Br. J. Pharmacol. (1990), **101**, 183–189

CI-977, a novel and selective agonist for the κ-opioid receptor

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1 CI-977 is a new, nonpeptide κ-opioid compound that has been synthesized and its pharmacological properties determined in a series of *in vitro* and *in vivo* rodent models.

2 In radioligand binding studies, with guinea-pig forebrain homogenates, CI-977 bound with high affinity to [^{3}H]-U69593-labelled κ-sites (K_i = 0.11 nM) but with low affinity to [^{3}H]-[D-Ala^2, MePhe^4, Gly-ol^5] enkephalin (DAMGO) labelled μ-sites (K_i = 99 nM) and [^{3}H]-[D-Pen^{2,5}]enkephalin (DPDPE) labelled δ-sites (K_i = 1.04 μM). CI-977 also bound with negligible affinity to [^{3}H]-(+)-3-(1-propyl-3-piperidinyl)phenol (3-PPP) labelled σ-sites (K_i = 1.9 μM) and [^{3}H]-1-(1-[2-thienyl]cyclohexyl)piperidine (TCP) labelled PCP sites (K_i > 10 μM).

3 CI-977 produced a potent inhibition of the electrically-evoked contractions of the guinea-pig ileum and rabbit vas deferens with IC_{50} values of 0.087 nM and 3.3 nM, respectively. The pK_B values for the opioid antagonists naloxone (7.6) and norbinaltorphimine (10.5) supported the κ nature of the CI-977-mediated effects in the smooth muscle assays.

4 CI-977 was a potent antinociceptive agent against a mechanical noxious stimulus in rats following intravenous, intramuscular, subcutaneous and oral administration. CI-977 was also effective against mechanical and chemical noxious stimuli in the mouse but ineffective against a thermal stimulus. The antinociceptive effects produced by CI-977 were completely reversed by naloxone (1 mg kg^{-1}, s.c.).

5 At doses close to those required to produce antinociception, CI-977 also caused a naloxone-reversible diuresis and inhibition of locomotor activity.

6 The *in vitro* and *in vivo* pharmacological profile of CI-977 demonstrates that it is a potent and selective agonist at the κ-opioid receptor.

Introduction

The κ-opioid receptor can be clearly distinguished from either the μ or δ sub-types by various *in vitro* biochemical and pharmacological models (Chang, 1984; Paterson *et al.*, 1984). In addition, with the main exception of antinociception, activation of the κ-receptor *in vivo* has been associated with a distinct behavioural profile. Thus, κ-agonists have been shown to cause sedation and diuresis at doses close to those producing antinociception (Leighton *et al.*, 1987; Dykstra *et al.*, 1987) but, more importantly, would also appear not to cause emesis, constipation, respiratory depression or to have a dependence liability; effects particularly associated with occupation of the μ-receptor (Martin, 1984).

These characteristics have been established principally through the use of compounds from the recent arylacetamide series such as U50488, U69593 and PD117302, which have demonstrated suitable selectivity for the κ-receptor (Von Voigtlander *et al.*, 1983; Lahti *et al.*, 1985; Clark *et al.*, 1988).

Previously, it had been difficult to ascribe a definitive pattern of behaviour to a specific action at the κ-receptor. This was mainly a consequence of the poor selectivity of the prototype benzomorphan ligands, such as ketocyclazocine, which were used in the original classification of the receptor (Martin *et al.*, 1976). These compounds have been shown to interact with comparable potency at μ- and δ-opioid, (Kosterlitz *et al.*, 1981; Gillan & Kosterlitz, 1982) as well as non-opioid (Zukin & Zukin, 1981; Su, 1982; Tam, 1983) types of receptor.

Nevertheless, despite this lack of selectivity, benzomorphans such as ethylketocyclazocine and bremazocine have maintained a higher affinity and/or potency at the κ-receptor than many of the arylacetamides (Lahti *et al.*, 1985; Leighton *et al.*, 1987; Clark *et al.*, 1988).

In the present study we describe the pharmacological characteristics of CI-977, a novel compound from the arylacetamide series that is under clinical evaluation at present. CI-977 exhibited the highest affinity yet described for an arylacetamide at the κ-receptor and, in addition, had the highest potency and efficacy in a series of *in vitro* and *in vivo* functional models.

A preliminary account of the results obtained in this study has been communicated to the British Pharmacological Society (Hunter *et al.*, 1990).

