The effects of sham and full spinalization on the systemic potency of μ - and κ -opioids on spinal nociceptive reflexes in rats

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1 Flexor withdrawal reflexes to noxious mechanical pinch stimuli were recorded as single motor unit activity in α -chloralose anaesthetized rats, by means of tungsten bipolar electrodes inserted percutaneously into hindlimb flexor muscles. The relative spinal and supraspinal contributions to μ - and κ -opioid agonists in inhibiting these spinal reflexes, together with possible potency changes elicited by surgical trauma, were examined by comparing their relative potencies in spinally unoperated, sham spinalized and spinalized rats.

2 The noxious stimuli, which were of comparable intensity in the three groups, elicited similar mean firing rates of the motor units in all groups. This indicates that the excitability levels in the reflex pathway were not greatly affected by either sham or actual spinalization.

3 The μ -agonists morphine and fentanyl, and the κ -agonist U-50,488H, inhibited the reflexes in a dose-dependent manner, when administered intravenously in a log₂ cumulative dose regime.

4 The surgery of sham spinalization had little effect on the potency of morphine and fentanyl, whereas it doubled the potency of U-50,488.

5 Spinalization did not affect the potency of morphine. In contrast it decreased the potency of fentanyl 2–4 fold and that of U-50,488 approximately 6 fold.

6 The effects of all agonists were antagonized by naloxone. Dose-dependence studies indicating that antagonism of U-50,488H required about 5 times the dose of naloxone that antagonized morphine.

7 The data suggest that surgical trauma to the spinal column and/or dura mater triggers supraspinal mechanisms that significantly enhance the potency of κ - but not μ -agonists.

8 It is concluded that most of the effects of systemic morphine on spinal reflexes are mediated, under all three conditions tested, by direct effects in the spinal cord. In contrast, the inhibition of reflexes by U-50,488H is mediated at both spinal and supraspinal levels, the latter being enhanced in the presence of surgical trauma. The differences between morphine and fentanyl remain unexplained.

Keywords: μ opioid; κ opioid; nociception; spinal reflexes; U-50,488H; fentanyl; morphine

Introduction

Morphine and other μ -opioids are effective analgesics and act at all levels of the neuraxis to reduce nociceptive responses, as indicated by numerous studies performed with a variety of local administration techniques, or with systemic administration with lesions of various nuclei or pathways (Yaksh, 1984; Yaksh & Noueihed, 1985; Millan *et al.*, 1989).

The relative degree to which the effects of systemic opioid agonists are produced directly at spinal rather than at supraspinal sites still remains unclear. Many reports have concluded that morphine increases the threshold in nociceptive tests primarily through supraspinal mechanisms. For example, systemic morphine was less effective in increasing the threshold in the tail flick test (which is generally accepted as a spinal reflex) in spinally transected as compared with intact rats (Advokat & Burton, 1987; Sinclair *et al.*, 1988). Lesions of the dorsolateral funiculus or of brainstem nuclei reduced the effectiveness of systemic morphine in this test (Murfin *et al.*, 1976; Basbaum *et al.*, 1977; Yaksh & Rudy, 1977; Hayes *et al.*, 1978; Barton *et al.*, 1980).

Other studies indicate that the spinal cord is relatively more important in mediating the effects of systemic μ -opioids (Yaksh & Noueihed, 1985; Millan *et al.*, 1989). Some experiments have even failed to find any supraspinal influence of systemic morphine on nociceptive responses of dorsal horn cells (Le Bars *et al.*, 1979; 1980; Chitour *et al.*, 1986). Many reports indicate that morphine has antinociceptive effects following local spinal administration (Yaksh & Noueihed, 1985; Sinclair *et al.*, 1988).

