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AGONIST AND ANTAGONIST PROPERTIES OF BUPRENORPHINE, A NEW ANTINOCICEPTIVE AGENT

A. COWAN[1], J.W. LEWIS & I.R. MACFARLANE
Department of Pharmacology, Reckitt & Colman, Dansom Lane, Kingston-upon-Hull HU8 7DS

1 Buprenorphine is a highly lipophilic derivative of oripavine. In rodent antinociceptive assays (writhing, tail pressure), buprenorphine had an action which was rapid in onset and of long duration; it was 25–40 times more potent than morphine after parenteral injection and 7–10 times more potent after oral administration.

2 The log dose-response relationship for buprenorphine was curvilinear in mouse and rat tail flick tests with the antinociceptive effect decreasing at higher, non-toxic doses.

3 Tolerance developed to the antinociceptive activity of buprenorphine in mice.

4 No signs of abstinence were observed on naloxone challenge or after abrupt withdrawal in monkeys receiving buprenorphine chronically for one month.

5 Buprenorphine antagonized the antinociceptive actions of morphine in mouse and rat tail flick tests but was an ineffective antagonist in the rat tail pressure test.

6 Buprenorphine precipitated signs of abstinence in morphine-dependent mice and monkeys but not in morphine-dependent rats.

7 Buprenorphine produced Straub tails in mice. This effect was not antagonized when the animals were pretreated with naloxone. However, in the rat tail pressure test high doses of diprenorphine antagonized established antinociceptive effects of buprenorphine.

8 It is concluded that buprenorphine represents a definite advance in the search for a narcotic antagonist analgesic of low physical dependence potential.

Introduction

In the search for alternative analgesics to morphine, interest has continued to focus on those compounds that possess both antinociceptive and narcotic antagonist properties. Much research has been sustained by the conviction that a certain balance of these two activities will produce an efficacious analgesic that lacks both the physical dependence potential of morphine and the psychotomimetic profile of cyclazocine. This belief has been reinforced during the pharmacological evaluation of buprenorphine, N-cyclopropylmethyl-7α-(1-*S*-hydroxy,1,2,2-trimethyl-propyl)-6,14-*endo*ethano-6,7,8,14-tetrahydronororipavine (RX 6029-M), a new narcotic antagonist with potent, long-lasting antinociceptive actions that are not associated with an ability to induce primary physical dependence in monkeys. The similarity in chemical structure between buprenorphine and the potent narcotic antagonist, diprenorphine (M5050), is shown in Figure 1.

[1] Present address: Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pa. 19140, U.S.A.

A preliminary communication on the agonist and antagonist effects of buprenorphine in animals was given at the First International Conference on Narcotic Antagonists, Warrenton, U.S.A. (November, 1972). In the present paper, the antinociceptive and narcotic antagonist properties of buprenorphine are fully described and the physical dependence liability of this new analgesic is assessed.

Animals

The experiments were carried out on albino mice (MFI/Ola, 18–24 g), male Sprague Dawley albino rats in the weight ranges indicated below, monkeys (*Erythrocebus patas*, 4–10 kg) and baboons (*Papio anubis*, 4–7 kg).

Compounds

The following compounds were dissolved or diluted in 0.9% w/v NaCl solution (saline) and the doses calculated in terms of the free base: buprenorphine hydrochloride (mol. wt. of base is 467.6) and

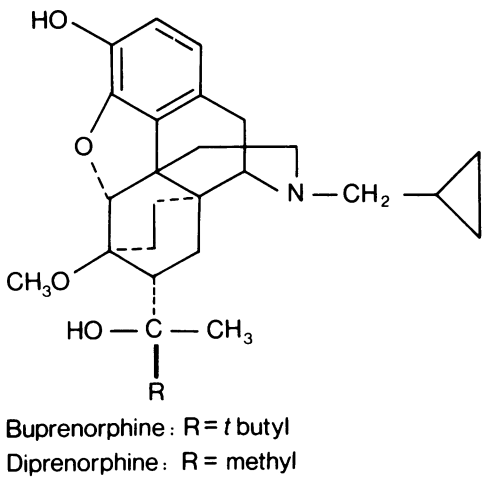

Figure 1 Structural diagrams of buprenorphine and diprenorphine.

diprenorphine hydrochloride (both Reckitt & Colman), morphine sulphate, B.P. (Macfarlan Smith), nalorphine hydrobromide, B.P. (Burroughs Wellcome), naloxone hydrochloride (Endo), pentazocine lactate (Sterling-Winthrop) and phenazocine hydrobromide (Smith & Nephew).

The free base of cyclazocine (Sterling-Winthrop) was dissolved in a minimal amount of 0.1 N HCl, the pH adjusted to 5.0 with $NaHCO_3$ solution and made up to volume with saline. Phenylquinone (2-phenyl-1,4-benzoquinone, Eastman) was dissolved in 5% w/v ethanol in distilled water.

