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Pharmacological properties of JDTic: a novel κ-opioid receptor antagonist

Ivy Carroll[a,*], James B. Thomas[a], Linda A. Dykstra[b], Arthur L. Granger[b], Richard M. Allen[b], James L. Howard[c], Gerald T. Pollard[c], Mario D. Aceto[d], Louis S. Harris[d]

[a]*Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, North Carolina, United States*
[b]*Departments of Psychology and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States*
[c]*Howard Associates, Research Triangle Institute, Research Triangle Park, North Carolina, United States*
[d]*Department of Pharmacology and Toxicology, School of Medicine, Virginia Commonwealth University, Richmond, Virginia, United States*

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Abstract

Biological studies were conducted on (3*R*)-7-Hydroxy-*N*-{(1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl}-1,2,3,4-tetrahydro-3-isoquinoline-carboxamide (JDTic), the first potent κ-selective opioid receptor antagonist not derived from an opiate class of compounds. In the mouse tail-flick test, JDTic, administered subcutaneously (s.c.), blocked anticociceptive activity for up to 2 weeks. When JDTic was administered either s.c. or p.o. 24 h before the selective KOP (κ)-opioid receptor agonist, enadoline, AD_{50s} of 4.1 and 27.3, respectively, were obtained. A time-course study of JDTic versus enadoline indicated significant antagonist p.o. activity up to 28 days. In contrast, JDTic, s.c., failed to antagonize the analgesic effects of the selective MOP (μ)-opioid receptor agonist, sufentanil. In the squirrel monkey shock titration antinociception test, JDTic given intramuscularly (i.m.) shifted the *trans*-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl] cyclohexyl) benzeneacetamide (U50,488) dose–effect curve to the right. In the U50,488-induced diuresis rat test, JDTic, s.c., suppressed diuretic activity with a greater potency than that of nor-binaltorphimine (nor-BNI). Thus, JDTic is a potent long- and orally acting selective κ-opioid antagonist.

Keywords: κ-Opioid receptor; Antagonist; JDTic; Antinociception; Tail-flick; Diuresis

1. Introduction

In recent years, KOP (κ)-opioid receptor agonists have received considerable attention, especially with the interest in their potential as analgesics with reduced side effects relative to MOP (μ)-opioid receptor agonists (Szmuszkovicz, 1999). Although numerous agonists selective for the κ-opioid receptor have been synthesized, very few pure opioid receptor antagonists selective for the κ subtype have been reported. The two most studied κ-opioid receptor-selective antagonists are nor-binaltorphimine (nor-BNI) and 5'-guanidinonaltrindole (GNTI), which were developed by Portoghese and coworkers (Jones et al., 1998; Jones and Portoghese, 2000; Portoghese et al., 1987b, a; Stevens et al., 2000). Both compounds are derived from naltrexone and depend on the *N*-cyclopropylmethyl group for their antagonist properties.

Research has suggested that κ-opioid receptors are involved in a number of biological processes. Mague et al. (2003) reported that i.c.v. administration of the κ-opioid receptor antagonists nor-BNI and GNTI dose-dependently decreased immobility in the forced-swim test (FST) and suggested that these κ-opioid receptor antagonists possessed antidepressant-like effects. These authors also suggested that their findings were consistent with the hypothesis that cAMP response element binding protein (CREB) mediated induction of dynorphin, an endogenous κ-opioid receptor agonist (Chavkin et al., 1982) in the nucleus accumbens, triggered immobility behavior in the FST.

* Corresponding author. Tel.: +1 919 541 6679; fax: +1 919 541 8868.
E-mail address: fic@rti.org (I. Carroll).

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Recently, McLaughlin et al. (2003) reported that a cocaine-conditioned place preference (CPP) response was increased threefold in C57BL/6 mice preexposed to repeated forced-swim stress. The increased cocaine CPP response was blocked by the κ-opioid receptor antagonist nor-BNI and was not seen in mice lacking the prodynorphin gene. The increased cocaine CPP response could be due to released dynorphin. Other studies have also suggested that κ-opioid receptor antagonism may also be involved in eating disorders (Jewett et al., 2001), irritable bowel syndrome (Hawkes et al., 2002), constipation (Choi and Billings, 2002), traumatic brain injury (Vink et al., 1991), and opioid, cocaine, and alcohol abuse (Rothman et al., 2000).

