Br. J. Pharmac. (1983), **79**, 731–736

Determination of receptors that mediate opiate side effects in the mouse

Ann. G. Hayes[1] & Michael B. Tyers[1]

Pharmacology Department, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE.

1 The effects of μ and κ-opiate receptor agonists were studied in a variety of tests in the mouse designed to correspond to clinical side-effects in man. These included sedation, decrease in pupil diameter, Straub tail, decrease in body temperature, decrease in respiratory rate and inhibition of gut propulsion.

2 The μ-receptor agonists tested produced opiate side-effects in the mouse at doses between 2.4 and 34 times higher than their antinociceptive doses in the abdominal constriction test. Their ranked orders of potency in producing these effects were very similar to their order of antinociceptive potency.

3 In contrast, the κ-receptor agonists only produced opiate side-effects at doses between 29 and >2500 times higher than their antinociceptive doses. There was no correlation between the potency ratios in these tests and in the abdominal constriction test.

4 It is concluded that μ-receptor agonists may produce both their antinociceptive effects and opiate side-effects by interacting with the μ-receptor. The κ-receptor agonists have previously been shown to produce antinociception via the κ-receptor, but the opiate-like side-effects which appear with some of the drugs at much higher doses are probably due either to interaction with the μ-receptor or to some other non-specific action.

Introduction

There is now considerable evidence from both *in vitro* and *in vivo* studies that opiate receptors exist in differing forms (Gilbert & Martin, 1976; Martin Eades, Thompson, Huppler & Gilbert, 1976; Lord, Waterfield, Hughes & Kosterlitz, 1977). Martin *et al.* (1976) identified differing profiles of activity for opiates in the chronic spinal dog showing that drugs acting as agonists on the μ-receptor were antinociceptive, suppressed the abstinence syndrome in morphine-withdrawn dependent dogs and decreased body temperature, respiratory rate and pulse rate. In contrast, κ-receptor agonists, which were also antinociceptive, did not suppress morphine abstinence and had little effect on body temperature, respiratory rate or pulse rate; but they did produce sedation. Differing profiles of antinociceptive activity with opiates selective for μ- and κ-receptors can also be identified in conscious animals (Tyers, 1980; Skingle & Tyers, 1980). Thus antinociceptive tests such as the hot plate and tail immersion (55°C and 50°C) tests are sensitive only to drugs that interact with μ-receptors, whilst other tests such as abdominal constriction in the mouse, paw pressure in the rat and toothpulp stimulation in the dog are sensitive to both μ- and κ-receptor agonists.

The purpose of the present study was to determine, in the conscious mouse, the role of μ- and κ-opiate receptors in producing several different parameters of opiate action, which may correspond to clinical side-effects in man. In particular, the effects of a range of opiate drugs, with differing selectivities for μ- and κ-receptors have been determined on normal behaviour, pupil diameter, body temperature, respiratory rate and gastrointestinal propulsion.

Methods

Mice (AH/1/ICI-derived) of either sex and weighing 18–22 g were used. Drugs were administered subcutaneously and 30 min later the following parameters were determined sequentially in the following order in the same animals: Straub tail, body temperature, pupil diameter, respiratory rate, rotarod latency and inhibition of gut propulsion. The order of testing was arranged such that there was no significant in-

[1]Present address: Pharmacology Department, Glaxo Group Research Limited, Ware, Herts.

teraction between tests. The acetylcholine-induced abdominal constriction test was carried out in a different set of mice. Each test is described in detail below.

Individual tests were carried out using dose-groups of 6 mice. Data for calculation of ED_{50} values and dose-response curves were accumulated from 2 or 3 individual tests carried out on different days (less than 7 days apart), such that final dose-groups comprised 12 or 18 animals. To eliminate cage interaction, mice were randomized into cages such that each cage contained animals receiving different treatments. Animals and drug solutions were colour coded such that the operators were unaware of which treatment the animals were receiving. In each test, 2 or 3 drugs were compared with saline controls given in the same dose-volume (0.2 ml per 20 g body wt). The data obtained were computed to determine, where applicable, ED_{50} values and 95% confidence limits, regression slopes, linearity and potency ratios using the methods of Finney (1964).

