Br. J. Pharmac. (1983), **78**, 659–663

Antinociceptive actions of morphine and buprenorphine given intrathecally in the conscious rat

Rosamunde M. Bryant, Jean E. Olley & M.B. Tyers

Neuropharmacology Department, Glaxo Group Research Ltd., Ware, Hertfordshire and Department of Pharmacology, University of Bradford, West Yorkshire

1 The antinociceptive effects of morphine and buprenorphine given intrathecally and subcutaneously have been compared in the conscious rat.

2 In the paw pressure test, when given subcutaneously buprenorphine 0.001–0.1 mg/kg s.c., was approximately 100 times more potent than morphine 0.1–3 mg/kg s.c., but in the hot plate test, buprenorphine 0.03–3.0 mg/kg s.c., produced a bell-shaped dose-response curve of low maximum effect and was about equipotent with morphine 0.03–3 mg/kg s.c.

3 When given intrathecally buprenorphine 10 μg and morphine, 10–60 μg, were approximately equipotent in both paw pressure and hot plate tests. Furthermore, morphine produced these effects at 1/25th of the minimum effective parenteral dose while the dose of buprenorphine exceeded the parenteral dose.

4 It is concluded that the predominant site of the analgesic action of buprenorphine is supraspinal. The significance of these findings in relation to the role of spinal opiate receptors is discussed.

Introduction

Morphine injected into the spinal subarachnoid space produces a naloxone-reversible focal analgesia in animals (Yaksh & Rudy, 1976; Wang, 1977) and man (Wolfe & Nicholas, 1979; Behar, Olshwang, Magora & Daidson, 1979). Very few studies have been carried out with other opioid analgesic drugs. Yaksh and Rudy briefly studied single doses of pentazocine and other benzomorphans on tail-flick reaction latencies in the rat. However, this test is insensitive to the antinociceptive actions of these agents even when they are given subcutaneously (Tyers, 1980). Thus the results obtained for intrathecal injections could not demonstrate whether or not these agonists were effective at spinal level. Buprenorphine has been classified as a partial agonist on μ-opiate receptors (Martin, Eades, Thompson, Hupplier & Gilbert, 1976). However, more recently in antinociceptive tests it has been shown that buprenorphine exhibits a profile as a selective κ-opiate receptor agonist and has partial agonist effects on the μ-opiate receptor only at significantly higher doses (Tyers, 1980; Skingle & Tyers, 1980). The present study was carried out to determine whether buprenorphine has a spinal site of analgesic action in the rat.

Methods

Male rats (AH-PVG/C) weighing 220 to 270 g were used. Rats were anaesthetized with pentobarbitone sodium (60 mg/kg i.p.) and mounted in a stereotaxic frame. Intrathecal cannulation was achieved by implanting catheters into the subarachnoid space using a method similar to that described by Yaksh & Rudy, (1976). The catheter was constructed from PP10 polythene tubing (0.28 mm i.d., 0.61 mm o.d.) with a collar made from PP60 (0.86 mm i.d., 1.27 mm o.d.) tubing welded 50 mm from the rostral end. The caudal end of the catheter was trimmed to a length of 85 mm from the collar to ensure that placement of the catheter tip was between vertebrae T13 and L1. The external rostral end of the catheter was sealed until required by inserting a short length of crimped 26G stainless steel tubing. The catheter was inserted into the spinal subarachnoid space so that the collar was just caudal to 2 anchor screws (10BA × 3/16 inch) located in the interparietal bones and secured with acrylic dental cement. Following recovery from the anaesthetic, animals were housed individually.

The antinociceptive activities of morphine and buprenorphine were determined for both intrathecal

and subcutaneous routes of injection. Antinociceptive activities were determined using the paw pressure, tail immersion and hot-plate tests as described previously (Tyers, 1980). In the hot-plate test, response latencies to both 'front paw lick' and 'hind paw lick' end-points were recorded in order to determine whether antinociception was limited to caudal areas. Each rat was subjected to each test in sequence, i.e. paw pressure threshold (g) determinations were followed by tail-flick latency (50°C) and then by hot-plate (55°C) reaction latencies (s).

For evaluations following subcutaneous administration, experiments were performed with dose-groups of 12 rats. Experiments were carried out blind so that the operator was unaware of the treatments the animals had received. Animals receiving the same treatment were randomly allocated to different cages so that each contained 3 rats receiving different treatments. Doses of morphine, 0.03–3.0 mg/kg, buprenorphine, 0.001–10.0 mg/kg and saline were administered subcutaneously in a dose-volume of 1 ml/kg 30 min before antinociceptive testing. In experiments to determine the effects of naloxone on the antinociceptive effects produced by these drugs, naloxone, 1.0 mg/kg i.p., was given 15 min before administration of a single submaximal dose of each test drug.

