Published in final edited form as:

Pharmacol Biochem Behav. 2012 June ; 101(4): 581–587. doi:10.1016/j.pbb.2012.03.006.

The Long-Lasting Effects of JD_{Tic}, a Kappa Opioid Receptor Antagonist, on the Expression of Ethanol-Seeking Behavior and the Relapse Drinking of Female Alcohol-Preferring (P) Rats

Gerald A. Deehan Jr.¹, David L. McKinzie², F. Ivy Carroll³, William J. McBride¹, and Zachary A. Rodd¹

¹Department of Psychiatry, Institute of Psychiatric Research, Indiana School of Medicine, and Department of Psychology, Purdue School of Science, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46202

²Eli Lilly and Company, Research Triangle Park, NC 27709

³Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC 27709

Abstract

The current study assessed the effects of the selective kappa opioid antagonist JD_{Tic} on alcohol (EtOH) -seeking behavior, EtOH relapse, and maintenance responding for EtOH. Adult alcohol-preferring (P) rats were trained in 2-lever operant chambers to self-administer 15% EtOH (v/v) on a fixed-ratio 5 (FR-5) and water on a FR-1 schedule of reinforcement during 1-hr sessions. After 10 weeks, rats underwent extinction training for seven sessions. Rats were then maintained in their home cages for 3 weeks without EtOH access. All rats received an injection (s.c.) of 0, 1, 3, or 10 mg/kg JD_{Tic} (n = 11–14/group) after the first week of the home cage period. Rats were then tested using the Pavlovian Spontaneous Recovery paradigm (PSR; an animal model of alcohol-seeking) for four sessions during which, responses on the EtOH and water levers were recorded but did not produce their respective reinforcer. Following PSR testing rats were returned to their home cages without access to EtOH for one week prior to the start of EtOH relapse testing. To examine EtOH relapse responding, rats were returned to the operant chambers and the EtOH (FR5) and water (FR1) levers were active. Finally, rats were then tested over 17 operant sessions to assess the effects of JD_{Tic} on maintenance responding for EtOH. Rats received 0, 1, 3, or 10 mg/kg JD_{Tic} (counterbalanced from the initial experiment) 30 minutes prior to the initial maintenance session. JD_{Tic} administered 14 and 25 days prior to testing dose-dependently reduced the expression of an EtOH PSR and relapse responding. In contrast, JD_{Tic} did not alter EtOH responding under maintenance conditions. Overall, the results of this study indicate that different mechanisms mediate EtOH self-administration under relapse and maintenance conditions and kappa opioid receptors are involved in mediating EtOH-seeking behavior and relapse responding but not on-going EtOH self-administration.

© 2012 Elsevier Inc. All rights reserved.

Address Correspondence to: Gerald A. Deehan Jr. Ph.D., gdeehan@iupui.edu, Indiana University School of Medicine, Institute of Psychiatric Research, 791 Union Drive, Indianapolis, IN 46202-4887 USA.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

ethanol self-administration; ethanol relapse; ethanol-seeking; Pavlovian Spontaneous Recovery; JDTic; kappa opioid receptor

1. Introduction

The neurological mechanisms that underlie the dysfunctional behaviors associated with alcohol (EtOH) abuse and alcoholism are complex. Despite extensive preclinical research that has evaluated numerous neurotransmitter systems and proof-of-concept clinical trials to determine the efficacy of putative treatments in high EtOH drinking populations, relatively few pharmacological treatments for EtOH dependence have been approved for use in the United States and throughout the world (Johnson and Ait-Daoud, 2000). Among the most studied approved pharmacotherapeutic agents for alcohol addiction is the non-selective opioid antagonist naltrexone which, has been reported to improve abstinence rates, decrease the impulse to initiate drinking, reduce the hedonic effects of alcohol, and reduce alcohol-induced positive mood (O'Malley et al., 1992; Volpicelli et al., 1992; Davidson et al., 1996). Although effect sizes relative to placebo responses in clinical trials have been modest, the therapeutic utility of naltrexone has consistently been demonstrated. For instance, recent data from the COMBINE study found that naltrexone significantly increased the length of abstinence in EtOH-dependent individuals (Anton et al., 2006) and reduced the probability that participants would fall into the consistent heavy drinking trajectory (Gueorguieva et al., 2010).

Preclinical data indicate that non-selective opioid antagonists robustly decrease free-choice EtOH consumption and/or the operant self-administration of EtOH in several alcohol-preferring rodent lines. For instance, Sable et al. (2006) reported that naltrexone was efficacious at reducing the expression of EtOH-seeking, relapse drinking, and maintenance EtOH self-administration during operant testing in both adolescent and adult alcohol-preferring (P) rats. Similarly, naloxone reduced EtOH intake in high-alcohol-drinking (Froehlich et al., 1990), P (Badia-Elder et al., 1999; Overstreet et al., 1999) and Alko-Alcoholic (Sinclair, 1990) rats. Naloxone also reduced EtOH intake in P rats under relapse conditions (i.e., after 2-weeks of EtOH deprivation; Badia-Elder et al., 1999). Naltrexone treatment has also been shown to reduce operant responding for EtOH (Middaugh et al., 1999; Middaugh et al., 2000), the consumption of EtOH (Middaugh & Bandy, 2000; Middaugh et al., 2003), as well as the expression of EtOH place conditioning (Middaugh & Bandy, 2000) in the EtOH-preferring C57BL/6 mouse line. Both naltrexone and naloxone bind to the three main opioid receptors (Mu: MOR, Delta: DOR, and kappa: KOR) and are believed to decrease EtOH intake by blocking EtOH-stimulated increases in endogenous opioid activity within the brain (for review see Gianoulakis, 2001). Although both naltrexone and naloxone possess a higher affinity for the MOR, studies utilizing selective opioid receptor subtype ligands have identified a significant contribution of each opioid receptor with regard to EtOH intake and reward (Di Chiara et al., 1996; Koob et al., 2003; Walker et al., 2011).

