The ethanol metabolite acetaldehyde induces water and salt intake via two distinct pathways in the central nervous system of rats

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ARTICLE INFO

ABSTRACT

The sensation of thirst experienced after heavy alcohol drinking is widely regarded as a consequence of ethanol (EtOH)-induced diuresis, but EtOH in high doses actually induces anti-diuresis. The present study was designed to investigate the introduction mechanism of water and salt intake after heavy alcohol drinking, focusing on action of acetaldehyde, a metabolite of EtOH and a toxic substance, using rats. The aldehyde dehydrogenase (ALDH) inhibitor cyanamide was used to mimic the effect of prolonged acetaldehyde exposure because acetaldehyde is quickly degraded by ALDH. Systemic administration of a high-dose of EtOH at 2.5 g/kg induced water and salt intake with anti-diuresis. Cyanamide enhanced the fluid intake following EtOH and acetaldehyde administration. Systemic administration of acetaldehyde with cyanamide suppressed blood pressure and increased plasma renin activity. Blockade of central angiotensin receptor AT1R suppressed the acetaldehyde-induced fluid intake and c-Fos expression in the circumventricular organs (CVOs), which form part of dipsogenic mechanism in the brain. In addition, central administration of acetaldehyde together with cyanamide selectively induced water but not salt intake without changes in blood pressure. In electrophysiological recordings from slice preparations, acetaldehyde specifically excited angiotensin-sensitive neurons in the CVO. These results suggest that acetaldehyde evokes the thirst sensation following heavy alcohol drinking, by two distinct and previously unsuspected mechanisms, independent of diuresis. First acetaldehyde indirectly activates AT1R in the dipsogenic centers via the peripheral renin-angiotensin system following the depressor response and induces both water and salt intake. Secondly acetaldehyde directly activates neurons in the dipsogenic centers and induces only water intake.

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1. Introduction

After drinking large amounts of alcohol, many people experience thirst (Penning et al., 2012). It is widely believed that the thirst sensation can be attributed to alcohol- or ethanol (EtOH)-induced diuresis (Wang et al., 1991a). EtOH has been reported to reduce vasopressin (AVP) release from the nerve terminals of the posterior pituitary, resulting in increased urine formation (Eisenhofer and Johnson, 1982; Wang et al., 1991b). However, although low doses induce diuresis, urine volume is reduced rather than increased by large doses of EtOH (Pohorecky, 1985). This suggests that diuresis is not always the most important underlying factor for thirst sensation after heavy-alcohol drinking. In the condition known as hangover, in which subjects experience nausea, vomiting and dizziness in addition to thirst, the symptoms are thought to be elicited by acetaldehyde, a metabolite of EtOH and a toxic substance (Penning et al., 2012). However, acetaldehyde is not usually considered to be the factor that causes thirst after alcohol drinking because acetaldehyde has no effect on AVP release from the

posterior pituitary (Hashimoto et al., 1985).

Acetaldehyde dilates blood vessels and lowers blood pressure (Satoh et al., 2008). Such depressor responses induce renin secretion from the kidneys, resulting in an increase in plasma angiotensin II (AngII) concentration (Wagner et al., 2010). AngII activates neurons in the dipsogenic centers that are situated in the circumventricular organs (CVOs) of the brain, including the organum vasculosum of the lamina terminalis (OVLT) and the subfornical organ (SFO), increasing both water and salt intake (Fitzsimons, 1998; McKinley et al., 2003). In addition, in vivo and in vitro studies have reported direct effects of acetaldehyde on neurons in brain regions protected by the blood–brain barrier (Melis et al., 2009). The CVOs lack a blood–brain barrier so circulating acetaldehyde may act directly on the neurons in the CVOs and induce thirst sensation. Here, we hypothesized that acetaldehyde, a thirst-inducing factor after heavy alcohol drinking, acts in ways that are independent of diuresis.

While EtOH has both anesthetic and sedative effects that might mask the behavioral responses induced by EtOH, the anesthetic and sedative effects of acetaldehyde are very weak (Weight et al., 1991; Stubbs and Rubin, 1993). These differences confer a great advantage on acetaldehyde when investigating thirst-related behavior after heavy alcohol drinking. For this reason we used acetaldehyde instead of EtOH in most of the experiments in the present study. Meanwhile, acetaldehyde is quickly degraded in vivo by aldehyde dehydrogenase (ALDH) (Isse et al., 2005). To avoid the problems associated with degradation and to maintain effective concentrations of acetaldehyde in the body similar to those after EtOH administration (or drinking) (Tsukamoto et al., 1989; Kinoshita et al., 2001), the ALDH inhibitor cyanamide was administered together with the acetaldehyde. In this study, we used a multidisciplinary approach combining behavioral, molecular biological and physiological approaches to support the hypothesis that acetaldehyde acts indirectly by activating AT1R in the dipsogenic centers and also activates neurons in the dipsogenic centers directly.