For evaluations following intrathecal administration, rats were randomly placed in groups of 6 for each drug or control group. Experiments were carried out using a 6 × 6 latin square, crossover design in which the order of the doses was randomized. Experiments were carried out blind to the operator and at least 2 days were allowed between treatments. On each test day, pre-drug nociceptive thresholds were determined for each rat in the paw pressure, tail-immersion (50°C) and hot-plate (55°C) tests 60 min before drug administration as described above. In other studies the peak antinociceptive effects of buprenorphine and morphine were determined to be 30 min after administration; therefore, this pretreatment time was used throughout. The effects of naloxone (1 mg/kg i.p.) pretreatment on the antinociceptive activities of intrathecally administered single doses of buprenorphine, 10 μg and morphine, 60 μg were determined using an 8 × 8 latin square, crossover design experiment. Naloxone or saline controls were administered intraperitoneally 15 min before intrathecal administration of the analgesic drugs. Intrathecal injections were given over a 60 s period in a dose volume of 5 μl flushed in with 10 μl artificial CSF.

On completion of the intrathecal experiments, 2 groups of 8 rats were injected with a saturated solution of Evans blue dye to determine the extent of diffusion. Placement of the catheter tip was confirmed in all of the rats both radiologically, following injection of 50 μl of a contrast medium (Myodil, Glaxo), and by dissection.

Drugs and materials

For subcutaneous injections morphine sulphate (MacFarlan Smith Ltd) was dissolved in 0.9% w/v NaCl solution (saline) and buprenorphine (Reckitt & Colman Ltd.) was dispensed from 5 ml ampoules containing 5 mg/ml of the hydrochloride salt in 5% dextrose solution and was diluted in saline. For intrathecal injections drugs were prepared in a balanced ion solution containing NaCl 7.46, KCl 0.19, $MgCl_2$ 0.19, and $CaCl_2$ 0.14 g/l in distilled water.

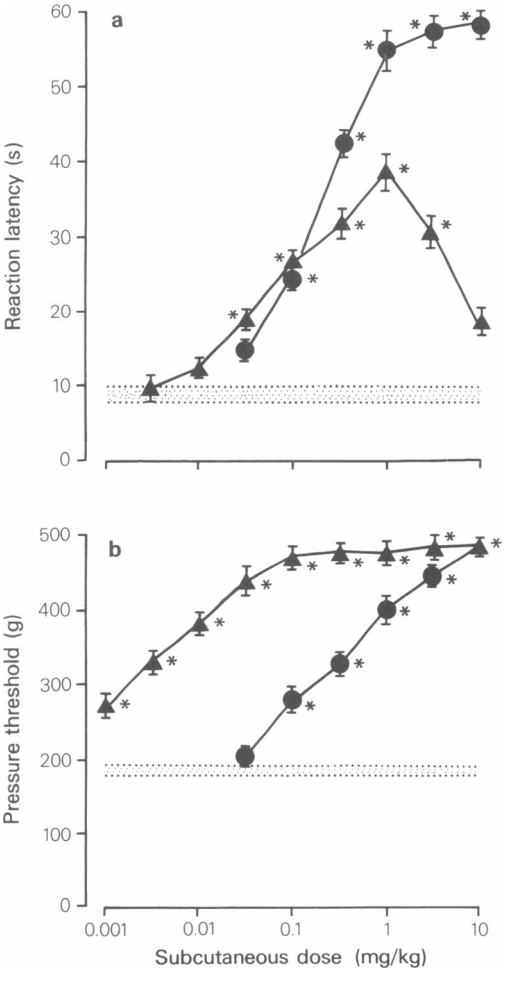

Figure 1 Antinociceptive effects of morphine (●) and buprenorphine (▲) given subcutaneously in the hot-plate (55°C) (a) and paw pressure (b) tests in the conscious rat. Data are means (± s.e., $n = 12$) analysed using Student's t test (*$P < 0.01$); stippled areas are placebo-treated groups.