Recently, the KOR system has gained attention for its role in addiction to several drugs of abuse including EtOH (Shippenberg et al., 2007; Wee & Koob, 2010). The KOR system is believed to function in the rewarding properties of EtOH, mainly through the mediation of DA signaling in the reward pathway. Specifically, KORs are located pre-synaptically on dopamine (DA) neurons in the nucleus accumbens (ACB) near the DA transporter, and serve to regulate the release and uptake of DA (Svingos et al., 2001). KORs are selectively sensitive to the dynorphin class of opioid peptides and studies have shown that acute EtOH

exposure produces an enduring increase in dynorphin levels and an increased sensitivity of KORs within the ACB (Lindholm et al., 2000; Lindholm et al., 2007). Manipulation of the KOR system, via local or systemic administration of KOR agonists, reduces basal levels of extracellular DA in the ACB (Spanagel et al., 1990) and inhibits cocaine- or morphine-evoked DA release in the ACB (Maisonneuve et al., 1994; Spanagel et al., 1994). Conversely, selective KOR antagonists, such as nor-BNI, increase extracellular DA levels in the ACB (Spanagel et al., 1992) and decrease EtOH self-administration in EtOH-dependent rats (Walker & Koob, 2008; Walker et al., 2011). Rats that are not EtOH-dependent fail to exhibit similar decreases in EtOH self-administration when administered KOR antagonists (Doyon et al., 2006; Walker & Koob, 2008) which suggests that the DYN and KOR systems become functionally unregulated during the development of EtOH-dependence (Przewlocka et al., 1997; Lindholm et al., 2000; Lindholm et al., 2007; Walker & Koob, 2008). Interestingly, rodents genetically predisposed to consume EtOH exhibit differences in the expression of opioid receptors, as well as brain tissue levels of opioid peptides, which, have been postulated to contribute to their high EtOH intake and preference (Fadda et al., 1999; McBride & Li, 1998; Murphy et al., 2002). However, relatively little attention has focused on the effect of selective KOR antagonism on EtOH self-administration in the P line of rats.

JDTic is the first potent, selective KOR antagonist not derived from naltrexone or opiate compounds (c.f., Carroll et al., 2004). JDTic has subnanomolar affinity for the KOR and is functionally selective for the KOR at levels over 500-fold and 16,000-fold than the MOR and the DOR respectively (Thomas et al., 2001). *In vivo* studies have shown that JDTic blocks KOR agonist-induced diuresis and antinociception for 2–4 weeks depending on the species and assay used (Carroll et al., 2004). In addition to the long-lasting pharmacological properties, JDTic exhibits antidepressant and anxiolytic-like effects, and has demonstrated efficacy in substance abuse and relapse models in male rats (Beardsley et al., 2005, 2010; Carroll et al., 2005; Knoll et al., 2007; Jackson et al., 2010).

The intent of this study was to examine the effects JDTic on operant EtOH self-administration, relapse, and EtOH-seeking behavior of P rats. Specifically, alcohol-seeking was tested through Pavlovian Spontaneous Recovery (PSR), the recovery of responding, in the absence of the previously trained reward, which, is observed following a period of rest after extinction (Domjan and Burkard, 1982; Macintosh, 1977). PSR is a measure of the relative strength of reinforcer-seeking behavior. In P rats, the expression of an EtOH PSR is enhanced following periadolescent alcohol drinking, exposure to EtOH odor during PSR testing, and EtOH priming (Rodd-Henricks et al., 2002a, b). Thus, the PSR model is an established animal model of craving-like behavior (c.f., Rodd et al., 2004). In addition, EtOH relapse responding, as well as maintenance responding, for EtOH were assessed. It was hypothesized that JDTic would act to decrease EtOH-seeking behavior, EtOH relapse responding, as well as the EtOH maintenance responding of P rats.

2. Materials and Methods

2.1 Animals

Adult female P rats from the 55th – 57th generations weighing 250–325g at the start of the experiment were used (n = 49). Rats were maintained on a 12-hr reversed light-dark cycle (lights off at 0900 hr). Food and water were available *ad libitum* throughout the experiment, except during operant testing. The animals used in these experiments were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the Indiana University Institutional Animal Care and Use Committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory*

Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council 1996).

Female rats were used in the current study because of availability. Past studies have indicated that male and female P rats express comparable levels of EtOH-seeking in the PSR model, and in response to cannabinoid agents of EtOH-seeking (Getachew, 2010). Estrus cycle determination was not tested in the current experiment. Vaginal smear testing is stressful to female rats during the initial days of estrus testing (Sfikakis et al., 1996). Vaginal smears in P rats can alter numerous alcohol-related behaviors (Rodd unpublished findings). Females in estrus may display greater drug-seeking behavior (Kippin et al., 2005), but testing for estrus also enhances drug-seeking behaviors in unselected rats (Nofrey et al., 2008). Therefore, estrus cycle was allowed to be a random factor that acted to increase within group variance.

