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Melanocortin activity in the amygdala influences alcohol intake

D.A. York *, S. Boghossian, M. Park-York

Center for Advanced Nutrition, Utah State University, United States
Department of Biology, Utah State University, United States

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ABSTRACT

Melanocortins have been reported to affect alcohol intake through actions in the hypothalamus thought to be mediated by melanocortin MC4 receptors. Since these receptors are expressed in a number of amygdala regions, we have explored their role in the regulation of alcohol intake in both alcohol-preferring (P) and non-preferring (NP) rats. Injections were made at the border of the central amygdala nucleus and the basolateral amygdala. The MC3/MC4R agonist MTII reduced alcohol and food intake but increased water intake while the selective MC4R antagonist HS014 only increased food and water intake. The MC3/MC4R antagonist SHU9119 increased food and water but had little effect on alcohol intake. However, when the SHU9119 stimulation of food intake was prevented by pair-feeding, SHU9119 induced a large and prolonged decline in alcohol intake that was paralleled by an increase in water intake. These effects were only observed in P rats. We conclude that melanocortin activity in the amygdala can alter the selective preference for water and alcohol independent of effects on food intake.

1. Introduction

Alcohol related health and behavioral problems are major public health and societal challenges. Development of treatment and preventive strategies are dependent upon a full understanding of the neurobiological basis of alcohol ingestive behavior and the environmental factors that impact these regulatory systems. Numerous signaling systems have been implicated in the regulation of alcohol intake including NPY, the melanocortins, galanin and the endocannabinoids.

Alcohol preferring rats show derangements in their melanocortin signaling system that include an increased ratio of AgRP/POMC expression in the arcuate nucleus and increased levels of MC3 receptors in a number of brain regions (Lindblom et al., 2002). A decreased level of α-MSH has also been reported in the arcuate nucleus of rats given an alcohol containing diet (Navarro et al., 2008). Ventricular injections of the melanocortin agonist MTII attenuated alcohol consumption in rats and mice (Navarro et al., 2003; Polidori et al., 2006), an effect opposed by the MC3/MC4R inverse agonist AgRP which alone can stimulate alcohol intake (Navarro et al., 2005). As this effect is still present in MC3R null mice, it suggests that it is the MC4Rs within the hypothalamus that are regulating alcohol intake (Navarro et al., 2005).

The effects of NPY on alcohol ingestion are less clear. There have been reports of no effects to icv injections (Badia-Elder et al., 2001; Katner et al., 2002) but increased or decreased consumption after PVN injections of NPY agonists or antagonists respectively (Gilpin et al., 2004; Lucas and McMillen, 2004; Thiele et al., 2004). While these reports focused on the hypothalamic centers, intra-amygdala administration of an NPY Y1 receptor antagonist decreased ethanol intake (Schroeder et al., 2003) whereas a NPY antisense vector increased and a NPY encoding vector decreased ethanol preference (Primeaux et al., 2006a).

Part of the difficulty in clearly defining the effects of neuropeptides on alcohol ingestion is the necessity to separate the response from other behavioral effects including feeding and anxiety. Anxiolytic behavior has been linked to increased alcohol intake in humans and rodents (Hirani et al., 2005; Polivy and Herman, 1976). NPY injections to the Central Amygdala Nucleus (CeA) also suppress anxiety (Primeaux et al., 2005) and only suppress alcohol intake in anxious rats (Primeaux et al., 2006a). Galanin, both icv and into the PVN, increases alcohol intake both in the presence and absence of food (Lewis et al., 2004; Rada et al., 2004). Increased intake of high fat diets is also associated with increased preference for alcohol (Carrillo et al., 2004; Pekkanen et al., 1978) and galanin also increases dietary fat intake (Leibowitz, 2007; Tempel et al., 1988).

Abbreviations: AgRP, agouti-related protein; AP/L/V, anterior–posterior/lateral/ventral; CeA, central nucleus of amygdala; GABA, gamma amino butyric acid; icv, intracerebroventricular; MC3R, melanocortin 3 receptor; MC4R, melanocortin 4 receptor; αMSH, alpha melanocyte stimulating hormone; MTII, melanotan II; NP, alcohol non-preferring; NPY, neuropeptide Y; P, alcohol-preferring; POMC, proopiomelanocortin.

* Corresponding author. Center for Advanced Nutrition, Utah State University, 4715 Old Main Hill, Logan, UT 84322-4715, United States. Tel.: +1 435 797 2578; fax: +1 435 797 1114.

E-mail addresses: david.york@usu.edu (D.A. York), Stephane.Boghossian@usu.edu (S. Boghossian), MieJung.park@usu.edu (M. Park-York).

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These data, together with observations that galanin expression in the PVN is increased by both high fat diets and alcohol intake (Leibowitz, 2007) suggests a close interaction between galanin, dietary fat and alcohol consumption (Leibowitz, 2005). NPY onto the CeA also decreased the selection of dietary fat (Primeaux et al., 2006b) and alcohol (Primeaux et al., 2006a), again illustrating the link between alcohol and fat intake.

