NeuroToxicology 54 (2016) 153–160

Contents lists available at ScienceDirect

NeuroToxicology

Full length article

Effects of chronic alcohol consumption, withdrawal and nerve growth factor on neuropeptide Y expression and cholinergic innervation of the rat dentate hilus

Pedro A. Pereira[a,b,*], João P. Rocha[a], Armando Cardoso[a,b], Manuel Vilela[a], Sérgio Sousa[a], M. Dulce Madeira[a,b]

[a] Department of Anatomy, Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal
[b] Center for Health Technology and Services Research (CINTESIS), Faculty of Medicine, University of Porto, Rua Dr. Plácido da Costa, 4200-450 Porto, Portugal

ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 16 March 2016
Accepted 8 April 2016
Available online 30 April 2016

Keywords:
Dentate gyrus
Prolonged alcohol intake
Alcohol withdrawal
Neuropeptide Y
Acetylcholine
Nerve growth factor

ABSTRACT

Several studies have demonstrated the vulnerability of the hippocampal formation (HF) to chronic alcohol consumption and withdrawal. Among the brain systems that appear to be particularly vulnerable to the effects of these conditions are the neuropeptide Y (NPY)-ergic and the cholinergic systems. Because these two systems seem to closely interact in the HF, we sought to study the effects of chronic alcohol consumption (6 months) and subsequent withdrawal (2 months) on the expression of NPY and on the cholinergic innervation of the rat dentate hilus. As such, we have estimated the areal density and the somatic volume of NPY-immunoreactive neurons, and the density of the cholinergic varicosities. In addition, because alcohol consumption and withdrawal are associated with impaired nerve growth factor (NGF) trophic support and the administration of exogenous NGF alters the effects of those conditions on various cholinergic markers, we have also estimated the same morphological parameters in withdrawn rats infused intracerebroventricularly with NGF. NPY expression increased after withdrawal and returned to control values after NGF treatment. Conversely, the somatic volume of these neurons did not differ among all groups. On other hand, the expression of vesicular acetylcholine transporter (VAChT) was reduced by 24% in ethanol-treated rats and by 46% in withdrawn rats. The administration of NGF to withdrawn rats increased the VAChT expression to values above control levels. These results show that the effects of prolonged alcohol intake and protracted withdrawal on the hilar NPY expression differ from those induced by shorter exposures to ethanol and by abrupt withdrawal. They also suggest that the normalizing effect of NGF on NPY expression might rely on the NGF-induced improvement of cholinergic neurotransmission in the dentate hilus.

1. Introduction

Health problems related to the harmful use of alcohol remain a serious public health concern (Colom et al., 2014; Rao et al., 2015; World Health Organization, 2014). It is well established that chronic alcohol consumption can severely affect brain structure and function (Crews and Nixon, 2009; Fadda and Rossetti, 1998; Harper, 2009; Madeira and Paula-Barbosa, 1999), and that the hippocampal formation (HF), a limbic area profoundly involved in cognitive functions (Aggleton and Brown, 2006; Eichenbaum, 2001), is particularly vulnerable to the effects of ethanol exposure (Korkotian et al., 2015; Matthews and Morrow, 2000; Miki et al., 2008; Paula-Barbosa et al., 1993). Specifically, studies in rodents have shown that lengthy periods of alcohol intake lead to a variety of morphological and neurochemical changes in the HF, including neuronal loss (Cadete-Leite et al., 1988a, b; Lukoyanov et al., 1999; Paula-Barbosa et al., 1993; Walker et al., 1980), structural alterations at dendritic and synaptic levels (Cadete-Leite et al., 1988b, 1989a, b), and changes in the gamma-aminobutyric acid (GABA)ergic (Andrade et al., 1992; Cadete-Leite et al., 1997a) and cholinergic (Arendt et al., 1988; Cadete-Leite et al., 1995, 1997b)

* Corresponding author at: Department of Anatomy, Faculty of Medicine, University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal.
E-mail addresses: pedroper@med.up.pt (P.A. Pereira), rocha.p.p.joao@gmail.com (J.P. Rocha), cardosoa@med.up.pt (A. Cardoso), manelvilela8@gmail.com (M. Vilela), sergioalvesdesousa7@gmail.com (S. Sousa), madeira@med.up.pt (M. D. Madeira).

http://dx.doi.org/10.1016/j.neuro.2016.04.007
0161-813X/

innervation of several components of the HF, and that these effects are further aggravated by ethanol withdrawal (Cadete-Leite et al., 1988a, 1997a,b; Lukoyanov et al., 1999; Paula-Barbosa et al., 1993).