2.2 Operant Procedures

Ethanol self-administration was conducted in standard two-lever operant chambers (Coulbourn Instruments) that were contained within ventilated, sound-attenuated environmental boxes. Within each chamber, two levers were located on the same wall 15 cm above a metal grid floor and 13 cm apart. A dipper trough was located immediately below each lever where a 0.1 ml dipper cup was presented to deliver response-contingent fluid. A small dipper trough light illuminated for the 4 s period while the dipper was raised. All operant chamber functions were controlled by a personal computer which, also recorded all lever responses and dipper presentations. Levers associated with 15% (v/v) EtOH or water were counterbalanced among rats but remained constant for each animal. Operant sessions were 60 min in duration and were conducted daily. Without any prior training, exposure to the experimental set-up, or access to EtOH, rats were placed into the operant chambers. The EtOH and water levers were maintained on a fixed-ratio 1 (FR1) schedule of reinforcement for the first 5 weeks. Subsequently, the reinforcement schedule on the EtOH lever was increased to FR3 in weeks 6 and 7 and to FR5 in weeks 8–10. Water was always reinforced on an FR1 schedule. The number of responses on the EtOH and water levers and the number of EtOH and water reinforcements were recorded. Following approximately 10 weeks of EtOH self-administration, P rats underwent extinction training whereby responses on. Rats were exposed to extinction training for 7 sessions. After extinction training, rats were maintained in the home cages for 21 days (imposed abstinence period). All rats were then returned to the operant chambers for PSR testing whereby both levers and dippers were once again active but EtOH and water remained absent. Rats were exposed to the PSR testing for 4 sessions.

2.3 The Effects of JDITic on responding for EtOH during the PSR test and during relapse and maintenance self-administration

JDITic was provided by Research Triangle Institute (Research Triangle Park, North Carolina). The drug was dissolved in saline. Seven days after the final extinction session (14 days prior to PSR testing), rats received a single s.c. injection of 0, 1, 3, or 10 mg/kg JDITic ($n = 11\text{--}14/\text{group}$) and were returned to their home cages and left undisturbed, except for normal husbandry activities, for the next 2 weeks. Rats then underwent 4 PSR test sessions after which, they were maintained in their home cages for an additional 7 days. Rats were then transferred to the operant chambers with both EtOH and water available for 60-min sessions to examine EtOH self-administration under relapse conditions. Note that the first EtOH relapse session occurred 25 days after the single JDITic administration.

Following EtOH relapse testing and an additional 16 consecutive daily sessions of operant access to EtOH and water, the effects of JDITic on EtOH maintenance responding was

tested. Rats were administered a second dose of JD_{Tic} (0, 1, 3, or 10 mg/kg s.c.) 30 min prior to the first maintenance operant test session. Operant responding data were recorded for 17 consecutive sessions (days) following JD_{Tic} administration. Drug treatment groups for the second JD_{Tic} injection were counterbalanced based upon initial drug assignment. For example, rats receiving the 1 mg/kg JD_{Tic} dose at the first administration were equally divided between all 4 dose groups during maintenance testing.

2.4 Statistical Analyses

Operant responding (60 min) data were analyzed with a mixed factorial ANOVA with a between-subject factor of Dose and a repeated-measure of Session. For the PSR experiments, the baseline measure for the factor of Session was the average number of responses on the EtOH lever for the last 3 extinction sessions. For the deprivation studies, the baseline measure for the factor of Session was the average number of responses on the EtOH lever for the 3 sessions immediately prior to deprivation. Baseline measure for the maintenance experiment was the 3 sessions immediately prior to JD_{Tic} testing. When appropriate, Tukey's b post-hoc comparisons were performed to determine individual differences.

3. Results

3.1 Effects of JD_{Tic} Administration on EtOH Lever Responding in the PSR Test—Under Relapse Conditions

P rats were self-administering on average 2.4 ml of 15% EtOH (estimated 1.1 ± 0.1 g/kg/session) prior to the start of extinction training. JD_{Tic} administered 14 days prior to testing, dose-dependently reduced responding on the EtOH lever during PSR testing. Examining the number of responses on the lever previously associated with the delivery of EtOH (Fig. 1a) indicated a significant effect of Session ($F_{4, 42} = 25.5$; $p < 0.001$), Dose ($F_{3, 45} = 6.9$; $p < 0.001$), and a Session by Dose interaction ($F_{12, 132} = 3.1$; $p < 0.001$). Decomposing the interaction term by performing individual ANOVAs on each session indicated that there was a significant effect of Dose during the initial PSR session ($F_{3, 45} = 13.5$; $p < 0.001$). Post-hoc comparisons indicated that rats administered saline responded more during the initial PSR test session compared to rats administered 1, 3, or 10 mg/kg JD_{Tic}. There were no significant effects of dose during the other 3 PSR test sessions (p values > 0.37). Rats administered saline or 1 mg/kg JD_{Tic} exhibited significantly higher levels of responding on the EtOH lever during the initial PSR test session compared to extinction baseline levels (p values < 0.047). The low levels of responding on the water lever was significantly altered during PSR testing (Fig. 1b; Session ($F_{4, 42} = 3.7$; $p = 0.01$), Dose ($F_{3, 45} = 1.3$; $p = 0.27$), Session by Dose interaction ($F_{12, 132} = 2.1$; $p = 0.023$). However, these effects were observed because of an increase in responding on the water lever during the 2nd PSR session by rats administered 10 mg/kg JD_{Tic}, but t -tests revealed that this level of responding was not significantly different from baseline ($p > 0.52$).

JD_{Tic} administered 25 days prior to relapse testing reduced responding on the EtOH lever during the first relapse session (Fig. 2a). Examining the number of responses on the EtOH lever indicated a significant effect of Session ($F_{4, 42} = 8.1$; $p < 0.001$) and a Session by Dose interaction ($F_{12, 132} = 3.4$; $p < 0.001$). Decomposing the interaction term by performing individual ANOVAs on each session indicated that there was a significant effect of Dose during the initial two relapse sessions (p values < 0.05). Post-hoc comparisons indicated that rats administered saline responded more during the initial relapse test session compared to rats administered 1, 3, or 10 mg/kg JD_{Tic}. Post-hoc comparisons indicated that there were no significant differences between groups during the 2nd reinstatement session. There were no significant differences during the 3rd and 4th reinstatement sessions (p values > 0.31). In

saline treated rats, the estimated EtOH intake during the 1st reinstatement session was greater than that observed prior to deprivation (1.7 ± 0.2 vs. 1.1 ± 0.1 g/kg). In the JD_{Tic} treated rats, the estimated EtOH intake during the 1st reinstatement session was reduced compared to that observed prior to deprivation (0.7 ± 0.2 vs. 1.1 ± 0.1 g/kg). Statistically, an analysis performed on estimated g/kg paralleled the findings observed for EtOH level responding.

