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Spinal antinociceptive actions of μ- and κ-opioids: the importance of stimulus intensity in determining 'selectivity' between reflexes to different modalities of noxious stimulus

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1 In electrophysiological experiments in spinalized rats, μ- and κ-opioids were tested intravenously on the responses of single motoneurones to electronically controlled, alternating noxious heat and noxious pinch stimuli. The effects of μ- and κ-opioids were compared with those of the general anaesthetic α-chloralose and the dissociative anaesthetic/PCP ligand ketamine.

2 The κ-opioids U-50,488 (0.5–16 mg kg^{-1} i.v.) and tifluadom (0.05–1.6 mg kg^{-1} i.v.) had very similar actions to the μ-opioid fentanyl (0.5–16 μg kg^{-1} i.v.). Thus all three agonists reduced thermal and mechanical nociceptive reflexes in parallel and in a dose-dependent manner, but only so long as neuronal responses to the alternating stimuli elicited similar excitability levels in the neurone under study. Ketamine (0.5–16 mg kg^{-1} i.v.) had similar actions to the opioids whereas α-chloralose (20 mg kg^{-1} i.v.) had very little effect on neuronal responsiveness.

3 Apparently 'selective' depressions by both μ- and κ-opioids could be orchestrated by a deliberate mismatch of the intensities of alternating noxious heat and pinch stimuli; as measured by neuronal firing rate, the weaker of the responses to either type of stimulus was invariably reduced to a greater degree.

4 Similar 'selectivity' could be demonstrated for both μ- and κ-ligands when the weaker and stronger responses were of the same modality, being applied by the same pincher device but with alternating applied force.

5 It is concluded that the 'selective' spinal actions of κ-opioids seen in non-thermal over thermal behavioural models of nociception is likely to be related to the relative intensities, rather than the modalities, of the noxious stimuli used. The validity of the interpretation of results obtained in such behavioural studies is discussed.

Introduction

For several years there has been evidence that opioids which act preferentially on the κ- subclass of opioid receptors (Martin *et al.*, 1976) are less effective in suppressing responses to thermal than responses to non-thermal noxious stimuli. Most of this evidence comes from behavioural experiments in intact animals in which the reflex output from the spinal cord is monitored. This effect of κ-agonists was investigated more formally by Tyers (1980) and Upton *et al.* (1982) who reported that with κ- (but not μ-) preferring ligands, appreciably higher doses were required to suppress responses in the rat tail flick and hotplate tests than responses in inflamed paw and writhing tests. Since in these studies drugs were administered systemically, it was not clear to what extent the effects observed were mediated at supraspinal, rather than spinal, sites. Subsequently,

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however, there have been various studies in which the intrathecal route of drug administration has been used in chronically-implanted rats (Yaksh & Rudy, 1976) and several such studies have again indicated a markedly lesser effectiveness of κ- as compared with μ-agonists on thermally evoked reflexes (e.g. Schmauss *et al.*, 1983; Bryant *et al.*, 1983; reviewed by Yaksh & Noueihed, 1985; Millan, 1987). Other studies have, however, indicated that an apparent selectivity of opioid action may be related to the stimulus intensities used (Vonvoigtlander *et al.*, 1983; Hayes *et al.*, 1987; Millan, 1989).

However, such behavioural data are not without problems of interpretation. Thus between the various animal behavioural tests used, both the intensity and the temporal synchronization of the nociceptive inputs are inevitably different. Drug potency differences may thus reflect either differential drug effectiveness on different pathways activated by different inputs, or may alternatively reflect varying effectiveness on responses of different magnitudes and/or degrees of synchrony in a common pathway. It is not clear to what degree the μ/κ differences quoted above relate to these aspects. In this context it is relevant that in the rat, much of the sensory input arising from both thermal and non-thermal noxious stimuli is thought to be mediated by a common class of primary afferent, the polymodal nociceptor (e.g. Lynn & Carpenter, 1982). There is a difficulty in explaining how activation of different sub-classes of spinal opioid receptor could have highly 'selective' effects on different modalities of nociceptive information carried to the spinal cord largely by the same class of primary afferent.

