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A CLASSIFICATION OF OPIATE RECEPTORS THAT MEDIATE ANTINOCICEPTION IN ANIMALS

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1 To investigate the opiate receptors that mediate antinociception, the activity profiles of opioid analgesic drugs have been determined against different nociceptive stimuli in the mouse and rat.

2 In tests that employ heat as the nociceptive stimulus, μ-opiate receptor agonists, such as morphine, pethidine and dextropropoxyphene, had steep and parallel dose-response curves and were capable of achieving maximum effects. In addition, the antinociceptive potency ratios of these drugs in heat tests were similar to those for analgesia in man.

3 The κ-agonists, such as ethylketazocine, nalorphine, Mr2034 and pentazocine, were essentially inactive against heat nociception except at doses that caused sedation and motor incapacitation.

4 In the writhing and paw pressure tests both μ- and κ-agonists produced steep and parallel dose-response curves.

5 It is concluded that both μ- and κ-opiate receptors mediate antinociception in animals and that the interactions of analgesic drugs with these receptors may be classified in terms of their antinociceptive activities against qualitatively different nociceptive stimuli.

Introduction

There is considerable evidence that opiate receptors exist in differing forms. Martin, Eades, Thompson, Huppler & Gilbert (1976) and Gilbert & Martin (1976) showed that opioid drugs could be classified in terms of their effects on several parameters in the chronic spinal dog as being either morphine-like (μ-receptor), nalorphine-like (κ-receptor) or N-allylnormetazocine-like (σ-receptor). Hutchinson, Kosterlitz, Leslie, Terenius & Waterfield (1975), Lord, Waterfield, Hughes & Kosterlitz (1976) and Kosterlitz & Leslie (1978) have identified a further receptor in the mouse vas deferens for the binding sites of the naturally occurring opioid peptides. This latter receptor was designated the δ-receptor. The functions of these different receptors in relation to the many pharmacological actions of opioid drugs remains somewhat obscure.

On measuring the antinociceptive activities of opioid analgesic drugs in animals it has been observed that certain drugs that have potent analgesic activity in man such as nalorphine (Lasagna & Beecher, 1954) and pentazocine (Archer, Albertson, Harris, Pierson, Bird, Keats, Telford & Papadopoulos, 1962) are essentially inactive in hot plate and radiant heat tail flick tests in rodents. In contrast, the antinociceptive activities of these drugs can readily be detected in writhing tests in the mouse (Taber, Greenhouse & Irwin, 1964) and against tooth pulp stimulation in the dog (Skingle & Tyers, 1979).

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The aim of the present study was to investigate the differences between antinociceptive tests and to identify the opiate receptors that mediate the antinociceptive actions of opioid analgesic drugs.

Methods

The methods used to evaluate antinociceptive activity were selected to include tests which employed different types of noxious stimuli, i.e., heat, chemical, and pressure.

Tests in mice (male, AHM/I/ICI-derived, 18 to 22 g) and rats (male, AH random bred Hooded, 35 to 80 g) were all carried out under essentially the same experimental conditions. Individual tests were carried out using dose-groups of 6 animals. Data for the calculation of ED_{50} values were accumulated from 2 or 3 individual tests carried out on different days (< 7 days apart), such that the final dose-groups comprised 12 or 18 animals respectively. To eliminate cage interaction the animals were randomized into different cages such that each cage contained animals receiving different treatments. Because tests normally lasted for a full working day this latter procedure also randomized treatments with respect to time of day and thus eliminated any bias associated with temporal cycles of pain sensitivity. Animals and drug solutions were colour coded such that the operators were unaware of the treatments the animals were receiving. In each test, one or more drugs were compared with mor-

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phine and a placebo control in which 0.9% w/v NaCl solution (saline) was given in the same dose-volume.

In all tests, drugs were given subcutaneously 30 min before testing for antinociceptive activity. Drugs were dissolved in saline and injected in a dose volume of 0.2 ml/20 g body wt. The results presented refer to the free acid/base equivalent.

Antinociceptive activities (ED_{50}), 95% confidence limits, regression slopes, linearity and potency ratios, where applicable, were calculated by methods of Finney (1964).

Acetylcholine-induced writhing in the mouse

Tests were carried out to determine the inhibitory effects of the test drugs against writhing induced by acetylcholine in the mouse. Thirty minutes after subcutaneous injection of drug or placebo, mice were injected with acetylcholine, 3 mg/kg intraperitoneally. The number of writhes occurring in the first 5 min thereafter was recorded. A writhe was defined as a contraction of the abdominal muscles accompanied by an extension of the hind limbs. The ED_{50} value was defined as the dose of test drug capable of reducing by 50% the number of writhes occurring in placebo-treated mice.

Hot plate test in the mouse

In the hot plate test, reaction times of mice placed on a copper plate heated to a mean (±range) temperature of 55 ± 0.2°C were determined. A 'front paw lick' was taken as the nociceptive response at which time the animals were rapidly removed from the hot plate; 60 s was taken as the maximum reaction time. The ED_{50} value was arbitrarily defined as the dose of test drug capable of elevating the reaction time to twice that determined for placebo-treated animals.

