-way analyses of variance for repeated measures. Separate ED_{50} values and 95% confidence limits were also calculated for the dose-response lines generated for the two heating rates. ED_{50} values that were derived from dose-response lines that did not significantly depart from parallelism (Tallarida & Murray, 1987) were directly compared and considered to be significantly different if confidence limits did not overlap.

Drugs and intrathecal injections

The following drugs were purchased from Research Biochemicals Incorporated: μ-opioid agonist DAMGO ([D-Ala^2, N-Me-Phe^4, Gly-ol^5-]-enkephalin), δ_1-opioid agonist DPDPE ([D-Pen^{2,5}]-enkephalin), δ_2 agonist DSLET ([D-Ser^2] Leu-enkephalin-Th^6, the κ-opioid agonist U50488 (*trans*(±)-3,4, dichloro-N-, ethyl-[2−1−pyrrolindyl)-cyclohexyl]-benzeneacetamide methanesulphonate), the μ antagonist naloxonazine (bis-[5-4,5-epoxy-3,14-dihydroxy-17-(2-prpenyl)-morphinan-6-ylidene]hydrazine), the δ_1 antagonist NTI (naltrindole hydrochloride), and the δ_2 antagonist NTII (naltrindole isothiocyanate). Morphine sulphate was purchased from Lilly.

All drugs were dissolved in 0.9% saline and were injected i.t. in a volume of 10 μl followed by 10 μl of saline to ensure drug delivery into the subarachnoid space at the level of lumbar enlargement. Solutions were injected at a rate 10 μl min^{-1}. All experimental procedures were in accordance with the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain, and were reviewed and approved by the University of Illinois Animal Care Committee.

Results

Intrathecal injection of either the κ agonist U50488 up to 500 nmol, any of the 3 antagonists alone or saline vehicle were without effect on foot withdrawal responses evoked by high (Aδ-mediated) or low (C-mediated) rate skin heating (data not shown). In contrast, i.t. injection of either of the μ agonists DAMGO or morphine produced significant (ANOVA,

Table 1 Drugs used for assessing Aδ/C opiate antinociception

Drug	n *(6 rats/group)*	*Antagonist used*	*Dose range* (nmol)
DAMGO (μ agonist)	24	None	0.1–1.0
Morphine (μ agonist)	30	None	0.5–5.0
DPDPE (δ_1 agonist)	18	None	10.0–50.0
DSLET (δ_2 agonist)	24	None	3.0–10.0
U50488 (κ agonist)	12	None	100.0–500.0
DAMGO (μ agonist)	6	Naloxonazine (μ)	1.0 with 2.0 antag.
Morphine (μ agonist)	6	Naloxonazine (μ)	5.0 with 2.0 antag.
DPDPE (δ_1 agonist)	6	NTI (δ_1)	50.0 with 200 antag.
DSLET (δ_2 agonist)	6	NTII (δ_2)	10.0 with 100 antag.
Naloxonazine (μ antag.)	6	NA	2.0
NTI (δ_1 antag.)	6	NA	200.0
NTII (δ_2 antag.)	6	NA	100.0
Saline (vehicle)	6	NA	NA

NA = not applicable.

$P<0.05$), dose-dependent antinociception for both Aδ- and C fibre-mediated responses (Figure 1, Table 2). However, the slopes of the dose-response curves for the two types of responses were significantly different (Table 2), indicating that the potencies of the drugs for Aδ and C mediated responses could not be directly compared. In both cases, the slope of the dose-response curve for Aδ-mediated responses was much greater than that for C fibre mediated responses (Table 2). However, the slopes of the lines within a response type were quite similar and a comparison of the lines indicate that