Methods

Radioligand binding assays

Guinea-pig forebrain homogenates were prepared as previously described (Smith *et al.*, 1989; Meecham *et al.*, 1989). Briefly, membrane homogenates were resuspended in 50 mM Tris-HCl (5 mM Tris-HCl for the [^{3}H]-TCP assay), to a final concentration of 10 mg ml^{-1} wet weight of original tissue. Membrane aliquots (400 μl) were added to a final incubation assay volume of 500 μl consisting of a single concentration of radioligand and at least six increasing concentrations of unlabelled ligand. The concentrations of the opioid radioligands were approximately equal to the respective equilibrium dissociation constants (K_D) determined by saturation analysis in a separate series of experiments (Meecham *et al.*, 1989). The radioligands used (K_D in parentheses) were the κ-selective [^{3}H]-U69593 (1.0 nM), the μ-selective [^{3}H]-[D-Ala^2, MePhe^4, Gly-ol^5] enkephalin (DAMGO; 0.4 nM) and the δ-selective [^{3}H]-[D-Pen^{2,5}] enkephalin (DPDPE; 2.0 nM). The non-specific binding was determined as that remaining in the presence of 1 μM naloxone. [^{3}H]-(+)-3-(1-propyl-3-piperidinyl)- phenol (3-PPP; 1 nM) and [^{3}H]-1-(1[2-thienyl]cyclohexyl)- piperidine (TCP; 1 nM) were used to label the σ and phencyclidine binding sites, respectively. Non-specific binding was defined by 5 μM pentazocine ([^{3}H]-(+)-3-PPP) and 5 μM phencyclidine ([^{3}H]-TCP). The K_D values for [^{3}H]-(+)-3-PPP (30 nM) and [^{3}H]-TCP (7 nM) were determined in a preliminary series of experiments. All radioligands were incubated at 25°C for 80 min, with the exception of [^{3}H]-TCP (30 min), following which the assay was terminated by rapid filtration through Whatman GF/B glass fibre filter strips on a Brandel M-48 cell harvester. The filters were then washed with

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3 × 4 ml ice-cold 50 nM Tris-HCl and the radioactivity determined by liquid scintillation spectrometry.

Analysis of binding data

The competition binding data were analysed by ALLFIT (DeLean *et al.*, 1978) and the K_i values calculated from the Cheng-Prusoff (1973) equation: $K_i = IC_{50}/[1 + L/K_D]$, where the IC_{50} represents the concentration which inhibited 50% of the radioligand specific binding and L and K_D represent the assay concentration and equilibrium dissociation constant for the radioligand respectively.

In vitro *smooth muscle preparations*

The guinea-pig ileum and rabbit vas deferens bioassay preparations were set up as described by previous studies from this laboratory (Clark *et al.*, 1988; Meecham *et al.*, 1989).

Control log dose-response curves and those in the presence of antagonist were analysed by ALLFIT (DeLean *et al.*, 1978) and dose-ratios calculated at the IC_{50} level. The pK_B (negative log of the equilibrium dissociation constant) values for either the non-selective opioid antagonist, naloxone, or the more κ-selective antagonist norbinaltorphimine (nor-BNI) (Portoghese *et al.*, 1987; Birch *et al.*, 1987) were then determined by the method initially defined by Arunlakshana & Schild (1959).

In vivo *antinociception models*

The methods used to evaluate antinociceptive activity in tests employing different types of noxious stimuli, i.e. mechanical, chemical and thermal, were performed as previously described (Leighton *et al.*, 1987). The effects of test compounds on nociceptive pressure (mechanical) thresholds were determined in either the rat (70–100 g, Wistar males, Interfauna, Huntingdon, U.K.) paw pressure or mouse (20–25 g, CFLP males, Interfauna) tailclip models. The effect of test compounds on chemically- and thermally-induced nociceptive thresholds were determined by use of the mouse acetylcholine-induced abdominal constriction and hotplate models, respectively (Leighton *et al.*, 1987).

Route of administration

For all compounds under investigation in the present study, animals were dosed by either the intravenous (i.v.), subcutaneous (s.c.) or oral (p.o.) route of administration in a manner previously described (Leighton *et al.*, 1987).

In addition, CI-977 was also assessed for antinociceptive activity in the rat paw pressure test following intramuscular dosing. Animals were dosed by this route 5 min before testing with a dose volume of 0.01 ml per 100 g injected into the large muscle group at the back of the thigh.

To determine the time course of antinociceptive activity rats were dosed with CI-977 either intramuscularly or orally and the nociceptive response to a mechanical (paw pressure) stimulus determined at one of the following time intervals: 5, 10, 15, 30, 45, 60, 120, 180, 240, 300 or 360 min after dosing. Of the rats used none was tested more than once.

Additional in vivo *tests*

Locomotor activity Male mice (CFLP, 35–50 g, Interfauna, Huntingdon) were group housed under conditions of constant temperature (21 ± 1°C) and humidity on a fixed 12 h light 12 h dark cycle with food and water available *ad libitum*. Locomotor activity was measured by an automated activity monitoring system consisting of twelve clear polycarbonate cages each bi-sected by a single infrared beam (Marshall *et al.*, 1987). Test compounds were administered subcutaneously in a dose volume of 1 ml per 100 g body weight and immediately after dosing the animals were placed individually in the cages of the activity monitoring system. Locomotor activity was measured for 1 h following the placement of the animals in the activity monitor. All experiments were timed to start at the beginning of the dark period of the light/dark cycle. A group size of nine animals was used for all treatments.