Paw pressure test in the rat

The effects of test drugs on nociceptive pressure thresholds of both yeast-inflamed and non-inflamed hind paws were determined in rats. Initial nociceptive thresholds were determined for both hind paws in all of the rats using an 'Analgesymeter' (Ugo Basile, Milan). Each rat received an injection of 0.1 ml of 20% w/v suspension of Brewer's yeast (CWE Ltd., Cawston, Norfolk) given subcutaneously into the subplantar surface of the right hind paw and then returned to their test cages. Test drugs were injected subcutaneously 3.5 h after the yeast injection. Nociceptive thresholds were re-determined 30 min later, i.e. 4 h after the yeast injections. The nociceptive response was usually a shrill vocalisation which was clearly distinguishable from that which often occurred with normal handling. A few animals did not vocalize but reacted to the stimulus with strong attempts to withdraw their paws. Either reaction was taken as the nociceptive response.

For calculation of ED_{50} values, results relate to the mean pre-yeast nociceptive responses, called the 'mean 0 h responses', determined for both paws in all of the rats in the test. For yeast-inflamed paws the ED_{50} value was defined as the dose of test drug that raised the nociceptive threshold to a value half-way between the mean hyperesthesia determined for the yeast-inflamed paws of placebo-treated rats and the 'mean 0 h response'. For non-inflamed paws the ED_{50} was arbitrarily defined as the dose of test drug that raised the nociceptive threshold by 100 g above the 'mean 0 h response'.

Tail immersion tests in the rat

In the tail immersion tests the noxious stimulus was hot water maintained at either 50 or 55°C. Nociceptive reaction times, which were the times taken for the rats to 'flick' their tails or to withdraw them from the hot water, were determined. The ED_{50} value was arbitrarily defined as the dose of test drug that raised the reaction time to a value twice that determined for placebo control animals.

Drugs

The following drugs were used: morphine hydrochloride, codeine phosphate, pethidine hydrochloride (MacFarlan Smith); dextropropoxyphene (Dista); AH7921 [3,4-dichloro-*N*[(1-(dimethylamino) cyclohexyl)methyl]benzamide hydrochloride] (Glaxo Group Research Ltd.); (±)-ethylketazocine methanesulphonate, (±)-ketazocine methanesulphonate; pentazocine as the free base (Sterling-Winthrop); buprenorphine hydrochloride (Reckitt and Colman); Mr2034 as the free base [(−)-α-(1R, 5R, 9R)-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6, 7-benzomorphan], Mr1353 [(±)-α-5, 9-dimethyl-2-(3-methylfurfuryl)-2′-hydroxy-6, 7-benzomorphan methane-sulphonate] (Dr H. Merz, Boehringer-Ingelheim); nalorphine hydrobromide (Burroughs Wellcome); acetylcholine chloride (Sigma).

Results

Hot plate and tail immersion tests

The antinociceptive activities (ED_{50}) of the analgesic drugs tested in the hot plate (55°C) test in the mouse and tail immersion (50 and 55°C) tests in the rat are given in Table 1. Dose-response curves are shown in Figures 1 to 3. In these tests morphine, codeine, D-propoxyphene, pethidine and AH7921 produced

steep dose-response curves and reached maximum effects without causing any observable motor incapacitation (Figure 1). On lowering the temperature in the tail immersion test from 55°C to 50°C there was no significant change in the antinociceptive potency of these drugs. Buprenorphine, 0.5 to 5.0 mg/kg was effective in the hot plate test but produced a shallower dose-response curve than morphine and did not reach maximum effect (Figure 2a). Higher doses of buprenorphine, 5 to 20 mg/kg, in the hot plate test caused less effect, producing a bell-shaped dose-response curve. In the tail immersion (55°C) test (Figure 2) buprenorphine, 0.3 to 3.0 mg/kg, produced rather variable effects with shallow dose-regression and a low maximum (Figure 2b). In the lower temperature tail immersion (50°C) test buprenorphine, 0.06 to 0.5 mg/kg, produced a more consistent effect; it was about 4 times more potent than in the higher temperature test, but the dose-response curve was much shallower than that for morphine reaching only 40% of maximum. Furthermore, as in the hot plate test, the highest dose of buprenorphine, 0.5 mg/kg, produced less effect than the next lower dose of 0.25 mg/kg.

The benzomorphans, ethylketazocine and Mr1353, produced steep dose-response curves in both the hot plate and tail immersion tests (Figure 2 and 3) but in contrast to the effects of morphine the effective doses of these drugs also produced marked motor incapacitation, i.e., the animals were clearly unable to respond to the nociceptive stimulus. Pentazocine increased hot plate reaction latency and caused sedation in mice but had no effect in the tail immersion tests in the rat (Figure 2).

Similarly, ketazocine and Mr2034 produced very shallow dose-response curves in the hot plate test in the mouse as well as motor incapacitation (Figure 3a and 2a) but were inactive at 80 and 20 mg/kg respectively in both tail immersion tests in the rat. Nalorphine, 0.1 to 80 mg/kg was inactive in the hot plate and tail immersion tests and had no observable effects on behaviour.

