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The contributions of μ-, δ- and κ-opioid receptors to the actions of endogenous opioids on spinal reflexes in the rabbit

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1 Spinal reflexes in the rabbit are suppressed tonically by endogenous opioids. The contributions made to this suppression by μ-, δ- and κ-opioid receptors have been investigated by studying the actions of a range of opioid antagonists and agonists on reflexes evoked by sural nerve stimulation in the ankle extensor gastrocnemius medialis (g.m.), and in the knee flexor semitendinosus (s.t.).

2 When given at a total dose of 88.5 μg kg^{-1} i.v., either of the universal opioid receptor antagonists (−)-naloxone and (−)-quadazocine enhanced the g.m. response to more than 7 times the pre-drug control values, and the s.t. reflex to 1.5 times controls. The effects of quadazocine were stereospecific. The selective δ antagonist ICI 174864 (3.5 mg kg^{-1} i.v. total) also augmented the g.m. reflex but only to twice pre-drug controls.

3 The μ-agonists fentanyl (100 μg kg^{-1}) and morphine (50 mg kg^{-1}) suppressed both g.m. and s.t. reflex responses to less than half control levels by a naloxone-reversible mechanism.

4 The κ-agonists bremazocine (50 μg kg^{-1} total), tifluadom (100 μg kg^{-1}), ethylketocyclazocine (200 μg kg^{-1}) and U50488H (1 mg kg^{-1}) potentiated the g.m. reflex and had variable effects on the s.t. response. Naloxone usually added to the facilitatory actions of these drugs. κ-Opioid receptor agonists also caused a profound, naloxone-reversible depression of arterial blood pressure.

5 It may be concluded that the endogenous opioid-mediated suppression of spinal reflexes in the rabbit is mediated mainly, if not exclusively, through μ-receptors. There are no known endogenous ligands which are specific for the μ-receptor, so in the present case it seems that selectivity is determined by the receptor population rather than by the ligand.

Introduction

In the spinalized rabbit, electrical stimulation of the sural nerve elicits a short latency reflex in the α motoneurones of the ipsilateral ankle extensor gastrocnemius medialis (g.m.), which is stereospecifically potentiated by low doses of the opioid antagonist naloxone (Catley *et al.*, 1983). The effects of naloxone were confined to ipsilateral extensor responses as the reflex evoked by sural nerve stimulation in the knee flexor semitendinosus (s.t.) was only weakly enhanced by the opioid antagonist. The results of this study suggested that endogenous opioids modulate the excitability of reflex pathways between skin and ipsilateral extensor motoneurones. A number of other investigators have demonstrated facilitatory effects of opioid antagonists on different reflexes in a variety of species (McClane & Martin, 1967; Goldfarb & Hu, 1976; Bell & Martin, 1977; Roppolo *et al.*, 1983; Duggan *et al.*, 1984) and it appears that the control of activity in reflex pathways is one function of the opioids present in the spinal cord.

Opioids exert a wide range of effects on neurones by interacting with at least three types of receptor known as μ, δ, and κ (see Kosterlitz, 1985 for review), all of which are present in rabbit spinal cord (Meunier *et al.*, 1983). Naloxone has only ten times greater affinity for the μ-receptor than for either the δ- or κ-sites (see Kosterlitz, 1985), so it is not possible to assign the effects of this drug on reflexes to an action at any particular opioid receptor. The present study was designed to investigate the types of opioid receptor involved in the suppression of reflexes in the rabbit. The role of δ-opioid receptors was evaluated by comparing the actions of the highly selective δ-receptor antagonist ICI 174864 (Cotton *et al.*, 1983) with those of the universal antagonists (−)-naloxone and (−)-quadazocine (formerly WIN 44441–3; Ward *et*

al., 1983). The contribution of κ-receptors was more difficult to assess because there is no selective antagonist for these sites. Therefore, the actions of two μ-agonists morphine and fentanyl (see Magnan *et al.*, 1982) were compared with those of a range of drugs which have been reported to be κ-receptor agonists:- the non-selective agents bremazocine (Römer *et al.*, 1980), ethylketocyclazocine (Ekc, see Magnan *et al.*, 1982), and tifluadom (Römer *et al.*, 1982) and the highly selective agonist U50488H (Vonvoigtlander *et al.*, 1983). Some of these data have already appeared in abstracts (Clarke & Ford 1985; 1986; Clarke *et al.*, 1986).

Methods

The experiments were performed on 61 rabbits of either sex, weighing between 2.1 and 3.6 kg. Anaesthesia was induced by intravenous injection of methohexitone sodium (Brietal, Eli Lilly, 20 mg initially), and maintained after tracheal cannulation by halothane (May & Baker or Fluothane, ICI) in oxygen or oxygen and nitrous oxide (30:70). One carotid artery and one jugular vein were cannulated and the second carotid artery was ligated. End tidal CO_2 and arterial blood pressure were monitored in all rabbits and the ECG and heart rate were recorded in most animals. Core temperature was maintained at 38° ± 0.5°C by a thermostatically-controlled heating blanket. A laminectomy was performed at the thoraco-lumbar junction and the spinal cord was divided. The rabbits were then decerebrated to the pre-collicular level by suction and anaesthesia was discontinued. The animals were paralysed with gallamine triethiodide (Flaxedil, May & Baker) and artificially ventilated with room air at a rate which kept the end-tidal CO_2 below 5.5% (i.e. a P_{CO_2} of 40 mmHg).