2. Methods

2.1. Animals

The study was conducted on male Wistar rats (7–10 weeks old for in vivo experiments and 3–4 weeks old for in vitro experiments). Rats of different ages were used intentionally for technical reasons to render the different types of experiments easier to conduct. AVP-enhanced green fluorescent protein (eGFP) Wistar transgenic rats were bred and maintained as described previously (Suzuki et al., 2009). All rats were housed in plastic cages under regular light/dark conditions (the lights were on from 8:00 AM to 8:00 PM). The temperature was maintained at 23 ± 1 °C. The rats had access to water and laboratory pellets ad libitum, except during experimental procedures. The handling and care of animals used in these experiments were in accordance with NIH recommendations for the humane use of animals. All experimental procedures were reviewed and approved by the appropriate animal experiment committees of Kyushu Dental University and University of Occupational and Environmental Health. All efforts were made to minimize animal suffering and to reduce the number of animals used. All AVP-eGFP rats were screened by polymerase chain reaction analysis of genomic DNA extracted from rat-ear biopsies (Suzuki et al., 2009).

2.2. Surgery and intracerebroventricular (i.c.v.) injections

A 24-gauge stainless-steel guide cannula was implanted into each rat under sodium pentobarbital anesthesia (60 mg/kg, i.p. injection) for i.c.v. injection of drugs, as described previously (Hirase et al., 2008; Miyahara et al., 2012). The cannulae were implanted into the lateral cerebral ventricles 0.7 mm caudal to the bregma, 1.4 mm to the left of the midline, and 2.0 mm below the dura. Test solutions were injected i.c.v. after a minimum of seven days following the surgery. Drugs were injected in a volume of 4 μl over a period of 30 s with a 25-μl Hamilton syringe (Hamilton, Reno, NV, USA) attached to polyethylene tube (PE10, Becton, Dickinson & Co., MD, USA). The positions of the tips of i.c.v. cannulae were verified by injecting methylene blue (Sigma–Aldrich, MO, USA) through the cannulae, followed by serial sectioning to confirm tip position.

2.3. Measurements of fluid intake using two-bottle test

The assessments of fluid intake were performed using two-bottle test, with one bottle filled with water and the other with 0.3 M NaCl solution. The amounts of water and salt intake were measured by subtracting the weight of each bottle (50 ml) at the end of each period from that at the beginning, (to the nearest 0.01 g). In both cases, the specific gravities of the solutions were considered one. The results were expressed as ml/100 g body weight. Animals were acclimated to the presence of the two-bottles for more than 3 days.

2.4. Measurement of urine volume

The measurement of urine volume was performed using a metabolic cage (3701D000, Tecniplast, Buguggiate, Italy). All animals were accustomed to being housed in the cages with water (and 0.3 M NaCl) bottles for more than 2 h for 3 d before testing. On the day of the experiment, they were moved to the cage with fluid solutions but without laboratory pellets 2 h before drug administration. Changes in urine volume after the relevant drug administration were measured either in the presence of or in the absence of the fluid solutions. Urine volume was expressed as ml/100 g body weight.

2.5. Measurement of blood pressure

Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). Blood pressure was monitored with a catheter filled with heparin (250 U/ml) in isotonic saline inserted into the right femoral artery. The catheter was connected to a pressure-transducer (Nihon Kohden, Tokyo, Japan). The blood pressure and heart rate were recorded with the PowerLab system (sampling rate: 200 Hz, ADInstruments, NSW, Australia). Changes in the mean blood pressure or mean heart rate were expressed as percentage changes every 2 min from the average values of the blood pressure or the heart rate for 6 min before the intraperitoneal (i.p.) or i.c.v. injections of acetaldehyde began.

2.6. Measurement of plasma renin activity, sodium level and osmolality

Rats were decapitated 45 min after i.p. administration of acetaldehyde and blood samples were collected from the trunk into EDTA-coated vials. Plasma was separated from the blood samples by centrifugation at 3500 g for 15 min at 4 °C and stored in a deep freezer until measurements were made. Plasma renin activity was measured with a standard double antibody radioimmunoassay method (SRL, Tokyo, Japan). Plasma sodium level and osmolality were measured using a sodium ion electrode (SRL, Tokyo, Japan) and a Fiske osmometer (ONE–TEN, MA, USA), respectively.