Acetylcholine-induced writhing and paw pressure tests

The antinociceptive activities (ED_{50}) of the analgesic drugs tested in the acetylcholine-induced writhing test in the mouse and inflamed and non-inflamed paw pressure tests in the rat are given in Table 2. Dose-response curves are shown in Figures 4 to 6.

With the exception of ketazocine and nalorphine, all drugs tested produced parallel dose-response curves and reached maximum effects when tested in the acetylcholine-induced writhing and paw pressure tests. The responses to ketazocine, 0.1 to 10 mg/kg, in the writhing test (Figure 6a) were similar to those for the other drugs but in the paw pressure tests dose-response curves were shallow and did not reach maximum effect (Figure 6b, c). Nalorphine, 0.03 to 1.0 mg/kg, in the writhing test achieved only 75% inhibition (Figure 5a) and in the yeast inflamed and non-inflamed paw pressure tests the dose-response curves reached a ceiling at 35 and 55% of maximum respectively. Higher doses (> 1.0 mg/kg) of nalorphine in the

Table 1 Antinociceptive potencies of opioid analgesic drugs against heat-induced nociception in the mouse and rat

	Antinociceptive potencies ED_{50} mg/kg subcutaneously (95% confidence limits)		
	Hot plate (55°C) test	*Tail immersion tests (rat)*	
Drug	*(mouse)*	*50°C*	*55°C*
Morphine	1.7 (1.1–2.2)	0.7 (0.3–1.5)	0.6 (0.3–1.2)
Codeine	15.3 (5.6–39.7)	10.1 (5.9–16.6)	16.1 (5.5–42)
D-Propoxyphene	11.3 (5.5–22.7)	4.1 (1.9–8.3)	6.2 (2.9–12.9)
Pethidine	7.8 (2.9–19.8)	5.4 (2.7–10.3)	6.0 (1.9–17.6)
AH7921	1.8 (1.4–2.1)	0.6 (0.3–1.0)	0.8 (0.3–1.6)
Nalorphine	Inactive 0.1–80	Inactive 0.1–80	Inactive 0.1–80
Ketazocine	†~ 10	Inactive 1.0–80	Inactive 1.0–80
Pentazocine	28.1 (1.5–5.6)	Inactive 1.0–40	Inactive 1.0–40
Buprenorphine	*1.5 (0.1–39.0)	*0.09 (0.01–0.4)	*0.4 (0.1–3.5)
Ethylketazocine	0.41 (0.2–0.9)	1.3 (0.17–6.1)	1.4 (0.23–6.7)
Mr2034	†~ 3.0	Inactive 0.1–9.0	Inactive 0.1–9.0
Mr1353	20.0 (1.4–143)	7.3 (3.1–16.0)	5.7 (2.9–10.7)

Drugs were given subcutaneously, 30 min before evaluation for antinociceptive activity. Dose-response curves are given in Figures 1–3. * Values for buprenorphine were calculated from the rising linear portions of the dose-response curves.

† Shallow dose-response curves, only the highest doses were significant, $P < 0.05$ (see Figures 2 and 3).

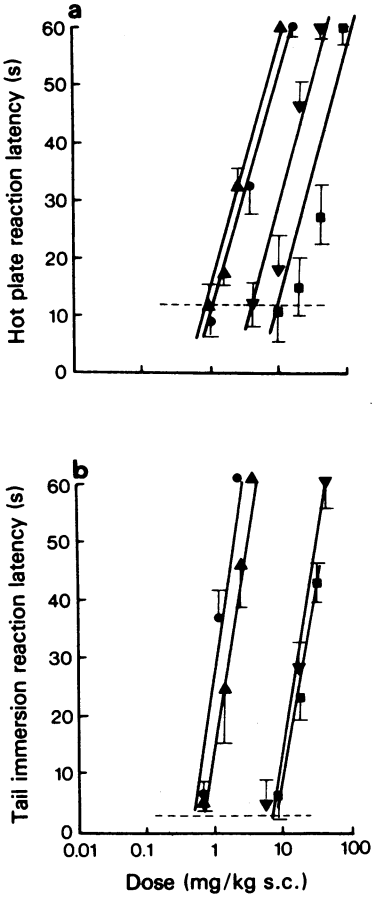

Figure 1 Effects of morphine (▲), AH7921 (●), pethidine (▼) and D-propoxyphene (■) on nociceptive reaction latencies in the hot plate (55°C) test in the mouse (a) and tail immersion (55°C) test in the rat (b). Broken lines are responses of placebo-treated animals; vertical lines show s.e. mean.

