Pharmacology, Biochemistry and Behavior 98 (2011) 112–119

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

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

ARTICLE INFO

Article history:
Received 5 October 2010
Received in revised form 18 November 2010
Accepted 8 December 2010
Available online 15 December 2010

Keywords:
MC3/MC4 receptors
SHU9119
MTII
Amygdala
Alcohol preference
Water intake

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).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.12.010

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.

2.5. Data analysis

We used appropriate ANOVA with repeated measures (time) when necessary to identify treatment effects, followed by Tukey's test for individual comparisons. A p value of <0.05 was taken as statistically significant.

3. Results

3.1. Effect of MTII on alcohol intake

We hypothesized that the MC3/4 receptor agonist MTII would inhibit alcohol intake in P rats that were consuming significant quantities of alcohol. As we were expecting an inhibitory effect we did not study the response of NP rats to MTII as their alcohol intake was already very low. However, MTII had no effect on alcohol, water or food intake in P rats that had been fasted overnight (data not shown). When the experiment was repeated in satiated rats (Fig. 1) MTII suppressed alcohol intake and food intake and total caloric intake in the first 24 h, the effect first becoming evident after 12 h. However, while food intake and total caloric intake returned to control levels by 48 h, alcohol intake and preference was not restored fully until 72–96 h (Fig. 2). Water intake was not affected by MTII in the first 24 h but showed a large increase at 48–72 h when food intake was also increased.

3.2. Effect of SHU9119 on alcohol intake

We hypothesized that the MC3/4 receptor antagonist SHU9119 would increase alcohol intake as well as food intake and that this response may be more evident in NP rats than P rats as P rats have higher expression of the MC3/4 inverse agonist AgRP (Lindblom et al., 2002). P and NP rats had similar total daily caloric intake. While NP rats consumed around 1% energy as alcohol, the alcohol intake of P rats was equivalent to approximately 16% of daily energy intake. The MC3/4R antagonist increased food intake and water intake in both P and NP rats, the responses having similar time courses and becoming statistically significant after 12 h (Fig. 3) and remaining elevated for 48 h in NP rats and 72 h in P rats (Fig. 4). Alcohol intake was not affected by SHU9119 in the initial 24 h although water intake increased in line with the food intake such that alcohol preference was significantly reduced in SHU9119-treated P rats between 12 and 24 h. During the second post injection day alcohol intake of P rats was reduced and water intake increased leading to a significant reduction in alcohol preference (Fig. 4). There were no effects on alcohol preference in NP rats.

In order to separate the effects of SHU9119 on alcohol intake from those on food intake, we repeated this experiment but prevented the SHU9119-induced hyperphagia in the first 72 h by pair feeding the rats to the intake of the control saline injected rats. We hypothesized that, in the absence of hyperphagia, that SHU9119 would increase alcohol intake to satisfy the demand for additional caloric intake. In contrast to expectations, there was no effect on alcohol intake in the first 24 h but there was a stimulation of water intake in P rats after 12 h that led to a significant reduction in alcohol preference (Fig. 5). The increase in water intake in SHU9119 treated P rats was large and prolonged, lasting for 8 days before returning to control levels (Fig. 6). Conversely, alcohol intake was reduced, but this response was not evident until day 3 but it did not return to normal control levels until day 10. As a consequence, alcohol preference was suppressed by SHU9119 in P rats. A transient one day increase in food intake in the initial 24 h after pair feeding was stopped was observed in SHU9119 treated rats. No significant effects of SHU9119 were observed in the NP rats.

3.3. Effect of HS014 on alcohol intake

As SHU9119 is not selective between MC3 and MC4 receptors, we studied the effects of the selective MC4R antagonist HS014 on food,