Urine output The methodology for measuring the effect of compounds on urine output in rats was as described in previous studies from this laboratory (Leighton *et al.*, 1987). Briefly, male, normally hydrated Wistar rats (250–350 g) were injected subcutaneously with a test substance and then placed individually in metabolism cages. Urine output was then determined over a subsequent 6 h period. Group sizes of 6 animals were used throughout.

Materials

The following drugs were obtained from external sources: ethylketocyclazocine (Sterling-Winthrop), bremazocine (Sandoz), MR2034 ((−)-α-(1**R**,5**R**,9**R**)-5,9-di-methyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6,7-benzomorphan, Boehringer-Ingelheim), DAMGO (Cambridge Research Biochemicals), DPDPE (Bachem), naloxone hydrochloride (Sigma) and U69593 ((5α, 7α, 8β)-(−)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5) dec-8-yl]benzeneacetamide, Upjohn Co.).

U50488 (*trans*-(±)-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methanesulphonate; spiradoline (5α,7α,8β)-(±)-3,4-dichloro-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]benzeneacetamide mesylate); PD117302 ((±)-*trans*-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide monohydrochloride) and CI-977 ((5**R**)-(5α,7α,8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide monohydrochloride) were all synthesized in the Department of Medicinal Chemistry, Parke-Davis Research Unit, Cambridge, U.K.

The following radioligands were used: [^{3}H]-U69593 (40–60 Ci mmol^{-1}), [^{3}H]-(+)-3-PPP (80–110 Ci mmol^{-1}), and [^{3}H]-TCP (40–60 Ci mmol^{-1}) were all purchased from New England Nuclear. [^{3}H]-DAMGO (40–60 Ci mmol^{-1}) and [^{3}H]-DPDPE (25–50 Ci mmol^{-1}) were both obtained from Amersham International.

Results

Radioligand binding studies

CI-977 produced a concentration-dependent inhibition of specific [^{3}H]-U69593 (κ) binding with a K_i value of 0.11 nM (Table 1). CI-977 also inhibited specific [^{3}H]-DAMGO (μ) and [^{3}H]-DPDPE (δ) binding with K_i values of 99.6 nM and 1036 nM, respectively (Table 1). The μ/κ and δ/κ ratios were 905 and 9420, respectively.

In comparison ethylketocyclazocine (EKC, 0.21 nM), bremazocine (0.12 nM) and MR2034 (0.09 nM) had κ affinities comparable to CI-977, but were considerably less selective with μ/κ ratios between 3.4 and 4.9 and δ/κ ratios between 9.2 and 42 (Table 1). In contrast, U50488 (0.69 nM), U69593 (0.67 nM), PD117302 (0.5 nM) and, to a lesser extent spiradoline (0.35 nM), had lower affinities for the κ-sites, and only U69593 (3672) had a higher μ/κ ratio, compared with CI-977 (Table 1). However, the arylacetamides in general had substantially higher κ selectivity than the benzomorphans with μ/κ and δ/κ ratios increasing from 125 (spiradoline) and 9418 (CI-977), respectively.

CI-977 was found to inhibit specific [^{3}H]-(+)-3-PPP binding to σ sites with a K_i value of 1.9 μM but had no detectable affinity (>10 μM) for the [^{3}H]-TCP-labelled phencyclidine (PCP) binding sites (Table 2). U50488 (1.04 μM), spiradoline (1.06 μM), U69593 (>10 μM) and PD117302 (1.2 μM) all had low affinities similar to CI-977 for the σ-site while bremazocine (496 nM), MR2034 (287 nM) and EKC (495 nM) were found to have higher affinities for this site (Table 2). At the PCP site, bremazocine (156 nM), MR2034

Table 1 Inhibition constants (K_i) obtained for CI-977 and standard opioid ligands at κ, μ and δ binding sites in guinea-pig forebrain membranes

