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Pharmacological activities of optically pure enantiomers of the κ opioid agonist, U50,488, and its cis diastereomer: evidence for three κ receptor subtypes

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De Costa et al. (FEBS Lett. 223, 335; 1987) recently described the synthesis of optically pure enantiomers of (±)-trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (U50,488). In the present study we examined the in vitro opioid receptor selectivity of (−)-(1S,2S)-U50,488, (+)-(1R,2R)-U50,488 and (±)-cis-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (the cis diasteromers of U50,488), as well as their pharmacological activities in rhesus monkeys. Using [^{3}H]5α,7α,8β-(−)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro (4,5)dec-8-yl]-phenyl-benzeneacetamide ([^{3}H]U69,593) to label κ binding sites of guinea pig membranes, the apparent dissociation constants of the enantiomers of U50,488 were 0.89 and 299 nM, for the (S,S) and (R,R) enantiomers, respectively. The (−)-cis and (+)-cis diastereomers had apparent K_ds of 167 and 2715 nM, respectively. Binding surface analysis of the interaction of (−)-(1S,2S)-U50,488 with κ binding sites labeled by [^{3}H]bremazocine resolved two binding sites at which (−)-(1S,2S)-U50,488 had K_ds of 30 and 10485 nM, respectively. The (±)-cis, (−)-cis and (+)-cis diastereomers of U50,488 (1 μM) did not inhibit [^{3}H]bremazocine binding. Rhesus monkeys were trained to discriminate ethylketocyclazocine (EKC) and saline. All compounds tested substituted completely for EKC. The order of potency was (−)-(1S,2S)-U50,488 > (±)-U50,488 > (±)-cis diastereomer of U50,488 > (+)-(1R,2R)-U50,488. In tests of analgesia, (−)-(1S,2S)-U50,488 was 2-4 times more potent than (±)-U50,488, while (±)-cis diastereomer of U50,488 and (+)-(1R,2R)-U50,488 were inactive at the highest doses tested (32 mg/kg). Taken collectively, these data indicate that the pharmacologically active enantiomer of U50,488 is (−)-(1S,2S)-U50,488, and provide preliminary evidence for three subtypes of κ binding sites in guinea pig brain.

Opiate receptors; κ Opioid receptors; U-50,488; U-69,593; (Enantiomers, cis diastereomer of U-50,488)

1. Introduction

(±)-Trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (U50,488) is a highly selective agonist at the κ opioid receptor (Von Voigtlander et al., 1983). The pharmacological profile of κ opioid receptor agonists includes activity as diuretics (Leander, 1983), as anticonvulsants (Von Voigtlander and Lewis, 1988), as analgesics (Von Voigtlander et al., 1983), and perhaps as mu receptor antagonists (Porreca and Tortella, 1987; Sheldon et al., 1987). Other data suggest that κ opioid agonists in man are psychotomimetic (Pfeiffer et al., 1986), and that κ agonists may be beneficial in the treatment of acute brain and spinal cord injury (Hall et al., 1987). Most studies employing U50,488 have used

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the racemate. The relative contributions of the two enantiomers to the observed pharmacological effects is unknown, and complicates the interpretation of results, especially since it is well known that enantiomers often produce different and sometimes opposite effects (Ariëns, 1987; 1986). For this reason De Costa et al. (1987) synthesized optically pure enantiomers of U50,488.

