Br. J. Pharmacol. (1992), **106**, 783–789

GR94839, a κ-opioid agonist with limited access to the central nervous system, has antinociceptive activity

[1]H. Rogers, P.J. Birch, S.M. Harrison, †E. Palmer, †G.R. Manchee, *D.B. Judd, *A. Naylor, *D.I.C. Scopes & A.G. Hayes

Departments of Neuropharmacology, †Biochemical Pharmacology and *Medicinal Chemistry, Glaxo Group Research Ltd, Ware, Herts SG12 0DP

1 The pharmacological profile of GR94839, a κ-opioid agonist with limited access to the central nervous system, has been investigated. Its antinociceptive activity has been compared with that of GR103545, a centrally-penetrating κ-agonist and ICI204448, the previously described peripherally-selective κ-agonist.

2 GR94839 was a potent agonist in the rabbit vas deferens *in vitro* assay for κ-opioid receptors (IC_{50}: 1.4 ± 0.3 nM; $n = 6$), but had limited activity at μ- or δ-opioid receptors.

3 In the mouse abdominal constriction test, GR94839 was 238 fold more potent when given i.c.v. (ED_{50}: 0.008 (0.004–0.029) mg kg^{-1}; $n = 18$) than when s.c. (ED_{50}: 1.9 (0.7–3.1) mg kg^{-1}; $n = 30$). In comparison, GR103545 was equipotent when given i.c.v. or s.c.

4 After intravenous administration, the maximum plasma to brain concentration-ratio attained by GR94839 was 18 compared with 2 for GR85571, a structurally-related κ-agonist that is centrally-penetrating.

5 GR94839 inhibited the 2nd phase of the rat formalin response at doses 7 fold lower than those required to inhibit the 1st phase (ED_{50} vs 1st phase: 10.2 (6.7–17.1) mg kg^{-1}, s.c.; ED_{50} vs 2nd phase: 1.4 (1.0–1.8) mg kg^{-1}, s.c.; $n = 18$). GR103545 was equipotent against the two phases.

6 Intraplantar administration of the opioid antagonists, norbinaltorphimine (100 μg) or naltrexone (1 μg), reversed the antinociceptive effect of systemic GR94839 (3 mg kg^{-1}, s.c.) against the 2nd phase of the formalin response and intraplantar injection of GR94839 (30–100 μg) selectively inhibited the 2nd phase.

7 GR94839 and ICI204448 reversed the hyperalgesia in the zymosan-inflamed rat paw at doses (ED_{50} GR94839: 2.0 (1.1–3.2) mg kg^{-1}, s.c.; ED_{50} ICI204448: 1.2 (0.8–1.7) mg kg^{-1}, s.c.), lower than those required to raise the noxious pressure threshold in the non-inflamed paw (ED_{50} GR94839: 16.4 (8.6–46.7) mg kg^{-1}, s.c.; ED_{50} ICI204448: 68.0 (22.1–32000) mg kg^{-1}, s.c.). GR103545 raised the noxious presure threshold in the inflamed and non-inflamed paws at the same doses.

8 GR94839 was sedative in the rat rotarod test (ED_{50}: 35 (12–245) mg kg^{-1}, s.c.) at doses higher than those required to inhibit the 2nd phase of the formalin response or reverse hyperalgesia in the zymosan-inflamed rat paw. The doses were comparable to those that inhibited the 1st phase of the formalin response and raised the noxious pressure threshold in the non-inflamed paw.

9 The results suggest that GR94839 is a selective κ-agonist which has antinociceptive activity against inflammatory pain at doses that produce limited central effects. These antinociceptive effects are probably mediated at peripheral opioid receptors.

Keywords: κ-Opioid agonist; antinociception; peripheral opioid receptor

Introduction

There is evidence to suggest that κ-opioid receptors are present on the peripheral terminals of primary afferent neurones (Stein *et al.*, 1989) and that activation of these receptors reduces hyperalgesia in a rat model of arthritic pain. A κ-opioid agonist, ICI204448, which has limited access to the central nervous system has been described previously by Shaw and colleagues (1989), however, the analgesic profile of this compound has not been investigated in detail. A κ-opioid agonist with limited access to the central nervous system could be antinociceptive against inflammatory pain but lack the adverse side-effects, e.g. sedation and dysphoria, of a centrally-penetrating κ-agonist. This paper describes the pharmacological profile of a second peripherally-selective κ-agonist, GR94839, including its activity in nociceptive tests with an inflammatory pain component.

GR94839 is a κ-opioid receptor agonist, closely related in structure to the potent κ-agonist, GR103545 (Hayes *et al.*, 1990). GR103545 has the behavioural profile of a centrally-penetrating κ-agonist, having antinociceptive activity in a variety of tests and producing sedation and diuresis (Hayes *et al.*, 1990). In the present study pharmacological and biochemical data suggest that GR94839 is a potent κ-agonist but, unlike GR103545, has limited access to the cental nervous system. The antinociceptive activity of GR94839 is compared with GR103545 and ICI204448. Also, the plasma and brain concentrations of GR94839 are compared with a centrally-penetrating κ-agonist, GR85571. The structures of the κ-agonists are shown in Figure 1. A preliminary communication of these results has been made (Hayes *et al.*, 1991).

Methods

Isolated tissue preparations

Vasa deferentia were removed from Californian rabbits (weight range 2.5–3.5 kg), Random Hooded rats (weight range 150–200 g) and Golden Syrian hamsters (weight range

[1] Author for correspondence.