Ligand	κ [^3H]-*U69593* K_i values (nM)	μ [^3H]-*DAMGO* K_i values (nM)	μ/κ	δ [^3H]-*DPDPE* K_i values (nM)	δ/κ
CI-977	0.11 ± 0.02 (6)	99.6 ± 4.3 (9)	905	1036 ± 77 (7)	9418
PD117302	0.50 ± 0.05 (6)	399 ± 90 (5)	798	7200 ± 920 (4)	14400
U50488	0.69 ± 0.04 (4)	435 ± 95 (2)	630	9200 ± 1400 (2)	13333
U69593	0.67 ± 0.04 (7)	2460 ± 157 (5)	3672	9900 ± 810 (4)	14776
Spiradoline	0.35 ± 0.06 (5)	43.7 ± 3.9 (6)	125	4530 ± 300 (6)	12943
Bremazocine	0.12 ± 0.006 (3)	0.46 ± 0.06 (3)	3.8	1.1 ± 0.1 (3)	9.2
MR2034	0.09 ± 0.02 (3)	0.31 ± 0.06 (5)	3.4	3.8 ± 0.2 (4)	42
EKC	0.21 ± 0.03 (5)	1.02 ± 0.25 (4)	4.9	7.0 ± 1.6 (4)	33
DAMGO	164 ± 16 (3)	0.55 ± 0.05 (3)	—	177 ± 14 (3)	—
DPDPE	>10000 (4)	790 ± 84 (4)	—	1.4 ± 0.2 (4)	—

Each value represents the mean ± s.e.mean for the number of experiments shown in parentheses, with each experiment performed in triplicate. EKC = ethylketocyclazocine.

(1.5 μM) and EKC (7.2 μM) had low, but demonstrable affinities while all arylacetamide ligands had no detectable affinity up to a concentration of 10 μM (Table 2).

In vitro *smooth muscle preparations*

CI-977 produced a concentration-dependent inhibition of the contractile responses to nerve stimulation of the guinea-pig ileum (GPI) and rabbit vas deferens (LVD) (Figure 1), with a full suppression of the nerve response at the highest doses tested. The IC_{50} values were 0.087 nM and 3.3 nM in the GPI and LVD respectively (Table 3).

The CI-977 responses were antagonized in a competitive manner by the relatively non-selective opioid antagonist naloxone and the κ-selective antagonist norbinaltorphimine (norBNI). The pK_B values (mean ± s.e. mean) following Schild plot analysis were 7.6 ± 0.1 (GPI) and 7.5 ± 0.1 (LVD) for naloxone and 10.5 ± 0.2 for nor-BNI in the ileum. Slope values in each case were not significantly different from unity ($P > 0.1$; Mann-Whitney U-Test).

The arylacetamide ligands, U50488, U69593, PD117302 and spiradoline, all produced responses qualitatively similar to CI-977 in both tissues but with IC_{50} values 10 to 40 fold lower in the GPI and 10 to 70 fold lower in the LVD (Figure 1).

In comparison, the benzomorphans EKC (IC_{50} = 0.26 nM), bremazocine (IC_{50} = 0.04 nM) and MR2034 (IC_{50} = 0.92 nM) all produced a full, concentration-dependent inhibition of contractions in the GPI while, in the LVD this was only achieved by EKC (Figure 1) with an IC_{50} of 22 nM (Table 3). The maximum response to MR2034 was only a partial inhibition of the nerve response with a range of 23–85%. The concentration of MR2034 that produced a response equivalent to 50% of the maximum effect of the drug was 18.4 ± 5.9 nM (n = 7). However, bremazocine produced a full inhibition in 7/19 tissues (IC_{50} = 10.1 nM; Table 3) but only a partial inhibition (Figure 1) in 12/19 tissues with a range of 25%–64%. In such tissues the concentration that produced a response equivalent to 50% of the maximum effect of the drug was 9.2 ± 2.1 nM.

Table 2 Inhibition constants (K_i) obtained for CI-977 and standard κ opioid ligands at σ and phencyclidine binding sites in guinea-pig forebrain membranes

Ligand	σ [^3H]-(+)-*3-PPP* K_i values (nM)	*Phencyclidine* [^3H]-*TCP* K_i values (nM)
CI-977	1916 ± 215 (5)	>10000 (3)
PD117302	1173 ± 236 (5)	>10000 (3)
U50488	1044 ± 146 (5)	>10000 (3)
U69593	>10000 (3)	>10000 (3)
Spiradoline	1056 ± 70 (5)	>10000 (3)
Bremazocine	496 ± 40 (4)	156 ± 12 (5)
MR2034	287 ± 42 (4)	1475 ± 56 (2)
EKC	495 ± 88 (5)	7180 ± 1090 (3)

Data represent the means ± s.e.mean for the number of experiments shown in parentheses.

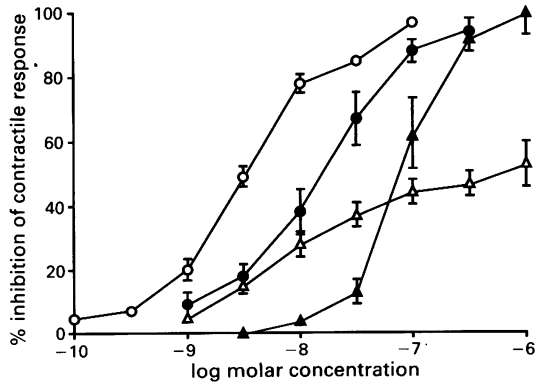

Figure 1 Concentration-dependent inhibitions of the electrically-induced contractions of the rabbit vas deferens by CI-977 (○), U62066 (●), U69593 (▲) and bremazocine (△). Each symbol represents the mean, and vertical lines the s.e.mean, of at least six independent preparations.

