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Effects of JDTic, a selective κ-opioid receptor antagonist, on the development and expression of physical dependence on morphine using a rat continuous-infusion model

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Abstract

JDTic, (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, is a potent and selective κ-opioid antagonist with a very long duration of action [Carroll, F.I., Thomas, J.B., Dykstra, L. A., Granger, A.L., Allen, R.M., Howard, J.L., Pollard, G.T., Aceto, M.D., Harris, L.S., 2004. Pharmacological properties of JDTic: A novel k-opioid receptor antagonist. Eur. J. Pharmacol. 501, 111–119.]. When given 24 h prior to a continuous 4-day infusion of morphine sulfate in rats, JDTic did not prevent the stereotypy that developed during the infusion of morphine. It had no effect on the dramatic loss of body weight associated with the abrupt withdrawal of morphine. However, it decreased the number of important withdrawal signs designated wet-dog shakes and facial rubs. These data suggest that JDTic may find some application in the treatment of opiate abuse.

Keywords: κ-Opioid receptor; JDTic; Morphine physical dependence; (Rat)

1. Introduction

It is now well established that the opioid system consists of three G-protein coupled receptors, MOP (μ), DOP (δ), and KOP (κ), which are stimulated by a family of endogenous opioid peptides (Contet et al., 2004). These receptors are widely expressed in the central nervous system where they mediate pain and mood-altering actions of opioids and modulate numerous other endogenous functions. Each receptor subtype plays a specific role in the pharmacological responses to opioids. While μ-opioid receptors play the dominant role in the control of pain, all three receptor subtypes are involved in the mediation of opioid analgesia (Aldrich, 2003). Mu- and δ-opioid receptor agonists both display reinforcing properties, while κ-opioid receptors modulate mood. Kappa-opioid receptor agonists show strong dysphoric and sedative effects (Aldrich, 2003), while κ-opioid receptor antagonists show antidepressant effects (Mague et al., 2003).

Mu-opioid receptors have been proposed as the major subtype involved in the development of expression of physical dependence (Aldrich, 2003). In 1986, Aceto and coworkers reported that beta-funaltrexamine (β-FNA), a μ-opioid receptor antagonist, blocked the development of physical dependence on morphine in rats. This finding provided some evidence that the μ-opioid receptor played a major role in the development of morphine-induced physical dependence. The absence of anti-nociceptive, as well as rewards effects and withdrawal symptoms in mice lacking the μ-opioid receptor gene demonstrated that μ-opioid receptors were involved (Gaveriaux-Ruff and Kieffer, 2002). The interplay of the μ- and κ-opioid systems in the expression of morphine-induced physical dependence was noted by Simonin et al. (1998). They showed that naloxone-precipitated withdrawal was attenuated in κ-opioid receptor-deficient but not wild type morphine-dependent mice.

These findings seem to be at odds with those of another study in which nor-binaltorphimine (nor-BNI) exacerbated withdrawal in morphine-dependent rats (Maldonado et al., 1992; Spanagel et al., 1994). These contrasting results could be explained by the non-selectivity of nor-BNI in vivo under the conditions used.

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We recently reported 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 new potent and more selective κ-opioid receptor antagonist (Carroll et al., 2004; Thomas et al., 2003). In this study, a continuous morphine-infusion assay in rats was used to investigate the effects of JDTic on the development and expression of morphine-induced physical dependence.

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.

