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Pharmacological profile of PD 117302, a selective κ-opioid agonist

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1 PD 117302, a new nonpeptide opioid compound shown in *in vitro* studies to be a selective κ-opioid agonist, has been evaluted *in vivo* for antinociceptive activity and other effects characteristic of κ-receptor activation.

2 Dose-related long lasting antinociception was produced by PD 117302 against a mechanical noxious stimulus in rats following intravenous, subcutaneous or oral administration.

3 PD 117302 was effective in raising the nociceptive threshold to mechanical and chemical but not to thermal noxious stimuli in the mouse. This effect was attenuated in animals pretreated with the opioid antagonist naloxone.

4 In addition to producing antinociception, PD 117302 also caused naloxone-reversible locomotor impairment and diuresis, effects that are typical of κ-agonists.

5 PD 117302 did not cause respiratory depression, inhibition of gastrointestinal motility or naloxone-precipated withdrawal jumping in mice, effects that are associated with actions at the μ-opioid receptor.

6 The pharmacological profile of PD 117302 *in vivo* is consistent with *in vitro* data suggesting that PD 117302 is a selective agonist at the κ-opioid receptor.

Introduction

Although many of the currently available opioid analgesics are potent and effective they are associated with undesirable and sometimes limiting side effects including respiratory depression (Popio *et al.*, 1978), constipation (Stewart *et al.*, 1978; Schulz *et al.*, 1979) and dependence liability (Martin *et al.*, 1965; Martin & Sloan, 1977). There is evidence that these effects are particularly associated with occupation of the μ-opioid receptor (Martin, 1984). It would be most desirable if an analgesic of comparable efficacy to the currently available opioid drugs could be developed that was devoid of these unwanted side effects.

Many authors have demonstrated that compounds with affinity for the κ-opioid receptor are capable of producing antinociception in animal models (Tyers, 1980; Freye *et al.*, 1983; Ward & Takemori, 1983, Steinfels & Cook, 1986) and the partial κ-agonist nalorphine has been shown to produce analgesia in man (Lasagna & Beecher, 1954). κ-Agonists seem to be devoid of constipating effects and unlike morphine, produce only a very mild withdrawal response in animals following cessation of long term treatment (Gmerek & Woods, 1986). Although κ-agonists do produce some respiratory depression this effect is much less marked than that produced by μ-agonists and a ceiling effect is observed as doses are increased (Gilbert & Martin, 1976; Martin *et al.*, 1976).

We have investigated the antinociceptive properties of a novel, selective κ-receptor agonist, PD 117302 (Clark *et al.*, 1987b) in a number of animal models. PD 117302 has also been evaluated in tests that may be predictive of side effects such as respiratory depression, constipating effects, dependence liability, sedation and diuresis. The properties of PD 117302 are compared with those of the κ-agonists U50488 and ethylketocyclazocine and the μ-agonist morphine.

Although κ-agonists appear to exhibit a different profile of antinociceptive activity from μ-agonists (Tyers, 1980) this does not always hold true. For example buprenorphine appears from such tests to act as a κ-agonist although it is in fact selective for the μ-receptor (Takemori *et al.*, 1986). It is therefore necessary to complement behavioural data with *in vitro* experiments to confirm that antinociception is κ-receptor-mediated. *In vitro* data for PD 117302 is included in a companion paper (Clark *et al.*, 1987a).

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Some of these results were presented in preliminary form to a meeting of the British Pharmacological Society (Leighton *et al.*, 1987).

Methods

The methods used to evaluate antinociceptive activity were selected to include tests which employed different types of noxious stimuli i.e. thermal, chemical and mechanical. Tests were performed on rats (70–100 g, males, Wistar, Interfauna, Huntingdon, UK) or mice (20–25 g, males, CFLP, Interfauna). All animals were housed at a constant temperature (21 ± 1°C) and humidity and maintained on a 12 h dark: 12 h light cycle. Standard laboratory chow (Labsure) and water were available *ad libitum* except for an overnight period before oral dosing when food was withdrawn. Individual tests were carried out with dose-groups of six animals and all analgesia testing was performed between 13 h 00 min and 17 h 00 min. All animals and drug solutions were coded and decoded with custom designed software run on a BBC microcomputer (Cairnduff *et al.*, 1985) such that operators were unaware of the treatments the animals were receiving. In each experiment all test compounds were compared with a reference compound, usually morphine, and with a saline control.

