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Evaluation of opioid-induced antinociceptive effects in anaesthetized and conscious animals

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1 The activity profiles of opioid agonists and non-steroidal analgesic agents have been compared against different nociceptive stimuli in the mouse and rat.

2 Opioid agonists, but not non-steroidal analgesic agents, inhibited reflex depressor responses evoked by visceral distension in anaesthetized rats. The ranked order of potency of opioids in the visceral distension reflex was identical to that observed in the mouse writhing assay.

3 Opioid-induced inhibition of reflex depressor responses and writhing was observed with ligands acting on μ- and κ-, but not δ-receptors. Antinociceptive activity of opioids in the rat cold water tail-flick assay was restricted to μ-receptor agonists.

4 Morphine- and ethylketocyclazocine (EKC)-induced inhibition of the visceral distension reflex was blocked by naloxone, but not by the quaternary opioid antagonist N-methylnalorphine.

5 Direct cardiovascular effects were observed with ligands for the μ- and κ-receptor. Blood pressure changes induced by morphine and Tyr.D-Ala.Gly.MePhe.Gly-ol (DAGOL), but not EKC, were blocked by N-methylnalorphine. Pretreatment with 16-methylcyprenorphine (M8008) antagonized morphine-, DAGOL- and EKC-induced cardiovascular effects, but not those of dynorphin-(1–13) or U50488.

6 It is concluded that reflex circulatory responses evoked by visceral distension in anaesthetized rats are a valid index for the evaluation of opioid-induced antinociception. A simultaneous assessment of cardiovascular effects of opioids was achieved.

Introduction

The application of mild, noxious stimuli may induce vocalization, body movements and reflex circulatory responses. The evaluation of analgesic agents, however, generally employs behavioural rather than autonomic manifestations to these stimuli. In contrast to behavioural responses, circulatory phenomena may be observed both in anaesthetized and decerebrate animals (Johannson, 1962). Response patterns mediated by the autonomic nervous system therefore appear to be independent of psychological factors (eg. stress).

Various models have been described in anaesthetized animals in which reflex cardiovascular changes have been evaluated following application of chemical (Downman *et al.*, 1948; Ferreira *et al.*, 1973; Moncada *et al.*, 1975) or thermal (Downman *et al.*, 1948) noxious stimuli. Distension of the intestines, a procedure known to cause visceral pain in man (Procacci *et al.*, 1979; Swarbrick *et al.*, 1980), has also been shown to evoke cardiovascular depressor responses and bradycardia during abdominal surgery (Folkow *et al.*, 1962). Similar circulatory effects occur following distension of the ileum (Lembeck & Skofitsch, 1982), renal pelvis (Brasch & Zetler, 1982) or duodenum (Moss & Sanger, 1987) in anaesthetized rats. Since both morphine (Lembeck & Skofitsch, 1982; Clark & Smith, 1985) and capsaicin (Lembeck & Skofitsch, 1982) abolish the depressor responses, it is possible that reflex decreases in blood pressure may offer objective, though indirect, criteria for the presence of nociception.

The antinociceptive effects of opioids on reflex cardiovascular phenomena may be influenced by factors such as species, model for assessment, the route of drug administration and the level of consciousness. Furthermore, the direct effects of opioids on blood pressure may complicate the quantitative evaluation of potential antinociceptive activity. The present study, therefore, was conducted to assess reflex circulatory responses, evoked by distension of an intestinal segment, as an index of nociception in anaesthetized rats. In this respect, opioids and non-steroidal analgesic agents were evaluated using the visceral distension reflex (VDR: Clark & Smith,

1985) and their potencies compared with those obtained in two behavioural antinociceptive models, namely a mouse writhing test (Smith *et al.*, 1985) and the rat cold water tail-flick assay (Pizziketti *et al.*, 1985). Ligands with relative selectivity for μ-, κ- or δ-opioid receptors were used to investigate the contribution of these multiple sites in opioid-induced effects.

