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Effect of the melanocortin receptor stimulation or inhibition on ethanol intake in alcohol-preferring rats

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ABSTRACT

It has been recently reported that acute intracerebroventricular injection of 1 nmol/rat of the non-selective melanocortin 3 and 4 receptor (MC3/4) agonist MTII reduces ethanol intake in female AA alcohol-preferring rats and alters opioid peptide levels in the ventral tegmental area of rats. To better understand the role of the MC system in the control of ethanol intake, we tested the acute and chronic effects of lateral ventricular (LV) injections of 0.01–1 nmol MTII, of 0.1–1 nmol of the MC3/4R receptor antagonist agouti related peptide (AgRP), and 0.1–0.5 nmol of the MC3/4R receptor antagonist SHU9119 on food, water, and 10% ethanol intake in Marchigian-Sardinian alcohol-preferring (msP) rats, which spontaneously ingest pharmacologically relevant quantities of ethanol both under short and long term access conditions. The data showed that with 2 h/day ethanol access, LV MTII injections reduced intake of food and ethanol intakes. When food, water, and ethanol were available ad libitum and 0.01 nmol MTII was given by daily LV injection, however, ethanol intake was reduced for only the first 2 days, whereas food intake was reduced for all 5 days of treatment. Finally, acute LV injection of neither AgRP nor SHU9119 affected ethanol intake under ad libitum conditions, although both antagonists significantly increased food and water intake. In conclusion, these data fail to support a role for endogenous MC3/4R in the control of spontaneous ethanol intake in the msP rat. MC3/4R agonism, however, reduced ethanol intake in association with reduced food intake, suggesting that MTII might reduce nutrient-related controls of ethanol intake rather than, or in addition to, reward-related controls of ethanol intake.

1. Introduction

Early studies on pro-opiomelanocortin (POMC), the common precursor of alpha melanocortin stimulating hormone (α-MSH) and the endogenous opioid peptide β-endorphin and its derivatives, suggested that melanocortin peptides antagonize the addictive properties of opiates [4]; later on, a down-regulation of melanocortin-4 receptor expression, in brain areas involved in opiate addiction, has been observed after morphine treatment [2] suggesting an active interaction between the melanocortin (MC) and the opioid systems. Interactions between the MC and the opioid systems also have been observed in feeding behavior [12]. For example, opioids suppress alpha-MSH-dependent satietymechanisms, and the orexigenic action of the endogenous MC 3 and 4 receptor (MC3/4-R) antagonist agouti related peptide (AgRP) is mediated via opioid dependent circuitry [2].

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There is strong evidence that the rewarding and reinforcing effects of ethanol are dependent upon the endogenous opioid system [10,15,19]. The opioid receptor antagonist naltrexone is currently the standard pharmacological treatment for alcoholism [17,18]. Naltrexone treatment reduces the number of drinks taken per day, alcohol craving and rates of alcohol relapse, with the clinical efficacy of naltrexone treatment limited mainly by patient compliance [19]. These effects are consistent with neuropharmacological evidence that activation of β-endorphinergic neurons of the arcuate nucleus (ARC) appear to be part of the mechanism by which ethanol stimulates reward [15]. These β-endorphin neurons project, in part, to the nucleus accumbens (NAC) where they may stimulate presynaptic δ opioid receptors on dopamine (DA) neurons so as to increase DA release. In contrast, other β-endorphin neurons project from the ARC to the ventral tegmental area (VTA), where they indirectly inhibit DA neurons via stimulation of μ opioid receptors on GABA interneurons [19].

It was recently reported that the non-selective melanocortin agonist MTII both reduced ethanol intake and increased enkephalin immunoreactivity levels in the VTA of the AA strain of alcohol-preferring rats [14]. Therefore, to better understand the role of the MC system in ethanol intake, we investigated the effects of intracerebroventricular (ICV) injections of MTII, AgRP and the synthetic MC3/4R antagonist SHU9119 in another strain of alcohol-preferring rats, the Marchigian-Sardinian (msP) alcohol-preferring rat, which consume pharmacologically relevant quantities of ethanol and are considered a good model for the study of alcohol intake [1,3].

