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Developmental lead exposure induces opposite effects on ethanol intake and locomotion in response to central vs. systemic cyanamide administration

Mara Soledad Mattalloni [a, b], Romina Deza-Ponzio [a, b], Paula Alejandra Albrecht [a, b], Liliana Marina Cancela [a, b], Miriam Beatriz Virgolini [a, b, *]

[a] IFEC - CONICET, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5016, Córdoba, Argentina
[b] Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5016, Córdoba, Argentina

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ABSTRACT

Lead (Pb) is a developmental neurotoxicant that elicits differential responses to drugs of abuse. Particularly, ethanol consumption has been demonstrated to be increased as a consequence of environmental Pb exposure, with catalase (CAT) and brain acetaldehyde (ACD, the first metabolite of ethanol) playing a role. The present study sought to interfere with ethanol metabolism by inhibiting ALDH2 (mitochondrial aldehyde dehydrogenase) activity in both liver and brain from control and Pb-exposed rats as a strategy to accumulate ACD, a substance that plays a major role in the drug's reinforcing and/or aversive effects.

To evaluate the impact on a 2-h chronic voluntary ethanol intake test, developmentally Pb-exposed and control rats were administered with cyanamide (CY, an ALDH inhibitor) either systemically or intracerebroventricularly (i.c.v.) on the last 4 sessions of the experiment. Furthermore, on the last session and after locomotor activity was assessed, all animals were sacrificed to obtain brain and liver samples for ALDH2 and CAT activity determination.

Systemic CY administration reduced the elevated ethanol intake already reported in the Pb-exposed animals (but not in the controls) accompanied by liver (but not brain) ALDH2 inactivation. On the other hand, a 0.3 mg i.c.v. CY administration enhanced both ethanol intake and locomotor activity accompanied by brain ALDH2 inactivation in control animals, while an increase in ethanol consumption was also observed in the Pb-exposed group, although in the absence of brain ALDH2 blockade. No changes were observed in CAT activity as a consequence of CY administration.

These results support the participation of liver and brain ACD in ethanol intake and locomotor activity, responses that are modulated by developmental Pb exposure.

1. Introduction

Developmental exposure to low doses of the non-essential metal lead (Pb) induces subtle neurobehavioral consequences that are noticeable later in life, including an enhanced vulnerability to drug addiction (Virgolini & Cancela, 2014). Interestingly, adult Pb-exposed animals evidenced attenuation in the pharmacological effects of ethanol (narcosis, lever press for the drug and pain responses) as well as higher ethanol consumption (Nation, Baker, Fantasia, Ruscher, & Clark, 1987; Nation, Dugger, Dwyer, Bratton, & Grover, 1991; Nation, Grover, & Bratton, 1991; Nation, Baker, Taylor, & Clark, 1986). Furthermore, in adolescent low-level developmentally Pb-exposed animals, we have reported a higher reactivity to the anxiolytic, motivational and hypnotic responses to the drug compared to non-exposed controls (Virgolini, Cancela, & Fulginiti, 1999). In addition, using a similar exposure scheme, we have recently demonstrated that Pb-exposed animals evidenced an enhanced ethanol intake and subsequent ethanol-induced locomotion, ascribing a critical role to brain ethanol metabolism in these responses. In effect, we showed that CAT pharmacological

* Corresponding author. IFEC - CONICET, Depto de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5016, Córdoba, Argentina. Tel.: +54 351 5353852x3162; fax: +54 351 4334420.
E-mail address: mvirgoli@fcq.unc.edu.ar (M.B. Virgolini).

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activation (3 nitropropionic acid) or inhibition (1,2,4 aminotriazole) resulted in parallel behavioral and biochemical changes in ethanol intake and CAT activity, respectively, in the Pb-exposed animals. We thus concluded that CAT-mediated ethanol oxidation (and brain ACD accumulation) is a putative mechanism involved in the heightened ethanol motivational effects observed in these animals (Mattalloni, De Giovanni, Molina, Cancela, & Virgolini, 2013).

As is well known, the enzyme alcohol dehydrogenase (ADH) presents low activity in the brain, by which catalase (CAT) and to a lesser extent cytochrome CYP2E1 catalyze central ethanol oxidation to acetaldehyde (ACD), whereas aldehyde dehydrogenase (ALDH) favors ACD oxidation to acetate, a step that is followed by acetyl CoA and CO_2 formation (Zimatkin, Pronko, Vasiliou, Gonzalez, & Deitrich, 2006). ALDH1A1 and ALDH2 are involved in ethanol-derived ACD oxidation to acetic acid, sharing a 68% amino acid similarity despite cytosolic ALDH1A1 having less affinity for ACD (Km 50–180 μM) than mitochondrial ALDH2 (Km < 1 μM) (Marchitti, Brocker, Stagos, & Vasiliou, 2008). Interestingly, a reported point mutation in ALDH2 (a glutamic acid substitution by a lysine in position 487 determining the ALDH2*2 variant) is responsible for the genetic susceptibility that leads to the "flushing syndrome" observed in East Asians as a consequence of systemic ACD accumulation (Higuchi, Matsushita, Murayama, Takagi, & Hayashida, 1995).

