

# Effects of intravenous $\mu$ and $\kappa$ opioid receptor agonists on sensory responses of convergent neurones in the dorsal horn of spinalized rats

Xiao-wei Dong,<sup>1</sup> Chris G. Parsons & <sup>2</sup>P. Max Headley

Department of Physiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD

**1** Electrophysiological experiments have been performed to assess the effects of intravenously administered  $\mu$  and  $\kappa$  opioid agonists on the responses to noxious thermal and mechanical and non-noxious tactile stimuli of single convergent neurones in laminae III–VI of the dorsal horn of spinalized rats anaesthetized with  $\alpha$ -chloralose.

**2** The  $\mu$  receptor agonists tested were fentanyl ( $1\text{--}16\ \mu\text{g kg}^{-1}$ ) and morphine ( $0.5\text{--}16\ \text{mg kg}^{-1}$ ) and the  $\kappa$ -receptor agonists U-50,488 ( $1\text{--}16\ \text{mg kg}^{-1}$ ) and tifluadom ( $0.1\text{--}1.6\ \text{mg kg}^{-1}$ ). Multiple drug tests were made on each cell so that compounds could be compared under closely comparable conditions.

**3** In one protocol, thermal and mechanical nociceptive responses of matched amplitudes were elicited alternately. Both  $\mu$  and  $\kappa$  agonists dose-dependently reduce the neuronal responses. Thermal nociceptive responses were as sensitive to the  $\kappa$  agents as were the mechanical nociceptive responses; the  $\mu$  agonists similarly reduced both types of response in parallel.

**4** In another protocol, nociceptive and non-nociceptive responses were elicited alternately to permit the degree of selective antinociception to be assessed. The  $\mu$  agonists were scarcely selective, fentanyl reducing nociceptive only slightly (but significantly at  $4\text{--}16\ \mu\text{g kg}^{-1}$ ) more than non-nociceptive responses. The  $\kappa$ -opioid agonist U50,488 reduced tactile responses somewhat more than nociceptive responses.

**5** The spontaneous discharge of these cells with ongoing activity was reduced to a significantly greater degree than the evoked responses; this is likely to have contributed to the non-selectivity of the reduction of the evoked responses.

**6** The results are discussed with respect firstly to previous reports that  $\kappa$  opioids are ineffective in tests of thermal nociception, and secondly to the likely spinal mechanisms by which opioid receptor agonists mediate antinociception.

**Keywords:** Opioids;  $\mu$ -opioid receptor agonists;  $\kappa$ -opioid receptor agonists; spinal cord; intravenous; convergent neurones; sensory responses

## Introduction

There is plentiful evidence that opioid analgesics have direct spinal actions that reduce the nociceptive responses of spinal neurones; this applies both to the motoneurones that mediate reflex and other motor outputs and to the neurones in the dorsal horn that are related to ascending projection pathways. There has however been considerable confusion as to which opioid receptors may be involved, which types of nociceptive input may be preferentially reduced, and which neuronal elements are the most affected.

Many reports have indicated that  $\kappa$  opioids are relatively ineffective on thermally-induced, as compared with mechanically or chemically-induced, spinal nociceptive reflexes (for reviews see Yaksh & Noueihed, 1985; Millan, 1990). We have recently shown in electrophysiological tests that such selectivity does not occur when care is taken to match the intensities of the peripheral stimuli used to elicit the reflexes, and when access to all spinal receptor sites is ensured by the use of systemic rather than topical administration of opioid agonists (Parsons & Headley, 1989a). Similar non-selectivity between thermal and non-thermal nociceptive reflexes is seen in standard behavioural reflex tests when similar care is taken to match the stimulus intensities (Millan 1989, 1990), at all but the strongest of stimulus intensities. There is however no direct evidence concerning the relative effectiveness of systemic  $\kappa$  opioids on thermal versus non-thermal nociceptive

responses of neurones in the spinal dorsal horn, although the data of Calthrop & Hill (1983) and of Fleetwood-Walker *et al.* (1988) suggest that there would be no great difference.

Studies in man indicate that morphine, whether administered systemically (Wikler, 1950) or spinally (Willer *et al.*, 1988; Chabal *et al.*, 1989), reduces pain sensations/nociceptive reflexes whilst having minimal effects on functions mediated by non-nociceptive afferents. It remains unclear how this selective analgesia is mediated, in terms of the precise spinal neuronal elements upon which the opioids may act. A specific problem arises from the finding that most dorsal horn neurones that respond to noxious stimuli also respond to non-nociceptive inputs: they are 'convergent', 'wide dynamic range', or 'multireceptive' in nature. The role of this population of convergent neurones in pain sensation remains under discussion (Besson & Chaouch, 1987; Dubner, 1989).

Various authors have reported that opioid receptor agonists can selectively reduce the nociceptive responses of such convergent neurones whilst leaving the responses to low threshold inputs relatively unaffected (see Kitahata & Collins, 1981; Duggan & North, 1984; Besson & Chaouch, 1987). Several such studies were performed with electrical rather than with 'natural', or adequate, stimulation of primary afferents, but it should be remembered that such stimulation of a subset of primary afferents cannot be expected to mimic the activation of receptors by adequate stimuli. Moreover many of the reports were of local rather than systemic administration of the opioid agonists, but as nociceptive and non-nociceptive afferents terminate, broadly speaking, in different laminae of the dorsal horn, local administration could result in selective effects as a result simply of access to some but not other neuronal elements. Of studies testing systemic opioids on

<sup>1</sup> Present address: Abt. Neurophysiologie, Max Planck Institut für Psychiatrie, 18a Am Klopferspitz, D-8033 Planegg-Martinsried, FRG.

<sup>2</sup> Author for correspondence.

responses to adequate peripheral stimuli, some have shown a rather poor degree of selectivity between nociceptive and non-nociceptive responses (Yaksh, 1978; Einspahr & Piercey, 1980). Notably, however, the systemic studies performed to date have been with opioid agonists acting primarily at  $\mu$  opioid receptors; there is no information on the selectivity of systemic  $\kappa$  opioids on convergent dorsal horn neurones in the spinal cord (but see Calthrop & Hill, 1983). Consequently the degree of selectivity between nociceptive and non-nociceptive responses of convergent spinal neurones, particularly in relation to the doses of opioid agonists that mediate behavioural analgesia, remains unclear.

