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Spinal antinociceptive actions and naloxone reversibility of intravenous μ - and κ -opioids in spinalized rats: potency mismatch with values reported for spinal administration

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1 The relative spinal effectiveness of μ - and κ -opioids has been assessed by their intravenous potencies on nociceptive responses (heat and/or pinch) of single motoneurons recorded in α -chloralose anaesthetized, spinalized rats.

2 The depressant actions of both μ - and κ -opioids were reversed by low intravenous doses of naloxone (10 to 100 $\mu\text{g kg}^{-1}$). When tested at a dose of 1 $\mu\text{g kg}^{-1}$ i.v., naloxone antagonized the effects of the μ -agonist morphine but had no effect on the κ -opioid U-50,488. This provides further support for the theory that the actions of μ - and κ -ligands were mediated at different subclasses of opioid receptor but highlights the difficulties in using antagonists with poor receptor selectivity to differentiate between μ - and κ -receptor-mediated effects *in vivo*.

3 The molar potency ratios of fentanyl:morphine:U-50,488:tifluadom for thermal and mechanical nociceptive responses were 620:1.0:0.74:5.7 and 520:1.0:0.56:7.7 respectively. These potency ratios, as well as the absolute potencies, agree well with those reported in several behavioural studies in which systemic administration of agonists was used in non-thermal tests.

4 The agonist potency values obtained in this study contrast with those reported for local spinal administration. By this route, the potency of lipophilic opioids (e.g. fentanyl, U-50,488 and tifluadom) relative to hydrophilic opioids (e.g. morphine) is much reduced, implying that activity of intrathecally administered opioids is more dependent on the physico-chemical properties of the agonists used than on the relative abundance in the spinal cord of functional opioid receptors of the μ - and κ -subtypes. This conclusion indicates that the results with locally applied opioids should not be used to assess spinal opioid receptor function.

Introduction

In the preceding paper (Parsons & Headley, 1989a) we have demonstrated that, following intravenous administration in spinalized rats, κ - as well as μ -opioids reduce spinal reflexes to thermal as well as to mechanical noxious stimuli. In contrast, much of the behavioural literature indicates that intrathecally administered κ -opioids are ineffective in tests of thermal nociception but are effective in models of visceral and mechanical nociception (see review by

Yaksh & Noueihed, 1985 and references quoted in Parsons & Headley, 1989a).

We have shown that at least some of this difference is likely to be related to a mismatch between the different stimulus intensities used in various behavioural models. However, the fact that intrathecally administered μ -opioids have frequently been reported to be equally effective in thermal, mechanical and visceral behavioural models of nociception (see Yaksh & Noueihed, 1985) indicates that variations of stimulus intensity cannot fully explain the reported μ/κ differences; other factors must also be involved.

It has already been shown that the ability of different μ -opioids to diffuse into the cord following local

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Table 1 Mean depression of mechanical and thermal nociceptive reflex responses by four opioids

Drug	Dose (kg ⁻¹)	Mechanical				Thermal				Relative molar potency	
		%Con	n	ED ₅₀	n	%Con	n	ED ₅₀	n		
Fentanyl	2 µg	44 ± 5	50	3.8 ± 0.5	40	520	47 ± 5	42	3.0 ± 0.4	40	620
Morphine	0.5–16 µg	38 ± 14	8	1.9 ± 0.7	6	1.0	55 ± 11	8	1.8 ± 0.6	5	1.0
	2 mg	53 ± 6	52	4.9 ± 0.6	31	0.56	56 ± 6	34	3.5 ± 0.6	28	0.74
U-50,488	0.5–16 mg	40 ± 7	26	0.34 ± 0.04	25	7.7	47 ± 11	12	0.43 ± 0.14	4	5.7
Tifluadom	0.2 mg										
	0.2–1.6 mg										

Depression of reflexes was quantified for individual motoneurons as a percentage of the mean control number of spikes per response. The mean effect of one selected dose of each opioid is shown as '% Con' in the upper row for that opioid. The lower row for each drug shows the ED₅₀ calculated for the subset of cells on which at least 3 doses were tested over the linear portion of the dose response curve, and within the dose ranges shown. See text for further details of analysis. The standard errors of the means, the numbers of cells tested (*n*) and the molar potencies relative to morphine = 1 are also shown.

spinal administration can vary enormously (Durant & Yaksh, 1986; Yaksh *et al.*, 1986) but there are no equivalent data for κ-ligands. It seems possible therefore, that there is also limited access to, and activation of, spinal κ-opioid receptors following intrathecal or epidural administration of κ-selective ligands such as U-50,488 and tifluadom. To investigate this possibility we have tested selective opioid agonists intravenously in spinalized animals so as to compare their relative and absolute potencies with those reported in behavioural tests following either local spinal or systemic administration.

Although the μ/κ selectivity of naloxone is poor (Lord *et al.*, 1977; Chavkin *et al.*, 1982; Hayes & Kelly, 1985), we attempted to determine the lowest intravenous dose capable of antagonizing presumed μ- and κ-opioid receptor-mediated events. Some of these data have been presented in a preliminary form (Parsons & Headley, 1988).