2.1. JDTic effects on morphine-induced physical dependence in rats

The experimental procedure was based on the method reported by Teiger (1974) and modified by Aceto et al. (2000). Briefly, adult male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were received, examined, and housed individually in stainless steel cages in a temperature-and humidity-controlled vivarium. The body weight range was 240–280 g when they were assigned to the study. After at least 3 days of acclimation, intraperitoneal (i.p.) cannulas were implanted. After each rat was anesthetized with pentobarbital (40 mg/kg i.p.), the lateral side of the lower left abdomen and the back of the neck was shaved, and exposed skin was cleansed with Povidone–Iodine Solution. Each rat was then fitted with a cannula (PE90 tubing, Clay Adams, NJ). The cannula was passed subcutaneously from the lateral side of the lower abdomen to the nape of the neck. The peritoneal end of the cannula was enclosed in silastic tubing to prevent foreign body reaction. It was introduced into the peritoneal cavity through a stab-wound entry site. The cannula was secured with sutures at both sites. Next, each rat was fitted with a harness consisting of a flat stainless steel plate fitted with a shoulder collar, a narrow strip of Velcro, and a spring coil. The collar was passed over the head of the rat, and the harness was secured by means of a strip of Velcro, which girdled the chest. The cannula was passed through the harness and spring coil and attached to a flow-through swivel (Instech Lab, Horsham, PA). The swivel allowed the rat to move about in its cage and eat and drink normally. An infusion pump (Harvard Apparatus, S. Natick, MA, Model-975) delivered the solutions in a volume of 8 mL every 24 h. The rats were allowed to recuperate from surgery for 5 days before starting the experiment.

2.1.1. Primary physical dependence study

Each rat was randomly allocated to a treatment regimen. Each subject also was randomly assigned to a cage on a rack. A synopsis of the experimental design and other particulars are presented in Table 1.

Overt behavioral observations were made at approximately the same time each day for 30 min while the rats were in their home cages. Then, stereotypy was scored. Next, each rat was disconnected from its tether, picked up and scored for irritability. These tests required less than 10 s each per rat. Each rat was subsequently weighed. Afterward, the rat was returned to its home cage and tethered. During withdrawal all withdrawal signs were noted for 30 min, however, the withdrawal signs designated wet-dog shakes and facial rubs (rubbing face with front paws) were quantified 24, 48 and 72 h after morphine was abruptly discontinued and vehicle was substituted for morphine. Irritability was scored as proposed (Teiger, 1974) as follows: 0 (remains tame when touched, and on being grasped and lifted); 1 (remains tame when touched, and on being grasped and lifted makes only a feeble attempt to wiggle free); 2 (remains tame when touched, but when grasped and lifted claws, bites, and/or vocalizes); and 3 (reacts to initial touch by vocalizing and biting, and attempts to grasp it by rolling over on its back and clawing). Stereotypy was evaluated using a modified scoring system (Johansson et al., 1992) that was

Table 1
Synopsis of experimental design of the rat-infusion study on the effects of JDTic pretreatment on the development and expression of morphine-induced physical dependence

Treatment regimens	Schedules (dose-mg/kg/day or 8 mL/kg/day)								N[d]
	Start[a] 24-hr pretreatment	Infusion-days 1–4				Withdrawal 24–72 h			
	JDTic (J) or vehicle (V)[b] s.c.	Morphine[c] (M) or Vehicle (V)[b] i.p.				V i.p.			
		1	2	3	4	24	48	72	
Vehicle control (V)	V	V	V	V	V	V	V	V	6
Morphine control (M)	V	50 M	100 M	200 M	200 M	V	V	V	7
JDTic (10 mg/kg, s.c.)+ M	(J) 10	50 M	100 M	200 M	200 M	V	V	V	7
JDTic (10 mg/kg, s.c.)+ V	(J) 10	V	V	V	V	V	V	V	6

[a] 24-h pretreatment, Vehicle (V) or JDTic (J), s.c.
[b] Sterile water, 8 mL/day.
[c] Dose of morphine sulfate.
[d] Number of subjects.