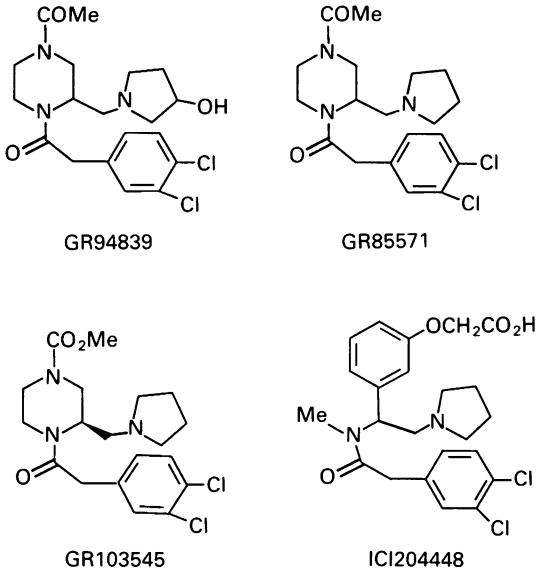

Figure 1 Chemical structures of the κ-opioid agonists: GR94839, GR85571, GR103545, and ICI204448.

250–300 g) and suspended between platinum electrodes in 5 ml organ baths containing Krebs-Henseleit solution of composition (mM): NaCl 118, $NaHCO_3$ 29, glucose 11, KCl 4.7, $CaCl_2$ 2.5 and KH_2PO_4 1.2, pH 7.4, gassed with 95% O_2:5% CO_2 at 37°C. Contractions were evoked by field-stimulation with a single square-wave pulse (width 0.5 ms; frequency 0.1 Hz; 1 V above maximum voltage) in rabbit or rat vasa deferentia, and a train of three similar pulses 200 ms apart in the hamster vas deferens. Cumulative concentration-response curves were constructed and the IC_{50} values (50% inhibition of the twitch height) ± standard error of the mean were determined where appropriate.

In vivo *experiments*

Abdominal constriction test Male CRH mice (weight range 14–22 g) were used. Intraperitoneal injections of acetylcholine 3 mg kg^{-1}, carba-prostacyclin 0.1 mg kg^{-1}, or acetic acid 0.6% (dose volume 10 ml kg^{-1}) were used to induce abdominal constrictions. The number of abdominal constrictions occurring 0–3.5 min post-acetylcholine, 0–10 min post-carba-prostacyclin and 5–20 min post-acetic acid injection were recorded. Kappa agonists were administered subcutaneously (dose volume 10 ml kg^{-1}) or directly into the cerebral ventricles (i.c.v.; dose volume 5 μl) 30 min prior to the observation period.

Determination of plasma and brain concentrations GR94839 or GR85571 were administered to male albino rats ($n = 2$; weight range 150–200 g) at 10 mg kg^{-1} i.v. via the tail vein. At various times post-dosing, animals were anaesthetized and exsanguinated via the dorsal aorta. The brain was then removed and homogenized with acetone, the samples centrifuged, and the supernatant removed. This was evaporated to dryness, resuspended in water and subjected to extraction on diol columns. Drug was extracted from the plasma by solid phase extraction with diol columns. The eluate was analysed by high performance liquid chromatography using a cyano spherisorb column and a mobile phase of sodium acetate (0.01 M, pH 4.2): acetonitrile (35:65 volume:volume).

Formalin-induced paw licking Male Random Hooded rats (weight range 50–80 g) were used. A solution of 5% formalin in phosphate-buffered saline (pH 7.4; dose volume 50 μl) was injected into the pad of one-hind-paw (intraplantar). A biphasic licking of the injected paw was observed, and the duration of licking was recorded 0–5 min (1st phase) and 15–30 min post-formalin injection (2nd phase). The 1st phase of the formalin response is thought to be due to direct activation of sensory nerve terminals by formalin and the 2nd phase is thought to involve local inflammation (Hunskaar & Hole, 1986). GR94839 or GR103545 were administered s.c. 30 min before each phase of licking in separate rats, and ICI204448 was injected 15 min before the 1st phase. The dose volume used was 4 ml kg^{-1}. In some experiments, drugs were administered intraplantar (dose volume 50 μl) immediately before the 1st or 2nd phases of licking.

Zymosan-induced hyperalgesia Male Random Hooded rats (weight range 80–120 g) or male Dunkin-Hartley guinea-pigs (weight range 150–220 g) were used. Intraplantar injection of 100 μl of a 20% suspension of zymosan in saline lowered the noxious pressure threshold of the injected paw at greater than 3 h post-zymosan in both guinea-pigs and rats. The noxious pressure threshold of each hind-paw was determined, before intraplantar injection of zymosan and at 5 h post-injection in rats and at 3 h and 4 h post-injection in guinea-pigs. Kappa-agonists were administered s.c. 4.5 h post-zymosan in rats (dose volume 4 ml kg^{-1}) and 3.5 h post-zymosan in guinea-pigs (dose volume 2 ml kg^{-1}).

Rotarod Male Random Hooded rats (weight range 80–120 g) were used. Rats were placed on a rotating horizontal rod which accelerated in a linear fashion from 0 to 50 rev min^{-1}. The score (proportional to the acceleration of the tread-mill) when the rat fell from the rod was recorded as a measure of the incapacitating effect of the drug. κ-Agonists or saline were administered 30 min before testing.

Experimental design and data analysis

Behavioural tests were performed on 6 or 8 animals/drug treatment. In some cases data have been pooled from more than one experiment. In all experiments animals were colour-coded and drug treatments randomized between groups. Observers were unaware of the drug treatments that animals had received. The methods of Finney (1964) were used to determine regression slopes, linearity and parrallelism for dose-response curves and also the ED_{50} values (50% reduction in response compared to saline pretreated animals) with 95% confidence limits. For the noxious pressure threshold experiments, the ED_{50} value in the inflamed paw (50% reversal of the fall in noxious pressure threshold) and the ED_{30} value in the non-inflamed paw (30% increase in noxious pressure threshold) were determined. The significance of drug effects was tested by a Student's unpaired t test ($P < 0.05$).

