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Protracted alcohol abstinence induces analgesia in rats: Possible relationships with BDNF and interleukin-10

Rebeca Vargas Antunes Schunck [a], Iraci L.S. Torres [b,*], Gabriela Laste [b], Andressa de Souza [b], Isabel Cristina Macedo [b], Marina Tuerlinckx Costa Valle [a], Janaína L.O. Salomón [c], Sonia Moreira [b], Jonnsin Kuo [b], Marcelo Dutra Arbo [d], Eliane Dallegrave [e], Mirna Bainy Leal [a,c,*]

[a] Graduate Program in Biological Sciences — Neuroscience, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre 90050-170, Brazil
[b] Pain Pharmacology and Neuromodulation Laboratory: Animal Models, Department of Pharmacology, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre 90050-170, Brazil
[c] Laboratory of Pharmacology and Toxicology of Natural Products, Department of Pharmacology, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre 90050-170, Brazil
[d] REQUIMTE, Toxicology Laboratory, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto 4050-313, Portugal
[e] Department Farmaco Sciences, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre 90050-170, Brazil

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ABSTRACT

Exposure to ethanol alters the expression of brain-derived neurotrophic factor (BDNF) in central regions such as, the hippocampus, cortex and striatum. Moreover, chronic alcohol intake is known to induce selective neuronal damage associated with an increase in the inflammatory cascade, resulting in neuronal apoptosis and neurodegeneration. In the present study, we investigated the nociceptive response after 24 h of protracted alcohol abstinence. Rats were submitted to a model of alcohol withdrawal syndrome and the nociceptive response was assessed by the tail-flick and the hot plate tests. In addition, we evaluated BDNF and interleukin-10 (IL-10) in the cerebral prefrontal cortex, brainstem and hippocampus of rats after protracted alcohol abstinence. Male adult Wistar rats were divided into three groups: non-treated group (control group), treated with water (water group), and alcohol (alcohol group). The water and alcohol administrations were done by oral gavage and were performed over three periods of five days of treatment with two intervals of two days between them. Alcohol (20% w/v) was given at 4 g/kg of body weight. There was a significant effect of treatment in the tail-flick and hot plate latencies with greater latencies in alcohol-treated rats after 10 days of abstinence. There was a significant increase in the prefrontal cortex BDNF levels in the alcohol group in relation to the water group, after 11 days of alcohol abstinence. In addition, alcohol withdrawal induced a significant increase in the hippocampus, prefrontal cortex and brainstem IL-10 levels compared with control group. Thus, the present study demonstrates that protracted alcohol withdrawal produced an analgesic effect indexed via increased nociceptive threshold. We suggest that these effects could be related to the increased levels of BDNF and IL-10 observed in the central nervous system.

1. Introduction

Data from the World Health Organization (WHO) suggest that approximately 2 billion people drink alcohol in the world. Excessive alcohol intake can result in alcohol dependence, which is currently one of the most prevalent neuropsychiatric diseases (Rocchitta et al., 2012; Spanagel et al., 2013).

Alcohol exposure can result in a wide range of adaptive responses in neurons, changes in brain function, and significant brain damage (Kril et al., 1997; Sutherland et al., 2014). Acute and chronic alcohol actions and withdrawal effects contribute to excessive drinking and relapse; alcohol produces analgesia followed by hyperalgesia after withdrawal (Gatch, 2009). Egli et al. (2012) hypothesize that pain sensitivity alterations by alcohol, such as analgesia and or withdrawal-induced hyperalgesia, contribute to alcohol misuse and alcohol addiction (Egli et al., 2012). However, the mechanisms involved in this process have not been fully elucidated. Interleukins (IL) and brain derived neurotrophic factor (BDNF) could be valid biomarkers to investigate such effects. In pain processing, BDNF acts at the spinal and supra-spinal modulating the nociceptive response (Pezet and McMahon, 2006). It has been reported that exposure to alcohol alters the expression of BDNF in regions of the central nervous system (CNS) such as the hippocampus, cortex and striatum (Hensler et al., 2003; McGough et al.,

* Corresponding authors at: Departamento de Farmacologia — ICBS, UFRGS, Rua Sarmento Leite, 500 sala 202, 90050-170 Porto Alegre, RS, Brazil.
E-mail addresses: iracitorres@gmail.com (I.L.S. Torres), mirnablufrgs@gmail.com (M.B. Leal).

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2004). Additionally, this neurotrophin is involved in cell survival and differentiation of specific areas of the CNS, as well as in regulating neuronal connectivity (Nandi and Fitzgerald, 2005). Besides, BDNF has been implicated in the development of alcohol addiction due to its role in the regulation of synaptic plasticity (Davis, 2008).

Moreover, chronic alcohol intake is known to induce selective neuronal damage associated with increased oxidative–nitrosative stress and the activation of an inflammatory cascade resulting in neuronal apoptosis and neurodegeneration (White, 2003). Ethanol intoxication has also been shown to have immunomodulatory properties (Szabo and Mandrekar, 2009). Acute and chronic ethanol exposures can facilitate infections as result of modulation of the immune status. A previous study showed that rats submitted to chronic alcohol intoxication inhibited immune reactions mainly mediated by T helper 1 (Th1) cells, increasing corticosterone levels, reducing T-lymphocyte acetylcholinesterase activity and blood concentrations of IL-2, IL-4, IL-10, and increasing IL-6 levels (Zabrodskii et al., 2011).