Methods

Electrophysiological experiments were performed on α-chloralose anaesthetized, spinalized rats according to the protocols and analytical techniques described in the preceding papers (Parsons & Headley, 1989a). Motoneurons were activated by a variety of 'natural' peripheral stimuli, including noxious heat and pinch and innocuous tap and vibration. Drugs tested intravenously in cumulative dose regimes were the μ-agonists fentanyl and morphine, the κ-agonists U-50,488 and tifluadom and the opioid antagonist naloxone. Drug doses refer to fentanyl base, U-50,488 methane sulphionate and tifluadom, morphine

and naloxone hydrochlorides. Morphine, fentanyl and naloxone were obtained from commercial sources.

In tests with naloxone the protocols were varied as follows. When attempting to antagonize the actions of all opioids except morphine the depressant effects of the agonist and the subsequent time course for full recovery of responses were first established in the absence of naloxone. When this time course was sufficiently long, as was often the case with U-50,488, then the same dose regime of agonist was repeated prior to the subsequent i.v. administration of one or more doses of naloxone. Thereafter responses were compared with the control agonist test. When the time course for recovery was very rapid, as was normally the case with fentanyl and tifluadom, then the same dose regime of agonist was repeated but was preceded by a single dose of naloxone (see e.g. Figure 4). The agonist was then tested again at regular 30 min intervals until full recovery from the naloxone antagonism was achieved. Systemic morphine has a long half life so that recovery cannot easily be observed in electrophysiological experiments. Morphine was therefore tested as the last opioid agonist in any one experiment and its effects were always challenged with the subsequent administration of one or more doses of naloxone.

Results

Recordings were made from a total of 173 motoneurons in 99 α-chloralose anaesthetized, spinalized rats. On several neurones noxious heat and pinch stimuli were alternated; on other cells however, noxious stimuli were alternated with innocuous

cutaneous stimuli (see Parsons & Headley, 1989b) and on a few cells only a single modality of peripheral stimulus was tested.

Comparison of the relative potencies of μ - and κ -agonists on nociceptive reflexes

Depression of reflexes on any individual cell was quantified as a percentage of the control number of spikes per response (mean of 3 pre-drug responses). The relative potencies of the opioid agonists were analysed in two ways. Firstly, the percentage depression was averaged for each dose of each drug. Secondly, when 3 or more doses of opioid had been tested over the 'linear' region of the dose-response curve, the ED_{50} was calculated by linear regression analysis (see Parsons & Headley, 1989a). Since the criteria for ED_{50} estimation could not be met on all cells, both forms of analysis are presented in Table 1; the considerable differences in sensitivity between individual cells are reflected in the s.e.mean values.

Noxious pinch

Fentanyl was tested on individual cells in a log-dose regime (but over variable dose-ranges) between 0.5 and $16 \mu\text{g kg}^{-1}$ i.v. Its potency on reflexes to noxious pinch was assessed in tests on 67 motoneurons recorded in 47 rats. The potency of fentanyl on mechanical nociceptive reflexes of one such cell is shown in Figure 1a. With 40 of the neurons tested with this μ -agonist a sufficient number of cumulative doses were given to calculate an ED_{50} on mechanical nociceptive reflexes for that cell. The pooled data are presented in Table 1.

Because morphine is the standard against which other opioids are frequently tested, this μ -agonist was compared with fentanyl (and U-50,488) on responses to noxious pinch of 9 motoneurons recorded in 9 rats. In the example in Figure 1 the effects of morphine were completely reversed by naloxone $20 \mu\text{g kg}^{-1}$. When the ED_{50} data from these i.v. tests are pooled, fentanyl is 500 times more potent than morphine against motoneuronal responses to noxious pinch (see Table 1).

The actions of the κ -agonist U-50,488 (0.5 to 16mg kg^{-1} i.v.) were tested on the mechanical nociceptive responses of 63 motoneurons in 40 rats and the example in Figure 1 illustrates that when tested at a cumulative dose of 4mg kg^{-1} , U-50,488 reduced the responses to noxious pinch to a similar degree as the subsequent administration of morphine over the same cumulative dose-range.

The κ -agonist tifuladom was tested in 24 rats at 0.1 to 1.6mg kg^{-1} i.v. on the nociceptive responses of 31 motoneurons to noxious pinch and showed similar actions to the other opioids. Tifuladom was

of intermediate potency between U-50,488 and fentanyl (Table 1 and Figure 4).

In these tests on mechanical nociceptive reflexes the molar potency ratios of fentanyl:morphine:U-50,488:tifuladom, based on the ED_{50} estimates, were thus, 520:1.0:0.56:7.7. The larger pool of data when all drug tests are included provide similar relative potency values (Table 1).