Methods

Visceral distension reflex

Reflex depressor responses, evoked by visceral distension, were studied using a modification of the model originally described by Lembeck & Skofitsch (1982). In brief, male Wistar rats (220–450 g) were anaesthetized with 10% w/v ethylcarbamate (urethane; 1.1 g kg^{-1} i.p.) in saline and placed supine on a heated (37 ± 0.5°C) operating table. A jugular vein was then cannulated and 1% w/v α-chloralose (50 mg kg^{-1}) administered. Systemic blood pressure (BP) was monitored continuously (Statham P23AC transducer) from a common carotid artery and heart rate recorded (Grass Polygraph Model 7D) using a tachograph triggered by the BP signal. The trachea was also intubated, but respiratory parameters were not measured. Following mid-line incision of the peritoneum, the small intestine was located and a segment (10 cm) of jejenum/proximal ileum ligated at both ends. The segment was then cannulated with polyethylene tubing (i.d. 2.5 mm) connected to a saline-filled pressure transducer (Statham P23Db) and infusion pump. The ligated segment was carefully replaced *in situ* and the abdomen closed with sutures.

To evoke the VDR, intraluminal pressure was increased to 100 mbar by rapid infusion (20 ml min^{-1}) of warmed (37°C) saline. After 5 s distension at 100 mbar, intraluminal pressure was returned to resting levels (0 mbar) by opening a valve at body level. This procedure was repeated at 6 min intervals throughout the experiment. Four control distensions were performed before commencement of each study. Rats producing a VDR < 14 mmHg were rejected. Vehicle or drugs were administered intravenously in a dose-volume of 1 mg kg^{-1} at mid-cycle (i.e. 3 min before elevation of intraluminal pressure) and effects on the VDR monitored for a further 30 min. As tolerance may result from repeated administration of opioid agonists, rats received a single dose of these compounds. In opioid antagonist studies, drugs were administered intravenously 12 min before agonists.

Drug-induced inhibition of the reflex depressor responses was calculated by [(pre-dose VDR − post-dose VDR)/pre-dose VDR] × 100%. Pre-dose VDR represents the depressor response (mmHg) immediately before drug administration. Post-dose VDR represents the smallest depressor response occurring within the 30 min observation period. Antinociceptive activity was expressed in terms of an ED_{50}, defined as the dose of drug reducing by half the magnitude of the control depressor response.

Direct effects of opioid agonists on resting blood pressure were calculated from [(pre-dose mean BP − post-dose mean BP)/pre-dose mean BP] × 100%. Mean BP was determined as diastolic BP + 1/3(systolic BP-diastolic BP).

Mouse writhing assay

The abdominal constriction (writhing) assay in conscious mice was based on a modification (Smith *et al.*, 1985) of the model originally described by Hendershot & Forsaith (1959). Female CD1 mice (18–32 g) were injected with phenylbenzoquinone (PBQ: 2.5 mg kg^{-1} i.p.) in a dose-volume of 10 ml kg^{-1}, 30 min after the subcutaneous administration of either vehicle or test compound. After 10 min exposure to PBQ, 'writhes' were counted for a period of 2.5 min. Vehicle and drugs were administered in a dose-volume of 10 ml kg^{-1}. Antinociceptive activity was expressed in terms of an ED_{50}, defined as that dose of drug which induced a 50% reduction in the number of writhes observed after vehicle administration. In antagonist studies, 16-methylcyprenorphine (M8008: 2.0 mg kg^{-1}) was injected subcutaneously 15 min before the opioid agonists.

Rat cold water tail-flick assay

The rat cold water tail-flick assay was based on that described by Pizziketti *et al.* (1985). Male Wistar rats (159–200 g) were restrained gently but firmly and the lower half of their tails immersed in a bath of cold (0–1°C) water. The time-latency of tail removal from the water was measured. Animals failing to respond after 60 s of tail immersion were discarded. Latencies were measured 30 min before and after the subcutaneous injection of vehicle or drugs. The dose-volume used in these experiments was 10 ml kg^{-1}. After dosing, a cut-off time of 90 s was used. Antinociceptive activity was expressed in terms of an ED_{50}, defined as the dose to give a 50% potentiation of the initial reaction time.