It has therefore been important to perform experiments which allow more accurate control over some of the relevant parameters. Because of variations in drug effectiveness between cells, all data in this study were obtained from cells responding alternately to noxious heat and noxious pinch stimuli. We now report that in electrophysiological experiments on spinalized rats, systemically administered κ-opioids are equally effective against thermal and mechanical nociceptive spinal reflexes so long as the stimuli are controlled such as to elicit similar excitability levels in the neurone under study. Altering the relative intensities of these alternating stimuli can, however, result in apparent, but false, 'selectivity'. The findings are discussed in relation to the intensity of the peripheral stimuli used in behavioural tests. Some of the results have been presented previously in a preliminary form (Headley *et al.*, 1984; 1987c; Parsons *et al.*, 1986). Data on the relative potencies of the agonists, and on their selectivity between nociceptive and non-nociceptive reflexes, are presented in accompanying papers (Parsons & Headley, 1989; Parsons *et al.*, 1989).

Methods

Animal preparation

Some of the experimental details have been published elsewhere (Headley *et al.*, 1987b). Rats were anaesthetized throughout experiments with α-chloralose (100 mg kg^{-1}, i.p., initially), supplemented with halothane (1 to 2% in O_2) during surgery. Respiration was spontaneous; inspired air was enriched with O_2. Tracheal, carotid and jugular cannulae were inserted and then a dorsal laminectomy was performed from lumbar 6 to thoracic 10 vertebrae. The spinal cord was transected at the level of thoracic 10 vertebra. Blood pressure was monitored continuously and experiments were terminated if systolic blood pressure fell below 100 mmHg (13 kPa) for any sustained period (i.e. other than transient drug-induced effects). In later experiments end tidal CO_2 was also monitored with an ADC FM1 fast response CO_2 analyser.

Animals were immobilized in a recording frame and core temperature was maintained at 37°C by a combination of a lamp-warmed paraffin pool over exposed dorsal tissues and by a thermistor controlled blanket. Fluid therapy (Haemaccel: Hoechst; or isotonic saline) was given intravenously at about 80 ml kg^{-1} 24 h^{-1}. In later experiments, animals received 1 mg kg^{-1} i.v. of betamethasone (a short-lasting corticosteroid) prior to surgery; in some cases they were given a blood transfusion at the end of surgery.

Peripheral somatosensory stimuli

The natural peripheral stimuli used in these experiments were noxious pinch and noxious heat of the ipsilateral hind paw and/or toes; both were controlled electronically for constancy of intensity, duration and repetition rate throughout any drug test. Pinch stimuli (15–20 s) were, in the majority of experiments, delivered by a pair of pneumatically driven Allis tissue forceps (Brown *et al.*, 1984); no attempt was made to monitor the force applied, but similar stimuli were judged to be painful when tested on the experimenters. In later experiments a different pincher device, which delivered stimuli over a 3 mm^2 area, incorporated a strain gauge which indicated that a force of 0.3–1.5 newtons (depending on site) was required to elicit sustained nociceptive responses. Noxious thermal stimuli (ramped from 37°C to 45–50°C over 10–15 s and held for a further 15–20 s) were delivered to glabrous skin of the ipsilateral plantar foot and/or toes via a copper contact thermode (1.5 cm^2) with feedback control. With all cells (except those tested as in Figure 6) noxious heat

and noxious pinch stimuli were alternated in a regular cycle of 3–4 min and their intensities were adjusted with considerable care so that they evoked matched responses in terms of firing rates of the motoneurone under study. The alternated stimuli were delivered in close spatial proximity in the neuronal receptive field.

Neuronal recording

Extracellular recordings of nociceptive responses were made with silver wire hook electrodes from single motoneurone axons in fine filaments of ventral roots L4–L6. Spike conformation was monitored on a digital storage oscilloscope so as to help ensure that the activity of only a single spike was analysed throughout any comparative series of drug tests. The receptive field properties of neurones were carefully mapped and noted.

A pen recorder displayed continuous traces of (1) neuronal firing rate in 2 second epochs, (2) integrated spike counts for stimulus-related epochs, (3) the mechanical force applied by the pincher and (4) the temperature of the contact thermode. In later experiments, integrated spike counts elicited by pinch stimuli were divided into early and late components as the initial adaptive phase of a response to a noxious mechanical stimulus presumably contains a greater non-nociceptive component than the later sustained phase. This late response was then used for further quantitative analysis of mechanical nociceptive reflexes. The integrated spike counts were sent to a microcomputer for online computations of drug effects (see Headley *et al.*, 1985); these effects were expressed as a percentage of control, where the control was the mean of the three response cycles immediately preceding the drug administration. It is these percentage figures based on spike counts (rather than on firing rates) that have been used in the analysis presented below.

Drugs, drug administration and analysis

Drugs were selected on the basis of three criteria: (1) specificity for the receptor subtype under study, (2) suitability for systemic administration and (3) half lives that are short enough to allow multiple drug testing on any one cell. Fentanyl was chosen as the μ-agonist; for its receptor selectivity in binding tests see Magnan *et al.* (1982). U-50,488H (*trans*-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide; Upjohn) and tifluadom (1-methyl-2(3-thienylcarbonyl)-aminomethyl-5-(2-fluorophenyl)-H-2,3-dihydro-1,4-benzodiazepine; Sandoz) were chosen as κ-agonists; their receptor selectivity has been demonstrated in a variety of different models (e.g. Römer *et al.*, 1982a,b; Leander, 1983; Vonvoigtlander *et al.*, 1983; Burkard, 1984; Hayes *et al.*, 1985; 1986). Drug doses refer to fentanyl and ketamine bases, tifluadom hydrochloride and U-50,488 methane sulphonate. Fentanyl (Sublimaze, Janssen) and ketamine (Ketalar; Parke-Davis) were obtained from commercial sources.

Drugs were administered intravenously in a logarithmic (base 2 for agonists; normally base 10 for naloxone) cumulative dose regime, starting with a dose which was found to be near threshold on most cells, and reaching a maximum of $16\,\mu g\,kg^{-1}$ for fentanyl, $1.6\,mg\,kg^{-1}$ for tifluadom and $16\,mg\,kg^{-1}$ for U-50,488H and ketamine. The interval between doses was 3 to 6 min depending on the time necessary for particular drugs to produce their peak effects (assessed from previous single dose tests). The effects of every drug test were quantified with three measurements on the spike counts (1) the peak effect on neuronal response amplitude, expressed as a percentage of control values, (2) the maximal recovery from this effect and (3) the time taken for 50% recovery of responses. Drug effects were only accepted for analysis if recovery of responses by at least half of the maximum drug effect occurred over a time course compatible with the known kinetics of the drug under study. In the interests of speed of recovery the cumulative dose regime was halted once a drug had reduced the evoked responses to below 25% of control. This strategy permitted both μ- and κ-agonists to be tested under closely comparable conditions on most of the cells examined.

The fact that the complete dose-response regime was not followed on all cells has the disadvantage that traditional mean dose-response analysis cannot be performed; the apparent dose-response curves are shallower than they would be because the higher doses were tested only on the more resistant cells. Nonetheless, mean percentage reductions at particular drug doses are presented graphically in Figures 2 and 4 because they do provide a valid comparison of mean drug effectiveness against responses to the two stimulus modalities. In addition, ED_{50} values were calculated for those individual cells on which it was possible to perform a linear regression analysis on the depression of responses by at least 3 different doses tested over the 'linear' region of the dose-response curve (from 25–75% of control values). On 10 cells, these estimated ED_{50}s were compared with those obtained from a curve fitting programme (written by Dr R. Barlow) and were found to agree closely. The individual ED_{50} values were then averaged to give a mean ED_{50} for each drug.

Any differences between μ- and κ-opioids on thermal and mechanical nociceptive reflexes were tested for statistical significance on pooled data with

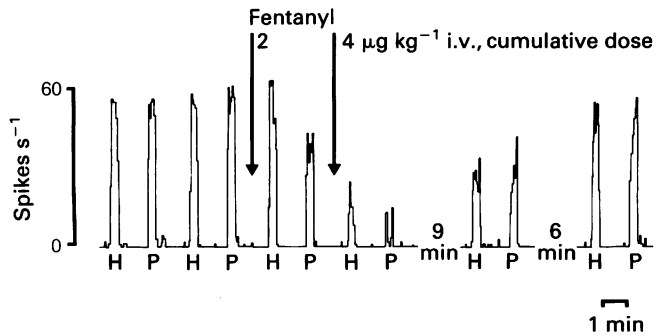

Figure 1 Effect of fentanyl on the responses of a rat motoneurone to alternating thermal and mechanical noxious stimuli. The neurone was activated by noxious heat (H; from a baseline of 37°C to 49.8°C for 30 s) of the ipsilateral hind paw and pinch (P; for 15 s) of toe 5 ('little' toe), alternating in a regular 3 min cycle. Fentanyl was administered in two divided doses of 2 $\mu g kg^{-1}$ i.v. Full recovery from the effects of fentanyl was evident after 21 min. Multireceptive motoneurone recorded in lumbar 5 ventral root (VR L5) of an α-chloralose anaesthetized, spinalized rat.

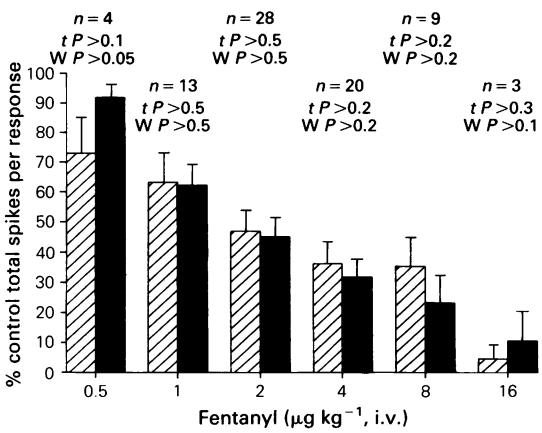

Figure 2 Pooled, paired data from tests with i.v. fentanyl on the responses of 31 motoneurones to alternating noxious heat (solid columns) and noxious pinch (hatched columns) stimuli. The mean effect of fentanyl on the total number of spikes counted in each stimulus-related epoch was determined as a percentage of control values (ordinate scale) and was plotted against dose in $\mu g kg^{-1}$, i.v. (abscissa scale). Error bars represent standard errors of the mean (s.e.mean). The number of cells tested (n) at each dose are given above each column of the histogram. Any difference in the pooled paired data at each dose was analysed for statistical significance using both parametric (paired Student's t) and non-parametric (Wilcoxon) tests. The two tests gave very similar results; two tail probabilities of the null hypothesis (HO) were greater than 0.05 for both tests at all doses. These values are presented above each column of the histogram (W P = Wilcoxon; t P = Student's t).

both parameteric (two tail paired Student's t test) and non-parametric tests (two tail Wilcoxon matched-pairs signed-ranks test; see Siegel & Castellan, 1988).

Results

The results presented below were obtained in experiments on 68 cells responding to alternating heat and pinch stimuli, recorded in 46 Wistar rats (210–600 g) of either sex. The i.v. administration of opioid agonists is presumed to have had effects mediated by a direct action within the spinal cord (see Discussion).

The μ-agonist fentanyl

As predicted, fentanyl reduced thermal and mechanical nociceptive reflexes in parallel and in a dose-dependent manner. This is illustrated in Figure 1 for a single motoneurone responding to alternating noxious heat and pinch stimuli. Figure 2 illustrates pooled data for the 31 cells tested in this way and shows that this non-selectivity of fentanyl was consistent over the whole dose-range tested and that any small differences between means of heat and pinch responses never reached statistical significance (both Wilcoxon and paired Student's t test; null hypothesis (HO) $P > 0.05$ for all doses).

Fentanyl was tested over a sufficiently wide dose range to calculate an ED_{50} for both the thermal and mechanical nociceptive responses of 8 individual cells. The mean ED_{50} on heat responses was

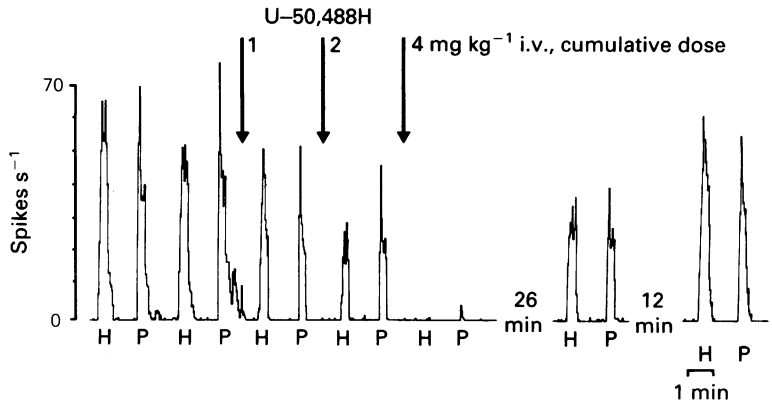

Figure 3 Effect of U-50,488 on the responses of a rat motoneurone to alternating thermal and mechanical noxious stimuli. The neurone was activated by noxious heat (H; to 48°C for 30 s) of the plantar surface of the ipsilateral hind paw and pinch (P; for 15 s) of toe 5, alternating in a regular 3 min cycle. U-50,488 was administered in divided doses ($1 + 1 + 2\,mg\,kg^{-1}$). Responses had recovered by approximately 50% some 30 min after the last dose of U-50,488 and the maximum recovery was evident 15 min later still. Multireceptive motoneurone recorded in VR L5 of an α-chloralose anaesthetized, spinalized rat.