Noxious heat

All four opioid agonists showed a similar potency on reflexes to noxious heat as on those to noxious pinch (see also Parsons & Headley, 1989a). Fentanyl (51 cells in 39 rats) was again almost three orders of magnitude more potent than morphine (10 cells in 10 rats); this is illustrated for an individual motoneuron in Figure 1. The potencies of U-50,488 (46 cells in 32 rats) and morphine were similar when tested on thermal nociceptive responses, as is again shown in Figure 1. The relative potencies of morphine, U-50,488 and fentanyl were consistent on all 9 neurones tested with all 3 agonists. The intermediate potency of tifuladom (16 cells in 13 rats) on both thermal and mechanical nociceptive reflexes is shown for one cell in the top trace of Figure 4.

The pooled data presented in Table 1 illustrate that, when tested on thermal nociceptive reflexes, the molar potency ratios of fentanyl:morphine:U-50,488:tifuladom (620:1.0:0.74:5.7) were very similar to those found for mechanical nociceptive reflexes.

Comparison of ED_{50} potency and neuronal firing rate

A notable feature in electrophysiological studies is the variability in the potency of agonists between cells. In view of the influence of firing rate on drug efficacy (Parsons & Headley, 1989a), the inter-cell variability in ED_{50} values might depend on variations in firing rates during sensory responses. However, linear regression analysis of the dependence of ED_{50} values on control firing rates showed only a weak correlation with U-50,488 on pinch (slope = $0.0904 \text{mg kg}^{-1} \text{Hz}^{-1}$, $r = 0.455$, HO $P = 0.011$; Spearman rank coeff. = 0.423, HO $P = 0.088$; Kendall rank coeff. = 0.316, HO $P = 0.008$, $n = 31$). For U-50,488 on heat ($n = 28$) and fentanyl on both heat ($n = 40$) and pinch ($n = 40$) there was no correlation between ED_{50} and control firing rate (coeffs. < 0.15 and HO $P > 0.3$ for all three tests).

Actions of the opioid antagonist naloxone

Effects on nociceptive reflexes in opioid-naive animals Before using naloxone to antagonize the actions of any exogenously administered opioid

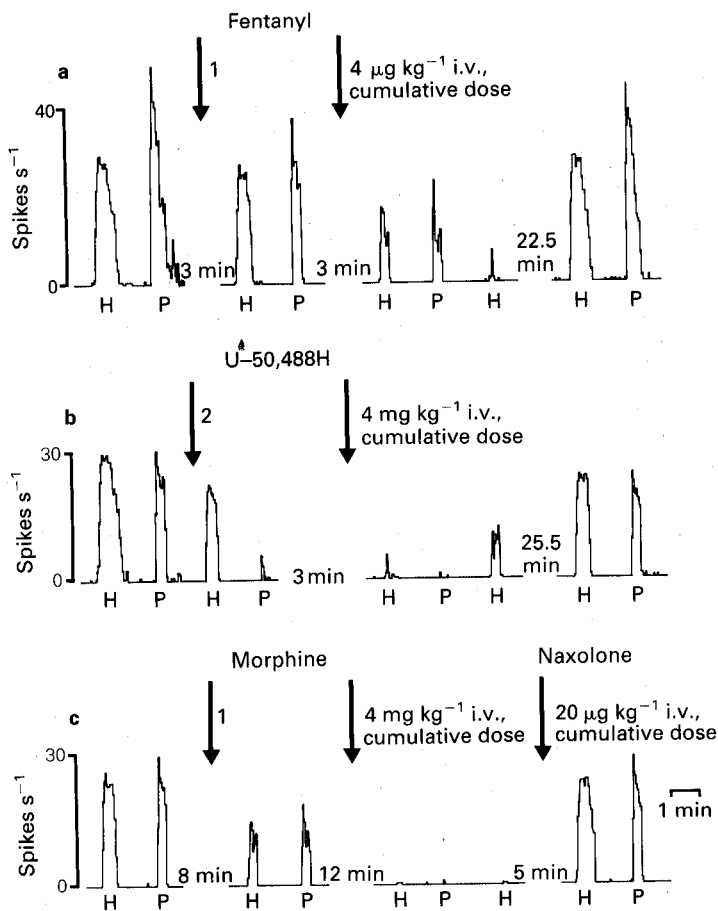


Figure 1 Comparison on a single motoneurone of the intravenous potencies of the μ -agonists fentanyl and morphine and the κ -agonist U-50,488. Responses were elicited by alternating noxious heat (H) of the ipsilateral plantar foot to 50°C for 30 s and noxious pinch (P) of toe 5 for 15 s. Trace (a) shows that fentanyl reduced nociceptive reflexes after divided doses of 1 + 1 + 2 $\mu\text{g kg}^{-1}$ i.v.; second 1 $\mu\text{g kg}^{-1}$ dose not shown. Trace (b) shows similar actions on the same cell of U-50,488 with a dose regime of 2 + 2 mg kg^{-1} i.v. Trace (c) shows the effects of morphine administered in divided doses of 0.5 + 0.5 + 1 + 2 mg kg^{-1} i.v.; first and third doses not shown. The depressant actions of morphine were rapidly reversed by the subsequent administration of naloxone 20 $\mu\text{g kg}^{-1}$ i.v. Recording in VR L5 of an α -chloralose anaesthetized, spinalized rat.