Drugs and solutions

Drugs were freshly made up in saline for each experiment, and administered in the dose volumes indicated. A control group of animals injected with an appropriate volume of saline was included in each experiment. Drugs were obtained from the following sources: acetylcholine (Sigma), carba-prostacyclin (Cayman Chemical Co. Inc.), [D-Ala^2, MePhe^4, Gly(ol)^5] enkaphalin (DAMGO, Bachem), [D-Pen^2,D-Pen^5] enkephalin (DPDPE, Bachem), ethylketocyclazocine methane sulphonate (EKC; Sterling-Winthrop), ICI204448 (**R,S**)-3-[1-[[(3,4-dichlorophenyl) acetyl] methylamino]-2-(pyrrolidinyl) ethyl] phenoxyacetic acid hydrochloride, Cambridge Research Biochemicals), morphine hydrochloride (Macfarlan-Smith), naltrexone hydrochloride (Endo). GR85571 (4-acetyl-1-[(3,4-dichlorophenyl) acetyl] -2 - (1-pyrrolidinylmethyl) piperazine), GR94839 (4-acetyl-1-(3,4-dichlorophenyl)acetyl]-2-[(3-hydroxy-1-pyrrolidinyl) methyl] piperazine), GR103545 ((**R**)-methyl 4-[(3,4-dichlorophenyl)acetyl]-3- (1-pyrrolidinylmethyl) -1-piperazinecarboxylate fumarate), M8008 (16-methylcyprenorphine), and norbinaltorphimine (norBNI) were all synthesized in the Department of Medicinal Chemistry, Glaxo Group Research Ltd, Ware.

Results

Field-stimulated vasa deferentia

In the rabbit vas deferens assay for κ-opioid receptors, GR94839 was a potent agonist, inhibiting the electrically-evoked twitch with an IC_{50} value of 1.4 ± 0.3 nM ($n = 6$). For comparison, the κ-agonist, ethylketocyclazocine had an IC_{50} value of 36 ± 8 nM ($n = 6$). Pre-equilibration of the tissue with naloxone (300 nM) for 30 min caused a rightward shift of the dose-response curve to GR94839 ($n = 4$). The pK_B value for naloxone antagonizing GR94839 was 7.5 ± 0.1 ($n = 4$), which is consistent with an action of GR94839 on κ-receptors. In the rat vas deferens assay for μ-opioid receptors, GR94839 at a dose of 100 μM reduced the twitch height by 42% ($n = 2$) whereas the selective μ-agonist [D-Ala^2,MePhe^4,Gly^5-ol]enkephalin (DAMGO) had an IC_{50} value of approximately 7 μM ($n = 2$). In the hamster vas deferens assay for δ-opioid receptors, GR94839 had an IC_{50} value of approximately 13 μM ($n = 2$) compared with approximately 0.12 μM for the selective δ-agonist, D-Pen^2,D-Pen^5]enkephalin (DPDPE; $n = 2$). Thus, *in vitro*, GR94839 was a potent κ-agonist with limited activity at μ- or δ-receptors.

In vitro experiments

Mouse abdominal constriction test In the mouse abdominal constriction test, GR94839 had EC_{50} values of 1.9 (0.7–3.1) mg kg^{-1}, s.c. ($n = 30$) and 0.16 (0.08–0.57) μg ($n = 18$) which is equivalent to approximately 0.008 mg kg^{-1}, i.c.v. Thus, the ratio of ED_{50} values after subcutaneous administration and i.c.v. administration was 238. In contrast, GR103545 had very similar ED_{50} values following subcutaneous (0.015 (0.005–0.025) mg kg^{-1}) and i.c.v. administration (0.010 (0.005–0.020) mg kg^{-1}), giving a subcutaneous to i.c.v. ED_{50} ratio of 1.5 ($n = 6$). ICI204448 was more potent given i.c.v. than subcutaneously with ED_{50} values of 0.14 (0.04–0.41) mg kg^{-1}, i.c.v. and 4.4 (2.5–7.4) mg kg^{-1}, s.c. ($n = 6$) with a subcutaneous to i.c.v. ED_{50} ratio of 31. GR94839 also inhibited carba-prostacyclin-induced and acetic acid-induced abdominal constrictions with ED_{50} values of 0.8 (0.3–1.7) and 4.1 (2.6–6.7) mg kg^{-1}, s.c. respectively ($n = 6$).

The inhibition produced by GR94839 at a dose of 10 mg kg^{-1}, s.c. was reversed by a co-injection of the opioid antagonist, naltrexone (10 mg kg^{-1}, s.c.) i.e. mean number of abdominal constrictions: saline 8.0 ± 0.7; naltrexone 8.7 ± 1.4; GR94839 0 ± 0; GR94839 + naltrexone 7.7 ± 1.1 ($n = 6$).

Plasma and brain concentration-time profiles of GR94839 and GR85571 The plasma and brain concentrations of GR94839 were measured up to 5 h following an i.v. injection at 10 mg kg^{-1} (Figure 2). Peak levels of GR94839 observed in plasma (1282 ng ml^{-1} at 15 min) were significantly higher than those observed in brain tissue (103 ng g^{-1} at 30 min). The maximum plasma:brain concentration ratio attained by GR94839 was 18, 15 min after injection. GR85571 has a similar *in vitro* potency to GR94839 (IC_{50} in rabbit vas deferens: 10 nM; $n = 2$) but has an ED_{50} in the mouse acetylcholine-induced abdominal constriction test of 0.08 (0.04–0.15) mg kg^{-1}, s.c. ($n = 6$), suggesting that it readily penetrates into the central nervous system. The concentrations of GR85571 achieved in brain and plasma following a dose of 10 mg kg^{-1} i.v. were very similar throughout the time-course and similar to the plasma levels attained by GR94839. The maximum plasma:brain concentration ratio attained was 2, 30 min after injection. These findings are consistent with GR94839 having limited access to the central nervous system.

