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PD117302: a selective agonist for the κ-opioid receptor

[2]C.R. Clark, B. Birchmore, N.A. Sharif, [1]J.C. Hunter, R.G. Hill & J. Hughes

Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge, CB2 2QB

1 A new nonpeptide κ-opioid compound, a cyclohexyl benzeneacetamide derivative (PD117302), has been synthesized and its affinity for the different types of opioid receptor determined. The ability of PD117302 to modify the activity of the electrically-stimulated guinea-pig ileum and rabbit vas deferens has also been evaluated.

2 In binding studies using guinea-pig brain homogenates, unlabelled PD117302 had a high affinity (K_i = 3.7 nM) at [^{3}H]-etorphine labelled κ sites and a low affinity at [^{3}H]-[D-Ala^2, MePhe^4, glyol^5]-enkephalin ([^{3}H]-DAGOL) labelled μ sites (K_i = 408 nM) and [^{3}H]-SKF 10047 labelled σ sites (K_i = 1.8 μM). In bioassay studies, PD117302 was a potent agonist, producing a maximum inhibition of the electrically-evoked contractions of the guinea-pig ileum (IC_{50} = 1.1 nM) and rabbit vas deferens (IC_{50} = 45 nM) which was naloxone-reversible.

3 In guinea-pig brain, [^{3}H]-PD117302 bound to a high-affinity opioid binding site with a K_D of 2.7 nM and a B_{max} of 3.4 pmol g^{-1} wet weight. The B_{max} was found to be less than 50% of the B_{max} values for [^{3}H]-etorphine and [^{3}H]-bremazocine suggesting that [^{3}H]-PD117302 may be a specific ligand for a subtype of κ receptor. [^{3}H]-PD117302 also bound with micromolar affinity to a non-opioid binding site.

4 Kinetic studies found that [^{3}H]-PD117302-specific binding to the high affinity site was saturable, reaching equilibrium within 20 min at 4°C, and reversible, with a half-life of dissociation of 3.9 min.

5 Several unlabelled compounds with high affinities for the [^{3}H]-etorphine labelled κ binding site, had comparable affinities when competing for the [^{3}H]-PD117302-specific high affinity binding site. In contrast, DAGOL, [D-Ala^2, D-Leu^5] enkephalin (DADLE) and [D-Pen^2, D-Pen^5] enkephalin (DPDPE) had no significant effect on [^{3}H]-PD117302 binding, suggesting minimal interaction with μ and δ binding sites.

6 In autoradiography studies [^{3}H]-PD117302 binding sites were found throughout the brain with the greatest density in the striatum, cerebral cortex (layers V–VI), substantia nigra, and the molecular layer of the cerebellum. Lowest levels were found in the granular layer of the cerebellum, thalamus and cerebral cortex (layers I–IV).

Introduction

In the mammalian central nervous system (CNS), multiple opioid receptors, namely μ, δ and κ, have been implicated in the mediation of opioid antinociceptive activity (Gilbert & Martin, 1976; Tyers, 1980; Ward & Takemori, 1983; Upton *et al.*, 1983).

κ-Opioid agonists are relatively potent analgesics (Martin *et al.*, 1976; Woods *et al.*, 1979) but, unlike μ-selective compounds, possess fewer opioid side-effects such as dependence liability, respiratory depression and constipation. This has led to the search for κ-selective agonists as safe and effective analgesics.

Several putative κ-selective ligands have been described in the literature, but despite low nanomolar affinity at the κ binding site most of these compounds have a similar potency at the μ binding site, a recent exception being the Upjohn compound [^{3}H]-U69593 (Lahti *et al.*, 1985). However, radiolabelling, characterization and light microscopic visualization of κ-opioid receptors with non-selective radioligands has been made possible because μ and δ binding sites can be preferentially blocked with selective unlabelled ligands (Gillan *et al.*, 1980; Kosterlitz *et al.*, 1981; Gillan & Kosterlitz, 1982; Kosterlitz & Paterson, 1985). Thus, [^{3}H]-bremazocine (Kosterlitz *et al.*, 1981; Foote & Maurer, 1982; Robson *et al.*, 1984), [^{3}H]-etorphine (Audigier *et al.*, 1982) and [^{3}H]-ethylketocyclazocine (EKC) (Gillan *et al.*, 1980; Pasternak,

[1] Author for correspondence.

[2] Present address: Warner Lambert/Parke-Davis Co., (Pharmaceutical Research Division), 2800 Plymouth Road, Ann Arbor, Michigan 48105 U.S.A.

1980) have been used to study the properties and regional distribution of κ receptors in either membrane homogenates and/or by autoradiography.