In our studies we define κ binding sites as the residual binding sites remaining after suppression of μ and δ binding sites (Kosterlitz et al., 1981). Initial binding experiments using [^{3}H](−)-bremazocine ([^{3}H]BRM) to label κ binding sites of guinea pig brain indicated that (−)-(1S,2S)-U50,488 was much more potent than (+)-(1R,2R)-U50,488 in displacing binding (De Costa et al., 1987). In a more detailed study, this was confirmed using [^{3}H]5α,7α,8β-(−)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]phenylbenzeneacetamide ([^{3}H]U69,593) to label κ binding sites (Lahti et al., 1985; Rothman et al., 1988b). In this paper we examine the interaction of (±)-cis-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (abbreviated (±)-cis-I) and its enantiomers (abbreviated (+)-cis-I and (−)-cis-I) with κ binding sites labeled by [^{3}H]U69,593 and [^{3}H]BRM. The pharmacological activity of (±)-cis-I and the two enantiomers trans-U50,488 were also studied in rhesus monkeys using a drug discrimination paradigm and a test for analgesia. The results demonstrate that in tests of analgesia and drug discrimination, (−)-(1S,2S)-U50,488 is more potent than (+)-(1R,2R)-U50,488, and further, although (±)-cis-I substitutes for ethylketocyclazocine (EKC) in the rhesus monkey, it is not an antinociceptive agent.

2. Materials and methods

2.1. Pharmacological studies

2.1.1. Subjects

Four male and two female rhesus monkeys (*Macaca mulatta*) weighing between 6 and 10 kg were housed individually and allowed free access to water. The two male monkeys in the drug discrimination experiment were maintained at 85% of their free-feeding weights by regulating the number of Purina Monkey Chow biscuits they received daily. The four monkeys in the tail withdrawal experiments were allowed free access to food in the home cage. All monkeys received fresh fruit and vitamins occasionally.

2.1.2. Drug discrimination procedure

Drug discrimination experiments were conducted in sound-attenuated, ventilated isolation chambers equipped with two response levers located on either side of a food receptacle and within easy reach of a seated monkey. An array of stimulus lights was located directly above the response levers and a dispenser that could deliver food pellets was located outside the chamber.

Monkeys were seated in Plexiglas primate chairs during daily experimental sessions that lasted 2 h or less. Both monkeys had been trained to discriminate between s.c. injections of EKC (0.0032 mg/kg) and saline. The multiple-cycle training and testing procedures used in the present study have been described elsewhere (Bertalimo et al., 1982). Training sessions consisted of between two and six discrete 15 min cycles; each cycle consisted of a 10 min time-out period during which the chamber was dark and lever presses had no programmed consequence, and a 5 min response period during which the stimulus lights were illuminated and 100 consecutive responses on the injection-appropriate lever (left lever, EKC-appropriate; right lever, saline-appropriate) resulted in delivery of 10 Noyes 300 mg banana-flavored pellets. When 100 correct responses were made within 5 min, the stimulus lights were extinguished for the remainder of the response period. On some training days a single injection of EKC was administered prior to one of the cycles and that cycle was followed by a single non-injection (sham) cycle during which only responding on the EKC-appropriate lever produced food. The number of saline injection cycles that preceded the EKC injection cycle varied across days and on some training days sham or saline was administered prior to all cycles.

Test sessions were identical to training sessions except that 100 consecutive responses on either level produced 10 food pellets, saline always was

administered prior to the first cycle, and drug was administered prior to subsequent cycles. Increasing doses of drug were administered so that the cumulative dose increased by 1/2 or 1/4 log unit per cycle (Bertalimo et al., 1982).

2.1.3. Tail withdrawal procedure and apparatus

The tail withdrawal procedure used in the present study to measure antinociception was similar to that described previously (Dykstra and Woods, 1986). During weekly 3 h sessions, monkeys were restrained loosely at the neck and arms while seated in Plexiglas primate chairs. For tests of tail withdrawal latency, the lower 10-12 cm of the shaved tail was immersed in a thermos containing water at 40, 50 or 55 °C. The latency until the tail was withdrawn from the thermos was recorded for each monkey at each temperature. If the tail was not withdrawn within 20 s (cut-off latency) the experimenter removed the tail from the thermos and a latency of 20 s was recorded.