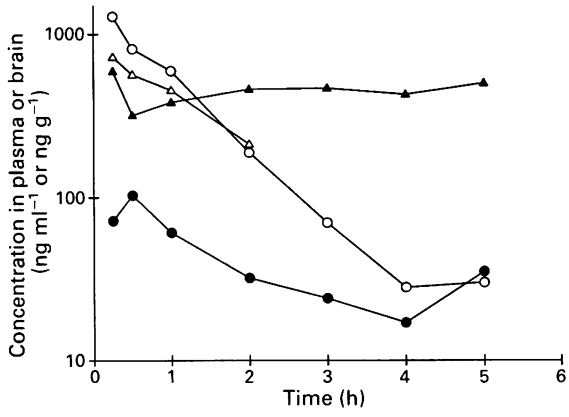

Figure 2 A comparison of the plasma and brain concentration-time profiles for GR94839 and GR85571. Rats were dosed ($n = 2$/drug) with either GR94839 (circles) or GR85571 (triangles) at 10 mg kg^{-1}, i.v. and killed at various times post-dosing, shown on the abscissa scale. The concentrations of drug in the plasma (ng ml^{-1}; open symbols) or the brain (ng g^{-1}; closed symbols) were measured by h.p.l.c. and are shown on the ordinate scale. See Methods for further technical details.

Rat formalin-induced paw licking GR94839 (1–10 mg kg^{-1}, s.c.) caused a significant decrease in the duration of licking recorded in the 2nd phase of the formalin response (Figure 3) with the highest dose virtually abolishing licking. However, the same doses had a lesser effect on licking in the 1st phase (Figure 3), and a dose of GR94839 30 mg kg^{-1}, s.c., was required to cause a marked inhibition of the 1st phase. The ED_{50} values for GR94839 vs the 1st and 2nd phases of the formalin response are given in Table 1. ICI204448 (1–100 mg kg^{-1}, s.c.) caused a decrease of the 2nd phase of the formalin response at slightly lower doses than inhibited the 1st phase (Table 1). In contrast, the centrally-penetrating κ-agonist, GR103545 was equi-effective against both phases (Table 1).

The inhibition of the 2nd phase by GR94839 (3 mg kg^{-1}, s.c.) was reversed by co-administration of naltrexone (0.3 mg kg^{-1}, s.c.; duration of licking in the 2nd phase: saline 153 ± 18 s; GR94839 19 ± 5 s; naltrexone 172 ± 18 s; GR94839 + naltrexone 141 ± 24 s; $n = 6$). However, co-administration of the μ/δ-opioid receptor antagonist, M8008 (Smith, 1987; Birch *et al.*, 1988) at a dose of 1 mg kg^{-1}, s.c., caused no significant reversal of the inhibition of the 2nd phase by the same dose of GR94839 (duration of licking in the 2nd phase: saline 136 ± 13 s; GR94839 37 ± 5 s; M8008

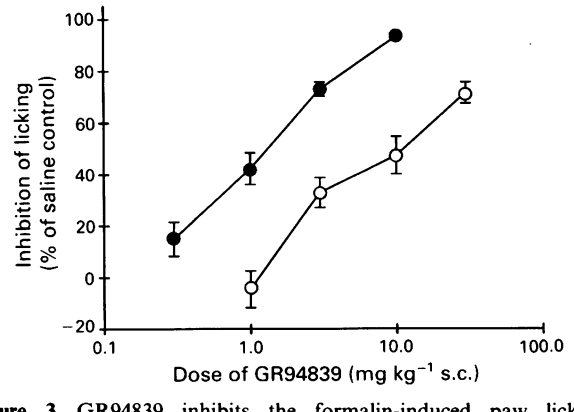

Figure 3 GR94839 inhibits the formalin-induced paw licking observed 0–5 min (○) or 15–30 min post-formalin (●) in a dose-dependent manner. Ordinate scale: inhibition of licking expressed as a percentage of licking in saline dosed rats. Abscissa scale: dose of GR94839 mg kg^{-1}, s.c. Rats were given GR94839 s.c. 30 min before intraplantar injection of formalin ($n = 18$).

Table 1 A comparison of the ED_{50} values for inhibition of the 1st and 2nd phases of the rat formalin response

κ-Agonist	ED_{50} mg kg^{-1} s.c., (95% confidence levels) 1st phase	2nd phase	1st/2nd	(n)
GR94839	10.2 (6.7–17.1)	1.4 (1.0–1.8)	7.3	(18)
GR103545	0.0004 (0.0002–0.0013)	0.0004 (0.0002–0.0005)	1	(6)
ICI204448	49% ↓ at 30 mg kg^{-1}	8.9 (5.4–36.5)	≃3.3	(6)

134 ± 14 s; GR94839 + M8008 68 ± 9 s; $n = 12$). M8008 (1 mg kg^{-1}, s.c.; co-injection) reversed the inhibition of the 2nd phase by the μ-agonist, morphine (duration of licking in 2nd phase: saline 163 ± 11 s; M8008 131 ± 30 s; morphine 0 ± 0 s; morphine + M8008 164 ± 17 s ($n = 6$)).

To see whether GR94839 could inhibit the 2nd phase of the formalin response by a local action in the paw, GR94839 (30–100 μg; $n = 6$–12) was injected into the same paw as formalin immediately before either phase of the formalin response. Following intraplantar injection of saline the duration of licking in the 2nd phase was 147 ± 10 s, which was reduced to 109 ± 15 s by GR94839 at a dose of 30 μg and to 55 ± 10 s ($P < 0.05$ vs saline) at a dose of 100 μg. The same doses of GR94839 had no effect on the duration of paw licking when injected into the contralateral paw (GR94938 30 μg: 155 ± 12 s; GR94839 100 μg: 142 ± 9 s). GR94839 (30–100 μg; $n = 6$) also failed to inhibit licking in the 1st phase of the formalin response when injected into the ipsilateral paw immediately before formalin injection (saline: 75 ± 6 s; GR94839 30 μg: 75 ± 3; GR94839 100 μg: 73 ± 8 s).