ligand it was important to test whether it had any actions in opioid-naïve rats, as would be expected if there were any tonically released opioid peptides in these preparations. Naloxone was therefore tested on the responses to noxious pinch of 14 motoneurons in 14 opioid-naïve rats and on the responses to noxious heat with 10 of these cells. Neither response was affected to any discernible degree by doses of naloxone of 100 $\mu\text{g kg}^{-1}$ i.v. or higher. This is illustrated for a single motoneurone in Figure 2; naloxone was administered i.v. in divided doses to a cumulative total of 400 $\mu\text{g kg}^{-1}$ and had no effect on either the thermal or the mechanical nociceptive

responses. This lack of effect was consistent; in pooled data from tests at 100 $\mu\text{g kg}^{-1}$ i.v., responses to heat were 115% (± 7 , $n = 10$) and those to pinch were 97% (± 4 , $n = 14$) of control values. Naloxone was tested at ten times this dose (1 mg kg^{-1}) in 6 cells but still had no discernible effect; responses to heat were 111% (± 18 , $n = 3$) and to pinch were 90% (± 17 , $n = 6$) of control.

Antagonism of μ - and κ -opioids The above control studies indicate that any reversal of exogenous opioid depressions by low doses of naloxone in our preparations should be mediated by direct antagonism of the exogenous drug rather than by other

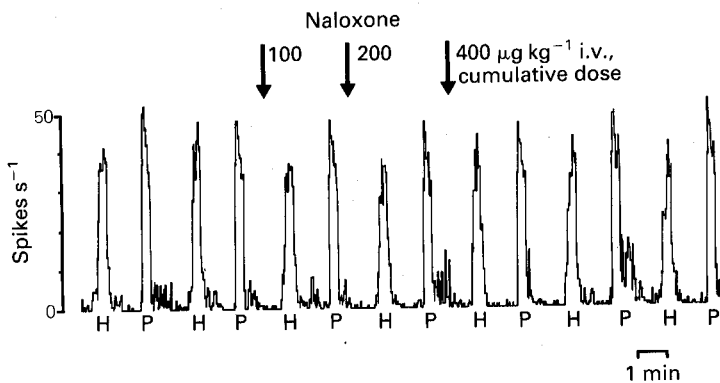


Figure 2 Lack of effect of naloxone on motoneuronal nociceptive responses in an opioid-naive rat. The multi-receptive motoneurone was recorded in VRL5 and was activated by noxious heat (H) of the ipsilateral plantar foot to 48°C for 30s and pinch (P) of toe 5 for 15s alternating in a regular 3 min cycle. α -Chloralose anaesthetized, spinalized rat.

mechanisms. Naloxone was therefore tested for antagonism of the actions of μ - and κ -agonists. The results are summarised in Table 2.

Due to inter-cell variability, the cumulative doses of fentanyl required to reduce responses to noxious pinch (to a mean of 23% (± 13) of control; $n = 6$) varied from 4 to 16 $\mu\text{g kg}^{-1}$ i.v. The actions of this μ -agonist were consistently and fully reversed (by 105% ± 5) by naloxone 20–200 $\mu\text{g kg}^{-1}$ (see Table 2).

A dose of naloxone generally considered low (100 $\mu\text{g kg}^{-1}$ i.v.) was tested for antagonism of the κ -opioid U-50,488 (2 to 16 mg kg^{-1} i.v.) on 10 motoneurons responding to alternating noxious heat and

pinch, and on 3 responding to alternating noxious and non-noxious stimuli. Figure 3 illustrates the reversal by naloxone 100 $\mu\text{g kg}^{-1}$ i.v. of the depression of nociceptive reflexes by U-50,488 2 mg kg^{-1} i.v. The antagonism of U-50,488 by this dose of naloxone was consistent for both thermal and mechanical nociceptive responses of all the cells tested (see pooled data in Table 2.)

Naloxone 100 $\mu\text{g kg}^{-1}$ i.v. was also tested for antagonism of tifluadom on 8 motoneurons. As with U-50,488, this dose was sufficient to antagonize substantially the actions of the κ -agonist. Figure 4 illustrates for a single neurone the antagonism of

Table 2 Antagonism by various doses of naloxone of the spinal actions of μ - and κ -opioids on nociceptive reflex responses of single motoneurons

