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Involvement of brain catalase activity in the acquisition of ethanol-induced conditioned place preference

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Abstract

It has been suggested that some of the behavioral effects produced by ethanol are mediated by its first metabolite, acetaldehyde. The present research addressed the hypothesis that catalase-dependent metabolism of ethanol to acetaldehyde in the brain is an important step in the production of ethanol-related affective properties. Firstly, we investigated the contribution of brain catalase in the acquisition of ethanol-induced conditioned place preference (CPP). Secondly, the specificity of the catalase inhibitor 3-amino-1,2,4-triazole (AT) was evaluated with morphine- and cocaine-induced CPP. Finally, to investigate the role of catalase in the process of relapse to ethanol seeking caused by re-exposure to ethanol, after an initial conditioning and extinction, mice were primed with saline and ethanol or AT and ethanol and tested for reinstatement of CPP. Conditioned place preference was blocked in animals treated with AT and ethanol. Morphine and cocaine CPP were unaffected by AT treatment. However, the reinstatement of place preference was not modified by catalase inhibition. Taken together, the results of the present study indicate that the brain catalase-H_2O_2 system contributes to the acquisition of affective-dependent learning induced by ethanol, and support the involvement of centrally-formed acetaldehyde in the formation of positive affective memories produced by ethanol.

Keywords: Mice; Place conditioning; Ethanol metabolism; Brain catalase; Acetaldehyde; Priming; Reinstatement

1. Introduction

It has been repeatedly suggested that some of the behavioral effects of ethanol are mediated by its first metabolite, acetaldehyde [28,38,47]. When centrally administered, acetaldehyde induces a wide range of behavioral effects that are similar to those induced by ethanol. Even more, these effects on behavior seem to be partially mediated through VTA dopamine neuronal activity because acetaldehyde increases the firing rate and the burst firing of VTA-DA neurons in rats [24]. Acetaldehyde is self-administered in the ventricular system of the brain [9] and in the posterior Ventral Tegmental Area [41], suggesting that acetaldehyde possesses reinforcing properties. In support of this notion, it has also been demonstrated that acetaldehyde produces place preference after intra-cerebroventricular administration [46]. In addition, central administration of this ethanol metabolite produces behavioral activation [14] and may act as a mediator in the oral self-administration of alcohol [10].

In the brain, the catalase–H_2O_2 complex is the main pathway responsible for the conversion of ethanol to acetaldehyde [4,5,26,52]. In fact, it has been shown that ethanol is metabolized to acetaldehyde in rodent brain homogenates [4,5,26] as well as in neural tissue cultures by the catalase–H_2O_2 system [27]. In addition to this *in vitro* data, it has been reported that *in vivo* ethanol administration effectively protects brain catalase from several inhibitors [7,13]. The protection of catalase by ethanol constitutes indirect evidence for the oxidation of ethanol by the peroxidatic activity of catalase in the brain *in vivo* [7,13].

Interestingly, brain catalase activity has been involved in several of the behavioral effects of ethanol [for review see [22,38,47,52]]. Indeed, the catalase inhibitor 3-amino-1,2,4-

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triazole (AT) reduced voluntary ethanol consumption in mice [30] and rats [2,42,48]. Also, the administration of the catalase inhibitors AT, sodium azide or sodium cyanamide either blocked or partially reduced the stimulation of locomotor activity induced by acute ethanol [3,23,43,44]. Conversely, treatments that enhanced the brain catalase–H_2O_2 system increased the motor stimulation induced by ethanol [15,16,35,46]. Moreover, recently, catalase inhibition has been shown to attenuate the enhancement of social recognition produced by ethanol in mice [33]. Taken together, all these studies suggest that brain catalase plays an important role in mediating ethanol-induced behavior. However, there is a lack of knowledge concerning the role of brain catalase in the affective memories induced by ethanol.

The conditioned place preference (CPP) procedure provides a reliable measure for assessing the positive affective memories of drugs [12,49,50]. This learning paradigm has been used to analyze the acquisition of a conditioned positive memory to ethanol or to assess the effect of a "priming" injection of the drug on the reinstatement of an extinguished CPP [32].