$3.5\,\mu g\,kg^{-1}$ (± 0.8, s.e.mean) which was not significantly different to the mean ED_{50} on mechanical responses, $3.6\,\mu g\,kg^{-1}$ (± 1.0) (Wilcoxon test HO, $P = 0.76$). It is notable that the mean ED_{50} values calculated from these data on a subset of cells were not very different from the apparent mean ED_{50} values that could be predicted from all the pooled data presented in Figure 2.

Intravenous fentanyl can produce large transient reductions of blood pressure and elevations of end tidal CO_2, especially if given rapidly. Although this did not have any effect on the recorded signal it was important to test the possibility that the effects of fentanyl on nociceptive responses were secondary to its hypotensive effects (see Duggan *et al.*, 1978). Amyl nitrite vapour was therefore introduced into the oxygen line in doses sufficient to produce similar reductions in blood pressure as those seen with the largest doses of fentanyl. On 12 cells amyl nitrite transiently reduced the systolic blood pressure by 50 to 80 mmHg (6.5–10.5 kPa). However, thermal and mechanical nociceptive responses were minimally affected, being altered to means of 110% (± 4) and 115% (± 5) of control respectively.

The κ-agonists U-50,488H and tifluadom

Both of the κ-opioids reduced responses to thermal noxious stimuli, on most cells at doses at the lower end of those used in behavioural tests of nociception (e.g. Römer *et al.*, 1982a,b; Vonvoigtlander *et al.*, 1983; Hayes *et al.*, 1986; 1987; Leighton *et al.*, 1988).

A more specific question is the relative effectiveness of κ-ligands on matched reflexes to alternating heat and pinch stimuli. This aspect was studied on a total of 27 cells in 19 rats with U-50,488 H (0.5–$16\,mg\,kg^{-1}$ i.v.) and on 16 cells in 12 rats with tifluadom (0.05–$1.6\,mg\,kg^{-1}$ i.v.). Figure 3 shows an example of a cell which was activated alternately by noxious heat and noxious pinch stimuli, the intensity of which had been adjusted during the pre-drug control period so as to evoke responses with well-matched firing rates. It is readily apparent that, under these conditions, this selective κ-agonist was equally effective against responses to both forms of noxious stimulus.

The pooled data for all 27 cells is shown in Figure 4. Whilst the mean effects of the higher doses of U-50,488 are probably an underestimate of this κ-agonist's true potency (see Methods) it is nonetheless clear that, at all doses, U-50,488 was similarly effective against thermal and mechanical nociceptive responses (Wilcoxon and Student's t tests, HO, $P > 0.05$ at all doses).

The mean ED_{50} on heat responses of 10 cells was $4.5\,mg\,kg^{-1}$ (± 1.2) which was not significantly different from the mean ED_{50} on mechanical responses, $5.8\,mg\,kg^{-1}$ (± 1.4) (Wilcoxon test, HO, $P = 0.18$). As with the μ-agonist fentanyl, the calculated mean ED_{50} values for U-50,488 were not dissimilar to the apparent mean ED_{50} values estimated from the pooled data for all cells, as shown in Figure 4.

U-50,488 produced varying degrees of hypertension associated with an increase in pulse pressure and a decrease in end tidal CO_2. In several rats,

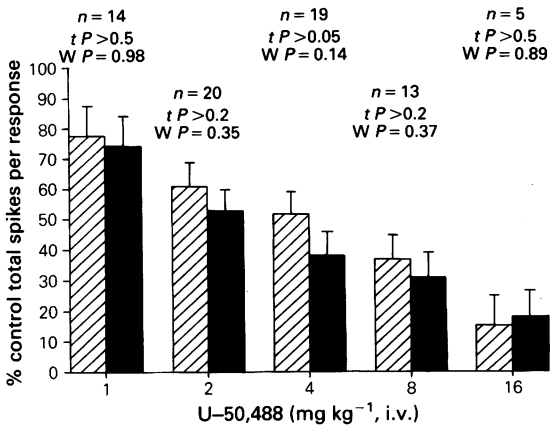

Figure 4 Pooled, paired data from tests with i.v. U-50,488 on responses of 27 motoneurones to alternating noxious heat (solid columns) and pinch (hatched columns) stimuli. Data presented as in Figure 2.

large 1.5–2 ml i.v. boluses of plasma expander were injected rapidly so as to produce a similar transient elevation of blood pressure as was produced by U-50,488. In no case did the plasma expander have a substantial effect on reflex responses.