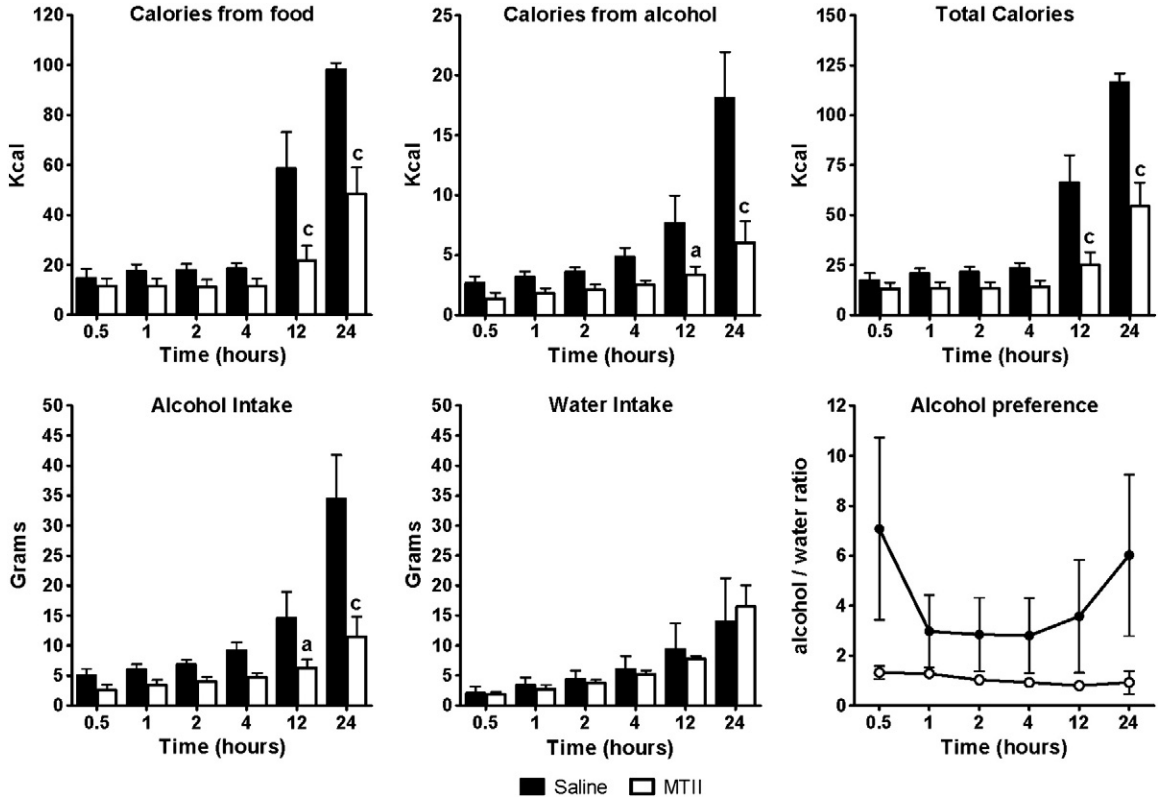

Fig. 1. Effect of MTII on food, water and alcohol intake and alcohol preference of satiated Alcohol-Preferring (P) rats during the first 24 h after injection. Values represent Means ± SEMs for 8 rats per group. a $p<0.05$, c $p<0.001$ compared to respective saline control.

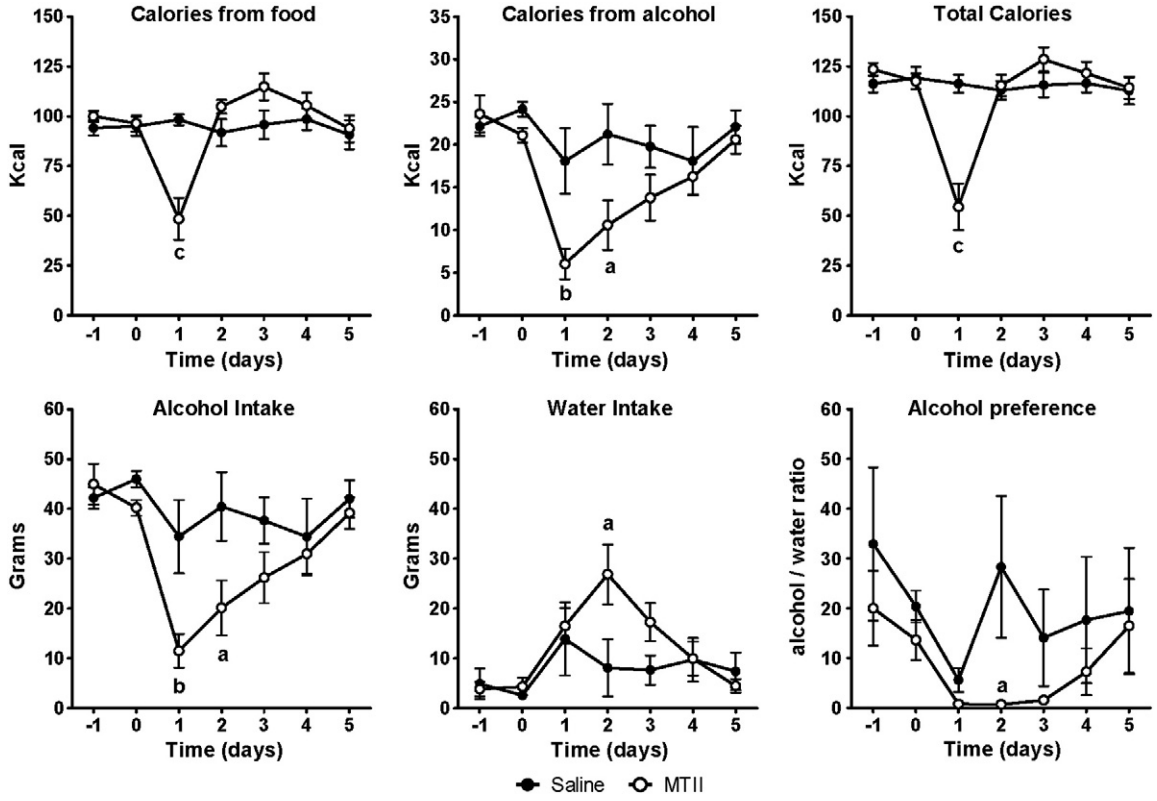

Fig. 2. Long-term response of intra-amygdala MTII on food, water and alcohol intake and alcohol preference of Alcohol-Preferring (P) rats. Values represent Means ± SEMs for 8 rats per group. a $p<0.05$, b $p<0.01$, c $p<0.001$ compared to respective saline control.