This manuscript focuses on the role of the melanocortin system within the amygdala to influence alcohol intake. The amygdala is targeted since it has a unique role in anxiety and reinforcement and anxiolytic behavior is linked to alcohol ingestion. Both P and NP rats were studied since P alcohol-preferring rats display more anxiolytic behavior than NP rats (Primeaux et al., 2006a; Stewart et al., 1993) and since differences in hypothalamic melanocortin signaling have been reported between P and NP rats (Lindblom et al., 2002; Primeaux et al., 2006a; Stewart et al., 1993). A reduction in anxiety might be expected to reduce alcohol intake. The amygdala has a wide expression of melanocortin MC4 receptors (Kishi et al., 2003; Mountjoy et al., 1994) and melanocortins are known to affect alcohol intake and anxiolytic behaviors (Chaki and Okuyama, 2005; Zarrindast et al., 2008). In addition, we have shown previously that melanocortin actions in the CeA affect dietary fat intake (Boghossian et al., 2010). MTII, the MC3/MC4R non-specific agonist, injected onto the CeA selectively inhibits dietary fat intake when rats can choose between high fat and low fat diets, whereas the antagonist (SHU9119) or inverse agonist (AgRP) promote fat intake (Boghossian et al., 2010). The effects are dose-related and prolonged, lasting between 3 and 4 days in response to a single injection. To our knowledge, this is the first report of the effects of melanocortins within the amygdala on alcohol ingestion.

2. Material and methods

2.1. Animals

Two cohorts of male 6 week old alcohol-preferring (P) and non-preferring (NP) rats (Bell et al., 2005) were used in these studies. They were supplied by Dr. Richard Bell (Indiana University School of Medicine). The first cohort (body weights at time of surgery 299.8 ± 6.2 and 269.2 ± 8.5 g for P and NP rats respectively) was used for the MTII experiment and the initial SHU91119 experiment. The second cohort (body weights at time of surgery 284.2 ± 7.7 and 310.6 ± 5.8 g for P and NP rats respectively) was used for the 2nd SHU9119 experiment and the HS014 experiment. There was a minimum of 7 days between the end of one experiment and the start of the next experiment. Rats were housed individually in hanging wire mesh cages to facilitate accurate measurement of spilled food, in a temperature (22–24 °C) and light controlled (lights off 1900–0700 h) room. They were fed a standard laboratory chow diet *ad libitum*. Experimental protocols involving the animals were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Utah State University.

2.2. Animal surgery

Indwelling 26 g stainless steel unilateral cannulas were stereotaxically implanted and aimed at the basolateral–CeA boundary of the amygdala using standard procedures that have been previously described (Boghossian et al., 2009, 2010). Coordinates were determined from the rat atlas of Paxinos and Watson (1982) (coordinates [AP/L/V to bregma] −2.8/−4.6/−6) and confirmed by ink injection in a group of rats in a pilot experiment. This site was chosen as the CeA and basolateral amygdala are rich in MC4Rs (Kishi et al., 2003; Mountjoy et al., 1994). Cannulas were implanted at 8 weeks of age. Rats were anesthetized with Nembutal (0.1 ml/100 g body weight) and placed in a stereotaxic frame. Each cannula was secured in place with 3 anchor screws and dental acrylic and occluded with a 26 gauge wire stylet. The injector was designed to project 1 mm beyond the guide cannula tip. Each rat received an injection of the analgesic drug Carprofen (Rimadyl® 5 mg/kg, *s.q.*) before returning to their home cage. Animals were allowed to recover for 7 days to their preoperative weight before starting adaptation to alcohol.

2.3. Adaptation to alcohol

Both alcohol-preferring (P) and non-preferring (NP) rats were adapted to drinking ethanol by following the protocol described by Resch and Simpson (2008) in which rats were provided with 2 bottles in their home cage, one containing water the other ethanol. For the initial experiment, the ethanol concentration was increased serially from 2.5% to 10% in 4 steps of increasing concentration at 3 day intervals. We found alcohol non-preferring rats adapt to alcohol equally as well by this method as with the sucrose-fading method (Files et al., 1997) so we used this uniform method for both groups of rats. Only P rats that achieved a consistent alcohol intake >6 g/kg body weight/day were included in the experiments. In addition, we found that alcohol intake of P rats was maximal on the 7.5% solution, so this concentration was used for all experiments and for alcohol adaptation of subsequent animals we used only 3 steps up to the 7.5% concentration.

2.4. Experimental protocols

Certain aspects were common to all experiments. Rats were provided with a choice of water or 7.5% alcohol to drink. Drugs in saline vehicle were infused in a total volume of 1 μl delivered over a 1 min period into the amygdala at the basolateral–CeA boundary. Infusion cannulas were left in place for a further minute to prevent back diffusion. Rats were then returned to their home cages and food, water and alcohol intake were measured over the times identified in each experiment. We have previously investigated the volume of diffusion of a 1 μl injection in the amygdala (Boghossian et al., 2009), from which we would expect the injections to diffuse into both the CeA and the basolateral amygdala regions.

2.4.1. Effect of MTII on alcohol preference

Two experiments were performed in P rats adapted to alcohol. The first in rats that were food and alcohol deprived overnight, the second in rats that were satiated. The effects of MTII (0.5 nmol) or saline vehicle on food, water and alcohol intake were measured after 0.5, 1, 2, 4, 12 and 24 h and daily thereafter for a further 5 days.

2.4.2. Effect of SHU9119 on alcohol preference

P and NP rats adapted to alcohol were used. SHU9119 (1 nmol) or saline vehicle were injected into the basolateral/CeA border region of the amygdala of satiated animals. Food, water and alcohol intake were measured after 1, 2, 4, 12 and 24 h and daily thereafter for a further 5 days.

2.4.3. Effect of SHU9119 on alcohol preference in rats pair-fed to their controls

The previous experiment was repeated in a second group of P and NP rats except that the hyperphagic response to SHU9119 was prevented by pair-feeding the SHU9119 injected rats to their saline controls for the initial 3 days after injections.

2.4.4. Effect of HS014 on alcohol preference

The experiment followed the same protocol as the SHU9119 experiment in rats fed *ad libitum* except that HS014 (1 nmol) or saline vehicle were injected.