It is noteworthy that ACD, ethanol's first metabolite, has opposite effects in the organism. In addition to the well-known aversive consequences of its accumulation in the periphery, centrally-formed ACD has positive reinforcing properties (Correa et al., 2012; Israel, Quintanilla, Karahanian, Rivera-Meza, & Herrera-Marschitz, 2015; Quertemont, Tambour, & Tirelli, 2005). In effect, ACD can be self-administered both orally (Peana, Muggironi, & Diana, 2010) and into VTA (Rodd-Henricks et al., 2002). Moreover, ADH inhibition prevents the ability of ethanol (but not ACD) to increase spontaneous firing activity of dopamine (DA) neurons in the VTA (Foddai, Dosia, Spiga, & Diana, 2004) and ERK phosphorylation in the nucleus accumbens (NAc) shell (Vinci et al., 2010), as well as ethanol-induced acquisition of conditioned place preference (Peana et al., 2008). Furthermore, ethanol favors DA release in the NAc (McBride, Le, & Noronha, 2002; Quertemont & Didone, 2006), an effect that was prevented by CAT inhibition (Diana et al., 2008; Melis, Enrico, Peana, & Diana, 2007). On the other hand, ACD's aversive effects are the basis of the first pharmacological approaches (including drugs such as disulfiram and cyanamide -CY) that prevent alcohol consumption by peripheral ACD accumulation resulting from ALDH inhibition, a strategy that is associated with symptoms that discourage the individual from further consumption (Koppaka et al., 2012). Cyanamide (approved by the European Medicine Agency -EMA- as calcium carbimide: Temposil®) potently inhibits liver ALDH, but is less effective against the brain enzyme, raising questions on whether the drug or its metabolites are able to cross the blood-brain barrier. This drug exerts a preferential ALDH2 inhibition (Crabb, Matsumoto, Chang, & You, 2004) that peaks 1–2 h after drug administration, with 80% restoration of the activity occurring within 24 h, a feature that has limited its clinical use because of the short duration compared to disulfiram (Deitrich, Troxell, & Worth, 1976). The first studies in animal models showed that CY depressed ethanol intake (Sinclair, Lindros, & Terho, 1980) and locomotion, a role ascribed to brain ALDH inhibition, as systemic ACD accumulation was prevented by the concurrent administration of CY plus 4-methyl pyrazole (4-MP, an ADH inhibitor) (Spivak, Aragon, & Amit, 1987). Similarly, ethanol-induced locomotor activity was partially suppressed by peripheral CY administration, while 4-MP reversed (Escarabajal & Aragon, 2002; Tambour, Closon, Tirelli, & Quertemont, 2007), and aminotriazole (AT, a CAT inhibitor) potentiated this inhibition (Sanchis-Segura, Miquel, Correa, & Aragon, 1999). Furthermore, when ethanol was administered into the VTA, it was reported that CY enhanced ethanol-induced locomotion (Martí-Prats et al., 2013). Interestingly, in the single report in which CY has been administered both intracerebroventricularly and systemically in rats that had never consumed ethanol, the lower doses employed enhanced subsequent ethanol intake, regardless of the administration route, a result that the authors ascribed to the endogenously-generated ACD in both, brain and periphery. Intriguingly, the 1.0 mg CY i.c.v. dose "consistently suppressed alcohol intake and the 0.5 mg dose produced mixed effects on the self-selection of alcohol" (Critcher & Myers, 1987).

Thus, on the basis of the demonstrated implication of CAT activity (and brain ACD formation) in the facilitator effect of developmental Pb exposure on ethanol consumption, the present study attempted to unravel the role of ALDH2 in the modulation of ACD accumulation in these animals. To that end, we assessed the interplay between central and peripheral ACD accumulation, given that CY, when systemically administered, would predominantly inhibit liver rather than brain ALDH (Deitrich et al., 1976), while central CY administration would impact directly on brain ACD accumulation. Therefore, to evaluate the two perspectives on ethanol intake and subsequent locomotor activity, CY was administered both centrally and systemically. Moreover, ALDH2 and peroxisomal CAT activities in the liver, whole brain and relevant brain areas were assessed to evidence CY-induced differential enzymatic inactivation in the ethanol intake and stimulant properties of the drug in control and Pb-exposed animals.

2. Material and methods

2.1. Animals

Adult Wistar female rats (250–300 g), bred and raised at the Facultad de Ciencias Químicas vivarium, were mated in a 2/1 index. Pregnant females were housed two per cage and exposed to 220 ppm Pb (0.4 g/l Pb acetate Mallinckrodt, J.T. Baker; Argentina) or filtered tap water (which contains less than 5.0 μg/l Pb) until the pups were weaned at postnatal day 25 (PND 25), when Pb exposure was interrupted. Animals were maintained at 22 °C under a 12 h light/dark cycle, with free access to food (Batistella, Córdoba, Argentina) and water, or the Pb solution. All procedures were handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals, as approved by the Animal Care and Use Committee of the Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. The number of animals used for each experiment is indicated in the corresponding figures. Studies began in male pups at PND 35, which is considered the periadolescence period in rats, a time of particular vulnerability to drug addiction (Smith, 2003). Only one pup from each litter was used for each experimental condition, as suggested by Maurissen (2010), except for the ethanol intake tests in which two litter-mates were housed in one cage and considered a single experimental subject. This ensured that no isolation-related stress would interfere with voluntary ethanol intake, which may be a confounding factor particularly in juvenile rats, as reviewed in Anacker and Ryabinin (2010).

2.2. Ethanol intake and group conformation

Thirty-day-old pup males were housed two per cage with 2 h/day access (between 9.00 a.m. and 1.00 p.m.) to four tubes, two containing water and the other two increasing concentrations of ethanol according to the following scheme (v/v): days 1–4: 2%; days 5–8: 4%; days 9–12: 6%; days 13–16: 8%, and 10% from day 17