Life Sciences 108 (2014) 34–43

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

# Functional effects of alcohol withdrawal syndrome on peripheral sympathetic neurotransmission in vas deferens of adult rats

Guilherme Henrique Souza Bomfim, Luciana Ferreira Verde, Roberto Frussa-Filho, Aron Jurkiewicz *, Neide Hyppolito Jurkiewicz

*Department of Pharmacology, Federal University of São Paulo (Unifesp), Rua 3 de maio 100, 04044-020 São Paulo, SP, Brazil*

ARTICLE INFO

*Article history:*
Received 17 September 2013
Accepted 2 May 2014
Available online 14 May 2014

*Keywords:*
Alcohol withdrawal syndrome
Peripheral sympathetic neurotransmission
Catecholamines
Calcium channels
Vas deferens

ABSTRACT

*Aims:* Alcohol withdrawal syndrome (AWS) is characterized by a set of physiological modifications triggered by abrupt withdrawal and/or decreasing consumption of ethanol (EtOH), which may manifest 16–48 h after ceasing consumption. The relationship between the effects of AWS and central and peripheral sympathetic neurotransmission is unknown. This study investigates the possible mechanisms on the sympathetic system during periods of AWS.

*Main methods:* Male Wistar rats were treated with EtOH (6–10 g/kg/day/v.o. 5 days). Subsequently, 1 h, 24 h, 48 h and 120 h after administration of the last dose of EtOH, the animals were sacrificed, and their vas deferens (VD) were removed to perform the following evaluations: (a) concentration–effect curves of sympathetic agonist; (b) activity of $\alpha_2$-adrenoreceptor; (c) function of voltage-dependent calcium channels (Cav); and (d) release of endogenous catecholamines measured in real time coupled to HPLC.

*Key findings:* The results showed that the maximum effects of contraction were increased by agonists tested in at 24 h and 48 h EtOH withdrawal. The inhibitory affinity ($pIC_{50}$) of guanfacine was decreased 24 h EtOH withdrawal. The function of Cav was also decreased as $pIC_{50}$ values dropped 24 h and 48 h EtOH withdrawal. The release of catecholamines increased 48 h after EtOH withdrawal. It is suggested that AWS triggers hyperactivity in peripheral sympathetic neurotransmission.

*Significance:* The mechanisms underlying hyperactivity are possibly explained by a failure of autoregulation from catecholamines released by $\alpha_2$-adrenoreceptors and/or an increase of Cav function, which may be potential targets to attenuate the symptoms of AWS at the peripheral level.

© 2014 Published by Elsevier Inc.

## Introduction

Chronic use of ethanol (EtOH) is a common and serious condition that has become a public health problem because it results in approximately 2.5 million deaths worldwide (WHO, 2011). Approximately 70% of alcohol regular drinkers can manifest moderate alcohol withdrawal syndrome (AWS), and 5% progress to severe AWS (Saitz et al., 1995). AWS is characterized as a set of organic modifications triggered by abrupt withdrawal and/or decreasing consumption of EtOH, which may manifest 16 to 48 h after the cessation of EtOH consumption and can last up to 72 h (Morris et al., 2010; Eşel, 2006).

The neurochemical pathophysiology of AWS in the central nervous system demonstrates an imbalance of different excitatory systems (glutamatergic and sympathetic) involving inhibitory neurotransmitters (GABAergic), voltage-gated calcium channels (Cav), and neuropeptide/hormonal pathways (Kovács et al., 2002). Typical features of AWS, such as anxiety, tremors, agitation, tachycardia, diaphoresis, insomnia, delirium tremens and hypertension are ascribed to peripheral sympathetic hyperactivity (McKeon et al., 2008) and associated with increased plasma levels of adrenaline, noradrenaline and their metabolites (Kovács et al., 2002; Laso et al., 1990). These increased catecholamine plasma levels are attributed to decreased inhibitory activity of $\alpha_2$-adrenoreceptors ($\alpha_2$-AR) responsible for feedback on the release of neurotransmitters from central presynaptic neurons (Muzyk et al., 2011).