Drugs

Drugs used were buprenorphine hydrochloride (Temgesic; Reckitt & Coleman), dipyrone (Kodak),

dynorphin-(1–13) (Cambridge Research Biochemicals), ethylketocyclazocine methanesulphonate (EKC; gift from Stirling-Winthrop), indomethacin (Sigma), [Leu^5]enkephalin (Cambridge Research Biochemicals), 16-methylcyprenorphine (M8008; gift from Reckitt & Coleman), N-methylnalorphine chloride (Wellcome, U.K.), morphine hydrochloride (McFarlane-Smith), naloxone hydrochloride (gift from Endo), [D-Pen^2, D-Pen^5]enkephalin (DPDPE; Sigma), Tyr.D-Ala.Gly.MePhe.Gly-ol(DAGOL; Wellcome, U.K.), Tyr.D-Arg.Gly.Phe(4-NO_2).Pro.NH_2(BW443C; Wellcome, U.K.) and *trans*-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) - cyclohexy] - benzene - acetamide methanesulphonate (U50488, Upjohn). Saline (0.9% NaCl w/v) was the vehicle for all compounds except indomethacin, which was dissolved in 0.05% w/v $NaHCO_3$. All dose-levels refer to the base.

Statistics

All data are expressed as the mean ± s.e.mean. Analysis was carried out by linear regression for ED_{50} determinations and Student's t test for group comparisons of significance. Probability values $P < 0.05$ were considered significant.

Results

Effects on antinociceptive models

Distension of the jejenum/proximal ileum to 100 mbar evoked transient, reflex falls in BP which were consistent in each experiment (mean pre-dose value = 31 ± 1 mmHg; $n = 51$). During the distension-induced depressor responses, effects on heart rate were generally negligible. Saline (1.0 ml kg^{-1} i.v.) administration did not affect the magnitude of the reflex, whereas, in descending order of potency, buprenorphine, EKC, morphine and U50488 induced dose-related inhibition of the VDR (Figure 1) and ED_{50} values are given in Table 1. The peak inhibitory effect of the opioids usually occurred within 15 min of intravenous administration and was followed by a gradual recovery towards the pre-dose level. The opioid peptides [Leu^5]enkephalin (4.0 mg kg^{-1}), DPDPE (1.0 mg kg^{-1}) and dynorphin-(1–13) (1.0 mg kg^{-1}), however, were relatively ineffective, the mean post-drug VDR being 33 ± 6, 37 ± 8 and 24 ± 5 mmHg, respectively. The effects of DAGOL (0.001–1.0 mg kg^{-1}) and BW443C (0.1–1.0 mg kg^{-1}) on the reflex were difficult to quantify, as both compounds caused a prolonged hypotension following intravenous administration.

The non-steroidal anti-inflammatory agents indomethacin (2.0 mg kg^{-1} i.v.) and dipyrone (300.0 mg kg^{-1} i.v.) did not appear to influence the reflex (mean post-drug VDR 48 ± 5 and 40 ± 4 mmHg, respectively).

In the conscious mouse, buprenorphine, EKC, U50488, morphine, DAGOL and BW443C all demonstrated dose-related inhibition of the number of writhes induced by intraperitoneal administration of PBQ (control range 8.8–10.4 writhes per animal). The potencies of the opioids in this antinociceptive model are shown in Table 1 and the ranked order of potency was buprenorphine > EKC > morphine > DAGOL > U50488 > BW443C. [Leu^5]enkephalin (50.0 mg kg^{-1} s.c.) had negligible effects on PBQ-induced writhing (mean post-drug no. writhes 10.4). The non-steroidal analgesic agents indomethacin and dipyrone reduced writhing in a dose-related manner, indomethacin being equipotent with DAGOL and approximately 60 times more potent than dipyrone (Table 1).

In the conscious rat, the range of latencies for tail withdrawal from cold water was 29.5–40.0 s. In descending order of potency, buprenorphine, EKC, morphine and BW443C induced a dose-related increase in latency for removal of the tail from cold water and ED_{50} values are shown in Table 1. U50488 (10.0 mg kg^{-1} s.c.) and indomethacin (10.0 mg kg^{-1} s.c.) were inactive in this model, demonstrating post-drug tail-flick latencies of 35.6 and 38.3 s, respectively.