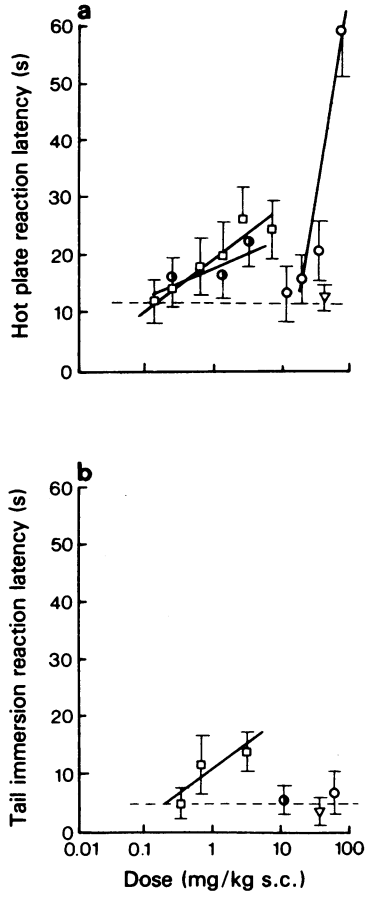

Figure 2 Effects of buprenorphine (□), Mr2034 (◑), nalorphine (▽) and pentazocine (○) on nociceptive reaction latencies in the hot plate (55°C) test in the mouse (a) and tail immersion (55°C) test in the rat (b). Broken lines are responses of placebo-treated animals; vertical lines show s.e. mean.

paw pressure tests produced less effect, and in the non-inflamed paw a dose of 3 mg/kg was not significantly different ($P > 0.05$) from placebo.

The potency differences for the actions of drugs in heat compared with non-heat tests ranged from one to six fold for morphine, codeine, D-propoxyphene, pethidine and AH7921; that is, these drugs were slightly more active in the pressure and writhing tests than in the hot plate and tail immersion tests. For the remaining drugs the potency differences ranged from 10 for Mr1353 which produced motor incapacitation in the mouse and rat at the same doses that prolonged reaction latencies, to greater than 400 (buprenorphine).

Statistical analyses

A principal components analysis was carried out on the antinociceptive data from rodent tests assuming that the log ED_{50}s were from a multivariant normal distribution. The aim of this analysis was to find a small number of linear combinations of the original 6 variables (tests) which could explain the majority of the total variance. Examination of the coefficients of these principle components revealed that 93% of the total variance fell within two components. The first (Y_1) explains 60% of the variance and is a measure of the overall antinociceptive activity in all of the tests. The second component (Y_2) accounts for 33% of the

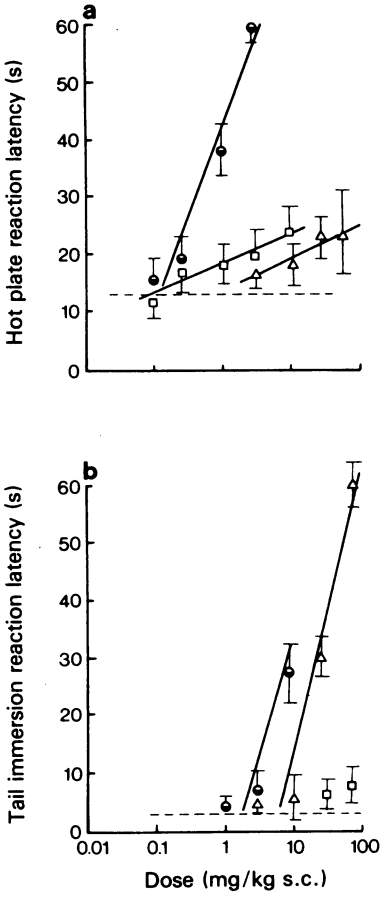

Figure 3. Effects of ethylketazocine (◒), ketazocine (□) and Mr1353 (△) on nociceptive reaction latencies in the hot plate (55°C) test in the mouse (a) and tail immersion (55°C) test in the rat (b). Broken lines are responses of placebo-treated animals; vertical lines show s.e. mean.

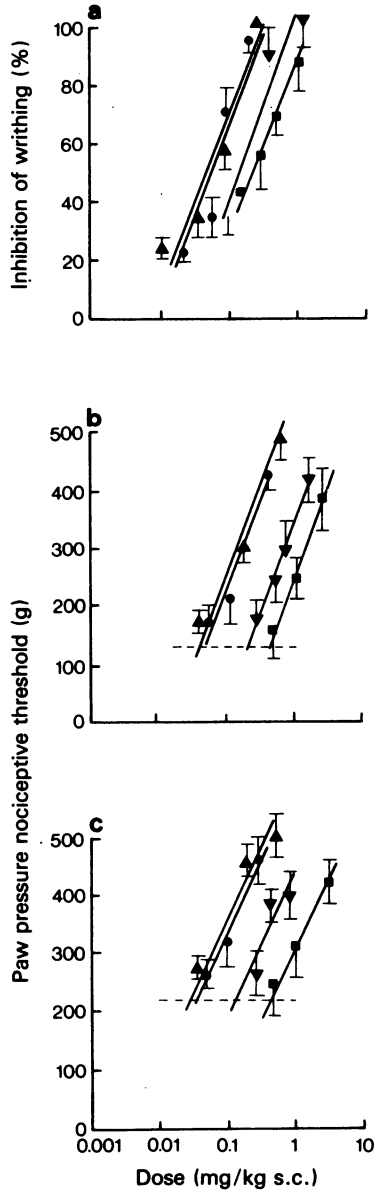

Figure 4 Antinociceptive actions of morphine (▲), AH7921 (●), pethidine (▼) and D-propoxyphene (■) in the acetylcholine-induced writhing test in the mouse (a) and in the inflamed (b) and non-inflamed (c) paw pressure tests in the rat. Broken lines are responses of placebo-treated animals; vertical lines show s.e. mean.