For these reasons we have now examined the degree to which  $\mu$  and  $\kappa$  opioid receptor agonists, administered systemically to spinalized rats, distinguish between sensory responses of convergent neurones in laminae III–VI of the rat spinal dorsal horn: potential selectivity was examined firstly between alternating thermal and mechanical nociceptive responses, and secondly between nociceptive and non-nociceptive responses. Some of the data have been presented elsewhere in preliminary form (Headley *et al.*, 1984; 1987; Headley & Dong, 1990).

## Methods

### Animal preparation

Experimental methods were similar to those detailed previously (Parsons & Headley, 1989a). Data were analysed from experiments on 40 spinalized Wistar-style male rats. Briefly, arterial, venous and tracheal cannulae were inserted under halothane anaesthesia, and a laminectomy performed between Th9–L4 vertebrae. The spinal cord was sectioned at Th10; where the dorsal vein was substantial, it was left intact together with a small wedge of dorsal column tissue.

After surgery, halothane was discontinued and anaesthesia was maintained with  $\alpha$ -chloralose (50–60 mg kg<sup>-1</sup>, i.v. initially, supplemented with doses of 20 mg kg<sup>-1</sup> approximately every hour). Some animals were ventilated after paralysis with pancuronium (1.5 mg kg<sup>-1</sup>, i.v. initially, supplemented as required to maintain an adequate (but not complete) level of muscle relaxation); in these animals end tidal CO<sub>2</sub> was monitored and maintained close to 4%. The adequacy of anaesthesia in paralysed animals was ensured by (a) the use of the relatively long-lasting anaesthetic  $\alpha$ -chloralose, administered strictly in the same dose regime used in non-paralysed animals; (b) the selection of pancuronium which, as well as causing less hypotension than gallamine in our rats, is relatively short-lasting in this species (Durant *et al.*, 1980), thereby permitting intermittent but direct monitoring of the degree of anaesthesia; and, most importantly, (c) by monitoring cardiovascular (blood pressure and heart rate) responses to noxious stimuli applied rostral to the spinalization (Flecknell, 1987). In all cases body temperature was maintained close to 37°C; blood pressure was monitored and experiments were terminated if systolic pressure fell below 100 mmHg.

### Peripheral stimuli and neuronal recording

Noxious thermal stimuli were applied to one hindlimb with a 1.5 cm<sup>2</sup> flat contact thermode with feedback control; the thermode was held at 37°C and then ramped to 46.0–48.8°C where it was held until the end of the 30 s stimulus. Noxious pinch stimuli were administered with pneumatically controlled forceps; in later experiments this was both feedback-controlled and calibrated to give 1–3.5 N applied over a tip area of 3 mm<sup>2</sup> for 15 s. Non-noxious mechanical stimuli, lasting 15–20 s, consisted of indenting the skin or moving the toes with a relay-operated device working at 3–20 Hz. In all cases stimulus intensity and duration were constant between applications and repetition rate was controlled in an automa-

ted cycle of two (lasting 3–3.5 min) or occasionally three stimuli (lasting 4–5 min). If inflammatory changes in response to the repeated noxious stimulation became detectable, the experiment was terminated.

Extracellular recordings of convergent neurones in laminae III–VI were made with three barrel glass micropipettes that contained 3.5 M NaCl for recording; quisqualate Na (5 mM in 200 mM NaCl pH 7.5) to permit activation of neurones and hence a distinction between somata and axons; and pontamine sky blue (2% in 0.5 M Na acetate) to permit histological verification of recording sites.

Neuronal activity, stimulus details and physiological data were recorded continuously on a pen recorder. Counts of evoked spike activity, in epochs related to the sensory stimuli, were analysed on-line to permit drug effects on each type of response to be expressed as a percentage of the last 3 pre-drug control values.

### Drugs, drug administration and analysis

The drugs tested were the  $\mu$ -opioids, fentanyl citrate (Sublimaze, Janssen) and morphine HCl; the  $\kappa$  opioids U-50,488 (*trans*-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methane sulphonate; Upjohn) and tifiuadom HCl (Sandoz); and the antagonist naloxone HCl (Sigma). (See Parsons & Headley, 1989a, for the reasons for selecting these agonists).

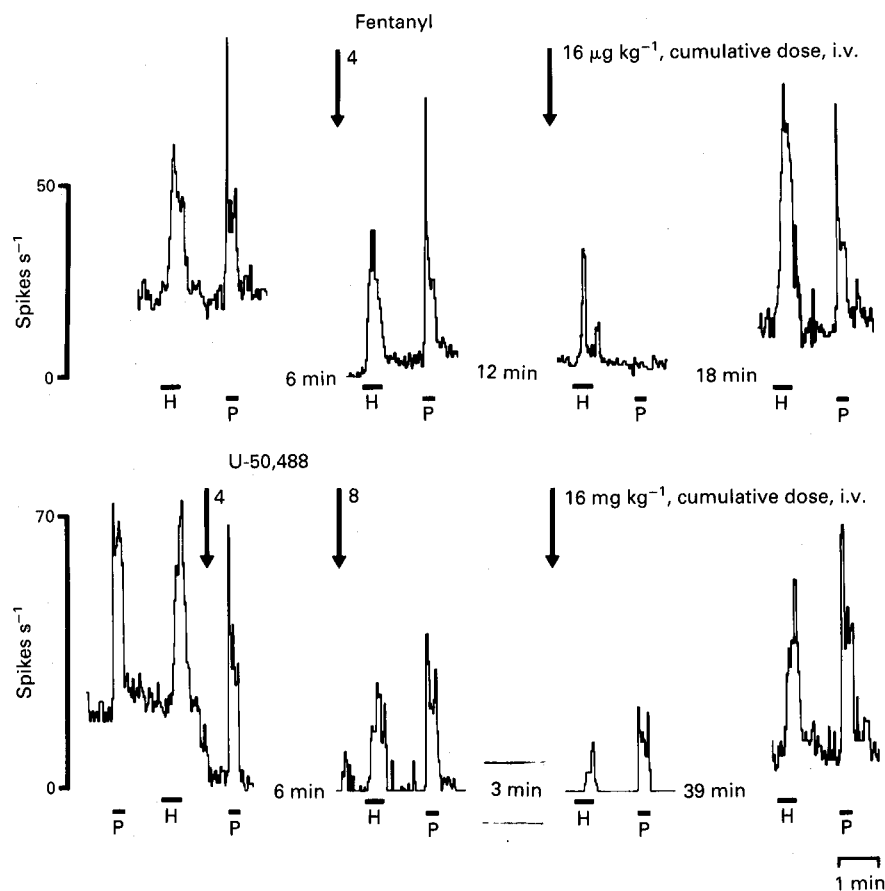
All opioid agonists were administered i.v. Injections were made over 30 s starting 1 min before the next evoked response; maximal effects are nearly always seen on the first subsequent response. Doses were incremented in a cumulative (log<sub>2</sub>) regime until evoked responses were reduced to below 25% control or the maximum dose compatible with monitoring subsequent recovery had been administered. The dose ranges used were fentanyl 1–16  $\mu$ g kg<sup>-1</sup>; morphine 0.5–16 mg kg<sup>-1</sup>; U-50,488 1–16 mg kg<sup>-1</sup>; and tifiuadom 0.1–1.6 mg kg<sup>-1</sup>. The opioid antagonist naloxone was given in doses of 1–50  $\mu$ g kg<sup>-1</sup>. (Doses refer to salts except for fentanyl base.) This regime permitted several drugs to be tested on most cells. In these experiments various anaesthetic agents were compared with the opioid agonists; data on the former will be presented elsewhere. Test data were only included for analysis either if (as was usual) recovery from the drug exceeded 50% of the initial drug effect or if the effect was reversed by naloxone (all cases tested).

