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Protracted withdrawal from ethanol and enhanced responsiveness stress: regulation via the dynorphin/kappa opioid receptor system

Kelli Gillett, Erin Harshberger, and Glenn R. Valdez

Department of Psychology, Grand Valley State University, Allendale, MI, USA

Abstract

Although recent work suggests that the dynorphin/kappa opioid receptor (DYN/KOR) system may be a key mediator in the stress-related effects of alcohol, the regulation of long-term changes associated with protracted withdrawal from ethanol via the DYN/KOR system has yet to be explored. The objective of the present study was to determine the role of the DYN/KOR system in the regulation of anxiety-related behaviors during an extended period of abstinence from ethanol in animals with a history of ethanol dependence. Male Wistar rats ($n = 94$) were fed an ethanol or control liquid diet for 25–30 days. Six weeks after its removal, rats were exposed to 20 minutes of immobilization, and the ability of the KOR-antagonist norbinaltorphimine (nor-BNI) (0–20 mg/kg, intraperitoneal [i.p.]) to attenuate the enhanced responsiveness to stress observed in rats chronically exposed to ethanol was investigated using the elevated plus maze. In addition, the ability of U50,488 (0–10 mg/kg, i.p.) to prime anxiety-like behavior during protracted withdrawal was also examined. Rats with a history of ethanol dependence showed a significant decrease in open-arm exploration after exposure to restraint, indicating an anxiety-like state, compared to similarly treated controls, an effect that was blocked by nor-BNI. nor-BNI also selectively decreased center time and open-arm approaches in ethanol-exposed rats. The highest dose of U50,488 decreased open-arm exploration and the total number of arm entries in ethanol-exposed and control rats. Although lower doses of U50,488 did not affect open-arm exploration in either group, the 0.1 mg/kg dose selectively decreased motor activity in the ethanol-exposed rats when compared to similarly pretreated controls. These findings further support the hypothesis that behaviors associated with withdrawal from ethanol are in part regulated by the DYN/KOR system, and suggest that these effects may be long-lasting in nature.

Keywords

kappa opioids; ethanol; protracted abstinence; stress; anxiety; withdrawal

Introduction

Withdrawal is one of the defining characteristics of alcohol dependence, and is often characterized by impaired physiological function and enhanced negative affect. These

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Address correspondence to: Glenn R. Valdez Grand Valley State University Department of Psychology 1 Campus Drive Allendale, MI 49401 USA Phone: (616) 331-3798 Fax: (616) 331-2480 valdezg@gvsu.edu.

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physiological abnormalities and mood disturbances experienced by many alcoholics also appear to be long-lasting in nature (Begleiter and Porjesz, 1979). For example, anxiety can persist for extended periods of time during abstinence following chronic alcohol consumption (Begleiter and Porjesz, 1979; Grant et al., 1987; Roelofs, 1985), and abstinent alcoholics may show symptoms of anxiety for months and even years after their last drink (De Soto et al., 1989; Roelofs, 1985). These symptoms are often associated with an increased risk of relapse (De Soto et al., 1989). Laboratory animals with a history of ethanol dependence also exhibit behaviors consistent with the presence of an anxiety-like state during protracted periods of abstinence, such as decreased cork gnawing (Rasmussen et al., 2001), decreased exploration of the open arms of the elevated plus maze (Rasmussen et al., 2001; Valdez et al., 2002b), and increased locomotor activity when placed in a familiar environment (Rasmussen et al., 2001). In addition, rats exposed to chronic ethanol exhibit, up to 6 weeks post-withdrawal, an enhanced responsiveness to a mild stressor when tested in the elevated plus maze; this stressor does not alter the behavior of control rats (Valdez et al., 2003). Furthermore, increased ethanol self-administration is seen up to 8 weeks post-withdrawal in the absence of physical withdrawal signs (Roberts et al., 2000; Valdez et al., 2002b), suggesting that increased ethanol consumption after protracted periods of abstinence is more likely due to negative affective-like states rather than physical withdrawal symptoms.