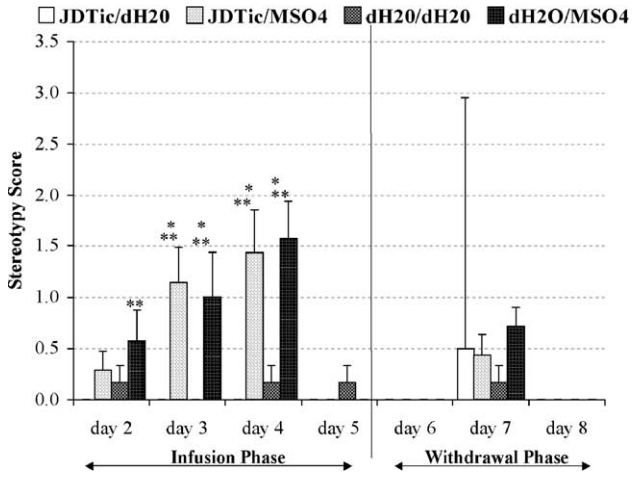

Fig. 1. Stereotypy in rats pretreated with JDTic or vehicle on Day 1, infused with morphine or vehicle on Days 2, 3, 4, and 5, and after abrupt withdrawal of morphine on Days 6, 7 and 8. *Significantly different from vehicle/vehicle group; **Significantly different from JDTic/vehicle group. $P=0.05$ or less. Vehicle controls: $N=6$ and morphine-treated rats: $N=7$.

originally proposed (Magos, 1969). A rat was scored: 0 if no repetitive behavior was noted; 1 for continuous sniffing; 2 for continuous licking of cage or grid or poking nose through the grid floor or mock biting; 3 for either stereotyped forceful biting of cage or grid or continuous gnawing on paws; and 4 for autophagia or gnawing or biting accompanied by tissue damage. Stereotypy was scored once during each observation period. A trained observer was "blinded" regarding treatment assignments during abrupt withdrawal.

2.1.2. Statistical analysis

When applicable, quantified data were assessed using repeated measures Analysis of Variance (ANOVA). If overall significance was found, Scheffe's test (post hoc test for any contrasts) was applied. Scored data for the signs, stereotypy, and irritability were analyzed using the nonparametric Kruskal–Wallis one-way ANOVA. When appropriate, post hoc comparisons were made using the Mann–Whitney U test. In all cases, significance was set at the 95% level. The StatView statistical package (Brainpower, Inc., Agoura Hills, CA) was used for these analyses.

2.2. Drugs

JDTic was synthesized as previously described (Thomas et al., 2003). Morphine was purchased from Mallinckrodt (St. Louis, MO).

3. Results

3.1. JDTic challenge in morphine-dependent rats

Except for the development of stereotyped behavior, which is discussed below, no other unusual overt signs were noted. Results with stereotypy are illustrated in Fig. 1. They were analyzed using the nonparametric Kruskal–Wallis one-way ANOVA. This test revealed significant differences among treatment groups on Days 3 ($H=9.965$, $p=0.0189$) and 4 ($H=10.714$, $p=0.0134$) only. During the infusion phase, stereotypy developed on days 3 and 4 in the JDTic/morphine and vehicle /morphine groups compared to controls Surprisingly, stereotypy was not observed on the last day of the infusion, Day 5. No significant activity was observed during withdrawal. These results indicate that JDTic had no effect on the development of sensitization to morphine.

Body weight data for the entire experiment are depicted in Fig. 2. Repeated measures (ANOVA) of all the data indicated no significant differences among treatment regimens ($F=0.597$, $p=0.6238$) and significant differences among days ($F=41.260$, $p=<0.0001$) and a significant treatment/day interaction ($F=31,016$; $p=<0.0001$). However, a repeated measures ANOVA of the period Day 5 (last day of the infusion) through 8 (last day of the experiment) was also done. It revealed a significant treatment regimen effect ($F=10.204$, $p=0.002$), a significant day effect (78.748, $p=<0.0001$), and a significant day x treatment regimen effect ($F=32.430$, $p=<0.0001$). Post hoc testing showed no significant values for all the contrasts on the day designated 0 (day prior to single dose of JDTic) and Day 1 (body weights 24 h after JDTic was given). By the end of Day 2 (first day of infusion), significant increases were found in all the groups receiving morphine (Vehicle/morphine and JDTic/morphine) compared to controls. The same results were obtained on Day 3. No significant comparisons were evident on infusion Days 4 and 5. Significant decreases in body weight were noted on Day 6 (24 h abrupt withdrawal) for the contrasts JDTic/vehicle and JDTic/morphine compared to controls. Significant decreases in body weight were also calculated on Days 7 and 8 (48 and 72 h abrupt withdrawal, respectively) for all the groups receiving morphine.