The fact that the full dose-response range was not tested on all cells precludes constructing standard dose-response curves; most of the pooled data are instead presented in bar graph form.

The quantitative analysis below is performed on the '% control' data for spike counts over the following stimulus-related epochs: heat (the entire 30 s stimulus); late pinch (excluding the first 5 s of the stimulus, which is presumed to contain a higher proportion of low threshold afferent induced activity); tap/vibration (the entire 15–20 s stimulus); and spontaneous activity monitored over 60–80 s between evoked responses.

## Results

The results presented below were obtained in experiments on 9 convergent neurones responding to alternating noxious heat and pinch stimuli, and from 42 cells responding to alternating noxious and non-noxious stimuli. At least one  $\mu$  and one  $\kappa$  receptor agonist was tested under closely comparable conditions on 18 of these cells. The data were not sufficiently extensive to permit a statistically significant comparison between drugs and responses for each of spinal laminae III (5 cells), IV (14 cells), V (21 cells) and VI (11 cells); neurones in these laminae appeared to respond similarly to the test drugs, and data for all laminae have therefore been pooled.



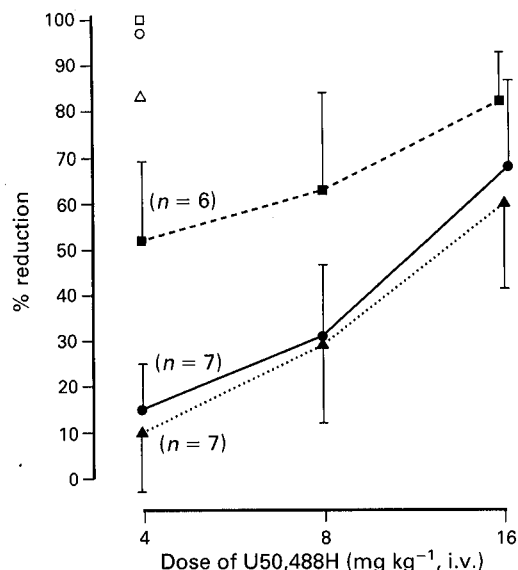
**Figure 1** Both the  $\mu$  opioid receptor agonist fentanyl and the  $\kappa$  agonist U-50,488, reversibly reduced thermal and mechanical nociceptive responses in parallel when tested on a single convergent neurone in lamina VI of the spinal dorsal horn of a chloralose anaesthetized spinalized rat. Note the greater sensitivity of the spontaneous as compared with the evoked activity. The cell was activated alternately by noxious heat (H; ramped from 37 to 48°C, applied to the plantar foot over 30 s every 3 min) and noxious pinch (P; toe 5 for 15 s). Fentanyl was tested at doses of 1, 2, 4, 8 and 16  $\mu\text{g kg}^{-1}$ ; some parts of the record have been omitted, as indicated. The lower record was obtained after an interval of 16 min; at this stage the cell had become somewhat more sensitive to both noxious stimuli. This record shows all three doses of U-50,488 tested on this cell.