In a study of the effects of the *N*-substituent analogs of the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (LY272922) class of opioid receptor antagonists, we recently reported in vitro results indicating that (3*R*)-7-Hydroxy-*N*-{(1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl] methyl}-2-methylpropyl}-1,2,3,4-tetrahydro-3-isoquinoline-carboxamide (JDTic) is a potent and selective κ-opioid receptor antagonist (Fig. 1). To our knowledge, JDTic is the first potent κ-opioid receptor antagonist not derived from the opiate class of compounds (Thomas et al., 1998b; Thomas et al., 1998a; Thomas et al., 2001; Thomas et al., 2003). A number of reports have shown that κ-opioid receptor antagonists derived from naltrexone, such as nor-BNI and GNTI, have low potency in vivo. The fact that JDTic met all five of Lipinski's requirements (Lipinski, 2000) for a compound to possess druglike properties suggested that JDTic may be a viable candidate as a pharmacotherapy for treatment of obesity and depression, as well as opioid and cocaine abuse and other central nervous system (CNS) disorders. In this study, we report that JDTic is a potent κ-opioid receptor antagonist in three different assays, (1) mouse antinociception produced by enadoline by either subcutaneous or oral routes, (2) monkey antinociception produced by *trans*-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]cyclohexyl)benzeneacetamide (U50,488), and (3) rat diuresis following administration of U50,488.

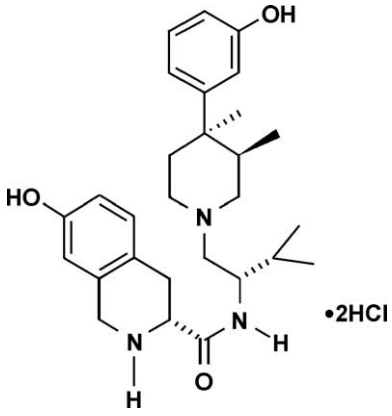

Fig. 1. Structure of JDTic.

2. Materials and methods

All animals received care according to "Guide for the Care and Use of Laboratory Animals," DHHS Publication, Revised, 1996. The animal care facilities were certified by the American Association for the Accreditation of Laboratory Care. These studies were approved by the Institutional Animal Care and Use Committees at Virginia Commonwealth University, Research Triangle Institute, or the University of North Carolina at Chapel Hill. Housing and experimental conditions were as nearly identical as possible at the three institutions.

2.1. JDTic κ- and μ-opioid receptor antagonist effects in the mouse

2.1.1. General methods

Antinociception following JDTic administration was examined in ICR male mice (Harlan Sprague–Dawley, Indianapolis, IN) weighing 20–30 g. Experiments used the D'Amour and Smith procedure (D'Amour and Smith, 1941) as modified (Aceto et al., 1997; Dewey et al., 1970; Dewey and Harris, 1971). Briefly, the mouse's tail was placed in a groove, which contained a slit. A photoelectric cell was located under the slit. When the heat source or noxious stimulus was turned on, heat was focused on the tail, and the mouse responded by flicking its tail out of the groove. As a result, light passed through the slit and activated a photocell, which, in turn, terminated the recording timer. The heat source was adjusted to produce tail-flick latencies of 2–4 s under control conditions. Only mice meeting this criterion were used. Each animal was tested only once after the control reading. JDTic was given by the subcutaneously (s.c.) or the p.o. route. A stainless steel animal feeding needle attached to a syringe was used for p.o. injections. Six animals per dose were used.

It was important to learn whether or not JDTic, per se, had agonist properties, because if it did, the interpretation of subsequent antagonism studies could be compromised. Accordingly, JDTic was tested after pretreatment intervals of 20 or 40 min, 24 or 48 h, and 14 days.

In the κ-opioid antagonist studies, the mice were pretreated at various intervals with either an s.c. or p.o. AD80 of JDTic, and then challenged with an s.c. ED80 of enadoline and tested 20 min later. Effective dose values (ED or AD) were computer generated using a least squares linear regression analysis followed by calculation of the 95% confidence limits (Bliss, 1967). In the μ-opioid receptor antagonism studies, the mice were pretreated with an s.c. AD80 of JDTic and then were administered an ED80 of sufentanil and tested.