In summary, morphine significantly increased body weight on Days 2 and 3. Although the body weights of rats receiving JDTic/morphine were higher each day than those of rats receiving vehicle/-morphine during the infusion phase, the difference was not significant. Nevertheless, this trend is

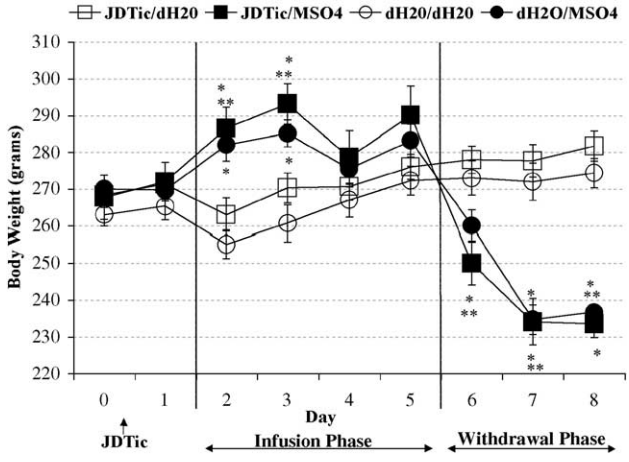

Fig. 2. Body weight in rats pretreated with JDTic or vehicle on Day 1, infused with morphine or vehicle on Days 2, 3, 4, and 5, and after abrupt withdrawal of morphine on Days 6, 7, and 8. *Significantly different from vehicle/vehicle group; **Significantly different from JDTic/vehicle group. $P=0.05$ or less. Vehicle controls: $N=6$ and morphine-treated rats: $N=7$.

probably related to an antidiuretic effect associated with κ-opioid receptor antagonists. However, during withdrawal the JDTic/morphine group lost as much weight as the vehicle/morphine controls. Thus, JDTic was without effect on this aspect of withdrawal.

The data for the withdrawal sign designated wet-dog shakes are illustrated in Fig. 3A. ANOVA of the data on Day 5 (end of infusion) through Day 8 (72 h withdrawal) indicates significant differences for treatment regimens ($F=15.920$, $p<0.0001$) and days ($F=25.762$, $p<0.0001$) and a day/treatment interaction ($F=10.015$, $p<0.0001$). Scheffe's post hoc test indicated no significant differences for any of the treatment contrasts on Days 5, 6, or 8. However, on Day 7 (48 h post withdrawal), significant differences ($p=0.05$ or less) were found for the following treatment comparisons: (1) vehicle/morphine group compared to vehicle/vehicle group, (2) JDTic/vehicle group compared to vehicle/morphine group, (3) JDTic/morphine group compared to vehicle/morphine group, and (4) JDTic/morphine group compared to vehicle/vehicle group. JDTic significantly lowered the number of wet-dog shakes expressed during withdrawal.