#### Effects of $\mu$ and $\kappa$ opioids on spontaneous activity

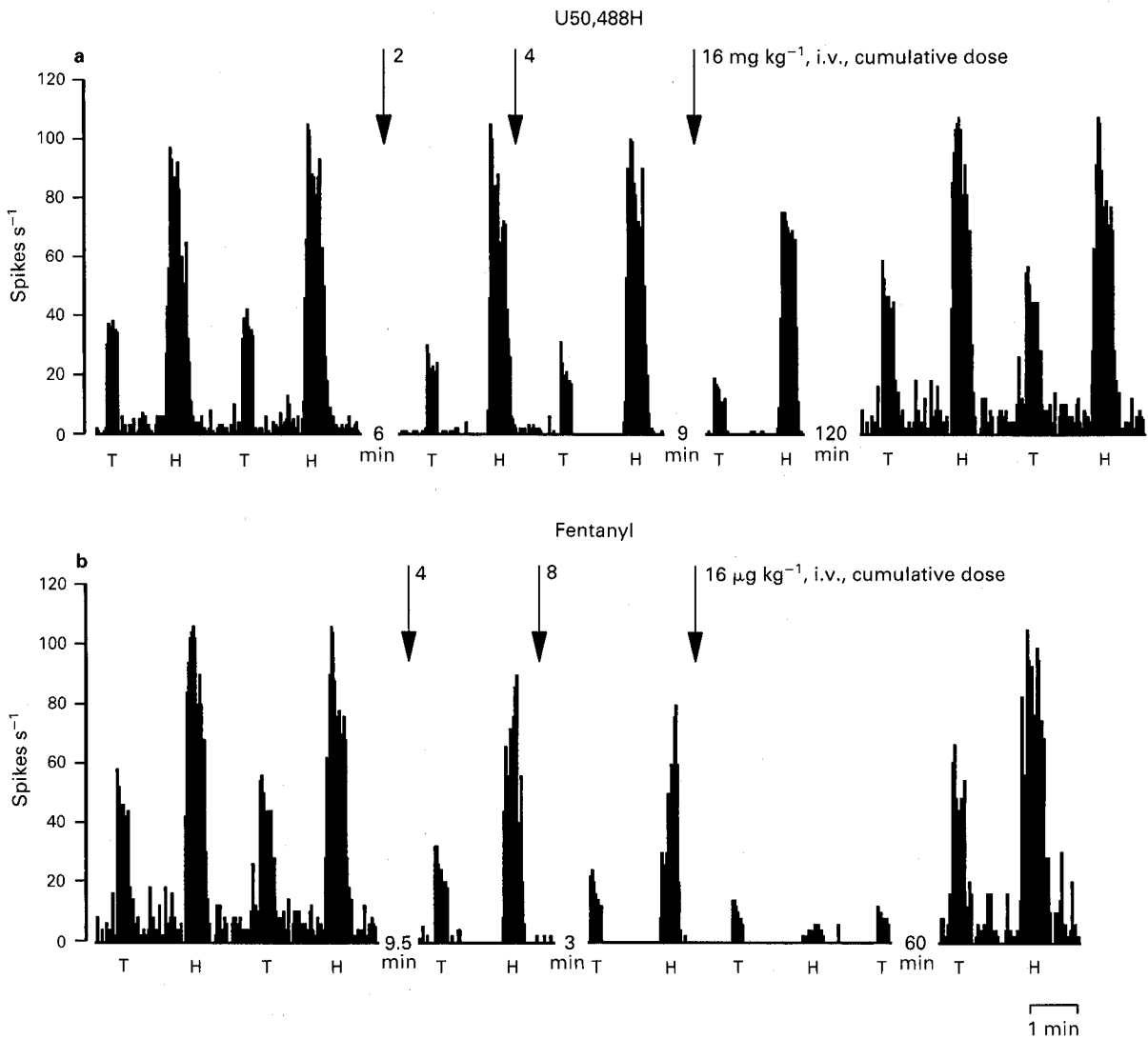
The protocol followed is illustrated in Figure 1 which shows tests with both fentanyl and U-50,488 on the same convergent neurone recorded in lamina VI. This cell had a relatively high level of spontaneous discharge, and it can be seen that both  $\mu$  and  $\kappa$  receptor agonists reduced this ongoing activity at doses lower than those that substantially reduced the evoked responses (i.e. the area under the trace for each response). Not all convergent neurones show such high levels, or indeed any, spontaneous activity and consequently the percentage reduction by the agonists was variable between cells. Figures 2, 4 and 6 show pooled data for the effects of the  $\mu$  and  $\kappa$  receptor agonists on such spontaneous activity and demonstrate that the spontaneous activity of convergent neurones, when present, was reduced by both types of agonist. This reduction occurred at doses appreciably lower than those that had clear effects on either nociceptive or non-nociceptive evoked responses; by the same token the reduction of spontaneous activity was significantly greater than that of evoked responses at all doses tested (Figures 4 and 6).

#### Effects on alternating thermal and mechanical nociceptive responses

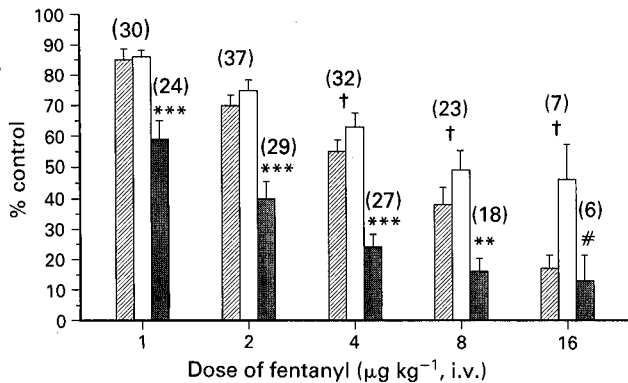
A significant finding is that thermal and non-thermal nociceptive responses, which were carefully matched in terms of evoked firing rate, were reversibly reduced in parallel by the  $\kappa$  as well as the  $\mu$  opioid. This parallel reduction of thermal and



**Figure 2** Dose-responses curves of pooled data from a total of 9 neurones tested with U-50,488 on responses to alternating thermal (triangles) and mechanical (circles) noxious stimuli, and on the spontaneous activity (squares), recorded in the manner illustrated in Figure 1. Seven cells (filled symbols) were tested at all three doses; one of these was not spontaneously active. Two other cells (open symbols) were sufficiently sensitive at 4  $\text{mg kg}^{-1}$  for further doses not to be administered. Statistical tests (Wilcoxon matched pairs) between the nociceptive response types gave *P* values greater than 0.10 at all doses.



**Figure 3** Relative non-selectivity of the  $\kappa$  agonist U50,488 (a) and the  $\mu$  receptor agonist fentanyl (b) on alternating nociceptive and non-nociceptive responses of a convergent neurone in lamina VI. T: tap stimulus to toes 2–4, at 3 Hz for 15 s; H: heat to toes 2–4 for 30 s, ramped from baseline 37°C and then held at 47.5°C. For clarity the last two stimulus cycles on the upper trace are repeated at the start of the lower trace.