To establish that the inhibition of the 2nd phase by intraplantar injection of GR94839 was a receptor-mediated effect and not due to local anaesthesia, the effect of pretreatment with systemic opioid antagonists was examined. Naltrexone (0.3 mg kg^{-1}, s.c.; 30 min pretreatment) reversed the effect of intraplantar GR94839 (duration of licking in 2nd phase: saline 172 ± 12 s; GR94839 100 μg 84 ± 10 s; GR94839 + naltrexone 144 ± 21 s; $n = 6$). M8008 at a dose of 1 mg kg^{-1}, s.c., had no effect on the inhibition of licking by intraplantar GR94839 (duration of licking in 2nd phase: saline 110 ± 19 s; M8008 136 ± 6 s; GR94839 67 ± 19 s; GR94839 + M8008 61 ± 12 s; $n = 6$).

Intraplantar injection of the non-selective opioid antagonist, naltrexone (1 μg; $n = 18$) or the κ-receptor selective antagonist, norbinaltorphimine (100 μg; $n = 6$), immediately before the 2nd phase of licking, reversed partially, but significantly, the inhibition of the 2nd phase of the formalin response by systemic GR94839 (3 mg kg^{-1}, s.c.; 15 min before formalin; Figure 4). Injection of the antagonists alone into the formalin-injected paw had no effect on the duration of licking in the 2nd phase, and injection of antagonist into the contralateral paw did not reverse the antinociceptive effects of systemic GR94839.

Zymosan-induced hyperalgesia In the rat, GR94839 (0.3–10 mg kg^{-1}, s.c.) reversed significantly the hyperalgesia observed in the zymosan-injected paw (Figure 5). Higher doses of GR94839 (10–30 mg kg^{-1}, s.c.) were required to raise significantly the noxious presure threshold in the normal paw. The ED_{50} value for GR94839 in the inflamed paw and the ED_{30} value in the non-inflamed paw are given in Table 2. ICI204448 also reversed the hyperalgesia in the inflamed paw at lower doses than raised the noxious pressure threshold in the non-inflamed paw (Table 2), whereas for GR103545 the ED_{50} values for both paws were similar. Thus, at doses similar to those that inhibit the 2nd phase of the formalin response GR94839 reversed the zymosan-induced hyperalgesia.

In the guinea-pig, GR94839 (0.3–10 mg kg^{-1}, s.c.) raised the noxious pressure threshold in the inflamed and non-inflamed paws equally (ED_{50} inflamed paw: 1.4 (0.2–2.6) mg kg^{-1}, s.c.; ED_{30} non-inflamed paw: 1.0 (0.2–2.6) mg kg^{-1}, s.c. ($n = 16$). GR103545 was also equipotent in raising the noxious pressure threshold in the inflamed and non-inflamed paws (ED_{50} inflamed paw: 0.4 (0.2–1.1) μg kg^{-1}, s.c.; ED_{30} non-inflamed paw: 0.12 (0.01–0.67) μg kg^{-1}, s.c. ($n = 16$)).

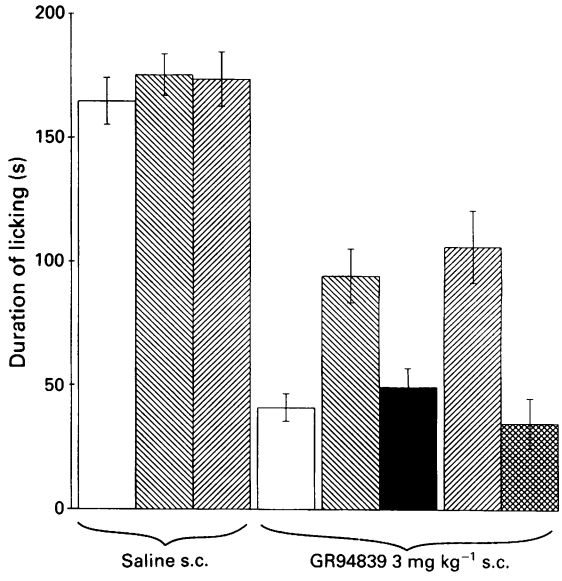

Figure 4 Intraplantar injection of naltrexone or norbinaltorphimine antagonizes the inhibition of the 2nd phase of the formalin response by systemic GR94839. Rats were injected with GR94839 3 mg kg^{-1} or saline s.c. (as indicated beneath the histogram) 15 min before intraplantar injection of formalin. Fifteen min after formalin injection saline or the opioid antagonists were injected intraplantar either ipsilateral or contralateral to the formalin injection ($n = 6$–18). Open columns indicate an ipsilateral saline injection; left-hatched columns, ipsilateral naltrexone 1 μg; and right-hatched columns, ipsilateral norbinaltorphimine 100 μg. The solid column indicates a contralateral injection of naltrexone 1 μg and the cross-hatched column a contralateral injection of norbinaltorphimine 100 μg. Ordinate scale: duration of licking 15–30 min post-formalin. *$P < 0.05$ vs saline s.c. and ipsilateral saline intraplantar. **$P < 0.05$ vs GR94839 3 mg kg^{-1}, s.c. and ipsilateral saline intraplantar.

Rat rotarod GR94839 (10–100 mg kg^{-1}, s.c.) decreased the rotarod score. The rats were considered to be mildly sedated at these doses. ED_{50} values for GR94839 and also ICI204448 and GR103545 are given in Table 3. The doses of GR94839 that had sedative effects were similar to those that decreased the duration of licking in the 1st phase of the rat formalin response and raised the noxious pressure threshold in the non-inflamed paw of the rat.

