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The neurokinin-1 receptor mediates escalated alcohol intake induced by multiple drinking models

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ABSTRACT

We have previously demonstrated that the neurokinin-1 receptor (NK1R) is upregulated in the central nucleus of the amygdala of alcohol preferring (P) rats and that this receptor mediates escalated alcohol consumption in this strain. However, it is unclear if non-genetic models of escalated consumption are also mediated by NK1R signaling, and if so, what brain regions govern this effect. In the experiments presented here, we use two methods of inducing escalated alcohol intake in outbred Wistar rats: yohimbine pretreatment and intermittent alcohol access (Monday, Wednesday, and Friday availability; 20% alcohol). We found that escalated alcohol consumption induced by both yohimbine injection and intermittent access is attenuated by systemic administration of the NK1R antagonist L822429. Also, when compared to continuous alcohol access or access to water alone, NK1R expression was increased in the nucleus accumbens (NAC) and dorsal striatum, but not the amygdala. Escalated consumption induced by intermittent access was attenuated when the NK1R antagonist L822429 was infused directly into the dorsal striatum, but not when infused into the NAC. Taken together, these results suggest that NK1R upregulation contributes to escalated alcohol consumption that is induced by genetic selection, yohimbine injection, and intermittent access. However there is a dissociation between the regions involved in these behaviors with amygdalar upregulation contributing to genetic predisposition to escalated consumption and striatal upregulation driving escalation that is induced by environmental exposures.

1. Introduction

In 2014, 16.3 million adults aged 18 and older had an alcohol use disorder, representing 6.8% of the United States population (SAMHSA, 2015b). Additionally, alcohol misuse cost the United States economy $249 billion dollars in 2010 (Sacks et al., 2015). In order to alleviate these burdens, the FDA has approved three medications to treat alcohol use disorders: acamprosate, disulfiram, and naltrexone (SAMHSA, 2015a). These medications have been shown to reduce alcohol consumption and increase abstinence, but these effects are moderate and inconsistent (Winslow et al., 2016). Because of these inconsistencies, it is important to investigate other potential treatment options.

One potential target for a novel treatment is the neurokinin-1 receptor (NK1R) and its primary endogenous ligand, substance P (SP). SP is historically recognized for its effects in the periphery, but has been implicated in both the stress response and ethanol consumption (Ebner and Singewald, 2006; Schank, 2014; Schank et al., 2012b). Specifically, our group has found that the SP/NK1R system mediates stress-induced alcohol seeking but does not affect cue-induced alcohol seeking (Ayanwuyi et al., 2015; Schank et al.,

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2011, 2014, 2015). Additionally, we and others have observed an effect of this system on escalated consumption, but not baseline alcohol intake (Augier et al., 2014; Ayanwuyi et al., 2015; Barson et al., 2015; Schank et al., 2011, 2013; Thorsell et al., 2010).

NK1Rs are located in a wide range of brain regions such as the striatum, amygdala, and hippocampus, all of which are involved in the regulation of affective disorders (Commons, 2010; Rigby et al., 2005). In examining the role of the NK1R in these regions in stress-elicited alcohol seeking and escalated consumption, we have found that upregulation of the NK1R in the central nucleus of the amygdala (CeA) of alcohol preferring (P) rats drives their escalated alcohol consumption (Schank et al., 2013). In contrast, stress-induced reinstatement of alcohol seeking in outbred Wistar rats appears to be driven by the actions of the NK1R in the NAC shell (Schank et al., 2015).

In addition to selective breeding for high alcohol preference, as utilized in generating the P rat and other preferring strains, escalated consumption of alcohol can be induced in outbred animals by manipulating the access schedule or injecting a stress-inducing pharmacological agent. Specifically, intermittent access in the two-bottle choice paradigm, which consists of cycles of drinking and abstinence, leads to a rapid increase in ethanol consumption whereas continuous access to ethanol does not. This method, first reported by Wise and colleagues (Wise, 1973), and rediscovered by Bartlett and colleagues more recently (Simms et al., 2008), has been adopted as a reliable method to induce escalated consumption in preclinical alcohol research. This method has significant translational value, as two of the FDA-approved treatments for alcohol use disorder, acamprosate and naltrexone, decrease alcohol consumption in rats with intermittent access to alcohol, but not in rats given continuous access to alcohol (Simms et al., 2008). In regards to pharmacological induction of escalated alcohol consumption, injection of the anxiogenic compound yohimbine, an α2 adrenergic antagonist that increases noradrenergic output, induces increased rates of alcohol self-administration (Le et al., 2005, 2013; Williams et al., 2016). While our previous work demonstrates a role of the NK1R in the CeA in escalated consumption of P rats, it is unclear if this receptor contributes to escalated consumption induced by the non-genetic models described above, and if so, what brain regions govern this effect.