Opioid antagonist studies on antinociception

Intravenous administration of saline (1.0 ml kg^{-1}), naloxone (0.9 mg kg^{-1}), M8008 (0.92 mg kg^{-1}) and N-methylnalorphine (8.6 mg kg^{-1}) did not affect the magnitude of the VDR. Pretreatment with naloxone (0.9 mg kg^{-1}), antagonized morphine- and EKC-induced inhibition of the VDR (Table 2). In addition, inhibitory effects of morphine and EKC on the VDR were significantly reduced by M8008 (0.92 mg kg^{-1} i.v.), but not by N-methylnalorphine (8.6 mg kg^{-1} i.v.). The marginal inhibitory effects of U50488 (0.79 mg kg^{-1} i.v.) on the VDR were not significantly reduced by either naloxone or M8008. At higher dose-levels of U50488 (2.37–7.9 mg kg^{-1} i.v.), prolonged hypotension was observed even in the presence of naloxone and M8008 (Table 3). Under these conditions, the effects of opioid antagonists on U50488-induced inhibition of the reflex could not be determined.

In PBQ-induced writhing, M8008 (2.0 mg kg^{-1} s.c.) significantly antagonized the antinociceptive effects of DAGOL, morphine and BW443C, the dose-ratios and 95% confidence limits being 3.2 (2.4–4.3), 5.0

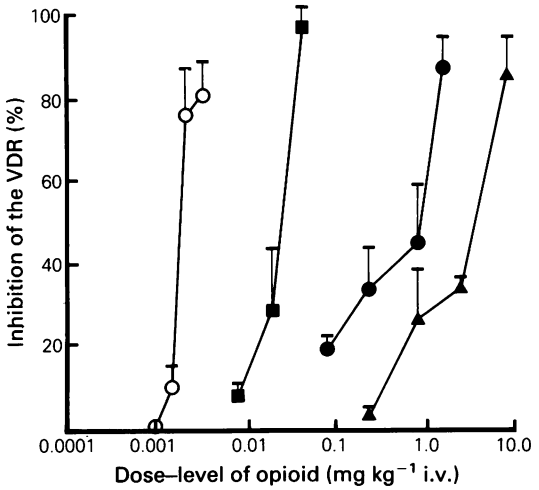

Figure 1 The effect of buprenorphine (○), ethylketocyclazocine (■), morphine (●) and U50488 (▲) on the magnitude of depressor responses (VDR) induced by visceral distension in urethane/chloralose anaesthetized rats. Results are the mean of 3–7 experiments. Vertical lines represent the s.e.mean.

(3.9–6.3) and 3.8 (2.8–5.3), respectively. Effects of EKC and U50488 were not reduced by M8008, dose-ratios and 95% confidence limits being 1.6 (1.3–2.0) and 1.1 (0.8–1.4), respectively.

Direct effects of opioids on resting blood pressure

The resting BP of rats before drug administration was 122 ± 2 mmHg (range 98–167 mmHg; $n = 65$). Bolus injections of saline (1.0 ml kg^{-1} i.v.) had a negligible effect on BP. Intravenous administration of morphine (0.04–0.81 mg kg^{-1}), U50488 (0.24–7.9 mg kg^{-1}) and dynorphin-(1-13) (1.0 mg kg^{-1}) decreased resting BP (Table 3). In contrast, EKC caused a dose-related hypertension. Buprenorphine, DPDPE and the non-steroidal analgesic agents were devoid of cardiovascular effects. Effects of morphine, dynorphin-(1–13) and [Leu^5]enkephalin were generally transient, whereas BW443C and DAGOL produced a more prolonged hypotension. The duration of U50488-induced depressor effects increased with dose (0.24–2.39 mg kg^{-1} i.v.), but was relatively transient at the highest dose-level (7.9 mg kg^{-1} i.v.) due to the appearance of a secondary pressor effect (28 ± 13%; $n = 3$). In general, the opioid agonists possessed steep dose-response curves and a plateau of cardiovascular effects at higher dose-levels.