Experimental sessions began with several exposures to 40 °C water. Four monkeys were tested consecutively and the time between immersions for individual monkeys was 5 min. It has been reported that 40 °C water does not produce tail withdrawal in rhesus monkeys (Dykstra and Woods, 1986; Dykstra et al., 1987) and in the present study monkeys generally did not withdrawn their tails from 40 °C water. However, if a monkey failed to keep its tail in 40 °C water for 20 s on at least three of four immersions, that animal was not tested further. In the subsequent pre-test component tails were immersed in 40, 50 and 55 °C water. The order in which the three temperatures were presented varied among subjects. If the latencies for tail withdrawal in the pre-test component were at or near 20 s for 40 °C water and less than 5 s for 55 °C water, monkeys received the test compound.

The test was identical to the pre-test, except that monkeys received s.c. injections of drug 10 min prior to tail immersion. The time between immersions for individual subjects was 5 min and the order in which temperatures were presented varied among subjects and across cycles. The inter-injection interval was 30 min and a maximum of four doses were studied in a single session (i.e. 2 h total time). The cumulative dosing procedure was similar to that described above.

2.2. Receptor binding assays

Guinea pig brain membranes were pretreated with the site directed acylating agents 2-(4-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanatobenzimidazole · HCl (BIT) and N-phenyl-N-[1-(2-(4-isothiocyanato)phenethyl)-4-piperidinyl]-propanamide · HCl (FIT) to deplete the membranes of μ and δ receptors (Rothman et al., 1985). κ Binding sites were labeled with both [^{3}H]U69,593 and [^{3}H]BRM. The [^{3}H]U69,593 binding assay proceeded as previously described (Rothman et al., 1988b). Briefly, incubations were for 60 min at 37 °C in 50 mM Tris-HCl, pH 7.4, containing 3 mM $MnCl_2$, 0.1 mg/ml bovine serum albumin, and a protease inhibitor cocktail containing bacitracin (100 μg/ml), bestatin (10 μg/ml), leupetin (4 μg/ml), chymostatin (2 μg/ml) and captopril (1 μg/ml). Non-specific binding was defined using 1 μM U69,593. The [^{3}H]BRM binding assay proceeded according to published protocols (Rothman et al., 1985), except that the assay was conducted without NaCl. Non-specific binding was defined using 1 μM (−)-bremazocine, [^{3}H][D-Ala^2,D-Leu^5]enkephalin ([^{3}H]DADL) and [^{3}H]6β-fluoro-6-desoxy-oxymorphone ([^{3}H]FOXY) binding assays were conducted, using rat brain membranes, as previously described (Rothman et al., 1988a,c). In these assays, the non-specific binding was defined using 10 μM levallorphan. Proteins were measured as described (Lowry et al., 1951).

Binding surfaces were generated and analyzed as previously described (Rothman et al., 1988d; Rothman, 1986). Briefly, two concentrations of ^{3}H ligand were each displaced by varying concentrations of test drugs. The combined data were fit to either a one site or two site binding model using MLAB (Knott et al., 1972) for the best-fit parameter estimates. MLAB fits the data according to a weighted non-linear least squares curve fitting algorithm.

2.3. Chemicals

[^{3}H]FOXY (SA = 53 Ci/mmol), [^{3}H]DADL (SA = 46.9 Ci/mmol) and [^{3}H](−)-bremazocine

(specific activity 21.3 Ci/mmol) were supplied by New England Nuclear Corp. [^{3}H]U69,593 (specific activity 40 Ci/mmol) was purchased from Amersham Corp. The enantiomers of U50,488 were synthesized as described previously (De Costa et al., 1987). The sources of the other chemicals can be found in previous publications (Rothman et al., 1988c).

3. Results

3.1. Drug discrimination studies

Monkeys trained to discriminate between s.c. injections of 0.0032 mg/kg EKC and saline generalized to EKC in a dose-related manner with the smallest dose of EKC that produced complete generalization being 0.0018 mg/kg (data not shown). (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488, (±)-U50,488 and (±)-cis-I substituted completely for the EKC discriminative stimulus in that all four drugs produced responding exclusively in the EKC appropriate lever (fig. 1, left panel). The order of potency as discriminative stimuli was (−)-(1S,2S)-U50,488 > (±)-U50,488 > (±)-cis-I > (+)-(1R,2R)-U50,488. At doses that produced EKC-appropriate responding, none of the drugs altered rates of lever pressing (data not shown). There was insufficient quantities of (+)-cis-I and (−)-cis-I for in vivo testing.