Oral dosing

Animals were dosed with a blunt ended enterogastric needle using a dose volume of 1 ml per 100 g for rats and 0.2 ml per 20 g for mice. Test compounds were dissolved in water or suspended in 0.25% w/v carboxymethylcellulose. All dosing was performed 60 min before analgesia testing.

Subcutaneous dosing

Compounds were dissolved in 0.9% NaCl w/v (saline) or suspended in 0.25% w/v carboxymethylcellulose. The dose volume used was 0.4 ml per 100 g for rats and 0.2 ml per 20 g for mice. Animals were subjected to analgesia testing 30 min after dosing.

Intravenous dosing

Animals were dosed by this route 5 min before testing with a dose volume of 0.1 ml per 100 g injected into a tail vein. All compounds were dissolved in water for injection BP.

The rat paw pressure test

The effects of test compounds on nociceptive pressure thresholds were determined in rats using an 'Analgesymeter' (Ugo Basile, Milan). The Analgesymeter was fitted with a single weight such that the maximum pressure exerted on the paw was 500 g. The pressure on the paw was increased at a rate of 32 g s^{-1} until a nociceptive response was seen. This nociceptive response was characterized either by a shrill vocalisation or by a vigorous attempt to withdraw the paw. The MPE_{50} dose was computed (using software as described above) as that required to produce a response midway between that seen in control animals and the maximum possible effect.

Acetylcholine-induced writhing in the mouse

Tests were performed to determine the inhibitory effects of test compounds against a chemically induced nociceptive response. The test used was the acetylcholine-induced writhing test. At a suitable interval after dosing with drugs or vehicle, treated mice were injected intraperitoneally with acetylcholine (0.25 mg per mouse). The number of writhes occuring within 5 min of acetylcholine injection was recorded. These writhes were characterized by a contraction of the abdominal muscles accompanied by an extension of the hind legs. The MPE_{50} value was defined as the dose capable of reducing by 50% the number of writhes compared to those occurring in the vehicle-treated mice.

Tail clip test in the mouse

This test was performed to determine the efficacy of test compounds against a pressure stimulus in the mouse. The test consisted of placing a 3.5 cm artery clip (Dieffenbach-Serrafine, Harvard) a distance of 3–4 cm from the tip of the tail. Any attempt made by the animal to remove the clip was taken as the nociceptive response. The clip was promptly removed at this point and the time taken to respond recorded. A cut off time of 40 s was used in this test to prevent tissue damage. The MPE_{50} was taken as the point on the dose-response curve corresponding to the dose required to produce a response equivalent to (40 + control)/2 s.

Hotplate test in the mouse

The effects of compounds on the reaction times of mice placed on a hot plate thermostatically maintained at 55°C were determined. The time at which mice displayed a nociceptive response, licking the front paws or fanning the hind paws, was recorded and the animal was removed from the hotplate at this time; 40 s was set as the cutoff time in this test to avoid damage to the paws. The MPE_{50} was taken as the dose required to increase the reaction latency to (control + 40)/2 s i.e. to produce a response midway between the control response and the cutoff time.

Determination of the time course of the antinociceptive effect

Rats were dosed orally or subcutaneously as described above and the nociceptive response to a mechanical stimulus (paw pressure) determined at one of the following time intervals; 30, 60, 120, 180, 240, 300 or 360 min after dosing. None of the rats used was tested more than once.

Respiratory depression

Male Wistar rats (70–100 g) were randomly assigned to treatment groups (6 per group) and then colour coded for identification. Animals were dosed subcutaneously according to the experimental code 30 min before testing. Respiratory rate was measured with a pneumotachygraph transducer system comprising an HSE model 0000 Fleisch tube connected to an HSE SP2040D differential pressure transducer. Respiratory flow was recorded via an HSE bridge amplifier and the signal from this used to trigger an HSE rate coupler. Each rat was placed in a 1 litre side armed conical flask (which served as a whole body plethysmograph) 5 min before testing. At the start of the 1 min test period the flask was connected to the Fleisch tube and the neck of the flask was closed to restrict air flow to that through the Fleisch tube. Results are expressed as the dose required to reduce the respiratory rate by 25% (MPE_{25}).

Measurement of urine output

Male Wistar rats (250–300 g) were housed individually in metabolism cages for the 6 h study period. These animals were in a normally hydrated state and were allowed free access to food and water during the experiment. Animals were injected subcutaneously with the test compound or with vehicle immediately before being placed in the metabolism cages. Urine from each animal was collected via a funnel into a graduated tube. The volume of urine produced was determined at 2 hourly intervals for a period of 6 h. A group of six animals per dose level was used.