The other κ-ligand tested was tifluadom and it produced similar parallel reductions as did U-50,488 (see Parsons *et al.*, 1989, Figure 4). When tested at 0.4 mg kg^{-1} on 9 cells, thermal and mechanical nociceptive responses were reduced similarly to 32% (±11) and 42% (±13) of control respectively. ED_{50} values could only be calculated for both types of nociceptive response on 4 of these cells; the mean ED_{50} values were 0.43 mg kg^{-1} (±0.14) and 0.24 mg kg^{-1} (±0.06) for noxious heat and noxious pinch stimuli respectively (Wilcoxon test, HO, $P = 0.28$). Tifluadom was dissolved in 5–10% ethanol for all these tests. The intravenous administration of equivalent and/or higher doses of this vehicle had no effect on nociceptive responses.

Controls with α-chloralose and ketamine

α-Chloralose was the anaesthetic of choice in these experiments on rats as it has been reported to have relatively weak effects on polysynaptic reflexes (e.g. Shimamura *et al.*, 1968). When tested at the dose which we normally gave approximately every hour to supplement anaesthesia during the recording period, i.e. 20 mg kg^{-1} i.v., α-chloralose had relatively little effect on nociceptive reflexes compared with other anaesthetics (Headley *et al.*, 1987a). Any depressant actions were often slow in onset with the peak effect occurring up to 10 min following intravenous administration (see Collins *et al.*, 1983). When tested quantitatively on 41 of the 68 rat motoneurones of this study, α-chloralose (20 mg kg^{-1}) reduced responses to noxious heat and pinch to means of 75% (±3) and 80% (±3) of control respectively. These results imply that any fluctuations of anaesthetic depth during the tests reported above are unlikely to have compromised the tests with the opioid agonists. Recent data indicate that α-chloralose does reduce spinal nociceptive reflexes in decerebrate rats (Hartell & Headley, 1989) but, importantly, fentanyl is equally effective in decerebrate and α-chloralose anaesthetized animals (unpublished).

As previously reported (Headley *et al.*, 1987b), sub-anaesthetic doses of ketamine reduced thermal and mechanical nociceptive reflexes in parallel. Thus, when compared on 16 cells tested with at least one opioid agonist, the ED_{50} values for ketamine versus noxious heat and noxious pinch were 3.4 mg kg^{-1} (±0.8) and 3.3 mg kg^{-1} (±0.7) respectively (Wilcoxon, HO, $P = 0.78$).

Effects of altered stimulus intensity

It is clear from these experiments that μ- and κ-opioids had very similar effects in depressing motoneuronal reflexes to different modalities of noxious stimulus. Under our conditions we were able to control closely the excitability levels of the motoenurone under study and, as has been stressed above, we were careful to match the firing rates during the thermal and mechanical nociceptive responses. If, however, the firing rates were not accurately matched, then apparent drug selectivity was observed. This is illustrated in Figure 5. The upper trace illustrates a test in which the stimulus intensities were adjusted correctly so as to match the neuronal firing rates evoked by the alternating heat and pinch stimuli; in this case a cumulative dose regime of U-50,488 reduced responses to both stimuli absolutely in parallel. However, a subsequent test was performed on the same neurone after the position of the thermal stimulator was adjusted such that the noxious thermal stimulus evoked a higher firing rate than the noxious mechanical stimulus (lower trace). The κ-agonist U-50,488, administered in an identical dose regime, now 'selectively' depressed the responses to noxious pinch (to 2% of control) whilst having little effect on the responses to noxious heat (79% of control).

An apparently selective depression of thermal over mechanical nociceptive responses was just as easy to orchestrate by altering the relative stimulus intensities in the appropriate fashion. Similar apparently selective actions of μ-opioids and other agonists could also be arranged at will.