With respect to the low water lever responding (Fig. 2b), there were significant effects of Session ($F_{4, 42} = 6.5$; $p < 0.001$) and a Session by Dose interaction ($F_{12, 132} = 1.8$; $p = 0.049$) under relapse conditions. The significant differences observed were based upon an increase in water responding, primarily during sessions 4 and 5, in rats administered 10 mg/kg JD_{Tic}. However, responding for water was low and this late experimental effect is most likely a spurious result produced by a floor effect. To date, reports have consistently indicated that administration of KOR agents do not alter water self-administration (June et al., 1998; 2004; Walker and Koob, 2008; Nealey et al., 2011).

3.2 Effects of JD_{Tic} Administration on EtOH Maintenance Responding

Administration of JD_{Tic} did not alter EtOH self-administration during maintenance testing. Examining the number of responses on the EtOH lever (Fig. 3a) for 17 consecutive sessions following the single administration of JD_{Tic} revealed no significant effect of Session ($F_{17, 19} = 1.25$; $p = 0.32$) Dose ($F_{3, 35} = 1.0$; $p = 0.39$), or a Session by Dose interaction ($F_{51, 63} = 1.2$; $p = 0.18$). During this time period, administration of JD_{Tic} did not alter water responding (Fig. 3b; all p values > 0.14). The estimated intake prior to JD_{Tic} administration (estimated 1.2 ± 0.1 g/kg/session) did not differ from the estimated intake following JD_{Tic} treatment (estimated 1.3 ± 0.3 g/kg/session; all p values > 0.44).

4. Discussion

The data indicate selective effects of JD_{Tic} on different aspects of EtOH self-administration behavior. A single injection of JD_{Tic} (all doses tested) 14 days prior to testing reduced the expression of EtOH-seeking in P rats, as measured in the PSR test. Additionally, the same single injection of JD_{Tic} (given 25 days before) suppressed responding on the EtOH lever under relapse conditions. To our knowledge, this is the first example of JD_{Tic} demonstrating a long-lasting effect on EtOH-seeking or EtOH relapse behavior. Previous research demonstrated that JD_{Tic} is pharmacologically active for greater than 21 days following a single subcutaneous injection (Beardsley et al., 2005) and up to 28 days following intra-gastric administration (Carroll et al., 2004). However, for the current research, it is uncertain whether the single administration of JD_{Tic} reduced EtOH-seeking and EtOH relapse behavior by preventing the development of the biological basis of these two phenomena, reducing the expression of these behaviors through a reduction in lever responding, or a combination of the two (development/expression). Given that JD_{Tic} did not significantly decrease operant responding on the water lever during both PSR and relapse testing nor did it produce a significant decrease in responding on the EtOH or water levers during maintenance responding, it would seem that the effect of JD_{Tic} on EtOH-seeking and EtOH-relapse is altering the biological basis of these two phenomena.

The lack of an effect of JD_{Tic}, at any dose, on maintenance responding for EtOH is similar to past findings that the KOR antagonist nor-binaltorphimine (nor-BNI) failed to alter operant responding for EtOH in rats and monkeys that were in a non-dependent state (Doyon et al., 2006; Williams and Woods, 1998). However, research has shown that nor-BNI does reduce operant responding for EtOH in dependent animals (Walker & Koob, 2008; Walker et al., 2011). The selective efficacy of nor-BNI was in contrast to that of naltrexone which, also reduced EtOH intake in non-dependent rats (Walker & Koob, 2008).

Further, microinjections of KOR agents into the nucleus accumbens shell reduced EtOH consumption in rats with prior vapor chamber exposure to EtOH (Nealey et al., 2011). Thus, it has been hypothesized that the KOR system may not be integral to all processes associated with EtOH reward, but rather undergoes alterations as part of the neuroadaptations underlying EtOH dependence (Walker & Koob, 2008). It is not known if P rats under the current operant conditions developed EtOH dependence. However, ongoing research has made progress in elucidating the distinct neurochemical system(s) that contribute to EtOH-seeking and/or EtOH relapse. For instance, Rodd et al. (2006) reported that activation of the metabotropic glutamate (mGlu) 2/3 receptors inhibited the expression of EtOH-seeking as well as EtOH relapse behavior but did not alter maintenance EtOH self-administration (Rodd et al., 2006). This finding relates directly to the current research as KORs mediate the release of excitatory glutamate in the shell region of the ACB (Hjelmstad and Fields, 2001). On the other hand, exposure to an orexin-1 receptor antagonist selectively decreased EtOH relapse behavior immediately following abstinence but did not affect EtOH-seeking (Dhaer et al., 2010). Thus, the mechanisms underlying EtOH-seeking behavior and EtOH relapse behavior appear to be separable, and support the idea that different mechanisms may mediate relapse and ongoing alcohol drinking. Further research will be needed to tease apart the complex interaction between contributory neurochemical systems.