2. Method

2.1. Animals and housing

Male msP rats, the product of 39 generations of genetic selection at the University of Camerino from Sardinian alcohol-preferring rats of the 13th generation bred at the University of Cagliari, Italy (weighing 360–380 g at the beginning of the experiments) were individually housed in hanging stainless steel cages with grid floors in a room with an artificial 12:12 h light/dark cycle (dark onset at 9:00 a.m.) and a constant temperature of 20–22 °C. They were offered free access to chow pellets (Mucedola Diets, Settimo Milanese, Italy) in hoppers hung on the cage wall, to tap water and to a 10% (v/v) solution of ethanol. The positions of the ethanol and water graduated (50 ml capacity) sipper tubes were alternated daily, at the beginning of the dark phase. Only animals that had an ethanol:water preference greater than 90% and an ethanol intake of at least 6 g/kg/day were used for the experiments.

2.2. Surgery

Rats were food deprived overnight and anesthetized by intramuscular injection of Tiletamine chlorahydrate (200 mg/kg) and Zolazepam chlorahydrate (200 mg/kg) (Laboratoires Virbac, Carros, France). A prophylactic dose of Rubrocillin 200 μl/rat (Farmaceutici Gellini Spa, Aprilia, Italy) was also given by intramuscolar injection. Then, a 22-gauge guide cannula for ICV injections was stereotaxically implanted into either the right lateral ventricle, 1 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 2 mm ventral to the surface of the skull [13] or into the third ventricle (3 V), 1 mm posterior to bregma, 1 mm lateral to the sagittal suture and 7.5 mm ventral from the surface of the skull, with an inclination angle of 10°. A stainless-steel obturator of the same length was placed into the guide cannula at the end of surgery. A 30-gauge injector that was 2.5 mm longer than the guide cannula was used for ICV injections.

2.3. Drugs

MTII and AgRP mouse recombinant peptides was purchased from Calbiochem (Inalco, S.p.a., Milano, Italy) SHU 9119 (Bachem, Italy) and dissolved in saline.

2.4. ICV cannula validation

The patency of the ICV cannulas was checked by injecting angiotensin II (AII; Sigma, 100 ng in 1 μl of saline for LV injections and AII 10 ng for 3 V injections) during the second week of postoperative recovery. Only rats that drank at least 5 ml of water within 15 min of the AII injection were included in the experiments [6]. At the end of behavioral testing, 1 μl of Indian ink was injected, the rats were sacrificed by CO_2 inhalation within 10 min, and the brains were dissected to evaluate the distribution of ink. Only rats in which ink was distributed thorough the right lateral ventricle were included in the data analyses.

2.5. Data analysis

Data are presented as mean ± S.E.M. In acute injections the ANOVA was performed as one factor (treatment) analysis for each time separately. In chronic injections the ANOVA was performed as multifactorial analysis with a between-within model of repeated measures (treatments as between factor and time as within factor). When the ANOVA revealed a significant level a *t*-test (pairwise comparisons) was performed. Analyses were done using Systat 10.0 (SPSS Inc. Chicago, IL). Differences were considered significant when $p < 0.05$.

3. Experimental procedure

3.1. Effect of acute MTII injection into the LV and 3 V on ethanol intake of alcohol-preferring rats given limited access to ethanol

Male rats with LV or 3 V cannulas had access to 10% ethanol for 2 h/d beginning at dark onset and were adapted to the ICV injection procedure 1 h prior to ethanol access. Tests began only when ethanol intake was stable and between 0.6 and 0.8 g/kg/2 h. LV injections of MTII (0.1 and 1 nmol/rat; 6 and 13 rats, respectively) or 3 V injections of MTII (0.1 nmol/rat; seven rats) were then tested in a latin square design with an interval between treatment of at least 4 days. Ethanol and water