Antinociceptive tests

In the writhing test, each dose of compound was administered to different groups of 10 female mice at various times before challenge with phenylquinone (2 mg/kg, i.p.). Five min later, the number of abdominal writhing movements made by each mouse was counted for 10 minutes. The dose of compound required to reduce the incidence of writhing by 50% was calculated by comparison with saline-injected controls (Hendershot & Forsaith, 1959). The development of tolerance to analgesics was monitored by this procedure. Thus, 2 groups of 240 mice were injected subcutaneously twice daily, at 09 h 00 min and 16 h 00 min, for 7 consecutive days with either buprenorphine (0.90 mg/kg i.e. 100 x the antinociceptive ED_{50} dose at 2 h, the time of peak effect) or morphine (56 mg/kg i.e. 100 x the ED_{50} dose at 0.5 h, the time of peak effect). Regression lines for antinociceptive effect were obtained daily at 10 h 00 min, with 24–30 mice from each group.

In the tail flick test, the noxious stimulus was hot water maintained at either 45, 55 or 65°C. Only mice and rats (120–150 g) withdrawing their tails from the water within 2 s were used. Immediately after selection, test compounds were administered intraperitoneally and 30 min later reaction times were again noted. On the basis of control data, those mice or rats failing to withdraw their tails within 7.5 s (45°C), 5.0 s (55°C) or 3.0 s (65°C), respectively, were termed 'non-responders'. Antagonism of the antinociceptive effect of morphine was also studied with this procedure. Either saline or test compound was given intraperitoneally to groups of 10 mice or rats at various times before subcutaneous injection of the antinociceptive ED_{90} dose of morphine. Reaction times (to water maintained at 55°C) were noted 30 min later. The AD_{50} and AD_{80} values are defined as the doses of antagonist required to reduce by 50% and 80%, respectively, the number of animals no longer responding to a noxious stimulus following treatment with the ED_{90} dose of morphine.

Groups of 10 rats (40–60 g) were used in the version of the tail pressure test described by Boura & Fitzgerald (1966). For antagonism studies, rats received the ED_{90} dose of morphine (s.c.) followed immediately by either saline or antagonist (s.c.). The analgesic test took place 30 min later.

Direct dependence test

Six patas monkeys were injected subcutaneously at 09 h 00 min, 16 h 00 min and 22 h 30 min for 30–32 consecutive days with increasing doses of buprenorphine (1.5–3.0 mg/kg in 2 monkeys and 3.0–12.5 mg/kg in 4 monkeys). The development of physical dependence was monitored by challenging each primate with naloxone (2 mg/kg, s.c.) on days 14 and 28 and looking for the signs of abstinence listed by Deneau & Seevers (1964). Saline was injected during the week following abrupt withdrawal of buprenorphine and the animals were frequently observed through a one-way mirror for signs of withdrawal.

Tests with morphine-dependent animals

The method described by Cowan (1976) was used to obtain an extended dose-response curve for buprenorphine as a morphine antagonist in the mouse jumping test.

Groups of 8 rats (140–170 g) were implanted in the dorsal subcutaneous tissue with a morphine pellet (75 mg morphine base, 2 mg polyvinylpyrrolidone and 1 mg magnesium stearate) on day 1 and again 48 h later. A further 24 h later each rat was weighed then challenged subcutaneously with antagonist or saline. Animals were placed in individual open plastic containers (43 cm long, 30 cm wide and 17 cm high) and observed over 10 min for signs of abstinence (Bläsig, Herz, Reinhold & Zieglgänsberger, 1973).

Food and water were removed and the weight of each rat was recorded 3 and 24 h later.

Five non-withdrawn, morphine-dependent patas monkeys that had received daily injections of the narcotic at 09 h 00 min, 16 h 00 min (6 mg/kg, s.c.) and 22 h 30 min (12 mg/kg) for 15 months were injected with buprenorphine (0.01–10 mg/kg, s.c.) at 11 h 00 minutes. The resulting abstinence syndromes were classified according to the protocol described by Deneau & Seevers (1964).

Straub tail and catalepsy tests

Thirty min after subcutaneous injection of buprenorphine, mice ($n=10$) were individually observed for 30 s and those with tails elevated >45° to the horizontal were scored as showing a positive Straub effect. In antagonism studies, the antagonist was given subcutaneously to groups of 10 mice, 15 min before the various doses of buprenorphine.

The cataleptic effects of buprenorphine and morphine were estimated 0.5, 1.5 and 2.5 h after subcutaneous administration to groups of 20 rats (120–150 g). The number of animals leaving both hind legs over a horizontal metal rod (4 cm above bench level) for longer than 45 s was recorded.