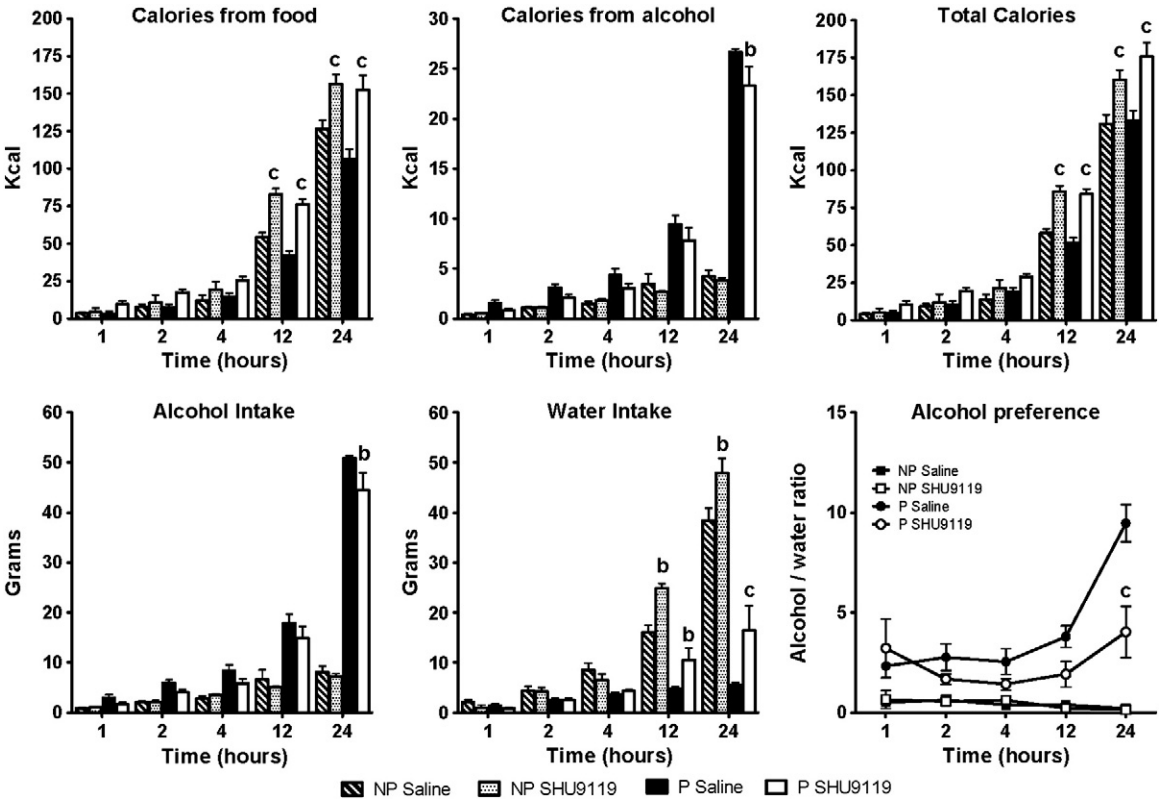

Fig. 3. Effect of SHU9119 on food, water and alcohol intake and alcohol preference of Alcohol-Preferring (P) and Non-Preferring (NP) rats during the first 24 h after injection. SHU9119 or saline vehicle was injected unilaterally into the amygdala at the border of the CeA and Basolateral areas. Values represent Means ± SEMs for 8 rats per group. b $p<0.01$, c $p<0.001$ compared to respective saline control.

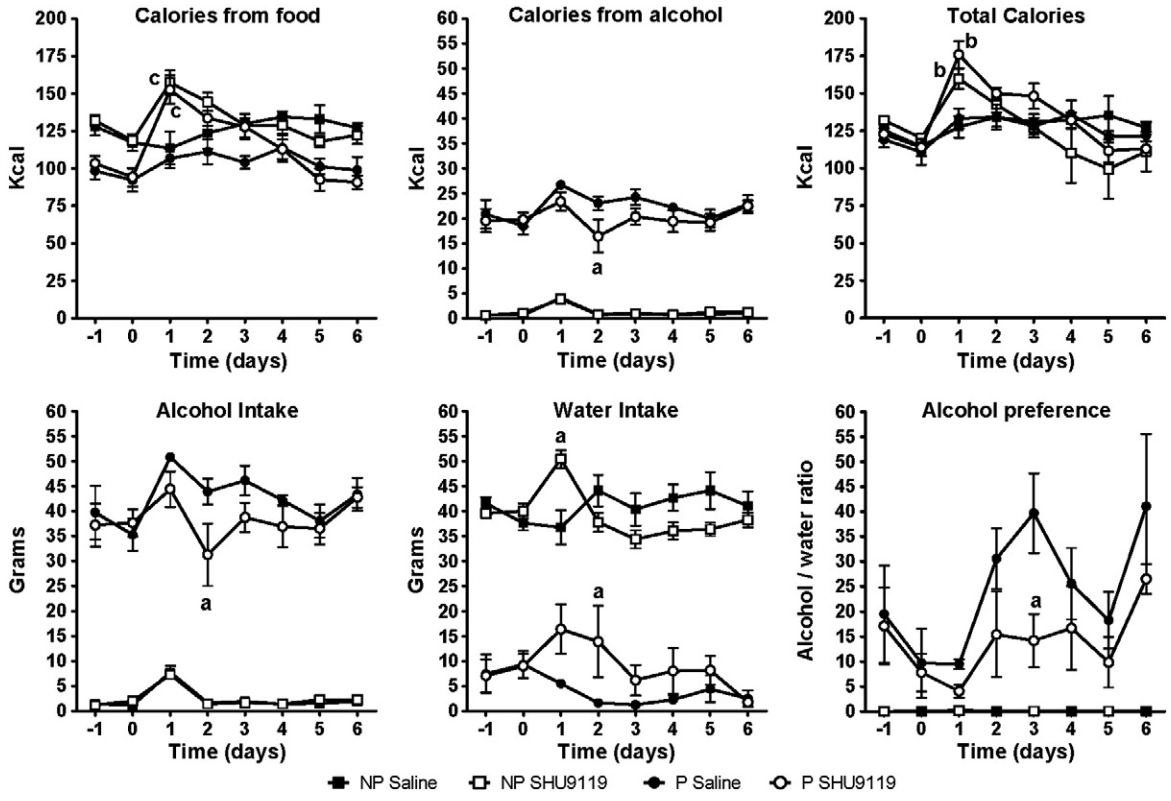

Fig. 4. Effect of SHU9119 on daily food, water and alcohol intake and alcohol preference of Alcohol-Preferring (P) and Non-Preferring (NP) rats. These animals are those described in Fig. 3. Values represent Means ± SEMs for 8 rats per group. a $p<0.05$, b $p<0.01$, c $p<0.001$ compared to respective saline control.

alcohol and water intake in P and NP rats. HS014 had a transient orexigenic effect in both P and NP rats that lasted just for the initial 24 h (Figs. 7 and 8). The effect was greater in P rats that eat less food than the NP rats. Likewise there was a significant stimulation of water intake in P rats within the first 24 h. There was a tendency towards a reduction in alcohol intake in the P rats during the first 48 h but this effect did not reach statistical significance. As the changes in water and alcohol intake were small, there were no significant changes in alcohol preference.