Recent work suggests that the DYN/KOR system may be a key mediator in the stress-related effects of alcohol (Walker et al., 2012). The KOR-antagonist nor-BNI reduces forced-swim stress-induced increases in ethanol intake, and the KOR-agonist U50,488 potentiates ethanol-conditioned place preference and ethanol drinking in mice (Sperling et al., 2010). nor-BNI also attenuates the characteristic increase in anxiogenic-like behavior in the elevated plus maze observed in rats experiencing acute ethanol withdrawal (Valdez and Harshberger, 2012). Increases in stress-related behavior are observed following injections of U50,488, an effect that is preventable by pretreatment with nor-BNI, suggesting that similar mechanisms are involved in the production of ethanol withdrawal- and KOR agonist-induced changes in behavior (Valdez and Harshberger, 2012). KOR antagonism also reduces the characteristic increase in ethanol self-administration in rats following exposure to chronic ethanol vapor without affecting ethanol drinking in control rats (Nealey et al., 2011; Walker and Koob, 2008; Walker et al., 2011), suggesting that KORs may regulate the negative reinforcing properties of ethanol. Additionally, mice subjected to social-defeat stress show increased ethanol consumption following U50,488 treatment whereas this KOR agonist did not affect ethanol consumption in mice that did not experience social defeat (Kudryavtseva et al., 2006). Although these studies suggest that the DYN/KOR system is involved in mediating the acute effects of ethanol-related stress, the role of KORs in mediating behavioral changes associated with protracted periods of abstinence has yet to be explored.

The objective of the present experiments was to determine the role of the DYN/KOR system in the regulation of anxiety-related behaviors during protracted withdrawal from ethanol. In the first study, the ability of nor-BNI to block the enhanced responsiveness to a mild stressor observed in rats with a history of ethanol dependence was examined following a protracted period of abstinence in the elevated plus-maze. In addition, a second experiment was conducted to determine if rats with a history of ethanol dependence would be more responsive to the stress-inducing effects of the KOR agonist U50,488 at doses lower than those that have previously been shown to induce anxiety-related behaviors in non-dependent rats. These experiments sought to provide further insight into the role of the DYN/KOR system in the long-term changes observed during protracted periods of abstinence from ethanol.

Materials and Methods

Animals

Male Wistar rats (Charles River, Kingston, NY; $n = 94$) were used in this experiment. Body weights were 200–250 g and age was approximately 60 days at the start of the experiments. Rats were group-housed (3 per cage) with food and water available *ad libitum* except during liquid diet administration, and were weighed daily. Rats were maintained on a 12-hour light/dark cycle (lights on at 10:00 PM). Procedures met guidelines of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication number 85–23, revised 2011) and were approved by the Institutional Animal Care and Use Committee of Grand Valley State University.

Ethanol Liquid Diet

Details of a similar (8.7% v/v) liquid-diet procedure have been reported previously (Schulteis et al., 1996). Briefly, food and water were removed and replaced with a nutritionally complete liquid diet. The ethanol diet consisted of a chocolate-flavored, liquid nutritional supplement (Mead Johnson, Inc., Evansville, IN), vitamin fortification and mineral salt mixture (0.3 and 0.5 g/100 mL respectively; ICN Nutritional Biochemicals, Aurora, OH), distilled water, and 95% ethanol diluted to a final concentration of 10% (v/v). Sucrose was isocalorically substituted for ethanol in the control diet. Rats receiving the ethanol liquid diet received *ad libitum* access to the diet. In order to control for caloric intake, control rats were pair-fed a daily ration equivalent to the prior mean daily intake of the ethanol group. Diets were prepared and administered at the onset of the dark cycle for 25–30 days.

Immobilization Stress

Each rat was restrained in a clear Plexiglas® tube (Kent Scientific, Torrington, CT) for 20 minutes prior to testing in the elevated plus maze. Previous studies have shown that a similar duration of restraint is sufficient to induce an anxiogenic-like response in rats with a history of ethanol dependence without affecting the behavior of non-dependent animals (Valdez et al., 2003).