Reflex responses were initiated by electrical stimulation of the cut left sural nerve at between 5 and 6 times threshold and recorded from the ipsilateral g.m. and s.t. muscle nerves to be quantified as the voltage/time integral (area) according to methods previously described (Catley *et al.*, 1983). No reflexes were recorded for at least one hour after cessation of anaesthesia. Responses were recorded alternately from g.m. and s.t. every 2 min throughout each experiment, and were allowed to stabilize over a control period of 30 to 70 min before any drugs were given. The threshold for each reflex was recorded every 10 min: this was done by turning the stimulator voltage to zero and gradually increasing the output until a consistent response could be detected in the relevant neurogram. The thresholds for reflexes were expressed in multiples of the threshold of the most excitable axons in the sural nerve (which is abbreviated hereafter to T_{su}).

Drugs were given in increasing doses at 24 min intervals after the reflexes had stabilized. The absolute sizes of control responses in the g.m. nerve were widely variable between rabbits: in the present experiments the range was 6 to 880 μV.ms and the data were therefore normalized. In each experiment the effects of any one treatment were assessed by taking the mean of all responses recorded after that treatment and before the application of the next one, and expressing this value as a percentage of the mean of the control responses. Cumulative dose-effect curves were constructed regardless of any inactivation of materials that may have occurred between doses. This approach was adopted because there is little information on the disposition and metabolism of many of these drugs in the rabbit, and the only quantities which could be determined with certainty were the actual amounts administered.

The following drugs and regimes were used: (−)-naloxone hydrochloride was made up in Ringer-Dale solution or 0.9% w/v NaCl solution (saline) to either 1 or 2 mg ml^{-1} and was given in doses of 0.5, 1, 2, 5, 10, 20, 50, 100 and 200 μg kg^{-1} (i.e. a total dose of 388.5 μg kg^{-1}) to 11 rabbits: one other animal was started at 5 μg kg^{-1}. (−)-Quadazocine methane sulphonate was dissolved in 5% dextrose at 5 mg ml^{-1} and diluted from this stock. It was given in doses of 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 μg kg^{-1} (a total dose of 888.5 μg kg^{-1}) i.v. to 6 rabbits. In 3 animals (+)-quadazocine 500 μg kg^{-1} was given before the (−)-enantiomer. ICI 174864 (the arginine salt) was made up to a strength of 4 mg ml^{-1} in saline with a few drops of 0.1 M $NaHCO_3$ and given to 3 rabbits in doses of 0.5, 1, 2 and 3 mg kg^{-1} (total 6.5 mg kg^{-1} i.v.). (−)-Quadazocine (500 μg kg^{-1}) was given 1 h after the last dose of ICI 174864. Morphine sulphate was obtained in commercial solutions of 10 and 30 mg ml^{-1}. It was given to a total of 9 animals in doses of 1, 9, 40, 50, 400 and 500 μg kg^{-1} proceeding to 1 and 3 mg kg^{-1} (total 5 mg kg^{-1}) to 4 animals: in five other animals further doses of 5, 10 and 30 mg kg^{-1} (total 50 mg kg^{-1}) were given and in three a final dose of 50 mg kg^{-1} was used, giving a total dose of 100 mg kg^{-1}. Fentanyl (citrate: Sublimaze, Janssen) was obtained in the commercial preparation of 50 μg ml^{-1} and given in doses of 0.5, 0.5, 1, 3, 5, 10, 30 and 50 μg kg^{-1} (total 100 μg kg^{-1}) to 6 rabbits. (±)-Bremazocine hydrochloride was dissolved to 1 mg ml^{-1} in 5% dextrose and administered in doses of 0.5, 0.5, 1, 3, 5, 40 and 50 μg kg^{-1} (total 100 μg kg^{-1}) to 6 rabbits: in three animals further doses were given up to 0.5–2 mg kg^{-1}. (±)-Ethylketocyclazocine methane sulphonate (Ekc) was made up in 5% dextrose to 10 mg ml^{-1} and given in doses of 1, 4, 5, 40, 50, 100 and 300 μg kg^{-1} (total 500 μg kg^{-1}) to 6 rabbits: in three animals further doses were given up to 1–5 mg kg^{-1}. (±)-Tifluadom hydrochloride was dissolved in distilled water to 0.5 mg ml^{-1} (warmed to 38°C and continuously agitated) and given in doses of

1, 4, 5, 10, 30, 50, 100, 300 $\mu g\,kg^{-1}$ (total 500 $\mu g\,kg^{-1}$) to 6 rabbits, and in 4 animals the dose was increased to 1 $mg\,kg^{-1}$. (±)-U50488H was prepared in 5% dextrose to a strength of 20 $mg\,ml^{-1}$ and given in doses of 10, 40, 50, 400 and 500 $\mu g\,kg^{-1}$ proceeding to 4 $mg\,kg^{-1}$ (total 5 $mg\,kg^{-1}$), in 6 rabbits. Pentazocine lactate (Fortral, Sterling-Winthrop) was obtained in the commercial solution of 30 $mg\,ml^{-1}$ and given to one rabbit in doses ranging from 0.1 to 10 $mg\,kg^{-1}$.

After the last dose of each agonist drug, naloxone was given in a dose of 250 $\mu g\,kg^{-1}$. Each drug injection was flushed in with 1 ml of Ringer solution. It should be noted that large quantities (up to 5 ml) of 5% dextrose solution or 1 M $NaHCO_3$ had no effect on either reflex when given alone.

The following drugs were gifts from the sources named: quadazocine, Ekc and naloxone (Sterling-Winthrop Research, Guildford, Surrey); bremazocine and tifluadom (Dr D. Römer, Sandoz, Basle, Switzerland); ICI 174864 (N,N, diallyl-Tyr-aminoisobutyric acid-aminoisobutyric acid-Phe-Leu-OH; (Mr R. Cotton, ICI, Alderley Park, Cheshire); and U50488H (*trans*-3,4-dichloro-N-methyl-(2-(1-pyrrolidinyl) cyclohexyl) benzeneacetamide; the Upjohn Company, Kalamazoo, Michigan, U.S.A.).