4. Discussion

The experiments described in this manuscript have for the first time identified effects of melanocortin signaling in the amygdala on alcohol ingestion. The MC3/MC4R agonist MTII reduced both food and alcohol intake although the reduction in alcohol intake lasted longer than the reduction in food intake. Surprisingly, the MC3/4 receptor antagonist SHU9119 also reduced alcohol intake. This response was short lived and relatively small in rats that were allowed to eat *ad libitum* and showed the expected hyperphagic response to SHU9119, but the reduction was large and prolonged in rats in which the hyperphagia was prevented by pair feeding. The more selective MC4R antagonist HS014 produced a short-lived hyperphagia and a small reduction in alcohol intake during the first 48 h. These effects were only observed in the P rats that showed a large preference for alcohol.

Interpretation of these experimental data is difficult, particularly in identifying whether the melanocortin effects are selective on alcohol ingestion and independent of the pathways through which melanocortins regulate energy (food) intake. Indeed, the inhibition of alcohol intake in response to MTII could reflect the inhibitory effect of

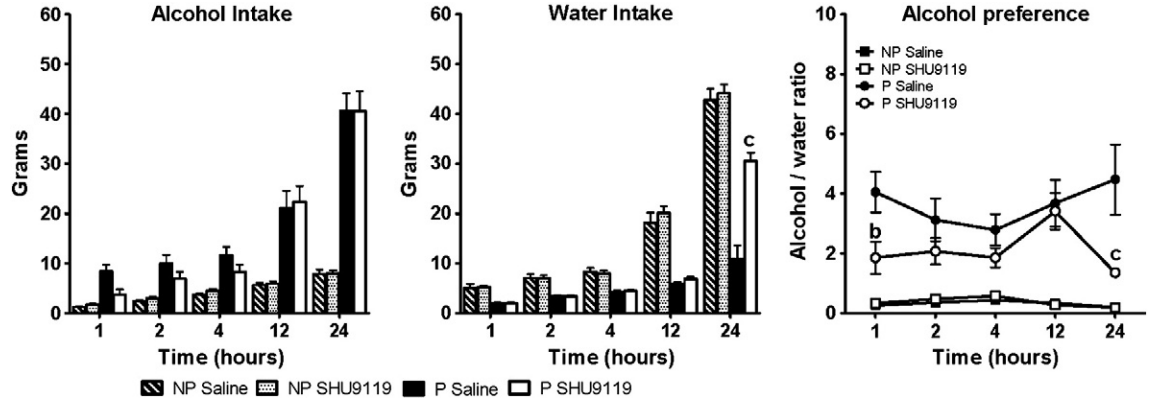

Fig. 5. Effect of SHU9119 on water and alcohol intake and alcohol preference of Alcohol-Preferring (P) and Non-Preferring (NP) rats that were pair fed to their respective control rat during the first 24 h after injection . SHU9119 or saline vehicle was injected unilaterally into the amygdala at the border of the CeA and Basolateral areas. SHU9119 injected rats were pair fed to the intakes of saline injected rats of the same group. Values represent Means ± SEMs for 8 rats per group. b $p<0.01$, c $p<0.001$ compared to respective saline control.