Alcohol-seeking (i.e., craving) and relapse possess a significant motivational learning component (Heinz et al., 2009). Several studies have shown that neutral discrete and contextual stimuli that have been previously associated with alcohol, serve to enact behaviors that were associated with alcohol consumption (i.e. operant responding) without alcohol being present or available (for review see: Heinz et al., 2009). Research attempting to correlate such learned associations with neurological functioning has shown motivational drug learning/associations to be heavily dependent on neural signaling within the extended mesolimbic DA reward pathway (Spanagel & Weiss, 1999). Within the mesolimbic pathway, there are several secondary neurochemical/receptor systems that have been shown to alter DA release, in turn altering drug related behaviors (for review see: McBride et al., 1999). For instance, KORs have been found to regulate the release of DA within the ACB. KOR agonists have been found to decrease DA efflux in the ACB (DiChiara & Imperato, 1988; Donzanti et al., 1992; Spanagel et al., 1992) whereas a limited amount of research suggests that KOR antagonists, such as nor-BNI, increase basal DA levels in the ACB (Maisonneuve et al., 1994; Spanagel et al., 1992). Furthermore, animals repeatedly exposed to EtOH exhibit an enduring increase in DYN tissue levels (Lindholm et al., 2000) as well as a greater sensitivity to nor-BNI compared to control animals (Lindholm et al., 2007). With respect to the current data, it is possible that JD_{Tic} reduced EtOH-seeking and -relapse by altering the mesolimbic DA system via KOR functioning, which, in turn altered learned EtOH associations/behaviors. However, it is unlikely that mediation of DA signaling by the KOR is solely responsible for the decrease in both EtOH-relapse and -seeking observed in the current study.

The KOR system has been hypothesized to oppose the action of the MOR system in a modulatory manner (Nealey et al., 2011; Walker & Koob, 2008; Wee & Koob, 2010). The action of KOR agonists in inhibiting DA release within the ACB is in direct contrast to the action of MOR agonists which increase accumbal DA levels through direct action within the ACB (Di Chiara & Imperato, 1998) as well as the disinhibition of DA neurons in the VTA (Johnson & North, 1992). This concept of KOR functioning opposing MOR functioning is an important focus in the field of addiction research as it is theorized that the primary reinforcement of virtually every major drug of abuse within the opioid system occurs, either directly or indirectly, through the MOR system (for review see: Contet et al., 2004). Much like major drugs of abuse, MOR agonists produce reinforcing effects and are readily self-administered directly into the VTA (Devine & Wise, 1994) and produce conditioned place

preferences in rodents (for review see: Shippenberg et al., 1992). On the other hand, KOR agonists reduce the rewarding properties of intra-cranial self-stimulation (Todtenkopf et al., 2004) and produce place aversions (Mucha & Herz, 1985). However, recent research suggests that the KOR system does possess a therapeutic benefit beyond modulating the activity of the MOR system on DA release. Clinical research has found that the combined pharmacotherapy of buprenorphine (partial MOR agonist/KOR antagonist) and naltrexone is more efficacious in patients suffering from opiate addiction than naltrexone alone (Gerra et al., 2006). The combination of naltrexone and buprenorphine significantly decreases the MOR activity while maintaining KOR antagonism (Gerra et al., 2006) further supporting a role for selective KOR antagonism in drug addiction treatment.

The current study showed that JDTC was effective at decreasing EtOH-seeking and EtOH relapse in an animal model for alcoholism. While this effect did not carry over to EtOH maintenance responding, it is believed that JDTC may represent a novel pharmacological compound that will aid in the development of treatments for individuals that are EtOH-dependent and/or suffering from alcoholism. A large majority of individuals suffering from alcoholism, that attempt abstinence, suffer relapse while attempting detoxification (Heinz et al., 2009). The profile of a KOR antagonist such as JDTC would be efficacious in the population of human alcoholics in many ways. Both stress and depression contribute to EtOH craving as well as relapse rates in human alcoholics (Farren & McElroy, 2010; Sinha, 2007). Given the anxiolytic and antidepressive properties of KOR antagonists (Knoll & Carlezon, 2010), such a pharmacological profile would be advantageous for treating comorbid dysphoric states associated with alcohol dependence and perhaps prevent stress-precipitated alcohol relapse. Additionally, since JDTC, as well as other KOR antagonists possess an extended period of action and are in some cases additive in nature, the compliance of individuals taking the drug may be higher due to the fewer number of doses needed. Thus, the development of a pharmacotherapy based on the profile of JDTC/KOR antagonists might possess a greater ability to decrease relapse rates in individuals suffering from alcoholism/alcohol abuse.

Highlights

>The Kappa opioid receptor mediates some alcohol related behaviors.>We investigated the effect of JDTC on alcohol related behaviors.>JDTC decreased alcohol seeking and relapse but not maintenance responding.>The Kappa opioid receptor mediates alcohol seeking and relapse but not maintenance.>Kappa opioid receptors represent therapeutic targets for the treatment of alcoholism.

Acknowledgments

This research was supported by the National Institutes of Health grants AA07611, AA07462, and AA10721 from the National Institute on Alcohol Abuse and Alcoholism, and DA09045 from the National Institute on Drug Abuse. The authors would like to thank the Research Triangle Institute (Research Triangle Park, North Carolina) and Eli Lilly for generously supplying the JDTC to be used in this research. The authors would also like to thank Tylene Pommer for her expert technical assistance with the completion of this research.

References

- Anton RF, O'Malley SS, Ciraulo DA, Cisler RA, Couper D, Donovan DM, et al. Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. *JAMA*. 2006; 295:2003–2017. [PubMed: 16670409]
- Badia-Elder NE, Mosemiller AK, Elder RL, Froehlich JC. Naloxone retards the expression of a genetic predisposition toward alcohol drinking. *Psychopharmacology (Berl)*. 1999; 144:205–212. [PubMed: 10435386]