Antinociception was calculated as percent maximum possible effect (% MPE)=(test latency−control latency)/(10 s−control latency)×100 for each dose tested. Cutoff time was 10 s. Analysis of variance (ANOVA) and Fisher's PLSD post hoc test were used to evaluate the results of the

JDTic-Enadoline time-course study. Significance was set at P=0.05. The StatView statistical package (Brainpower, Agoura Hills, CA) was used for these analyses.

Percent antagonism for each pretreatment interval was calculated as 1−(JDTic+enadoline or sufentanil ED80 MPE)/(enadoline or sufentanil ED80 MPE)×100. Vehicle controls corresponding to each time point were also tested.

2.2. JDTic versus U50,488-induced antinociception in squirrel monkeys

2.2.1. Subjects

Three adult male squirrel monkeys (*Saimiri sciureus*) weighing between 0.70 and 0.95 kg were pair-housed in a climate-controlled colony room maintained on a 12-h light/dark cycle (7:00 AM to 7:00 PM). All monkeys had free access to water and were maintained at their free-feeding weights through a high-protein monkey diet supplemented with fresh fruit. No monkey had received any drugs in the month prior to beginning these experiments.

2.2.2. Apparatus

Each monkey was seated in a Plexiglas primate restraint chair and held in place by a waist support with its tail secured by a small stock (Dykstra, 1985). Electrical shock was administered via two hinged brass plates that rested on a shaved portion of the monkey's tail. The tail was coated with a noncorrosive electrode paste (EKG Sol) to provide a low-resistance electrical contact. Each chair was enclosed within a ventilated sound-attenuating chamber. The chamber was illuminated by a 10-W bulb during experimental sessions. A lever was positioned on the right side of the front panel. When the lever was pressed with a downward force of at least 0.15 N, an audible click was heard, and the response was recorded. Experimental events, including control of shock intensity, were controlled using software and hardware from Med Associates (St. Albans, VT) through a microcomputer located in the adjacent room.

2.2.3. Behavioral procedure

The shock titration procedure previously described (Dykstra and Massie, 1988) was used. At the start of each experimental session, the shock intensity began at 0.01 mA and increased in 30 intervals to a maximum shock level of 2.0 mA. Monkeys received continuous shock during 15-s trial periods. If a monkey did not respond five times (FR5) during that 15-s period, the shock was increased one interval and maintained at that level for another 15-s period. When a monkey met the FR5 requirement, the shock was immediately terminated for a 15-s time-out period, after which the shock resumed at the next lower intensity. During time-out periods, chamber lights stayed on, but responding had no experimental consequence. If, at the highest shock intensity, the FR5 requirement was not met in any of five consecutive 15-s shock periods, the session was automatically terminated.

Each control session consisted of four 15-min periods, during which the shock titration procedure was in effect. A 10-min interval preceded each 15-min shock period. During this 10-min interval, the chamber was dark and responding had no consequence. Control sessions were typically conducted on Monday, Wednesday, and Thursday. Test sessions usually occurred on Tuesdays and Fridays. For test sessions, the intercomponent interval was extended to 20 min, and a fifth component was added.

2.2.4. Pharmacological procedure

U50,488 and JDTic were dissolved in distilled water, with doses expressed in terms of the salts. Distilled water was used as the vehicle injection. U50,488 and JDTic were administered intramuscularly (i.m.) into the calf muscle (injection volume=1.0 ml/kg).

The U50,488 dose–effect curve was obtained in the following way: first, an injection of distilled water was given at the beginning of the experimental session, and responding was examined during the next 15-min FR titration period. Then, the first dose of U50,488 was administered, and its effects were examined during the next 15-min FR titration period. The dose of U50,488 was increased prior to each of the next three 15-min FR5 periods, with the total dosage increasing by one quarter or one-half log unit.

Dose–effect curves were obtained in each monkey for U50,488 alone (0.3–10 mg/kg) and then for U50,488 following administration of 1.0 mg/kg of JDTic. The U50,488-alone dose–effect curve was examined twice, with the two determinations spaced 3 days apart. Four days later, each monkey was administered 1.0 mg/kg of JDTic, and the U50,488 dose–effect curve was then redetermined at four time points: 2 h and 3, 7, and 10 days subsequent to the administration of JDTic.

2.2.5. Data analysis

Two dependent variables were extensively analyzed in this study: median shock level (mA) and response rate during shock (RR, responses/s). Median shock level was expressed as the shock intensity, below which the monkey kept the shock 50% of the time. Response rates were determined for each monkey by dividing the total number of responses during shock by the total amount of time spent in shock. Data from the first 5 min of each FR5 titration period were eliminated from analysis to avoid the measurement of responding during a warm-up period.