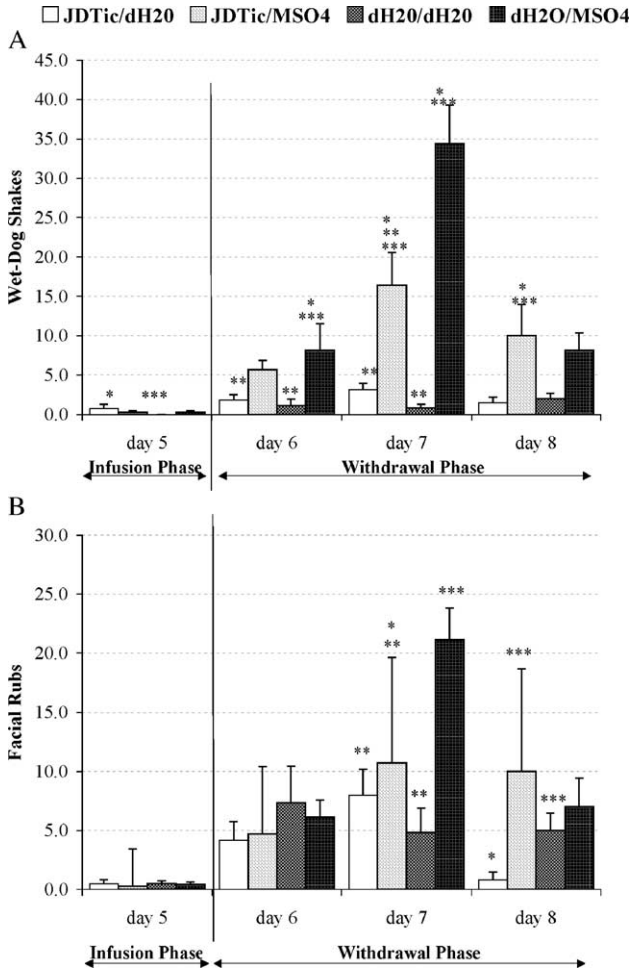

Fig. 3. (A) Wet-dog shakes in rats pretreated with JDTic or vehicle on Day 1, infused with morphine or vehicle on Days 2, 3, 4, and 5, and after abrupt withdrawal of morphine on Days 6, 7 and 8. (B) Facial rubs in rats pretreated with JDTic or vehicle on Day 1, infused with morphine or vehicle on Days 2, 3, 4, and 5, and after abrupt withdrawal of morphine on Days 6, 7, and 8. *Significantly different from vehicle/vehicle group; **Significantly different from vehicle/morphine group; ***Significantly different from JDTic/vehicle group. $P=0.05$ or less. Vehicle controls: $N=6$ and morphine-treated rats: $N=7$.

The results obtained with the withdrawal sign designated facial rubs are depicted in Fig. 3B. ANOVA of the data encompassing Day 5 (end of infusion) through Day 8 (72 h withdrawal) showed significant differences for treatment regimens ($F=7.969$, $p<0.0009$); days ($F=22.230$, $p<0.0001$) and the day/treatment interaction ($F=4.412$, $p<0.0002$). There were no significant differences among comparisons of treatment contrasts for Days 5 and 6. The Scheffe test revealed significant differences ($p=0.05$ or less) on day 7 for the following contrasts: (1) JDTic/vehicle compared to vehicle/morphine, (2) JDTic/morphine compared to vehicle/morphine, and (3) vehicle/morphine compared to vehicle/vehicle. In addition, comparison of the data for the contrasts JDTic/vehicle compared to JDTic morphine also indicated significant differences on Day 8. JDTic significantly reduced the number of facial rubs expressed during abrupt withdrawal in rats physically dependent on morphine.

Regarding irritability, some withdrawal-induced irritability was detected (Day 6 or 24-h withdrawal); and the tied p-value for the Kruskal–Wallis test was 0.0282, indicating a significant difference among treatments. However, the Mann–Whitney test (post hoc) failed to show a significant difference between any of the contrasts including JDTic/morphine and vehicle/morphine indicating a lack of effect by JDTic on this parameter.