Table 1 Effects of naloxone on the antinociceptive activities of morphine and buprenorphine given subcutaneously in the rat

Treatments (mg/kg s.c.)	*Mean (± s.e.) nociceptive thresholds*			
	Reaction latency (s) *in hot-plate (55°C) test*		*Pressure threshold* (g) *in paw pressure test*	
	Pre-drug	Post-drug	Pre-drug	Post-drug
Saline/saline	7.5 ± 0.3	7.7 ± 0.5	150 ± 6	146 ± 10
Naloxone/saline	8.7 ± 0.9	8.0 ± 1.5	170 ± 8	148 ± 12
Saline/morphine (0.3)	7.6 ± 0.5	20.6 ± 0.9	180 ± 4	374 ± 8
Naloxone/morphine (0.3)	8.6 ± 0.6	8.9 ± 0.5*	168 ± 6	158 ± 8*
Saline/buprenorphine (0.1)	8.2 ± 0.5	22.7 ± 1.9	156 ± 10	386 ± 16
Naloxone/buprenorphine (0.1)	8.4 ± 0.6	9.6 ± 0.5*	154 ± 8	188 ± 14*

Naloxone (1.0 mg/kg i.p.) or saline was given 15 min before morphine or buprenorphine. Nociceptive responses were determined 60 min before and 30 min after subcutaneously dosing with morphine, buprenorphine or saline. *$P < 0.001$ Student's t test comparing post-drug data for naloxone and corresponding saline pretreated groups.

Naloxone hydrochloride (Endo) was dissolved in saline. Control injections contained the appropriate vehicle(s). All doses in the text are expressed as the parent compound equivalent. Student's t test was used to determine the significance of effects between the treatment and the comparable control group.

Results

Subcutaneous administration

Following subcutaneous administration in the hot-plate (55°C) test (Figure 1a) morphine 0.03–1.0 mg/kg, caused dose-dependent, significant ($P < 0.01$) increases in reaction latencies. In the same test buprenorphine 0.03–1.0 mg/kg s.c., also caused dose-dependent, significant ($P < 0.01$) increases but the slope was less than that for morphine. Furthermore, higher doses of buprenorphine, 3 and 10 mg/kg, had progressively less effects on reaction latencies than at 1.0 mg/kg. In the paw pressure test, morphine, 0.1 mg/kg s.c., caused dose-dependent, significant ($P < 0.01$) increases in nociceptive pressure thresholds and was therefore of similar potency in both hot-plate and paw pressure tests. Buprenorphine, 0.001–0.1 mg/kg s.c., also caused dose-dependent increases in nociceptive pressure thresholds with the maximum and slope of the dose-response curve not significantly different from that for morphine (Figure 1b). Saline did not induce significant effects in either test.

Thus the analgesic drugs displayed differing profiles in the two tests. Buprenorphine was some 125 times more active than morphine in the paw pressure test although approximately equipotent in the hot-plate test. Furthermore, in the hot-plate test the dose-response curve for buprenorphine was bell-shaped and had a lower maximum than that for morphine whereas in the paw pressure test it was apparently sigmoidal and fully effective.

In further experiments the antinociceptive effects of morphine, 0.3 mg/kg s.c., and buprenorphine, 0.1 mg/kg s.c., were significantly ($P < 0.001$) reduced by naloxone, 1.0 mg/kg i.p., in both hot-plate and paw pressure tests (Table 1).

Intrathecal administration

In the hot-plate test, morphine 3–60 μg intrathecally, produced dose-dependent increases in reaction latencies to 'hind-paw lick' which were significant ($P < 0.005$) at 30 and 60 μg (Figure 2a). Very high doses of buprenorphine (10 μg intrathecally) were needed to increase the reaction latencies. Neither drug affected the 'forepaw lick' response latency indicating that the antinociceptive effects were probably limited to the caudal part of the body. Evaluation of the antinociceptive activity using the tail immersion test generated similar data (Figure 2c). In the paw pressure test morphine 10–60 μg intrathecally, and buprenorphine 10 μg intrathecally, caused significant ($P < 0.05$) increases in nociceptive pressure thresholds 30 min after administration (Figure 2b). Nociceptive thresholds in saline-treated catheterized rats were not significantly different from those of normal animals. Therefore, in contrast to the effects of buprenorphine and morphine following subcutaneous administration, the antinociceptive effects of these drugs in the hot-plate, tail immersion and paw pressure tests following intrathecal administration were very similar. In further experi-