Recently, PD117302, a novel non-peptide compound selective for the κ opioid receptor was synthesized in our laboratories (Clark *et al.*, 1987) and radiolabelled with tritium. The aims of this study were: (1) to define the κ receptor binding parameters, pharmacological properties and the discrete localization of [^{3}H]-PD117302 binding sites in guinea-pig brain and peripheral tissues; (2) to determine the efficacy of unlabelled PD117302 in comparison with recognized opioid compounds in the electrically-stimulated guinea-pig ileum and rabbit vas deferens bioassay preparations.

Preliminary results from this study have been communicated to the British Pharmacological Society (Birchmore *et al.*, 1987).

Methods

Guinea-pig brain homogenate preparation

Male Dunkin-Hartley guinea-pigs (250–350 g) (Interfauna plc, UK) were killed by cervical dislocation. Whole brains minus cerebellae were removed rapidly and individual brain regions dissected. The tissues were homogenized in 10 vol. Tris-HCl (50 mM, pH 7.4 at 4°C) with a Brinkman Polytron (setting 6 for 15 s) and the homogenate centrifuged at 49,000 *g* for 10 min at 4°C. The resultant pellets were resuspended in 10 vol of fresh 50 mM Tris-HCl buffer and incubated at 37°C for 45 min. After this time the homogenate was recentrifuged as above, the supernatant discarded and the pellets resuspended in ice-cold buffer at a concentration of 10 mg original wet weight ml^{-1}.

Receptor binding assays

Membrane aliquots (1.88 ml) were incubated in a final volume of 2 ml with competing unlabelled compound and either [^{3}H]-PD117302 (1 nM), [^{3}H]-etorphine (0.5 nM) or [^{3}H]-bremazocine (0.2 nM) to label κ receptors, [D-Ala^2, MePhe^4, glyol^5] enkephalin ([^{3}H]-DAGOL; 1 nM) to label μ receptors or [^{3}H]-SKF10047 (norallylmetazocine, 1 nM) to label σ binding sites (Su, 1982). In experiments involving either [^{3}H]-etorphine or [^{3}H]-bremazocine, binding was in the presence of 500 nM unlabelled DAGOL and DADLE ([D-Ala^2, D-Leu^5] enkephalin) to block μ and δ receptors respectively and [^{3}H]-SKF10047 binding was in the presence of 1 μM etorphine to block all opioid receptors. Non-specific binding of [^{3}H]-PD117302 was defined as the binding persisting in the presence of 1 μM unlabelled etorphine, though initial studies used 10 μM unlabelled U50488. Etorphine (1 μM) was used for defining non-specific binding in all other opioid receptor assays. SKF10047 (100 μM) was used to define the non-specific binding of [^{3}H]-SKF10047 binding to σ sites. The assays were conducted at 4°C and terminated after 2.5 h by rapid filtration through Whatman GF/B glass fibre filters (presoaked in 0.1% polyethyleneimine and 1 μM unlabelled PD117302 for the [^{3}H]-PD117302 assays) on a Brandell M-48 cell-harvester. The filters were washed with 3 × 2 ml of ice-cold 50 mM Tris HCl buffer and the radioactivity determined by liquid scintillation spectrometry.

Autoradiography studies

Guinea-pig brains were frozen in dry-ice onto aluminium chucks using water or plastic embedding material. Following equilibration at − 18°C for 2–24 h, sections (10 μM) were cut at − 15°C using a Brights freezing microtome and thaw-mounted on dust-free, gelatin-coated glass microscope slides. These slides were stored at − 20°C for up to 2 weeks. This storage period did not affect the κ receptor binding on the brain sections (unpublished results). For receptor binding assays, slide-mounted sections were thawed at room temperature and then pre-incubated at 37°C for 30–45 min in order to degrade endogenous opioids. The slides were laid flat on glass rods and allowed to cool to 4°C in a cold-room. The sections were covered evenly with 1 ml of 50 mM Tris HCl, pH 7.4 at 4°C containing 5 nM [^{3}H]-PD117302. Adjacent sections were exposed to the same concentration of the label containing 100 μM U50488 to define the non-specific binding. The assays were terminated after 90 min by draining the assay mixture and rinsing the slides in 500 ml of ice-cold 50 mM Tris HCl (pH 7.4) for 15–20 min and dipping (2 s) the slides into ice-cold deionized water to remove buffer salts. The washed and labelled sections were then dried rapidly under a stream of cool dry air, dessicated overnight and then apposed to a sheet of tritium-sensitive film (LKB, Ultrofilm), together with brain paste standards containing known amounts of radioactivity, in an X-ray cassette in the dark. After three months the autoradiograms were developed in Kodak D-19 (3 min at 18°C), fixed in Kodafix (3 min) and washed in water for 15–20 min. The autoradiograms were analysed on the Quantimet 920 image analysing system (Sharif *et al.*, 1986).

Briefly, adjacent non-specific binding sections were re-aligned on the outlines of the total binding sections and the specific binding determined by automatic digital subtraction of the former from the latter images. Quantification of the data was by reference to a series of tritiated brain paste standards using a natural log plot of absorbance versus radioactivity.