Statistical evaluation

Quantal data were subjected to logit analyses using Bliss 17, a computer programme written by Professor D.J. Finney (see Finney, 1971). Figures in parentheses in the Tables refer to the 95% confidence limits of the AD_{50} or ED_{50}.

Results

The potencies of buprenorphine and reference analgesics in the phenylquinone test are presented in Table 1. The slope values of the narcotic antagonist analgesics did not differ significantly from that of morphine at 30 minutes. A fast onset of action was noted for buprenorphine; the subcutaneous ED_{50} value obtained at 5 min (0.019 mg/kg, 0.008–0.043, 95% confidence limits) was close to the optimal value obtained at 2 h (0.008 mg/kg, 0.005–0.013). In contrast to findings from other procedures (see below),

Table 1 The potencies of analgesics against abdominal constriction responses induced by phenylquinone in mice

Compound	*Median effective antinociceptive dose*				
	ED_{50} (mg/kg, s.c.)			*ED_{50} (mg/kg, orally)*	
	0.5 h	*Slope*	*2.0 h*	*1.0 h*	*2.0 h*
Buprenorphine	0.014 (0.007–0.027)	1.3	0.008 (0.005–0.013)	0.39 (0.25–0.63)	0.24 (0.14–0.39)
Cyclazocine	0.22 (0.11–0.42)	1.3	4.6 (2.1–10)	5.2 (2.9–9.4)	27 (19–39)
Morphine	0.36 (0.22–0.60)	1.8	2.4 (1.4–4.0)	2.6 (1.4–4.9)	9.6 (6.3–14)
Pentazocine	4.3 (2.6–7.1)	1.9	>60	59 (46–76)	>60

Different groups of mice were used at each time interval. The slopes of the antinociceptive regression lines, computed from the readings at 0.5 h, are expressed as logits/$\log_e$ dose. Figures in parentheses refer to 95% confidence limits.

Table 2 The antinociceptive potencies of analgesics in the mouse and rat tail flick tests

Compound	*Mouse tail flick test ED_{50} (mg/kg, i.p.)*	*Rat tail flick test ED_{50} (mg/kg, i.p.)*
Buprenorphine	2.4(0.25–22)	1.6(0.71–3.6)
Morphine	3.8(2.0–7.4)	9.5(4.8–19)
Pentazocine	>30	>30

The noxious stimulus was water maintained at 55°C. In both tests, the log dose-response curve for buprenorphine was curvilinear. The so-called ED_{50} values for buprenorphine were calculated from that part of the curve where the antinociceptive effect increased with dose. Figures in parentheses refer to 95% confidence limits.

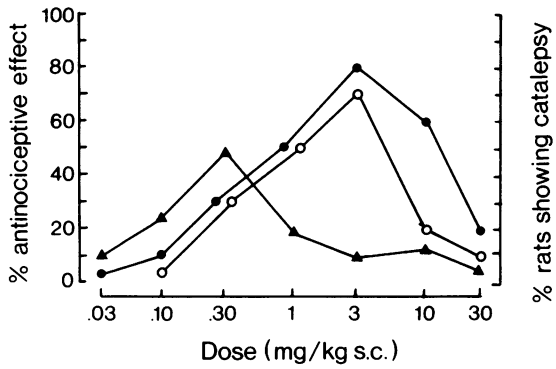

Figure 2 Dose-response curves obtained with buprenorphine (i) in the rat tail flick test using water at 45°C (●) or 55°C (○) as the noxious stimulus and (ii) in a catalepsy test (▲) where those rats that left both hind legs over a horizontal rod >45 s were scored as showing a positive effect. Ten rats were used for each point in the analgesic test; 20 rats were used per point in the catalepsy test. All readings were taken 30 min after injection.

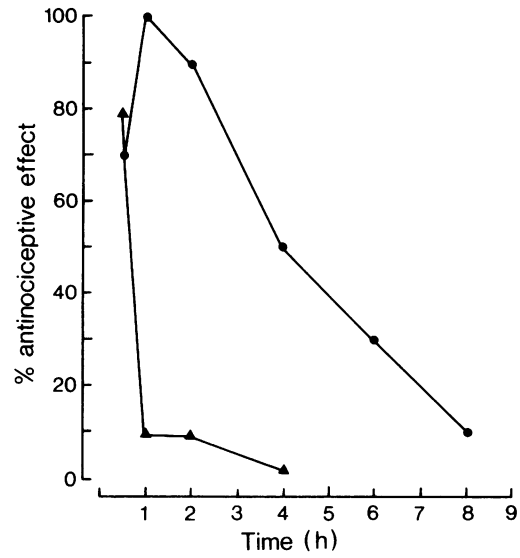

Figure 3 Comparison of the lengths of action of buprenorphine (●) and morphine (▲) as antinociceptive agents in the rat tail pressure test. The ED_{80} agonist doses (previously estimated to be 0.046 and 1.0 mg/kg, s.c. at 0.5 h for buprenorphine and morphine, respectively) were injected into different groups of 10 rats at zero time. Each group was used at only one time interval. The number of rats in each group that did not respond to the noxious stimulus at specified times (abscissa scale) is plotted on the ordinate scale.

buprenorphine maintained a 100% effect after high doses (0.10–100 mg/kg) in the phenylquinone test.