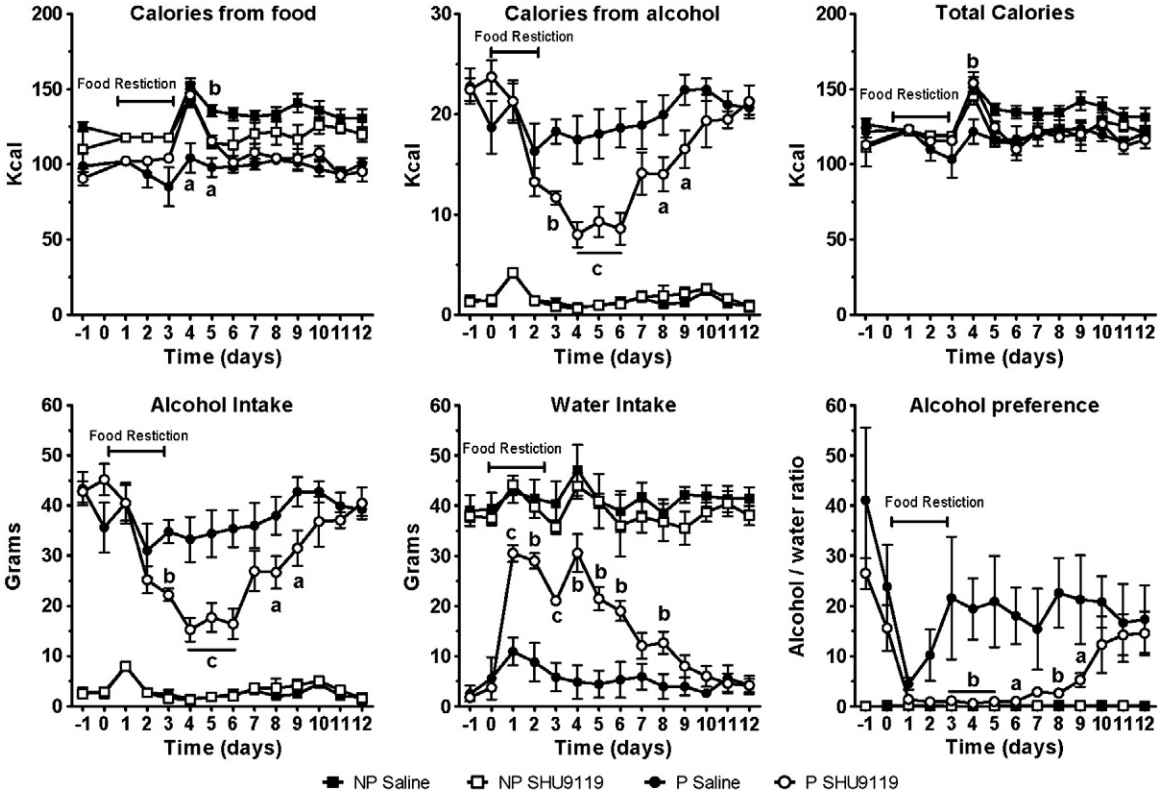

Fig. 6. Effect of SHU9119 on food, water and alcohol intake and alcohol preference of Alcohol-Preferring (P) and Non-Preferring (NP) rats that were pair fed to their respective control rat for 3 days after injection . SHU9119 or saline vehicle was injected unilaterally into the amygdala at the border of the CeA and Basolateral areas. SHU9119 injected rats were pair fed to the intakes of saline injected rats of the same group for 3 days. Values represent Means ± SEMs for 8 rats per group. a $p<0.05$, b $p<0.01$, c $p<0.001$ show statistical difference between saline and SHU9119 rats at that time point.

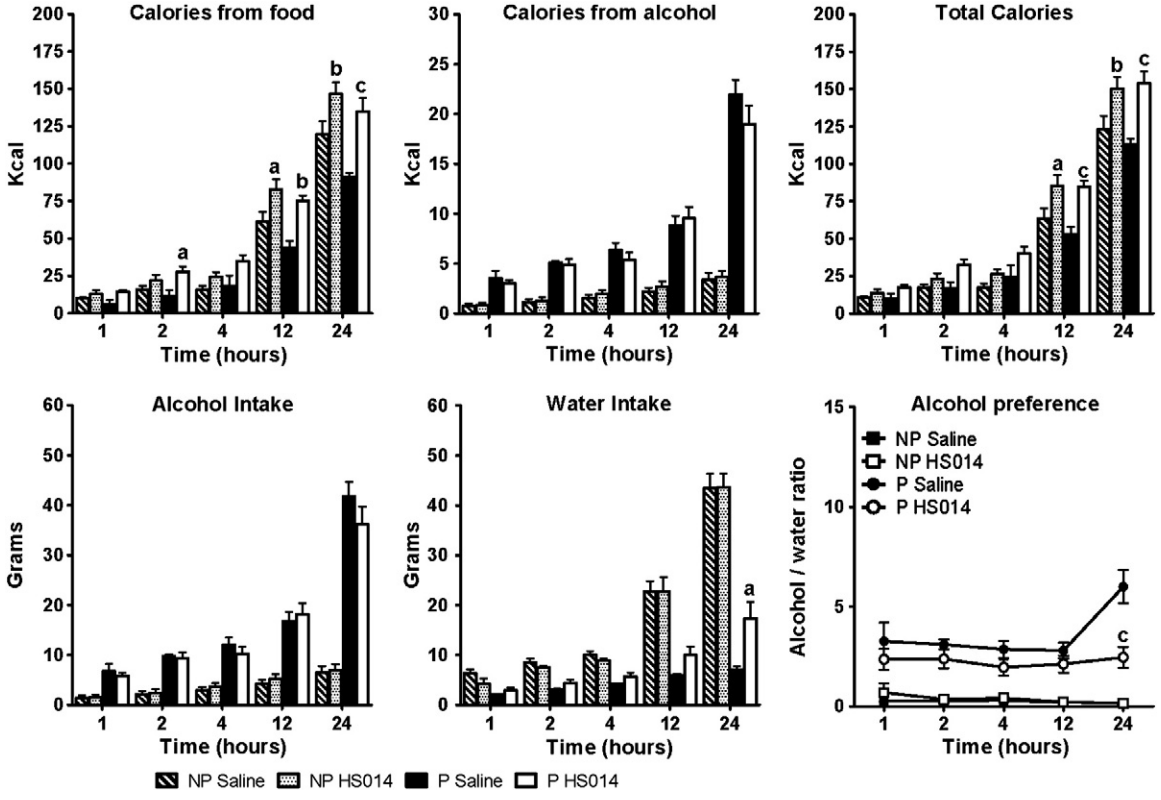

Fig. 7. Effect of HS014 on food, water and alcohol intake and alcohol preference of Alcohol-Preferring (P) and Non-Preferring (NP) rats during the first 24 h after injection. HS014 or saline vehicle was injected unilaterally into the amygdala at the border of the CeA and Basolateral areas. Values represent Means ± SEMs for 8 rats per group. a $p<0.05$, b $p<0.01$, c $p<0.001$ compared to respective saline control.