Isolated tissue preparations

Guinea-pig ileum Male Dunkin-Hartley guinea-pigs were killed by cervical dislocation, the ileum removed 10 cm proximal to the caecum and placed in Tyrode buffer. The ileum was then flushed with buffer and 2–3 cm segments suspended in 10 ml organ baths containing buffer at 37°C bubbled with a mixture of 95% O_2 and 5% CO_2.

Rabbit vas deferens Male Californian white rabbits (1.5 to 2 kg) were killed by cervical dislocation, the vasa deferentia quickly removed and placed in Mg^{2+}-free Krebs solution. The vasa were then suspended in 2.5 ml organ baths containing buffer bubbled with 95% O_2:5% CO_2 at 37°C.

Both preparations were allowed to equilibrate for 30 min under a resting tension of 0.5 g and then stimulated either transmurally (ileum) or co-axially (vas) at supramaximal voltage, 0.15 Hz and with a pulse width of 0.5 ms. Control log dose-response curves and those in the presence of naloxone were calculated at the IC_{50} level, i.e. the concentration of agonist producing 50% inhibition of the contractions evoked by electrical stimulation. The pA_2 values for naloxone were then determined from Schild plots as previously described (Arunlakshana & Schild, 1959).

H.p.l.c. analysis of [^{3}H]-PD117302

The stability and purity of [^{3}H]-PD117302 was periodically checked by high performance liquid chromatography (h.p.l.c.). Aliquots (20 μl) of the radioligand were injected onto a C_{18} μ Bondapak reverse-phase h.p.l.c column with a mobile phase consisting of 65% methanol, 35% water and 0.08% trifluoroacetic acid (TFA). The flow rate was 1.5 ml min^{-1}. The column eluate was first directed through a Pye Unicam PV4020 ultra-violet detector at 220 nm and then through a radioactivity flow detector (Radiomatic Instruments).

Analysis of binding data

The receptor binding data were analysed by either 'KINETIC' (Elsevier Biosoft) for the association and dissociation rate constants or by 'LIGAND' (Munson & Rodbard, 1980) for the competitive inhibition data. The inhibition constant (K_i) was calculated using the Cheng & Prusoff (1983) equation:

$$K_i = IC_{50}/(1 + L/K_D).$$

Preparation of [^{3}H]-PD117302

The tritiated form of PD117302 was prepared by the route described for the unlabelled compound (Clark *et al.*, 1987) except that [^{3}H]-pyrrolidine was used instead of unlabelled pyrrolidine. Essentially, the [^{3}H]-pyrrolidine derivative was prepared by reaction of the acid chloride with [^{3}H]-*trans*-N-methyl-2-(1-pyrrolidinyl) cyclohexanamine. The acid chloride was freshly prepared and the former compound was prepared by catalytic reduction of the corresponding 3-pyrroline derivative using tritium gas. The product was purified by reverse-phase thin-layer chromatography and converted to the hydrochloride salt by addition of ethanolic hydrochloric acid. All these procedures were performed by Amersham International. The radioligand was stored at −20°C under nitrogen. The specific activity of [^{3}H]-PD117302 was 24.5 Ci $mmol^{-1}$ (1 mCi ml^{-1}).

Materials

[^{3}H]-PD117302 was custom-synthesized and supplied by Amersham International (U.K.). All other radioligands were also obtained from Amersham. The following unlabelled drugs were used: Tifluadom (Kali-Chemie Pharma); ethylketocyclazocine (Sterling Winthrop); etorphine (C. Vet), bremazocine (Sandoz); morphine sulphate (Savory and Moore); all peptides (Bachem); U69593 ((5α, 7α, 8β)-(−)-N-methyl-N-(7-(1-pyrrolidinyl)-1-(oxaspiro (4,5) dec-8-yl) benzene acetamide, Upjohn Co); U50488 (*trans*-(−)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]-benzeneacetamide), U62066 (5α, 7α, 8β)-(±)-3,4-dichloro-N-methyl-N-(7-1-pyrrolidinyl)-1-(oxaspiro (4,5) dec-8-yl) benzeneacetamide) and PD117302 (prepared at Parke-Davis Research Unit, Cambridge).

Results

Purity and stability of [^{3}H]-PD117302

The chemical structure of PD117302, (±)-*trans*-N-methyl-N-[2- (1-pyrrolidinyl)-cyclohexyl] benzo [b] thiophene-4-acetamide is shown in Figure 1. Analysis of [^{3}H]-PD117302 by reverse-phase high performance liquid chromatography (h.p.l.c.) showed that the radioligand was stable over a period of at least three months (data not shown).

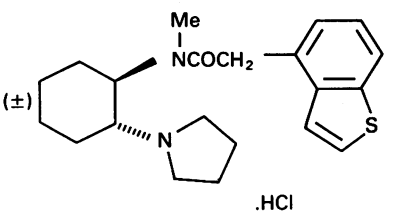

Figure 1 Structure of PD117302.