Discussion

It has been shown previously that opioid agonists selective for the μ,κ-, and δ-opioid receptor subtypes can mediate antinociception in a rat model of arthritis by activation of

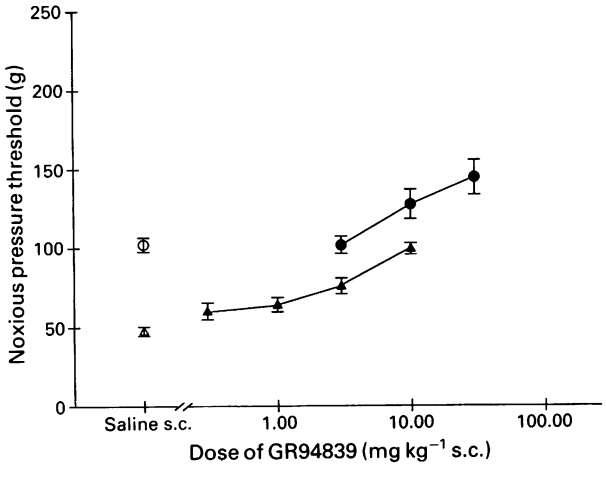

Figure 5 GR94839 raises the noxious pressure threshold in the zymosan-inflamed and the normal paw of the rat. Zymosan was injected into one hind-paw and the noxious pressure threshold of the zymosan-injected paw (triangles) and the non-injected paw (circles), was measured 5 h post-injection. GR94839 (closed symbols) or saline (open symbols) was administered s.c., 4.5 h post-zymosan ($n = 18$). Ordinate scale: paw pressure threshold (g). Abscissa scale: dose of GR94839 mg kg^{-1}, s.c.

opioid receptors in the periphery (Stein *et al.*, 1989). These experiments suggest that an opioid agonist with limited access to the CNS could be analgesic without possessing the undersirable effects of centrally-acting opioids, e.g. for μ-opioids, dependence and respiratory depression and for κ-opioids, sedation and dysphoria. Peripherally-selective μ- or δ-agonists could have effects on gastrointestinal function (Birch *et al.*, 1986; Tavani *et al.*, 1990), thus a peripherally-selective κ-agonist may be the best candidate for a novel analgesic. The pharmacological and biochemical profile described for the κ-agonist, GR94839, in the present study suggests that it has both limited access to the CNS and can mediate antinociception at a peripheral site and may thus have potential as an analgesic.

Two sets of experiments strongly suggest that GR94839 has limited access to the central nervous system. In the mouse acetylcholine-induced abdominal constriction test, GR94839 was weakly active when given subcutaneously, but underwent a marked enhancement of potency when it was given i.c.v. The lack of potency after systemic administration was not dependent on the agent used to induce abdominal constriction, and the effects were opioid receptor-mediated as they were naltrexone reversible. In fact the effect seen after systemic administration was probably due to penetration of GR94839 into the central nervous system, because the potency correlated poorly with the nanomolar potency the compound has for κ-receptors in the rabbit vas deferens, whereas the potency i.c.v. correlated well. In contrast, the potent, centrally penetrating κ-agonist, GR103545 (Hayes *et al.*, 1990), was highly effective and equipotent at inhibiting acetylcholine-induced abdominal constrictions after subcutaneous or i.c.v. administration. Co-administration of GR94839 with acetylcholine failed to inhibit abdominal constrictions, suggesting that the site of action of GR94839 is distant from the site of injection and as the pretreatment time for intraperitoneal injection of GR94839 was increased the ability of GR94839 to inhibit abdominal constrictions increased (data not shown). This could suggest that GR94839 gains slow access to its site of action i.e. the central nervous system. These findings together suggest that the ratio of the subcutaneous and i.c.v. ED_{50} values for GR94839 and ICI204448 in the abdominal constriction test, 238 and 31 respectively, reflects the ability of the compounds to enter the CNS.

Table 3 A comparison of the ED_{50} values in the rat rotarod test for sedation

κ-Agonist	*Rotarod test* (ED_{50} mg kg^{-1}, s.c.)	(n)
GR94839	35 (12–245)	(6)
GR103545	0.0017 (0.0013–0.0023)	(8)
ICI204448	Inactive at 30 mg kg^{-1}	(6)

Measurement of the plasma and CNS levels of GR94839 following i.v. administration in the rat, showed that the levels of GR94839 attained in the CNS were always lower than the levels of GR85571, a structurally similar but centrally-penetrating κ-agonist. This finding is direct evidence for the inability of GR94839 to accumulate within the CNS. The lack of penetration of GR94839 into the CNS may be due to its lower lipophilicity: the log P of GR94839 is 1.25, compared with 3.14 for GR103545 and 2.47 for GR85571.

It has been suggested that opioid analgesics are more effective at peripheral opioid receptors in the presence of inflammation (Ferreira & Nakamura, 1979; Stein *et al.*, 1988; 1989; Bartho *et al.*, 1990). For this reason, the antinociceptive activity of GR94839 was investigated in two nociceptive tests that had an inflammatory pain component: the rat formalin-induced licking test and the zymosan-induced mechanical hyperalgesia model. In the rat formalin test, a biphasic response has been described both behaviourally (Dubuisson & Dennis, 1977; Hunskaar *et al.*, 1985; Wheeler-Aceto *et al.*, 1990) and electrophysiologically (Banna *et al.*, 1986; Heapy *et al.*, 1987; Dickenson & Sullivan, 1987). The first phase is thought to be due to a direct activation of C-fibres because it is only inhibited by high doses of non-steroidal anti-inflammatory drugs but is inhibited by centrally-acting opioids (Dickenson & Sullivan, 1987; Shibata *et al.*, 1989). The 2nd phase is thought to have a peripheral inflammatory component because it is selectively inhibited by indomethacin and steroids (Hunskaar & Hole, 1986; Hunskaar *et al.*, 1987; Shibata *et al.*, 1989), and bradykinin (B_2) antagonists (Haley *et al.*, 1989), although other studies suggest it can in part be attributed to central plasticity (Dickenson & Sullivan, 1987; Coderre *et al.*, 1990).