variance and clearly distinguishes the heat and non-heat tests. The remaining four components are less important. The principle component scores for each compound are given in Table 3. High values for Y_1 indicate high overall activity while high values for Y_2 indicate relatively high non-heat; heat activity. Values for Y_2 clearly distinguish μ-agonists (morphine, codeine, pethidine, AH7921, D-propoxyphene) and κ-agonists (nalorphine, buprenorphine and the benzomorphans tested).

Discussion

The classification of opiate receptors from data obtained in the chronic spinal dog (Martin *et al.*, 1976) is based on the pharmacological profiles of a range of opioid analgesic drugs on several parameters. Of these parameters, two were included to determine the antinociceptive activities of the drugs. These were the 'flexor reflex' response to a pressure stimulus and

the 'skin twitch' response latency to a radiant heat stimulus. Drugs classified as μ-opiate receptor agonists were about equally effective against both pressure and radiant heat stimuli while drugs that had high activity on κ-opiate receptors were effective against pressure nociception but less effective or inactive against radiant heat nociception. The benzomorphan SKF10047 (N-allynormetazocine), a σ-receptor agonist, was only very weakly active against the flexor reflex in the dog and was inactive in the phenylquinone-writhing test in the mouse (Pearl & Harris, 1966). It is unlikely therefore, that σ-receptors are involved in antinociception.

The results obtained in the present study show that the interactions of opioid analgesic drugs with their receptors may also be defined in terms of their antinociceptive activity profiles against different noxious stimuli in the conscious mouse and rat. The antinociceptive tests used may be divided into two categories according to their sensitivities to opioid analgesic drugs. The hot plate and tail immersion tests, in which heat is the nociceptive stimulus form one category and writhing and pressure tests form another.

Morphine-like compounds, including D-propoxyphene, pethidine, codeine and AH7921 are μ-opiate receptor agonists. That is, they strongly and completely suppress the abstinence syndrome in withdrawn morphine-dependent monkeys and/or dogs (Deneau & Seevers, 1964; Martin *et al.*, 1976). In the isolated ileum of the guinea-pig and mouse vas deferens preparations, their potency ratios are the same as that for morphine (Hughes, Kosterlitz & Leslie, 1975). Results obtained in the present study show that the μ-opiate receptor agonists are approximately equipotent in heat and non-heat antinociceptive tests. In these tests they produced steep dose-response curves and were able to reach maximum effect. In addition, the ranked order of potency for these drugs in all of the animal tests studied is the same as the order of potency for their analgesic potencies in man. This indicates that for μ-agonists, antinociceptive activities in both heat and non-heat tests are predictive of analgesic potencies in man.

The remaining drugs in this study are predominantly agonists on the κ-receptor. That is, they do not substitute for morphine in the dependent monkey (Villarreal & Seevers, 1972; Swain & Seevers 1974; 1976); in the chronic spinal dog they have a profile of action that differs from that of the μ-agonists (Martin *et al.*, 1976) and in the mouse isolated vas deferens they are more potent than in the guinea-pig ileum relative to morphine; in both preparations more naloxone is required to antagonize their effects than those of the μ-agonists (Hutchinson *et al.*, 1975). Results obtained in the present study indicate that in antinociceptive tests the κ-agonists are significantly more effective against writhing and pressure noxia than against heat-induced nociception. These drugs are also very potent against tooth pulp stimulation in the dog (Skingle & Tyers, 1979; 1980). Of the drugs tested, the most selective agonist on κ-receptors was the benzomorphan, Mr2034. Ethylketazocine, pentazocine and Mr1353 were also more potent against responses to non-heat stimuli than against heat-

Table 2 Antinociceptive potencies of opioid analgesics drugs against chemical and pressure-induced pain in the mouse and rat

	Antinociceptive potencies ED_{50} mg/kg subcutaneously (95% confidence limits)		
	Acetylcholine writhing (mouse)	*Inflamed paw pressure (rat)*	*Normal paw pressure (rat)*
Morphine	0.45 (0.2–0.9)	0.43 (0.3–0.5)	0.50 (0.3–0.8)
Codeine	5.0 (2.0–9.4)	2.1 (1.2–3.1)	3.6 (2.1–5.2)
D-Propoxyphene	1.5 (0.7–3.2)	2.7 (1.6–4.6)	10.2 (4.8–21.8)
Pethidine	2.9 (1.9–3.8)	1.1 (0.6–1.7)	2.0 (1.1–3.4)
AH7921	0.59 (0.5–0.8)	0.57 (0.5–0.7)	0.67 (0.5–0.8)
Nalorphine	*0.12 (0.06–0.3)	*0.08 (0.01–0.2)	*0.30 (0.05–1.7)
Ketazocine	0.29 (0.1–0.8)	*0.12 (0.01–0.3)	*0.37 (0.1–0.9)
Pentazocine	1.4 (0.5–4.0)	1.6 (0.9–2.5)	1.9 (1.0–3.3)
Buprenorphine	0.004 (0.001–0.008)	0.001 (0.0005–0.002)	0.001 (0.0005–0.004)
Ethylketazocine	0.12 (0.04–0.3)	0.08 (0.04–0.13)	0.14 (0.07–0.27)
Mr2034	0.06 (0.02–0.16)	0.05 (0.03–0.08)	0.07 (0.04–0.1)
Mr1353	1.1 (0.4–3.0)	0.72 (0.3–1.2)	1.1 (0.5–1.9)