3.2. Tail withdrawal

The latency for tail withdrawal from 40, 50 and 55°C water was temperature-dependent. The average latencies for tail withdrawal during pre-test components was 17.7, 1.7 and 1.3 s for 40, 50 and 55°C water, respectively. Because the average latencies obtained with 40°C water were > 17 s during all pre-test components as well as during all test components, data with 40°C water are not

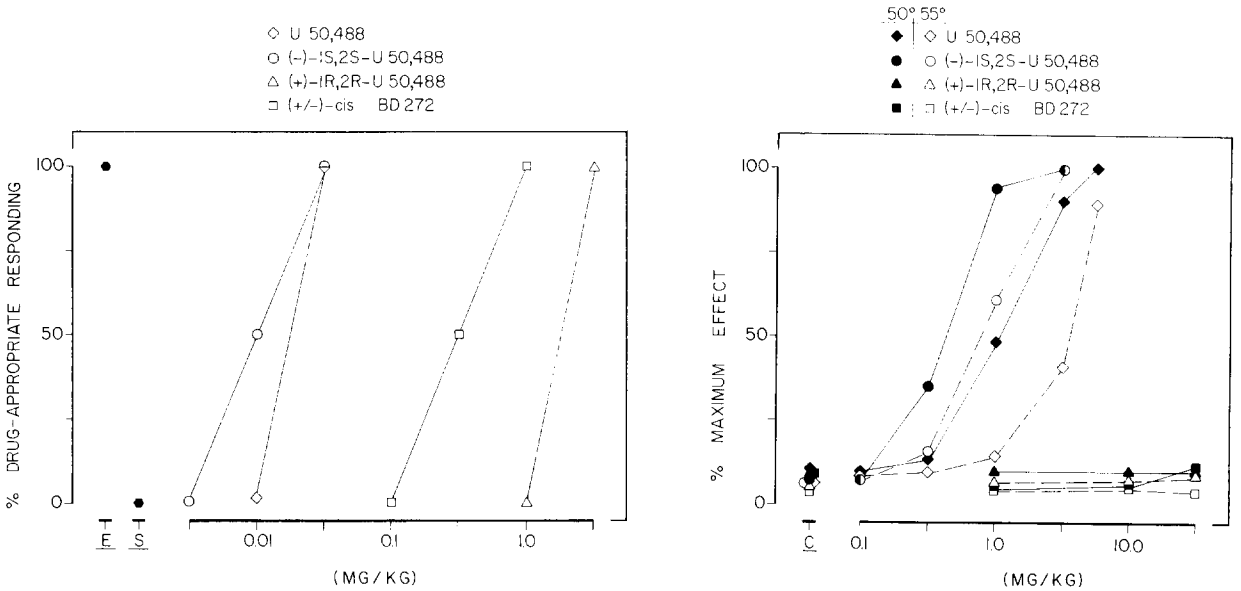

Fig. 1. Discriminative stimulus effects (left panel) and effects on tail withdrawal latencies (right panel) of U50,488 (diamonds), (−)-(1S,2S)-U50,488 (circles), (+)-1R,2R-U50,488 (triangles) and (±)-cis-I (BD272) (squares). Ordinates: left panel, average percentage of responses on the ethylketocyclazocine (EKC) lever for two monkeys; right panel, average latency for tail withdrawal from 50°C (closed symbols) and 55°C (open symbols) water for four monkeys, expressed as a percentage of the maximum possible latency (i.e. 20 s). Abscissas: both panels, dose in mg/kg body weight. Points above E and S in the left panel represent discriminative stimulus effects of the training dose of EKC (0.0032 mg/kg) and saline, respectively. Points above C in the right panel represent the average latency for tail withdrawal from 50°C (closed symbols) or 55°C (open symbols) water prior to the administration of drug (pre-test component).