In the present study, it was found that not only did systemic GR94839 inhibit the 2nd phase of the formalin response at doses 7 fold lower than those required to inhibit the 1st phase, but local injection of GR94839 selectively inhibited the 2nd phase. Together the data suggest that GR94839 is having its antinociceptive action at a peripheral locus during the 2nd phase of the formalin response. The

Table 2 A comparison of the ED_{50} values to raise the noxious pressure threshold in the zymosan-inflamed or non-inflamed paws of the rat

κ-Agonist	*Non-inflamed* (ED_{50}, mg kg^{-1})	*Inflamed* (ED_{30}, mg kg^{-1})	*Non-inf. /Inf.*	(n)
GR94839	16.4 (8.6–46.7)	2.0 (1.1–3.2)	8.2	(18)
GR103545	0.0011 (0.0008–0.0016)	0.0012 (0.0008–0.0017)	1	(24)
ICI204448	68.0 (22.1–32000)	19.5 (13.3–32.7)	3.5	(8)

antinociception was mediated by opioid receptor activation (rather than local anaesthesia) because it was reversed by systemic naltrexone. The peripherally-selective κ-agonist, ICI204448 also showed some selectivity (approximately 3 fold) for the 2nd phase of the formalin response but was less potent than GR94839. GR103545, in contrast, was equipotent versus both phases of the licking response, consistent with the previously demonstrated ability of κ-opioids to inhibit the formalin response at a central site (Calcagnetti *et al.*, 1988; Pelissier *et al.*, 1990). The opioid receptor activated is probably a κ-receptor because the inhibition by intraplantar GR94839 was not blocked by the μ/δ-antagonist, M8008, at selective doses (Birch *et al.*, 1988). Further evidence for a peripheral activation of κ-receptors by GR94839 was obtained when the inhibition of the 2nd phase of the formalin response was reversed by local injection of naltrexone, and the κ-selective opioid antagonist, norbinaltorphimine. The present findings are in agreement with previous studies (Abbott, 1988; Haley *et al.*, 1990) where a peripheral inhibition of the formalin response by κ-opioids was demonstrated, although we were not able to demonstrate an inhibition of the 1st phase on local administration (Haley *et al.*, 1990). This difference between the studies may be due to the intensity of spinal cord nerve activity not being directly proportional to the behavioural response.

In a second model of nociception associated with inflammation, the rat zymosan-induced mechanical hyperalgesia model, GR94839 caused a significant reversal of inflammation-induced mechanical hyperalgesia at doses that had no significant effect on the noxious pressure threshold in the non-inflamed paw. The doses that reversed the hyperalgesia were similar to the doses that inhibited the 2nd phase of the formalin-induced paw licking, whereas the doses that raised the non-inflamed paw noxious pressure threshold corresponded with the doses that inhibited the 1st phase of the formalin response. The latter were presumably central effects, as these doses are mildly sedative and impair performance in the rat rotarod test. ICI204448 showed less selectivity against the noxious pressure threshold of the inflamed paw than GR94839 and was less potent, whereas GR103545 was highly effective and equipotent at raising the noxious pressure threshold in both paws. Thus, in a second pain model with an inflammatory component GR94839 and ICI204448 have peripheral antinociceptive activity.

The mechanism by which GR94839 mediates antinociception in the periphery has not been rigorously investigated in the present study. Kappa opioids are known to have a diuretic action at a peripheral site (Leander, 1983). However, the local action of GR94839 in the formalin test would exclude this systemic action from mediating the antinociceptive effect. κ-Opioids have been shown to inhibit electrically-evoked plasma protein extravasation in the hind-paw of rats (Bartho & Szolcsanyi, 1981; Smith & Buchan, 1984). Also, a recent study showed that the enhanced antinociceptive activity of morphine against inflammatory pain was eliminated by capsaicin-pretreatment (Bartho *et al.*, 1990). Both studies suggest that the opioid receptors are located on sensory nerve terminals, and indeed κ-opioids have been shown to reduce the spontaneous discharge of sensory afferents in the inflamed knee joint of the cat (Russell *et al.*, 1987).

The doses of GR94839 required locally or systemically to inhibit the 2nd phase of formalin-induced licking and the zymosan-induced hyperalgesia were higher than one would predict from the *in vitro* potency of the compounds and the doses required to inhibit acetylcholine-induced abdominal constriction after i.c.v. administration. This may reflect the density of opioid receptors peripherally or the efficiency of coupling of peripheral receptors to second messenger systems. Unfortunately, the relatively high doses of GR94839 that are required to activate peripheral κ-receptors compared with central receptors may have resulted in the inability to demonstrate a selective effect of GR94839 in the inflamed paw of the guinea-pig. The guinea-pig has a higher density of κ-receptors in the CNS than the rat, and is thought to resemble man in this respect (Mansour *et al.*, 1988). A significantly greater κ-receptor reserve in the central nervous system of the guinea-pig than the periphery, could mean that lower concentrations of GR94839 are required to elicit central effects than peripheral effects. As man resembles the guinea-pig in having abundant central κ-receptors (Mansour *et al.*, 1988), a compound possessing a better separation of central and peripheral effects than GR94839 would be required to be an effective analgesic in the absence of central side-effects.

References

ABBOTT, F.V. (1988). Peripheral and central antinociceptive actions of ethylketocyclazocine in the formalin test. *Eur. J. Pharmacol.*, **152**, 93–98.

BANNA, N.R., SAADE, N.E., ATWEH, S.F. & JABBUR, S.J. (1986). Prolonged discharge of wide dynamic range spinal neurons evoked by formaldehyde injected in their cutaneous receptive fields. *Exp. Neurol.*, **93**, 275–278.

BARTHO, L., STEIN, C. & HERZ, A. (1990). Involvement of capsaicin-sensitive neurones in hyperalgesia and enhanced opioid antinociception in inflammation. *Naunyn-Schmiederbergs Arch. Pharmacol.*, **342**, 666–670.

BARTHO, L. & SZOLCSANYI, J (1981). Opiate agonists inhibit neurogenic plasma extravasation in the rat. *Eur. J. Pharmacol.*, **73**, 101–104.

BIRCH, P.J., HAYES, A.G., SHEEHAN, M.J. & TYERS, M.B. (1988). In vivo studies with the opioid antagonist 16-methyl cyprenorphine. *J. Pharm. Pharmacol.*, **40**, 213–214.