Elevated Plus Maze

The elevated plus maze (Med Associates, St. Albans, VT) was made of dark Plexiglas® and consisted of 4 arms, each 50 cm long $\times$ 10 cm wide. The 2 enclosed arms had dark walls 40 cm high, and the 2 open arms had ledges 0.5 cm high. Lighting on the open arms was 1.5–2.0 lux. The maze was elevated to a height of 50 cm. Individual animals were handled for approximately 10–20 minutes daily prior to testing. Because exposure to the elevated plus maze itself has been proposed to be a stressor (Lister, 1987), this handling procedure was used to prevent a floor effect under which anxiogenic-like effects of ethanol withdrawal or U50,488 may not be detected. Prior handling history has been shown to affect open-arm exploration under baseline conditions (Andrews and File, 1993; Valdez et al., 2002a). Testing occurred 2–3 hours after the onset of the dark cycle.

Rats were placed individually onto the center of the apparatus facing the closed arm, and time spent on and entries onto each arm were recorded automatically by photocell beams and monitored by a computer for 5 minutes. The maze was cleaned with water and dried after each trial. The traditional temporal measures, as well as behavioral measures of anxiety and general motor activity, were analyzed. Open-arm preference, as indicated by the ratio of open-arm to total arm time and entries, has been proposed to relate inversely to anxiety (Cruz et al., 1994). Arm entries, defined as all 4 limbs entering the arm of a maze, have been proposed as an index of locomotor activity (Cruz et al., 1994). In addition, behaviors that

have been proposed to reflect risk assessment, such as time spent in the center of the maze and number of open-arm approaches, were also assessed (Rodgers and Johnson, 1995). Decreases in time spent in the center of the maze and number of open-arm approaches, defined as the entry of the 2 front limbs into the open arm, have also been proposed to inversely relate to anxiety (Rodgers and Johnson, 1995). These behavioral measures have been shown to be able to detect the anxiolytic effects of compounds that may not be detected by traditional measures of anxiety (Griebel et al., 1997; Setem et al., 1999). Each rat was naïve to the apparatus.

Drugs and Injections

U50,488 ([*trans*]-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclo-hexyl] benzeneacetamide; Tocris Biosciences, Ellisville, MO) and nor-binaltorphimine (nor-BNI; Tocris Biosciences, Ellisville, MO) were dissolved in 0.9% saline solution for i.p. injections.

Experimental procedure

To examine the enhanced responsiveness to stress via KORs during protracted withdrawal from ethanol, rats were given access to an ethanol or control liquid diet. Following removal of the diet, rats were left undisturbed for 6 weeks with the exception of daily handling and routine husbandry. At the conclusion of this 6-week period, rats received i.p. injections of nor-BNI (20 mg/kg) or saline. This dose of nor-BNI has previously been shown to decrease ethanol self-administration (Walker and Koob, 2008) and anxiety-like behavior (Valdez and Harshberger, 2012) in ethanol-dependent rats. After a 24–30 hour period following the injections, the rats were given 20 minutes of immobilization stress and then examined in the elevated plus maze. The pretreatment period was chosen because previous research has shown that nor-BNI is most selective for KORs at 24 hours after administration as opposed to earlier times (Endoh et al., 1992).

A second experiment was conducted to determine the effects of direct activation of the KOR system on anxiety-like behavior in rats with a history of ethanol dependence. Rats were given access to either an ethanol or control liquid diet and were left undisturbed for 6 weeks following its removal as described. At the end of this 6-week period, rats received injections of the KOR agonist U50,488 (1.0–10 mg/kg, i.p.) or saline 10 minutes prior to testing in the elevated plus maze. This dose range was chosen in order to determine if rats with a history of ethanol dependence would be more responsive to the stress-inducing effects of U50,488 at doses lower than those that have previously been shown to induce anxiety-related behaviors in non-dependent rats (Smith et al., 2012; Tejeda et al., 2012; Valdez and Harshberger, 2012).

Data Analysis

Data were analyzed by using a 2-way analysis of variance (ANOVA) with diet (ethanol or control), antagonist dose (20 mg/kg nor-BNI or vehicle), or agonist dose (0.1–10 mg/kg U50,488 or vehicle) as between-subjects factors. Fisher's test was used for *post hoc* analysis as warranted.