shown. (±)-U50,488 and (−)-(1S,2S)-U50,488 produced dose-related increases in tail withdrawal latencies from 50 and 55°C water (fig. 1, right panel). (−)-(1S,2S)-U50,488 was 2-4 times more potent than (±)-U50,488 and both compounds were more potent as antinociceptive agents with 50°C water than with 55°C water. (+)-(1R,2R)-U50,488 and (±)-cis-I, up to a dose of 32.0 mg/kg, failed to increase tail withdrawal latencies above pre-test values (triangles and squares, respectively, right panel, fig. 1). Limitations on the solubility of (+)-(1R,2R)-U50,488 and (±)-cis-I precluded administration of doses larger than 32.0 mg/kg.

3.3. Ligand binding data

The relatively potent ability of (±)-cis-I to substitute for EKC in the drug discrimination studies (ED_{50} about 0.5 mg/kg) and its virtual inactivity in the test for antinociception, led us to examine the interaction of (±)-cis-I and its enantiomers with κ binding sites labeled by [^{3}H]BRM and [^{3}H]U69,593.

Binding surface analysis of the interaction of (−)-(1S,2S)-U50,488, (+)-(1R,2R)-U50,488 and (+)-tifluadom with [^{3}H]BRM binding revealed the existence of two binding sites distinguished by the inhibitory potency of (−)-(1S,2S)-U50,488 (fig. 2, table 1). Whereas BRM had similar K_ds for both sites (0.46 and 0.81 nM), (−)-(1S,2S)-U50,488 had K_ds of 30.2 and 10485 nM for site 1 and site 2, respectively. (+)-(1R,2R)-U50,488 bound to both sites with very low affinity. (+)-Tifluadom had K_ds of 0.95 and 113 nM for site 1 and 2,

TABLE 1

Best-fit parameter estimates of the two site model. The binding surfaces reported in fig. 2 (78 data points) were fit to the two site competitive model. The best-fit parameter estimates are reported above, and generated the lines in fig. 2. The sum-of-squares was 0.152. When the data were fit to a one site model, the sum-of-squares was 0.779, which was significantly worse ($P < 0.001$). The binding parameters of (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488 were used to predict the displacement which would be observed for (±)-U50,488. As shown in in fig. 2C, the lines generated by these binding parameters fit the observed data well. Each parameter value is ±S.D.

Parameter	Site 1	Site 2
B_{max} (fmol/mg protein)	172±17	221±22
Bremazocine (K_d, nM)	0.46±0.10	0.81±0.16
(−)-(1S,2S)-U50,488 (K_d, nM)	30.2±9.0	10485±2831
(+)-(1R,2R)-U50,488 (K_d, nM)	14568±4785	4340±1196
(+)-Tifluadom (K_d, nM)	0.95±0.31	112±27