Antinociception in the rat

CI-977 caused a dose-dependent elevation in the paw pressure (mechanical) nociceptive threshold with MPE_{50} (dose producing a response that was 50% of the maximum possible effect

Table 3 A comparison of IC_{50} (nM) values obtained for CI-977 and reference κ-opioid ligands in the guinea-pig ileum (GPI) and rabbit vas deferens (LVD)

Compound	*GPI* (IC_{50}, nM)	*LVD* (IC_{50}, nM)
CI-977	0.087 ± 0.007 (31)	3.3 ± 0.5 (19)
PD117302	1.01 ± 0.14 (16)	58.0 ± 10.0 (11)
U50488	1.64 ± 0.09 (5)	222 ± 74 (4)
EKC	0.26 ± 0.10 (5)	22.4 ± 4.0 (4)
Spiradoline	0.88 ± 0.1 (13)	28.7 ± 4.4 (12)
U69593	3.4 ± 0.9 (7)	89.8 ± 12.1 (6)
Bremazocine	0.042 ± 0.016 (5)	10.1 ± 3.3 (7)[a]
MR2034	0.92 ± 0.07 (7)	—[b]

Each value represents the mean ± s.e.mean for the number of experiments shown in parentheses.

[a] Bremazocine produced a full inhibition of the nerve response in seven experiments. In a further twelve experiments it produced only a partial inhibition (range 22–64%) of the nerve response (see text).

[b] — MR2034 produced only a partial inhibition of the nerve response (range 23–85%) in all experiments (n = 7) (see text).

of the test) values of 0.02 and 1.8 mg kg^{-1} following intravenous (i.v.) and oral (p.o.) administration, respectively. (Figure 2a; Table 4).

The MPE_{50} values (95% confidence limits in parentheses) for CI-977 following intramuscular (i.m.) and subcutaneous (s.c.) administration were 0.07 (0.03–0.1) and 0.08 (0.04–0.1) mg kg^{-1}, respectively.

Following oral administration the antinociceptive effect for CI-977 remained significantly ($P < 0.05$, Dunnett's t test) above control levels for three hours at an MPE_{50} dose (2 mg kg^{-1}, p.o.) and for five hours at a dose (20 mg kg^{-1}, p.o.) ten fold higher than the MPE_{50} dose (Figure 3a). In comparison, at slightly below the MPE_{50} dose, CI-977 (0.03 mg kg^{-1}, i.m.) had a duration of action lasting 30 min which was prolonged with increasing dose to approximately two hours at four times (0.3 mg kg^{-1}, i.m.) the MPE_{50} dose (Figure 3b).

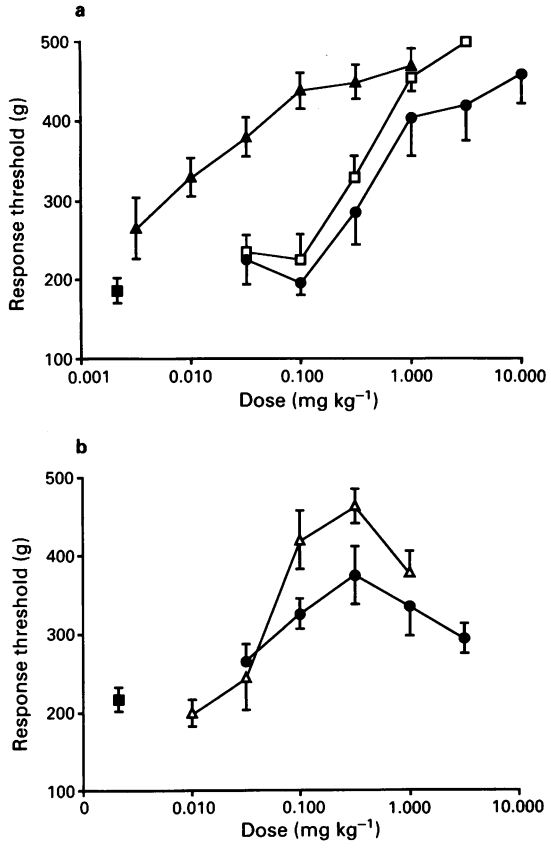

Figure 2 Dose-response curves for (a) CI-977 (▲), spiradoline (□) and U69593 (●) and (b) MR2034 (△) and bremazocine (●) in the rat paw pressure test. All compounds were given by the intravenous route of administration 5 min before testing. Each data point represents the mean, and vertical lines the s.e.mean, for groups of at least six animals. (■) Represents controls.