Drugs were given subcutaneously, 30 min before assessment of antinociceptive activity. Dose-response curves are shown in Figures 4–6. * Values for nalorphine and ketazocine were calculated from the rising linear portions of the dose-response curves.

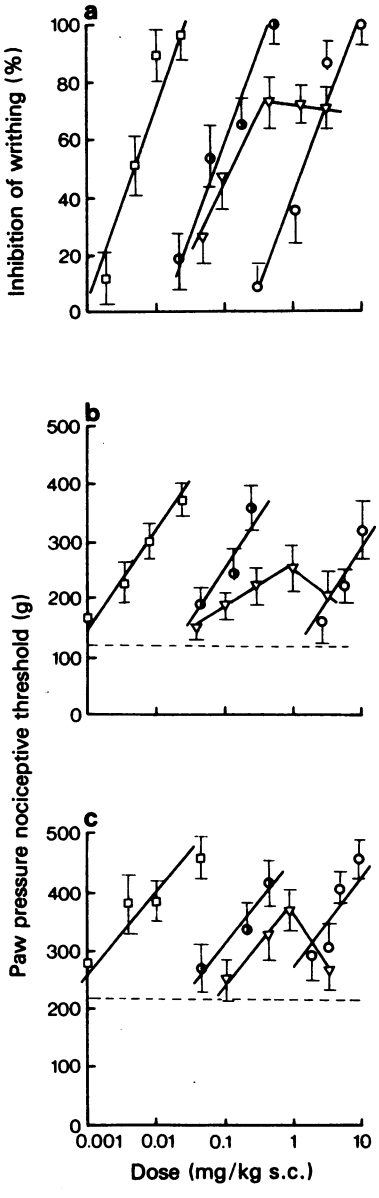

Figure 5 Antinociceptive actions of buprenorphine (□), Mr2034 (◑), nalorphine (▽) and pentazocine (○) in the acetylcholine-induced writhing test in the mouse (a) and in the inflamed (b) and non-inflamed (c) paw pressure tests in the rat. Broken lines are responses of placebo-treated animals; vertical lines show s.e. mean.

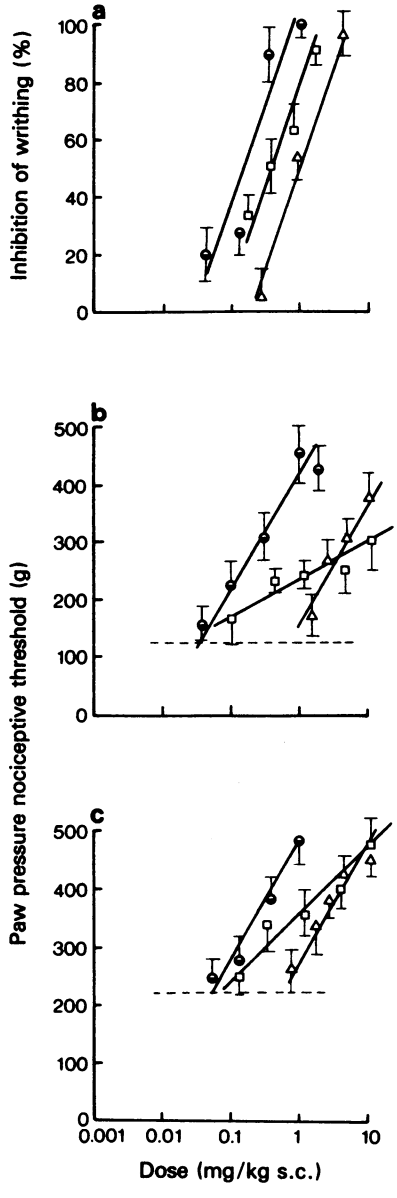

Figure 6 Antinociceptive effects of ethylketazocine (⊖), ketazocine (□) and Mr1353 (△) in the acetylcholine-induced writhing test in the mouse (a) and in the inflamed (b) and non-inflamed (c) paw pressure tests in the rat. Broken lines are responses of placebo-treated animals; vertical lines show s.e. mean.

induced nociception. But for these latter drugs the potency differences between heat and non-heat tests were less than for Mr2034. However, it is important to stress that the doses of these latter benzomorphans that increased reaction latencies in the hot plate and tail immersion tests also caused marked sedation, ataxia and, at the higher doses, loss of righting reflex. These animals were unable to respond to the heat stimulus and therefore the activities of these drugs in heat tests were most probably an indication of their

effects on motor coordination rather than on nociception. Therefore, for these drugs, particularly ethylketazocine, any actions on μ-receptors in heat tests would be masked by their depressant properties. Similar observations were reported by Harris & Rosenberg (1967) who showed that the activities of pentazocine and cyclazocine in the hot plate test were due to sedation or motor in-coordination as measured using an inclined screen.