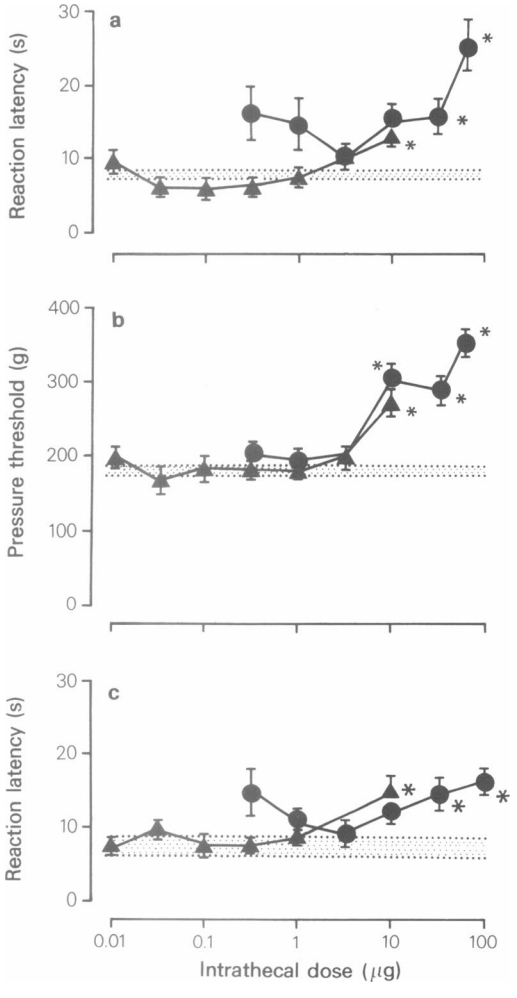

Figure 2 Antinociceptive effects of morphine (●) and buprenorphine (▲) given intrathecally in the hot-plate (55°C) (a), tail immersion (50°C) (b) and paw pressure (c) tests in the conscious rat. Data are means ($n = 12$) analysed using Student's t test (*$P < 0.01$). Vertical lines show s.e. mean. Stippled areas are placebo-treated groups.

ments the antinociceptive effects of both morphine, 60 μg intrathecally, and buprenorphine, 10 μg intrathecally, were inhibited by pretreatment with naloxone, 1 mg/kg i.p. (Table 2).

Post mortem and radiological examination of the catheter tip locations showed that 98% were sited between T12 and L2. Histological examination of the cord revealed no observable damage or inflammation, although in some cases adhesion had occurred between the catheter and the arachnoid mater in the lumbar and thoracic regions.

The results of the dye diffusion showed that 10 min after intrathecal injection, the dye had diffused rostrally in the subarachnoid space to the lower cervical vertebrae (C5 to C7), and caudally only as far as L3. After 30 min the dye had travelled only as far as C4 and C5 with no additional diffusion caudally. Dye was never observed in the brain at either 10 or 30 min pretreatment.

Discussion

Considerable interest has been shown recently in the spinal site of the analgesic action of morphine. Analgesia has been demonstrated following subarachnoid injections of morphine in both animals and man. Furthermore, opiate receptors have been shown to exist on primary afferent nerve terminals in the dorsal horn of the spinal cord (La Motte, Pert & Snyder, 1976) and may mediate inhibition of substance P released from these terminals (Jessell & Iversen, 1977). However, it is unlikely that the spinal cord is the major site of the analgesic action of morphine when it is administered parenterally.

In the present study, in order to evaluate the antinociceptive activity of analgesics on the spinal cord, administration directly into the spinal subarachnoid space of conscious rats was achieved by means of a chronically implanted catheter. It was possible to position the catheter tips accurately at the thoraco-lumbar border and dye diffusion studies indicated limited rostral diffusion so that the drugs would be available to thoracic and parts of the cervical and lumbar regions of the cord but not the brain.

Morphine, when injected into the spinal subarachnoid space caused naloxone-reversible antinociception against both thermal and pressure stimuli. The effect was maximal 30 min after injection and was similar to that observed by others. The effects of morphine were demonstrable at 1/25 of the minimal effective parenteral dose. In contrast, buprenorphine was relatively weak when given intrathecally. The effect, at a dose of 10 μg, which exceeds the effective parenteral dose per body weight of buprenorphine, was possibly due to diffusion away from the site of injection. It was interesting that morphine and buprenorphine given intrathecally were approximately equipotent in both thermal and mechanical nociception tests whereas in the paw pressure test following subcutaneous injection buprenorphine was approximately 100 times more active than morphine. An explanation for the relatively weaker effect of buprenorphine given intrathecally compared with the subcutaneous route in the paw pressure test may be that spinal analgesia is only mediated via μ-receptors. Buprenorphine has been characterized as a partial agonist on μ-receptors with a potency similar to that of morphine (Martin *et al.*, 1976; Tyers, 1980). However, the greater potency of buprenorphine when

Table 2 Effects of naloxone on the antinociceptive activities of morphine and buprenorphine given intrathecally (i.th.) in the rat