Rotarod test

Mice were randomly allocated to treatment groups and colour coded as described for the antinociception testing. Drugs were also coded so that experiments were performed 'blind'. Thirty minutes after subcutaneous dosing mice were placed individually on an accelerating rotarod (Ugo Basile, Milan). The time taken for each mouse to fall off the rotarod was recorded.

Inhibition of gastrointestinal motility

Fasted mice were treated subcutaneously with test compound or vehicle. After 30 min they were dosed orally with a suspension of powdered charcoal in gum acacia (0.3 ml per mouse). Twenty minutes later the mice were killed and the distance that the charcoal meal had travelled down the intestine from the stomach was measured.

The distance travelled by the charcoal meal was calculated as a percentage of the length of the intestine between the stomach and the caecum. The MPE_{50} was taken as the dose required to reduce the distance travelled to 50% of that for the vehicle only treated animals.

Naloxone jumping test

Mice were randomly allocated into groups of 6 animals per cage. Each group of animals received subcutaneous injections of either test compound or vehicle using the following dosing schedule; day 1, 1 × dose (dose = 100 × antinociceptive MPE_{50} previously determined in the mouse acetylcholine writhing test); day 2, 1.5 × dose; day 3, 2.5 × dose; day 4, 4 × dose; day 5, 4 × dose. Animals were injected twice daily (9 h 00 min and 18 h 30 min). On day 5 animals received the morning injection only; 5 h later the animals were colour coded and randomized so that the observer was blind to the treatment received by each animal. These mice were challenged with a subcutaneous injection of naloxone (20 mg kg^{-1}) and then placed in a clear perspex tube. The number of stereotyped jumps occurring over a 5 min period following the naloxone injection was recorded.

Drugs

The following drugs were used: morphine sulphate (McCarthy), ethylketocyclazocine (Sterling-Winthrop), U50488 hydrochloride (*trans*-3,4-dichloro-N-methyl-N-(2 (1-pyrrolidinyl)cyclohexyl)-benzeneacetamide, synthesized at Parke-Davis), naloxone hydrochloride (Dupont), acetylcholine chloride (Sigma) and PD 117302 hydrochloride ((±)-*trans*-N-methyl-N[2-(1-pyrrolidinyl) -cyclohexyl]benzo [b] thiophene-4-acetamide, Parke-Davis). All doses quoted refer to the base.

Results

Antinociception in the rat

PD 117302 was found to produce antinociception in the rat as determined in the paw pressure test following administration by the intravenous, oral or subcutan-

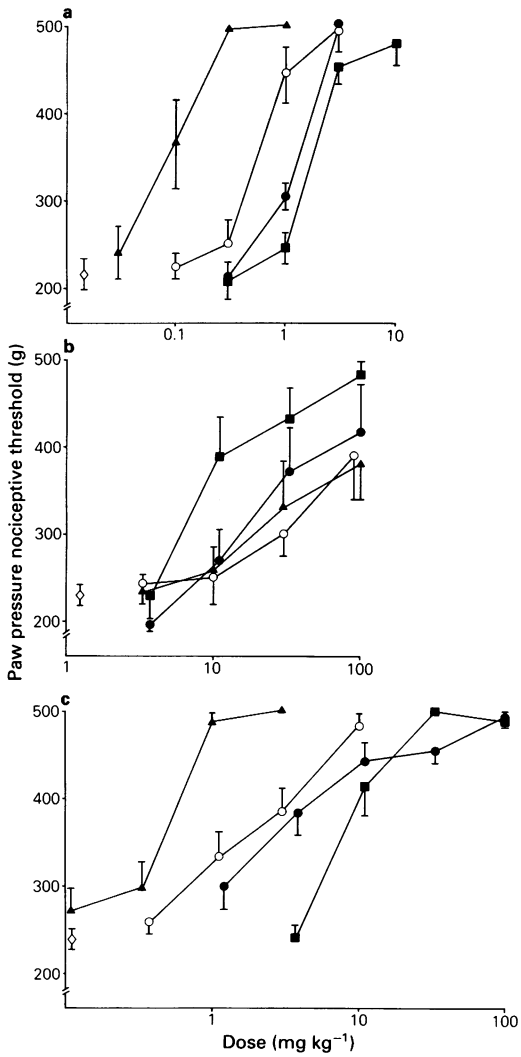

Figure 1 The effects of PD 117302 (●), U50488 (■), ethylketocyclazocine (▲) and morphine (○) on the nociceptive threshold in the rat paw pressure test following (a) intravenous, (b) oral or (c) subcutaneous administration; (◇) represents the response in a group of saline-treated animals. Values are shown as mean with s.e.mean indicated by vertical lines. $n = 6$ rats per dose level.