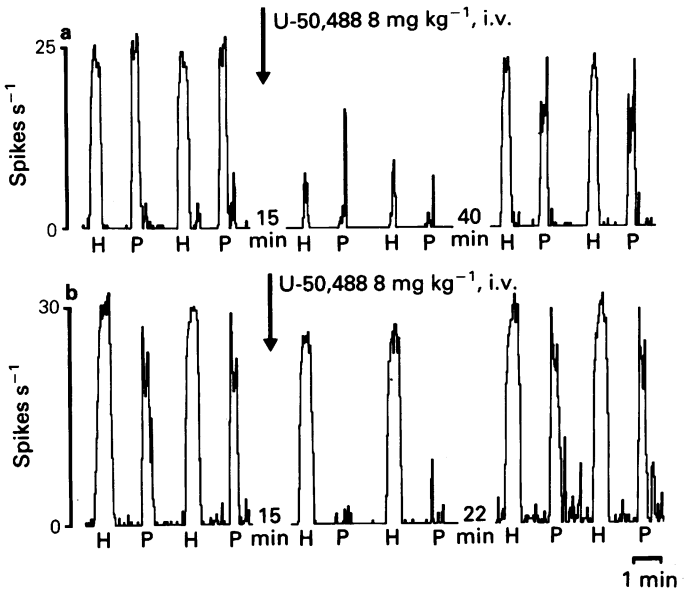

Figure 5 Effect of stimulus intensity in determining the selectivity of U-50,488 between responses to noxious pinch and those to noxious heat. The motoneurone was activated by noxious heat (H; to 49.5°C for 30 s) of the plantar foot and pinch (P; for 15 s) of toe 5 alternated in a regular 3 min cycle. Trace (a) illustrates the parallel reduction of responses to noxious heat and noxious pinch by U-50,488. The κ-agonist was administerd in divided doses (2 + 2 + 4 mg kg^{-1} i.v.) over 15 min. Trace (b) shows neuronal responses of the same cell tested later in the experiment with the same dose regime of U-50,488 but after the thermal stimulus had been altered, by a slight movement of the thermode, so as to evoke a greater neuronal firing rate. In this case U-50,488 was apparently 'selective' in depressing responses to noxious pinch over those to noxious heat. Multireceptive motoneurone in VR L4 of an α-chloralose anaesthetized, spinalized rat.

A critical test for the importance of stimulus strength was therefore to test for 'selectivity' between responses to different intensities of the *same* modality of noxious stimulus. Figure 6 illustrates one such test for a motoneurone responding to noxious pinch stimuli of two differing intensities, applied alternately by the same pincher unit to the same site. Both fentanyl and U-50,488 'selectively' reduced reflexes to the lower intensity noxious pinch stimulus whilst having less effect on the responses to the higher intensity stimulus. Thus, when quantified as the total number of spikes elicited by each stimulus, fentanyl reduced the smaller reflex responses to 12% of control whereas the larger reflex was only reduced to 70% of control values. U-50,488H had similar differential actions on this cell; the smaller and larger reflexes were reduced to 11% and 84% of control values respectively.

Discussion

It is most likely that the site of action of the i.v. administered opioids in this study is entirely within the spinal cord. Supraspinal actions should have been eliminated by the spinal section. Peripheral actions seem unlikely since, in monoarthritic rats, intraplantar naloxone (1–40 μg per paw) does not affect the antinociceptive actions of [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAGOL), [D-Pen^2, D-Pen^5]enkephalin (DPDPE) or U-50,488 in the uninflamed paw (Stein *et al.*, 1989). Moreover, in 'normal' rats, quaternary naltrexone (up to 40 mg kg^{-1}, s.c.) fails to antagonize the elevation of paw pressure threshold and tail flick latency produced by systemic μ- and κ-opioids (Millan *et al.*, 1989). In the present study care was taken to avoid the induction of inflammation and experiments were terminated if this did occur.

The results on non-thermal nociceptive responses presented in this paper are in broad agreement with a number of behavioural and electrophysiological studies showing that systemically administered μ and κ-opioids are all capable of reducing spinal reflexes to such stimuli.

On the other hand, the non-selectivity in our experiments of κ-opioids between thermal and mechanical nociceptive responses is in sharp contrast to the selective reduction of mechanical/visceral over

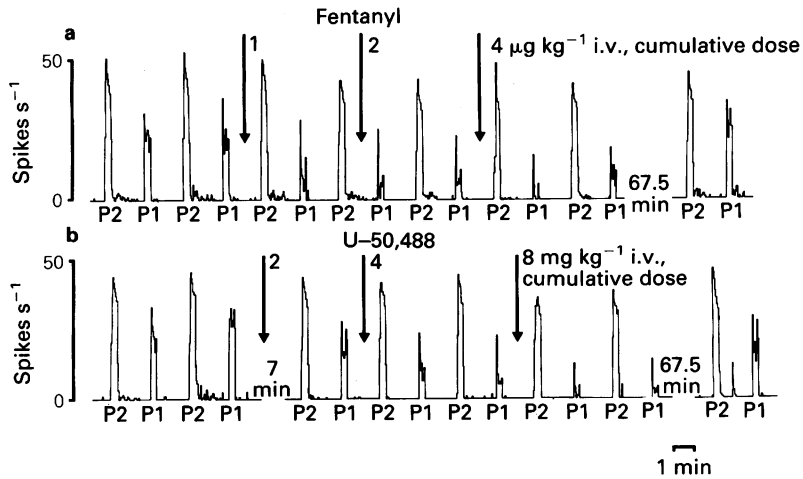

Figure 6 Differential reduction by μ- and κ-opioids of motoneuronal responses to noxious pinch stimuli alternated between different intensities. Noxious 15 s pinch stimuli to toe 5 were alternated in a regular 3.5 min cycle between P1 (0.3 newtons) and P2 (1.1 newtons). Trace (a) illustrates the greater sensitivity of responses to the lower intensity pinch stimulus to the depressant actions of fentanyl and trace (b) shows similar effects of U-50,488 on the same cell. Multireceptive motoneurone recorded in VR L4 of an α-chloralose anaesthetized, spinalized rat.