Calcium ($Ca^{2+}$) plays an important role in the regulation of smooth muscle contraction of the rat vas deferens (VD). This ion enters the cytoplasm either from outside the cell through voltage-gated calcium (Cav) L-type channels or from internal calcium stored by the sarcoplasmic reticulum (Burt et al., 1996). Furthermore, the involvement of L-type Cav channels in the increase of peripheral sympathetic hyperactivity in AWS is not fully elucidated. Chronic exposure to EtOH is related

* Corresponding author at: Rua 3 de maio, 100, São Paulo, SP, Brazil. Tel.: +55 11 5576 4449 voip 1044; fax: +55 11 6676 4449.
*E-mail addresses:* henrique_epm@hotmail.com (G.H.S. Bomfim), aron.farm@epm.br (A. Jurkiewicz).

http://dx.doi.org/10.1016/j.lfs.2014.05.002
0024-3205/© 2014 Published by Elsevier Inc.

to increases in functional Cav, and this may contribute to signs of AWS (Walter and Messing, 1999). However, upregulation of L-type Cav channels was observed during AWS, and this could contribute to neuronal hyperexcitability (Colombo et al., 1995; Littleton et al., 1990).

Previous studies from our laboratory showed that acute administration of EtOH (Silva Júnior et al., 2012) and amphetamine, fluoxetine or sibutramine (Jurkiewicz et al., 2012) affects the sympathetic nervous system, causing changes in cytosolic $Ca^{2+}$ and contractility in young rat vas deferens. Studies of AWS showed the occurrence of hyperactivity in various central neurotransmitter systems, mainly in sympathetic neurotransmission (Eşel, 2006). However, the relationship between the central effects of AWS and the peripheral alterations that are triggered remains unknown. Therefore, due to the few studies that emphasize the influence of AWS at the peripheral level, we decided to investigate possible mechanisms and consequences of AWS on sympathetic neurotransmission in the VD in adult animals.

## Materials and methods

### *Animals*

Male Wistar rats (strain colony INFAR), 90 days old, from the animal house of the "Instituto Nacional de Farmacologia-INFAR" of the Federal University of São Paulo (UNIFESP) were used in each experiment and maintained under controlled temperature (22 ± 1 °C) with a 12 hour dark/light cycle and food and water ad libitum. Animals were treated in accordance with the Guide for Care and Use of Laboratory Animals (2011). The present study was approved by the Animal Research Ethics Committee from UNIFESP with protocol number 1168/11.

### *Treatment*

The animals were randomly separated into (1) a control group (drug-free) and (2) an ethanol-treated group. The control group received drug-free vehicle (water) in equal volumes to that of ethanol-treated group. All groups received concomitant supplementation with 1.0 mL/day/v.o. multivitamins and oligominerals (Clusivol® — obtained from Wyeth Pharmaceutical Co., Ltd., SP, Brazil).

Ethanol-treated rats were subjected to induction of ethanol physical dependence by intragastric ethanol administration for 5 days by gavage (modified from Majchrowicz, 1975). On the first day, ethanol-treated animals received two daily administrations of 3 g/kg (EtOH solution of 30% v/v) with an interval of 7 h between each dose, totaling 6 g/kg/day/v.o. On the second day, ethanol was administered with the same time interval between each dose at doses of 3 g/kg (EtOH solution of 30% v/v) and 4 g/kg (EtOH solution of 20% v/v), totaling 7 g/kg/day/v.o. On the third day, two doses of 4 g/kg were administered (EtOH solution of 20% v/v), totaling 8 g/kg/day/v.o. On the fourth day, ethanol-treated animals received 4 g/kg (EtOH solution of 20% v/v) and 5 g/kg (EtOH solution of 25% v/v), totaling 9 g/kg/day/v.o. Finally, on the last day, two doses of 5 g/kg (EtOH solution of 25% v/v) were administered with the same time interval between each dose, for a total of 10 g/kg/day/v.o.