Nalorphine could also be classified as a κ-agonist but compared with Mr2034 the dose-response curves were shallow and lower maximum effects were obtained. Martin *et al.* (1976) obtained a similar result for nalorphine in the chronic spinal dog on the flexor reflex response. Thus, results obtained in the present study support the concept that nalorphine is a partial agonist on κ-receptors. One finding that is difficult to explain is that higher doses of nalorphine in the paw pressure test consistently produced lesser effects than lower doses. This bell-shaped dose-response curve is similar to that found for buprenorphine in heat tests and for inhibition of gut propulsion (Cowan, Lewis & MacFarlane, 1977).

Ketazocine was only weakly active against heat-induced nociception at doses that also caused sedation. In the paw pressure test ketazocine was more potent than against heat nociception but produced a shallow dose-response curve with low maximum effect. However, in the acetylcholine-writhing test in the mouse the dose-response curve was parallel to that of Mr2034 and reached maximum effect. It may be that ketazocine is also a partial agonist on κ-receptors but has a higher intrinsic activity than nalorphine.

Buprenorphine, at doses devoid of sedative activity, produced a shallow dose-response curve with low maximum effect in the hot plate and tail immersion tests. This finding is consistent with the classification of buprenorphine as a partial agonist on μ-receptors (Martin *et al.*, 1976). However, buprenorphine was much more potent (50 to 400 times) against non-heat than against heat noxia suggesting that this drug is predominantly an agonist on κ-receptors. Further evidence that the predominant antinociceptive actions of morphine and buprenorphine are mediated via different opiate receptors is shown when these drugs are injected into the spinal subarachnoid space in rats (Byrant & Tyers, 1979). By this route, low doses of morphine are antinociceptive in hot plate and paw pressure tests but buprenorphine is inactive until the intrathecal dose exceeds that which is active by the subcutaneous route.

The results obtained show that heat and non-heat antinociceptive tests in rodents distinguish between μ- and κ-opiate receptors. Nalorphine, buprenorphine and the benzomorphans tested appear to have antinociceptive actions that are mediated predominantly through interactions with κ-opiate receptors. The ranked order of potency for these drugs is the same in each of the non-heat tests and for their analgesic potencies, where known, in man. There is no correlation between the antinociceptive potency ratios in heat and non-heat tests.

The extent of interaction, if any, of the μ-agonists with κ-receptors cannot be determined from these data. In terms of antinociception it may be that the agonist actions on μ- and κ-receptors selectively inhibit responses to heat and non-heat noxia respectively. If this were so, then because the ranked order of potency is the same in each of the tests for the μ-agonists, they would be expected to be equally efficacious on both μ- and κ-receptors. This seems unlikely in view of the widely differing chemical structures of the μ-agonists.

It is more likely that interaction with either receptor can inhibit nociception arising from the non-heat stimuli employed here while interaction with μ-receptors is necessary for inhibition of nociception arising from heat stimuli. This postulate would also imply that there are at least two neural pathways which either inhibit or convey nociception arising from heat and non-heat stimuli. Experiments with opiate receptor antagonists and receptor binding studies are needed to confirm these concepts.

In contrast to some of the results presented here, several investigators have shown that the antinociceptive actions of analgesic drugs with dual agonist and antagonist actions may be evaluated in hot plate (O'Callaghan & Holtzman, 1975), tail flick (Gray,

Table 3 Principle component scores for the analgesic drugs in rodent tests

Drug	*Principle components* Y_1*	Y_2†
Morphine	1.18	0.56
Codeine	−4.51	1.73
D-Propoxyphene	−3.76	0.65
Pethidine	−2.93	1.47
AH7921	0.74	0.37
Nalorphine	−3.47	8.32
Ketazocine	−4.06	7.75
Pentazocine	−5.92	5.13
Buprenorphine	9.50	6.03
Ethylketazocine	2.79	2.70
Mr2034	−0.72	8.30
Mr1353	−2.50	2.53

* High values of Y_1 indicate high overall activity.
† High values of Y_2 indicate relatively high non-heat: heat activity. Y_2 clearly distinguishes μ- and κ-agonists.

Osterberg & Scuto, 1970) and tail immersion (Sewell & Spencer, 1976) tests providing that the temperature is lowered to give a less intense nociceptive stimulus. However, even in these tests, the antinociceptive potencies reported for these drugs were still considerably lower than their analgesic potencies in man or indeed in other non-heat antinociceptive tests. In the present study, of all the drugs tested, only buprenorphine was slightly more potent in the lower than in the higher temperature tail immersion test.