Measurement of antinociceptive activity

The effect of drugs on acetylcholine-induced abdominal constriction was determined as described previously (Tyers, 1980).

Measurement of Straub tail reaction

Straub tail is a typical reaction to the administration of morphine, in which the tail becomes rigid and erect across the back of the animal. The mice were scored for presence or absence of this reaction and the ED_{50} value was defined as the dose producing Straub tail in 50% of the mice.

Body temperature measurement

Body temperature was measured using an oesophageal probe. The ED_{50} value was defined as the dose of drug capable of reducing body temperature to 2°C below that of the control group.

Pupil diameter measurement

The diameters of both pupils of each mouse were measured to the nearest 25 μm with a Beck Multimax stereoscopic microscope. The ED_{50} value was defined as the dose of test drug that increased pupil diameter to double the value of the saline-treated group.

Measurement of respiratory rate

Respiratory rate was measured by the method of Crossland, Horsfall, Oxenham, Shaw & Turnbull (1977). To obtain a steady baseline the mean respiratory interval was measured three times without removing the animal's snout from the barrel. The third reading only was recorded. The reciprocal of the respiratory interval was calculated to obtain the respiratory rate. The ED_{50} value obtained for this measure was defined as the dose of test drug capable of depressing the respiratory rate of the control group by 25%.

Measurement of rotarod reaction latency

Mice were placed on a rotating horizontal rod which accelerated in a linear fashion from 0 to 50 rev min^{-1} over 5 min. The time taken for each mouse to fall from the rod was determined as a measure of the incapacitating effect of the drug. The ED_{50} value was defined as the dose of test drug capable of decreasing the reaction latency to half that obtained for placebo-treated mice.

Measurement of inhibition of gut propulsion

Mice were dosed orally with 0.3 ml of a 3% w/v suspension of powdered crystalline carbon. Twenty minutes later, the mice were killed and the small intestine removed. The distance the carbon bolus had travelled along the small intestine was measured and expressed as a percentage of the total length of the small intestine for each mouse. The ED_{50} value was defined as the dose of drug that reduced the distance moved by the carbon bolus to half that obtained for the vehicle-treated control group.

Drugs

The following drugs were used: morphine hydrochloride; codeine phosphate; pethidine hydrochloride (MacFarlan Smith); AH 7921 (3,4-dichloro-N [(1-dimethylamino) cyclohexyl methyl]) benzamide hydrochloride) (Glaxo Group Research Ltd.); Mr 2034 as the free base [(−)-α-(1R,5R,9R)-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2-hydroxy-6,7-benzomorphan] (Dr H. Merz, Boehringer-Ingelheim); nalorphine hydrobromide (Burroughs Wellcome); (±)-ethylketazocine methanesulphonate; (±)-ketazocine methanesulphate; pentazocine as the free base (Sterling-Winthrop); buprenorphine hydrochloride (Reckitt & Colman); naloxone hydrochloride (Endo); bremazocine (Sandoz).

Results

The potencies of the opiate drugs in producing the various opiate side-effects and their antinociceptive potencies in the acetylcholine-induced abdominal