thermal nociceptive responses reported for κ-opioids in many (e.g. Tyers, 1980; Upton *et al.*, 1982; Bryant *et al.*, 1983; Schmauss *et al.*, 1983; Ward & Takemori, 1983; Schmauss & Yaksh, 1984; Schmauss 1987; see Yaksh & Noueihed, 1985) but by no means all behavioural studies (Han & Xie, 1982; Piercey *et al.*, 1982; Kaneko *et al.*, 1983; Przewłocki *et al.*, 1983a,b; Vonvoigtlander *et al.*, 1983; Herman & Goldstein, 1985; Spampinato & Candeletti, 1985).

We propose that the experimental differences can be explained, at least in part, by the limitation in nearly all behavioural tests of spinal antinociception, that the different stimuli used have not been matched in intensity so as to elicit reflex responses of similar magnitude in terms of muscular force and/or latency, and hence also in terms of spinal neuronal excitability. In addition, comparisons are often made between behavioural reflexes elicited from anatomically separate areas. In our experiments great care was taken to adjust the intensities of alternating thermal and mechanical noxious peripheral stimuli so as to match the strength of responses in terms of neuronal firing rates, and the peripheral stimuli were placed close together in the neuronal receptive field.

On those occasions when the firing rates in response to alternating noxious thermal and mechanical stimuli were not accurately matched, the weaker response was invariably reduced to a greater extent than the stronger response, regardless of whether μ- and κ-opioids were tested. The weaker response was similarly affected when different intensities of the same modality of noxious stimulus were alternated on the same cell. The weaker stimuli were still considered to be noxious in that they were aversive when tested on the experimenter. Moreover, if the less intense stimuli were not noxious then the resulting responses would, in fact, be expected to be less rather than more sensitive to the opioids tested (see also Parsons & Headley, 1989). It is clear from these results that stimulus intensity can be a crucial factor in the assessment of the selectivity of the antinociceptive effects of opioids. It is because of the importance of this that the examples illustrated are of the firing rates of the cells, although the analysis has been made on the basis of spikes elicited per response.

The relative strength of reflexes in behavioural tests cannot be assessed in terms of neuronal firing rate and therefore cannot easily be matched and quantified in this way (but see Kawakita & Funakoshi, 1987). However, judging by the reported latency and vigour of motor responses, the intensity of stimuli in the tail flick test results in considerably greater motoneuronal discharge rates than occurs in other tests such as the paw pressure or writhing tests; it would therefore be expected to be less sensitive to depression by opioids. Interpretation of the similar relative resistance of hot plate responses to κ-opioids is complicated by the probable supraspinal effects of opioids on this more complex response.

Stability of the background excitability of neurones is clearly also important in determining drug effects. In this regard, the choice of α-chloralose anaesthesia in these experiments was supported by (1) the stable baseline responses of single motoneurones recorded for up to 10 h under this anaesthetic and (2) the relative lack of effect of α-chloralose when supplementary doses were administered i.v.

In conclusion, these experiments clearly demonstrate that stimulus intensity is an important factor in determining the thermal/non-thermal selectivity of spinal opioids. Thermal/non-thermal selectivity reported in behavioural tests with κ-agonists is therefore likely to be related to differences in the stimulus intensities used. In contrast, μ-opioids with full agonist activity have more often been reported to be equally effective on thermal and non-thermal nociceptive reflexes whether following local spinal or systemic administration (but see Hayes *et al.*, 1987). It therefore seems likely that factors other than stimulus intensity are also important in determining the relative activity of opioid agonists on reflexes to different modalities of noxious stimulus. These factors may include variable access to opioid receptors following local spinal administration, and the efficacy of the agonists tested in a tissue which may have limited reserves of the opioid receptor subtype under study. These two points will be addressed in a following paper (Parsons *et al.*, 1989), as will the possibility that the spinal actions of intravenous U-50,488 and tifluadom in our experiments were mediated at μ-opioid receptors.

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