eous route. Results obtained in the intravenous test are shown in Figure 1a in comparison with similar dose-response curves to morphine, ethylketocyclazocine (EKC) and U50488. All four compounds produced steep parallel dose-response curves with relative potencies EKC > morphine > PD 117302 > U50488. Following oral administration all four compounds produced antinociception although EKC was

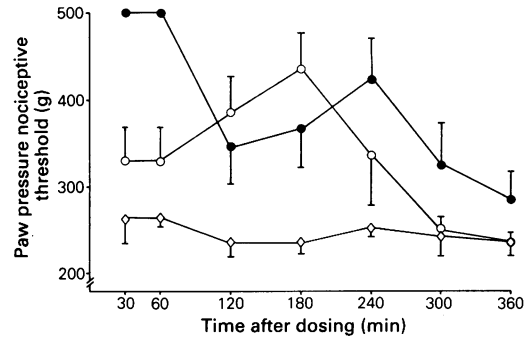

Figure 2 Time course of the antinociceptive effect seen following oral administration of PD 117302 (●), U50488 (■) or morphine (▲); (◇) represents responses seen in a group of saline-treated controls. $n = 6$ rats per time interval. Data are presented as means with s.e.mean indicated by vertical line.

poorly absorbed and very high doses relative to the effective intravenous dose were required. The order of potency following administration by this route was U50488 > PD 117302 > EKC > morphine; results are shown in Figure 1b.

Figure 1c shows the dose-response curves obtained with PD 117302, U50488, morphine and EKC following subcutaneous administration. The order of potency for administration via this route was the same as that following intravenous administration.

Results from the oral time course study, as shown in Figure 2, reflect the very rapid absorption of PD 117302 from this site of administration, peak antinociceptive effects being observed within the first 30 min after dosing. This antinociceptive effect was maintained above control levels for the duration of the study (6 h). The second peak in the antinociceptive effect, consistently seen at around 3 h after dosing, probably reflects reabsorption from the bile as pharmacokinetic studies (unpublished data) show that PD 117302 is rapidly excreted into the bile. Morphine is absorbed much more slowly following oral administration with the peak effect occurring 3 h after dosing. In the morphine-treated group, paw pressure responses had returned to control levels by 5 h after dosing. Results of the time course of effect following subcutaneous administration are also shown (Figure 3) demonstrating the longer duration of action of PD 117302 compared to U50488.

Antinociception in the mouse

As can be seen from the results presented in Table 1, morphine is effective in raising the nociceptive threshold to chemical, mechanical and thermal noxious stimuli. PD 117302 together with U50488 is active in

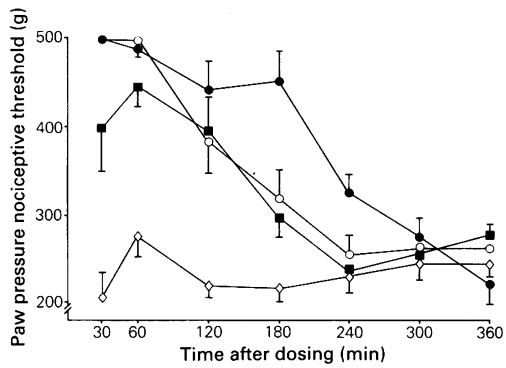

Figure 3 Time course of the antinociceptive effect seen following subcutaneous administration of PD 117302 (●), U50488 (■) or morphine (○); (◇) represents saline-treated controls. $n = 6$ rats per time interval. Data are presented as mean with s.e.mean indicated by vertical lines.

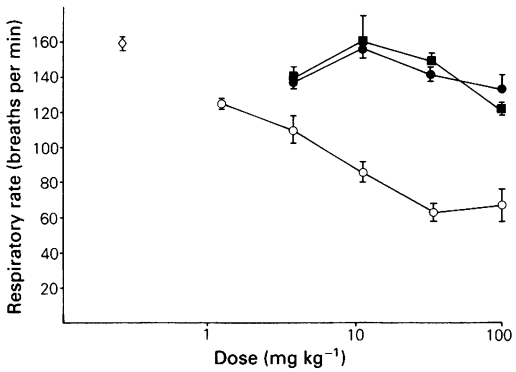

Figure 4 The effects of subcutaneously administered PD 117302 (●), U50488 (■) and morphine (○) on respiratory rate in the conscious rat; (◇) represents the saline-treated control group. Results are shown as mean with s.e.mean indicated by vertical lines. $n = 6$ rats per group.