Results

Naloxone, quadazocine and ICI 174864

(+)-Quadazocine (500 $\mu g\,kg^{-1}$) had no effect on the amplitude, latency or threshold of either the sural-g.m. or sural-s.t. reflexes (Figure 1). (−)-Quadazocine and (−)-naloxone caused dose-dependent increases in the sizes of both sural-g.m. and sural-s.t. responses (Figures 1 and 2). The size of the g.m. reflex was significantly greater than control when the total dose of each drug reached 1.5 $\mu g\,kg^{-1}$ (paired t tests, $P<0.01$ for both: these doses are 4.1 nmol and 3.1 $nmol\,kg^{-1}$ for naloxone and quadazocine, respectively), and the response in s.t. was increased to significant levels when the total dose of each drug reached 18.5 $\mu g\,kg^{-1}$ (51 and 39 $nmol\,kg^{-1}$: paired t tests, $P<0.025$ in both cases). There were significant decreases in the threshold and latencies for both extensor and flexor reflexes with naloxone and quadazocine (Figure 2). For the g.m. reflex the control values for these variables were significantly greater in the naloxone-treated animals than in those receiving quadazocine (unpaired t test, $P<0.05$), but after administration of the opioid antagonists the latencies in both groups approached the same value and were not significantly different from each other (unpaired t test, $P>0.05$, see Figure 2). The thresholds of both reflexes were not significantly different from T_{su} in the presence of saturating doses of (−)-quadazocine or naloxone (unpaired t test, $P>0.05$, Figure 2).

Although the sural nerve was cut, it was possible to evoke reflex activity in g.m. and s.t. motoneurones by mechanical stimulation of the heel. In untreated rabbits it was necessary to apply a rough scraping or pinching stimulus to elicit these responses. In animals treated with naloxone or quadazocine, there was a higher level of spontaneous discharge in the motor nerves and the motoneurones of both g.m. and s.t. discharged in response to even the lightest brushing of the fur over the heel (e.g. Figure 3).

The δ antagonist ICI 174864 enhanced the sural-g.m. reflex to 216% of controls when given at 3.5 $mg\,kg^{-1}$ (total dose). No further increase was seen when the dose was increased to 6.5 $mg\,kg^{-1}$. The effects of this drug were no longer apparent between 26 and 48 min after the last injection. There were no changes in the sural-s.t. response (Figure 2). When the animals that had previously received ICI 174864 were given (−)-quadazocine (500 $\mu g\,kg^{-1}$) the g.m. reflex increased in size to 792 ± 140% of pre-drug levels, the threshold fell to a mean of $1.3 \pm 0.3 \times T_{su}$ and the latency decreased to a mean of 5.7 ± 0.27 ms. The corresponding values for the s.t. response were 128 ± 14%, $1.2 \pm 0.2 \times T_{su}$ and 5.2 ± 0.55 ms. None of these values was significantly different from the results obtained when using saturating doses of (−)-quadazocine alone (unpaired t tests, $P>0.05$).

Fentanyl and morphine

Fentanyl and morphine were inhibitory to both reflexes, but not to the same extent. Fentanyl suppressed the g.m. response to 5.6% of control levels when the total dose was 100 $\mu g\,kg^{-1}$, and there were concomitant increases in the threshold and latency of the reflex (Figure 4). The same dose of fentanyl depressed the s.t. reflex to 33% of controls and also significantly increased the threshold and latency of this response (Figure 4, paired t tests, $P<0.025$ and 0.05). The approximate ED_{50}s for fentanyl were 20 $\mu g\,kg^{-1}$ (total dose) for the g.m. response and 50 $\mu g\,kg^{-1}$ total for the flexor reflex. The half-life of fentanyl was very short, in the order of 10 or 12 min, which means that this material is even more potent than it appears from Figure 4.

Morphine was between 500 and 1000 times less potent than fentanyl on a molar basis (Figure 4), and could not suppress the g.m. reflex to the same level that was achieved with fentanyl. Moreover, morphine did not distinguish between the extensor and flexor reflexes (Figure 4), the approximate ED_{50}s being 10 $mg\,kg^{-1}$ total for both responses. The increases in thresholds and latencies were not statistically significant (paired t test, $P>0.05$). Doses of morphine from 10 to 100 $\mu g\,kg^{-1}$ caused a small but significant increase in the size of the g.m. reflex ($P<0.05$ for all