Individual ED_{50} values were calculated using a modified procedure (Tallarida and Murray, 1987). Only the ascending portion of the group dose–effect curve was used in the analysis. The maximal effect was determined by subtracting the average median shock level for a control water injection from 1.8 mA, the maximum possible median shock level recorded if a monkey did not respond for five 15-s FR periods, resulting in termination of the experimental session.

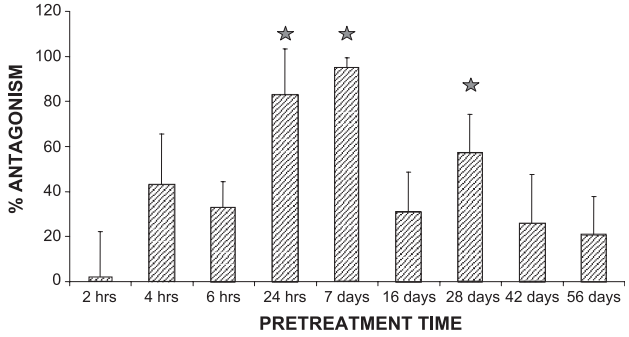

Fig. 2. Time-course study in which 54 mice (6 mice per pretreatment group) were pretreated with the κ-opioid antagonist JDTic (p.o., AD_{80}) at the times indicated on the abscissa. Then, as the appropriate pretreatment time elapsed, the mice were given enadoline, the selective κ-opioid agonist (s.c., ED_{80}), and tested 20 min later. Results are expressed as percent antagonism of enadoline-induced antinociception. Each column represents the means±S.E.M. Asterisk indicates a significant difference from the 2-h group ($P \leq 0.05$).

2.3. Effects of JDTic and nor-BNI on U50,488-induced diuresis in rats

The method was adapted from Leander (1983a,b). A total of 48 (16 in the first experiment and 32 in the second) male Sprague–Dawley-derived rats (Charles River Laboratories, Raleigh, NC) arrived at 250–275 g and were housed two per cage on a regular 12/12 light/dark cycle. Food and water were available ad libitum except during the time in the metabolism cages. Rats were habituated to the animal quarters for at least 1 week prior to use. On experimental days, they were brought to the metabolism suite approximately 1 h before injection. They weighed between 275 and 420 g at the time of testing.

U50,488, nor-BNI, and JDTic were prepared in distilled water and injected at 1 ml/kg. Each rat received two s.c. injections back-to-back and was immediately placed into a standard metabolism cage. The first injection was vehicle, JDTic or nor-BNI, and the second injection was vehicle or U50,488 at 10 mg/kg. In subsequent weeks, only vehicle was given as the first injection. The weight in grams of urine output was measured hourly for 5 h.

Four groups (N=4) were run in the first experiment. The injections were vehicle+vehicle, vehicle+U50,488, and JDTic 17 or 100 mg/kg+U50,488. Eight groups (N=4) were run in the second experiment. The injections were vehicle+vehicle, vehicle+U50,488, JDTic 0.3, 1, or 3 mg/kg+U50,488, and nor-BNI 0.3, 1, or 3 mg/kg+U50,488. Because the two experiments used identical procedures and there was no difference between the control groups, the control data were combined; thus, N=8 for vehicle+U50,488 and N=4 for other groups.

2.4. Drugs

JDTic was synthesized as previously described (Thomas et al., 2001). The structure is shown in Fig. 1. U50,488, enadoline hydrochloride, sufentanil citrate, and nor-BNI were supplied by the National Institute on Drug Abuse (Washington, DC).