mechanical or chemical nociceptive tests but is virtually inactive against a thermal stimulus. EKC was also inactive at the doses tested (up to 3.0 mg kg^{-1}) in this hotplate test. The antinociceptive effects of PD 117302 in the tail clip test were shown to be attenuated by naloxone pretreatment (15 min pretreatment with 10 mg kg^{-1}, s.c naloxone. Subsequent experiments confirmed that this effect could also be reduced by pretreating animals with lower doses of naloxone).

Table 1 The effects of PD 117302, U50488, ethylketocyclazocine (EKC) and morphine in the mouse tailclip, hotplate and acetylcholine writhing tests

		MPE_{50} (mg kg^{-1})		
	n	*Tail clip*	*Hotplate*	*Writhing*
Morphine	3	1.7 ± 0.7	7.3 ± 1.3	0.3 ± 0.1
EKC	1	NT	> 3.0	0.2
U50488	4	6.4 ± 3.6	> 100	1.0 ± 0.1
PD 117302	6	2.2 ± 0.6	> 100	0.8 ± 0.1
PD 117302 + naloxone	1	29.2	NT	1.8

NT = not tested
Results are shown as mean MPE_{50} values (see text) ± s.e.mean. n = number of separate experiments; in each experiment 6 mice were used per dose level.

Respiratory depression

The depression of respiratory rate produced by morphine is shown in Figure 4. Using the protocol described above the subcutaneous MPE_{25} was determined as 1.9 mg kg^{-1}. Doses of PD 117302 and U50488 up to 100 mg kg^{-1} (s.c.) did not depress respiratory rate to the MPE_{25} level.

Rotarod performance

PD 117302, U50488, EKC and morphine all produced sedation (locomotor incapacitation) as determined by the ability of mice to maintain their position on an accelerating rotarod (Figure 5a). Following subcutaneous administration, EKC was shown to be more potent than PD 117302 which in turn was more potent than U50488. The ratio of the sedative dose (MPE_{50}) to antinociceptive dose (MPE_{50}, mouse writhing test) was found to be much greater for morphine (ratio = 50) than for the κ-agonists tested (ratio was approximately 5 for PD 117302, U50488 and EKC). These results indicate that there is a smaller separation between the antinociceptive and sedative doses of κ-agonists than there is for the μ-agonist morphine.

This sedative effect of PD 117302 was attenuated in animals pretreated with naloxone as shown in Figure 5b, confirming that it is an opioid receptor-mediated effect.

Diuretic effects

The effects of PD 117302 were compared with those of the κ-agonist U50488. Results are shown in Figure 6. The diuresis seen with PD 117302 and U50488 was of short duration, the rate of urine production being maximal during the first 2 h measurement interval.

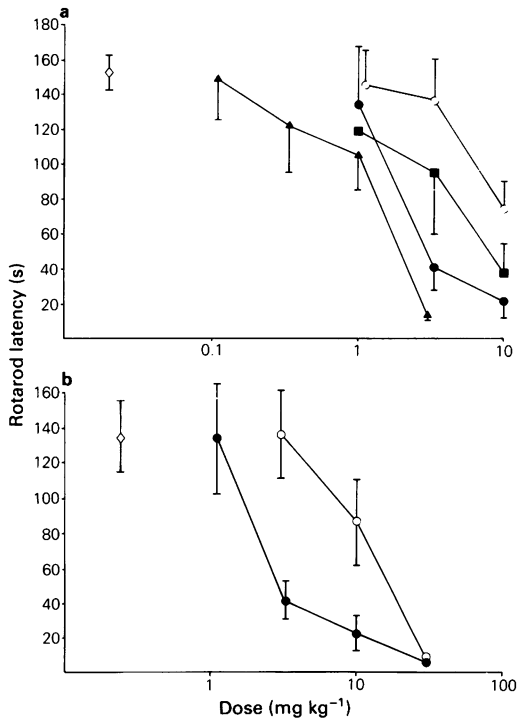

Figure 5 (a) The effects of PD 117302 (●), U50488 (■), ethylketocyclazocine (▲) and morphine (○) on rotarod latency in the mouse; (◇) represents the saline-treated control group. (b) The effects of naloxone (10 mg kg^{-1}, s.c. 15 min pretreatment time) on the reduction in rotarod latency produced by PD 117302; (●) saline pretreatment + PD 117302; (○) naloxone pretreatment + PD 117302. Data are presented as mean with s.e.mean indicated by vertical lines. $n = 6$ mice per group.