The aim of the present research was to investigate the role of brain catalase-dependent metabolism of ethanol to acetaldehyde in the acquisition and reinstatement of ethanol-induced CPP. The primary goal of the first set of experiments was to study, using an unbiased procedure, whether the acquisition of ethanol CPP could be blocked by AT, a catalase inhibitor. Another two studies were designed to address the specificity of the effects of AT on CPP induced by morphine and by cocaine. Finally, in another experiment, we examined whether the reinstatement of ethanol CPP by a priming injection of ethanol could be blocked by AT treatment.

2. Materials and methods

2.1. Subjects

Male albino Swiss (IOPS Orl) mice were shipped from CERJ-Janvier (Spain) at 4 weeks of age, and allowed to acclimatize in the animal colony for 4 weeks before experimentation. They were housed in groups of three or four per cage with lab chow and water continuously available in the home cage. Room temperature was maintained at 21 ± 2 °C with a 12-h/12-h light/dark cycle. All experimental procedures were conducted during the light phase from 10:00 to 14:00 h (lights on from 8:00–20:00 h/and from 13:00–1:00 h). Animal procedures were performed in accordance with the European Community Council directive (86/609/ECC).

2.2. Apparatus

Place conditioning and preference testing were conducted in four black acrylic chambers (30 cm long × 15 cm wide × 20 cm high) contained in an evenly-illuminated and sound-attenuated enclosure. This enclosure consisted of a double-layer Plexiglas compartment elevated 5 cm above the floor. No divider was used in the place-conditioning chambers, so animals had access to the entire box. Conditioned stimuli were provided by the tactile cues of the interchangeable floor halves placed beneath each chamber. The grid floor was constructed from 2.3 mm stainless steel rods mounted 6.4 mm apart on acrylic rails; the holed floor consisted of perforated stainless steel sheet metal (16 ga) containing 6.4 mm round holes on 9.5 mm staggered centers. This combination of floor textures was selected based on previous studies showing that drug-naïve control mice spend about half their time on each floor type during the preference test [18,19,25]. All experiments were recorded via a computerized video tracking system (SMART; Spontaneous Motor Activity Recording & Tracking. Letica S.A., Spain).

2.3. Drugs

3-amino-1,2,4-triazole obtained from Sigma-Aldrich Química, S.A. (Spain) was dissolved at a concentration of 1 g/10 ml. Morphine sulphate and cocaine hydrochloride were dissolved at a concentration of 16 mg/10 ml and 20 mg/10 ml respectively (Sigma-Aldrich Química S.A. Spain). The injection volume used was 10 ml/kg. Ethanol solutions at a concentration of 20% v/v were diluted from an initial stock of ethanol (96%) (Panreac Química, S.A., Spain). The ethanol injection volume was 12.5 ml/kg. All solutions were dissolved on a daily basis in saline (0.9%). Drugs were administered by intra-peritoneal route (IP).

2.4. General procedure

All experiments consisted of three phases: habituation (1 session), conditioning (8 sessions) and preference testing: CPP I (1 session). Experiment 4 also includes a phase of extinction (4 sessions), and a phase of reinstatement (1 session). Each one of these three phases was followed by 1 session of preference testing (CPP II and III). In all experiments a 2-day break separated the first and the last four conditioning sessions. Four days before the habituation phase, the mice were handled and injected with saline for two days to reduce the novelty and stress associated with handling and injection. For each experiment, four animals were conditioned simultaneously. The procedure of the preference testing was identical in all experiments.

2.4.1. Ethanol place conditioning: experiments 1 and 4

2.4.1.1. Acquisition of ethanol CPP

2.4.1.1.1. Habituation. On the habituation day, the animals received one saline injection (IP). Ten minutes after the injection, the subjects were placed in the conditioning chamber on a smooth paper floor for 5 min to become habituated to the experimental apparatus and injection procedures. In this phase, the mice were not exposed to the distinctive floor textures to avoid the development of latent inhibition, which could occur if the animals were exposed to the conditioned stimulus in the absence of the unconditioned stimulus.

2.4.1.1.2. Conditioning. Conditioning began the day after habituation. During the conditioning phase, the mice were randomly assigned to one of two conditioning subgroups (CS+: