Kappa opioid regulation of reward seeking during acute and protracted withdrawal from ethanol

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Introduction
Alcoholism is a social and public health issue that leads to over 75,000 lives lost and over $200 billion in costs annually in the United States alone (Bouchery, Harwood, Sacks, Simon, & Brewer 2011). As defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) diagnostic criteria, alcoholism includes tolerance, withdrawal, drinking to avoid or alleviate withdrawal symptoms, and repeated yet unsuccessful attempts to control alcohol consumption (DSM-IV 2000). Because withdrawal tends to be accompanied by symptoms such as depression and reward seeking, periods of abstinence typically result in high relapse rates, making alcoholism difficult to manage over the long term (Cloninger 1987).

Adding to this challenge is the fact that the underlying neuropharmacological mechanisms correlated with alcoholism, depression and reward seeking, are not completely understood. Knowing the behaviors and the underlying brain physiology that accompany alcohol dependence and withdrawal may lend some insight into how to effectively treat dependence.

One system that has recently been implicated in regulating depressive-like and reward-seeking behaviors during alcohol withdrawal is the dynorphin (DYN)/kappa-opioid receptor (KOR) system. For example, the KOR antagonist nor-binaltorphimine (nor-BNI) has been shown to decrease dependence-induced ethanol self-administration without impacting ethanol drinking in non-dependent rats (Walker & Koob 2008). nor-BNI also reduced increases in ethanol consumption caused by forced swim stress (Sperling, Gomes, Sypek, Carey, & McLaughlin 2010), as well as attenuated the usual decrease in open arm exploration of the elevated plus maze (Valdez & Harshberger, 2012), a behavior that is putatively indicative of an anxiety-like state (Pellow, Choplin, File, & Briley 1985). Administration of another KOR antagonist, JDTic, has been shown to decrease alcohol self-administration and cue-induced reinstatement of alcohol seeking (Schank et al., 2012). The KOR agonist U50,488 also potentiates ethanol drinking and ethanol-conditioned place preference (Sperling et al. 2010).

The purpose of this study was to examine the influence of the KOR antagonist nor-BNI on reward-seeking behavior in ethanol dependent adult rats. These behaviors were studied by measuring responding for a saccharin solution in an operant paradigm. Preference for sweet substances has previously been correlated with elevated ethanol consumption (Dess, Badia-Elder, Thiele, Kiefer, & Blizard 1998) as well as a vulnerability toward other drugs of abuse (Carroll, Thomas, Dykstra, Granger, Allen, & Howard 2008; Holtz & Carroll 2013; Radke, Holtz, Gewirtz, & Carroll 2012). Based on the findings of previous work, we hypothesized that we would observe a change in reward seeking during both acute and protracted withdrawal from ethanol and that administration of nor-BNI would reverse this effect.

Materials and Methods

Animals and Housing
Male Wistar rats (n=8) (Portage, MI, USA) weighing 150-200 g upon arrival were used in this experiment. Animals were group housed (2-3 per cage) with food and water available ad libitum, except for 3 days of water restriction at the initiation of saccharin self-administration training. Lights were on a 12-hour reverse light/dark cycle (lights on at 8:00 PM). Animals were allowed to habituate to colony housing for 7-14 days prior to testing and were handled and weighed regularly. 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.
Saccharin Self-Administration Training

Standard operant chambers (Med Associates, St. Albans, VT, USA) housed in sound attenuated, ventilated cubicles were used. Syringe pumps (Med Associates, St. Albans, VT, USA) dispensed either water or 0.1% saccharin into two stainless steel drinking cups mounted 4 cm above the grid floor. Two levers were located 4.5 cm to either side of the drinking cups. Fluid delivery and recording of operant responding was controlled by a computer. A continuous reinforcement (FR1) schedule was used, and each response resulted in delivery of 0.1 ml of a 0.1% saccharin solution or 0.1 ml of water. The levers were counterbalanced between subjects, with half of the subjects receiving saccharin when responding on the left lever and the other half receiving saccharin when responding on the right lever. Water was concurrently available when responding on the opposite lever. Animals were experimentally naïve prior to the beginning of the training. Operant sessions were 30 min in duration and occurred daily until the animals showed at least an 80% preference for the saccharin solution over water.