Buprenorphine was marginally more potent than morphine in the mouse and rat tail flick tests when water at 55°C was used as the noxious stimulus; in both cases, the maximal effect of pentazocine was only 40% and so ED_{50} values could not be calculated (Table 2). The efficacy (maximal effect) of buprenorphine was increased when the noxious stimulus was set at 45°C (Figure 2). Under these conditions pentazocine was weakly active, a 50% response being obtained. Buprenorphine was less efficacious than morphine in mice and rats; the maximal effect of the new compound, with water at 65°C, was only a 50% suppression of the flick reflex.

The log dose-response lines for morphine at 45, 55 and 65°C were of the typical sigmoid shape. In contrast, those for buprenorphine at each of these temperatures, and in both mice and rats, were bell-shaped with the antinociceptive effect decreasing at higher, non-toxic doses. A similar bell-shaped curve was obtained with pentazocine at 45°C. It is of interest that the dose of buprenorphine (3 mg/kg) producing the maximal antinociceptive effect in rats was 10 times higher than the dose causing the maximal cataleptic effect in this species (Figure 2). Thus, in view of the bell-shaped dose-response curve for catalepsy, only 10% of the animals were classified as being cataleptic at the peak antinociceptive dose level.

Buprenorphine was more potent as an antinociceptive agent than morphine and, especially, pentazocine in the rat tail pressure test (Table 3). A rapid onset of action occurred since the ED_{50} value at 5 min was 0.053 mg/kg (0.016–0.18). There was no evidence of a bell-shaped dose-response curve in this procedure; a consistent 100% antinociceptive effect

Table 3 The antinociceptive potencies of analgesics in the rat tail pressure test

Compound	*ED_{50} (mg/kg, i.p.)*	*ED_{50} (mg/kg, orally)*
Buprenorphine	0.016(0.011–0.024)	0.35(0.16–0.77)
Morphine	0.66(0.26–1.6)	3.6(1.6–8.1)
Pentazocine	8.8(3.5–22)	35(16–76)

Antinociceptive effects were recorded 0.5 h after intraperitoneal injection and 1.0 h after oral administration. Figures in parentheses refer to 95% confidence limits.

was obtained at 30 min with animals receiving higher doses of buprenorphine (0.10–100 mg/kg). The duration of action of buprenorphine (based on the time taken for the ED_{80} dose to be effective in only 20% of the rats) was 9 or 13 times longer than that of morphine, the choice depending on which of two possible times is taken as zero for buprenorphine (Figure 3).

Two morphine-like properties of buprenorphine were demonstrated in the phenylquinone-induced writhing test. Tolerance developed to the anti-nociceptive effect of buprenorphine, the onset being slower than with morphine. Also, cross tolerance was shown to exist between the two analgesics; this was more pronounced in mice pretreated with morphine (Table 4). Despite these findings, multiple injections of buprenorphine did not induce physical dependence in monkeys as judged by the absence of signs of abstinence on naloxone challenge and also after abrupt withdrawal of buprenorphine.

The morphine antagonist activity of buprenorphine was most clearly demonstrated using the mouse tail flick test. Buprenorphine was as potent as naloxone at the time of peak effect (Table 5) and had a duration of action approximately 10 times that of naloxone (Table 6). The maximum antagonistic effect obtained with buprenorphine, however, was only 80% and this was maintained over the dose-range 0.10–100 mg/kg.

Table 4 Development of tolerance to the antinociceptive actions of buprenorphine and morphine and possible cross tolerance between these compounds in the mouse phenylquinone-writhing test

No. of injections of analgesic	*Antinociceptive ED_{50} (mg/kg, s.c.)*	
(a) Tolerance	*Buprenorphine*	*Morphine*
Saline control	0.013(0.005–0.036)	0.60(0.40–0.90)
2	0.013(0.005–0.034)	1.1(0.68–1.7)
4	0.028(0.016–0.049)	3.3(1.7–6.7)
6	0.083(0.052–0.13)	6.9(4.3–11)
8	0.13(0.078–0.21)	8.2(6.0–11)
14	5.6(3.2–9.8)	7.7(3.7–16)
(b) Cross tolerance		
14 (morphine)	0.39(0.21–0.74)	
14 (buprenorphine)		6.0(4.1–8.7)

Each analgesic was injected at 09 h 00 min and 16 h 00 min daily for 7 days. The dose level was 100 × antiwrithing ED_{50}: morphine (56 mg/kg) and buprenorphine (0.90 mg/kg). Antinociceptive regression lines were obtained at 10 h 00 min daily. Figures in parentheses refer to 95% confidence limits.