General binding characteristics of [^{3}H]-PD117302

[^{3}H]-PD117302-specific binding to guinea-pig brain membranes represented 40% of total binding when the non-specific binding (NSB) was determined as binding in the presence of excess unlabelled etorphine (1 μM)

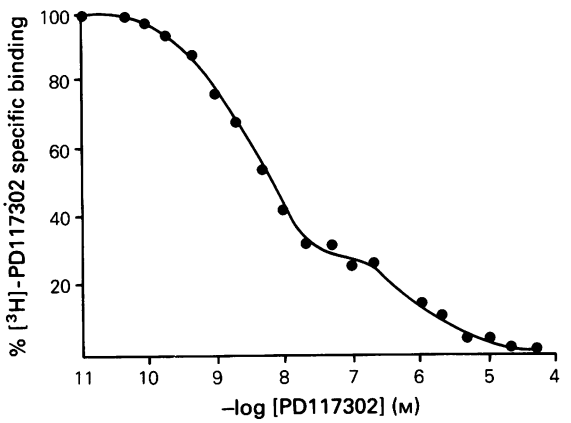

Figure 2 Competitive inhibition of [^{3}H]-PD117302 binding to guinea-pig forebrain membranes by unlabelled PD117302 using 100 μM unlabelled U50488 to define non-specific binding. Each point represents the mean of three individual experiments. The standard error of the mean was always within 15% of the mean.

and 62% of total binding when the NSB was determined as binding in the presence of either excess unlabelled PD117302 (10 μM) or U50488 (10 μM). Analysis of the concentration-response curve for PD117302 in competition with [^{3}H]-PD117302 revealed that the interaction of [^{3}H]-PD117302 with guinea-pig membranes was consistent with binding to two sites of differing affinities (Figure 2). The equilibrium dissociation constant, K_D, and apparent maximum binding capacity, B_{max}, for the high affinity site were 0.95 ± 0.02 nM and 1.98 ± 0.31 pmol g^{-1} wet weight, respectively. The K_D and B_{max} for the lower affinity site were 1.1 ± 0.2 μM and 373 ± 73 pmol g^{-1} wet weight, respectively. However, using a wide range of opioid compounds in competition with 1 nM [^{3}H]-PD117302, only unlabelled PD117302 and the structurally similar U50488 displaced the radioligand from both high and low κ affinity sites. In contrast, etorphine, naloxone and proxorphan displaced [^{3}H]-PD117302 from only the high affinity site, while SKF10047 (up to 100 μM) did not inhibit binding from either site. Since these results implied that the lower affinity site was non-opioid, in all subsequent analysis of [^{3}H]-PD117302 binding to the high affinity site, 1 μM etorphine was used to estimate the non-specific binding.

Under these conditions, unlabelled PD117302 produced a concentration-dependent competitive inhibition of [^{3}H]-PD117302 (1 nM)-specific binding

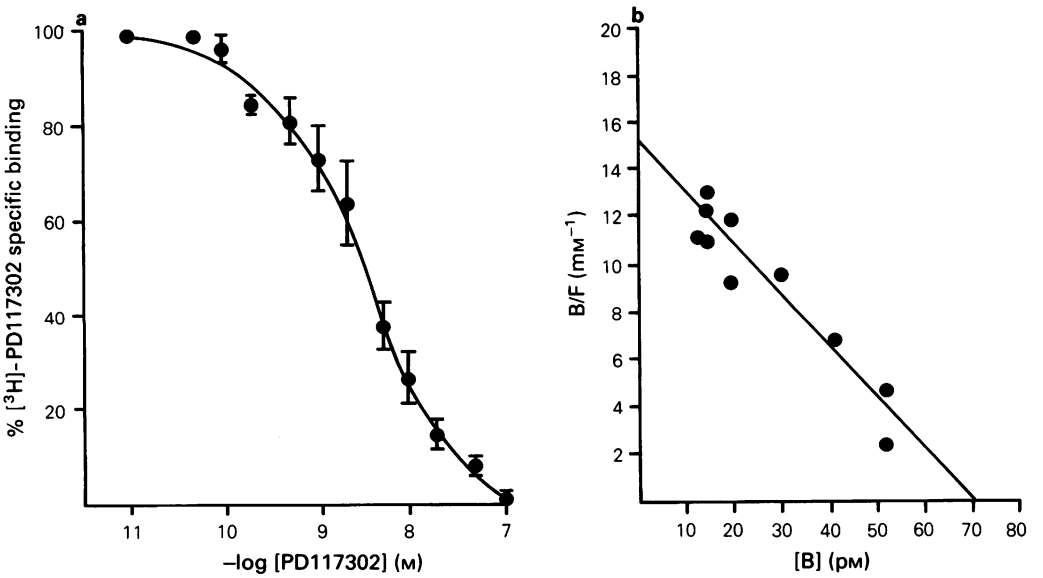

Figure 3 (a) Competitive inhibition of [^{3}H]-PD117302 specific binding by unlabelled PD117302 using 1 μM unlabelled etorphine to define non-specific binding. Each point represents the mean of six experiments; vertical lines indicate s.e. (b) Scatchard transformation of the specific binding data.