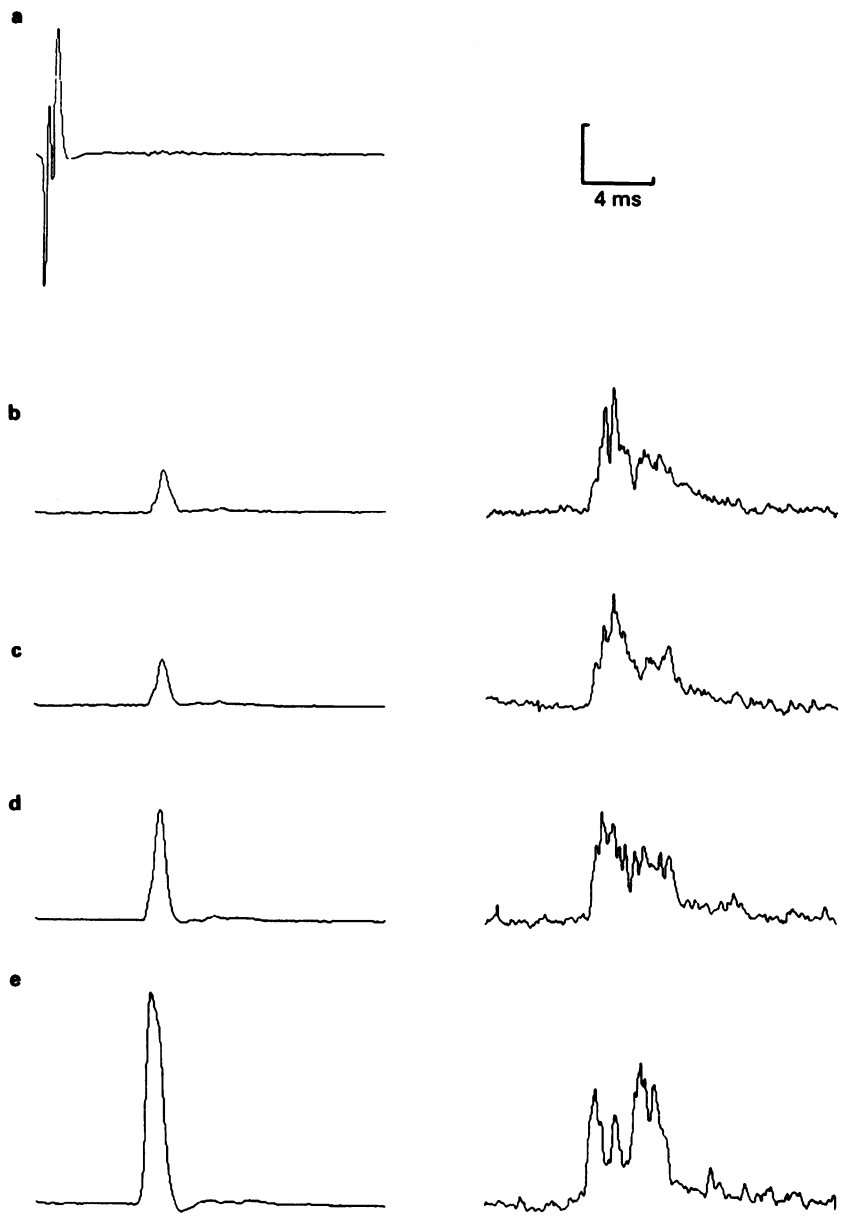

Figure 1 A demonstration of the stereospecificity of quadazocine. (a) Shows the sural volley evoked by stimulation at 6 times threshold: only an Aβ wave is visible. Below (b – e) are reflex responses to that volley, recorded from the ankle extensor gastrocnemius medialis (g.m., left) and from the knee flexor semitendinosus (s.t., right). These were taken in (b) the control period, (c) after (+)-quadazocine 500 $\mu g\,kg^{-1}$ i.v., (d) after (−)-quadazocine 5 $\mu g\,kg^{-1}$ and (e) after (−)-quadazocine 50 $\mu g\,kg^{-1}$. All records are the average of 8 sweeps and the stimulus was applied at the beginning of each trace. The voltage scale is 400 μV for the sural volley, 200 μV for the g.m. records and 50 μV for s.t.

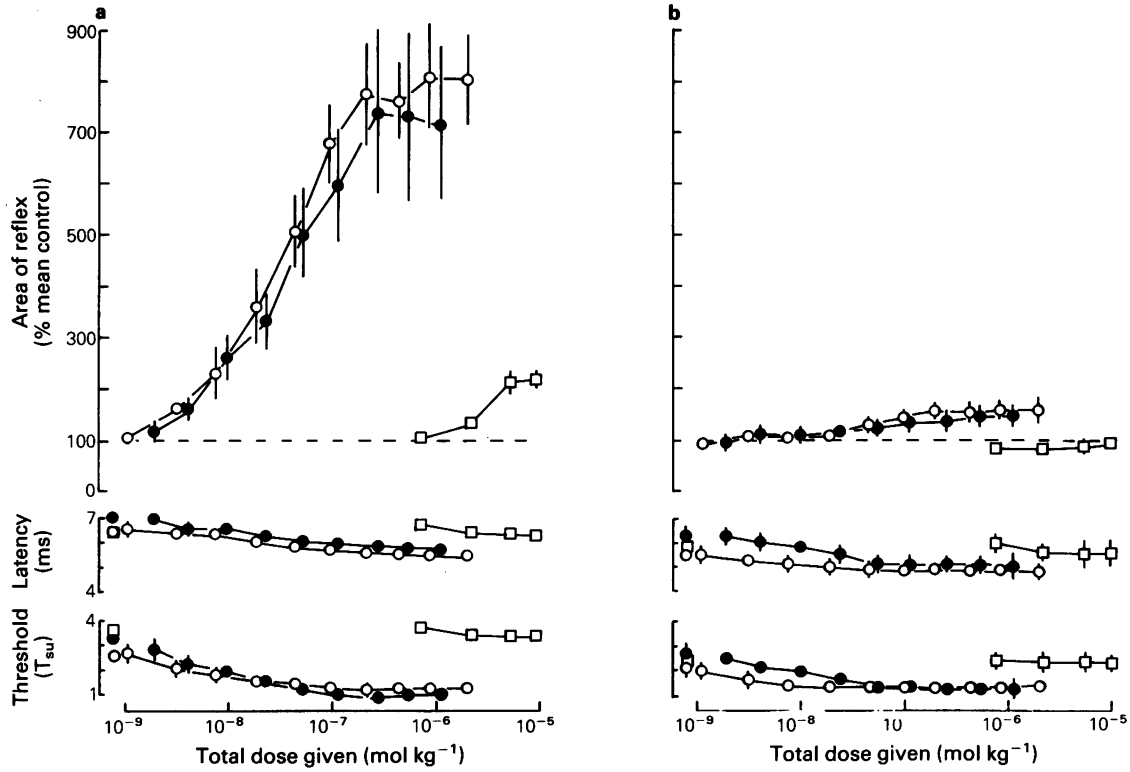

Figure 2 Cumulative dose-effect curves for (−)-quadazocine (○), (−)-naloxone (●) and ICI 174864 (□) on the magnitude (area), latency and thresholds of the sural-g.m. (a) and sural-s.t. (b) reflexes. The unjoined points at the far left of the threshold and latency graphs are the control values. All points are the means with vertical lines showing s.e.means.

doses; paired *t* tests).