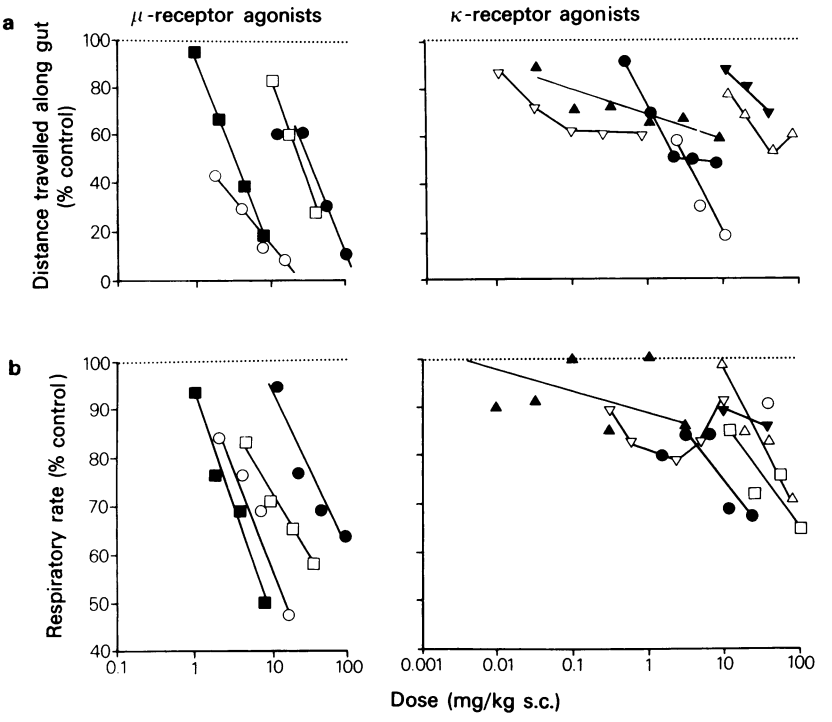

Figure 1 Dose-response curves showing mean effects of μ- and κ-agonists on (a) gastrointestinal propulsion and (b) respiratory rate in the conscious mouse. Symbols are for μ-agonists: (○) morphine, (●) codeine, (□) pethidine, (■) AH 7921; and for κ-agonists: (●) Mr 2034, (▼) nalorphine, (○) ethylketazocine (□) ketazocine, (△) pentazocine, (▽) buprenorphine and (▲) bremazocine.

constriction test are shown in Table 1. For all the parameters measured the μ-agonists, morphine, codeine, pethidine and AH 7921, produced steep dose-response curves with morphine and AH 7921 approximately equipotent and more potent than pethidine and codeine. Doses that produced side effects were similar to those producing antinociception. The remaining drugs, which have been characterized previously as selective agonists for the κ-receptor, produced steep, parallel dose-response curves in the abdominal constriction test, but their effects in the tests for opiate side-effects were more varied. Thus, they generally had little or no effect on pupil diameter, Straub tail and body temperature. The exceptions were high doses of buprenorphine, 0.5–5.0 mg kg^{-1} subcutaneously, which produced Straub tail, and high doses of pentazocine, 10–90 mg kg^{-1} subcutaneously, which produced mydriasis and hypothermia. Gastrointestinal propulsion was inhibited by Mr 2034, 0.3–10 mg kg^{-1}, pentazocine, 10–90 mg kg^{-1}, nalorphine, 30–60 mg kg^{-1}, buprenorphine, 0.01–0.1 mg kg^{-1}, ethylketazocine, 2–10 mg kg^{-1} and bremazocine, 0.1–8 mg kg^{-1} (all subcutaneously); but the dose response curves were shallow and generally reached a lower maximum effect than that achieved by the μ-agonists (Figure 1a). Similarly, respiratory rate was slowed by the κ-agonists, but again the dose-response curves tended to be shallower than those for the μ-agonists (Figure 1b). Buprenorphine, 0.3–10 mg kg^{-1} subcutaneously, produced a bell-shaped dose-response curve for this measure, with a low maximum effect.

In the rotarod test (Table 1), Mr 2034, 0.8–25 mg kg^{-1}, ethylketazocine, 0.3–10 mg kg^{-1}, bremazocine, 0.1–8 mg kg^{-1} and pentazocine, 10–80 mg kg^{-1} produced dose-related decreases in rotarod latencies. The dose-response curves for these drugs showed similar slopes and maxima to those obtained for the μ-agonists. Very high doses of nalorphine, 40 mg kg^{-1}, had no effect on rotarod latencies. Ketazocine, 12.5–100 mg kg^{-1} produced a dose-related decrease in rotarod reaction latencies but the slope of the dose-response curve was shallow and the maximum depression achieved was 25%. Buprenorphine, 0.3–10 mg kg^{-1} produced a bell-shaped dose-response curve similar to that obtained for depression of respiratory rate.