(Figure 3a). Scatchard transformation of these data, (Figure 3b), was linear with a Hill coefficient not significantly different from unity, indicating that [^{3}H]-PD117302 was binding to a single population of sites. The K_D was 2.7 ± 0.6 nM ($n = 6$) with a B_{max} of 3.4 ± 0.9 pmol g^{-1} wet weight ($n = 6$). Application of the Cheng-Prusoff (1973) equation gave a K_i value for unlabelled PD117302 of 2.4 ± 0.5 nM ($n = 6$). Affinity constant and receptor capacity values were also obtained for [^{3}H]-etorphine and [^{3}H]-bremazocine. The K_D for [^{3}H]-etorphine was 0.6 nM and the B_{max} 7.2 pmol g^{-1} wet weight. The K_D for [^{3}H]-bremazocine was 0.2 nM and the B_{max} 7.9 pmol g^{-1} wet weight.

Kinetic studies of [^{3}H]-PD117302 binding showed that it was time-dependent, reaching a plateau within 15 min which was maintained for 120 min (Figure 4a). The specific binding was linearized according to the pseudo first-order rate equation (Weiland & Molinoff, 1981). A plot of log ($B_{eq}/B_{eq} - B_t$) versus time is shown in Figure 4a, where B_{eq} is the amount of [^{3}H]-PD117302 specifically bound at equilibrium and B_t is the amount specifically bound at time t. This resulted in a K_{obs} value of 0.37 ± 0.04 min^{-1}. [^{3}H]-PD117302 specific binding was reversible as shown by the rapid dissociation of the radioligand following addition of an excess volume of 1 μM unlabelled etorphine (Figure 4b). A plot of log (B_t/B_o) versus time (Figure 4b) resulted in a dissociation rate constant, k − 1, of 0.18 ± 0.04 min^{-1} ($t_{\frac{1}{2}}$ = 3.9 min). From the slope of the pseudo-first order association reaction, K_{obs}, and the dissociation rate constant, k − 1, the association rate constant, k + 1, was calculated to be 1.6 × 10^8 M^{-1} min^{-1}. From the respective association and dissociation rate constants the K_D (k − 1/k + 1) was calculated as 1.13 nM.

Pharmacological specificity of [^{3}H]-PD117302-specific binding

Several compounds with high affinity for κ receptors, such as bremazocine, etorphine, ethylketocyclazocine (EKC), U50488 and tifluadom, had comparable affinities when competing for the [^{3}H]-PD117302 specific high-affinity binding site and [^{3}H]-etorphine labelled κ binding sites (Table 1). In contrast, compounds with a high affinity for μ and δ receptors, such as DAGOL, DPDPE and DADLE respectively, had no significant effect, in concentrations up to 1 μM, on [^{3}H]-PD117302 specific binding.

The affinity of unlabelled PD117302 when competing with [^{3}H]-etorphine binding to κ receptors (K_i = 3.17 nM) was markedly higher than its affinity for [^{3}H]-DAGOL labelled μ receptors (K_i = 408 nM) or [^{3}H]-SKF 10047 labelled σ receptors (K_i = 1.8 μM).