Agonist	Naloxone dose	Pinch			Heat		
		Pre-naloxone %Con	Reversal %Rev	n	Pre-naloxone %Con	Reversal %Rev	n
Fentanyl	20–200	23 \pm 13	105 \pm 5	6	8 \pm 6	127 \pm 12	4
Morphine	100	6 \pm 6	103 \pm 15	5	0 \pm 0	117 \pm 27	3
	50	20 \pm 10	88 \pm 11	3	2 \pm 7	96 \pm 27	3
	20	15 \pm 9	74 \pm 8	4	3 \pm 2	81 \pm 17	5
	10	15 \pm 6	58 \pm 14	5	16 \pm 9	76 \pm 19	9
	1				13 \pm 3	57 \pm 24	5
U-50,488	100	14 \pm 5	79 \pm 14	10	14 \pm 4	89 \pm 13	13
	50				17 \pm 9	90 \pm 9	5
	10				7 \pm 3	60 \pm 11	8
	1				10 \pm 4	6 \pm 4	5
Tifluadom	100	12 \pm 6	81 \pm 10	8	5 \pm 3	71 \pm 5	5

See 'Methods' for protocol. The range of cumulative i.v. doses of the μ - and κ -agonists giving the pre-naloxone reductions of reflex responses (% Con) were as follows: fentanyl 4–16 $\mu\text{g kg}^{-1}$; morphine 0.5–8 mg kg^{-1} ; U-50,488 0.5–16 mg kg^{-1} ; tifluadom 0.2–1.6 mg kg^{-1} . The effects of naloxone (1–200 $\mu\text{g kg}^{-1}$ i.v., as indicated) were quantified as the percentage reversal of the agonist-induced depression (% Rev), such that 100% indicates complete reversal of the effect of the agonist. Means (% Con, % Rev), standard errors of these means and numbers of cells tested (n) are indicated.

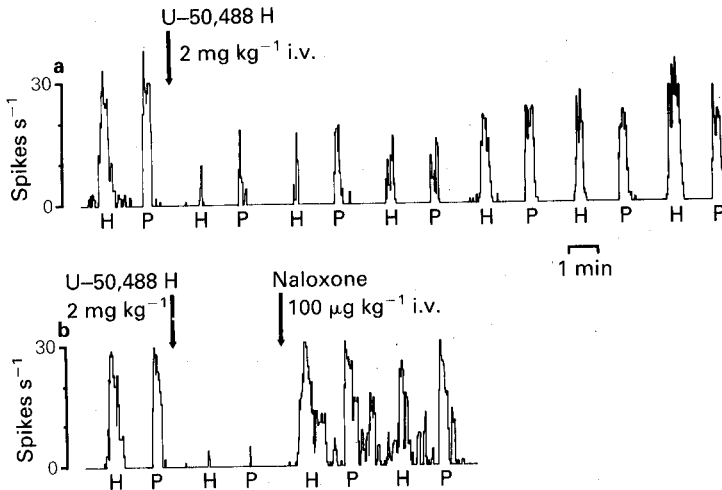


Figure 3 Naloxone antagonism of the depressant actions of U-50,488 on motoneuronal thermal and mechanical nociceptive responses. The motoneurone was recorded in VRL5 and was activated by noxious heat (H) of the ipsilateral plantar foot to 48.8°C for 30 s and pinch (P) of toe 5 for 15 s alternating in a regular 3 min cycle. Trace (a) shows the depressant actions of U-50,488 2 mg kg⁻¹ i.v. on nociceptive responses and the subsequent time course for recovery of responses from this effect. Trace (b) shows data recorded from the same cell later in the experiment. U-50,488 2 mg kg⁻¹ i.v. reduced responses to the same degree as previously and this effect was rapidly reversed by the subsequent administration of naloxone 100 µg kg⁻¹ i.v. α -Chloralose anaesthetized, spinalized rat.

tifluadom by naloxone 100 µg kg⁻¹; full recovery from this antagonism was not seen with subsequent tifluadom tests until 153 min post naloxone (see 'Methods'). This implies that a lower dose of naloxone might have been sufficient to antagonize the effects of tifluadom. The mean depressions by tifluadom and reversals by naloxone are presented in Table 2.

The effectiveness of a dose of 100 µg kg⁻¹ i.v. naloxone in antagonizing κ - as well as μ -effects prompted us to test even lower doses of i.v. naloxone. Despite the poor μ/κ selectivity of naloxone, morphine and U-50,488 were differentially sensitive to antagonism by naloxone. This is illustrated in Figures 5 and 6. The top trace of Figure 6 shows the selective actions of U-50,488 1 mg kg⁻¹ i.v. in depressing responses to noxious heat to a greater degree than those to innocuous vibration, and the subsequent time course for recovery from this effect. (The selectivity of both μ - and κ -ligands between reflexes to noxious and non-noxious stimuli will be discussed in greater detail in the subsequent paper: Parsons & Headley, 1989b). The bottom trace shows data recorded from the same cell later in the experiment. The prior administration of naloxone 1 µg kg⁻¹ i.v. had no effect on the antinociceptive actions of U-50,488 but a subsequent dose of 9 µg kg⁻¹ i.v. (cumulative 10 µg kg⁻¹ i.v.) completely reversed the effects of the κ -ligand. Table 2 shows pooled dose-response data for naloxone antagonism

of the actions of μ - and κ -ligands. These data show both the dose-dependency of naloxone and the greater effectiveness of naloxone on μ compared with κ -ligands.