The opioid antagonists naloxone (0.9 mg kg^{-1} i.v.) and M8008 (0.92 mg kg^{-1} i.v.) did not affect resting BP. The quarternary opioid antagonist N-methylnalorphine (8.6 mg kg^{-1} i.v.), however, caused a transient decrease in BP (15 ± 2%; $n = 14$). As shown in Table 3, naloxone blocked the cardiovascular effects of morphine (0.04 and 0.81 mg kg^{-1} i.v.), EKC (0.08 mg kg^{-1} i.v.), dynorphin-(1–13)

Table 1 Antinociceptive activity of opioid agonists and non-steroidal analgesic agents in the visceral distension reflex (VDR), phenylbenzoquinone (PBQ)-induced writhing and tail-flick assays

	Antinociceptive activity: (ED_{50} mg kg^{-1})		
Compound	*VDR* (i.v.)	*PBQ-writhe* (s.c.)	*Tail-flick* (s.c.)
Buprenorphine	0.0018 (0.0015–0.0020)	0.011 (0.009–0.014)	0.007 (0.003–0.013)
EKC	0.02 (0.015–0.025)	0.06 (0.05–0.08)	0.27 (0.13–0.49)
Morphine	0.50 (0.22–1.41)	0.43 (0.37–0.50)	0.81 (0.01–1.87)
DAGOL	?	0.87 (0.68–1.09)	NT
U50488	2.40 (1.56–4.43)	0.96 (0.82–1.12)	NE @ 10.0
BW443C	?	2.76 (2.28–3.30)	14.0 (no limits)
Indomethacin	NE @ 2.0	0.72 (0.50–0.90)	NE @ 10.0
Dipyrone	NE @ 300	42.6 (35.0–53.3)	NT

At least 3 animals per dose group and a minimum of 3 doses were used for each ED_{50} determination. Figures in parentheses represent 95% confidence limits.
DAGOL- and BW443C-induced inhibition of the VDR could not be determined due to profound effects on the resting blood pressure (see results).
NE = no effect; NT = not tested.

Table 2 Effect of pretreatment with either saline (1.0 ml kg^{-1} i.v.), naloxone (0.9 mg kg^{-1} i.v.), N-methylnalorphine (8.6 mg kg^{-1} i.v.) or M8008 (0.92 mg kg^{-1} i.v.) on opioid-induced inhibition of the visceral distension reflex (VDR)

Opioid	*Dose* (mg kg^{-1})	*Saline*	% *decrease VDR* (mean ± s.e.mean) *Naloxone*	*M8008*	*N-methylnalorphine*
Morphine	0.08	19 ± 2 (3)	8 ± 4 (5)	—	—
	0.81	45 ± 14 (5)	13 ± 7* (4)	6 ± 1* (4)	53 ± 10 (4)
U50488	0.79	27 ± 12 (4)	7 ± 4 (3)	8 ± 5 (5)	—
	7.90	87 ± 3 (3)	—	—	—
EKC	0.08	96 ± 4 (4)	15 ± 8** (3)	6 ± 3** (4)	96 ± 4 (3)

Figures in parentheses are the number of experiments in each group. Asterisks denote a significant difference between saline and antagonist-pretreated animals. *$P < 0.05$; **$P < 0.01$.

(1.0 mg kg^{-1} i.v.) and U50488 (0.79 mg kg^{-1} i.v.). Naloxone and M8008 also blocked the pressor, but not depressor effects induced by a high dose of U50488 (7.9 mg kg^{-1} i.v.). In single dose studies, morphine- and DAGOL-, but not EKC-induced cardiovascular effects, were antagonized by N-methylnalorphine (8.6 mg kg^{-1} i.v.). M8008 (0.92 mg kg^{-1} i.v.) blocked the effects of morphine, DAGOL and EKC, but not those of dynorphin-(1–13) or U50488.

Discussion

Distension of the jejenum/proximal ileum to 100 mbar induced transient depressor responses in approximately 90% of urethane/chloralose anaesthetized rats. This figure represents an improvement over that of 72% obtained by Clark & Smith (1985), where urethane alone was employed as the anaesthetic agent.