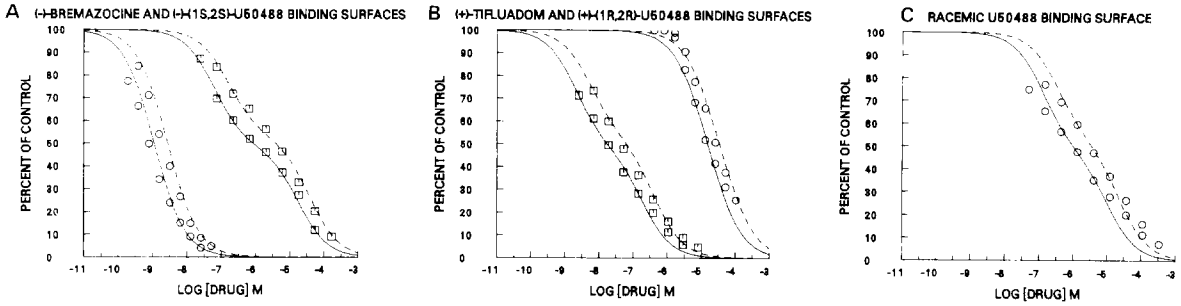

Fig. 2. (A) Two concentrations of [^{3}H](−)-bremazocine (0.52 and 2.1 nM) were each displaced by eight concentrations of (−)-bremazocine (circles) and (−)-(1S,2S)-U50,488 (squares). The solid and dashed lines are those predicted by the best-fit parameter estimates for the 0.52 and 2.1 nM displacement curves (table 1). Each point is the mean of two determinations which differed by less than 10%. (B) Two concentrations of [^{3}H](−)-bremazocine (0.52 and 2.1 nM) were each displaced by eight concentrations of (+)-(1R,2R)-U50,488 (circles) and (+)-tifluadom (squares). The solid and dashed lines are those predicted by the best-fit parameter estimates for the 0.52 and 2.1 nM displacement curves (table 1). Each point is the mean of two determinations which differed by less than 10%. (C) Two concentrations of [^{3}H]bremazocine were each displaced by eight concentrations of (±)-U50,488. The binding parameters of (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488 were used to generate the lines in the figure. Each point is the mean of two determinations which differed by less than 10%.

TABLE 2

Interaction of (±)-cis-I and its enantiomers with μ, δ and κ binding sites. (A) The ability of test drugs at a concentration of 1 μM to inhibit the binding of [^{3}H]BRM (2.6 nM), [^{3}H]DADL (2.1 nM) and [^{3}H]FOXY (2.4 nM) to rat brain membranes is reported. The inhibitory potency (±)-cis-I (1 μM) was also determined using [^{3}H]BRM to label κ binding sites of guinea pig brain membranes. Each value is the mean of two determinations which differed by less than 5%. (B) As described in Methods, [^{3}H]U69,593 (2.2 nM) was displaced by the enantiomers of (±)-cis-I. The displacement curves were fit to the two parameter logistic equation (Rodbard et al., 1976) for the IC_{50}, and the K_is calculated using the equation: $K_i = IC_{50}/(1+l/K_d)$. [a] The K_is on the enantiomers of U50,488 reported in another publication (Rothman et al., 1988b) are included to facilitate comparison of the results.

(A) Drug (1 μM)	Percent of control			
	[^{3}H]BRM (guinea pig)	[^{3}H]BRM (rat)	[^{3}H]DADL (rat)	[^{3}H]FOXY (rat)
(±)-Cis-I	108	95	94	94
(−)-(1S,2S)-U50,488	N.D.	83	73	64
(+)-(1R,2R)-U50,488	N.D.	88	89	61
(B) Drug	**K_i (nM)**			
(−)-Cis-I	167 ± 4			
(+)-Cis-I	2715 ± 130			
(−)-(1S,2S)-U50,488 [a]	0.89 ± 0.05			
(+)-(1R,2R)-U50,488 [a]	299 ± 16			

respectively. (±)-U50,488 was a weak displacer of [^{3}H]BRM binding (fig. 2C). The binding parameters obtained for (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488 accurately predicted the (±)-U50,488 binding surface. (±)-Cis-I, at concentrations of 1 μM, failed to significantly inhibit [^{3}H]BRM binding (table 2A) using brain membranes prepared from either guinea pig or rat. (+)-Cis-1 and (−)-cis-I at 1 μM also failed to inhibit [^{3}H]BRM binding to guinea pig brain membranes (data not shown).

Other experiments tested the hypothesis that (±)-cis-I might interact at μ or δ binding sites (table 2A). At a concentration of 1 μM (±)-cis-I did not significantly inhibit either [^{3}H]FOXY or [^{3}H]DADL binding. At 1 μM, both (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488 weakly inhibited [^{3}H]DADL binding, fairly potently inhibited (approximately 40%) [^{3}H]FOXY binding, and consistent with the data reported in fig. 2, weakly inhibited [^{3}H]BRM binding.