Contradictory results have also been obtained with κ -receptor agonists concerning their relative spinal and supra-

spinal actions (Millan, 1990). In paw-pressure, tail flick or hot-plate tests, intrathecal administration of selective κ -opioids produced markedly less antinociception than when injected either intravenously (i.v.) or intracerebroventricularly (Stevens & Yaksh, 1986; Leighton *et al.*, 1988; but see Wilcox & Hwang, 1984) except in some tests of visceral chemical nociception (Schmauss & Yaksh, 1984). On the other hand Millan (1989) clearly demonstrated that intrathecal κ -agonists reduced reflexes to noxious pinch and moderate heat stimuli, and intrathecal naloxone reduced the effects of systemic κ - (and μ -) agonists (Millan *et al.*, 1989). In electrophysiological tests local administration had variable effects between cells (Knox & Dickenson, 1987; Hope *et al.*, 1990). With systemic administration to spinalized rats, however, the selective κ -opioid U-50,488H caused consistent and naloxone-reversible reductions of the nociceptive responses of both motoneurons (Parsons & Headley, 1989; Parsons *et al.*, 1989) and dorsal horn neurones (Headley *et al.*, 1989; Dong *et al.*, 1991; see also Calthrop & Hill, 1983).

It was appropriate to clarify this situation. We have now made direct comparisons of the potencies of the μ -opioids, morphine and fentanyl, and the κ -opioid, U-50,488H, on withdrawal reflexes evoked in rats by noxious mechanical pinch stimuli. Drugs were administered intravenously so as to ensure the adequate access of the test opioids to all relevant receptors (see Parsons *et al.*, 1989). The potencies of the opioid agonists were compared between intact and spinalized rats so as to assess the contribution of direct spinal effects to the overall inhibition of these nociceptive reflexes. Because it has been shown that surgery to the spinal column can enhance the direct spinal potency of opioids and anaesthetics on nociceptive reflexes (Hartell *et al.*, 1990; Hartell & Headley, 1991) it was important to include a sham-operated group when comparing intact versus spinalized animals.

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A preliminary account of some of these data has appeared elsewhere (Herrero & Headley, 1990).

Methods

Animal preparation

Details of most of the methods have been described previously (Hartell & Headley, 1990). Male Wistar rats (300–380 g) were anaesthetized with halothane in O_2 and cannulae were implanted in the trachea, one carotid artery and one jugular vein. In one group (spinally unoperated) no further surgery was performed. In the other two experimental groups, a small laminectomy was performed, with infiltration of lignocaine with adrenaline, from thoracic 10 to 8 vertebrae, and the dura mater was opened. In one group (sham spinalization) no further surgery was performed and the incision was closed. In the other (spinalized group) the spinal cord was transected at thoracic segment 8 or 9 using cautery to minimise bleeding. When a larger vein was present at this level it was left intact, together with a wedge of dorsal column tissue for support, so as not to compromise the venous drainage from the cord.

Halothane was discontinued and α -chloralose was used as the maintenance anaesthetic (50 mg kg^{-1} , i.v. initially and 20 mg kg^{-1} , i.v. approximately every hour). Blood pressure was monitored continuously and experiments were terminated if systolic pressure fell below 100 mmHg. Core temperature was maintained at $37^\circ\text{C} \pm 0.5^\circ\text{C}$. All animals were left for at least 1 h after surgery before recording was started.

Stimulus presentation and recording systems

Nociceptive reflexes were elicited by use of one of two pneumatically-driven pincher devices. Most experiments were performed with a device to which a constant pneumatic pressure was applied, but from which no precise measure of applied force was obtained. In later experiments a different unit permitted a constant force to be pre-calibrated (2.5 N applied between 4 mm diameter, smooth surfaced jaws). Stimuli were applied to one of the two most lateral toes for 15 s every 3 min. Receptive fields were restricted to the foot area. The stimulus force used was always well above that required with these devices for eliciting withdrawal in conscious rats (N.A. Hartell, unpublished observations) but below that causing swelling of the foot with repeated application.

Bipolar recording electrodes were inserted percutaneously into hindlimb flexor muscles (Hartell & Headley, 1990). Extracellular recordings of single motoneurone reflex activity were made from single motor units (s.m.u.). Unit firing rate (see Figure 2), counts of spikes in stimulus related epochs and blood pressure were recorded on a chart recorder. The quantitative analysis presented below is based on counts of spikes evoked during the last 10 s of each pinch stimulus (see Parsons & Headley, 1989).