Table 4 Comparison of the antinociceptive effect of CI-977 with other κ-agonists in the rat paw pressure test

	MPE_{50} (mg kg^{-1}) i.v.	p.o.
CI-977	0.02 (0.008–0.043)	1.8 (1.5–2.4)
PD117302	1.41 (0.90–2.20)	25.3 (14.6–43.9)
Spiradoline	0.38 (0.16–0.87)	48.5 (29.9–78.7)
U69593	0.67 (0.32–1.28)	18.4 (4.8–71.0)
U50488	1.96 (1.67–2.29)	9.6 (4.6–19.8)
Bremazocine	—[a]	>30
EKC	0.09 (0.02–0.37)	>30
MR2034	0.07 (0.04–0.13)	>30

Each value represents the mean with 95% confidence limits in parentheses for groups of at least six animals.
[a] — The maximum antinociceptive response to bremazocine was lower (60%) than the maximum possible effect in this test. The dose of bremazocine that produced a response equivalent to 50% of the maximum effect of the drug was 0.096 mg kg^{-1} (see text).

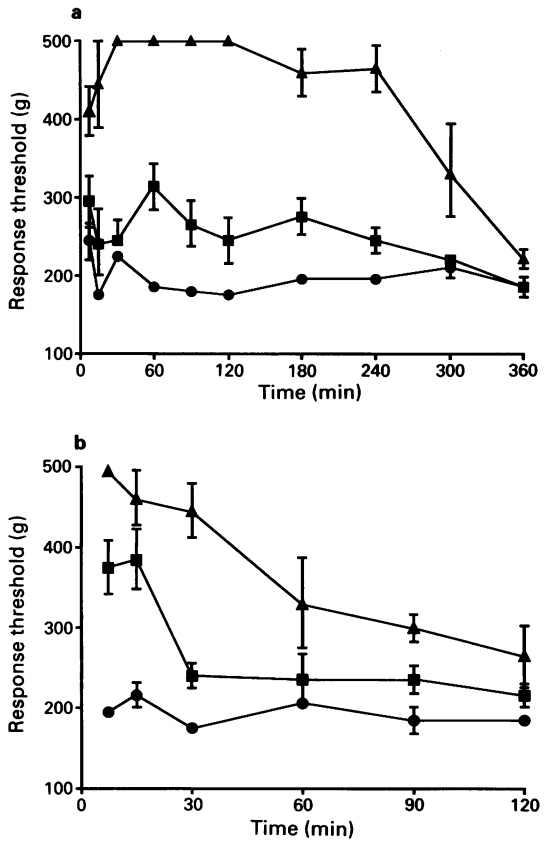

Figure 3 Duration of action of CI-977 in the rat paw pressure test following (a) oral administration, (●) control, (■) 2 mg kg^{-1} CI-977, (▲) 20 mg kg^{-1} CI-977 and (b) intramuscular administration, (●) control, (■) 0.03 mg kg^{-1} CI-977, (▲) 0.3 mg kg^{-1} CI-977. Group sizes of six were used throughout with no animal being tested more than once. Data are shown as mean and vertical lines indicate s.e.mean.

Spiradoline (Figure 2a), U69593 (Figure 2a), PD117302 and U50488, following i.v. and oral administration, all produced a dose-dependent increase in the nociceptive threshold qualitatively similar to CI-977, with maximum antinociception at the highest doses tested. However, the MPE_{50} values for these compounds (Table 4) were between 20 (spiradoline) and 100 (U50488) fold less potent (i.v.) and between 10 (U69593) and 26 (spiradoline) fold less potent (p.o.) than CI-977.

Following i.v. administration, MR2304 (Figure 2b) and EKC also produced maximum antinociception in the paw pressure test but were 3 to 5 fold less potent than CI-977. In contrast, the antinociception produced by bremazocine (i.v.) reached a plateau that was approximately 60% of the maximum possible effect attainable in this model (Figure 2b). The dose (mean with 95% confidence limits) of bremazocine that produced a response equivalent to 50% of the maximum effect of the drug was 0.096 (0.04–0.21) mg kg^{-1}. Following oral administration, none of the benzomorphans produced antinociception at doses up to 30 mg kg^{-1} (Table 4).

The opioid nature of the antinociceptive effects of all agents was confirmed by complete reversibility with naloxone (15 min pretreatment with 1 mg kg^{-1}, s.c.).

Antinociception in the mouse

CI-977 produced a dose-dependent increase in the nociceptive threshold in the acetylcholine-induced abdominal constriction (chemical) and tailclip (mechanical) nociception models with MPE_{50} values of 5 and 30 μg kg^{-1} (s.c.) respectively. In con-

trast it was ineffective up to 10 mg kg^{-1} (s.c.) in the mouse hotplate (thermal) test (Table 5).