All effects of fentanyl and morphine were completely reversed by naloxone given at a dose of 250 μg kg^{-1}: increasing the dose to 500 μg kg^{-1} produced no further effects. The sizes and thresholds of the responses were not significantly different from those obtained in the presence of a saturating dose of naloxone given alone (unpaired *t* tests, $P < 0.05$ for all parameters).

Bremazocine, tifluadom, ethylketocyclazocine and U50488H

The κ-agonists all produced enhancement rather than depression of the extensor response (Figure 5). Bremazocine was the most potent, giving maximum potentiation to 465% of controls with a total dose of 50 μg kg^{-1}. Tifluadom caused the response to increase to a mean of 417% with a total dose of 100 μg kg^{-1}, and the maximum effect of Ekc was to augment the reflex to 333% at 200 μg kg^{-1} (total dose). The maximal effects of these three drugs were not significantly different from each other (unpaired *t* tests). U50488H was significantly (unpaired *t* test, $P < 0.025$) less effective than the other three drugs, the response reaching only 236% of controls with a total dose of 1 mg kg^{-1} (Figure 5). In the one rabbit to which it was given, pentazocine also increased the g.m. response to 650% of controls with a total dose of 5 mg kg^{-1}. The increases in the size of the response were associated with a tendency for the thresholds and latencies to decrease but only rarely did these become statistically significant (Figure 5). Bremazocine, when given at doses above 1 mg kg^{-1} (2 rabbits), ekc when given at more than 200 μg kg^{-1} and tifluadom at doses greater than 100 μg kg^{-1} caused depression of the sural-g.m. response but not to below pre-drug control levels. With tifluadom and Ekc this effect was of short duration and was often followed by further enhancement of the response.

Bremazocine and tifluadom both enhanced the s.t. response, Ekc had no significant effect and with

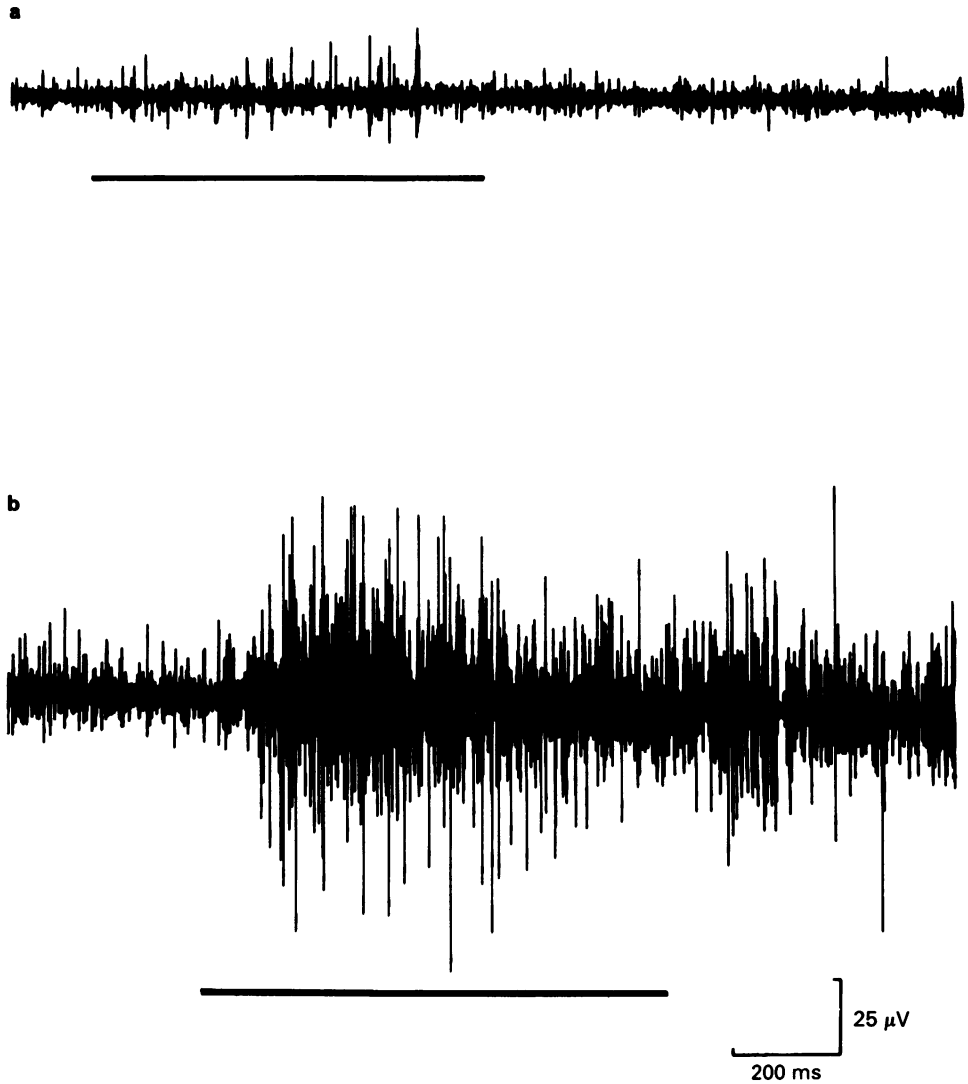

Figure 3 Activity recorded from the whole g.m. muscle nerve in response to light mechanical stimulation of the heel. The signal was filtered between 500 Hz and 5 kHz for these recordings. Each trace is a single sweep which was stored on a Gould OS4202 digital storage oscilloscope and plotted on a Bryans 2400 X-Y recorder. The stimulus was a single stroke using the long axis of a soft brush (Gallenkamp BU-620, 25 × 3 mm), which lasted for the approximate period indicated by the black bar. (a) Recorded in the pre-drug control period, (b) was recorded 3 min after the administration of a single dose of naloxone (100 $\mu g\,kg^{-1}$ i.v.): note that spontaneous activity was also increased after naloxone.

