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Is catalase involved in the effects of systemic and pVTA administration of 4-methylpyrazole on ethanol self-administration?

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ABSTRACT

The oxidative metabolism of ethanol into acetaldehyde involves several enzymes, including alcohol dehydrogenase (ADH) and catalase-hydrogen peroxide (H_2O_2). In this regard, while it is well known that 4-methylpyrazole (4-MP) acts by inhibiting ADH in the liver, little attention has been placed on its ability to interfere with fatty acid oxidation-mediated generation of H_2O_2, a mechanism that may indirectly affect catalase whose enzymatic activity requires H_2O_2. The aim of our investigation was twofold: 1) to evaluate the effect of systemic (i.p. [intraperitoneal]) and local (into the posterior ventral tegmental area, pVTA) administration of 4-MP on oral ethanol self-administration, and 2) to assess *ex vivo* whether or not systemic 4-MP affects liver and brain H_2O_2 availability. The results show that systemic 4-MP reduced ethanol but not acetaldehyde or saccharin self-administration, and decreased the ethanol deprivation effect. Moreover, local intra-pVTA administration of 4-MP reduced ethanol but not saccharin self-administration. In addition, although unable to affect basal catalase activity, systemic administration of 4-MP decreased H_2O_2 availability both in liver and in brain. Overall, these results indicate that 4-MP interferes with ethanol self-administration and suggest that its behavioral effects could be due to a decline in catalase-H_2O_2 system activity as a result of a reduction of H_2O_2 availability, thus highlighting the role of central catalase-mediated metabolism of ethanol and further supporting the key role of acetaldehyde in the reinforcing properties of ethanol.

Introduction

The oxidative metabolism of ethanol into its first by product, acetaldehyde, can occur in several organs and involve multiple enzymes, including peripheral NAD^+-alcohol dehydrogenase (ADH) and brain catalase-hydrogen peroxide (H_2O_2) (Aragon & Amit, 1992; Bradford, Forman, & Thurman, 1993; Bradford, Seed, Handler, Forman, & Thurman, 1993; Handler & Thurman, 1988; Koechling & Amit, 1994; Lieber, 2004). In recent years, a number of contributions brought support to the suggestion that ethanol-derived acetaldehyde plays a critical role in the motivational properties of ethanol (Correa et al., 2012; Peana & Acquas, 2013; Peana, Rosas, Porru, & Acquas, 2016; Peana et al., 2017) and, in this regard, we have recently shown that pre-treatment with 4-methylpyrazole (4-MP), a competitive inhibitor of human ADH (Blomstrand & Theorell, 1970), reduces blood acetaldehyde levels in rats after a single intragastric administration of ethanol (Peana et al., 2008). Furthermore, administration of 4-MP reduces acetaldehyde levels without increasing voluntary ethanol drinking (Lindros & Sinclair, 1979), decreases ethanol-induced conditioned place preference (Peana et al., 2008), decreases ethanol-elicited stimulation of spontaneous firing of dopaminergic neurons in the posterior ventral tegmental area (pVTA) (Foddai, Dosia, Spiga, & Diana et al., 2004), as well as ethanol-elicited stimulation of dopamine release (Melis, Enrico, Peana, & Diana, 2007) and of extracellular signal regulated kinase (ERK) phosphorylation (Vinci et al., 2010) in the nucleus accumbens shell, a brain structure crucial for the rewarding properties of drugs of abuse (Di Chiara & Bassareo, 2007). All this notwithstanding, it was found that the inhibition of ADH decreases brain acetaldehyde concentrations

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(Jamal et al., 2007), a finding contrary to what one would expect since brain ADH isoforms are not active on short-chain alcohols such as ethanol (Bühler, Pestalozzi, Hess, & Wartburg, 1983; Deng & Deitrich, 2008; Raskin & Sokoloff, 1972; Tabakoff & von Wartburg, 1975). Indeed, the pivotal role played by ADH in the mediation of the effects of ethanol-derived acetaldehyde was questioned (Blomstrand, Kager, & Lantto, 1973; Bradford, Forman, et al., 1993; Bradford, Seed, et al., 1993), as it was suggested that the contribution of ADH to peripheral ethanol metabolism had been overestimated based on some experiments where 4-MP showed *in vivo* a very low rate of ethanol elimination, leading to the conclusion that catalase, but not ADH, is mainly responsible for ethanol's metabolism (Blomstrand et al., 1973). Certainly, peripheral (liver) (Bradford, Forman, et al., 1993; Bradford, Seed, et al., 1993; Handler & Thurman, 1988) and central catalase-H_2O_2 (Aragon & Amit, 1992; Gill, Menez, Lucas, & Deitrich, 1992; Hamby-Mason, Chen, Schenker, Perez, & Henderson, 1997; Zimatkin & Buben, 2007; Zimatkin, Pronko, Vasiliou, Gonzalez, & Deitrich, 2006) play an important role in the conversion of ethanol into acetaldehyde (Fig. 10). Accordingly, some authors suggested that liver metabolism of ethanol is mediated predominantly by catalase-H_2O_2 (Bradford, Forman, et al., 1993; Bradford, Seed, et al., 1993; Handler & Thurman, 1988) located mainly in peroxisomes, whereby H_2O_2 that originates also from the metabolism of fatty acids via peroxisomal oxidation (Handler & Thurman, 1988; Handler, Bradford, Glassman, Ladine, & Thurman, 1986) is needed for such catalase enzymatic activity (Fig. 10). Interestingly, 4-MP inhibits acyl-CoA synthase (Bradford, Forman, et al., 1993; Bradford, Seed, et al., 1993), an enzyme essential to initiating the process of fatty acid oxidation (Fig. 10). Therefore, by blocking this oxidation, the generation of H_2O_2 in the peroxisomes could be inhibited and, accordingly, synthesis of catalase-H_2O_2 could be indirectly prevented. In this regard, it is important to note that a negative, indirect, interference with the catalase-H_2O_2 metabolic pathway has been shown to impair different phases of oral ethanol self-administration (Peana, Muggironi, Fois, & Diana, 2013), of voluntary ethanol intake (Ledesma, Baliño, & Aragon, 2014), and of ethanol-stimulated locomotion (Ledesma & Aragon, 2012). Interestingly, Dawidek-Pietryka, Dudka, and Jagiełło-Wójtowicz (2003) also reported that 4-MP acts as a catalase competitive inhibitor *in vitro*.