Treatments (μg i.th.)	*Mean (± s.e.) nociceptive thresholds*					
	Reaction latency (s)				*Pressure thresholds* (g) *in paw pressure test*	
	Hot-plate (55°C) *test*		*Tail immersion* (50°C) *test*			
	Pre-drug	Post-drug	Pre-drug	Post-drug	Pre-drug	Post-drug
Saline/vehicle	9.4 ± 0.8	8.5 ± 0.7	8.2 ± 0.4	8.4 ± 0.5	156 ± 6	172 ± 10
Naloxone/vehicle	7.2 ± 0.7	8.6 ± 0.9	8.1 ± 0.7	7.4 ± 0.6	154 ± 6	164 ± 8
Saline/morphine (60)	8.9 ± 0.7	25.1 ± 0.4	7.7 ± 0.4	16.7 ± 2.3	166 ± 6	342 ± 22
Naloxone/morphine (60)	7.5 ± 0.5	11.5 ± 2.8*	8.5 ± 0.4	8.6 ± 0.6*	156 ± 6	192 ± 12
Saline/buprenorphine (10)	9.0 ± 0.9	14.0 ± 2.1	8.5 ± 0.5	16.5 ± 2.2	160 ± 8	254 ± 28
Naloxone/buprenorphine (10)	8.4 ± 0.6	8.0 ± 1.4*	8.6 ± 0.7	9.1 ± 0.3*	156 ± 10	178 ± 14*

Naloxone (1.0 mg/kg i.p.) or saline, was given 15 min before morphine or buprenorphine. Nociceptive responses were determined 60 min before and 30 min after intrathecal dosing with morphine, buprenorphine or vehicle. *$P < 0.01$ Student's *t* test comparing post-drug data for naloxone and corresponding saline pretreated groups.

given subcutaneously in the paw pressure test has been attributed to a selective action on κ-opiate receptors (Tyers, 1980; Skingle & Tyers, 1981). This suggests that κ-receptors are not involved in spinally-mediated antinociception. It is unlikely that the relative lack of effect of buprenorphine given intrathecally was due to poor penetration of the pia mater and neural tissues of the dorsal horn, since buprenorphine is more lipophilic than morphine and should therefore diffuse more rapidly.

In man, epidural buprenorphine 0.2 mg has been reported to produce analgesia and sedation in the treatment of post-operative pain after elective caesarian section (Srivastava, 1982). However, this dose of buprenorphine is the same as the effective parenteral dose and the analgesic effect may be due to an action at another distant site. Alternatively, this high dose of buprenorphine may be sufficient for it to interact with μ-opiate receptors.

These results indicate that the predominant site of the analgesic action of buprenorphine in the rat is supraspinal. For example, discrete injections of buprenorphine into the median raphe nucleus (Bryant, Olley & Tyers, 1982) and cerebral ventricles (unpublished data) in the rat induce marked analgesia in which buprenorphine is approximately 1000 times more potent than morphine in pressure and chemical nociception tests. Therefore the median raphe nucleus is certainly a more important site for the analgesic action of buprenorphine than the spinal cord. These results also support the concept that buprenorphine and morphine produce analgesia through interactions with different opiate receptors.

Correspondence to M.B.T., Glaxo, please.

References

BEHAR, M., OLSHWANG, D., MAGORA, F. & DAVIDSON, J.T. (1979). Epidural morphine in treatment of pain, *Lancet*, **i**, 527–528.

BRYANT, R.M., OLLEY, J.E. & TYERS, M.B. (1982). Involvement of the median raphe nucleus in antinociception induced by morphine, buprenorphine and tilidine in the rat. *Br. J. Pharmac.*, **77**, 615–624.

JESSELL, T. & IVERSEN, L.L. (1977). Opiate analgesics inhibit substance P release from rat trigeminal nucleus. *Nature*, **268**, 549–551.

LAMOTTE, C., PERT, C.B. & SNYDER, S.H. (1976). Opiate receptor binding in primate spinal cord: distribution and changes after dorsal root section, *Brain Res.*, **112**, 407–412.

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.

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.

SRIVASTA, S. (1982). Epidural buprenorphine for post-operative pain relief. *Anaesthesia*, **37**, 699.

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

WANG, J.K. (1977). Analgesic effect of intrathecally administered morphine, *Reg. Anaesthesia*, **4**, 2–3.

WOLFE, M.J. & NICHOLAS, A.D.G. (1979). Selective epidural analgesia. *Lancet*, **ii**, 150–151.

YAKSH, T.L. & RUDY, T.A. (1976). Analgesia mediated by a direct spinal action of narcotics. *Science N.Y.*, **192**, 1357–1358.

(*Received August 16, 1982.*)