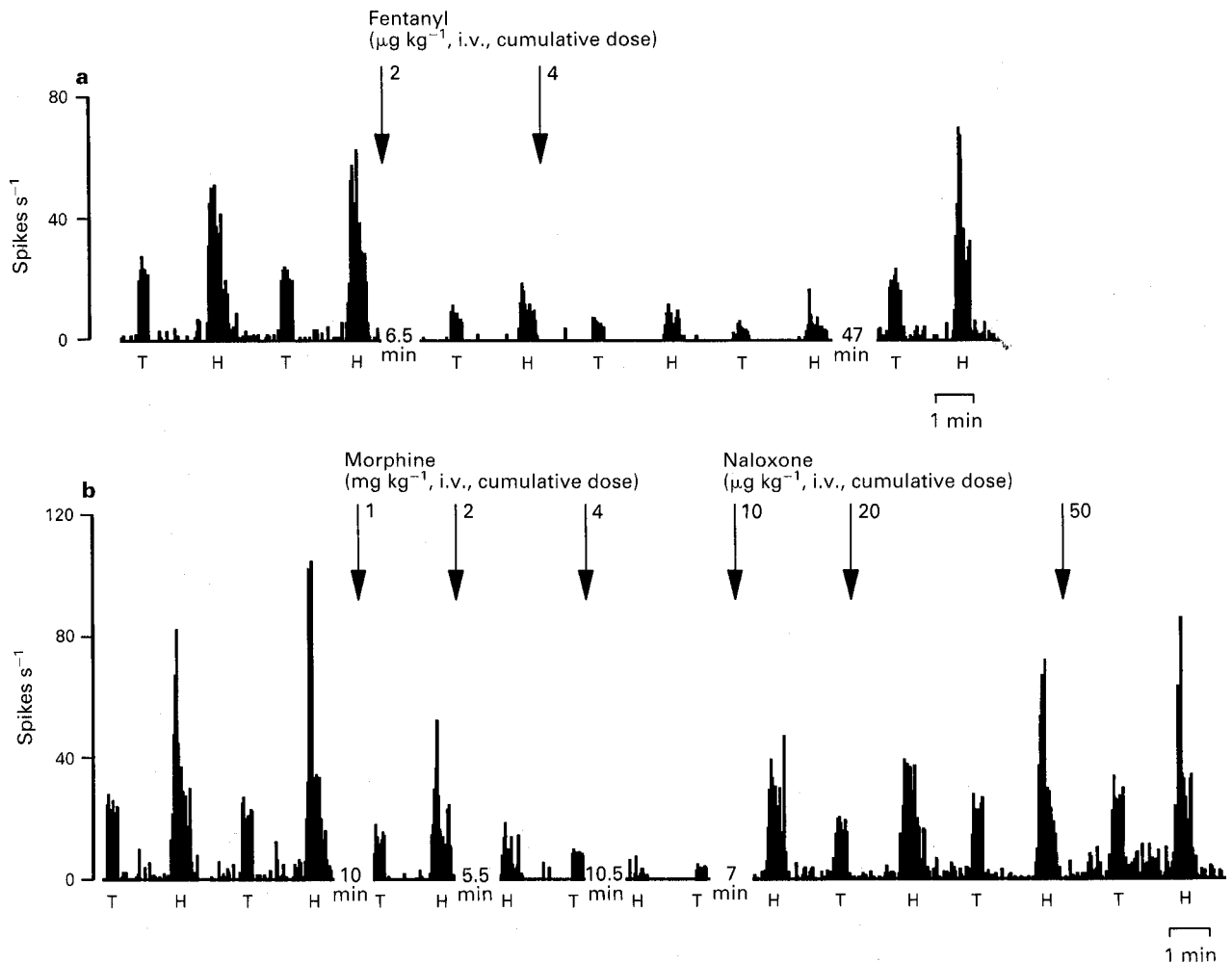


**Figure 4** Pooled data from 40 neurones on which fentanyl was tested i.v. on alternating nociceptive (hatched columns) and non-nociceptive responses (open columns) according to the protocol shown in Figure 3. Not all doses were tested on all cells; numbers tested are shown above the bars. The effects on spontaneous activity are also shown (stippled columns) for those cells showing such activity (numbers shown above the stippled columns). Statistical tests: Wilcoxon (two tailed) tests were performed for each dose between the two evoked responses: †  $P < 0.05$ . Mann-Whitney U tests were performed between spontaneous and evoked activity: \*\*  $P < 0.005$ , \*\*\*  $P < 0.001$  between spontaneous and both evoked responses; #  $P < 0.05$  between spontaneous and non-nociceptive responses.

mechanical nociceptive responses was a consistent observation with the  $\kappa$  as well as the  $\mu$  receptor agonists. This is shown for the pooled data of tests with U-50,488 in Figure 2. The benzodiazepine  $\kappa$  ligand, tifluadom, similarly reduced the thermal and mechanical responses in parallel on the 4 neurones tested; at 0.4 mg kg<sup>-1</sup> responses were reduced to 38% ± 20 (mean ± standard error of mean) and 28% ± 18 of control respectively. The large standard error values for both  $\kappa$  ligands indicate the variability of drug potency between cells, but, as indicated by the mean values, and in Figure 1 on any one cell, the two types of nociceptive response were reduced to a very similar degree. The  $\mu$  receptor agonist fentanyl showed a similar pattern on the paired nociceptive responses of the three cells tested: at 4 μg kg<sup>-1</sup> it reduced thermal and non-thermal nociceptive response to means of 19% control (±12 and ±15 respectively).

*Effects on alternating nociceptive and non-nociceptive responses*

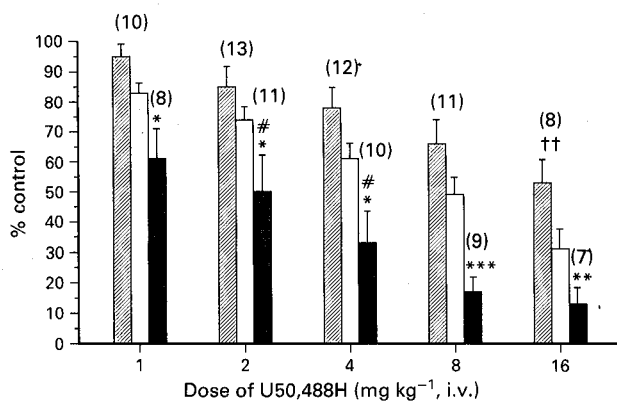
Whereas care was taken, when noxious stimuli were alternated, to achieve similar firing rates for the two types of response, the properties of many convergent neurones preclude achieving this when noxious and non-noxious stimuli are alternated: many cells will fire more vigorously to the more intense stimulus. Moreover the pattern of the evoked discharge is different for the two types of response (Steedman



**Figure 5** Fentanyl (a) and morphine (b) tested with cumulative i.v. doses on the same cell responding to alternating noxious and non-noxious stimuli. Both  $\mu$  receptor agonists reduced the responses non-selectively, and the effect of morphine was reversed by naloxone. Noxious heat (H; 37°C baseline ramped to 48.8°C) was applied to toe 2 of the ipsilateral hindlimb; the non-noxious stimulus was tapping toe 5 at 3 Hz. The morphine test was performed 90 min after the fentanyl test; during this period the cell became progressively more sensitive to the noxious stimulus, although no peripheral inflammation was detectable. Cell in lamina V of an  $\alpha$ -chloralose anaesthetized and spinalized rat.