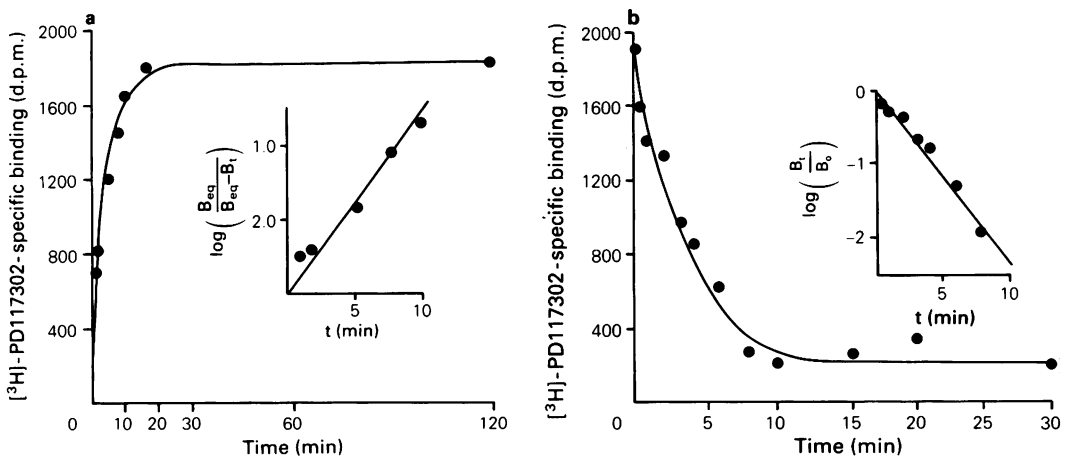

Figure 4 Kinetic analysis of [^{3}H]-PD117302 binding to guinea-pig forebrain membranes. (a) Time course for association of [^{3}H]-PD117302 binding. Membranes were incubated at various time intervals with 1 nM [^{3}H]-PD117302 at 4°C in the absence (total) or presence (non-specific) of 1 μM unlabelled etorphine. Specific binding was then calculated by subtracting non-specific from total binding. The data are representative of three such experiments. Inset, a plot of the pseudo first-order kinetics of association for specific [^{3}H]-PD117302 binding. (b) Dissociation of specific [^{3}H]-PD117302 binding. The membranes were incubated with [^{3}H]-PD117302 for 2.5 h at 4°C. At time zero, 1 μM etorphine was added to initiate dissociation, then the amount of [^{3}H]-PD117302 specifically bound to the membranes was measured at various time intervals. The data are representative of three such experiments. Inset, the initial dissociation of [^{3}H]-PD117302 binding was linearized by the first-order dissociation rate equation.

Table 1 Pharmacological characterization of [^{3}H]-PD117302-specific binding in comparison with [^{3}H]-etorphine

Compound	*[^{3}H]-PD117302* K_i (nM)	*[^{3}H]-etorphine* K_i (nM)
PD117302	2.4 ± 0.5	3.7 ± 0.4
Etorphine	0.9 ± 0.1	1.2 ± 0.2
EKC	0.9 ± 0.2	0.6 ± 0.2
Bremazocine	0.3 ± 0.1	0.6 ± 0.2
Tifluadom	0.9 ± 0.1	3.1 ± 0.9
Naloxone	4.9 ± 1.1	7.1 ± 1.0
DAGOL	$>10^{-6}$	$>10^{-6}$
DADLE	$>10^{-6}$	$>10^{-6}$
DPDPE	$>10^{-5}$	$>10^{-5}$

Each value represents the mean ± s.e.mean of the inhibition constants (K_i) calculated from between 3 and 4 experiments. The K_D values were respectively 2.7 nM for [^{3}H]-PD117302 and 0.6 nM for [^{3}H]-etorphine.

Regional distribution of [^{3}H]-PD117302-specific binding

The distribution of [^{3}H]-PD117302-specific binding sites was investigated in homogenates of several guinea-pig brain regions (Table 2). The highest concentration of [^{3}H]-PD117302-specific binding sites was in the striatum followed by cerebral cortex, cerebellum, amygdala, hypothalamus and thalamus.

Quantitative autoradiography studies

The discrete regional distribution of specific [^{3}H]-PD117302 binding sites in the guinea-pig brain, as determined by quantitative autoradiography, is shown in Table 3 and Figure 5. A high density (>100 amol mm^{-2}) of [^{3}H]-PD117302 sites was found in the cerebral cortex (layers V-VI), striatum (head and tail), cerebellum (molecular layer) and substantia nigra, while a medium-high density (70–100 amol mm^{-2}) was associated with the nucleus accumbens, hippocampus (molecular layer), striatum (body), globus pallidus, hypothalamus, and inferior colliculus. The lowest levels of [^{3}H]-PD117302 binding sites (<70 amol mm^{-2}) were found in the cerebellum (granular layer), thalamus, superior colliculus, central gray, septum and cerebral cortex (layers I–IV).

Table 2 Regional distribution of [^{3}H]-PD117302-specific binding in guinea-pig

Tissue/region	*Specific binding* (pmol g^{-1} wet weight)
Striatum	1.33 ± 0.38
Cerebral cortex	1.23 ± 0.16
Cerebellum	0.79 ± 0.17
Amygdala	0.52 ± 0.04
Hypothalamus	0.44 ± 0.11
Thalamus	0.39 ± 0.02

Concentration of [^{3}H]-PD117302 used in these experiments was approximately 1 nM. Data represent the mean ± s.e.mean from 3 experiments.

In vitro *bioassay preparations*

PD117302 produced a concentration-dependent inhibition of the contractions induced by electrical stimulation of the guinea-pig ileum (IC_{50} = 1.1 ± 0.1 nM, n = 41; Table 4) and rabbit vas deferens (IC_{50} = 44.5 ± 14.1 nM, n = 3) which was naloxone-reversible. In the guinea-pig ileum, the pA_2 value (7.78) for naloxone against PD117302 was compared to values obtained against other opioid compounds (Table 4). It was not significantly different (P > 0.05) from those values obtained against the κ-

Table 3 Quantitative autoradiography of [^{3}H]-PD117302 binding sites in guinea-pig brain