Results

Rats fed the ethanol liquid diet consumed an average of 11.20 g/kg per day (SD = 3.77, range = 2.95–30.70 g/kg per day) over the course of the liquid diet administration. This amount of ethanol intake has previously been shown to induce physical withdrawal signs and pharmacologically relevant blood ethanol levels in rats (Macey et al., 1996; Valdez et al., 2004). Although the average intake per animal was based on total intake per cage, there were no significant differences in body weights, suggesting that wide variability in

consumption between animals housed in the same cages was unlikely. There were also no significant differences between ethanol liquid diet-exposed rats and control liquid diet-exposed rats in average body weight (ethanol mean = 260.87 g, SD = 19.83; control mean = 253.58 g, SD = 14.86) and the amount of fluid consumed (ethanol mean = 33.65 mL, SD = 11.67; control mean = 33.41 mL, SD = 8.43) over the course of the liquid diet administration.

There was a significant interaction between diet and nor-BNI dose on percentage of time spent exploring the open arms, $F(1, 24) = 7.19, p < .05$ (Figure 1A). Following 20 minutes of restraint, rats fed the ethanol liquid diet displayed a characteristic decrease in percent open arm time compared to controls. Pretreatment with 20 mg/kg nor-BNI attenuated this effect, causing rats with a history of ethanol dependence to explore the open arms at a level comparable to that of controls, $p < .05$ (Fisher's Test). There was no significant interaction between diet and nor-BNI administration on the percentage of open-arm entries. In addition, there were no significant effects of diet or nor-BNI administration on total number of arm entries (Figure 1B). Although there were no significant overall effects of diet or nor-BNI administration on center time or open-arm approaches, further analysis revealed that nor-BNI decreased both of these measures in rats with a history of ethanol dependence. Pretreatment with 20 mg/kg nor-BNI led to a decrease in time spent in the center in rats fed an ethanol liquid diet compared to vehicle-treated controls, $p < .05$ (Fisher's Test, Figure 1C). nor-BNI also decreased open-arm approaches in rats with a history of ethanol dependence compared to controls, $p < .05$ (Fisher's Test, Figure 1D).

Although there was no significant interaction between diet and U50,488 dose on open-arm exploration in the elevated plus maze, injection with 10 mg/kg U50,488 significantly decreased the percentage of time spent in the open arms in both ethanol-exposed and control rats $F(3,58) = 5.24, p < .01$ (Figure 2A). There was also a significant interaction between diet and U50,488 dose on the total number of arm entries $F(3,58) = 2.92, p < .05$ (Figure 2B). Further analysis revealed that rats with a history of ethanol dependence had fewer total arm entries when injected with 0.1 mg/kg U50,488, compared to 0.1 and 1.0 mg/kg U50,488 pretreated controls and vehicle-injected ethanol-exposed rats, $p < .05$ (Fisher's Test). Ethanol-exposed and control animals receiving 10 mg/kg U50,488 also had fewer total arm entries when compared to these groups, $p < .05$ (Fisher's Test). There were no significant effects of diet or U50,488 dose on time spent in the center of the elevated plus maze (Figure 2C). Although there were no significant interactions between diet and U50,488 dose on the number of open-arm approaches, there was a significant main effect of diet on this measure, $F(1,58) = 7.10, p < .01$ (Figure 2D). *Post hoc* analysis demonstrated that ethanol-exposed rats pretreated with 0.1 and 1.0 mg/kg U50,488 showed fewer open-arm approaches compared to controls, $p < .05$ (Fisher's Test).