In contrast, initial experiments indicated that 1 μM (±)-cis-I inhibited [^{3}H]U69,593 binding to guinea pig brain membranes. We therefore examined the interaction of (−)-cis-I and (+)-cis-I with [^{3}H]U69,593 binding. As reported in table 2B, these agents had apparent K_ds of 167 and 2715 nM, respectively. Previous studies established that (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488 had apparent K_ds of 0.89 and 299 nM, respectively (Rothman et al., 1988b). Thus the enantiomers of (±)-cis-I exhibit about the same degree of enantioselectively as the enantiomers of U50,488, but with K_ds about one order of magnitude higher.

4. Discussion

Although U50,488 (a racemic compound) has been used as a selective κ agonist for several years, few studies have examined the pharmacological activity of its enantiomers, (−)-(1S,2S)-U50,488, (+)-(1R,2R)-U50,488, (−)-cis-I and (+)-cis-I. The major finding of this paper is that the biological activity related to activation of κ receptors by U50,488 appears to reside primarily in the (−)-(1S,2S)enantiomer. In drug discrimination studies (fig. 1), (−)-(1S,2S)-U50,488 is about 100 times more potent than (+)-(1R,2R)-U50,488. Similarly, in a test of analgesia, whereas (−)-(1S,2S)-U50,488 is active (ED_{50} less than 1 mg/kg), (+)-(1R,2R)-U50,488 is virtually inactive. The racemate, U50,488, was active in both paradigms,

having a potency intermediate between that of its two enantiomers.

An interesting finding to emerge from this study is that (±)-cis-I potently substituted for EKC in the drug discrimination experiments, yet was inactive in the tail withdrawal tests. This finding prompted the examination of the interaction of (±)-cis-I and its two enantiomers with μ, δ and κ binding sites in vitro. As described previously (Kosterlitz et al., 1981), we define a κ binding site as the residual [^{3}H]BRM binding remaining after suppression of μ and δ binding sites.

Previous studies demonstrated that, using membranes depleted of μ and δ binding sites by pretreatment with the site-directed acylating agents BIT and FIT respectively, [^{3}H]BRM labels a population of non-μ, non-δ binding sites with some of the characteristics of κ receptors (Rothman et al., 1985). The experiments reported here demonstrate the resolution of two [^{3}H]BRM binding sites distinguished by the inhibitory potency of (−)-(1S,2S)-U50,488. The site sensitive to (−)-(1S,2S)-U50,488 (K_d = 31 nM), is 44% of the total number of binding sites. This, and the observation that the K_d of (−)-(1S,2S)-U50,488 for site 2 is about 10 μM, explains the low potency with which (−)-(1S,2S)-U50,488 displaces [^{3}H]BRM binding from rat brain membranes pretreated with BIT and FIT (fig. 2C).

In contrast, (−)-(1S,2S)-U50,488 binds about ten times more potently to the population of non-μ, non-δ binding sites labeled by [^{3}H]U69,593 (K_d about 2 nM). This, and the fact that pretreatment of membranes with the acylating agent 1S,2S-trans-2-isothiocyanato-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexylbenzeneacetamide (deschloro U50,488 isothiocyanate) (De Costa et al., 1989) completely eliminates [^{3}H]U69,593 binding without altering [^{3}H]BRM binding strongly suggests that [^{3}H]U69,593 and [^{3}H]BRM label different populations of κ binding sites, at which (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488 exhibit similar degrees of enantioselectivity. Taken collectively, these data support the hypothesis that guinea pig brain has three subtypes of κ binding sites, distinguished by the inhibitory potencies of (−)-(1S,2S)-U50,488.