Region	*Specific binding* (amol/mm^2)
Cerebral Cortex	
Layers I–IV	55 ± 17 (3)
Layers V–VI	147 ± 3 (3)
N. acumbens	99 ± 6 (3)
Striatum	
Head	103 ± 16 (3)
Body	97 ± 11 (3)
Tail	146 ± 2 (3)
Globus Pallidus	84 ± 6 (3)
Septum	61, 49
Hypothalmus	82, 93
Thalamus	65, 79
Hippocampus	
Molecular Layer	94 ± 7 (3)
Oriens Layer	53 ± 11 (3)
Central Gray	96, 54
Superior Colliculus	63, 92
Inferior Colliculus	85, 97
Substantia Nigra	107 ± 16 (3)
Cerebellum	
Molecula Layer	117, 168
Granular Layer	72, 81

Data represent mean ± s.e.mean of values from number of animals shown in parentheses. Individual values are shown for less than three animals.

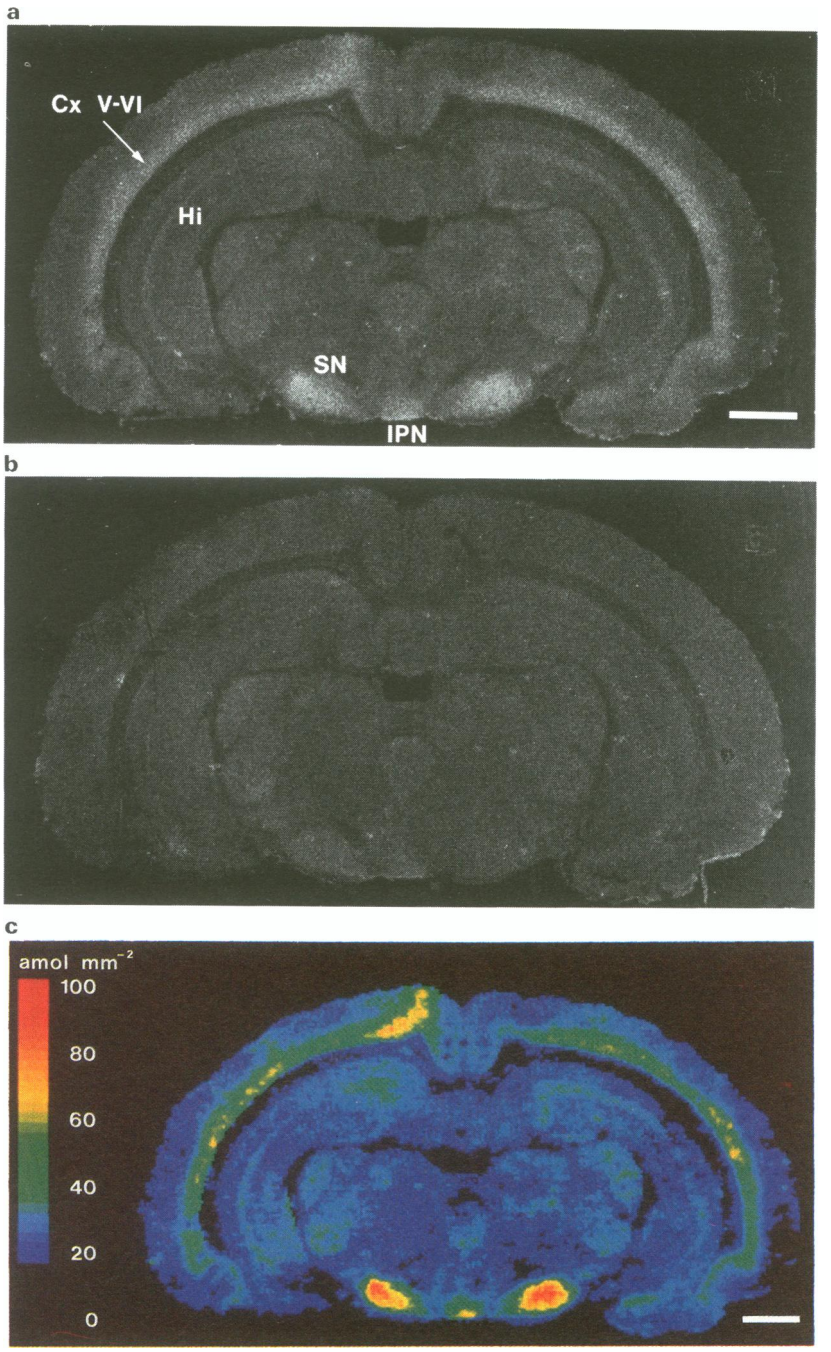

Figure 5 Autoradiographic localization of (a) total [^{3}H]-PD117302 binding sites in a coronal section from guinea-pig brain (bregma − 2.0 mm). Cx = cerebral cortex, Hi = hippocampus, SN = substantia nigra and IPN = interpeduncular nucleus. (b) Autoradiographic image from an adjacent section following incubation of [^{3}H]-PD117302 in the presence of 1 μM unlabelled etorphine. (c) Specific [^{3}H]-PD117302 binding in guinea-pig brain. Using a series of ^{3}H-labelled brain paste standards (see Methods), [^{3}H]-PD117302 binding is quantified and the specific binding obtained by digital subtraction of the non-specific binding from the total binding. Scale bar = 1 mm.