Discussion

The antinociceptive actions of μ - and κ -opioids in our experiments were evident at doses similar to, or slightly lower than, those found to be effective following systemic administration in spinally intact rats in behavioural tests of thermal, mechanical and visceral nociception. However, it should be noted that in the present study all drugs were administered intravenously and, as a result, at any given dose, higher concentrations may have reached the opioid receptors under study than if, as in most behavioural studies, they had been injected via an intraperitoneal or subcutaneous route.

The variation in the potency of each opioid agonist between cells was not strongly dependent on differences in control firing rates. This initially surprising result does not, however, contradict our previous data (Parsons & Headley, 1989a). Firstly, although there were differences in the firing rates attained during pre-drug control responses, this reflected variation in the peak firing rates obtainable from individual cells rather than variation in the stimulus intensities used, this being particularly so

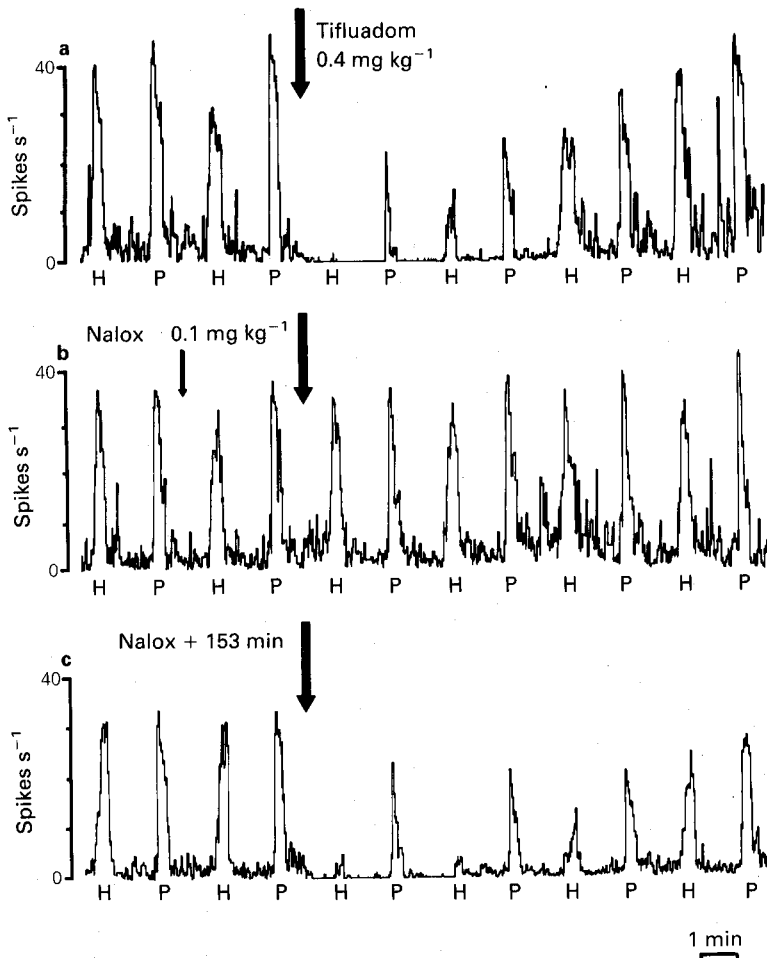


Figure 4 Naloxone (Nalox) antagonism of the depressant actions of tifluadom on motoneuronal thermal and mechanical nociceptive responses. The cell was activated by noxious heat (H) and pinch (P) stimuli alternating in a regular 3 min cycle. See text for details of protocol. Multireceptive motoneurone recorded in VRL5 of α -chloralose anaesthetized, spinalized rat.

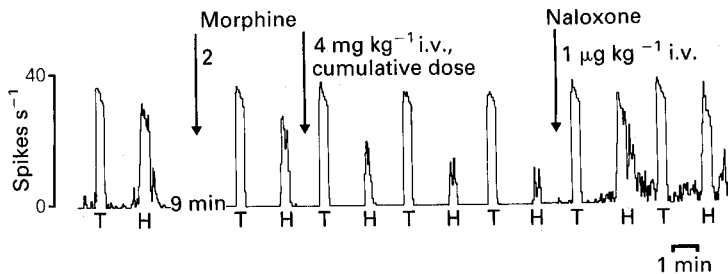


Figure 5 Antagonism of the antinociceptive actions of morphine by very low doses of naloxone. This single VRL4 motoneurone was activated by noxious heat (H) of the ipsilateral plantar foot to 48.5°C for 30 s and by non-noxious tap (T) of toe 5 at 8 Hz for 20 s, alternating in a regular 190 s cycle. Morphine 4 mg kg⁻¹ i.v. cumulative dose selectively depressed responses to noxious heat and this effect was rapidly reversed by the intravenous administration of naloxone at 1 µg kg⁻¹. α -Chloralose anaesthetized, spinalized rat.

