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N-acetylcysteine prevents behavioral and biochemical changes induced by alcohol cessation in rats

Ricardo Schneider Jr. [c,*], Carolina Ferreira Santos [a], Vanessa Clarimundo [a], Carla Dalmaz [b,c], Elaine Elisabetsky [a,c], Rosane Gomez [a,c]

[a] Departamento de Farmacologia, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Sarmento Leite, 500/202, Porto Alegre, RS 90050170, Brazil
[b] Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Sarmento Leite, 500/202, Porto Alegre, RS 90050170, Brazil
[c] Programa de Pós-Graduação em Neurociências, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Sarmento Leite, 500/202, Porto Alegre, RS 90050170, Brazil

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ABSTRACT

N-acetylcysteine (NAC), a glutamate-modulating agent with antioxidant and anti-inflammatory properties, has been considered as a potential anti-addictive drug. Beneficial effects were reported for cocaine, cannabis, and tobacco addicts, but the effect of NAC in alcoholics or in alcohol animal models is unknown. The aggravation of alcohol withdrawal symptoms, such as anxiety, has been associated with increased levels of serum corticosterone and leptin. Thus, the aim of this study was to assess the effects of NAC on anxiety, as well as corticosterone and leptin serum levels, after cessation of chronic alcohol treatment in rats. Male Wistar rats were treated with 2 g/kg ethanol, twice daily, by gavage for 30 days; control animals received an appropriate dose of glucose to balance caloric intake. Rats were treated for 4 days with NAC (60 and 90 mg/kg, intra-peritoneally [i.p.]) or saline after alcohol cessation. Twenty-four hours after the last treatment, rats were exposed to a 5-min session in the open-field test (OF). Corticosterone and leptin serum levels were determined by ELISA in samples collected within 30 min after the OF. Results showed that rats were hypoactive (decreased rearing, peripheral, and total crossings), and that corticosterone and leptin levels were increased 5 days after alcohol cessation. Four days of NAC prevented the behavioral and biochemical changes brought about by alcohol cessation. We suggest that, in addition to the anti-addictive properties reported for other drugs of abuse, NAC is potentially useful in the management of alcohol withdrawal.

Introduction

Alcohol dependence is a relapsing disorder, which shares three distinct phases with other drug addictions: anticipation-preoccupation (including craving), intoxication, and withdrawal-negative affect (Koob & Le Moal, 2001). Alcohol dependence deregulates responses to stress and appetite, contributing to the negative emotional state associated with withdrawal (Engel & Jerlhag, 2014; Gilpin & Koob, 2008). The withdrawal syndrome in alcohol-dependent individuals is a well-characterized set of signs and symptoms, usually manifested 24–48 h after the last drink, and considered to be a key factor in the maintenance of the addiction cycle as well as for relapse (Koob & Le Moal, 2001; McKeon, Frye, & Delanty, 2008). These manifestations of alcohol withdrawal have been especially related to decreases in dopamine and GABA (Gilpin & Koob, 2008; Sanna et al., 2003), along with increases in glutamate activity (Gass & Olive, 2008; Kalivas, 2008). The syndrome frequently includes anxiety, tremors, agitation, delirium, and eventually seizures (McKeon et al., 2008). In rodents, it may be manifested as anxiety, decrease of exploratory behavior, and hypoactivity (Fukushiro et al., 2012; Kliethermes, 2005; Slawecki & Roth, 2004).

A growing body of evidence relates stress, anxiety, and glutamate (Popoli, Yan, McEwen, & Sanacora, 2011). The role of anxiety and stress in withdrawal is well documented (Sinha, 2012). Increased glutamatergic activity on cortico-striatal circuitry has been related to drug-seeking behavior and relapse (Gass & Olive, 2008; Kalivas, 2008). N-acetylcysteine (NAC), a cysteine pro-drug with glutamatergic properties, acts through the cystine/glutamate antiporter located in astrocytes, ultimately restoring extracellular glutamate concentrations and synaptic glutamate activity, via tonic activation of $mGluR_{2/3}$ receptors (Berk, Malhi, Gray, & Dean, 2013). Of specific relevance to drug-seeking behavior and relapse, it has

* Corresponding author. Departamento de Farmacologia, ICBS, UFRGS, Rua Sarmento Leite, 500/202, Porto Alegre, RS 90050170, Brazil. Tel./fax: +55 51 3308 3121.
E-mail address: quimtox@gmail.com (R. Schneider).

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been reported that a single administration of NAC normalized glutamate levels in the anterior cingulate cortex in cocaine-dependent patients (Schmaal, Veltman, Nederveen, van den Brink, & Goudriaan, 2012).

In addition to its actions on brain glutamate, NAC exhibits antioxidant properties by stimulating the cysteine/cystine cycle, promoting glutathione (GSH) synthesis, and preventing oxidative stress and cellular damage (Berk et al., 2013). Additionally, NAC has anti-inflammatory properties, either by inhibiting the inflammatory response of microglia (Tsai et al., 2009) and/or by affecting the synthesis of pro-inflammatory cytokines of inflammatory pathways associated with psychiatric disorders, including drug addiction (Berk et al., 2013).