Based on these premises and in light of the interference of 4-MP on liver catalase-H_2O_2 activity (Bradford, Forman, et al., 1993; Dawidek-Pietryka et al., 2003), the aim of this investigation has been to evaluate the effects of 4-MP, after systemic or intra-pVTA administration, on oral ethanol self-administration in rats. Furthermore, to better understand the specificity of the effects of 4-MP on ethanol self-administration, we also extended our investigation to the effect of this compound on oral acetaldehyde and saccharin self-administration. Finally, additional experiments were carried out to evaluate *ex vivo* the consequences of systemic 4-MP treatment on liver and brain catalase activity and on the levels of H_2O_2.

Materials and methods

This study was carried out in accordance with Italian legislation (art. 31 D. Legs. 6, 2014), which allows experimentation on laboratory animals only after submission and approval of a research project to a welfare and health organization on animal experimentation of the University of Sassari (Italy) and to the Ministry of Health (Rome, Italy), and in accordance with European Council directives (n. 2007/526/CE) and in accordance with the "Guide for the care and use of laboratory animals" (Wolfensohn & Lloyd, 2003) as approved by the Society for Neuroscience (National Research Council, 1996). All possible efforts were made to minimize animal pain and discomfort and to reduce the number of experimental subjects.

Animals

Male Wistar rats (Envigo, Udine, Italy), weighing 125–150 g at the beginning of the experiment, were housed in pairs in Plexiglas® cages with tap water (provided by two bottles/cage) and food (Envigo, Udine, Italy) available *ad libitum*. The colony room was maintained under controlled environmental conditions (temperature: 22 ± 2 °C; humidity: 60–65%) on a 12/12-h light/dark cycle (lights on at 8:00 h). For the experiments of self-administration behavior and for the determination of liver and brain H_2O_2 levels, 50 and 16 ethanol-naïve rats were used, respectively. In particular, the rats for the behavioral experiments (n = 50) were divided into three groups: 24 were used for ethanol self-administration, 14 were used for acetaldehyde self-administration, and 12 were used for saccharin self-administration experiments. For the experiments on the maintenance phase and on the ethanol deprivation effect upon intra-pVTA administration of 4-MP, we tested the same rats (n = 24) under a random treatment order.

Drugs and chemicals

Ethanol solutions (v/v) were obtained by dilution (U.S. Pharmacopeia National Formulary, 1995) of ethanol (95%; Silvio Carta, Italy) with tap water (10% v/v ethanol solution contains 8.7 g of ethanol in 100 mL). Acetaldehyde (99.5% Sigma–Aldrich, Milan, Italy) was dissolved in tap water as w/v (0.2%). To avoid acetaldehyde evaporation, we prepared the solutions while keeping the preparation beaker on ice (0 °C). In addition, acetaldehyde was always used in this diluted form, for reducing surface tension (solvation). To evaluate the possibility of the formation of acetaldehyde polymers in our experimental setup, acetaldehyde solutions underwent nuclear magnetic resonance analysis at different time points (1, 4, and 6 h) after the solution was made. Saccharin (Sigma–Aldrich, Milan, Italy) was dissolved in tap water as w/v (0.2%). 4-MP and 3-amino-1,2,4-triazole (3-AT) were purchased from Sigma–Aldrich (Milan, Italy) and dissolved in saline solution. 4-MP for intra-pVTA treatment was dissolved in an artificial cerebrospinal fluid (PFC) (CMA Microdialysis, Holliston, Massachusetts, USA) prior to administration. The PFC solution consisted of 147.0 mmol/L NaCl, 2.7 mmol/L KCl, 2.5 mmol/L $CaCl_2$, and 0.85 mmol/L $MgCl_2$. The systemic doses of 4-MP (45.0, 67.5, and 90.0 mg/kg, i.p.) were administered 24 h before self-administration experiments or before determination of brain catalase activity. Intra-pVTA administration of 4-MP (1.25 or 5 mM) took place immediately before the self-administration session. 3-AT (1 g/kg, i.p.) was administered 90 min before anesthesia for sacrifice. Doses and timing of 4-MP and 3-AT were selected based on previously published reports (Ledesma & Aragon, 2012; Ledesma, Font, Baliño, & Aragon, 2013; Melis et al., 2007; Peana et al., 2015, 2008; Zimatkin et al., 2006). All solutions were freshly prepared before experiments.

Apparatus

Training and testing was conducted in modular operant chambers located in ventilated soundproof environmental cubicles (Med Associates Inc., St. Albans, VT, USA). Each chamber was equipped with a non-retractable drinking cup (capacity 0.50 mL) and two nose poke holes located 3 cm to the left and right of the cup. A white light placed above the active hole and a dim white light placed above the inactive hole were used as environmental stimuli. Only the active nose poke hole set off the dipper-delivering solution