The current study investigated the role of NK1R in escalated drinking induced by yohimbine injection in the operant self-administration paradigm and in escalated intake induced by intermittent access in the two-bottle choice paradigm. Furthermore, we assessed changes in NK1R expression in stress-sensitive brain regions following exposure to intermittent access and targeted the NK1R in specific regions using intracranial infusion of the NK1R antagonist L822429.

2. Materials & methods

2.1. Animals and housing

Adult male, Wistar rats (200–225 g at time of arrival) were individually housed on a reversed light cycle (lights on at 8:00 a.m.). The animals were acclimatized to housing conditions for one week and handled for an additional week before the start of experimentation. Food and water were provided *ad libitum* except where otherwise stated. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Georgia.

2.2. Operant self-administration

Rats self-administered alcohol (10% v/v) in standard operant chambers (MedAssociates) on an FR1 schedule with a 5 s timeout/cuelight presentation that followed alcohol delivery. Prior to initiation of alcohol self-administration, a saccharin fading procedure in the absence of the cue light was used to initiate lever pressing, as previously described (Schank et al., 2011, 2012a, 2013, 2014, 2015). Sessions were 30 min in duration and run once daily, five days per week. The rats reached stable lever pressing after 15–20 days at which point the rats were injected with either vehicle (2-hydroxypropyl β-cyclodextrin, 45% w/v) or the NK1R antagonist L822429 (30 mg kg, i.p. at 2 ml/kg). We have used this dose of L822429 in previous studies and have observed no non-specific or sedative effects in Wistar rats at this dose (Schank et al., 2011, 2013). Thirty minutes after these injections, rats were injected with vehicle (sterile water) or yohimbine (2.5 mg/kg, i.p. at 1 ml/kg). Self-administration sessions began 30 min after the yohimbine injections. Each rat received each treatment in a random, counterbalanced order with 2–3 days of self-administration without pretreatment between each treatment session.

2.3. Two-bottle choice

Continuous Access: Rats were given continuous access to one bottle of 20% alcohol and one bottle of water in their home cage for 4–5 weeks. Bottles were equipped with stainless steel drinking spouts and were placed on the wire lid of the home cage.

Intermittent Access: The intermittent access paradigm was carried out as described by de Guglielmo et al. (2016). Briefly, rats were given access to two bottles with stainless-steel drinking spouts, one of which contained water at all times. Rats were given access to 20% (v/v) alcohol in one of the two bottles on Monday, Wednesday, and Friday. All other days, both bottles contained water. This access schedule induces escalation and rats exposed to this procedure achieve blood alcohol levels as high as 100–150 mg/dl (Carnicella et al., 2009; Simms et al., 2008).

At the beginning of the dark cycle, bottles for all rats were weighed to calculate consumption. In order to avoid side preference, the position of the alcohol bottle was switched each time alcohol was available. Each rat was weighed weekly in order to calculate the grams of alcohol intake per kilogram of body weight. Two weeks before the treatment phase was to begin, bottles were weighed 2 h after bottle presentation in addition to the 24 h time point to establish a baseline measure for the first 2 h of the drinking session. This is a useful measure in the event that treatment effects are transient due to limited drug half-life.

An additional group of rats was presented with water in both bottles for the duration of the experiment and was used to establish control levels for NK1R gene expression.

2.4. Systemic L822429 - two bottle choice

After baseline-drinking rates reached stability, the treatment phase began. Rats were injected with L822429 (15 or 30 mg/kg) or vehicle (2-hydroxypropyl β-cyclodextrin, 45% w/v; i.p. at 2 ml/kg) 60 min before being given access to alcohol. Two hours after being presented with alcohol, the bottles were weighed. Each rat received each treatment in a random, counterbalanced order with 2–3 days of self-administration without pretreatment between each treatment session. Because a subset of these rats were the same as those used for gene expression measures, the group of rats receiving water only was also treated with the NK1R antagonist in the same manner, even though they did not have access to alcohol. After 2–4 days of alcohol access without pretreatment, all rats were sacrificed