This treatment was adapted from the classic protocol for induction of physical dependence to ethanol by Majchrowicz (1975), which is currently widely used for this type of study (Braconi et al., 2010; Cippitelli et al., 2010; Sharma et al., 2010; Faingold, 2008). Subsequently, at 1 h, 24 h, 48 h and 120 h after administration of the last doses of EtOH or vehicle, different animals were sacrificed by decapitation, which was followed by removal of the VD for functional experiments.

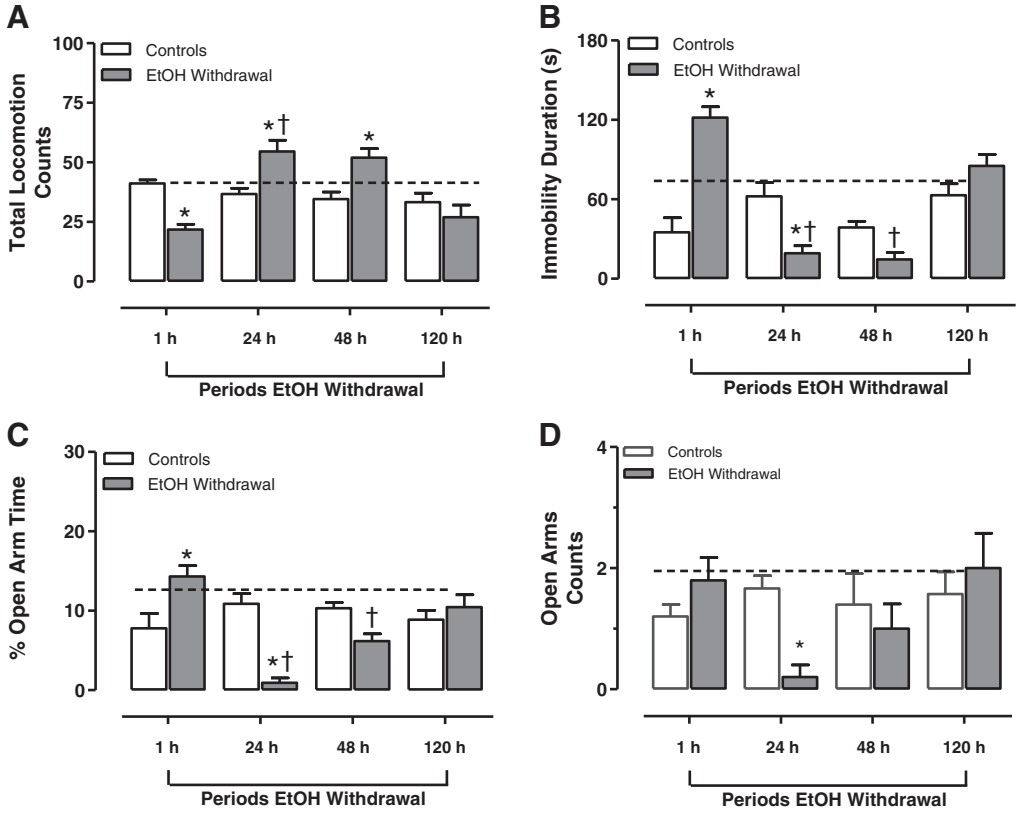


**Fig. 1.** (A) Histogram representing total locomotion counts and immobility duration (B) in the open-field (OF) test. (C) Percentages of open arm time and open arm counts (D) in the elevated plus maze (EPM) test of animals treated with EtOH (6–10 g/kg/day/v.o. for 5 days) or vehicle at different time points after withdrawal. Data represent the mean ± S.E.M. of at least 5 experiments. *$p < 0.05$ vs. control group; †$p < 0.05$ vs. 1 h EtOH withdrawal group.

Download English Version:

https://daneshyari.com/en/article/2551228

Download Persian Version:

https://daneshyari.com/article/2551228

Daneshyari.com