In accordance with the results of Lembeck & Sko-

Table 3 Effect of pretreatment with either saline (1.0 ml kg^{-1} i.v.), naloxone (0.9 mg kg^{-1} i.v.), N-methylnalorphine (8.6 mg kg^{-} i.v.) or M8008 (0.92 mg kg^{-1} i.v.) on opioid-induced cardiovascular effects

Opoid agonist	*Dose* (mg kg^{-1} i.v.)	*Saline*	% *change resting blood pressure* (mean ± s.e.mean) *Naloxone*	*M8008*	*N-methylnalorphine*
DAGOL	0.001	−13 ± 2 (3)	—	—	—
	0.003	−24 ± 10 (3)	—	—	—
	0.010	−38 ± 5 (3)	—	—	—
	1.000	−39 ± 3 (7)	—	−4 ± 2** (3)	−4 ± 1** (4)
Morphine	0.040	−19 ± 4 (6)	−3 ± 1** (5)	—	—
	0.080	−31 ± 3 (10)	—	—	—
	0.810	−30 ± 7 (5)	−1 ± 1** (4)	0 ± 0* (4)	0 ± 0* (4)
BW443C	0.100	−18 ± 7 (3)	—	—	—
	1.000	−49 ± 6 (3)	—	—	—
[Leu^5]enkephalin	0.100	−8 ± 4 (3)	—	—	—
	1.000	−19 ± 3 (3)	—	—	—
	4.000	−33 ± 2 (3)	—	—	—
U50488	0.240	−3 ± 3 (3)	—	—	—
	0.790	−27 ± 7 (6)	0 ± 0* (4)	−20 ± 4 (5)	—
	2.370	−26 ± 5 (4)	—	−45 ± 1 (3)	—
	7.900	−36 ± 1† (3)	−45 ± 1 (3)	−39 ± 1 (4)	—
Dynorphin-(1–13)	1.000	−24 ± 6 (3)	0 ± 0* (3)	−16 ± 1 (3)	—
EKC	0.020	+13 ± 3 (5)	—	—	—
	0.040	+15 ± 6 (4)	—	—	—
	0.080	+39 ± 6 (4)	−9 ± 3** (3)	−4 ± 4** (4)	+15 ± 9 (3)

Figures in parentheses are the number of experiments in each group. † In saline pretreated animals, U50488 (7.9 mg kg^{-1} i.v.) caused a secondary pressor effect of 28 ± 13%. Asterisks denote a significant difference between saline and antagonist pretreated animals. *$P < 0.05$; **$P < 0.01$.

fitsch (1982) and Clark & Smith (1985), the classical μ-receptor agonist morphine (Paterson *et al.*, 1983) decreased the magnitude of distension-induced depressor responses. Buprenorphine, a partial agonist on μ- and κ-receptors (Kajiwara *et al.*, 1986), EKC, a non-selective opioid agonist with some preference for the κ-receptor (Paterson *et al.*, 1983) and U50488, a selective κ-receptor agonist (Von Voigtlander *et al.*, 1983), also exhibited dose-related inhibition of the VDR. An essential criterion for the assessment of these opioids on cardiovascular reflexes is the maintenance of a stable resting BP. Although morphine and U50488 caused hypotension and bradycardia, inhibition of the VDR was still evident in animals where the BP had returned to pre-dose levels. However, the selective μ-receptor agonist DAGOL (Kosterlitz & Paterson, 1981; Handa *et al.*, 1981) exhibited a more prolonged hypotension and the time-course of this effect may have exceeded inhibition of reflex depressor responses. Similar problems were encountered in the quantitative evaluation of the opioid peptide BW443C, a postulated μ-receptor agonist that displays peripherally-mediated antinociceptive effects (Follenfant *et al.*, 1988). In contrast to U50488, dynorphin-(1–13) ($1\,mg\,kg^{-1}$ i.v.), an endogenous opioid peptide possessing *in vitro* agonist activity on κ-receptors (Oka *et al.*, 1982), did not appear to influence the reflex. Higher doses of dynorphin-(1–13) were not tested in this model due to the marked hypotensive effects of this peptide.