Discussion

Consistent with previous findings (Valdez et al., 2003), rats with a history of ethanol dependence showed a significant decrease in open-arm exploration of the elevated plus maze compared to controls when given 20 minutes of restraint, a stressor that does not induce behavioral changes in non-dependent animals. This effect was blocked by nor-BNI, suggesting that the enhanced responsiveness to stress observed following protracted periods of abstinence may be regulated in part by the DYN/KOR system. The total number of arm entries did not differ between groups, suggesting it was unlikely that this finding was due to changes in general motor activity. nor-BNI also selectively reduced risk-assessment behaviors in rats with a history of ethanol dependence, further supporting an anxiolytic profile for KOR antagonists. Injections of 0.1 and 1.0 mg/kg U50,488 did not lead to an anxiety-like response in rats with a history of ethanol dependence and non-dependent

controls, but the highest dose tested decreased open-arm exploration in both of these groups. These results suggest that while KOR antagonism may be sufficient to attenuate the increased responsiveness to stress observed during long-term withdrawal from ethanol, direct activation of the DYN/KOR system may not be needed to produce this anxiety-like state. However, lower doses of U50,488 decreased the total number of arm entries and open-arm approaches in ethanol-exposed rats compared to similarly pretreated controls. This finding indicates that while animals with a history of dependence may not be more susceptible to the stress-inducing properties of KOR agonists, ethanol-exposed rats may be more sensitive to other behavioral effects, such as motor suppression, of KOR agonists. Further investigation will aid in clarifying the role of the DYN/KOR system in protracted abstinence from ethanol.

Although nor-BNI injections decreased the anxiety-like response observed in rats with a history of ethanol dependence, low doses of U50,488 did not induce an anxiogenic-like effect in ethanol-exposed rats. One possible explanation for this finding may be that physical restraint and U50,488 may induce stress via different KOR subtypes. Various studies have provided evidence for the existence of multiple KOR subtypes (Clark et al., 1989; Nock et al., 1988), which appear to be activated differentially by arylacetamide and benzomorphan KOR agonists (Vonvoigtlander and Lewis, 1988; Zukin et al., 1988). Previous studies suggest that nor-BNI may be more effective in antagonizing the effects of benzomorphans than of the arylacetamides (Butelman et al., 1998; Powell and Holtzman, 1999). Taken together with these previous findings, it appears that U50,488 may activate a subpopulation of KORs that is not necessary in inducing stress-related behaviors during protracted withdrawal from ethanol. However, the ability of nor-BNI to reverse restraint-induced anxiogenic-like behaviors suggests that KORs are partially involved in regulating the enhanced responsiveness to stressful stimuli observed during protracted withdrawal.

The ability of nor-BNI to reverse anxiogenic-like behaviors despite the failure of U50,488 to induce anxiety-like behaviors may also be explained by ligand-directed signaling of KORs. Recent evidence suggests that KOR-mediated mitogen-activated protein kinase (MAPK) cascade activation may be important in the regulation of stress-related behaviors, but KOR ligands may differentially activate MAPK signaling pathways. For example, U50,488-induced conditioned place aversion seems to be dependent on p38 MAPK signaling (Bruchas et al., 2007a). However, the ability of nor-BNI to block the effects of KOR activation appears to be due to c-Jun N-terminal Kinase (JNK) MAPK phosphorylation (Bruchas et al., 2007b). Although U50,488 also leads to JNK phosphorylation, this activation occurs in a pertussis-toxin-sensitive manner (Kam et al., 2004) whereas nor-BNI induced JNK phosphorylation is insensitive to pertussis toxin (Bruchas et al., 2007b). Although purely speculative at this point, it is possible that restraint-induced anxiogenic-like behaviors observed in rats with a history of ethanol dependence may be regulated independently of KOR-activated p38 MAPK signaling and/or pertussis-toxin-sensitive JNK phosphorylation.

Alternatively, the finding that nor-BNI decreased the effects of a mild external stressor in rats with a history of ethanol dependence whereas low doses of U50,488 did not induce anxiety-like behaviors in ethanol-exposed rats may be explained by interactions between the DYN/KOR system and other stress-related neurotransmitter systems. For example, DYN and corticotrophin-releasing factor (CRF), one of the major regulators of stress in the body (Vale et al., 1981), appear to interact in various regions of the brain, including the hypothalamus, amygdala, nucleus accumbens, dorsal raphe nucleus, and hippocampus (Bruchas et al., 2009; Roth et al., 1983). KOR agonists stimulate the hypothalamic-pituitary-adrenal (HPA) axis in rodents (Laorden et al., 2000) and humans (Ur et al., 1997), which is primarily regulated by CRF release in the hypothalamus (Vale et al., 1983). DYN has also