3. Results

3.1. JDTic κ- and μ-opioid receptor antagonist effects in the mouse

To avoid possible confounding effects, it was considered important to rule out JDTic-induced antinociceptive activity in the tail-flick test. At doses of 1, 10, or 30 mg/kg, s.c., JDTic was devoid of agonist effects when given 20 or 40 min or 24 h before testing. In addition, at the highest dose tested, JDTic was totally devoid of activity 48 h and 14 days after its administration. In another experiment, separate groups of mice received one of several escalating doses of the antagonist JDTic by either the s.c. or p.o. route of administration. Then, 24 h later, they were given s.c. an ED_{80} of the agonist enadoline and tested 20 min later. The s.c. AD_{50} of JDTic was found to be 4.14 (2.55–6.78), whereas the p.o. AD_{50} of JDTic was 27.3 (16.7–44.7). The results of a time-course study with JDTic in combination with enadoline are illustrated in Fig. 2. Analysis of variance revealed significant overall antagonist activity [$F(8,45)=3.117$, $P=0.00699$]. Post hoc comparisons using Fisher's PLSD test

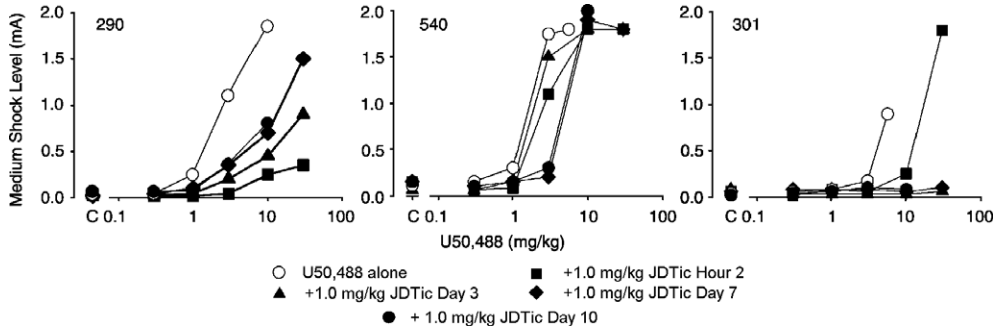

Fig. 3. Effects of U50,488 alone and in combination with 1.0 mg/kg JDTic on median shock level in three individual monkeys. Ordinate: median shock level in mA; abscissa: dose of U50, 488 mg/kg administered cumulatively.

Table 1
ED_{50} values for U50,488 alone and in combination with JDTic in the squirrel monkey opioid antagonist assay

	ED_{50} (mg/kg)				
	U50,488 and 1.0 JDTic				
Monkey	U50,488 alone	2 h	3 days	7 days	10 days
290	2.61	>30	>30	14.89	16.63
540	1.62	2.53	1.90	5.17	4.72
301	11.42	16.78	>30	>30	>30

indicated significant antagonist activity at 24 h (P=0.0016) and at 7 days (P=0.0004) and 28 days (P=0.0203) compared to the 2-h effect.

As a μ-opioid antagonist, JDTic, at doses of 1, 3, 10, 30, and 100 mg/kg, s.c., was without effect when given 24 h before an s.c. ED_{80} of sufentanil (data not shown).

3.2. JDTic antagonism of κ-opioid receptor agonist-induced antinociception in the monkey

A cumulative dose–effect curve for U50,488, alone and in combination with JDTic, was obtained in each monkey, and control median shock levels and rate of responding (RR) were determined from water injections. Control median shock levels ranged between 0.02 and 0.15 mA in individual monkeys, and RR ranged between 0.28 and 0.47 responses. Fig. 3 shows the effects of U50,488 on median shock level in individual monkeys. Dose–effect curves are shown for U50,488, alone and at four different time points following administration of 1.0 mg/kg JDTic.

When administered alone, U50,488 increased median shock levels dose-dependently, with peak increases occurring between 5.6 and 10 mg/kg. Several days following the second determination of the U50,488-alone dose-effect curve, JDTic (1.0 mg/kg) was administered and the U50,488 dose-effect curve was redetermined 2 h and 3, 7, and 10 days later. The results from the U50,488/JDTic combinations are also presented in Fig. 3. It can be seen that JDTic shifted the U50,488 dose–effect curve to the right, with shifts apparent up to 7 and 10 days following administration of JDTic. ED_{50} values are shown in Table 1.