Table 5 Antagonism of morphine by test compounds in the mouse tail flick test

Dosing interval(h) before morphine ED_{90}	*AD_{50} (mg/kg, i.p.)*		
	Buprenorphine	*Naloxone*	*Nalorphine*
0.25	0.058(0.024–0.14)	0.017(0.008–0.039)	3.1(1.2–7.8)
0.50	0.028(0.010–0.076)	0.035(0.016–0.076)	0.83(0.27–2.5)
1.0	0.019(0.008–0.043)	0.074(0.038–0.14)	2.6(1.0–6.5)
2.0	0.013(0.006–0.028)	0.14(0.063–0.32)	3.4(1.7–6.8)
4.0	0.016(0.006–0.043)	0.16(0.072–0.36)	5.2(2.3–12)

The noxious stimulus was water maintained at 55°C and the test took place 0.5 h after injection of morphine (50 mg/kg, s.c.). Figures in parentheses refer to 95% confidence limits.

Antagonism was also demonstrated when mice received buprenorphine 15 min before the ED_{90} dose of another narcotic agent, phenazocine (28 mg/kg). The AD_{50} value of 0.035 mg/kg (0.017–0.74) was similar to that obtained against morphine. Buprenorphine was much less potent in the corresponding rat tail flick test. Thus, when buprenorphine was injected immediately before morphine, the AD_{50} value at 30 min was only 2.5 mg/kg (1.3–4.5) whereas that of naloxone was 0.38 mg/kg (0.18–0.80).

Buprenorphine (0.001–10 mg/kg) was not a morphine antagonist in the rat tail pressure test; the AD_{50} value for naloxone was 0.17 mg/kg (0.060–0.45). When buprenorphine (3 mg/kg) was injected either 30 or 60 min before the ED_{50} dose of morphine a 100% antinociceptive effect was recorded 30 min later.

In the mouse jumping test there was no bell-shaped dose-response curve with buprenorphine when log dose was plotted against the number of mice jumping at least 6 times during 1 hour. The maximum antagonistic effect with high doses of buprenorphine (3–30 mg/kg) was only 70%, a finding in keeping with results from the mouse tail flick test. Surprisingly, buprenorphine was not an antagonist when given to morphine-dependent rats. In contrast to nalorphine (3 mg/kg), which precipitated the range of signs previously described (Bläsig, Höllt, Herz & Paschelke, 1976) and caused a significant ($P < 0.05$) weight loss over 3 h, buprenorphine (0.30–10 mg/kg) induced a dose-related weight loss over 3 h which was not significantly different from control results (Table 7). Buprenorphine was clearly a morphine antagonist in non-withdrawn, morphine-dependent monkeys since a dose as low as 0.1 mg/kg precipitated a moderate abstinence syndrome with such signs as shaking, yawning and fighting. Also, when 2 drug-naïve baboons were narcotized with etorphine/methotrimeprazine (Small Animal Immobilon, Reckitt & Colman, 0.11 mg/kg, s.c.) then injected 20 min later with buprenorphine (0.1 mg/kg, i.v.) in both cases there was a rapid reversal of the narcosis.

The Straub tail ED_{50} value for buprenorphine in mice was 0.16 mg/kg (0.060–0.75). It is noteworthy that pretreatment with large amounts of naloxone or diprenorphine (each at 1 and 10 mg/kg) did not significantly influence the position of the buprenorphine regression line. However, in the rat tail pressure test the buprenorphine regression line for antinociceptive effect was increasingly displaced to the right when diprenorphine (0.10, 0.30 or 1.0 mg/kg) was injected immediatley after buprenorphine (Table 8). A much larger dose of diprenorphine (e.g. 3 mg/kg rather than 0.30 mg/kg) was required to cause the same shift when this antagonist was injected 30 min after buprenorphine. It was thus more difficult to antagonize the antinociceptive effect of buprenorphine once it was well-established.

As indicated above, a bell-shaped log dose-response curve was obtained with rats in the catalepsy

Table 6 Duration of morphine antagonist activities of test compounds in the mouse tail flick test

Compound	*Duration (min) of effect of equi-antagonistic doses*
Buprenorphine	966
Diprenorphine	303
Naloxone	105

The noxious stimulus was water maintained at 55°C. Different groups of 10 mice were challenged with morphine (50 mg/kg, s.c.) at various times after the injection of the AD_{80} dose of each antagonist. The time intervals between 80% and 20% antagonism are compared for each compound.