U50488H the reflex decreased to below control levels but not in a dose-related manner (Figure 5). There were no significant effects on the threshold or latency of this reflex.

When naloxone (250 $\mu g\,kg^{-1}$) was given after tifluadom, Ekc or U50488H the g.m. and s.t. responses always increased further, whatever the final dose of the agonist had been. Increasing the dose of naloxone to 500 $\mu g\,kg^{-1}$ did not produce any further enhancement of reflexes. The effects of naloxone given after bremazocine depended upon the final dose of this drug. If the dose of bremazocine was restricted to 100 $\mu g\,kg^{-1}$, as it was in 3 animals, then the dose of naloxone 250 $\mu g\,kg^{-1}$ caused further enhancement of

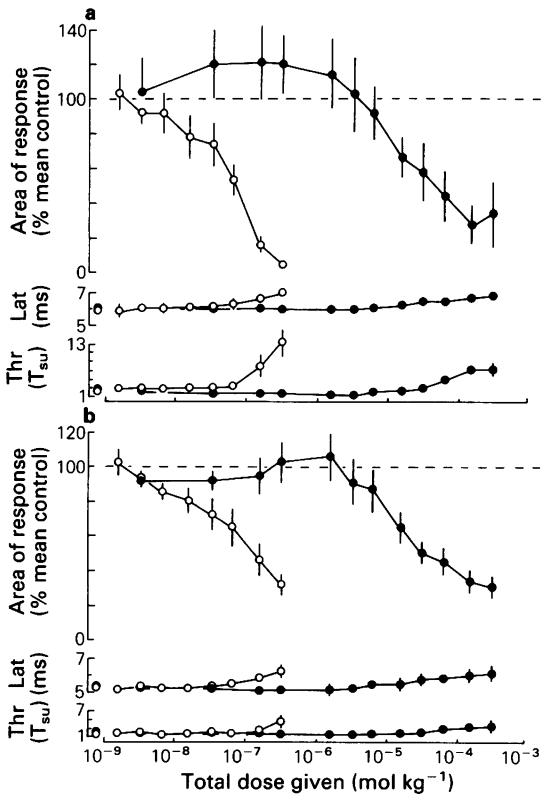

Figure 4 Cumulative dose-effect curves for the effects of fentanyl (○) and morphine (●) on the area, latency (Lat) and thresholds (Thr) of the gastrocnemius (a) and semitendinosus (b) reflexes. The unjoined points at the far left of the threshold and latency graphs are the control values. All points are the means and vertical lines show s.e.means.

the g.m. reflex response. If, on the other hand, the dose of bremazocine exceeded this 100 μg kg^{-1} level then doses of naloxone up to 1 mg kg^{-1} failed to produce a further increase in the size of the response. Otherwise, the size, thresholds and latencies for both g.m. and s.t. reflexes after naloxone were not significantly different from those obtained when saturating doses of naloxone or quadazocine were given alone.

Cardiovascular actions

None of the opioid antagonists had any consistent effects on cardiovascular function. Fentanyl appeared to cause a decrease in blood pressure but this was not statistically significant, whereas morphine had a clear hypotensive action (Table 1). Doses of morphine greater than 5 mg kg^{-1} did not produce further falls in blood pressure but were accompanied by transient episodes of rapidly increasing blood pressure and heart rate. Of the κ-opioids, tifluadom, Ekc and U50488H all reduced blood pressure to a lower level than either of the μ-agonists, and at high doses caused a profound bradycardia (Table 1). The hypotensive actions of κ-opioids could not be prevented by bilateral cervical vagotomy. In five animals, including the one given pentazocine, total cardiovascular collapse occurred with high doses of κ-opioids: otherwise all of the depressor effects of opioids were reversed by naloxone at 250 μg kg^{-1}.

Discussion

The sural-gastrocnemius and sural-semitendinosus reflexes

The object of this study was to obtain information on the opioid receptors involved in the tonic, opioid-mediated suppression of spinal reflexes in the rabbit as described by Catley *et al.* (1983). It is useful first to consider the nature of the responses studied. Although the usual pattern of reflexes evoked by stimulation of the skin of a limb is excitation of the flexors with concurrent inhibition of the extensors of that limb (Sherrington, 1910), it is well established that extensor motoneurones receive an excitatory input from skin areas directly overlying their target muscles (Hagbarth, 1952, Catley *et al.*, 1983). As the gastrocnemius muscle lies beneath skin which is innervated by the sural nerve, the sural-g.m. reflex is a manifestation of the latter rather specialized type of response, whereas sural-s.t. is an example of the more usual flexor reflex. More detailed discussion of the physiological significance of these reflexes and their differential control by endogenous opioids can be found in publications by Clarke (1982) and Catley *et al.* (1983).

The effects of opioid antagonists

The actions of (−)-naloxone, and (−)- and (+)-quadazocine were entirely consistent with previous observations on the sural-g.m. and sural-s.t. reflexes (Catley *et al.*, 1983). The data obtained with these drugs provide hard evidence that, in spinalized rabbits, endogenous opioids exert a tonic suppressive action on these reflexes which is mediated by stereospecific receptors. The availability of reasonable quantities of the inactive stereoisomer of quadazocine means that this drug will prove to be a useful substitute for naloxone in future studies of this type (see Duggan & Johnson, 1983).