3.3. JDTic Antagonism of κ agonist-induced diuresis in rats

U50,488 produces a marked increase in urine output in normally hydrated rats (compare the water+U50,488 groups to the water+water group in Fig. 4). The left-hand panel of the figure shows the dose-related antagonism of U50,488's effect by JDTic. During the 5 h immediately following the administration of JDTic+U50,488 (Week 0), JDTic significantly reduced the polyuria [$F(5,20)$=24.65, with 3, 17, and 100 mg/kg being different from water+U50,488 by Newman–Kuels]. AD_{50} values are shown in Table 2. The antagonism was still present 1 week after administration [$F(5,20)$=43.40, with all doses different from water+U50,488]; in fact, 17 and 100 mg/kg completely blocked the polyuria. The AD_{50} at 1 week was 0.41 mg/kg. The antagonism remained at 2 weeks [$F(5,20)$=37.62, with 1 mg/kg and larger doses different, and an AD_{50} of 1.64 mg/kg] and 3 weeks [$F(3,15$=6.37, with 3 mg/kg different]. Seventeen and 100 mg/kg could not be continued to Week 3 because of severe necrosis at the injection site, and no AD_{50} could be calculated.

The right-hand panel shows the dose-related antagonism effect of U50,488 by the known κ-opioid receptor antagonist nor-BNI. The antagonism was significant at Week 0 [$F(3,15)$=9.74, with 1 and 3 mg/kg different], Week 1 [$F(3,15)$=11.07, with 3 mg/kg different], Week 2 [$F(3,15)$=8.26, with 3 mg/kg different], and Week 3 [$F(3,15)$=5.23, with 3 mg/kg different]. Because of the restricted range of doses, no AD_{50}s were calculated.

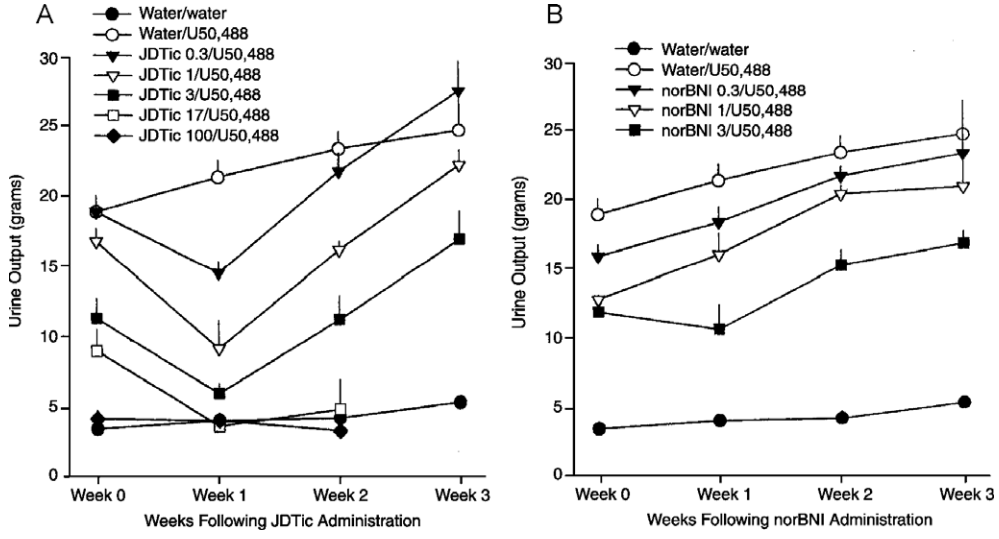

Fig. 4. (A) Antagonism of U50,488-induced urine output by various doses (mg/kg) of JDTic in rats. (B) Antagonism of U50,488-induced urine output by various doses (mg/kg) of nor-BNI. JDTic and nor-BNI were only administered once at Week 0.

Table 2
AD_{50} values for U50,488 antagonism by JDTic in rat diuresis assay

5 h Data AD_{50} to block U50,488	
Week 0	2.81 mg/kg
Week 1	0.41 mg/kg
Week 2	1.64 mg/kg
Week 3	>3 mg/kg

Comparison of the curves for JDTic and nor-BNI suggests that JDTic is more potent. Analysis of variance confirmed that JDTic at 0.3 to 3 mg/kg (0.54 to 5.39 μM/kg) produced greater antagonism than nor-BNI at the same mg/kg doses (0.41 to 4.08 μM/kg) at Weeks 1 and 2 [$F(1,14)=23.51$ and $F(1,14)=12.60$, respectively]. For Week 0, the antagonism by JDTic just missed being significantly greater than nor-BNI [$F(1,14)=4.15$, $P<0.06$]. The conclusion that JDTic is more potent than nor-BNI is tempered by the lack of knowledge of the time of peak effect of the two compounds and the restricted range of nor-BNI doses.