been found to modulate the effects of CRF on autonomic function (Overton and Fisher, 1989). In the basolateral amygdala, CRF increases KOR immunoreactivity (Bruchas et al., 2009; Land et al., 2008). Dynorphin and CRF neurons are also co-localized in the central nucleus of the amygdala (Reyes et al., 2008). The CRF-receptor antagonist D-Phe-CRF₍₁₂₋₄₁₎ decreases the enhanced responsiveness to stress observed in rats with a history of ethanol dependence in a manner similar to nor-BNI (Valdez et al., 2003). The CRF₁ receptor antagonist CP 154,526 also significantly attenuates KOR-agonist priming-induced reinstatement of cocaine seeking in squirrel monkeys (Valdez et al., 2007). Valdez et al. (2007) hypothesized that this finding may be due to downstream activation of stress-related mechanisms by KOR activation. When considered with these previous findings, it is possible that restraint stress led to a non-selective increase in activity of these stress-related mechanisms and that nor-BNI blocked the interactions between KORs and these other systems. In addition, the doses of U50,488 used in this study may have been insufficient to activate this interaction between the DYN/KOR system and other stress-related mechanisms. Further research into the relationship between the DYN/KOR system and other neurobiological mechanisms involved in regulating the behavioral stress response would aid in clarifying this issue.

The findings of the present study did not support the hypothesis that low doses of U50,488 would prime anxiety-like behavior in rats with a history of ethanol dependence, although the highest dose tested decreased open-arm exploration in both ethanol-exposed and control rats. However, injection with 0.1 mg/kg U50,488 led to a significant decrease in the total number of arm entries in rats with a history of ethanol dependence without altering this behavior in controls. This selective decrease suggests that although rats with a history of ethanol dependence may not be more susceptible to the stress-inducing effects of direct KOR activation during protracted abstinence, they may be more sensitive to other behavioral effects associated with KOR agonists. The total number of arm entries has been proposed as an indicator of general motor activity (Cruz et al., 1994), and KOR activation has previously been shown to have motor suppressive effects (Negus, 2004). Although all rats injected with 10 mg/kg U50,488 showed a decrease in general motor activity, control animals that received lower doses of U50,488 in this study did not show any changes in the total number of arm explorations. Interestingly, U50,488 decreased the number of open-arm approaches, which has also been proposed to indicate an anxiolytic-like state, in ethanol-exposed rats. However, it is likely that this effect may also be the result of general motor suppression given the observed decrease in the total number of arm entries. Additional research regarding the role of KORs during long-term withdrawal would provide further insight into this issue.

Another issue to consider in the present study is that rats with a history of ethanol dependence that were not exposed to restraint stress were not tested in this study. One could argue that the decrease in open-arm exploration was not due to an enhanced responsiveness to stress during protracted abstinence, but was simply the result of ethanol withdrawal itself. During ethanol withdrawal, rats consistently show decreased open-arm exploration in the elevated plus maze in the absence of exposure to an external stressor (Baldwin et al., 1991; Rasmussen et al., 2001; Rassnick et al., 1993; Valdez et al., 2002b; 2004; Valdez and Harshberger, 2012), an effect that lasts for up to 4 weeks following cessation of chronic ethanol exposure (Rasmussen et al., 2001; Valdez et al., 2002b). However, the severity of the anxiogenic-like state displayed in these animals appears to decrease over time (Valdez et al., 2002b). In addition, rats with a history of dependence previously tested under similar conditions did not show a decrease in open-arm exploration compared to controls when tested in the elevated plus maze in the absence of brief restraint stress (Valdez et al., 2003). These previous findings suggest that it is unlikely that the decrease in open-arm exploration observed in ethanol-exposed rats was simply due to withdrawal from ethanol alone.