The use of high doses (i.e. >5 μg kg^{-1}) of opioid antagonists has revealed the full and quite remarkable

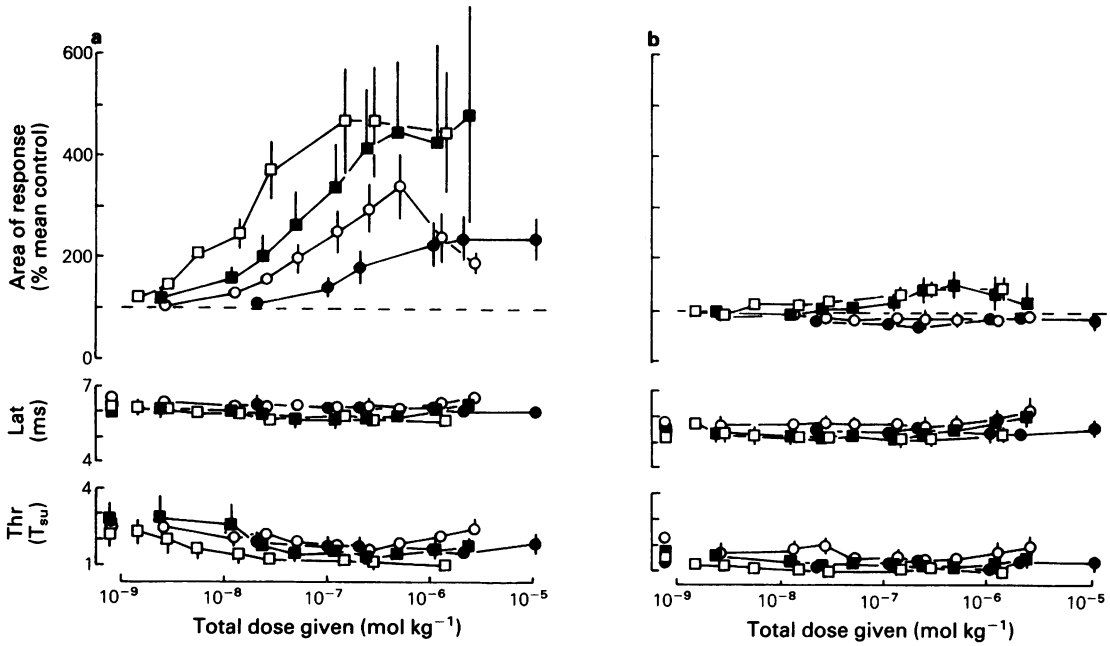

Figure 5 Cumulative dose-effect curves for the actions of bremazocine (□), tifluadom (■), ethylketocyclazocine (○) and U50488H (●) on the area, latency (Lat) and thresholds (Thr) of the sural-g.m. (a) and sural-s.t. (b) reflexes. The unjoined points at the far left of the threshold and latency graphs are the control values. All points are the means and vertical lines show s.e.means.

extent to which endogenous opioids suppress the g.m. reflex, and has confirmed that the influence of opioids on the flexor reflex is relatively weak. Naloxone and quadazocine also produced significant decreases in the thresholds to electrical stimulation of both reflexes, which were reflected in the increased sensitivity of motoneurones to light mechanical stimulation of the skin over the heel. This shows that one action of opioid antagonists is to release from inhibition the reflex drive from the largest diameter sural nerve afferents,

Table 1 The effects of μ- and κ-opioids on mean arterial blood pressure (MAP) and heart rate

Drug	*MAP* (mmHg)	*Heart rate* (beats min^{-1})
Control	61.4 ± 6.5	262 ± 16
Fentanyl 100 μg kg^{-1}	51.6 ± 5.6	245 ± 17
Control	63.8 ± 4.0	281 ± 20
Morphine 5 mg kg^{-1}	51.7 ± 1.5*	255 ± 33
Control	66.7 ± 4.8	246 ± 22
Bremazocine 100 μg kg^{-1}	44.5 ± 5.9**	245 ± 17
Control	73.5 ± 3.1	280 ± 10
Tifluadom 500 μg kg^{-1}	40.8 ± 5.4***	213 ± 11**
Control	66.3 ± 2.9	288 ± 29
Ethylketocyclazocine 500 μg kg^{-1}	27.5 ± 2.0***	230 ± 12**
Control	61.5 ± 3.2	258 ± 10
U50488H 5 mg kg^{-1}	34.0 ± 5.6**	194 ± 12*

All results are means ± s.e. Statistical comparisons were by paired *t* tests: *$P<0.05$; **$P<0.005$; ***$P<0.0005$, otherwise $P>0.05$.

and explains the decreases in latencies of the g.m. and s.t. responses obtained with these drugs. It is important to note that the responses of g.m. to selective stimulation of sural A δ fibres are also increased by naloxone (Catley *et al.*, 1983), and it seems that the endogenous opioids which act upon this reflex arc suppress responses evoked by all groups of myelinated afferent fibres. This is not consistent with the many reports which show that opioids selectively inhibit inputs to the spinal cord from small diameter or nociceptive afferents (see Duggan & North, 1984 for a review). However, it has been found that opioids suppress responses to all types of input when applied close to the cell bodies of neurones in laminae IV–V of the dorsal horn of the cat (Duggan *et al.*, 1977).

We can only speculate on the site of action of opioid antagonists in enhancing spinal reflexes. The work of Duggan & Zhao (1984) in the cat suggests that naloxone does not act directly at motoneurones, and there is no evidence for a tonic release of opioids onto the terminals of primary afferent fibres to produce presynaptic inhibition. It is most likely that endogenous opioids exert their actions on interneurones in the reflex pathways, but the location of these cells remains to be elucidated.