Drug administration and analysis of drug effects

Morphine hydrochloride (Richard Daniel), fentanyl citrate (Sublimaze, Janssen) and U-50,488H (*trans*-3,4-dichloro-N-methyl-N[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide, Upjohn) were dissolved or diluted in isotonic saline and administered i.v. in a constant volume of 0.3 ml. All agonists were injected in a logarithmic base₂ cumulative dose regime, starting with a dose of $1 \mu\text{g kg}^{-1}$ for fentanyl and 1 mg kg^{-1} for morphine and U-50,488H. Doses were increased at 6 min intervals until the evoked response was reduced to below 20% of control. Fentanyl and U-50,488 reach their peak effect within 3 min of i.v. injection, and morphine usually within 6 min, as was observed following the last dose in each cumulative regime (not illustrated; but see Parsons & Headley, 1989;

Parsons *et al.*, 1989). Most cells were tested with more than one opioid agonist. A subsequent drug was only administered after an interval of at least 30 min following recovery from the previous compound. The different agonists were therefore tested under closely comparable conditions.

Antagonism of morphine and U-50,488H was tested with cumulative i.v. doses of naloxone (Sigma) injected in a regime of 1, 10, 20, 50 and $100 \mu\text{g kg}^{-1}$ given at intervals of 6 min; administration was stopped when complete recovery of the response was obtained. Because U-50,488H is relatively short-lasting (see Figure 2) the degree of reversal by naloxone was assessed by comparing the recovery phase between two tests with U-50,488H, one without and one followed by naloxone (see Parsons *et al.*, 1989). Fentanyl was sensitive to naloxone but the dose-dependence of the antagonism was not studied. In some experiments fentanyl was used to assess whether naloxone had been adequately metabolized: it was injected at hourly intervals until its efficacy returned to pre-naloxone values.

Data are expressed as a percentage of control, where the control was taken as the mean of the three responses preceding the administration of the drug. Data were only accepted for analysis if the response recovered by at least 80% of the maximum effect of the drug, either spontaneously or following naloxone. Statistical significance was determined on pooled data by the non-parametric two-tailed Mann-Whitney U-test (Siegel & Castellan, 1988).

Results

General

Data were obtained from 84 s.m.u. in 63 rats. Spontaneous activity was low or absent in all experiments. The pre-drug neuronal firing rate during each response evoked by a noxious pinch stimulus (see Figure 2) was similar for the three groups: mean 20.6 ± 0.9 s.e.mean ($n = 48$) action potentials per second in spinally unoperated, 21.9 ± 1.7 ($n = 15$) in sham spinalized and 20 ± 2.2 ($n = 21$) in spinalized animals. All three opioid agonists reduced mechanical nociceptive reflexes in a dose-dependent and naloxone reversible manner.

Actions of the μ -agonists, fentanyl and morphine

Figure 1 shows pooled data of the effects of morphine in the three groups of animals. The mean ED_{50} was between 2 and 4 mg kg^{-1} and the dose necessary to depress the response below 20% of control was $4\text{--}8 \text{ mg kg}^{-1}$ in all three groups (intact: $n = 12$, sham: $n = 8$, spinalized: $n = 7$); there were no significant differences between groups. The effects of morphine were fully reversed by doses of $10\text{--}20 \mu\text{g kg}^{-1}$ of naloxone (Figure 1).

Figure 1b shows that fentanyl was similarly potent in spinally unoperated and sham spinalized rats (ED_{50} $2\text{--}4 \mu\text{g kg}^{-1}$) but that (unlike morphine) its potency was significantly reduced by spinalization (ED_{50} approximately $8 \mu\text{g kg}^{-1}$).