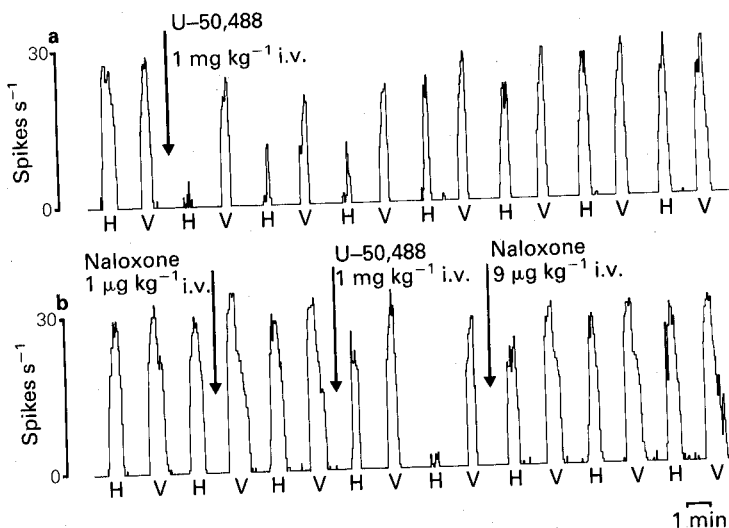


Figure 6 Dose-dependency of naloxone in antagonizing the selective antinociceptive actions of U-50,488 tested on motoneuronal responses to noxious and non-noxious stimuli. The cell was activated by noxious heat (H) of the ipsilateral plantar foot to 48°C for 30s and non-noxious vibration (V) of the hock at 8 Hz for 20s (stimulus similar to top stimulus in Figure 5 except that the probe was in constant contact with the skin). Stimulus cycle 190s. Trace (a) shows the selective antinociceptive effects of U-50,488 1 mg kg⁻¹ i.v. and the subsequent time course for recovery of nociceptive responses from this effect. Trace (b) shows data recorded from the same cell later in the experiment. U-50,488 was administered at the same dose of 1 mg kg⁻¹ i.v. but this time was preceded by a dose of naloxone 1 µg kg⁻¹ i.v. The effects of U-50,488 were unaltered by this dose of naloxone. However, the subsequent administration of naloxone 9 µg kg⁻¹ i.v. rapidly reversed the effects of U-50,488. α -Chloralose anaesthetized, spinalized rat.

with heat, which was usually held at noxious levels of between 46.5°C and 48.5°C to avoid frank tissue damage. Secondly, some of the data used for the above analysis were recorded from neurones responding to a single modality of noxious stimulus. In contrast, any difference in the firing rates evoked in single neurones by alternating noxious heat and noxious pinch should reflect genuine differences in the intensities of the noxious stimuli used; in this case we have clearly shown the importance of stimulus intensity in determining apparent drug 'selectivity' (Parsons & Headley, 1989a).

Relative potencies of μ - and κ -agonists

The potency ratios for fentanyl:morphine:U-50,488:tifluadom were similar to those reported in many behavioural studies (e.g. Irwin *et al.*, 1951; Janssen *et al.*, 1963; see Yaksh & Noueihed, 1985 and references in Parsons & Headley, 1989a). In contrast, following intra-cerebroventricular (i.c.v.), intrathecal or epidural administration, the relative potencies of these ligands have been found to be very different. Thus, when tested in both thermal and non-thermal models of nociception, the potency ratio for fentanyl:morphine is reduced to less than 5:1 (Herz &

Teschmacher, 1971; Durant & Yaksh, 1986; Yaksh *et al.*, 1986; see Yaksh & Noueihed, 1985). When tested on non-thermal nociceptive reflexes, the potency of intrathecal U-50,488 relative to morphine is markedly reduced to less than 0.03:1; the ratio is even wider for thermal nociceptive reflexes (Schmauss *et al.*, 1983b; Durant & Yaksh, 1986; Yaksh *et al.*, 1986; Schmauss, 1987; see Yaksh & Noueihed, 1985) and for some other synthetic κ -ligands e.g. buprenorphine (Bryant *et al.*, 1983). The same is the case when U-50,488 is applied topically in electrophysiological studies of C-fibre evoked activity of single dorsal horn cells (Dickenson & Sullivan, 1986; Knox & Dickenson, 1987).

The different potency ratios found between those studies using systemic as compared to those using topical administration seems to be related to the relative lipid partition coefficients of the compounds studied. Thus, following local spinal or i.c.v. administration, the more lipophilic drugs quickly gain access to the CNS but are also rapidly redistributed by the systemic circulation (see Cousins & Mather, 1984).

This highlights a disadvantage of using local administration of agonists or antagonists when assessing the relative roles of opioid receptors; namely that this technique does not allow any esti-

mate to be made of the relative drug access to the receptors under study. For most opioid drugs, diffusion from intrathecal or epidural sites into the cord has not been assessed. For the μ -agonists fentanyl and sufentanyl it is apparently very poor, for the effective dose intrathecally can exceed the effective systemic dose (Colpaert *et al.*, 1986; Durant & Yaksh, 1986; Yaksh *et al.*, 1986). That this is due to inadequate access to receptors rather than a lack of spinal μ -receptors is indicated by the generally accepted efficiency of the hydrophilic μ -agonist morphine given by the same route.

Despite these problems, and despite the absence of specific data for U-50,488 and tifuadom, the potencies of spinally administered κ -opioids relative to morphine are frequently taken as a measure of the effectiveness of spinal receptors for these agonists. Of particular relevance to this point are (1) reports of high lipid/water partition coefficients of drugs with very similar structures to U-50,488 (see Cheney *et al.*, 1985) and (2) the rapid time to peak effect and subsequent rapid redistribution to the systemic circulation of the selective κ -ligand PD117302 administered i.c.v. (Leighton *et al.*, 1988).

Differential receptor access by i.t. drugs would be heightened if spinal κ -receptors are located in deeper laminae than are μ -receptors, as is suggested by reports that microelectrophoretic administration of κ -agonists was more effective in deeper laminae than in superficial laminae of the spinal cord, whereas μ -agonists were effective in all laminae (Fleetwood-Walker *et al.*, 1988). A diffuse distribution of spinal κ -receptors is also supported by some (Członskowski *et al.*, 1983; Slater & Patel, 1983) but not by all (Morris & Herz, 1987) studies on the regional localisation of spinal κ -opioid binding sites. In contrast, spinal levels of the presumed endogenous κ -ligand dynorphin appear to be highest in the superficial dorsal horn (Vincent *et al.*, 1982).

Hayes *et al.* (1987) have recently reported that, following systemic administration in intact rats, μ - as well as κ -selective ligands are most effective in visceral models and are least effective in thermal models of nociception. Furthermore, this difference in sensitivity of visceral and thermal tests of nociception was greatest when used to test ligands with low intrinsic activity at the opioid receptors under study. Similar observations have been reported following intrathecal administration of partial agonists (Schmauss *et al.*, 1983a). This effect would be compounded in tissues with a low effective receptor reserve (see Miller *et al.*, 1986) and presumably also in tissues where there is poor receptor access by the drugs under study.

If this were the case for κ -receptors, then following intrathecal administration, the low concentrations of κ -opioids that might penetrate to deeper laminae

of the dorsal horn might activate κ -receptors sufficiently to reduce non-thermal but not the more vigorous thermal nociceptive reflexes. In contrast, the μ -opioids tested intrathecally may have a higher intrinsic activity at their receptors and/or there may be a higher μ -receptor reserve in more accessible superficial laminae of the spinal dorsal horn.

Potency of naloxone against μ - and κ -agonists

The fact that, in these experiments, naloxone administered to opioid-naive animals did not potentiate nociceptive reflexes to natural peripheral stimulation is in contrast to several reports that low doses of naloxone can potentiate electrically-evoked reflexes (see Duggan & North, 1984); this will be discussed in further detail elsewhere. This lack of effect does however allow us to interpret the reversal of exogenous opioid agonists by naloxone as an indication of competitive rather than physiological antagonism.

The results from our studies on the naloxone reversibility of the agonists tested therefore indicate that the antinociceptive actions of all the agonists were mediated via opioid receptors. The very low intravenous doses of naloxone required to reverse the effects of the κ -opioids U-50,488 and tifuadom illustrate the danger of using naloxone *in vivo* in attempts to distinguish the relative roles of opioid receptor subtypes. However, the fact that the depression of nociceptive responses by morphine was substantially antagonized by naloxone $1 \mu\text{g kg}^{-1}$ i.v. whereas $10 \mu\text{g kg}^{-1}$ i.v. or more was necessary to affect U-50,488 to a comparable degree, is compatible with the μ/κ selectivity of naloxone reported in many systems. This result therefore provides additional evidence that the actions of U-50,488 and tifuadom were mediated at κ -receptors. Further support for this conclusion is provided by the fact that μ - and κ -ligands had contrasting effects on cardiovascular and respiratory systems in our experiments (see Parsons & Headley, 1989a).

The potency ratios of morphine, fentanyl, U-50,488 and tifuadom in these electrophysiological tests on spinal nociceptive reflexes are very close to those reported in the literature following systemic administration in intact animals. This implies that spinal sites mediate a major contribution of the effects of systemic opioids on spinal reflexes, a finding not incompatible with a greater contribution of supraspinal than spinal sites to true analgesia. These potency ratios do, however, contrast sharply with those reported for epidural or intrathecal tests. This is likely to reflect differential spinal penetration following topical administration. In the previous paper (Parsons & Headley, 1989a) we have highlighted a further problem associated with many behavioural tests of nociception, namely that any differences of

stimulus intensities will result in apparent but false modality selectivity by the test drugs. Neither problem applies to our electrophysiological experiments and, despite the undoubted caveat of interpreting recordings obtained from acutely prepared animals, these electrophysiological results do have considerable credence. The generally held view, based primarily on behavioural data, that spinal μ - and κ -opioids have differential effects on reflexes to

thermal versus non-thermal stimuli, therefore needs radical reassessment.

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