MR2034 and U69593 were effective against both chemical and mechanical noxious stimuli but were 16 and 20 fold less potent, respectively, than CI-977 in the abdominal constriction test and 3 and 47 fold less potent, respectively, in the tailclip model. Bremazocine also caused a maximum increase in the nociceptive threshold in the abdominal constriction and tailclip tests but, in contrast to MR2034 and U69593, was equipotent with CI-977 in both models. All three compounds were inactive in the hotplate test (Table 5).

Spiradoline was effective against all three noxious stimuli including the thermal stimulus (MPE_{50} 13 mg kg^{-1}, s.c.). In the abdominal constriction and tailclip tests spiradoline was 20 and 40 fold less active, respectively, than CI-977 (Table 5).

Locomotor activity

All compounds tested produced a dose-dependent suppression of spontaneous locomotor activity (sedation) in mice with MPE_{50} values (95% confidence limits in parentheses) of 0.09 (0.03–0.23), 0.7 (0.2–3.6), 2.6 (0.6–10.2) and 6.8 (2.0–22.6) mg kg^{-1} (s.c.) for CI-977, spiradoline, PD117302 and U50488, respectively.

However, U69593 produced a biphasic effect consisting of a short lasting (30–40 min) period of hypoactivity (MPE_{50} = 2.4 (0.2–28.5) mg kg^{-1} s.c.) followed by rebound hyperactivity. All other compounds suppressed activity for the entire 60 min test period. The MPE_{50} values for bremazocine, EKC and MR2034 were 0.08 (0.04–0.15), 0.7 (0.3–1.7) and 1.5 (0.9–2.5) mg kg^{-1} (s.c.), respectively.

Diuretic effects

CI-977, spiradoline and U69593 all produced a potent diuresis in rats with MPE_{50} values (95% confidence limits in parentheses) of 0.16 (0.11–0.23), 0.65 (0.29–1.45) and 1.1 (0.4–2.6) mg kg^{-1} (s.c.), respectively. The diuresis observed with these agents was of short duration with the rate of production being maximal during the first 2 h measurement interval. The maximum volume of urine produced by each agent was 15.5 ± 1.2, 14.8 ± 1.1 and 14.1 ± 1.0 ml per 6 h for CI-977, spiradoline and U69593, respectively. Bremazocine (0.03–0.3 mg kg^{-1}, s.c.) and MR2034 (0.01–1.0 mg kg^{-1}, s.c.) also produced a diuretic response but with a maximum output of urine that was significantly below that of CI-977. The maximum volume of urine produced by bremazocine and MR2034 was 11.8 ± 0.7 and 8.2 ± 0.4 ml per 6 h, respectively. Since both benzomorphans appeared to be acting like partial agonists in this test an MPE_{50} value was not determined.

Discussion

In the present study it has been demonstrated that the novel arylacetamide CI-977 is a potent and selective agonist for the κ-opioid receptor.

In the *in vitro* opioid ligand binding and smooth muscle assays, CI-977 exhibited superior affinity and agonist potency, respectively, at the κ-receptor over the established arylacetamides. Moreover, the activity of CI-977 in each assay was either comparable to, or indeed, in the rabbit vas deferens, higher than the most potent benzomorphan ligand. Of the compounds tested only U69593 had a higher μ/κ selectivity than CI-977.

CI-977 would also appear to be more potent and of comparable selectivity to the more recently described ICI 197067 and its congeners (Costello *et al.*, 1988). The pK_B values for the non-selective opioid antagonist naloxone (Hutchinson *et al.*, 1975) and the κ-selective norbinaltorphimine (Portoghese *et al.*, 1987; Birch *et al.*, 1987) against CI-977 in the smooth muscle assays confirmed that the agonist effects of the compound were mediated through the κ-receptor.

In the rabbit vas deferens, a tissue with a lower κ-receptor reserve than the ileum (Miller *et al.*, 1986), there was, in most cases, an increase in the potency of CI-977 relative to the other agents, which would be consistent with it having a higher relative efficacy (Kenakin, 1984). This was further exemplified by comparing the ratio of agonist potency to receptor affinity which, as an empirical measure of receptor occupancy (Kenakin, 1984), was found to be between 3 and 10 fold lower for CI-977 relative to those agents observed to be full agonists in the rabbit vas deferens. However, the difference in efficacy was particularly apparent with bremazocine and MR2034 both of which failed to inhibit completely the contractions of the vas deferens at maximally-effective concentrations, indicative of partial agonism (Miller *et al.*, 1986; Verlinde & De Ranter, 1988). In contrast, in approximately 40% of vasa investigated, a sufficient κ-receptor reserve appeared to be present for bremazocine to act as a full agonist, such variability confirming previous observations (Verlinde & De Ranter, 1988).