Ethanol Liquid Diet Induced Dependence

Once the animals demonstrated an 80% preference for saccharin, they were placed on a liquid diet composed of distilled water, 95% ethanol (Sigma-Aldrich, Milwaukee, WI) diluted to a final concentration of 10% (v/v), a commercially available liquid supplement (Mead Johnson, Inc., Evansville, IN) and vitamins and minerals (0.3 and 0.5g/100 ml respectively; ICN Nutritional Biochemicals, Aurora, OH) (Macey, Schulteis, Neirichs, & Koob 1996). Control animals were placed on a similar diet with sucrose isocalorically substituted for ethanol. The animals in the ethanol condition were allowed free access to the diet, and the animals in the control condition were given the average intake of the ethanol condition animals on the previous day. This diet was administered daily for 28 days and has been shown to induce physical signs of dependence (Macey et al., 1996). No other food or water was available during the course of the liquid diet.

Drug Administration

Immediately following completion of the liquid diet, animals were injected with a saline solution and then tested for operant responding for saccharin 24 hours following this injection. Testing consisted of placing the animals into the operant boxes described above and recording the number of lever presses that occurred during a 30 min period. Following this initial test, rats were injected with nor-BNI (20 mg/kg i.p.) and were again tested 24 hours following injection. Because nor-BNI has been shown to have lasting effects for up to three weeks (Jones & Holtzman 1992), administration and testing of the effects of nor-BNI were repeated three weeks following its initial administration. In this way, protracted effects of withdrawal were measured. Furthermore, using each animal as its own control limited between-subjects variability and reduced the number of subjects required for the study.

Data Analysis

The statistical reliability of the results following removal of the diet were analyzed in the ethanol and control groups using separate analyses of variance (ANOVA) with time as the between subjects factor and nor-BNI dose as the within subjects factor. Data obtained prior to liquid diet exposure were analyzed using a paired t-test.

Results

Rats given the ethanol liquid diet consumed an average of 10.973 g/kg of alcohol per day (SD = 2.80, range = 9.30-12.1 g/kg per day) over the course of the liquid diet administration. These levels of ethanol consumption have previously been shown to induce pharmacologically relevant blood alcohol levels that induce physical symptoms of dependence and withdrawal (Macey et al., 1996). Although the average intake per animal was determined by the total consumption of fluid per cage, the average body weight of each animal did not significantly differ from each other. This suggests that significant differences in amount of fluid intake between animals housed together are unlikely. In addition, there were no significant differences in body weight between animals exposed to the ethanol diet versus similarly housed animals exposed to a control diet (ethanol mean = 367.00 g, SD 13.31; control mean = 379.50 g, SD = 19.13). Likewise, there were no significant differences between the amount of fluid consumed between the two groups during the entirety of the liquid diet (ethanol mean = 46.34 ml, SD = 1.89; control mean = 44.54 ml; SD = 2.60).

Prior to liquid diet exposure, there were no differences in saccharin responding between rats exposed to an ethanol diet and those given a control diet (see Figure 1). There was a significant effect of time of withdrawal on saccharin responding in ethanol dependent rats F(4, 39) = 7.46, p < 0.01 (see Figure 2a). Further analysis showed that saccharin responding decreased in saline-treated ethanol dependent rats during acute withdrawal (p < 0.05, Fisher’s Test). Although not statistically significant, there was a trend towards an increase in saccharin responding during protracted abstinence in rats with a history of ethanol dependence when injected with saline (p = 0.07, Fisher’s Test). nor-BNI reduced responding for saccharin during protracted abstinence compared to saline treated rats at this time point.

Rats exposed to a control diet showed no significant differences in levels of responding, with one exception (see Figure 3a). Analysis found that nor-BNI reduced responding three weeks after removal of the diet, as compared to saline treated animals (p < 0.05, Fisher’s Test). For both ethanol dependent and non-dependent animals, no significant differences in responding for water were observed.

Discussion

During acute withdrawal, ethanol dependent rats exhibited a decrease in saccharin self-administration, an effect that was not reversed by administration of nor-BNI. Although previous work has demonstrated that ethanol self-administration is decreased by nor-BNI during acute withdrawal (Walker & Koob 2008), the results of the current study suggest that KORs may not regulate self-administration of other reinforcers. However, during protracted withdrawal, ethanol dependent rats exhibited an increased reward seeking, and this effect was attenuated by nor-BNI. Taken together, these results suggest that long-term alterations in the DYN/KOR due to...
withdrawal from ethanol increase general reward-seeking behavior.