The actions of ICI 174864 could be construed as evidence that δ-opioid receptors mediate some of the effects of endogenous opioids on the g.m. reflex (Clarke & Ford, 1986). The δ-antagonist was roughly 500 times less potent than either of the two universal antagonists on a molar basis. Although this ratio might be expected from data obtained in receptor binding studies performed in Tris buffers (see Kosterlitz, 1985), the potency of ICI 174864 as a δ-antagonist has been found to be much closer to that of naloxone in binding studies performed in solutions containing sodium ions (Appelmans *et al.*, 1986), and in bioassays *in vitro* (Cotton *et al.*, 1983; Miller *et al.*, 1986). It was found to be more potent than naloxone when given subcutaneously to antagonize the effects of the selective δ-agonist [D-Pen^2, D-Pen^5] enkephalin applied intracerebroventricularly in the rat (Blackburn *et al.*, 1986). From these facts it would seem that the potency ratio between naloxone and ICI 174864 found in the present study was higher than would be expected, and it is likely that the doses of ICI 174864 used were large enough to have affected receptors other than δ-receptors. If these factors are taken into account, the present data suggest that δ-receptors make little or no contribution to the tonic actions of endogenous opioids in the sural-g.m. and sural-s.t. reflex pathways.

The effects of opioid agonists

The inhibitory actions of the μ-agonists, morphine and fentanyl, confirm that activation of μ-receptors causes suppression of reflex activity. The high potency of fentanyl in depressing the reflex discharges of motoneurones has been noted previously (Headley *et al.*, 1984), and the drug has been observed to have a beneficial effect in one case of spinal spasticity in man (Struppler *et al.*, 1983). That morphine produced less profound suppression of the g.m. reflex than did fentanyl suggests that it acted as a partial agonist on the receptors in this reflex arc. This probably arises from the low intrinsic activity of morphine (Smith & Rance, 1983; Miller *et al.*, 1986). Although there were differences between the reflexes in their sensitivity to fentanyl, the fact that μ-agonists suppressed both the g.m. and s.t. responses shows that neurones intercalated in the reflex pathways to both muscles carry opioid receptors of this type. As opioid antagonists selectively enhanced the extensor reflex, it is likely that the endogenous opioids involved in this system were released close to their site(s) of action in the spinal cord.

The effects of κ-opioids were completely unexpected: all of those used in the present study have been shown to depress the rat tail-flick reflex (Römer *et al.*, 1980; 1982; Vonvoigtlander *et al.*, 1983) and to suppress flexor reflex responses in spinalized dogs (Martin *et al.*, 1976) or rats (Headley *et al.*, 1984; 1985). It was therefore anticipated that these agents would also inhibit reflexes in the rabbit. Presumably the differences between the present data and those previously described are a consequence of studying different species and of the different types of stimuli used to evoke reflexes. The fact that κ-opioids enhanced the sural-g.m. reflex and did not suppress the response in s.t. shows that activation of these receptors cannot be important in the inhibitory effects of the endogenous opioids involved in this system, and raises the possibility that μ- and κ-receptors mediate opposing actions on the neurones in these reflex pathways. However, all of the κ-agonists used in this study have been shown to be antagonists against μ-receptors in isolated tissues (see Smith & Rance, 1983; Kosterlitz, 1985; Miller *et al.*, 1986), and might therefore have enhanced the sural-g.m. reflex by blockade of endogenous opioids acting at μ-receptors. We do not have sufficient evidence to reach a conclusion on whether the so-called κ-opioids potentiated reflexes by an agonist or an antagonist action, although the order of potency amongst these drugs in enhancing the sural-g.m. reflex was the same as that reported for their agonist effects in isolated tissues (Miller *et al.*, 1986). The development of a highly selective κ-antagonist would provide the best way of differentiating between the two possible modes of action of κ-opioids: the use of naloxone after giving these drugs in the present experiments simply replaced one form of facilitation with another and did not help to resolve this question. The inhibitory effects of κ-

opioids, which were seen with high doses of tifluadom and Ekc (but never with U50488H), may have been the result of residual μ-agonist activity (Hirning *et al.*, 1985; Hayes *et al.*, 1986). The suppressive actions of bremazocine appear to have been of a non-opioid nature.

The profound depression of the cardiovascular system produced by κ-opioids in the present experiments has also been seen in the pithed rabbit (Ensinger *et al.*, 1984; 1986; Szabo *et al.*, 1986). These authors have presented evidence that κ- and to a lesser extent δ-receptor activation reduces the release of noradrenaline from postganglionic sympathetic neurones, whereas μ-agonists are much less effective (Ensinger *et al.*, 1986; Szabo *et al.*, 1986). Such a mechanism would provide an adequate explanation for the results obtained in the present study. There are no reports of other species showing such sensitivity to κ-opioids and it is possible that this action is specific to the rabbit. The failure of opioid antagonists to have any consistent effects on blood pressure indicates that these peripheral receptors were not occupied by endogenous ligands in our preparation.

It is clear that the suppressive action of endogenous opioids on the sural-g.m. and sural-s.t. reflexes in the rabbit is mediated mainly, if not exclusively, through μ-receptors. None of the many endogenous opioids yet identified is completely selective for the μ-receptor (Kosterlitz, 1985), so it may be assumed that the neurones intercalated in these reflex pathways are influenced by opioid receptors which are predominantly of the μ-type. Other studies in the rabbit have revealed that single types of opioid receptors predominate in the cerebellum (Meunier *et al.*, 1983), in the vas deferens (Oka *et al.*, 1981), and on postganglionic sympathetic neurones (see Ensinger *et al.*, 1986). The interaction of non-selective ligands with specific groups of receptors is commonly found in other transmitter systems, and on current evidence this principle appears to apply generally to opioid transmission in the rabbit.

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