- Beardsley PM, Howard JL, Shelton KL, Carroll FI. Differential effects of the novel kappa opioid receptor antagonist, JD_{Tic}, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. *Psychopharmacology (Berl)*. 2005; 183:118–126. [PubMed: 16184376]
- Beardsley PM, Pollard GT, Howard JL, Carroll FI. Effectiveness of analogs of the kappa opioid receptor antagonist (3R)-7-Hydroxy-N-((1S)-1-[(3R,4R)-4-(3-hydroxyphenyl)-3,4dimethyl-1-piperidinyl]methyl)-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JD_{Tic}) to reduce U50,488-induced diuresis and stress-induced cocaine reinstatement in rats. *Psychopharmacology (Berl)*. 2010; 210:189–198. [PubMed: 20372878]
- Carroll FI, Harris LS, Aceto MD. Effects of JD_{Tic}, a selective kappa-opioid receptor antagonist, on the development and expression of physical dependence on morphine using a rat continuous-infusion model. *Eur J Pharmacol*. 2005; 524:89–94. [PubMed: 16236279]
- Carroll I, Thomas JB, Dykstra LA, Granger AL, Allen RM, Howard JL, et al. Pharmacological properties of JD_{Tic}: a novel kappa-opioid receptor antagonist. *Eur J Pharmacol*. 2004; 501:111–119. [PubMed: 15464069]
- Contet C, Kieffer BL, Befort K. Mu opioid receptor: a gateway to drug addiction. *Curr Opin Neurobiol*. 2004; 14:370–378. [PubMed: 15194118]
- Davidson D, Swift R, Fitz E. Naltrexone increases the latency to drink alcohol in social drinkers. *Alcohol Clin Exp Res*. 1996; 20:732–739. [PubMed: 8800392]
- Devine DP, Wise RA. Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *J Neurosci*. 1994; 14:1978–1984. [PubMed: 8158252]
- Dhaer R, Hauser SR, Getachew B, Bell RL, McBride WJ, McKinzie DL, Rodd ZA. The Orexin-1 Receptor Antagonist SB-334867 Reduces Alcohol Relapse Drinking, but not Alcohol-Seeking, in Alcohol-Preferring (P) Rats. *J Addict Med*. 4:153–159. [PubMed: 20871792]
- Di Chiara G, Acquas E, Tanda G. Ethanol as a neurochemical surrogate of conventional reinforcers: the dopamine-opioid link. *Alcohol*. 1996; 13:13–17. [PubMed: 8837928]
- Di Chiara G, Imperato A. Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. *J Pharmacol Exp Ther*. 1988; 244:1067–1080. [PubMed: 2855239]
- Domjan, M.; Burkhard, B. The principles of learning and behavior. Monterey, CA: Brooks Cole Publishing; 1982.
- Donzanti BA, Althaus JS, Payson MM, Von Voigtlander PF. Kappa agonist-induced reduction in dopamine release: site of action and tolerance. *Res Commun Chem Pathol Pharmacol*. 1992; 78:193–210. [PubMed: 1282270]
- Doyon WM, Howard EC, Shippenberg TS, Gonzales RA. Kappa-opioid receptor modulation of accumbal dopamine concentration during operant ethanol self-administration. *Neuropharmacology*. 2006; 51:487–496. [PubMed: 16781738]
- Fadda P, Tronci S, Colombo G, Fratta W. Differences in the opioid system in selected brain regions of alcohol-preferring and alcohol-nonpreferring rats. *Alcoholism Clin Exp Res*. 1999; 23:1296–1305.
- Farren CK, McElroy S. Predictive factors for relapse after an integrated inpatient treatment programme for unipolar depressed and bipolar alcoholics. *Alcohol Alcohol*. 2010; 45:527–533. [PubMed: 20855410]
- Froehlich JC, Harts J, Lumeng L, Li TK. Naloxone attenuates voluntary ethanol intake in rats selectively bred for high ethanol preference. *Pharmacol Biochem Behav*. 1990; 35:385–390. [PubMed: 2320646]
- Fuentealba JA, Gysling K, Magendzo K, Andres ME. Repeated administration of the selective kappa-opioid receptor agonist U-69593 increases stimulated dopamine extracellular levels in the rat nucleus accumbens. *J Neurosci Res*. 2006; 84:450–459. [PubMed: 16676328]
- Gianoulakis C. Influence of the endogenous opioid system on high alcohol consumption and genetic predisposition to alcoholism. *J Psychiatry Neurosci*. 2001; 26:304–318. [PubMed: 11590970]
- Georgiueva R, Wu R, Donovan D, Rounsaville BJ, Couper D, Krystal JH, et al. Naltrexone and combined behavioral intervention effects on trajectories of drinking in the COMBINE study. *Drug Alcohol Depend*. 2010; 107:221–229. [PubMed: 19969427]