& Zachary, 1990). These points should be borne in mind when interpreting the type of test reported below.

When the same group of opioid receptor agonists was tested in a similar preparation on alternating nociceptive and non-nociceptive reflex responses of single motoneurons



**Figure 6** Pooled data from 15 neurons on which U-50,488 was tested on alternating nociceptive and non-nociceptive responses. Data presented as in Figure 4. Wilcoxon matched pairs tests between the two types of evoked response: ††  $P < 0.01$ . Mann-Whitney  $u$  tests (a) between spontaneous and nociceptive responses: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$  and (b) between spontaneous and non-nociceptive responses: #  $P < 0.05$ .

(Parsons & Headley, 1989b), the selectivity shown by both  $\mu$  and  $\kappa$  opioids varied considerably between units, showing selective antinociception only when relatively lower doses were effective. This pattern was not seen with dorsal horn convergent neurones, which were less sensitive than moto-

neurones in terms of the reduction of responses at given intravenous doses of the opioid agonists. Figure 3 shows an example of a test with both  $\mu$  and  $\kappa$  agonists on a convergent neurone activated alternately by noxious heat and non-noxious tap stimuli. Figure 3b shows a test with fentanyl and indicates that the  $\mu$  agonist reduced both nociceptive and non-nociceptive responses; that is, it was not selectively antinociceptive on this neurone.

Pooled data for the effects of fentanyl on such alternating nociceptive and non-nociceptive responses are shown in Figure 4. On this population of convergent neurones the  $\mu$  receptor agonist reduced both responses, showing only weak selectivity which reached significance at only the higher doses tested (4–16  $\mu\text{g kg}^{-1}$ ; compare data in Figure 4 with those on motoneurone responses in Table 1 of Parsons & Headley, 1989b). The spontaneous activity of those neurones which had an ongoing discharge was reduced to a significantly greater degree.

In view of this non-selectivity by fentanyl, a limited number of tests were performed with morphine (0.5–16  $\text{mg kg}^{-1}$ , i.v.). In each case ( $n = 4$ ) morphine showed the same degree of non-selectivity as did fentanyl; an example of a direct comparison is shown in Figure 5. At 4  $\text{mg kg}^{-1}$ , thermal and tactile

responses were reduced to means of 13% ( $\pm 6$ ) and 21% ( $\pm 6$ ) control respectively. These non-selective effects of morphine were readily reversed by low i.v. doses of naloxone, as is shown in Figure 5.

The  $\kappa$  opioid U-50,488 displayed no selective antinociception at all. Figure 3a shows a test (same neurone as the fentanyl test) in which it reduced the non-nociceptive responses to an even greater degree than the responses to noxious heat. The pooled data shown in Figure 6 indicate that this was a consistent finding; at all doses tested, it reduced non-nociceptive responses somewhat more, although this difference only reached significance at the highest dose (16 mg kg<sup>-1</sup>).

## Discussion

The i.v. administration of the opioid receptor agonists used in this study should have ensured that all opioid receptors within the spinal cord were exposed to similar concentrations of the agents, a situation most suited to an assessment of the possible selective effects of opioids between the different types of sensory response of dorsal horn convergent neurones. The fact that  $\mu$  and  $\kappa$  agonists were often tested on the same neurone responding to the same stimuli permitted a direct comparison to be made between the actions of these agents. The site(s) of action of the opioid agonists should have been restricted by the spinalization to the spinal cord. The effectiveness of systemic  $\kappa$  opioids in such physiological tests contrasts with the low levels of  $\kappa$  binding sites reported in rat spinal cord; technical problems, however, have evidently contributed to the latter findings (Wood *et al.*, 1989).

### Thermal vs non-thermal nociceptive responses

To avoid the potential problems associated with using stimuli of differing intensities, care was always taken to adjust the stimulation devices so that firstly the stimuli were applied as close as possible within the peripheral receptive field of the neurone, and secondly the evoked firing rate of the neurone was similar for the two alternating response types. Under these conditions neither the  $\mu$  nor the  $\kappa$  agonists tested showed any selectivity between thermal and non-thermal nociceptive responses, just as was observed under similar conditions when reflex responses of single motoneurons were tested (Parsons & Headley, 1989a). The present results provide further evidence that the relative spinal inactivity of  $\kappa$  opioids that has been reported by many authors performing standard reflex tests of thermal nociception (see Yaksh & Noueihed, 1985), has resulted primarily from a combination of inadequate stimulus matching and inadequate access of the opioids from the site of topical administration to the relevant spinal receptors (Parsons & Headley, 1989a; Parsons *et al.*, 1989; Millan 1989; 1990).

At high stimulus intensities, even with matched withdrawal latencies,  $\kappa$  agonists are less effective against thermal than against mechanical nociceptive flexion reflexes (Millan, 1989). In the present experiments such high intensity thermal stimuli could not be tested because, under our conditions, repeating stimuli of above 49.5°C results relatively rapidly in inflammatory swelling. All thermal stimuli were therefore kept below this limit. Consequently there remains no electrophysiological evidence concerning this difference between  $\mu$  and  $\kappa$  opioids seen by Millan (1989) at high stimulus intensities.

### Nociceptive vs non-nociceptive responses

The general consensus from previous electrophysiological tests with spinal convergent neurones is that the opioids that have been tested systemically – and that act primarily at  $\mu$  receptors – showed a selective antinociception: the non-nociceptive responses were relatively unaffected. This is true whether comparisons were between responses to noxious and non-noxious

adequate peripheral stimuli, or between responses to electrical stimulation of fast- versus slowly conducting primary afferent fibres. There have however been some exceptions to this general finding. Most clearly, Yaksh (1978) and Einspahr & Piercy (1980) reported that systemic morphine was not selective on convergent neurones in the cat spinal cord, an effect not reported in various other tests of systemic  $\mu$  opioids in cats (Calvillo *et al.*, 1979; Headley *et al.*, 1987). Equivalent tests with systemic opioids appear not to have been performed either in rat or with  $\kappa$ -selective opioid agonists. Preliminary data with systemic  $\kappa$  agonists on convergent neurones in cats suggested that the only effect was a change in the threshold of the nociceptive response (Piercy *et al.*, 1982).