Considering the relevance of this issue, the aim of this study was to evaluate the nociceptive response of rats submitted to a model of alcohol withdrawal syndrome. In addition, we evaluated BDNF and IL-10 levels in the cerebral prefrontal cortex, brainstem and hippocampus of these animals.

2. Materials and methods

2.1. Chemicals

Alcohol (ethanol, Nuclear, Diadema, SP, Brazil) was diluted daily with distilled water to prepare a 20% w/v solution and administered by gavage in a volume of 4 g/kg of body weight. The BDNF levels were determined using a commercially available enzyme-linked immunosorbent (ELISA) assay kit (ChemiKine, Millipore, Billerica, MA, USA). IL-10 levels were evaluated using DuoSet enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA).

2.2. Animals

Male adult Wistar rats weighing 400–450 g were housed in groups of five in 49 × 34 × 16 cm polypropylene home cages. All animals were maintained under a standard 12-hour light/dark cycle (lights on at 07:00 a.m. and off at 07:00 p.m.) in a temperature-controlled environment (22 ± 2 °C). Animals had free access to water and chow. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (Application No. 23651 — Graduate Research Group at Universidade Federal do Rio Grande do Sul — UFRGS). They were compliant with Brazilian guidelines regulating the use of animals in research (Law No. 11.794), and adhered to the ethical and methodological standards of the Principles of Laboratory Animal Care (Laboratory Guide for the Care and Use of Animals, 8th ed., 2011). All possible measures were taken to minimize animal suffering and external sources of pain and discomfort. In addition, the minimum number of animals required to produce reliable scientific data was used.

2.3. Experimental design and alcohol exposure

Rats were habituated to the maintenance room for one week prior to the experiment. Subsequently, animals were randomly divided into three different groups of 8 to 15 animals each. According to DSM criteria 291.81, alcohol withdrawal is cessation of (or reduction in) alcohol use that has been heavy and prolonged. The model of administration was adapted from Overstreet et al. (2002). Animals were treated by oral gavage. The groups were as follows: water (oral gavage), alcohol (oral gavage) and a control group, without any administration. The oral gavage administration was performed in three periods of five days of treatment with two intervals of two days between them (Fig. 1). The administrations were done at the same time each day, between 10.00 a.m. and 12.00 a.m. The evaluations were performed 24 h and 10 (tail-flick test), 11 (hot plate test), and 12 (BDNF and IL-10 levels) days after alcohol cessation.

2.4. Nociceptive analysis

2.4.1. The tail-flick test

The tail-flick apparatus was described by D'amour and Smith (1941). Twenty-four hours before the experiment, the animals were exposed to the apparatus to familiarize them with the procedure because the novelty can itself induce antinociception (Netto et al., 1987). Rats were wrapped in a towel and placed on the apparatus (Ugo Basile, Varese, Italy); the light source positioned below the tail was focused on a point 2.3 cm rostral to the tip of the tail. Deflection of the tail activated a photocell and automatically terminated the trial. A cut-off time of 10 s was used to prevent tissue damage. The test was performed 24 h and 10 days after the last administration of water or alcohol (Fig. 1).

2.4.2. The hot plate test

The hot plate test was carried out to assess the effects of alcohol under the thermal nociceptive threshold (Woolfe and Macdonald, 1944). The surface of the hot plate was pre-heated and kept at a constant temperature of 55 ± 0.1 °C (Insight Equipments, São Paulo, Brazil). All rats were acclimated to the hot plate for 5 min, 24 h prior to testing, as, again; the novelty of the apparatus itself can induce antinociception (Netto et al., 1987). Animals were placed in glass funnel on the heated surface, and the time, in seconds, between placement of the rat and the first response (foot licking, jumping, or rapidly removing paws) was recorded as the latency of nociceptive response. The cut-off time was 20 s to avoid tissue damage. The test was performed 24 h and 11 days after the last administration of initial treatment with water or alcohol (Fig. 1).

2.5. Sample collection

After 12 days of alcohol withdrawal, four to six animals of each group were anesthetized with ketamine/xylazine (50 mg/kg and 10 mg/kg, respectively), and an experienced investigator euthanized the animals by decapitation. The prefrontal cortex, brainstem and hippocampus were separated on a cold surface and immediately frozen in liquid nitrogen and kept at −80 °C for subsequent analysis.

2.6. Analysis of BDNF and IL-10 immunocontent

The prefrontal cortex, brainstem and hippocampus were weighed and homogenized with a handheld homogenizer in Tris-buffered saline. The resulting homogenates were centrifuged for 10 min at 4500 rpm. Before specific analyses, the total amount of proteins was measured by the Coomassie Blue method using bovine serum albumin as a standard (Bradford, 1976). The BDNF levels were determined using a commercially available enzyme-linked immunosorbent (ELISA) assay kit (ChemiKine, Millipore, Billerica, MA, USA) according to the manufacturer's recommendations and expressed in pg/μg. IL-10 levels were evaluated using DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions and expressed as a pg/mg tissue protein.

2.7. Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS) version 18.0 (SPSS, Chicago, IL, USA). The interactions between factors (time and group) were analyzed using repeated-measures ANOVA test, followed by Student–Newman–Keuls (SNK) post hoc. Student's *t* test was performed for BDNF and IL-10 analysis. All results were expressed as means ± standard error of mean (SEM). Significance was set at 5% ($P < 0.05$).