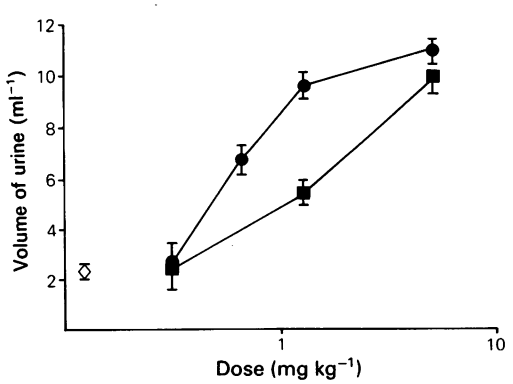

Figure 6 The effects of PD 117302 (●) and U50488 (■) on 6 h urine output following subcutaneous administration in the rat. $n = 6$ rats per dose level. Results are shown as mean with s.e.mean shown by vertical lines.

Table 2 The response of mice to naloxone challenge following 5 days of twice daily treatment with PD 117302, U50488, ethylketocyclazocine (EKC) or morphine

*Treatment**	*Number of jumps per animal* (× ± s.e.mean)	*Number of animals jumping from a group of 6*
PD 117302 (40–100 mg)	0	0
U50488 (40–100 mg)	0	0
EKC (10–40 mg)	1 ± 1	1
Morphine (40–160 mg)	29 ± 7	6
Saline	0	0

*All treatments were given subcutaneously using the dosing schedule described in the text.

Dependence inducing liability

The number of naloxone-precipitated jumps seen following 5 days treatment with PD 117302, U50488, EKC or morphine are shown in Table 2. Animals treated with morphine consistently showed a high number of jumps following naloxone challenge. No jumps were seen following treatment with PD 117302 or U50488 and only one mouse of a group of six jumped after 5 days treatment with EKC followed by naloxone-precipitated withdrawal.

Discussion

PD 117302 is a highly selective κ-receptor agonist (Clark *et al.*, 1987a) and displays the typical profile *in vivo* of such a class of compound. It is active in tests for antinociception in three different species, rat, mouse, and rhesus monkey (lowest effective dose = 0.3 mg kg^{-1} (s.c.) in the tail withdrawal test, Dykstra & Woods unpublished observations) and results obtained in the rat following oral administration indicate rapid absorption when given by this route. The same cannot be said of the prototypic κ-receptor agonist EKC which shows relatively poor oral bioavailability. When evaluated in antinociception tests employing differing nociceptive stimuli i.e. mechanical, thermal or chemical stimuli, PD 117302 was effective in increasing the nociceptive threshold to a mechanical and a chemical stimulus but was much less active against a thermal stimulus. This property is characteristic of compounds producing antinociception via an action at the κ-opioid receptor (Tyers, 1980) although this profile of activity is not associated exclusively with κ-agonists. PD 117302 has also been

evaluated in animal models for predicting possible side effects. The results indicate that although PD 117302 does not produce an inhibition of gastrointestinal motility, withdrawal jumping in mice or the significant degree of respiratory depression that is seen with morphine, it does display the diuretic and sedative effects in animals that have been described for other, less selective, κ-opioids (Slizgi & Ludens, 1982; Leander, 1983; Skingle *et al.*, 1985; Ukai & Kameyama, 1985).

The finding that the profile of activity seen with PD 117302 is very similar to that reported for bremazocine and other benzomorphan κ-agonists is as expected in view of the very similar pattern of distribution of binding sites in the brain obtained for [^{3}H]-PD 117302 using quantitative autoradiographic techniques (Clark *et al.*, 1987a) compared to results reported for [^{3}H]-bremazocine or [^{3}H]-EKC (Foote & Maurer, 1982; Goodman & Snyder, 1982; Robson *et al.*, 1984). The association of specific *in vivo* effects of PD 117302 with regions of the brain that show high levels of κ-receptor binding sites can only be speculative unless supported by more detailed behavioural studies following, for example, lesions of discrete brain regions or localised intracerebral injections of PD 117302, although it has been suggested on the basis of autoradiographic data alone that κ-receptors localised in the deep layers of the cerebral cortex are associated with the sedative effects of this class of compounds (Goodman & Snyder, 1982).

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