In conclusion, evidence has been presented to show that at least two opiate receptors mediate antinociception in animal tests. In addition, it has been shown that opioid analgesic drugs may be classified in terms of their profiles of antinociceptive actions against different noxia. Tests that employ heat as the nociceptive stimulus, such as the hot plate and tail immersion tests, are selective for μ-agonists and are insensitive to the antinociceptive actions of κ-agonists. Tests that employ pressure, chemical writhing or tooth pulp stimulation (Skingle & Tyers, 1979) are sensitive to both μ- and κ-agonists.

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References

ARCHER, S., ALBERTSON, N.F., HARRIS, L.S., PIERSON, A.K., BIRD, J.G., KEATS, A.S. TELFORD, J. & PAPADOPOULOS, C.N. (1962). Narcotic antagonists as analgesics. *Science*, **137**, 541–542.

BRYANT, R.M. & TYERS M.B. (1979). Antinociceptive actions of morphine and buprenorphine given intrathecally in conscious rats. *Br. J. Pharmac.*, **66**, 472–473P.

COWAN, A., LEWIS, J.W. & MACFARLANE, I.R. (1977). Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *Br. J. Pharmac.*, **60**, 537–545.

DENEAU, G.A. & SEEVERS, M.H. (1964). Drug Dependence. In *Evaluation of Drug Activites: Pharmacometrics*. Vol. I, ed. (Laurence, D.R. & Bacharach, A.L. pp. 167–179. London: Academic Press.

FINNEY, D.J. (1964). Statistical Method in Biological Assay. 2nd Ed. London: Griffin.

GILBERT, P.E. & MARTIN, W.R. (1976) The effects of morphine- and nalorphine-like drugs in the nondependent, morphine-dependent and cylazocine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **198**, 66–82.

GRAY, W.D., OSTERBERG, A.C. & SCUTO, T.J. (1970). Measurement of the analgesic efficacy and potency of pentazocine by the D'Armour and Smith method. *J. Pharmac. exp. Ther.*, **172**, 154–162.

HARRIS, L.S. & ROSENBERG, F.J. (1967). CNS effects of pentazocine and other analgesic antagonists. *Archs Biol. Med. Exper.*, **4**, 136–143.

HUGHES, J., KOSTERLITZ, H.W. & LESLIE, F.M. (1975). Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics. *Br. J. Pharmac.*, **53**, 371–382.

HUTCHINSON, M., KOSTERLITZ, H.W., LESLIE, F.M., WATERFIELD, A.A. & TERENIUS, L. (1975). Assessment in the guinea-pig ileum and mouse vas deferens of benzomorphans which have strong antinociceptive activity but do not substitute for morphine in the dependent monkey. *Br. J. Pharmac.*, **55**, 541–546.

KOSTERLITZ, H.W. & LESLIE, F.M. (1978). Comparison of the receptor binding characteristics of opiate agonists interacting with μ- or κ-receptors. *Br. J. Pharmac.*, **64**, 607–614.

LASAGNA, L. & BEEECHER, H.K. (1954). The analgesic effectiveness of nalorphine and nalorphine-morphine combinations in man. *J. Pharmac.*, **112**, 356–363.

LORD, J.A.H., WATERFIELD, A.A., HUGHES, J. & KOSTERLITZ, H.W. (1977). Endogenous opioid peptides: multiple agonist, and receptors. *Nature*, **267**, 495–499.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197**, 517–532.

O'CALLAGHAN, J.P. & HOLTZMAN, S.G. (1975). Quantification of the analgesic activity of narcotic antagonists by a modified hot plate procedure. *J. Pharmac. exp. Ther.*, **192**, 497–505.

PEARL, J. & HARRIS, L.S. (1966). Inhibition of writhing by narcotic antagonists. *J. Pharmac. exp. Ther.*, **154**, 319–323.

SEWELL, R.D.E. & SPENCER, P.S.J. (1976). Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. *Neuropharmac.*, **15**, 683–688.

SKINGLE, M. & TYERS, M.B. (1979). Evaluation of antinociceptive activity using electrical stimulation of the tooth pulp in the conscious dog. *J. Pharmac. Methods*, **2**, 71–80.

SKINGLE, M. & TYERS, M.B. (1980). Further studies on opiate receptors that mediate antinociception: tooth pulp stimulation in the dog. *Br. J. Pharmac.* (in press).

SWAIN, H.H. & SEEVERS M.H. (1974). Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. *Bull. Problems Drug Dependence*, **36**, *Addendum*, **1**, 1168–1195.

SWAIN, H.H. & SEEVERS, M.H. (1976). Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. *Bull. Problems Drug Dependence*, **38**, *Addendum 2*, 768–787.

TABER, R.I., GREENHOUSE, D.D. & IRWIN, S. (1964). Inhibition of phenylquinone-induced writhing by narcotic antagonists. *Nature*, **204**, 189–190.

VILLARREAL, J.E. & SEEVERS, M.H. (1972). Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. *Bull. Problems Drug Dependence* **34**, *Addendum* 7, 1040–1053.

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