Table 7 Relative weight losses shown by morphine-pelleted rats after challenge with test compounds

Compound	*Dose (mg/kg, s.c.)*	*Mean weight loss (g) (s.e. mean)* +3 h	+24 h
Saline	5.0 ml/kg	4.1 ± 1.1	30.3 ± 1.8
Nalorphine	3.0	7.0 ± 0.94*	28.4 ± 1.3
Buprenorphine	0.30	2.9 ± 0.37	26.9 ± 1.9
	1.0	3.9 ± 0.81	28.8 ± 0.90
	3.0	4.2 ± 0.69	28.4 ± 1.9
	10	5.3 ± 0.70	29.7 ± 1,0

Groups of 8 rats were implanted with 75 mg morphine pellets on day 1 and again on day 3. The rats were weighed on day 4, test compounds were then injected and further weighings took place 3 and 24 h later.
* Significantly different ($P < 0.05$) from control animals by the Mann Whitney U test.

Table 8 The effect of diprenorphine on the antinociceptive ED_{50} of buprenorphine in the rat tail pressure test

Treatment		*Buprenorphine ED_{50} (mg/kg, s.c.)*	*Relative potency*
0 min	*0 min*	*30 min*	
Buprenorphine	Saline	0.022(0.013–0.037)	1
Buprenorphine	Diprenorphine (0.10 mg/kg)	0.34(0.16–0.73)	17
Buprenorphine	Diprenorphine (0.30 mg/kg)	1.3(0.59–2.7)	77
Buprenorphine	Diprenorphine (1.0 mg/kg)	6.9(4.5–10)	313
0 min	*30 min*	*60 min*	
Buprenorphine	Saline	0.010(0.005–0.017)	1
Buprenorphine	Diprenorphine (1 mg/kg)	0.059(0.041–0.083)	6.8
Buprenorphine	Diprenorphine (3 mg/kg)	0.66(0.38–1.2)	77

Each regression line for buprenorphine was obtained with 30 rats, either in the presence or absence of subcutaneous diprenorphine. The slopes of these lines did not differ significantly from parallelism. Figures in parentheses refer to 95% confidence limits.

experiment (Figure 2). At the time of peak effect (30 min), the maximum cataleptic response to buprenorphine (0.30 mg/kg) was only 50%. In a similar experiment with morphine the ED_{50} value (at 30 min) was 10 mg/kg (6.0–18).

Discussion

Buprenorphine is a potent antinociceptive agent with a rapid onset and long duration of action in rodents. This new narcotic antagonist analgesic is 25–40 times more potent than morphine in mouse writhing and rat tail pressure tests after subcutaneous or intraperitoneal injection and 7–10 times more potent after oral administration. The positive results obtained with buprenorphine in rodent tail flick tests are noteworthy since reference narcotic antagonist analgesics are essentially inactive when the conventional tail flick (radiant heat) method is used (Dewey, Harris, Howes & Nuite, 1970). The antinociceptive action of buprenorphine was demonstrated in mice and rats by using a modified tail flick method where water at 45, 55 or 65°C served as the noxious stimulus (Cowan, Lewis & Macfarlane, 1971). The efficacy of buprenorphine was greater than that of pentazocine but less than that of morphine in this procedure. In contrast to the sigmoid-shaped log dose-percentage response lines obtained with morphine, the corresponding lines for buprenorphine (at 45, 55 and 65°C) and pentazocine (at 45°C) resembled an inverted U. On the other hand, the log dose-response relationship for buprenorphine was linear in the mouse writhing and rat tail pressure tests, that is, tests in which buprenorphine produced the maximum possible antinociceptive effect over a wide range of doses.

Biphasic dose-response curves have also been obtained with buprenorphine in tests involving different measures e.g. reduction of respiratory rate in mice or antagonism of gastrointestinal motility in rats (Cowan, Doxey & Harry, 1977); and induction of catalepsy in rats (see Results section). In these tests, and in the tail flick test, the maximum effect of buprenorphine occurred within the dose range 0.10–3.0 mg/kg. The finding that buprenorphine is less efficacious at 10 and 30 mg/kg than at 3 mg/kg in the rat tail flick test (Figure 2) might be thought due to high doses of the analgesic causing a general behavioural depression. This possibility is unlikely since the degree of catalepsy is of the same low order over the range 3–30 mg/kg in rats; moreover, it may be argued that depressed animals will take longer to react to the noxious stimulus and thus give enhanced rather than reduced, tail flick latencies.

At the receptor level, the biphasic dose-response curves obtained with buprenorphine may represent examples of non-competitive autoinhibition (Ariëns, van Rossum & Simonis, 1957). According to this theory, in certain systems buprenorphine would have an affinity not only for the so-called μ (morphine) receptor but also for a second, interdependent receptor, then as a result of an increasing interaction between buprenorphine and the second receptor, the intrinsic activity of the buprenorphine-μ complex

would become correspondingly less.