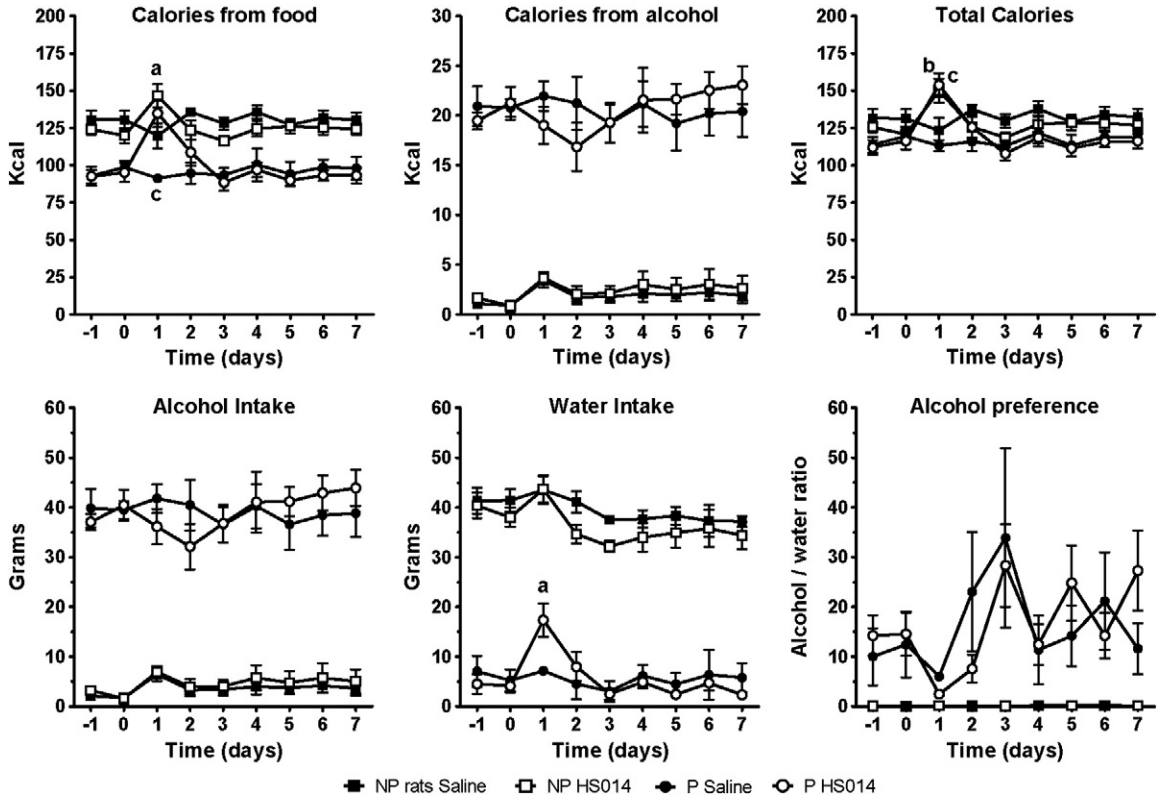

Fig. 8. Long term response of food, water and alcohol intakes and alcohol preference of Alcohol-Preferring (P) and Non-Preferring (NP) rats to a single intra-amygdala dose of HS014. Values represent Means ± SEMs for 8 rats per group. a $p<0.05$, b $p<0.01$, c $p<0.001$ indicates significant difference between saline and HS014 group at that time point.

melanocortin signaling in the CeA to reduce food intake as we and others have previously reported (Boghossian et al., 2010; Kask and Schioth, 2000). This would not be surprising as a number of other neuropeptides e.g., NPY, galanin, CCK and cannabinoids have been shown to affect both food and alcohol intake (Blednov et al., 2007; Gilpin et al., 2004; Kulkosky and Chavez, 1984; Leibowitz, 2007). However, this simple interpretation is questioned by the prolonged inhibition of alcohol intake over 72 h while food intake returned to normal levels by the second day, suggesting a selective effect of MTII on alcohol rather than a general effect on energy intake. Likewise, the small attenuation of alcohol intake after both SHU9119 and HS014 could be interpreted as being secondary to the increased energy intake in the form of chow. Once again this simple interpretation is questioned by the prolonged inhibition of alcohol intake in response to SHU9119 when the hyperphagic response was prevented for 3 days by pair feeding. However, the reduction in alcohol intake in response to SHU9119 was paralleled by a reciprocal increase in water intake. This was also the case in the smaller acute response to HS014. Further, the changes in water intake in response to SHU9119 and HS014 were evident 24 h before the reciprocal changes in alcohol intake. These data would suggest that the reduction in alcohol intake after antagonism of MC3/MC4 receptors in the amygdala is secondary to an enhanced intake and/or preference for water that appears to be independent of any increase in food intake. Melanocortin effects on water intake have been reported previously but the effects appear to be regionally specific, MTII inhibiting water intake in parallel with the reduction in food intake after injections in the brainstem but enhancing water intake after injection into the forebrain lateral ventricle (Grill et al., 1998). MTII also stimulated water intake in P rats in our study, but this increase was only evident 24 h after the reduction in alcohol intake suggesting a secondary effect. Our data suggest that the reduction in alcohol intake in response to melanocortin antagonists in the amygdala is secondary to the large stimulation of water intake and decreased preference for alcohol but that the small decrease in alcohol in response to the MC3/MC4R agonist MTII occurs prior to the water response and probably reflects a primary effect on energy intake.

An increased ratio of POMC/AgRP mRNA was reported in the arcuate nucleus together with increased MC3R ligand binding levels in a number of hypothalamic nuclei of alcohol-preferring AA rats while MC4R ligand binding was only elevated in the VMH (Lindblom et al., 2002). However, MTII reduced ethanol drinking in Mc3r-deficient mice (Navarro et al., 2005) suggesting that it is MC4Rs that regulate alcohol intake. In our studies, both P and NP rats were responsive to the antagonists SHU9119 and HS014 which increased food and water intake. However, only P rats were responsive to the SHU9119 stimulation of water intake when the hyperphagic response was prevented. This may reflect a ceiling effect in the NP rats that were drinking more than 4 times the level of water than the P rats although SHU9119 did increase water intake by 20% in NP rats when food was available *ad libitum*. Likewise the small effect of the specific MC4R antagonist HS014 compared to the large and prolonged response to the MC3/MC4R antagonist SHU9119 would suggest that the major effects on water and alcohol intake were mediated through MC3 receptors rather than MC4R.