- Heinz A, Beck A, Grusser SM, Grace AA, Wrase J. Identifying the neural circuitry of alcohol craving and relapse vulnerability. *Addict Biol.* 2009; 14:108–118. [PubMed: 18855799]
- Institute of Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals. 7th ed.. Washington D.C.: National Academy Press; 1996.
- Jackson KJ, Carroll FI, Negus SS, Damaj MI. Effect of the selective kappa-opioid receptor antagonist JDTC on nicotine antinociception, reward, and withdrawal in the mouse. *Psychopharmacology (Berl)*. 210:285–294. [PubMed: 20232057]
- Johnson BA, Ait-Daoud N. Neuropharmacological treatments for alcoholism: scientific basis and clinical findings. *Psychopharmacology (Berl)*. 2000; 149:327–344. [PubMed: 10867960]
- Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci.* 1992; 12:483–488. [PubMed: 1346804]
- June HL, Cummings R, Eiler WJ, Foster KL, McKay PF, Seyoum R, et al. Central opioid receptors differentially regulate the nalmefene-induced suppression of ethanol- and saccharin-reinforced behaviors in alcohol-preferring (P) rats. *Neuropsychopharmacology*. 2004; 29:285–299. [PubMed: 14603266]
- June HL, Grey C, Warren-Reese C, Durr LF, Ricks-Cords A, Johnson A, et al. The opioid receptor antagonist nalmefene reduces responding maintained by ethanol presentation: preclinical studies in ethanol-preferring and outbred Wistar rats. *Alcohol Clin Exp Res.* 1998; 22:2174–2185. [PubMed: 9884166]
- Knoll AT, Carlezon WA Jr. Dynorphin, stress, and depression. *Brain Res.* 1314:56–73. [PubMed: 19782055]
- Knoll AT, Meloni EG, Thomas JB, Carroll FI, Carlezon WA Jr. Anxiolytic-like effects of kappa-opioid receptor antagonists in models of unlearned and learned fear in rats. *J Pharmacol Exp Ther.* 2007; 323:838–845. [PubMed: 17823306]
- Koob GF, Roberts AJ, Kieffer BL, Heyser CJ, Katner SN, Ciccocioppo R, et al. Animal models of motivation for drinking in rodents with a focus on opioid receptor neuropharmacology. *Recent Dev Alcohol.* 2003; 16:263–281. [PubMed: 12638642]
- Lindholm S, Ploj K, Frank J, Nylander I. Repeated ethanol administration induces short- and long-term changes in enkephalin and dynorphin tissue concentration in rat brain. *Alcohol.* 2000; 22:165–171. [PubMed: 11163124]
- Lindholm S, Rosin A, Dahlin I, Georgieva J, Franck J. Ethanol alters the effect of kappa receptor ligands on dopamine release in the nucleus accumbens. *Physiol Behav.* 2007; 92:167–171. [PubMed: 17610914]
- Macintosh, JJ. Stimulus control: attentional factors. In: Honig, WK.; Staddon, JER., editors. *Handbook of operant behavior*. Englewood Cliffs, NJ: Prentice-Hall; 1977. p. 162-241.
- Maisonneuve IM, Archer S, Glick SD. U50,488, a kappa opioid receptor agonist, attenuates cocaine-induced increases in extracellular dopamine in the nucleus accumbens of rats. *Neurosci Lett.* 1994; 181:57–60. [PubMed: 7898771]
- McBride WJ, Li TK. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol.* 1998; 12:339–369. [PubMed: 10348615]
- McBride WJ, Murphy JM, Ikemoto S. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res.* 1999; 101:129–152. [PubMed: 10372570]
- Middaugh LD, Bandy AL. Naltrexone effects on ethanol consumption and response to ethanol conditioned cues in C57BL/6 mice. *Psychopharmacology (Berl)*. 2000; 151:321–327. [PubMed: 11026738]
- Middaugh LD, Kelley BM, Cuison ER Jr, Groseclose CH. Naltrexone effects on ethanol reward and discrimination in C57BL/6 mice. *Alcohol Clin Exp Res.* 1999; 23:456–464. [PubMed: 10195818]
- Middaugh LD, Lee AM, Bandy AL. Ethanol reinforcement in nondeprived mice: effects of abstinence and naltrexone. *Alcohol Clin Exp Res.* 2000; 24:1172–1179. [PubMed: 10968654]
- Middaugh LD, Szumlanski KK, Van Patten Y, Marlowe AL, Kalivas PW. Chronic ethanol consumption by C57BL/6 mice promotes tolerance to its interoceptive cues and increases extracellular dopamine, an effect blocked by naltrexone. *Alcohol Clin Exp Res.* 2003; 27:1892–1900. [PubMed: 14691376]

- Mucha RF, Herz A. Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. *Psychopharmacology (Berl)*. 1985; 86:274–280. [PubMed: 2994144]
- Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Lumeng L, Li TK. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behav Genet*. 2002; 32:363–388. [PubMed: 12405517]
- Nealey KA, Smith AW, Davis SM, Smith DG, Walker BM. kappa-opioid receptors are implicated in the increased potency of intra-accumbens nalmefene in ethanol-dependent rats. *Neuropharmacology*. 2011; 61:35–42. [PubMed: 21338616]
- O'Malley SS, Jaffe AJ, Chang G, Schottenfeld RS, Meyer RE, Rounsaville B. Naltrexone and coping skills therapy for alcohol dependence. A controlled study. *Arch Gen Psychiatry*. 1992; 49:881–887. [PubMed: 1444726]
- Overstreet DH, Kampov-Polevoy AB, Rezvani AH, Braun C, Bartus RT, Crews FT. Suppression of alcohol intake by chronic naloxone treatment in P rats: tolerance development and elevation of opiate receptor binding. *Alcohol Clin Exp Res*. 1999; 23:1761–1771. [PubMed: 10591592]
- Rodd ZA, Bell RL, Sable HJ, Murphy JM, McBride WJ. Recent advances in animal models of alcohol craving and relapse. *Pharmacol Biochem Behav*. 2004; 79:439–450. [PubMed: 15582015]
- Rodd ZA, McKinzie DL, Bell RL, McQueen VK, Murphy JM, Schoepp DD, McBride WJ. The metabotropic glutamate 2/3 receptor agonist LY404039 reduces alcohol-seeking but not alcohol self-administration in alcohol-preferring (P) rats. *Behav Brain Res*. 2006; 171:207–215. [PubMed: 16678921]
- Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, et al. Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats: I. Periadolescent exposure. *Alcohol Clin Exp Res*. 2002a; 26:1632–1641. [PubMed: 12436051]
- Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, et al. Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats: II. Adult exposure. *Alcohol Clin Exp Res*. 2002b; 26:1642–1652. [PubMed: 12436052]
- Sable HJ, Bell RL, Rodd ZA, McBride WJ. Effects of naltrexone on the acquisition of alcohol intake in male and female periadolescent and adult alcohol-preferring (P) rats. *Int J Adolesc Med Health*. 2006; 18:139–149. [PubMed: 16639868]
- Shippenberg TS, Herz A, Spanagel R, Bals-Kubik R, Stein C. Conditioning of opioid reinforcement: neuroanatomical and neurochemical substrates. *Ann N Y Acad Sci*. 1992; 654:347–356. [PubMed: 1321576]
- Shippenberg TS, Zapata A, Chefer VI. Dynorphin and the pathophysiology of drug addiction. *Pharmacol Ther*. 2007; 116:306–321. [PubMed: 17868902]
- Sinclair JD. Drugs to decrease alcohol drinking. *Ann Med*. 1990; 22:357–362. [PubMed: 2291844]
- Sinclair JD, Senter RJ. Development of an alcohol-deprivation effect in rats. *Q J Stud Alcohol*. 1968; 29:863–867. [PubMed: 5705408]
- Sinha R. The role of stress in addiction relapse. *Curr Psychiatry Rep*. 2007; 9:388–395. [PubMed: 17915078]
- Spanagel R, Almeida OF, Bartl C, Shippenberg TS. Endogenous kappa-opioid systems in opiate withdrawal: role in aversion and accompanying changes in mesolimbic dopamine release. *Psychopharmacology (Berl)*. 1994; 115:121–127. [PubMed: 7862883]
- Spanagel R, Herz A, Shippenberg TS. The effects of opioid peptides on dopamine release in the nucleus accumbens: an in vivo microdialysis study. *J Neurochem*. 1990; 55:1734–1740. [PubMed: 1976759]
- Spanagel R, Herz A, Shippenberg TS. Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc Natl Acad Sci U S A*. 1992; 89:2046–2050. [PubMed: 1347943]
- Spanagel R, Weiss F. The dopamine hypothesis of reward: past and current status. *Trends Neurosci*. 1999; 22:521–527. [PubMed: 10529820]