BIRCH, P.J., HAYES, A.G. & SPRAGGS, C.F. (1986). Studies on the δ-opioid receptor-mediated inhibition of I_{sc} in the isolated guinea-pig ileal mucosal preparation. *Br. J. Pharmacol.*, **89**, 833P.

CALCAGNETTI, D.J., HELMSTETTER, F.J. & FANSELOW, M.S. (1988). Analgesia produced by centrally administered DAGO, DPDPE and U50488H in the formalin test. *Eur. J. Pharmacol.*, **153**, 117–122.

CODERRE, T.J., VACCARINO, A.L. & MELZACK, R. (1990). Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. *Brain Res.*, **535**, 155–158.

DICKENSON, A.H. & SULLIVAN, A.F. (1987). Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurons. *Neurosci. Letts*, **83**, 207–211.

DUBUISSON, D. & DENNIS, S.G. (1977). The formalin test: quantitative study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. *Pain*, **4**, 161–174.

FERREIRA, S.H. & NAKAMURA, M. (1979). III Prostaglandin hyperalgesia: relevance of the peripheral effect for the analgesic action of opioid-antagonists. *Prostaglandins*, **18**, 201–208.

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

HALEY, J.E., DICKENSON, A.H. & SCHACHTER, M. (1989). Electrophysiological evidence for a role of bradykinin in chemical nociception in the rat. *Neurosci. Letts*, **97**, 198–202.

HALEY, J.E., KETCHUM, S. & DICKENSON, A.H. (1990). Peripheral κ-opioid modulation of the formalin response: an electrophysiological study in the rat. *Eur. J. Pharmacol.*, **191**, 437–446.

HAYES, A.G., BIRCH, P.J., HAYWARD, N.J., SHEEHAN, M.J., ROGERS, H., TYERS, M.B., JUDD, D.B., SCOPES, D.I.C. & NAYLOR, A. (1990). A series of novel, highly potent and selective agonists for the κ-opioid receptor. *Br. J. Pharmacol.*, **101**, 944–948.

HAYES, A.G., ROGERS, H., BIRCH, P.J., HARRISON, S.M., JUDD, D.B., NAYLOR, A. & SCOPES, D.I.C. (1991). Activation of peripheral opioid receptors by GR94839 mediates antinociception in the rat. *Fundamentals Clin. Pharmacol.*, **5**, 414.

HEAPY, C.G., JAMIESON, A. & RUSSELL, N.J.W. (1987). Afferent C-fibre and A-delta activity in models of inflammation. *Br. J. Pharmacol.*, **90,** 164P.

HUNSKAAR, S., BERGE, O.G. & HOLE, K. (1987). Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test. *Pain*, **25,** 125–132.

HUNSKAAR, S., FASMER, O.B. & HOLE, K. (1985). Acetylsalicylic acid, paracetamol and morphine inhibit behavioural responses to intrathecally administered substance P or capsaicin. *J. Neurosci. Methods*, **14,** 69–76.

HUNSKAAR, S. & HOLE, K. (1986). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain*, **30,** 103–114.

LEANDER, J.P. (1983). A kappa opioid effect: increased urination in the rat. *J. Pharmacol. Exp. Ther.*, **224,** 89–94.

MANSOUR, A., KHACHATURIAN, H., LEWIS, M.E., AKIL, H. & WATSON, S.J. (1988). Anatomy of CNS opioid receptors. *Trends Neurosci.*, **11,** 308–314.

PELISSIER, T., PAEILE, C., SOTO-MOYANO, R., SAAVEDRA, H. & HERNANDEZ, A. (1990). Analgesia produced by intrathecal administration of the κ-opioid agonist, U-50,488H on formalin-evoked cutaneous pain in the rat. *Eur. J. Pharmacol.*, **190,** 287–293.

RUSSELL, N.J.W., SCHAIBLE, H.-G. & SCHMIDT, R.F. (1987). Opiates inhibit the discharge of fine afferent units from the inflammed knee joint of the cat. *Neurosci. Letts*, **76,** 107–112.

SHAW, J.S., CARROLL, J.A., ALCOCK, P. & MAIN, B.G. (1989). ICI204448: a κ-opioid agonist with limited access to the CNS. *Br. J. Pharmacol.*, **96,** 986–992.

SHIBATA, M., OHKUBO, T., TAKAHASHI, H. & INOKI, R. (1989). Modified formalin test: characteristic biphasic pain response. *Pain*, **38,** 347–352.

SMITH, C.F.C. (1987). 16-Me cyprenorphine (RX 8008M): a potent opioid antagonist with some δ selectivity. *Life Sci.*, **40,** 267–274.

SMITH, T.W. & BUCHAN, P. (1984). Peripheral opioid receptors located on the rat saphenous nerve. *Neuropeptides*, **5,** 217–220.

STEIN, C., MILLAN, M.J., SHIPPENBERG, T.S., PETER, K. & HERZ, A. (1989). Peripheral opioid receptors mediating antinociception in inflammation. *J. Pharmacol. Exp. Ther.*, **248,** 1269–1275.

STEIN, C., MILLAN, M.J., YASSOURIDIS, A., & HERZ, A. (1988). Antinociceptive effects of μ- and κ-agonists in inflammation are enhanced by a peripheral opioid receptor specific mechanism. *Eur. J. Pharmacol.*, **155,** 255–264.

TAVANI, A., PETRILLO, P., LA REGINA, A. & SBACCHI, M. (1990). Role of peripheral μ-, and κ-opioid receptors in opioid-induced inhibition of gastrointestinal transit in rats. *J. Pharmacol. Exp. Ther.*, **254,** 91–97.

WHEELER-ACETO, H., PORRECA, F. & COWAN, A. (1990). The rat paw formalin test: comparison of noxious agents. *Pain*, **40,** 229–238.

(Received January 30, 1992
Revised March 19, 1992
Accepted March 23, 1992)