Table 4 A comparison of the potency of PD117302 with several opioid agonists in the electrically-stimulated of the guinea-pig ileum

Compound	IC_{50} (nM)	*Naloxone* pA_2
PD117302	1.07 ± 0.11 (41)	7.78 ± 0.09 (5)
U50488	1.63 ± 0.15 (20)	7.85 ± 0.13 (3)
EKC	0.46 ± 0.09 (5)	7.85 ± 0.05 (4)
Fentanyl	0.49 ± 0.04 (30)	8.38 ± 0.02 (3)
DAGOL	3.38 ± 0.17 (12)	8.63 ± 0.08 (4)

pA_2 values were determined according to the method of Arunklakshana & Schild (1959). Slopes of the individual Schild plots were not significantly different from unity.

selective compound U50488 and against ethylketocyclazocine (EKC).

Discussion

PD117302 is a novel non-peptide κ-opioid agonist which has been shown to possess potent, naloxone-reversible antinociceptive properties in the rat, guinea-pig and mouse (Clark *et al.*, 1987; Leighton *et al.*, 1987).

In the present study, [^{3}H]-PD117302-specific binding to guinea-pig forebrain membranes appeared to involve binding to both high and low affinity sites. However, it was apparent that, after competition studies with a number of unlabelled compounds, only the high affinity site was opioid in nature. The properties of the non-opioid low affinity site will be described elsewhere.

The close correlation between the affinities of several opioid compounds for the [^{3}H]-PD117302 high-affinity binding site and the [^{3}H]-etorphine labelled κ binding site indicated that [^{3}H]-PD117302, at the concentrations investigated, was selectively binding to κ sites. The high κ selectivity of PD117302 was supported by the low affinity of the selective μ and δ ligands DAGOL and DADLE or DPDPE respectively, in competition with [^{3}H]-PD117302 for the high affinity site. In addition, unlabelled PD117302 had a low, approximately micromolar, affinity for the [^{3}H]-DAGOL labelled μ, the [^{3}H]-DADLE labelled δ (data not shown) and the [^{3}H]-SKF 10047 labelled σ sites.

The advantage, therefore, of [^{3}H]-PD117302 over most of the currently available radioligands used to label κ binding sites such as [^{3}H]-etorphine and [^{3}H]-bremazocine (Fischel & Medzihradsky, 1985; Mansour *et al.*, 1986), was that, with a suitably low concentration of [^{3}H]-PD117302, no selective suppression of μ and δ sites was necessary and thus κ binding sites could be labelled with minimal cross-reactivity with other types of opioid receptor. One interesting feature of [^{3}H]-PD117302 labelled κ binding sites was that the B_{max} was found to be less than half the capacity of either [^{3}H]-etorphine or [^{3}H]-bremazocine labelled sites. This observation is similar to that found for [^{3}H]-U69593 (Lahti *et al.*, 1985, Hughes *et al.*, unpublished observations), and suggests that both [^{3}H]-PD117302 and [^{3}H]-U69593 selectively label a subtype of κ receptor.

The quantitative autoradiographic data showed that [^{3}H]-PD117302 binding sites were heterogeneously distributed in the guinea-pig central and peripheral nervous system with a similar pattern to that expected for a κ-selective compound. Thus, the highest density of binding sites was found in lamina V and VI of the cerebral cortex, the striatum and in the molecular layers of the hippocampus and cerebellum. This profile compares closely with quantitative observations obtained from autoradiographic studies using [^{3}H]-bremazocine and [^{3}H]-EKC to label κ binding sites (Foote & Maurer, 1982; Goodman & Snyder, 1982; Robson *et al.*, 1984) and also previous quantitative data obtained using [^{3}H]-etorphine and [^{125}I]-dynorphin (1–8) (Sharif *et al.*, 1986).

The data from the *in vitro* isolated tissue preparations further demonstrated the κ selectivity of PD117302. In the guinea-pig ileum and rabbit vas deferens, the latter being a tissue thought to contain only κ receptors (Oka *et al.*, 1980), PD117302 produced a full inhibition of the nerve induced contractile response which was naloxone-reversible. In the guinea-pig ileum, a preparation shown to contain both κ and μ receptors (Hutchison *et al.*, 1975; Lord *et al.*, 1977), the pA_2 for naloxone against PD117302 was consistent with a PD117302 interaction at κ receptors (Hutchison *et al.*, 1975; Lord *et al.*, 1977).

In summary, we have studied the biochemical and physiological properties of PD117302 in the guinea-pig and conclude that it is an opioid compound possessing a high affinity and selectivity for the κ-opioid receptor.

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