Tolerance developed to the antinociceptive actions of buprenorphine in the phenylquinone-writhing test. In this respect, buprenorphine is similar to morphine but differs from cyclazocine, nalorphine and pentazocine since Smits & Takemori (1970) found that marked tolerance did not occur with these narcotic antagonist analgesics in the same writhing test. The long duration of action of buprenorphine as an agonist in this test may have contributed to the apparent divergent results. Although buprenorphine can induce tolerance in the mouse, it is known (Cowan, 1974) that a dose of naloxone as high as 10 mg/kg, subcutaneously, precipitates only a very low incidence of repetitive jumping in this species when buprenorphine is run in the primary dependence test described by Saelens, Granat & Sawyer (1971). These results provide evidence of a dissociation of physical dependence from tolerance after multiple injections of buprenorphine to mice.

The finding that buprenorphine may not produce physical dependence in patas monkeys is in agreement with the conclusion from a direct dependence study carried out in rhesus monkeys at the University of Michigan (Swain & Seevers, 1975). Also, it has recently been found that buprenorphine (10 mg kg^{-1} day^{-1}) neither substitutes for morphine in morphine-dependent rats when the intraperitoneal infusion technique described by Teiger (1974) is used nor induces physical dependence during a 6-day infusion (50–100 mg kg^{-1} day^{-1}) in this species (Dewey, Harris & Ritter, 1975; Dr W.L. Dewey, personal communication). These observations are at variance with the report by Martin, Gilbert, Eades, Thompson & Huppler (1975) that buprenorphine partially suppresses the withdrawal syndrome in morphine-dependent chronic spinal dogs, and that naloxone precipitates signs of abstinence in chronic spinal dogs receiving multiple intravenous injections of buprenorphine (Martin, Eades, Thompson, Huppler & Gilbert, 1976). However, it is important to note, that in the dog experiments (a) buprenorphine precipitated signs of abstinence in non-withdrawn animals (b) the slope of the regression line for buprenorphine in the suppression study was significantly less than that of morphine and d-propoxyphene and (c) the abstinence syndrome precipitated by naloxone challenge emerged rather slowly and was classified as being 'mild'. Although no explanation is available at present for the different results obtained with mice, rats and monkeys as opposed to dogs it would seem reasonable to agree with the prediction of Martin *et al.* (1976) that buprenorphine may produce no more than a liminal (and perhaps clinically insignificant) degree of physical dependence in man.

In the present work, narcotic antagonism was demonstrated with buprenorphine in mice, rats, monkeys and baboons. Compared to naloxone in the mouse tail flick test, buprenorphine was equipotent and longer acting but less efficacious. Experience with the hot water version of the tail flick test using mice has suggested that the antagonistic potencies of many analgesics, particularly oripavine-thebaine derivatives, are overestimated in relation to values obtained from other procedures. Thus, buprenorphine is approximately 7 times less potent than naloxone in the corresponding rat tail flick test and 15 times less potent in the mouse jumping test (Cowan, 1976). Buprenorphine contrasts with naloxone in the latter test by again being less efficacious and (after a slow onset) having a longer duration of action. The profile of buprenorphine as a morphine antagonist is complex since antagonism was demonstrated in rats using the tail flick test but antagonism was absent in the tail pressure test; moreover, buprenorphine precipitated signs of abstinence in non-withdrawn, morphine-dependent monkeys but not in non-withdrawn, morphine-dependent rats. The result from the tail pressure test was not entirely unexpected since experience has shown that it is difficult to detect the antagonist component of narcotic antagonist analgesics that are potent agonists in the procedure.

Relatively large doses of diprenorphine were required to antagonize the antinociceptive action of buprenorphine in the rat tail pressure test. The surprising feature of the buprenorphine-diprenorphine interaction was the critical importance of the relative timing of the two injections. Thus, a ten-fold increase in the dose of diprenorphine was required to effect the same degree of antagonism when this compound was given 30 min after buprenorphine rather than at the same time. In contrast, the corresponding antagonism of morphine by diprenorphine was only slightly affected by the same difference in injection times (Cowan & Macfarlane, unpublished observation). The greater difficulty in displacing buprenorphine, when once established, from narcotic receptors and the slow dissociation of the compound from these sites (J.M. Hambrook & M.J. Rance, personal communication) are findings reminiscent of those described for methadone on isolated tissues (Kosterlitz, Leslie & Waterfield, 1975). In this context, it may be significant that buprenorphine and methadone are the most lipophilic of the widely studied analgesics.

Psychotomimetic episodes have occurred in men after receiving high doses of many narcotic antagonist analgesics e.g. pentazocine, nalbuphine (Jasinski, Martin & Hoeldtke, 1970; Jasinski & Mansky, 1972). Dysphoric effects have not been associated with buprenorphine during extensive clinical trials involving over 1500 subjects. Since these clinical studies have also shown buprenorphine to be an effective, long-lasting and safe analgesic, it is concluded that this new agent represents a definite advance in the search for a non-psychotomimetic, narcotic antagonist analgesic of low physical dependence potential.