Using thermal nociceptive assays, antinociception has been observed in mice after intracerebroventricular (Ward & Takemori, 1983; Chaillet *et al.*, 1984) or intrathecal (Yaksh & Rudy, 1977) administration of ligands for the δ-receptor. Such effects, however, could not be demonstrated following systemic administration of the selective δ-receptor agonist DPDPE (Mosberg *et al.*, 1983) or [Leu^5]enkephalin, a non-selective opioid agonist with preference for the δ-receptor (Lord *et al.*, 1977). These results may indicate that δ-receptor ligands, administered by either the intravenous or subcutaneous route, do not reach adequate concentrations in the CNS. Alternatively, δ-receptor agonists may be relatively inactive in tests employing mechanical and chemical stimuli. However, Rodriguez *et al.* (1986) demonstrated that intrathecal administration of DPDPE suppressed nociception in a rat paw pressure model, and that these effects were antagonized by pretreatment with the selective δ-receptor antagonist ICI 174,864 (Cotton *et al.*, 1984; Corbett *et al.*, 1984).

A comparison of the ED_{50} values of opioid agonists acting on μ- and/or κ-receptors demonstrated an identical ranked order of potency in the VDR and PBQ-induced writhing assay i.e. buprenorphine > EKC > morphine > U50488. These results support the view that nociceptive tests employing non-heat noxia are sensitive to μ- and κ-receptor agonists (Tyers, 1980). It would appear, therefore, that μ-, and κ-receptor agonists possess a similar profile in the VDR and PBQ-writhing assay, although direct cardiovascular effects of DAGOL prevented evaluation of the selective μ-receptor ligand on reflex depressor responses. However, effects of non-steroidal anti-inflammatory agents were not comparable. Of the present models, only the PBQ-induced writhing assay was sensitive to indomethacin and dipyrone.

The apparent inhibitory effects of non-analgesic compounds e.g. CNS depressants (Hendershot & Forsaith, 1959), have cast doubts on the reliability of the PBQ-induced writhing assay. Additional experiments therefore were performed using the rat cold water tail-flick assay, a model that detects opioid agonists but not antipsychotic or anxiolytic drugs (Pizziketti *et al.*, 1985). In these studies, the ranked order of potency of opioids was buprenorphine > EKC > morphine > BW443C. Hence, antinociceptive activity of opioids in the tail flick assay resembled that obtained in PBQ-writhing experiments. Although probably a μ-receptor agonist, the high doses of BW443C required to obtain antinociceptive effects in the tail-flick assay support recent findings that this opioid peptide is less potent where CNS penetration is required (Follenfant *et al.*, 1988). U50488 also failed to increase latency in the tail-flick assay, supporting the notion that κ-receptor agonists are relatively ineffective against thermally-induced nociception (Tyers, 1980). Thus antinociceptive effects of EKC in this model are more likely to result from interaction with μ-receptors. In agreement with Pizziketti *et al.* (1985), non-narcotic analgesic agents do not significantly affect tail-flick latency at dose-levels likely to inhibit synthesis of prostaglandins (Moncada *et al.*, 1975). The rat tail-flick and VDR models therefore appear to be specific assays for opioid-induced antinociceptive effects.

The relative contribution of μ- and κ-receptor subtypes in opioid-induced antinociception was further investigated using various opioid antagonists in the VDR and PBQ-induced writhing assays. Pretreatment with M8008 ($2.0\,mg\,kg^{-1}$ i.v.), a pure antagonist with *in vitro* selectivity for δ- and μ-receptors (Smith, 1987), caused a significant decrease in the DAGOL-, morphine- and BW443C- induced inhibition of writhing. In the presence of M8008, effects of U50488 were sustained, a result that clearly demonstrates a role of κ-receptors in inhibition of writhing. Some antagonism of EKC was apparent, though this failed to reach significance.

The opioid nature of morphine and EKC-induced inhibition of VDR was confirmed by their antagonism with naloxone. Furthermore, the cardiovascular effects of these opioid agonists were