Actions of the κ -agonist, U-50,488H

Typical effects of U-50,488H are illustrated in Figure 2 which shows s.m.u. recordings from three different animals, one in each experimental group, recorded under otherwise identical conditions. Pooled data are shown in Figure 3. In spinally unoperated animals the potency of this κ -opioid was similar to that of morphine (mean ED_{50} for both was $2\text{--}4 \text{ mg kg}^{-1}$). In sham spinalized animals U-50,488H was significantly more potent (mean ED_{50} approximately 1 mg kg^{-1}) whereas spinalization caused a 6 fold decrease in its potency (mean ED_{50} increased to approximately 8 mg kg^{-1}).

The ability of naloxone to antagonize the actions of U-50,488H was tested on a subset of these units (Figure 3b). Doses of $50\text{--}100 \mu\text{g kg}^{-1}$ of naloxone i.v. were effective in all

Figure 1 The effects of sham surgery and of spinalization on the potency of intravenous morphine (1–8 mg kg⁻¹, a) and fentanyl (1–16 μg kg⁻¹, b) in reducing reflex responses of single motoneurons to pinch stimuli: the spinally unoperated group is shown by open columns, the sham spinalized group by cross hatched columns and the spinalized group by solid columns. Naloxone (10–20 μg kg⁻¹, i.v.) was administered to most units immediately after the morphine tests and dose-dependently reversed the inhibition produced by morphine in all 3 groups. Error bars represent standard errors of the mean and the numbers above bars indicate the number of units recorded. Data from spinally unoperated and spinalized groups were compared statistically with those obtained from sham-spinalized rats. Significance was calculated by the Mann-Whitney U-test (* $P < 0.05$; ** $P < 0.01$).

three groups. These doses were about 5 times higher than those needed to reverse the effects of morphine (see Figure 1).

Discussion

The similarity of the firing rate during pinch stimuli for the three experimental groups suggests that tonic descending inhibition affects these nociceptive reflexes rather little under our control conditions. On the other hand the time spent looking for a stable unit was considerably shorter in spinalized rats, so that it appeared that the number of motoneurons that could be excited by the pinch stimuli was greater in this group, as might be expected from the lack of descending inhibition. The possibility that the intensity of the pinch stimuli was consistently different between the experimental groups is unlikely given the care that was taken to ensure constancy, between experiments, of the force applied.

The effects elicited by both morphine and U-50,488H were mediated by opioid receptors, for they were antagonized by low doses of naloxone. The five fold higher doses required to antagonize U-50,488H are consistent with the different sensitivity to naloxone of μ - and κ -opioid agonists as well as with binding site data (see Parsons *et al.*, 1989). Moreover the κ -agonist generally increased, whereas the μ -opioids reduced, arterial blood pressure. All these factors indicate that the agents were acting at different opioid receptors.

Our results suggest that under these experimental conditions, supraspinal actions do not contribute to the net suppression of spinal reflexes by morphine: this follows from the similar potency seen in spinally unoperated, sham spinalized and spinalized groups. This conclusion is similar to that obtained with systemic administration of morphine in other electrophysiological experiments (Le Bars *et al.*, 1979; 1980; Soja & Sinclair, 1983; Chitour *et al.*, 1986). Other authors, using behavioural tests of nociception, have however reported significant reductions of the effects of systemic morphine after producing lesions aimed at descending pathways (see Introduction).

These findings imply that some combination of the anaesthesia and the preparatory surgery may explain the greater effectiveness of morphine seen in electrophysiological recordings. In the present study the potency of morphine was not affected by the sham spinalization, suggesting that surgery *per se* was not the significant factor in the above studies. Various authors have suggested that the presence of general anaesthetics can influence the potency of opioids (see Advokat & Burton, 1987; Sinclair *et al.*, 1988). Whilst this is likely for the supraspinal actions of morphine, it is not the case for the direct spinal effects of μ -opioids: using the same technique as in the current study, Hartell & Headley (1989) found that the presence of α -chloralose (as used here) did not affect the direct spinal potency with which μ -opioids depressed nociceptive reflexes.