Ethanol dependent rats showed a significant decrease in saccharin self-administration during acute withdrawal. Previous work has found that animals will exhibit a decrease in preference for sweetened solutions following exposure to stress (Willner 1977; 2005). This is thought to model depression-linked anhedonia (Overstreet 2012). However, as previously mentioned, this effect was not influenced by administration of nor-BNI. This confounds previous work showing that nor-BNI attenuates decreased saccharin preference in male mice during protracted withdrawal (Morales, Anderson, Spear, & Varlinskaya 2013). The effect observed in our study may be due to the fact that the duration of exposure to the stressor (i.e., withdrawal from ethanol) may not have been long enough to induce significant changes in the DYN/KOR system. The authors of one study involving reward seeking for cocaine have suggested a similar idea, in that behavior observed may be dependent on the timeline of withdrawal (Kallupi, Wee, Edwards, Whitfield, Oleata, & Luu 2013).

During protracted withdrawal, however, ethanol-exposed animals showed an increase in saccharin self-administration. This contrasts the operant responding observed during acute withdrawal. One possible explanation for this finding could be that the DYN/KOR system is differentially affected by the duration or time period of ethanol withdrawal. While the behavior seen during acute withdrawal may reflect the anhedonic-like symptoms of depression, the increase in saccharin self-administration seen during protracted withdrawal may reflect heightened reward seeking due to self-medication. One study examining concurrent operant responding for ethanol and saccharin found that limited home cage access to saccharin later depressed responding for ethanol in alcohol-preferring rats (Toalston, Oster, Kuc, Pommer, Murphy, Lumeng, et al. 2008). In addition, high doses of sucrose intake have been shown to ameliorate the withdrawal symptoms of morphine dependence (Jain, Mukherjee, & Singh 2004). Another possibility is that prior exposure to nor-BNI on the part of the KOR system may influence later sensitivity of the receptor to that antagonist. Because nor-BNI, GNTI, and JDTic are all structurally diverse KOR antagonists, yet all have a similarly long duration of action, one proposed explanation for this duration of action is that of a conformational change on the part of the KOR (Carroll et al., 2004). However, this idea has not yet been explored in the case of nor-BNI.

Although not statistically significant, animals exposed to the control diet also showed a trend towards increased saccharin self-administration three weeks after its removal. Following administration of nor-BNI, saccharin responding returned to baseline levels, and this effect was unexpected, considering that these animals were not exposed to ethanol. One reason for this observed effect could be that nor-BNI generally reduces responding for a sweet substance. There is some literature on the ability of naltrexone - a general opioid antagonist - to reduce sucrose consumption in rats, following previous exposure to sucrose (Levine, Grace, Cleary, & Billington 2002; Michaels & Holtzman 2007). However, if that were the case, one would expect to see a similar result during the acute withdrawal condition, which did not occur. An alternative explanation for this effect may be that changes in diet acted as a mild stressor in itself, and indeed, previous work has shown that changes in diet may impact findings (South, Westbrook, & Morris 2012; Cottone, Sabino, Steardo, & Zorrilla 2009). This idea is supported by the fact that the increase in saccharin self-administration was reversed following administration of nor-BNI, which as mentioned previously, has been shown to reverse stress-induced behaviors. With regard to why this effect was not observed during the acute withdrawal condition, the KOR system may not have been adequately recruited by the time of the acute withdrawal condition. However, given the time that had passed three weeks later, perhaps this system of the brain had in fact been augmented.

In summary, these results suggest a differential influence of time of withdrawal on general reward-seeking behavior, and the DYN/KOR system may be more involved in regulating long-term alterations in reward seeking. Because of the dearth of literature available on the long-term effects of withdrawal, future studies will need to further examine this complex facet of alcohol dependence, as well as the underlying influence of the DYN/KOR system. The current findings suggest that time-sensitive KOR antagonism could be a promising avenue with which to treat alcohol dependence.
References


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Figure 1. Saccharin responding prior to liquid diet administration. Rats were trained to self-administer saccharin in a two-lever choice procedure with saccharin and water concurrently available. Data are the number of responses on the final day of saccharin self-administration training prior to liquid diet exposure.

Figure 2. Saccharin and water responding in ethanol dependent rats during acute and protracted withdrawal from ethanol. Rats were trained to self-administer saccharin and then were exposed to an ethanol liquid diet. A. Responding for saccharin decreased during acute withdrawal and increased during protracted withdrawal. nor-BNI reversed heightened saccharin self-administration during protracted withdrawal. B. Water responding was unaffected. * $p<0.05$, # $p=0.07$ Fisher’s Test.
Figure 3. Saccharin and water responding in control rats. Rats were trained to self-administer saccharin and then were exposed to a liquid diet with sucrose substituted for ethanol. A. nor-BNI reversed increased saccharin self-administration during protracted withdrawal. B. Water responding was unaffected. * p<0.05 Fisher's test.