- Svingos AL, Chavkin C, Colago EE, Pickel VM. Major coexpression of kappa-opioid receptors and the dopamine transporter in nucleus accumbens axonal profiles. *Synapse*. 2001; 42:185–192. [PubMed: 11746715]
- Thomas JB, Atkinson RN, Rothman RB, Fix SE, Mascarella SW, Vinson NA, Xu H, Dersch CM, Lu Y, Cantrell BE, Zimmerman DM, Carroll FI. Identification of the first trans-(3R,4R)- dimethyl-4-(3-hydroxyphenyl)piperidine derivative to possess highly potent and selective opioid kappa receptor antagonist activity. *J Med Chem*. 2001; 44:2687–2690. [PubMed: 11495579]
- Todtenkopf MS, Marcus JF, Portoghese PS, Carlezon WA Jr. Effects of kappa-opioid receptor ligands on intracranial self-stimulation in rats. *Psychopharmacology (Berl)*. 2004; 172:463–470. [PubMed: 14727002]
- Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP. Naltrexone in the treatment of alcohol dependence. *Arch Gen Psychiatry*. 1992; 49:876–880. [PubMed: 1345133]
- Walker BM, Koob GF. Pharmacological evidence for a motivational role of kappa-opioid systems in ethanol dependence. *Neuropsychopharmacology*. 2008; 33:643–652. [PubMed: 17473837]
- Walker BM, Zorrilla EP, Koob GF. Systemic kappa-opioid receptor antagonism by norbinaltorphimine reduces dependence-induced excessive alcohol self-administration in rats. *Addict Biol*. 2011; 16:116–119. [PubMed: 20579007]
- Wee S, Koob GF. The role of the dynorphin-kappa opioid system in the reinforcing effects of drugs of abuse. *Psychopharmacology (Berl)*. 2010; 210:121–135. [PubMed: 20352414]
- Williams KL, Woods JH. Oral ethanol-reinforced responding in rhesus monkeys: effects of opioid antagonists selective for the mu-, kappa-, or delta-receptor. *Alcohol Clin Exp Res*. 1998; 22:1634–1639. [PubMed: 9835275]

1a

1b

Fig. 1.

a. Depicts the mean ($\pm$ SEM) responses/session on the lever previously associated with the delivery of EtOH in P rats ($n = 11-14$ /group) given 0, 1, 3 or 10 mg/kg JDTic 14 days prior to the four PSR session. * indicates that vehicle treated rats responded significantly more on the EtOH lever during the 1st PSR session compared to extinction baseline levels and from all other groups.

b. Depicts the mean ($\pm$ SEM) responses/session on the lever previously associated with the delivery of water in P rats ($n = 11-14$ /group) given 0, 1, 3 or 10 mg/kg JDTic 14 days prior to the four PSR session.

2a

2b

Fig. 2.

a. Depicts the mean ($\pm$ SEM) responses/session by P rats on the EtOH lever under relapse conditions ($n = 8$ /group) ($n = 11-14$ /group) given 0, 1, 3 or 10 mg/kg JD_Tic 25 days prior to the first reinstatement session. * indicates that rats administered vehicle responded significantly more on the EtOH lever during the 1st reinstatement session compared to baseline levels and significantly more than all other rats.

b. Depicts the mean ($\pm$ SEM) responses/session on the water lever during reinstatement of EtOH and water in P rats ($n = 11-14$ /group) given 0, 1, 3 or 10 mg/kg JD_Tic 25 days prior to the first reinstatement session. * indicates that rats administered 10 mg/kg JD_Tic responded significantly more on the water lever during the 3rd and 4th reinstatement session compared to baseline levels and significantly more than all other rats.

3a

3b

Fig. 3.

- a. Depicts the mean ($\pm$ SEM) responses/session on the EtOH lever during reinstatement of EtOH and water in P rats ($n = 11-14$ /group) given 0, 1, 3 or 10 mg/kg JDTic 30 min prior to the first test session. JDTic did not alter EtOH self-administration during the 17 consecutive days of testing.
- b. Depicts the mean ($\pm$ SEM) responses/session on the water lever during reinstatement of EtOH and water in P rats ($n = 11-14$ /group) given 0, 1, 3 or 10 mg/kg JDTic 30 min prior to the first test session. JDTic did not alter water self-administration during the 17 consecutive days of testing.