Table 1 Potencies of opiate drugs in producing antinociception and opiate side-effects in the mouse

Drug	*Side effect activity ED_{50} (95% confidence limits) (mg/kg s.c.)*						
	Rotarod latency	*Pupil diameter*	*Straub tail*	*Body temperature*	*Respiratory rate*	*Inhibition of gut propulsion*	*ACh-induced abdominal constriction*
Morphine	14.2 (6.7–42.1)	5.8 (2.8–12.3)	6.3 (2.6–20.8)	20.9 (10.3–66.5)	4.2 (1.8–7.7)	1.2 (0.5–2.1)	0.45 (0.21–0.91)
Codeine	127.5 (58.7–443.9)	34.8 (18.2–66.6)	>100	56.4 (33.3–105.5)	35.7 (21.2–60.3)	24.3 (17.0–33.6)	4.0 (0.3–9.4)
Pethidine	65.8 (26.9–332.1)	13.6 (7.4–25.1)	37.9 (18.9–∞)	30.6 (19.2–55.0)	8.2 (3.7–13.6)	25.6 (18.1–41.1)	2.6 (1.3–5.5)
AH 7921	20.2 9.9–148.7)	18.4 (6.2–86.1)	5.8 (4.4–8.4)	11.4 (7.7–18.3)	2.5 (2.0–3.0)	3.3 (2.8–3.9)	0.55 (0.27–0.94)
Bremazocine	0.74 (0.34–1.64)	>8	>8	>8	>8	29.2 (4.9–)	0.008 (0.003–0.028)
Mr 2034	10.4 (5.2–29.1)	>16.7	>16.7	>16.7	4.9 (0.14–∞)	3.1 (2.0–6.3)	0.02 (0.006–0.05)
Nalorphine	>40	>40	>40	>40	>40	>40	0.16 (0.02–1.08)
Ethylketazocine	3.5 (1.3–9.2)	>40	>40	>40	>40	2.6 (1.6–4.0)	0.12 (0.04–0.3)
Ketazocine	>100	>100	>100	>100	36.6 (7.1–222.5)	—	0.25 (0.02–1.34)
Pentazocine	40.2 (20.7–111.9)	44.5 (17.6–121.6)	>80	68.3 (30.0–258.8)	61.4 (34.6–189.1)	121.2 (50.3–∞)	0.75 (0.17–2.34)
Buprenorphine	~1*	>10	2.2 (0.5–14.4)	>10	~2.5*	1.5 (0.5–7.8)	0.004 (0.001–0.008)

*Buprenorphine produces bell-shaped dose-response curves in these tests.
The ED_{50} levels were not reached but these doses produced maximum effects.

All of the effects produced by both groups of drugs were reduced significantly ($P < 0.05$) by a single high dose of naloxone, 1 mg kg^{-1} subcutaneously, given at the same time as the opiate. Dose ranging studies with naloxone have not been carried out as yet.

Discussion

In the experiments described here, morphine, pethidine, codeine and AH 7921 produced opiate side-effects in the mouse at doses similar to their antinociceptive doses. This suggests that morphine-like drugs produce both their antinociceptive effects and opiate side-effects by interacting with the same receptor, probably the μ-receptor.

Nalorphine, buprenorphine and the benzomorphans tested appear to have antinociceptive actions that are mediated predominantly through interaction with κ-receptors (Martin *et al.*, 1976; Skingle & Tyers, 1980; Tyers, 1980). Thus, the ranked order of potency of these drugs is the same in all antinociceptive tests employing chemical, pressure or toothpulp electrical nociceptive stimuli and for their analgesic potencies, where known, in man. These drugs are very much less potent in antinociceptive tests using heat as the noxious stimulus and there is no correlation between their antinociceptive potency ratios in heat and non-heat tests. It seems likely that such activity as they have in heat tests is due to an interaction with the μ-receptor. In the experiments described here, the κ-receptor agonists, with the exception of buprenorphine, were ineffective in producing Straub tail, as also reported for pentazocine, cyclazocine and nalorphine (Collier & Schneider, 1969). Buprenorphine, in agreement with results published by Cowan, Lewis & McFarlane (1977), did produce Straub tail, but only at relatively high dose levels. The κ-receptor agonists were also ineffective in decreasing body temperature or increasing pupil diameter in the mouse. Pickworth & Sharpe (1979) and Martin *et al.* (1976) also found little hypothermic effect with κ-receptor agonists in their respective dog models. But the latter authors observed some mydriatic effect with κ-receptor agonists in the dog, although the dose-response curves were much shallower than those obtained with morphine.

The κ-receptor agonists did produce some inhibition of gut propulsion and reduction in respiratory rate, but only at much higher doses than those producing an antinociceptive effect in the abdominal constriction test. This lack of potency in producing these opiate side effects has been noted by other groups (Martin *et al.*, 1976; Pickworth & Sharpe, 1979; Porreca, Raffa, Cowan & Tallarida, 1981). In our experiments, there was no correlation between the potency ratios of the κ-receptor agonists in these tests and their antinociceptive potency ratios. This suggests that κ-receptors play a minor role, if they are involved at all, in reducing gut motility or respiratory rate.

Martin *et al.* (1976) have suggested that sedation is a κ-effect in the chronic spinal dog. However, in the mouse, while some of the κ-agonists produced marked decreases in rotarod reaction latencies, there was no correlation between potency ratios in the rotarod test and antinociceptive potency ratios in the abdominal constriction test. Thus it seems unlikely that sedation is a κ-effect in the mouse, a conclusion similar to that arrived at by Pickworth & Sharpe (1979) in the conscious dog.

Of the drugs tested, Mr 2034, bremazocine, nalorphine, ketazocine and buprenorphine showed the best separation between antinociceptive and opiate side-effects, thus suggesting that they are the most selective κ-receptor agonists. Pentazocine showed less separation and produced dose-related effects in all of the tests. However, the potency ratios for opiate side-effects: antinoceptive effect were higher than for the μ-agonists. These results suggest that pentazocine is a less selective κ-agonist. A similar conclusion was reached using antinociceptive profiles (Tyers, 1980). Clinical data with pentazocine confirm this lack of selectivity; its side-effect profile in man being little better than with morphine, especially with regard to respiratory depression (Engineer & Jennett, 1972).

In conclusion, evidence has been presented which suggests that the undesirable effects of opiate analgesics in the mouse are due predominantly to an interaction with the μ-receptor, but κ-receptors may have a minor role to play in respiratory depression and inhibition of gastrointestinal propulsion.

We would like to thank Malcolm Skingle, Janine Thompson, Christine Evans, Debbie Murray, Vrajesh Patel, Susan Purdy and Julia Wilson for their expert technical assistance. Generous gifts of Mr 2034 (Boehringer-Ingelheim), ethylketazocine and ketazocine (Sterling-Winthrop), buprenorphine (Reckitt and Coleman), bremazocine (Sandoz) and naloxone (Endo) are gratefully acknowledged.

References

COLLIER, H.O.J. & SCHNEIDER, C. (1969). Profiles of activity in rodents of some narcotic and narcotic antagonist drugs. *Nature*, **224**, 610–612.

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.

CROSSLAND, N.J., HORSFALL, G.B., OXENHAM, S.T., SHAW, J.S. & TURNBULL, M.J. (1977). A simple device for measurement of respiratory rate in the mouse. *Br. J. Pharmac.*, **61**, 490–491 P.

ENGINEER, S. & JENNETT, S. (1972). Respiratory depression following single and repeated doses of pentazocine and pethidine. *Br. J. Anaesth.*, **44**, 795–801.

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 non-dependent and cyclazocine-dependent chronic spinal dog. *J. Pharmac. Exp. Ther.*, **198**, 66–82.

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

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

PICKWORTH, W.B. & SHARPE, L.G. (1979). EEG-behavioural dissociation after morphine- and cyclazocine-like drugs in the dog: further evidence for two opiate receptors. *Neuropharmac.*, **18**, 617–622.

PORRECA, F., RAFFA, R., COWAN, A. & TALLARIDA, R.J. (1981). Ethylketocyclazocine and morphine: a comparison of their efficacies on gastrointestinal transit (GIT) after central and peripheral administration to rats. *Fed Proc.*, **40**, 288.

SKINGLE, M. & TYERS, M.B. (1980). Further studies on opiate receptors that mediate antinociception: tooth pulp stimulation in the dog. *Br. J. Pharmac.*, **70**, 323–327.

TYERS, M.B. (1980). A classification of opiate receptors that mediate antinociception in animals. *Br. J. Pharmac.*, **69**, 503–512.

(*Received December 14, 1982. Revised February 24, 1983.*)