significantly reduced by naloxone ($1.0\,mg\,kg^{-1}$ i.v.) pretreatment. At similar dose-levels, naloxone has been shown to antagonize the analgesic effects of morphine, EKC and cyclazocine (Tallarida *et al.*, 1979). For studies with M8008, however, the dose-level ($1.0\,mg\,kg^{-1}$ i.v.) was within the range used by Birch & Hayes (1987) to differentiate between μ- and κ-mediated effects of opioids. Opioids known to act on μ-receptors e.g. morphine and DAGOL, caused dose-related hypotension. In addition, [Leu^5]enkephalin, but not DPDPE decreased BP, possibly indicating an action on μ- rather than δ-receptors. Although these opioid agonists differed in relative potency, peak hypotensive effects of [Leu^5]enkephalin, DAGOL and morphine were of comparable magnitude. It was found that M8008 blocked the direct cardiovascular effects of DAGOL and morphine, and antagonized morphine-induced inhibition of the VDR. However, cardiovascular effects of the κ-receptor agonist dynorphin-(1–13) were not reduced by M8008. In addition, the intravenous administration of U50488 ($0.79\,mg\,kg^{-1}$) caused a hypotensive response that was blocked by naloxone but not by M8008. Pretreatment with M8008 also had no significant effect on U50488-induced inhibition of the VDR. These results therefore provide further evidence for the selective antagonist effects of M8008 on μ-receptor sites. At the dose of $7.9\,mg\,kg^{-1}$ i.v., U50488 caused a transient hypotension followed by a relatively prolonged pressor effect. Pretreatment with naloxone and M8008 blocked the secondary hypertensive phase, but not the initial decrease in BP. Thus a high dose-level of U50488 appears to exert non-opioid effects on BP. In contrast to U50488, EKC caused a dose-related hypertension. Laurent & Schmitt (1983) have shown that opposite cardiovascular effects could be induced by activation of various opioid receptors in multiple sites of the CNS. However, the attenuation of EKC-induced cardiovascular effects and inhibition of the VDR by M8008 supports the results of the tail-flick studies where EKC appears to act on μ-receptors.

Using the PBQ-induced writhing model, Follenfant *et al.* (1988) have demonstrated antagonism of the effects of BW443C, but not morphine, by N-methylnalorphine, a quaternary opioid antagonist which acts preferentially at μ-receptor sites (Kobylecki *et al.*, 1982; Magnan *et al.*, 1982) and which has a limited penetration to the CNS (Smith *et al.*, 1982). In the current studies, peripherally mediated effects of ligands selective for the μ-receptor were observed on BP, but not on the VDR. Thus direct hypotensive effects of DAGOL and morphine were attenuated by N-methylnalorphine, a result that confirms previous investigations (Clark & Smith, 1985; Given *et al.*, 1986). However, a central site of action for BW443C-induced hypotension must also be considered. Chapple & Donoghue (1987) demonstrated that this opioid peptide may enter the CNS via the obex, a region of the brain stem where the blood-brain barrier is relatively permeable. It has been claimed that cardiovascular effects of EKC (Ensinger *et al.*, 1986; Szabo *et al.*, 1986) are peripherally mediated via κ-receptors located on sympathetic nerve terminals. However, in the present experiments EKC-induced hypertension appears to be mediated predominantly via μ-receptors.

In summary, the antinociceptive profiles of various opioid agonists were compared using an established assay, i.e. PBQ-induced writhing in mice, and two relatively novel techniques, the VDR and the cold water tail-flick in rats. Although different species, routes of drug administration and levels of consciousness were employed in these studies, each test system exhibited a similar ranked order of potency of the opioid agonists. The VDR and tail-flick assays, but not PBQ-induced writhing, appear to be specifically influenced by opioid antinociceptive agents. However, studies employing thermal stimuli do not detect κ-receptor agonists, whereas the VDR may be sensitive to this class of opioid. Continuous monitoring of BP in the anaesthetized rat enabled a simultaneous assessment of cardiovascular and antinociceptive effects of opioids.

A further advantage of using anaesthetized rats was the removal of psychological factors that may complicate analysis of opioid-induced effects in conscious animals. Peets & Pomeranz (1987) reached similar conclusions following morphine-induced suppression of a nocifensive reflex i.e. tail-flick electromyograms, in the anaesthetized rat. The VDR, therefore, may be considered as a valid index for the evaluation of analgesic effects of opioids. The model also has the potential for investigating central and peripheral roles of mediators thought to be involved in the nociceptive process e.g. substance P (Lembeck & Skofitsch, 1982) and 5-hydroxytryptamine (Moss & Sanger, 1988).

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