In the *in vivo* rodent nociception models, following systemic administration, against either a mechanical or chemical noxious stimulus, the relative potencies for antinociception followed a similar pattern to those obtained in the *in vitro* assays. CI-977 was clearly the most potent antinociceptive compound of the arylacetamide series in the rat paw pressure and mouse tailclip and writhing models. Moreover, CI-977 again had a higher potency than the benzomorphans, with the exception of bremazocine which was equipotent with CI-977 in both the mouse tests. However, in the paw pressure test the

Table 5 A comparison between the effects of CI-977 and other κ-agonists against different types of noxious stimuli in the mouse

	Tailclip	*Abdominal constriction* MPE_{50} (mg kg^{-1})	*Hotplate*
CI-977	0.03 (0.008–0.12)	0.005 (0.003–0.009)	>30
PD117302[a]	2.2	0.8	>100
Spiradoline	0.81 (0.21–3.2)	0.3 (0.08–1.2)	13.1 (9.2–18.7)
U69593	1.4 (0.63–3.3)	0.6 (0.03–0.11)	>30
U50488[a]	6.4	1.0	>100
Bremazocine	0.03 (0.005–0.138)	0.006 (0.002–0.016)	>30
MR2034	0.08 (0.01–0.54)	0.08 (0.0003–17.9)	>30
EKC[a]	NT	0.2	>30

Each value represents the mean, with 95% confidence limits in parentheses where appropriate, for groups of at least six animals.
NT = not tested.
[a] Mean MPE_{50} values taken from Leighton *et al.* (1987).

potency and efficacy of bremazocine was considerably lower, as shown by the low ceiling to its effect when compared with CI-977.

The additional ineffectiveness of CI-977 against a thermal noxious stimulus of the intensity applied in the mouse hotplate (55°C) was consistent with its profile as a κ-receptor agonist (Tyers, 1980; Leighton *et al.*, 1987).

Interestingly, although most of the arylacetamides and benzomorphans shared a similar lack of antinociceptive potency in this model, spiradoline was found to be active. However, rather than being an indication of increased efficacy at the κ-receptor, the activity of spiradoline is more likely to be mediated through the μ-receptor. Previous studies have demonstrated that the (+)-enantiomers of racemic benzeneacetamides have agonist potency and selectivity for the μ-receptor (Von Voigtlander & Lewis, 1988; Meecham *et al.*, 1989).

CI-977 would also appear to have a clear superiority in potency when compared to morphine particularly when this involves mechanical and chemical noxious stimuli. However, morphine, as would be expected of a μ-agonist, maintains its potency against the type of thermal stimulus intensity used in the hotplate test (Leighton *et* al., 1987).

The pronounced sedation and diuresis produced by CI-977 at doses close to the antinociceptive dose was again consistent with the expected profile for a κ-receptor agonist (Leander, 1983; Ukai & Kameyama 1985; Leighton *et al.*, 1987; Dykstra *et al.*, 1987; Peters *et al.*, 1987). Furthermore, CI-977 does not cause inhibition of gastrointestinal motility, a dependence liability or respiratory depression (unpublished observations), effects normally associated with actions at the μ-receptor (Martin, 1984). The rank order of potency for the sedative and diuretic properties was similar to that obtained for antinociception in the rat paw pressure test. Moreover, the partial agonist properties of both bremazocine and MR2034 were again demonstrated by the low ceiling nature of the volume of urine produced by each agent relative to CI-977.

It has been suggested that σ, as well as PCP, sites might play some role in the mediation of the dysphoric (or psychotomimetic) behaviour associated with some of the previous, non-selective benzomorphan κ-agonists (Martin *et al.*, 1976; Steinfels *et al.*, 1988; Tam *et al.*, 1988). In this respect the negligible affinity of CI-977 at either site could be considered advantageous and might, therefore, improve the therapeutic utility of this compound.

However, additional studies have suggested that κ-receptors themselves may be involved in the mediation of dysphoria (Pfeiffer *et al.*, 1986; Shearman & Stenfors, 1986; Shippenberg *et al.*, 1988). Consistent with this notion spiradoline has been shown to be clinically dysphoric (Peters & Gaylor, 1989). However, although spiradoline exhibited acceptable κ-selectivity in the *in vitro* tests, the selectivity of the compound *in vivo* remains questionable (Von Voigtlander & Lewis, 1988; Peters & Gaylor, 1989), perhaps due to its relative lack of potency in producing an antinociceptive response. It should, therefore, prove interesting to observe the clinical effects of a compound like CI-977 that maintains its selectivity for the κ-receptor in animal behavioural models.

In conclusion, CI-977 was the only arylacetamide to have both an affinity and potency at the κ-receptor that is comparable to the most potent benzomorphan ligands. In addition, it would appear that CI-977 may also have a higher relative efficacy. It remains to be observed whether these apparent advantages can be translated into a clinical analgesic efficacy that will be comparable to the currently-available μ-agents such as morphine.

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(Received February 14, 1990
Revised May 2, 1990
Accepted May 18, 1990)