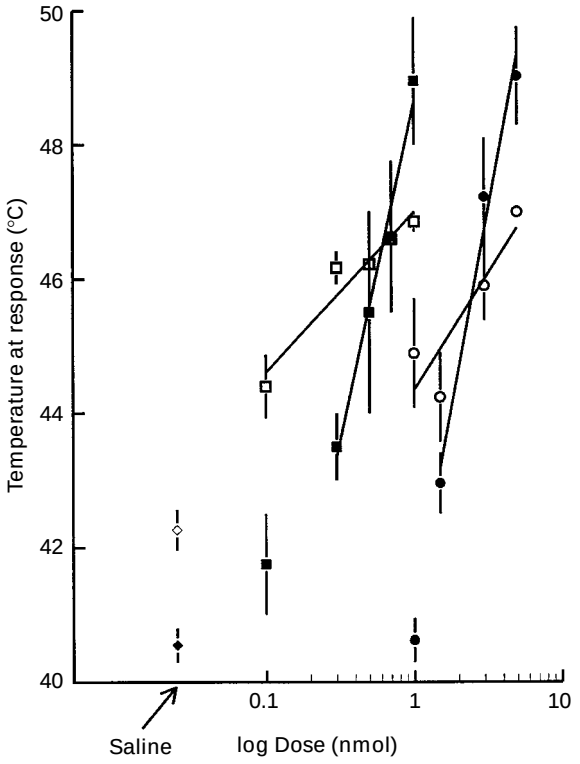

Figure 1 Dose-response relationships for the effects of intrathecally administered μ opioids on nociceptive responses to high (Aδ nociceptor) and low (C nociceptor) heating rates. Mean temperatures (°C) at response (see text) are plotted on the ordinate scale and doses (nmol) are plotted as a log scale on the abscissa scale. Vertical lines show s.e.mean. Mean response temperatures for six animals per dose of DAMGO with the high scale heating rate are represented by (●) and mean response temperatures with the low heating rate are represented by (○). Mean response temperatures for six animals per dose of morphine with the high heating rate are represented by (■) and mean response temperatures with the low heating rate are represented by (□). The dose-response lines represent least-square lines of best fit. The mean response temperature with the high heating rate of 6 rats after intrathecal injection of saline vehicle and (◇) the mean response temperature of 6 rats with the low heating rate after intrathecal injection of saline vehicle. Some error bars are obscured by the symbols in this and subsequent figures.