We now show that in rats, the  $\mu$  receptor agonists fentanyl and morphine, administered intravenously in spinalized animals, show only very weak selectivity between alternating nociceptive and non-nociceptive responses of convergent neurones in laminae III–VI. The  $\kappa$  agonist U-50,488 was effective on these neurones in that it depressed spontaneous and evoked activity, but it did not cause any degree of selective antinociception. The question therefore arises as to how these electrophysiological results relate to the behavioural data from both animals and man indicating that spinal opioid agonists can indeed be selectively antinociceptive.

### Significance of effects on spontaneous activity

A major contribution to the non-selectivity of the opioid agonists on evoked responses is likely to have been the marked reduction of spontaneous activity caused by low doses of the agents: such a decrease in neuronal excitability would result in non-selective reductions of all synaptic responses. It may be pertinent that DNIC (diffuse noxious inhibitory controls), which evidently involve a spinal relay utilizing an opioid peptide (Le Bars *et al.*, 1987), cause a non-selective inhibition of convergent neurone activity (Le Bars *et al.*, 1979). Whilst there is clear evidence for direct depressant effects of opioids on spinal neurones (Zieglansberger & Bayerl, 1976; Yoshimura & North, 1983), it is not possible to resolve from the current type of experiment whether such a depression was occurring on the cells under study or on cells earlier in the (probably) polysynaptic pathway mediating the ongoing and/or evoked synaptic activity.

Although we take care to keep the area of surgery well outside the receptive fields of the neurones studied, tonic nociceptive input to the recorded cell could still be generated by the surgical preparation of the animal (Duggan & North, 1984; Collins *et al.*, 1987). In acute preparations, nociceptive neurones have indeed been reported to have a higher spontaneous discharge than do non-nociceptive neurones (Surmeier *et al.*, 1989). As implied above, such tonic activity would enhance superimposed phasic responses such as those elicited in this study. It follows that even if the opioid agonists were selective in reducing nociceptive input onto the cell, the resulting decrease in tonic activity would cause the non-nociceptive inputs of these convergent neurones to be reduced as well as the nociceptive responses. Two other findings suggest that the effect of opioid agonists on surgery-induced ongoing activity contributes significantly to the reduction of evoked responses. Firstly, morphine has long been known to depress the spontaneous activity of nociceptive more than that of non-nociceptive cells (Kitahata & Collins, 1981). Secondly, the potency of fentanyl on spinal withdrawal reflexes is increased when the severity of preparatory surgery is increased in a controlled manner (Hartell *et al.*, 1990).

There is an alternative explanation for the apparent discrepancy between the non-selectivity seen with convergent neurones and the selectivity of the spinal analgesia that is seen behaviourally and clinically with opioid analgesics: namely that it is the balance of activity between populations of dorsal horn neurone that permits the interpretation of nociception by higher centres. This postulate has arisen from the proper-

ties of DNIC inhibitions (as discussed by Besson & Chaouch, 1987) and the same considerations would apply to the antinociception mediated by opioids. We have recently found that systemically administered opioid agonists can indeed have differential effects between different types of dorsal horn neurone (Headley & Dong, 1990); the data will be presented and the concept discussed in full elsewhere.

### Conclusion

The effects of opioid receptor agonists on convergent dorsal horn neurones are qualitatively similar to the effects they have on spinal reflexes tested under closely comparable conditions: both  $\mu$  and  $\kappa$  opioids are relatively non-selective in reducing

responses to noxious thermal, noxious mechanical and light tactile stimuli. The selectivity reported in other types of test (a) between nociceptive and non-nociceptive responses and (b), for  $\kappa$  opioids, between thermal and non-thermal responses, requires other explanations in terms of the technical limitations of the techniques employed, as well, perhaps, as of other sorts of neural interaction.

We should like to thank Upjohn for supplies of U-50,488 and Sandoz for tiufadom. The project was funded by the Medical Research Council and by the University of Bristol. We thank Mr Craig Turner and Ms Mel Watson for technical assistance.