When considered with previous work demonstrating that rats exposed to chronic ethanol show an enhanced responsiveness to a mild stressor during protracted abstinence (Valdez et al., 2003), our data further support the hypothesis that ethanol dependence may lead to long-term dysregulation of brain neurochemical systems involved in regulating stress (Koob, 2003). For example, rats with a history of ethanol dependence previously examined under this same model show increased CRF-like immunoreactivity in the amygdala 6 weeks following chronic ethanol exposure (Zorrilla et al., 2001). This increase may indicate a shift in the homeostatic set point of brain stress systems during ethanol dependence that persists after the acute withdrawal phase. The findings of the present experiments suggest that the DYN/KOR system may also become dysregulated following chronic ethanol exposure, leading to long-term changes in behavior.

The current study provides further evidence that the DYN/KOR system may be a key mediator in the stress-related effects of alcohol. Rats with a history of ethanol dependence demonstrated an enhanced responsiveness to a mild stressor when tested in the elevated plus maze during protracted abstinence, an effect blocked by nor-BNI. Lower doses of U50,488 did not lead to an anxiety-like response but selectively decreased motor activity in ethanol-exposed rats. These results suggest that while KOR antagonism may be sufficient to attenuate the increased responsiveness to stress observed during long-term withdrawal from ethanol, direct activation of the DYN/KOR system may not be necessary to produce stress-related behaviors. However, animals with a history of ethanol dependence may be more sensitive to other behavioral effects induced by KOR agonists. Overall, these findings indicate that the DYN/KOR system is likely involved in regulating the long-term behavioral changes associated with alcohol dependence.

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Figure 1.

KOR regulation of the enhanced responsiveness to stress observed in rats with a history of ethanol dependence during protracted withdrawal from ethanol. Rats were fed an ethanol or control liquid diet for 25–30 days. Six weeks after its removal, rats were pretreated with nor-BNI or vehicle 24–30 hours prior to testing in the elevated plus maze. Immobilization stress (20 minutes) took place immediately prior to testing. A. nor-BNI reverses the anxiogenic-like effects of mild restraint in rats with a history of ethanol dependence. The percentage of time spent exploring the open arms, as indicated by the ratio of open-arm to total arm time and entries, has been proposed to relate inversely to anxiety. $*p < .05$ compared to all other groups, Fisher's test. B. Effects of nor-BNI on total number of arm entries. Arm entries have been proposed as an index of locomotor activity. C. Effect of nor-BNI on time spent in the center of the elevated plus maze in rats with a history of ethanol dependence. A decrease in time spent in the center has been proposed as a measure of decreased risk-assessment behavior. $*p < .05$ compared to vehicle-treated controls, Fisher's test. D. nor-BNI decreases open-arm approaches in rats with a history of ethanol dependence. A decrease in open-arm approaches has been proposed as a measure of decreased risk-assessment behavior. $*p < .05$ compared to controls, Fisher's test.

Figure 2.

Effects of low dose U50,488 injections on behavior in the elevated plus maze in rats with a history of ethanol dependence during protracted withdrawal from ethanol. Rats were fed an ethanol or control liquid diet for 25–30 days. Six weeks after its removal, rats were pretreated with U50,488 (0.1–10 mg/kg, i.p.) or saline 10 minutes before testing in the elevated plus maze. A. Effects of U50,488 on open-arm exploration in the elevated plus maze. The percentage of time spent exploring the open arms, as indicated by the ratio of open-arm to total arm time and entries, has been proposed to relate inversely to anxiety. B. U50,488 decreases locomotor activity in rats with a history of ethanol dependence. Arm entries have been proposed as an index of locomotor activity. * $p < .05$ compared to controls treated with 0.1 or 1.0 mg/kg U50,488 and vehicle-treated ethanol rats, Fisher's test. C. Effects of U50,488 on center time in the elevated plus maze. Time spent in the center has been proposed as a measure of risk-assessment behavior. D. Effects of U50,488 on open-arm approaches in the elevated plus maze. A decrease in open-arm approaches has been proposed as a measure of decreased risk-assessment behavior. * $p < .05$ compared to controls, # $p < .05$ compared to controls treated with 0.1 or 1.0 mg/kg U50,488, Fisher's test.