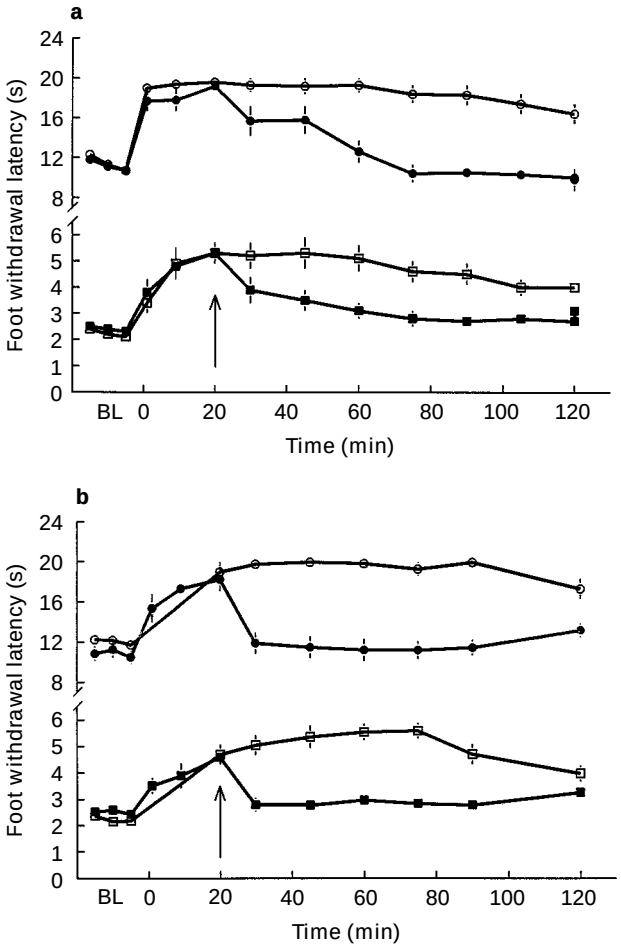

Figure 2 Time course of the effects produced by intrathecal injection of 1.0 nmol DAMGO (a) or 5.0 nmol morphine (b) and the effects of subsequent intrathecal injection of 2.0 nmol naloxonazine on the latencies of foot withdrawal responses to two different skin heating rates. In both (a) and (b), squares represent the effects of the agonist on mean latencies of Aδ-mediated responses to the high heating rate, while circles represent latencies of C nociceptor-mediated responses to the low heating rate. Either naloxonazine or saline vehicle was administered at the 20 min point, as indicated by the arrows. Naloxonazine (solid symbols) significantly (ANOVA, $P<0.05$) reduced the antinociceptive effects of DAMGO (a) and morphine (b) when compared to vehicle (open symbols) for both Aδ- and C nociceptor-mediated responses. Mean response latencies (s) are; plotted on the ordinate scale and the time (min) after drug injection is plotted on the abscissa scale. Three baseline (BL) control response latencies determined before drug injection are plotted before the zero time point on the abscissa scale. Each value represents the mean response latency (s) for six animals; vertical lines show s.e.mean.

Table 2 Effects of intrathecal opiates on Aδ/C mediated nociception

Drug	*Aδ*/C	*Antag. revers.?*	F *score*	ED_{50} (nmol)	*Slope*	*Aδ & C parallel?*
DAMGO (μ)	Aδ	Yes	3.68	0.52 (0.34–0.73)	10.69 (−1.17–22.5)	No
DAMGO (μ)	C	Yes	19.94	0.14 (0.12–0.16)	2.22 (1.20–3.25)	
Morphine (μ)	Aδ	Yes	29.98	2.10 (1.71–2.45)	10.64 (6.58–14.71)	No
Morphine (μ)	C	Yes	41.66	0.64 (0.57–0.71)	3.75 (2.61–4.89)	
DPDPE (δ_1)	Aδ	Yes	2.64	10.08 (9.46–10.67)	2.64 (−0.75–6.03)	Yes
DPDPE (δ_1)	C	Yes	2.46	8.96 (8.79–9.12)	2.06 (0.46–4.76)	
DSLET (δ_2)	Aδ	Yes	17.66	4.47 (3.71–5.2)	9.49 (4.71–14.28)	No
DSLET (δ_2)	C	Yes	32.59	2.08 (1.79–2.36)	2.82 (1.69–3.96)	
U50488 (κ)	Aδ	NA	NA	>500	NA	NA
U50488 (κ)	C	NA	NA	>500	NA	

Both ED_{50} and slope values are shown with confidence limits in parentheses.

DAMGO is 5–6 times more potent than morphine for both Aδ and C fibre-mediated responses. Figure 2 demonstrates the time course of experiments in which the μ-selective antagonist naloxonazine was administered i.t. 20 min after DAMGO (Figure 2a) or morphine (Figure 2b). In both cases, naloxonazine significantly ($P<0.05$) attenuated the antinociceptive effect of the agonist, providing additional evidence that the effects of the agonists were mediated by μ receptors.

Intrathecal application of the δ_1 agonist DPDPE also produced significant (ANOVA, $P<0.05$), dose-dependent antinociception for responses for both Aδ and C fibre-mediated responses (Figure 3, Table 2). The slope of these dose-response lines were not significantly different and a comparison of ED_{50} s indicates that DPDPE is approximately two times more potent on C-mediated responses (Table 2). The time-course shown in Figure 4a demonstrates that the antinociceptive effect of 50 nmol DPDPE was partially reversed ($P<0.05$) by i.t. application of 200 nmol of the δ_1 antagonist NTI, providing additional evidence that the antinociceptive effects of the agonist were, in fact, mediated by δ_1 receptors.

The effect of intrathecal injection of the δ_2 agonist DSLET was clearly different from that of DPDPE. DSLET produced significant antinociception for both Aδ and C mediated responses (ANOVA, $P<0.05$), but the slope of the dose-response curves for these two response types were clearly different (Figure 3, Table 2). As with the μ opioids, the slope of the dose-response effect for Aδ-mediated responses was significantly greater than that produced for C fibre-mediated responses. In addition, the i.t. injection of the selective δ_2 antagonist NTII significantly ($P<0.05$) attenuated the antinociceptive effect of DSLET, providing further evidence of δ_2 mediation of observed affects (Figure 4b).

Discussion

The purpose of this study was to compare the effects of intrathecal application of different classes of opiates on beha-

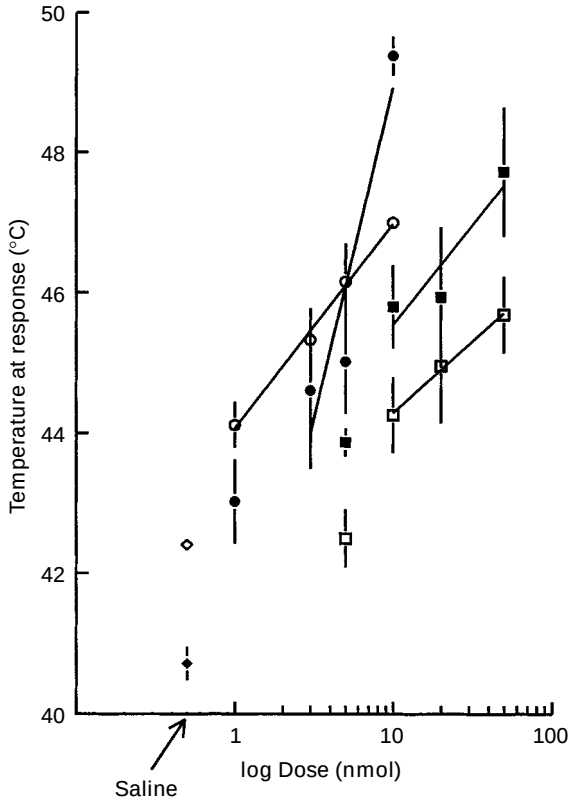

Figure 3 Dose-response relationships for the effects of intrathecally administered δ opioids on nociceptive responses to high (Aδ nociceptor) and low (C nociceptor) heating rates. Mean temperatures (°C) at response (see text) are plotted on the ordinate scale and doses (nmol) are plotted as a log scale on the abscissa scale. Mean response temperatures for six animals per dose of DPDPE with the high heating rate are represented by the solid circles and mean response temperatures with the low heating rate are represented by the open circles. Mean response temperatures for six animals per dose of DSLET with the high heating rate are represented by the solid squares and mean response temperatures with the low heating rate are represented by the open squares. The dose-response lines represent least-squares lines of best fit. The solid diamond represents the mean response temperature with the high heating rate of 6 rats after intrathecal injection of saline vehicle and the open diamond represents the mean response temperature of 6 rats with the low heating rate after intrathecal injection of saline vehicle. Vertical lines show s.e.mean; some error bars are obscured by the symbols in this and subsequent figures.

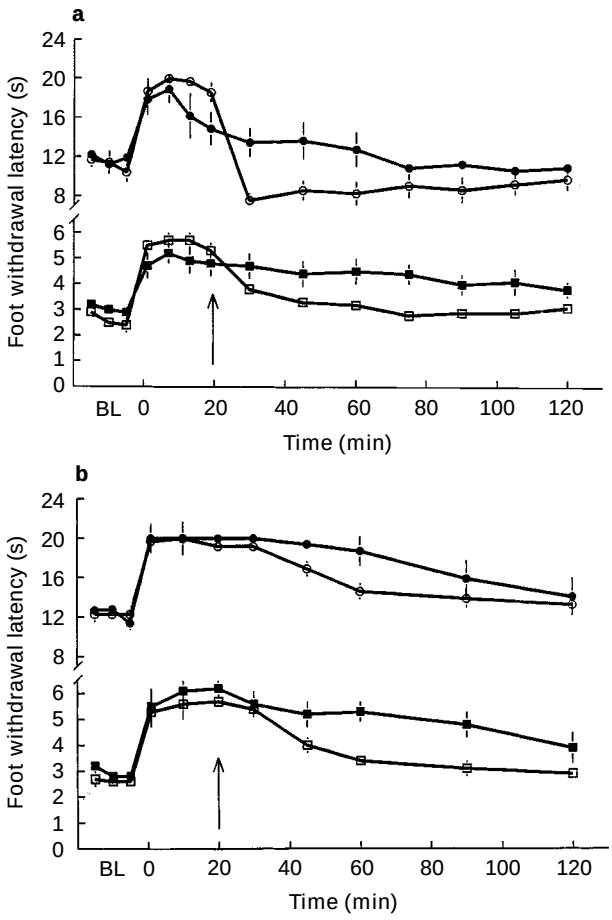

Figure 4 Time course of the effects produced by intrathecal injection of 50.0 nmol DPDPE (a) or 10.0 nmol DSLET (b) and the effects of subsequent intrathecal injection of 200.0 nmol naltrindole (NTI) after DPDPE (a) or 100 nmol naltrindole isothiocyanate (NTII) after DSLET (b) on the latencies of foot withdrawal responses to two different skin heating rates. In both (a) and (b), squares represent the effects of the agonist on mean latencies of Aδ-mediated responses to the high heating rate, while circles represent latencies of C nociceptor-mediated responses to the low heating rate. Either antagonist or saline vehicle was administered at the 20 min point, as indicated by the arrows. In both cases, the antagonist (solid symbols) significantly (ANOVA, $P<0.05$) reduced the antinociceptive effects of DPDPE (a) and DSLET (b) when compared to vehicle (open symbols) for both Aδ- and C nociceptor-mediated responses. Mean response latencies (s) are plotted on the ordinate scales and the time (min) after drug injection is plotted on the abscissa scales. Three baseline (BL) control response latencies determined before drug injection are plotted before the zero time point on the abscissa scale. Each value represents the mean response latency (s) for six animals; vertical lines show s.e.mean.

vioural responses evoked by the activation of Aδ or C fibre thermonociceptors. The κ agonist tested, U50488, was without affect on nociception of either type. This result is consistent with previous findings that, in general, i.t. κ opioids do not attenuate thermal nociception (Tyres, 1980; Leighton *et al*., 1988; Millan, 1989; 1990; Piercey & Einspahr, 1989), as opposed to mechanical and electrical stimulation (Knox & Dickenson, 1987). However, it is important to note that this was the first time the agonist class was tested on responses with known afferent physiology. Thus, it appears that i.t. application of κ opioids do not affect nociception mediated by either Aδ or C fibre thermonociceptors.

In contrast to the κ agonist, μ, δ_1 and δ_2 agonists produced dose-dependent, specific-antagonist-reversable antinociception for foot withdrawals evoked by either Aδ or C fibre nociceptors. However, there were important differences in the effect of these drugs on responses mediated by the two nociceptor types. For the δ_1 agonist DPDPE, the dose-response lines were parallel, suggesting that the drug works by similar mechanisms on Aδ and C mediated responses, and that the observed 2 fold difference in ED_{50}s is a valid comparison. This potency distinction may be due simply to differential access of the drug to receptors at various depths in the spinal cord. With intrathecal injection, a concentration gradient is established from the cerbrospinal fluid (CSF) toward the centre of the cord (Gregory *et al*., 1986). In addition, the distribution, and hence part of the potency of drugs within the spinal cord will depend on its lipid solubility and the local lipophilicity/hydrophylicity (McQuay *et al*., 1989). Thus, if those δ_1 receptors involved in inhibition of Aδ-mediated responses are located deeper, or in areas of lower drug solubility, on average, than those affecting C-mediated responses, the apparent potency would be less for those responses evoked by Aδ activation. That Aδ and C afferents terminate differentially within the dorsal horn has been clearly demonstrated (see Fitzgerald, 1989), indicating a possible anatomical substrate for the observed potency distinctions. Alternatively, differences in receptor densities at Aδ and C fibre sites of action may be responsible for differences in potency. However, the results clearly demonstrate that the δ_1 selective agonist DPDPE more potently attenuates C fibre-mediated nociception than that mediated by Aδ activation.

For the μ agonists, DAMGO and morphine, and the δ_2 agonist, DSLET, direct potency comparisons could not be made between the effects on Aδ-mediated and C-mediated nociception. In each case, the slopes of the dose-response lines for the Aδ-mediated responses were considerably greater than those for the C-mediated responses. Thus, although μ and δ_2 agonists affect both Aδ and C thermonociceptor-mediated responses, there may be important distinctions in the mechanisms by which these effects are produced. One possible distinction that might explain the observed differences is that the effects of these agonists are mediated, at least partly, presynaptically for C fibre mediated responses and postsynaptically for Aδ-mediated responses.