Our hypothesis derives additional support from the ligand binding and autoradiographic data published by Nock and associates (Nock et al., 1988a,b) that [^{3}H]U69,593 and [^{3}H]ethylketocyclazocine label different populations of κ binding sites. Although Zukin et al. (1988) suggested that guinea pig brain κ receptors are almost exclusively of the type sensitive to U50,488, our data suggest otherwise.

Both (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488 were equipotent in inhibiting [^{3}H]FOXY to μ binding sites. This observation supports the observations (Von Voigtlander and Lewis 1988) that (+)-(1R,2R)-U50,488 might possess weak μ agonist activity, illustrating again how different stereoisomers of the same drug can possess different pharmacological activities (Ariëns, 1987; 1986).

(±)-Cis-I, (+)-cis-I and (−)-cis-I (1 μM) failed in inhibit [^{3}H]BRM binding to guinea pig membranes (table 2A,B). For this reason we did not examine further the interactions of (−)-cis-I or (+)-cis-I. (±)-Cis-I did inhibit [^{3}H]U69,593 binding (data not shown). Additional experiments demonstrated that (−)-cis-I and (+)-cis-I had K_ds of about 167 and 2700 nM for the population of κ receptors labeled by [^{3}H]U69,593. The K_d of (−)-cis-I was intermediate between that of (−)-(1S,2S)-U50,488 (0.89 nM) and (+)-(1R,2R)-U50,488 (299 nM), consistent with the intermediate potency of (±)-cis-I observed in the drug discrimination studies. These in vitro results suggest that the discriminative cue is mediated by the population of κ receptors labeled by [^{3}H]U69,593. However, it is still necessary to show that this effect is naloxone reversible. Because (−)-cis-I and (+)-cis-I bind with reasonably high affinity to haloperidol-sensitive σ binding sites (K_ds of 81 and 221 nM, respectively), it remains a remote possibility that this binding site is involved in the pharmacological actions of the cis-enantiomers of U50,488 observed in this study (De Costa et al., in press).

The failure of (±)-cis-I to produce antinociception might be taken as evidence that different populations of κ receptors mediate the discriminative cue and antinociception. However, (+)-(1R,2R)-U50,488 was also inactive in the tail-withdrawal test, which is consistent with the observation that all compounds tested in this study

TABLE 3

Summary of results. The pharmacological data reported in this study are summarized and compared with the K_ds of test drugs for the κ binding site labeled by [^{3}H]U69,593. [a] These K_d values were calculated using the binding parameters obtained for the enantiomers (table 2B).

Drug	ED_{50} (mg/kg)			
	Drug discrimination	Antinociception		K_d (nM)
		50 °C	55 °C	
(±)-U-50,488	0.018	0.98	2.72	1.8 [a]
(±)-Cis-I	0.32	inactive	inactive	314 [a]
(−)-(1S,2S)-U50,488	0.01	0.36	0.79	0.89
(+)-(1R,2R)-U50,488	1.8	inactive	inactive	299

were ten to a hundred times more potent in the drug discrimination experiments than in the tests of analgesia. A more likely explanation for these observations is that the discriminative cue is activated at low fractional occupancy of the κ receptor, whereas higher fractional occupancy is required to produce antinociception.

Taken collectively, the results reported here, and summarized in table 3, suggest that the κ receptor-related pharmacological activity of U50,488 resides primarily in the (−)-(1S,2S)-enantiomer, and that (+)-(1R,2R)-U50,488 might possess weak activity at μ receptors, consistent with data published by Von Voigtlander and Lewis (1988). Examination of the interaction of the four stereoisomers of U50,488 with the population of non-μ, non-δ binding sites labeled by [^{3}H]BRM and those labeled by [^{3}H]U69,593 provides further evidence for the existence of at least three classes of κ binding sites in guiea pig brain, and suggest that the pharmacological actions of U50,488 result from activation of the κ receptor labeled by [^{3}H]U69,593. The structure-activity profile and functional significance of the κ binding sites labeled by [^{3}H]BRM are currently under investigation.

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