4. Discussion

The first selective κ-opioid receptor antagonist was the β-naltrexamine derivative triethyleneglycolnaltrexamine (TENA; Erez et al., 1982). Because its κ selectivities over μ- and δ-opioid receptors were only 4- and 2.5-fold, respectively, TENA did not prove to be a very useful compound for studying the κ-opioid receptor. This was followed by the development of nor-BNI as a more selective κ-opioid receptor antagonist (Takemori et al., 1988), whose in vitro κ/μ selectivities exceeded those of TENA. nor-BNI was also reported to have selective centrally mediated κ-opioid receptor antagonist effects in mice if the time separating the administration of nor-BNI and the κ agonist was at least 24 h (Endoh et al., 1992). The resulting κ-opioid receptor antagonist activity and selectivity lasted up to 28 days (Horan et al., 1992). Thus, under these conditions, nor-BNI is a κ-opioid receptor-selective antagonist.

More recently, GNTI was developed as a more potent and more selective κ-opioid receptor antagonist (Jones et al., 1998; Jones and Portoghese, 2000; Negus et al., 2002; Stevens et al., 2000) than nor-BNI. GNTI also was found to have a slow onset and long duration of action in studies with rhesus monkeys (Negus et al., 2002).

Zimmerman et al. (1993) reported the discovery of a structurally unique series of opioid receptor subtype nonselective pure antagonists based on *N*-substituted analogues of 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (LY272922). These compounds were novel, because unlike all other known opioid receptor antagonists, their intrinsic antagonist activity was not mediated by the structure of the *N*-substituent. In the case of the 4-(3-hydroxyphenyl)piperidines, the *trans*-3,4-dimethyl orientation is required for pure opioid receptor antagonist activity (Mitch et al., 1991; Mitch et al., 1993; Zimmerman et al., 1978; Zimmerman et al., 1985; Zimmerman and Leander, 1990; Zimmerman et al., 1993; Zimmerman et al., 1994). Although numerous *N*-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine analogs were studied (Zimmerman et al., 1993), none of the compounds showed selectivity for the κ-opioid receptor subtype.

As a strategy for obtaining κ-selective opioid antagonists, we designed a library of compounds using *N*-substituents of LY272922, which allowed incorporation of diversity elements while avoiding features resembling μ-favoring *N*-substituent structures previously identified (Thomas et al., 1998b; Thomas et al., 2001). Modification of lead compounds identified in this library led to the discovery of JDTic, an active κ-opioid receptor antagonist with greater potency and selectivity than nor-BNI and GNTI in the sulfur-35-guanosine-5'-0-(3-thio)-triphosphate ([^{35}S]GTPγS) in vitro functional assay (Thomas et al., 2001; Thomas et al., 2003). JDTic, with a K_e value of 0.01 nM (pA_2=10.46), was more potent than nor-BNI (K_e=0.04 nM) in the inhibition of 5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide (U69,595)-stimulated [^{35}S]GTPγS binding in cloned human κ-opioid receptors (Black et al., 2003; Thomas et al., 2003) (Table 3). JDTic showed no agonist activity at levels of 10 μM and possessed selectivity for κ-opioid receptor over μ- and DOP (δ)-opioid receptors of 341- and 7930-fold, compared to nor-BNI selectivity of 475- and 110-fold for the μ- and δ-opioid receptors. Thus, JDTic was more

Table 3
Comparison of K_e and/or pA_2 values from the [^{35}S]GTPγS binding assay for JDTic to nor-BNI and GNTI in cloned human μ-, δ-, and κ-opioid receptors

Compd	μ, DAMGO		δ, DPDPE		κ, U69,593		μ/κ	δ/κ
	K_e (nm)	pA_2	K_e (nm)	pA_2	K_e (nm)	pA_2		
Nor-BNI[a]	19		4.4		0.04		475	110
GNTI[b]		8.49		7.81		10.40		
JDTic[c]	3.41	8.50	79.3	ND[d]	0.01	10.46	341	7930

[a] Data taken from Black et al. (2003).
[b] Data taken from Jones and Portoghese (2000).
[c] Taken from Thomas et al. (2003).
[d] The pA_2 value could not be determined, because this compound is a noncompetitive antagonist at the δ-opioid site.

selective than nor-BNI for the κ-opioid receptor compared to the δ-opioid receptor but is slightly less selective for the κ-opioid receptor over the μ-opioid receptor in this in vitro functional test. The pA_2 values of 8.50 and 10.46 at the μ- and κ-opioid receptors for JDTic are almost identical to those of 8.49 and 10.40 for GNTI. The 79.3 K_e value of JDTic compared to a pA_2 value of 7.81 for GNTI at the δ-opioid receptor shows that neither compound has appreciable potency for the δ-opioid receptor.

In this study, JDTic was devoid of agonist activity when given 20 min or 24 h before testing. Importantly, JDTic in the mouse tail-flick test did antagonize κ-opioid receptor-mediated antinociception produced by enadoline, but had no effect on the antinociception produced by the selective μ-opioid receptor agonist sufentanil. Thus, JDTic is a potent and selective κ-opioid receptor antagonist in the mouse tail-flick assay and devoid of any antinociceptive activity. JDTic was shown to be orally active in the same assay.

In the squirrel monkey shock titration procedure (Dykstra and Massie, 1988), JDTic antagonized the antinociceptive effects of the κ-opioid agonist U50,488. JDTic also antagonized κ-opioid receptor-mediated diuresis induced by U50,488 with a potency greater than that of nor-BNI.

Similar to nor-BNI and GNTI, JDTic possessed a slow onset and long duration of antagonist activity in the mouse tail-flick test, the monkey shock titration procedure, and antagonism of U50,488-induced urine output in rats. The observation that the naltrexone-derived κ-opioid receptor antagonists nor-BNI and GNTI, as well as the 3,4-dimethyl-4-(3-hydroxyphenyl)piperidine-derived κ-opioid receptor antagonist JDTic, all have unusually long duration of action (21 days or longer in mice and rats) is particularly interesting. In the case of nor-BNI, it was suggested that the long-lasting activity might be due to resistance to metabolism, induction of a conformational receptor change, and slow distribution and passage across cell membranes (Horan et al., 1992). The fact that all three structurally diverse κ-opioid receptor antagonists show similar long duration of action is more consistent with a change in conformation of the receptor that renders it functionally inactive or a postreceptor event that has a memory.

Although the causes of drug addiction are multifaceted and complex, considerable research suggests that the opioid receptor system may be pathologically altered in human opiate addicts (Kreek et al., 2002). Chronic administration of opiates increases brain levels of the endogenous κ-opioid peptide dynorphin. This increase in dynorphin produces an imbalance in abstinent μ-opioid-dependent individuals and dysphoric mood states, which can result in the desire to take μ-opioid agonists to normalize mood (Rothman et al., 2000). This κ overdrive could be normalized by κ-opioid receptor antagonists; however, there are no clinically available selective κ-opioid receptor antagonists with which to test this hypothesis.

The potential effectiveness of a functional κ-opioid receptor antagonist for the treatment for opiate dependence was tested by conducting an open-label study of buprenorphine, which is a partial μ-opioid receptor agonist and κ-opioid receptor antagonist combined with the moderately selective μ-opioid receptor antagonist naltrexone to block the μ-opioid agonist effect (Rothman et al., 2000). Interestingly, administration of the mixed μ-opioid receptor agonist/κ-opioid receptor antagonist buprenorphine in combination with naltrexone improved positive responses in opioid-dependent individuals as compared to naltrexone alone (Rothman et al., 2000). Viewed collectively, these results suggest that a κ-opioid receptor-selective antagonist, such as JDTic may be useful for treating opiate addicts in withdrawal and possibly relapse. In addition, because nor-BNI and GNTI decreased immobility in the forced swim test (Mague et al., 2003) and clinical studies suggest that buprenorphine may be a useful antidepressant (Bodkin et al., 1995), κ-opioid receptor antagonists may represent a new class of antidepressants.

In summary, previous in vitro studies showed that JDTic was a potent κ-opioid receptor-selective antagonist in the [^{35}S]GTPγS binding assay. The results of this biological study corroborate that JDTic is an orally acting selective κ-opioid receptor antagonist with a long duration of action. The structural diversity of JDTic, GNTI, and nor-BNI and their comparable time-course of antagonist activity, suggests that the long duration of action may result from some type of change in the κ-opioid receptor that renders it functionally inactive. Obviously, additional studies will be needed to characterize this unusual property of κ-opioid receptor antagonists. Finally, JDTic should be considered for use as a pharmacotherapy for treating cocaine and opioid abuse, as well as depression.

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