It is clear that μ receptors are located both presynaptically on primary afferent terminals and postsynaptically on dorsal horn cells. Anatomical localization of μ receptors to presynaptic terminals and to postsynaptic cells has been demonstrated both autoradiographically (Fields *et al*., 1980; Besse *et al*., 1990) and, more recently, immunohistochemically (Arvidsson *et al*., 1995; Ji *et al*., 1995). In addition, Arvidsson *et al*. (1995) demonstrated that μ receptors did not co-localize with RT97 (a marker for myelinated afferents), indicating to these authors that μ receptors are localized to neurones that give rise to unmyelinated fibres. Similarly, Gamse *et al*. (1979) found a distinct capsaicin sensitivity of neurones possessing μ receptors, again suggesting a selectivity for C fibre afferent neurones. Glaum *et al*. (1995), recording intracellularly from neurones in lamina II of the rat spinal cord, found a clearly predominant presynaptic effect of DAMGO on neuronal excitability. As lamina II has been shown to be selectively or predominantly innervated by C as opposed to Aδ nociceptive afferents (see Fitzgerald, 1989), these data provide presumptive evidence for a pre- and not postsynaptic effect of μ opioids on C fibre nociception. Similarly Taddese *et al*. (1995), demonstrated an inhibitory effect of μ agonists on C but not Aδ tooth pulp afferent cell bodies. Thus, it appears that C afferents terminating in lamina II of the spinal cord are presynaptically inhibited by μ opioids. In contrast, Aδ afferents, at least in the trigeminal ganglion, are not inhibited by μ opioids.

Aδ-mediated nociception, in contrast, may be attenuated postsynaptically by μ opioids. Approximately 24% of μ receptors in the superficial dorsal horn are postsynaptic (Besse *et al*., 1990). Furthermore, both inhibitory and excitatory postsynaptic effects of μ opioids have been clearly demonstrated (Yoshimura & North, 1983; Hope *et al*., 1990; Magnuson & Dickenson, 1991). Administration of μ opioids inhibits responses of neurones in lamina I to both noxious stimulation in the periphery as well as local application of excitatory amino acids (Hope *et al*., 1990; Jones *et al*., 1990), providing clear evidence of postsynaptic effects in an area heavily innervated by Aδ nociceptive afferents (Mense & Prabhakar, 1986). Thus, there is substantial evidence indicating a postsynaptic location for some μ opioid antinociceptive effects. Although there is no direct evidence that these effects are on neurones receiving nociceptive innervation from Aδ and not C afferents, the apparent lack of presynaptic effects on Aδ afferents may indicate, by exclusion, a postsynaptic site of action of μ opioids on Aδ-mediated nociception.

Differences in the slope of the δ_2 opioid agonist on Aδ vs C fibre-mediated behavioural responses may indicate similar differences in the synaptic location of the observed effects. As with μ opioids, our results are consistent with those in the literature suggesting that the effect of δ_2 agonists on C activity are, at least partially, presynaptic (Glaum *et al*., 1995), whereas the effects on Aδ activity may be primarily postsynaptic. However, since it has only recently been determined that there are δ_2 receptors in the spinal cord (Sofuoglu *et al*., 1991; 1993; Stewart & Hammond, 1993; Glaum *et al*., 1995), additional experiments must be performed to support or refute this hypothesis.

In summary, our experimental results demonstrate that while intrathecal application of a κ opioid did not attenuate either Aδ or C thermonociceptor-mediated responses, μ, δ_1 and δ_2 opioid agonists are effective on both. The δ_1 selective agonist DPDPE was only slightly more potent on C fibre-mediated responses, and appeared to act by similar mechanisms on both response types, as indicated by parallel dose-response curves. Intrathecal administration of μ opioids on the other hand, produced clearly non-parallel dose-response curves for Aδ vs C fibre mediated responses, indicating that the mechanisms by which these drugs act may be different. An examination of the literature suggests that this difference may be the sites of action of the drugs in the spinal cord. Specifically, μ drugs may act presynaptically on C fibre afferent terminals, but postsynaptically on dorsal horn neurones to attenuate responses to Aδ input. Finally, the δ_2 selective drug DSLET produced a similar dose-response pattern to μ-selective drugs, possibly suggesting similar differences in the sites of action for Aδ and C thermonociceptor-mediated responses.

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