4. Discussion

There is impressive evidence that physical dependence is an important determinant in situations involving compulsive drug abuse, especially if withdrawal from a substance produces very unpleasant symptoms, as is the case with opioids. For the most part, the expression of physical dependence in the rat and humans involves secretory, autonomic and motor signs. We chose the signs wet-dog shakes and facial rubs in the rat model because they reflect the motor component of withdrawal and are associated with important brain areas common to rats and humans such as the locus coeruleus, nucleus raphe magnus and anterior hypothalamus. Furthermore, the neuroanatomical brain sites of motor signs linked with the expression of physical dependence in the rat were different from those associated with the rewarding properties of opiate dependence (Maldonado et al., 1992).

Studies with mice that were μ-opioid receptor deficient showed that the analgesic activity as well as other pharmacological properties associated with morphine such as reward, withdrawal, respiratory depression, and constipation were abolished (Kieffer, 1999). Somewhat surprisingly, μ-opioid receptor-deficient mice also did not show cocaine place preference and no longer found other abused drugs such as alcohol, nicotine, and delta-9-tetrahydrocannabinol (Δ^9-THC) rewarding (Contet et al., 2004). Support for the use of κ-opioid receptor antagonists in the treatment of substance abuse was provided by Gaveriaux-Ruff and Kieffer (2002). They noted that morphine withdrawal was attenuated in κ-opioid receptor-deficient mice.

Maldonado and others (Maldonado et al., 1992) precipitated a withdrawal syndrome in rats dependent on morphine after the i.c.v. administration of μ-, or δ̃, or κ-selective opioid receptor antagonists, D–phe–Cys–Tyr–O–trp–Arg–Thr–Pen–Thr–NH2 (CTAP), naltrindole and nor-BNI, respectively. These authors found that although μ-opioid receptors played a crucial role in the expression of central opiate dependence, nor-BNI elicited a significantly increased number of 5 of 14 withdrawal signs. Spanagel et al. (1994) also reported that nor-BNI given by the i.c.v. route of administration potentiated the number of most of the overt withdrawal signs (Spanagel et al., 1994). Along these lines, Cui et al. (2000) reported that intrathecal administration of nor-BNI to morphine-dependent rats resulted in exacerbation of the number of withdrawal signs designated wet-dog shakes, teeth chattering, and weight loss.

In our study, in contrast to the results reported above for nor-BNI, JDTic lowered significantly the wet-dog and facial rub scores in the JDTic-pretreated rats infused with morphine.

Several authors have reported that brain levels of dynorphin are elevated by chronic exposure to cocaine as well as amphetamines and morphine (Sivam, 1989; Smiley et al., 1990; Trujillo and Akil, 1989; Trujillo et al., 1990). It is possible that the hyperactivity of κ opioid receptor neurons may underline the motivational incentive to use drugs of abuse. Withdrawal from the drugs results in dysphoric mood abnormalities similar to that seen after administration of the κ-opioid agonists ketocyclazocine and cyclazocine (Kumor et al., 1986; Pfeiffer et al., 1986). This suggests that the increased levels of dynorphin resulting from the hyperactivity of the κ-opioid receptor system may contribute to the dysphoric mood abnormalities associated with cocaine withdrawal.

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 receptor 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 receptor agonists to normalize mood (Rothman et al., 2000). This κ-opioid overdrive could be normalized by κ-opioid receptor antagonists; however, at the present time there are no clinically available selective κ̃-opioid receptor antagonists on which to test this hypothesis. The potential effectiveness of a functional κ̃-opioid receptor antagonist in the treatment of opiate dependence was tested by conducting an open-label study using buprenorphine, which is a partial μ-opioid receptor agonist and κ̃-opioid receptor antagonist, combined with the moderately selective μ-opioid receptor antagonist naltrexone to block the μ̃-opioid receptor agonist effects (Rothman et al., 2000). In fact, the combination of buprenorphine and naltrexone helped ameliorate withdrawal in opioid-dependent individuals (Rothman et al., 2000).

Viewed collectively, these results suggest that a κ-selective opioid antagonist such as JDTic may be useful for treating opiate addicts in withdrawal and possibly prevent relapse.

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