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References

ARIËNS E.J., VAN ROSSUM, J.M. & SIMONIS, A.M. (1957). Affinity, intrinsic activity and drug interactions. *Pharmac. Rev.*, **9**, 218–236.

BLÄSIG, J., HERZ, A. REINHOLD, K. & ZIEGLGÄNSBERGER, S. (1973). Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. *Psychopharmacologia*, **33**, 19–38.

BLÄSIG, J., HÖLLT, V., HERZ, A. & PASCHELKE, G. (1976). Comparison of withdrawal precipitating properties of various morphine antagonists and partial agonists in relation to their stereospecific binding to brain homogenates. *Psychopharmacologia*, **46**, 41–51.

BOURA, A.L.A. & FITZGERALD, A.E. (1966). The pharmacology of N-(cyclopropylmethyl)-19-isopentyl-nororvinol hydrochloride. A potent and long lasting central depressant. *Br. J. Pharmac. Chemother.*, **26**, 307–321.

COWAN, A. (1974). Evaluation of the physical dependence capacities of oripavine-thebaine partial agonists in patas monkeys. *Adv. Biochem. Psychopharmac.*, **8**, 427–438.

COWAN, A. (1976). Use of the mouse jumping test for estimating antagonistic potencies of morphine antagonists. *J. Pharm. Pharmac.*, **28**, 177–182.

COWAN, A., DOXEY, J.C. & HARRY, E.J.R. (1977). The animal pharmacology of buprenorphine, an oripavine analgesic agent. *Br. J. Pharmac.*, **60**, 547–554.

COWAN, A., LEWIS, J.W. & MACFARLANE, I.R. (1971). Analgesic and dependence studies with oripavine partial agonists. *Br. J. Pharmac.*, **43**, 461–462P.

DENEAU, G.A. & SEEVERS, M.H. (1964). Drug dependence. In *Evaluation of Drug Activities: Pharmacometrics*, Vol. 1, ed. Laurence, D.R. & Bacharach, A.L. pp. 167–179. London: Academic Press.

DEWEY, W.L., HARRIS, L.S. & RITTER, K.S. (1975). Blockade of the development of morphine dependence and substitution studies in rats. *Pharmacologist*, **17**, 236.

DEWEY, W.L., HARRIS, L.S., HOWES, J.F. & NUITE, J.A. (1970). The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail flick and phenylquinone tests. *J. Pharmac. exp. Ther.*, **175**, 435–442.

FINNEY, D.J. (1971). The comparison of effectiveness. In *Probit Analysis*, 3rd edition, pp. 100–124. Cambridge: Cambridge University Press.

HENDERSHOT, L.C. & FORSAITH, J. (1959). Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and nonanalgesics. *J. Pharmac. exp. Ther.*, **125**, 237–240.

JASINSKI, D.R. & MANSKY, P.A. (1972). Evaluation of nalbuphine for abuse potential. *Clin. Pharmac. Ther.*, **13**, 78–90.

JASINSKI, D.R., MARTIN, W.R. & HOELDTKE, R.D. (1970). Effects of short- and long-term administration of pentazocine in man. *Clin. Pharmac. Ther.*, **11**, 385–403.

KOSTERLITZ, H.W., LESLIE, F.M. & WATERFIELD, A.A. (1975). Rates of onset and offset of action of narcotic analgesics in isolated preparations. *Eur. J. Pharmac.*, **32**, 10–16.

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.

MARTIN, W.R., GILBERT, P.E., EADES, C.G., THOMPSON, J.A. & HUPPLER, R.E. (1975). Progress report on the animal assessment program of the Addiction Research Center. *Proc. Comm. Problems of Drug Dependence*, 37th Meeting, 110–120.

SAELENS, J.K., GRANAT, F.R. & SAWYER, W.K. (1971). The mouse jumping test—a simple screening method to estimate the physical dependence capacity of analgesics. *Archs int. Pharmacodyn.*, **190**, 213–218.

SMITS, S.E. & TAKEMORI, A.E. (1970). Studies on the receptors involved in the action of the various agents in the phenylbenzoquinone analgesic assay in mice. *Br. J. Pharmac.*, **39**, 639–646.

SWAIN, H.H. & SEEVERS, M.H. (1975). Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. *Proc. Comm. Problems of Drug Dependence*, 37th Meeting, Addendum, 773–795.

TEIGER, D.G. (1974). Induction of physical dependence on morphine, codeine and meperidine in the rat by continuous infusion. *J. Pharmac. exp. Ther.*, **190**, 408–415.

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