Anti-addictive properties have been documented for NAC. In rodents, NAC reduces self-administration of cocaine (Reichel, Moussawi, Do, Kalivas, & See, 2011), nicotine (Ramirez-Niño, D'Souza, & Markou, 2013), and heroin (Zhou & Kalivas, 2008). Moreover, short-term treatment with NAC reduces cocaine reinstatement in rats (Amen et al., 2011) and craving in humans (LaRowe et al., 2013). NAC decreases marijuana (Gray, Watson, Carpenter, & Larowe, 2010) and cigarette use (Knackstedt et al., 2009), craving for cannabis (Gray et al., 2010) and cocaine (LaRowe et al., 2013), and the positive rewarding effects from cigarette smoking after short-term abstinence (Schmaal et al., 2011). Moreover, NAC treatment reduces cocaine craving (Amen et al., 2011) in abstinent subjects, but not in active users (LaRowe et al., 2013), suggesting a specific effect of NAC during withdrawal. However, there are no studies exploring the effects of NAC during alcohol withdrawal.

During alcohol withdrawal, corticotrophin-releasing factor (CRF) is excessively produced and released from the hypothalamus, activating the hypothalamic-pituitary-adrenal axis (HPA) and increasing cortisol/corticosterone secretion and anxiety in humans and animals (Stephens & Wand, 2012). Leptin is a hormone secreted primarily by the adipose tissue, classically associated with satiety (Ahima et al., 1996), as well as having been shown to play an important role in controlling the HPA (Ahima et al., 1996; Roubos, Dahmen, Kozicz, & Xu, 2012). Interestingly, there seems to be a correlation between serum leptin levels and craving in alcoholics and smokers during withdrawal (Aguiar-Nemer, Toffolo, da Silva, Laranjeira, & Silva-Fonseca, 2013; Kiefer, Jahn, Jaschinksi, et al., 2001). It has been suggested that increased leptin levels during withdrawal, by reducing the dopaminergic transmission in the mesolimbic system, may lead to intensified craving and maintenance of addictive behavior (Fulton et al., 2006).

The aim of this study was to investigate the effects of NAC in the behavioral and biochemical changes induced by alcohol cessation in rats.

Methods

Animals

Seventy-eight adult Wistar rats (~300 g) obtained from the university's own colony (CREAL-UFRGS) were housed in the Pharmacology Department animal facility, in polypropylene cages (5 rats/cage, 33 × 40 × 17 cm), under controlled environmental conditions (22 ± 2 °C, 12-h light/dark, lights on at 7:00 AM), with free access to water and food (Nuvilab, Colombo, Brazil). All procedures were performed according to international and local policies for experimental animal handling, and the study was approved by the Ethics Committee for Animal Experimentation (CEUA-UFRGS #23069).

Drugs and reagents

Ethanol (98%) (Nuclear, São Paulo, Brazil) was diluted to 20% (w/v) in a 3% glucose (D-Glucose, Nuclear, São Paulo, Brazil) solution. Control rats received an 8% glucose solution, matching the caloric intake of the alcohol groups. N-acetylcysteine (Sigma–Aldrich, St. Louis, USA) was diluted in saline. All solutions were prepared fresh daily.

Experimental design

Rats were assigned to two treatment groups ($n = 39$): 2 g/kg of alcohol or glucose (gavage, twice daily at 9:00 AM and 2:00 PM) for 30 days. Twenty-four hours after the last gavage, rats were further divided ($n = 13$) to be treated for 4 days (i.p., 9:00 AM–11:00 AM) with saline (NAC0) or 60 and 90 mg/kg NAC. Doses were chosen based on literature (Ramirez-Niño et al., 2013), but the treatment was repeated daily for 4 consecutive days because pilot experiments detected significant behavioral changes 5 days after alcohol cessation. Behavior in the open field was analyzed 24 h after the last NAC administration. The trunk blood was collected within 30 min after the open-field test.

Open-field test (OF)

Rats were habituated to the dimly lighted experimental room in their home cages for at least 30 min before the experiment. The open field consisted of a white wooden arena (100 × 100 × 50 cm) with the floor divided by black lines into 16 equal squares. Rats were individually placed in the center of the arena and the behavior was video-recorded for 5 min. After each session, the floor was cleaned with a wet paper towel. Videos were analyzed by a trained observer blinded to treatments, using a BASIC written software (Kevin Willioma, KD Ware Computer, Boston, MA). The frequency of peripheral, central, and total crossings, as well as rearing and grooming episodes were scored. Total activity was calculated by adding the total crossing with the frequency of rearing (Pähkla, Harro, & Rägo, 1996).

Corticosterone and leptin levels

The rats were euthanized by decapitation within 30 min after the open-field session. The trunk blood was collected in plastic tubes (Vacutainer, NC, USA), centrifuged at 1.4 × g, and the serum was stored at −80 °C until further processing. Serum corticosterone was extracted with ethyl acetate (3 times in 100 μL), diluted to 1:133, and measured with a commercial ELISA kit (Enzo Life Sciences International Inc., Plymouth Meeting, PA, USA).

The intra-assay variation was 4.3%, which is compatible with the manufacturer-expected percentage (6.6%) for the applicable concentration range. In order to minimize the inter-assay variation, all samples were processed at the same day. According to the manufacturer, the minimum detectable concentration is less than 20 pg/mL. For leptin, the serum was diluted 1:5 and measured with a commercial ELISA kit (Invitrogen, Grand Island, NY, USA). The intra-assay variation was 4.4%, which is compatible with the manufacturer-expected percentage (5.6%) for the concentration range used. The minimum detectable dose is 26.99 pg/mL. The corticosterone and leptin levels were measured in a microplate reader (PerkinElmer, Waltham, MA, USA) at 405 nm and 450 nm, respectively, according to the manufacturer's instructions.