The potency of the other μ -agonist tested, fentanyl, was surprisingly affected differently from morphine by spinalization: following spinal section, 2–4 times the dose was necessary to have the same effect as in sham-operated animals. This difference between morphine and fentanyl remains unexplained. It is hard to perceive how the much greater lipophilicity of fentanyl could differentially affect the responses between the three experimental groups following systemic administration. Whether the difference could relate to μ -receptor subtypes, the distribution of which is reported to be different in cord and supraspinal regions (see Paul *et al.*, 1989) remains to be assessed.

U-50,488H showed clear inter-group differences. Thus the surgery of sham-spinalization doubled the potency of this κ -opioid, whilst additional spinalization decreased the potency about 6 fold. As a result U-50,488H was only half as potent in spinalized as in unoperated spinally intact animals.

The simplest interpretation of the latter findings is that the surgery sensitizes some unidentified supraspinal centres to the actions of κ - (but not μ -) opioid agonists. It is possible that this is related to the selective increase of κ -receptors seen in the periaqueductal grey during arthritis induced by Freund's Complete Adjuvant (Millan *et al.*, 1987).

When the cord is transected this supraspinal effect is removed, leaving only the direct spinal actions of U-50,488H. This κ -agonist (unlike morphine) is evidently somewhat weaker at spinal compared with the supraspinal sites that control descending inhibitions (see also Takemori *et al.*, 1988; Millan *et al.*, 1989). The potency of U-50,488H in the present sample of spinalized rats (ED₅₀ 4–8 mg kg⁻¹) was similar to that in the larger sample analyzed previously (ED₅₀ 4.9 mg kg⁻¹, $n = 31$, Parsons *et al.*, 1989). These doses of U-50,488 are within the range observed to be effective in behavioural tests of nociception (see Parsons *et al.*, 1989; Millan, 1989). All these data indicate that κ -opioids are effective at the level of the spinal cord.

In summary, this study appears to be the first in which selective μ - and κ -opioids have been compared electrophysiologically for spinal vs. supraspinal actions; moreover it is one of very few studies in which sham spinalization was included. Neither surgical trauma nor spinalization affected the potency of morphine on the reflex tested, indicating that under these conditions morphine reduces spinal nociceptive reflexes by a direct action within the cord. The different effects of fentanyl, which was weaker in spinalized rats, suggests that morphine and fentanyl may activate different groups of μ -receptors with

Figure 2 Effects of the κ -opioid U-50,488H on reflex responses to noxious pinch stimuli. The figure shows traces of original records of the firing rate of single motor units in hindlimb flexor muscles in response to 15 s pinch stimuli (P) repeated every 3 min (the pen recorder was halted between stimuli). One example is shown for each of the experimental groups, spinally intact (a), sham spinalization (b) and spinalized (c). The traces illustrate the typical differences in the potency of this opioid between groups.

different spinal vs. supraspinal distributions. Supraspinally mediated inhibitions of spinal reflexes are activated by lower doses of the κ -agonist U-50,488 than those having direct spinal effects, but the latter still occur at behaviourally relevant doses. Trauma increased the potency of U-50,488, an effect which must be borne in mind in other studies involving surgical preparation.

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Figure 3 Effects of sham and actual spinalization on the potency of the κ -opioid agonist U-50,488 on spinal nociceptive reflexes. Pooled data presented as in Figure 1 for spinally unoperated (open columns), sham spinalized (solid columns) and spinalized groups of rats (hatched columns). Note that it was not necessary to test the higher doses in all groups. Pooled data from all tests are shown in (a); (b) shows the subset of units on which naloxone reversal of U-50,488 was assessed. The left panel indicates the effectiveness of U-50,488 on these units, and the right panel the reversal of these reductions by naloxone (10–100 $\mu\text{g kg}^{-1}$). Naloxone was similarly effective in the three groups despite the different doses of agonist administered. Data from spinalized and spinally unoperated animals were compared statistically with the data from sham-operated rats (Mann-Whitney U-test, * $P < 0.05$; ** $P < 0.01$).

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