Alcohol intake has been related to the anxiolytic state (Hirani et al., 2005; Polivy and Herman, 1976) and the intra-amygdalar NPY reduction in alcohol intake is associated with a reduction of anxiety (Primeaux et al., 2006a) suggesting that anxiety levels are predictive for alcohol consumption. P alcohol-preferring rats display more anxiolytic behavior than NP rats (Primeaux et al., 2006a; Stewart et al., 1993) so a reduction in anxiety might be expected to reduce alcohol intake. Microinjection of αMSH or MTII into hypothalamic sites or intraventicularly induces anxiogenic behavior (Chaki and Okuyama, 2005; Kokare et al., 2006). Likewise, injections of αMSH into the amygdala induced anxiety (Kokare et al., 2005). It is possible that the reduction in alcohol after the MC3/MC4R agonist MTII reflects an inhibitory effect on energy intake despite an increase in anxiety,

whereas the large prolonged reduction in alcohol intake after the MC4R antagonist SHU9119 may reflect a reduction in anxiety.

The orexigenic response to a single injection of SHU9119 icv or into the CeA is prolonged (Boghossian et al., 2010; Fan et al., 1997; Hagan et al., 2000). The mechanism that maintains this hyperphagic state for several days is unclear. However, the reduction in alcohol intake after SHU9119 and increase in water intake that are independent of any changes in food intake are even more prolonged, lasting for 8–9 days before returning to normal control levels. This contrasts with the orexigenic response to brain stem SHU9119 which is shortened by food deprivation (Grill et al., 1998). However, we did observe a small increment in feeding for one day when the pair feeding restriction was removed after 72 h. The large response to the MC3/MC4R antagonist indicates a role for endogenous melanocortin activity in the amygdala to regulate alcohol or water preferences.

In summary, our results show that melanocortin agonists and antagonists can regulate alcohol intake through actions on the MC3 and/or MC4 receptors in the amygdala. The effects were only observed in P rats. The inhibitory effects of both MC3/MC4R agonists and antagonists on alcohol intake suggests that the inhibitory effect of agonists may reflect their effect on energy intake whereas the prolonged inhibitory response to MC3/MC4R antagonist is independent of effects on food intake and is related to a stimulation of water intake. Our data suggests that melanocortin signaling within the amygdala has profound effects on water intake. Further studies are required to understand the pathways that are involved and the physiological importance of them.

Acknowledgements

This work was supported by funding from the Utah Science, Technology and Research (USTAR) funds.

References

Badia-Elder NE, Stewart RB, Powrozek TA, Roy KF, Murphy JM, Li TK. Effect of neuropeptide Y (NPY) on oral ethanol intake in Wistar, alcohol-preferring (P), and -nonpreferring (NP) rats. Alcohol Clin Exp Res 2001;25:386–90.

Bell RL, Rodd ZA, Murphy JM, McBride WJ. Use of selectively bred alcohol-preferring rats to study alcohol abuse, relapse and craving. In: Watson VPaR, editor. Comprehensive handbook of alcohol related pathology. New York: Academic Press, Elsevier Science; 2005. p. 1515–33.

Blednov YA, Cravatt BF, Boehm II SL, Walker D, Harris RA. Role of endocannabinoids in alcohol consumption and intoxication: studies of mice lacking fatty acid amide hydrolase. Neuropsychopharmacology 2007;32:1570–82.

Boghossian S, Lemmon K, Park M, York DA. High-fat diets induce a rapid loss of the insulin anorectic response in the amygdala. Am J Physiol Regul Integr Comp Physiol 2009;297:R1302–11.

Boghossian S, Park M, York DA. Melanocortin activity in the amygdala controls appetite for dietary fat. Am J Physiol Regul Integr Comp Physiol 2010;298:R385–93.

Carrillo CA, Leibowitz SF, Karatayev O, Hoebel BG. A high-fat meal or injection of lipids stimulates ethanol intake. Alcohol 2004;34:197–202.

Chaki S, Okuyama S. Involvement of melanocortin-4 receptor in anxiety and depression. Peptides 2005;26:1952–64.

Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 1997;385:165–8.

Files FJ, Denning CE, Hyytia P, Kiianmaa K, Samson HH. Ethanol-reinforced responding by AA and ANA rats following the sucrose-substitution initiation procedure. Alcohol Clin Exp Res 1997;21:749–53.

Gilpin NW, Stewart RB, Murphy JM, Badia-Elder NE. Neuropeptide Y in the paraventricular nucleus of the hypothalamus increases ethanol intake in high- and low-alcohol-drinking rats. Alcohol Clin Exp Res 2004;28:1492–8.

Grill HJ, Ginsberg AB, Seeley RJ, Kaplan JM. Brainstem application of melanocortin receptor ligands produces long-lasting effects on feeding and body weight. J Neurosci 1998;18:10128–35.

Hagan MM, Rushing PA, Pritchard LM, Schwartz MW, Strack AM, Van Der Ploeg LH, et al. Long-term orexigenic effects of AgRP-(83–132) involve mechanisms other than melanocortin receptor blockade. Am J Physiol Regul Integr Comp Physiol 2000;279:R47–52.

Hirani K, Sharma AN, Jain NS, Ugale RR, Chopde CT. Evaluation of GABAergic neuroactive steroid 3alpha-hydroxy-5alpha-pregnane-20-one as a neurobiological substrate for the anti-anxiety effect of ethanol in rats. Psychopharmacology 2005;180:267–78.

Kask A, Schioth HB. Tonic inhibition of food intake during inactive phase is reversed by the injection of the melanocortin receptor antagonist into the paraventricular nucleus of the hypothalamus and central amygdala of the rat. Brain Res 2000;887:460–4.