### References

- BESSON, J.-M. & CHAOUCH, A. (1987). Peripheral and spinal mechanisms of nociception. *Physiol. Rev.*, **67**, 67–186.
- CALTHROP, J. & HILL, R.G. (1983). The action of  $\kappa$ -agonists on the nociceptive responses of neurones in the medullary dorsal horn of the anaesthetized rat. *Life Sci.*, **33**, Suppl. 1: 541–544.
- CALVILLO, O., HENRY, J.L. & NEUMAN, R.S. (1979). Actions of narcotic analgesics and antagonists on spinal units responding to natural stimulation in the cat. *Can. J. Physiol. Pharmacol.*, **57**, 652–663.
- CHABAL, C., JACOBSON, L. & LITTLE, J. (1989). Intrathecal fentanyl depresses nociceptive flexion reflexes in patients with chronic pain. *Anesthesiol.*, **70**, 226–229.
- COLLINS, J.G., REN, K. & TANG, J. (1987). Lack of spontaneous activity of cutaneous spinal dorsal horn neurones in awake, drug free, spinally transected cats. *Exp. Neurol.*, **96**, 299–306.
- DUBNER, R. (1989). Introduction to section VII. In *Processing of Sensory Information in the Superficial Dorsal Horn of the Spinal Cord*. ed: Cervero, F., Bennett, G.J. & Headley, P.M. pp. 485–488. New York: Plenum.
- DUGGAN, A.W. & NORTH, R.A. (1984). Electrophysiology of opioids. *Pharmacol. Rev.*, **35**, 219–281.
- DURANT, N.N., HOUWERTJES, M.C. & CRUL, J.F. (1980). Comparison of the neuromuscular blocking properties of Org NC45 and pancuronium in the rat, cat and rhesus monkey. *Br. J. Anaesth.*, **52**, 723–730.
- EINSPAHR, F.J. & PIERCEY, M.F. (1980). Morphine depresses dorsal horn neurone responses to controlled noxious and non-noxious cutaneous stimulation. *J. Pharmacol. Exp. Ther.*, **213**, 456–461.
- FLECKNELL, P.A. (1987). *Laboratory Animal Anaesthesia*. London: Academic Press.
- FLEETWOOD-WALKER, S.M., HOPE, P.J., MITCHELL, R., EL-YASSIR, N. & MOLONY, V. (1988). The influence of opioid receptor subtypes on the processing of nociceptive inputs in the spinal dorsal horn of the cat. *Brain Res.*, **451**, 213–226.
- HARTELL, N.A., HEADLEY, P.M. & PARSONS, C.G. (1990). The degree of surgical intervention influences the potency with which injectable anaesthetics depress spinal nociceptive reflexes in the adult rat. *J. Physiol.*, **420**, 28P.
- HEADLEY, P.M. & DONG, X.-W. (1990). Two unexpected effects of intravenous mu and kappa agonists on different types of neurones in the spinal dorsal horn. In *New Leads in Opioid Research*. ed: van Ree, J.M., Mulder, A.H., Wiegant, V.M. & Griedanus, T.J. van W. Int. Congress Series, Vol. 914, pp. 61–62. Amsterdam: Excerpta Medica.
- HEADLEY, P.M., PARSONS, C.G. & WEST, D.C. (1984). Comparison of mu, kappa and sigma preferring agonists for effects on spinal nociceptive and other responses in rats. *Neuropeptides*, **5**, 249–252.
- HEADLEY, P.M., PARSONS, C.G. & WEST, D.C. (1987). Opioid receptor-mediated effects on spinal responses to controlled noxious natural peripheral stimuli: technical considerations. In *Fine Afferent Nerve Fibres and Pain*. ed. Schmidt, R.F., Schaible, H.G. & Vahle-Hinz, C. Weinheim, FRG: VCH Press.
- KITAHATA, L.M. & COLLINS, J.G. (1981). Spinal actions of narcotic analgesics. *Anesthesiol.*, **54**, 153–163.
- LE BARS, D., BOURGOIN, S., VILLANUEVA, L., CLOT, A.M., HAMON, M. & CESSÉLIN, F. (1987). Involvement of dorsolateral funiculi in the spinal release of Met-enkephalin-like material triggered by heterosegmental noxious mechanical stimuli. *Brain Res.*, **412**, 190–195.
- LE BARS, D., DICKENSON, A.H. & BESSON, J.-M. (1979). Diffuse noxious inhibitory controls (DNIC). II. Lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications. *Pain*, **6**, 305–327.
- MILLAN, M.J. (1989). Kappa-opioid receptor-mediated antinociception in the rat. 1. Comparative actions of mu- and kappa-opioids against noxious thermal, pressure and electrical stimuli. *J. Pharmacol. Exp. Ther.*, **251**, 334–341.
- MILLAN, M.J. (1990). Kappa opioid receptors and analgesia. *Trends Pharmacol. Sci.*, **11**, 70–76.
- PARSONS, C.G. & HEADLEY, P.M. (1989a). Spinal antinociceptive actions of  $\mu$ - and  $\kappa$ -opioids: the importance of stimulus intensity in determining 'selectivity' between reflexes to different modalities of noxious stimulus. *Br. J. Pharmacol.*, **98**, 523–533.
- PARSONS, C.G. & HEADLEY, P.M. (1989b). On the selectivity of intravenous  $\mu$ - and  $\kappa$ -opioids between nociceptive and non-nociceptive reflexes in the spinalised rat. *Br. J. Pharmacol.*, **98**, 544–552.
- PARSONS, C.G., WEST, D.C. & HEADLEY, P.M. (1989). Spinal antinociceptive actions and naloxone reversibility of intravenous  $\mu$ - and  $\kappa$ -opioids in spinalised rats: potency mismatch with values reported for spinal administration. *Br. J. Pharmacol.*, **98**, 533–544.
- PIERCEY, M.F., LAHTI, R.A., SCHROEDER, L.A., EINSPAHR, F.J. & BARSUHN, C. (1982). U-50488H, a pure kappa receptor agonist with spinal analgesic loci in the mouse. *Life Sci.*, **31**, 1197–1200.
- STEEDMAN, W.M. & ZACHARY, S. (1990). Characteristics of background and evoked discharges of multireceptive neurons in the lumbar spinal cord of the cat. *J. Neurophysiol.*, **63**, 1–15.
- SURMEIER, D.J., HONDA, C.N. & WILLIS, W.D. (1989). Patterns of spontaneous discharge in primate spinothalamic neurons. *J. Neurophysiol.*, **61**, 106–115.
- WIKLER, A. (1950). Sites and mechanisms of action of morphine and related drugs in the central nervous system. *Pharmacol. Rev.*, **2**, 435–506.
- WILLER, J.C., BERGERET, S., DE BROUCKER, T. & GAUDY, J.H. (1988). Low dose epidural morphine does not affect non-nociceptive spinal reflexes in patients with postoperative pain. *Pain*, **32**, 9–14.
- WOOD, M.S., RODRIGUEZ, F.D. & TRAYNOR, J.R. (1989). Characterisation of  $\kappa$ -opioid binding sites in rat and guinea-pig spinal cord. *Neuropharmacol.*, **28**, 1041–1046.
- YAKSH, T.L. (1978). Inhibition by etorphine of the discharge of dorsal horn neurons: effects on the neuronal response to both high and low threshold sensory input in the decerebrate spinal cat. *Exp. Neurol.*, **60**, 23–40.
- YAKSH, T.L. & NOUEIHED, R. (1985). The physiology and pharmacology of spinal opiates. *Annu. Rev. Pharmacol. Toxicol.*, **25**, 433–462.
- YOSHIMURA, M. & NORTH, R.A. (1983). Substantia gelatinosa neurones hyperpolarized *in vitro* by enkephalin. *Nature*, **305**, 529–530.
- ZIEGLGANSBERGER, W. & BAYERL, H. (1976). The mechanism of inhibition of neuronal activity by opiates in the spinal cord of the cat. *Brain Res.*, **115**, 111–128.

(Received September 7, 1990)

Revised January 6, 1991

Accepted January 16, 1991