Katner SN, Slawecki CJ, Ehlers CL. Neuropeptide Y administration into the third ventricle does not increase sucrose or ethanol self-administration but does affect the cortical EEG and increases food intake. Psychopharmacology 2002;160:146–54.

Kishi T, Aschkenasi CJ, Lee CE, Mountjoy KG, Saper CB, Elmquist JK. Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. J Comp Neurol 2003;457:213–35.

Kokare DM, Dandekar MP, Chopde CT, Subhedar N. Interaction between neuropeptide Y and alpha-melanocyte stimulating hormone in amygdala regulates anxiety in rats. Brain Res 2005;1043:107–14.

Kokare DM, Chopde CT, Subhedar NK. Participation of alpha-melanocyte stimulating hormone in ethanol-induced anxiolysis and withdrawal anxiety in rats. Neuropharmacology 2006;51:536–45.

Kulkosky PJ, Chavez MR. Sulphated cholecystokinin octapeptide inhibits ethanol consumption in the rat. Alcohol 1984;1:409–12.

Leibowitz SF. Regulation and effects of hypothalamic galanin: relation to dietary fat, alcohol ingestion, circulating lipids and energy homeostasis. Neuropeptides 2005;39:327–32.

Leibowitz SF. Overconsumption of dietary fat and alcohol: mechanisms involving lipids and hypothalamic peptides. Physiol Behav 2007;91:513–21.

Lewis MJ, Johnson DF, Waldman D, Leibowitz SF, Hoebel BG. Galanin microinjection in the third ventricle increases voluntary ethanol intake. Alcohol Clin Exp Res 2004;28:1822–8.

Lindblom J, Wikberg JE, Bergstrom L. Alcohol-preferring AA rats show a derangement in their central melanocortin signalling system. Pharmacol Biochem Behav 2002;72:491–6.

Lucas LA, McMillen BA. Effect of neuropeptide Y microinjected into the hypothalamus on ethanol consumption. Peptides 2004;25:2139–45.

Mountjoy KG, Mortrud MT, Low MJ, Simerly RB, Cone RD. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. Mol Endocrinol (Baltimore, Md) 1994;8:1298–308.

Navarro M, Cubero I, Knapp DJ, Thiele TE. MTII-induced reduction of voluntary ethanol drinking is blocked by pretreatment with AgRP-(83–132). Neuropeptides 2003;37:338–44.

Navarro M, Cubero I, Chen AS, Chen HY, Knapp DJ, Breese GR, et al. Effects of melanocortin receptor activation and blockade on ethanol intake: a possible role for the melanocortin-4 receptor. Alcohol Clin Exp Res 2005;29:949–57.

Navarro M, Cubero I, Knapp DJ, Breese GR, Thiele TE. Decreased immunoreactivity of the melanocortin neuropeptide alpha-melanocyte-stimulating hormone (alpha-MSH) after chronic ethanol exposure in Sprague–Dawley rats. Alcohol Clin Exp Res 2008;32:266–76.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1982.

Pekkanen L, Eriksson K, Sihvonen ML. Dietarily-induced changes in voluntary ethanol consumption and ethanol metabolism in the rat. Br J Nutr 1978;40:103–13.

Polidori C, Geary N, Massi M. Effect of the melanocortin receptor stimulation or inhibition on ethanol intake in alcohol-preferring rats. Peptides 2006;27:144–9.

Polivy J, Herman CP. Effects of alcohol on eating behavior: influence of mood and perceived intoxication. J Abnorm Psychol 1976;85:601–6.

Primeaux SD, Wilson SP, Cusick MC, York DA, Wilson MA. Effects of altered amygdalar neuropeptide Y expression on anxiety-related behaviors. Neuropsychopharmacology 2005;30:1589–97.

Primeaux SD, Wilson SP, Bray GA, York DA, Wilson MA. Overexpression of neuropeptide Y in the central nucleus of the amygdala decreases ethanol self-administration in “anxious” rats. Alcohol Clin Exp Res 2006a;30:791–801.

Primeaux SD, York DA, Bray GA. Neuropeptide Y administration into the amygdala alters high fat food intake. Peptides 2006b;27:1644–51.

Rada P, Avena NM, Leibowitz SF, Hoebel BG. Ethanol intake is increased by injection of galanin in the paraventricular nucleus and reduced by a galanin antagonist. Alcohol 2004;33:91–7.

Resch GE, Simpson CW. Glycyl-glutamine reduces ethanol intake at three reward sites in P rats. Alcohol 2008;42:99-106.

Schroeder JP, Olive F, Koenig H, Hodge CW. Intra-amygdala infusion of the NPY Y1 receptor antagonist BIBP 3226 attenuates operant ethanol self-administration. Alcohol Clin Exp Res 2003;27:1884–91.

Stewart RB, Gatto GJ, Lumeng L, Li TK, Murphy JM. Comparison of alcohol-preferring (P) and nonpreferring (NP) rats on tests of anxiety and for the anxiolytic effects of ethanol. Alcohol 1993;10:1-10.

Tempel DL, Leibowitz KJ, Leibowitz SF. Effects of PVN galanin on macronutrient selection. Peptides 1988;9:309–14.

Thiele TE, Sparta DR, Hayes DM, Fee JR. A role for neuropeptide Y in neurobiological responses to ethanol and drugs of abuse. Neuropeptides 2004;38:235–43.

Zarrindast MR, Sarahroodi S, Arzi A, Khodayar MJ, Taheri-Shalmani S, Rezayof A. Cannabinoid CB1 receptors of the rat central amygdala mediate anxiety-like behavior: interaction with the opioid system. Behav Pharmacol 2008;19:716–23.