The two aforementioned systems play important roles in the modulation of the HF function (Szilágyi et al., 2011; Teles-Grilo Ruivo and Mellor, 2013). In the rat, the cholinergic innervation of the HF is of dual origin, with some fibers originating from local interneurons and the remaining deriving mostly from cholinergic neurons of the medial septum and vertical limb of the diagonal band of Broca (Aznavour et al., 2002; Cadete-Leite et al., 1995, 1997b; Mesulam et al., 1983; Woolf and Butcher, 2011). Earlier investigations in this field have shown that the number of neurons of both cholinergic populations is decreased after chronic alcohol intake and alcohol withdrawal (Arendt et al., 1988; Cadete-Leite et al., 1995, 1997b, 2003). The HF also contains a heterogeneous population of GABAergic interneurons, with diverse morphological and physiological characteristics and different calcium-binding protein and neuropeptide content, that are involved in a variety of functions including the fine tuning of hippocampal circuits and the control of calcium homeostasis (Freund and Buzsáki, 1996; Hipólito-Reis et al., 2013; Houser, 2007; Szilágyi et al., 2011; Vreugdenhil et al., 2003). Among the neuropeptides expressed by these interneurons is neuropeptide Y (NPY, Freund and Buzsáki, 1996; Milner et al., 1997; Sperk et al., 2007), a neurotransmitter/neuromodulator that has been implicated in the regulation of a wide array of physiological functions and behaviors, including alcohol intake, withdrawal and neuronal excitability (for review, see Baraban, 2004; Borbély et al., 2013; Malva et al., 2012; Thorsell, 2007; Thorsell and Ehlers, 2006; Wettstein et al., 1995). It has been shown that ethanol treatment and withdrawal cause complex plastic alterations in the brain NPY-ergic system (for review, see Carvajal et al., 2006; Thiele and Badia-Elder, 2003; Thiele et al., 2004). These changes might be of particular importance because withdrawal from heavy alcohol consumption can trigger alcohol withdrawal seizures (Fadda and Rossetti, 1998; Jung and Metzger, 2010; Maier and Pohorecky, 1989; Tsai and Coyle, 1998; Walker et al., 1981) and there is evidence that NPY acts as an endogenous modulator of epileptic activity (Baraban, 2004; Malva et al., 2012; Scharfman and Gray, 2006; Vezzani and Sperk, 2004).

As such, as since the hilus of the dentate gyrus contains a large population of NPY-immunoreactive neurons (Sperk et al., 2007) and the expression of this neuropeptide in this region seems to be dependent on the cholinergic system (Milner et al., 1997, 1999), we sought to examine the effects of long-term alcohol consumption (6 months) and subsequent withdrawal (2 months) on the expression of NPY and on the cholinergic innervation of the rat dentate hilus. For that purpose, we have estimated the areal density and the somatic volume of NPY-immunoreactive neurons, and the density of the varicosities immunoreactive for vesicular acetylcholine transporter (VAChT). In addition, bearing in mind that alcohol consumption and withdrawal are associated with impaired nerve growth factor (NGF) trophic support (Fiore et al., 2009; Miller, 2004; Miller and Mooney, 2004; Miller et al., 2002) and that the delivery of exogenous NGF to rats alters the effects of those conditions on several cholinergic markers (Cadete-Leite et al., 2003; Lukoyanov et al., 2003; Paula-Barbosa et al., 2003; Pereira et al., 2014), we have also estimated the same morphological parameters in withdrawn rats that were intracerebroventricularly infused, during the last 12 days of the experiment, with NGF.

2. Materials and methods

2.1. Animals and treatments

A total of 20 ($n = 5$/group) male Wistar rats were used in this study. Animals were housed in a temperature- (22 °C) and humidity- (50%) controlled room under 12:12 h light/dark cycles, with lights on at 7:00 am. Solid diet (4RF21/C Mucedola, Milan, Italy) and water were freely available until rats were 2-months-old. At this age, rats were assigned to either pair-fed control (Madeira et al., 1997) or chronic ethanol-treated groups. Chronic ethanol-treated rats received a 20% (v/v) aqueous ethanol solution as their only available liquid source for 6 months. The ethanol concentration was progressively increased: starting with a 5% (v/v) solution, and rising by 1% per day to a final 20% (v/v) 2 weeks later. At the end of the period of ethanol administration (8 months of age), a subgroup of ethanol-treated rats was switched to tap water for a further 2 months (withdrawn group); this shift was performed gradually over a 2-week period by progressively reducing the ethanol concentration in the drinking solution by 1% per day. A subset of withdrawn rats was implanted intracerebroventricularly (i.c.v.; see details in Section 2.2.) with osmotic minipumps delivering 2.5S NGF (Prince Laboratories, Toronto, Canada). Because the i.c.v. infusion of vehicle (artificial cerebrospinal fluid supplemented with 0.1% bovine serum albumin; Sigma–Aldrich Company Ltd., Madrid, Spain) does not interfere with the central NPY-ergic (Cardoso et al., 2006) and cholinergic (Cadete-Leite et al., 2003) systems, vehicle-treated rats were not included in this study. In all groups, liquids consumed were supplemented with vitamins and minerals (Madeira et al., 1997).

The amount of ethanol solution consumed was recorded once per week. Blood samples (500 μl) were collected once per month, at 2 h after the beginning of both light and dark periods. The experiments were performed in accordance with European Communities Council Directive (2010/63/EU) of 22 September 2010 and Portuguese Act n°129/92. All efforts were made to minimize the number of animals used, and their discomfort and suffering.

2.2. Stereotaxic surgery and NGF treatment

Rats were anesthetized by sequentially injecting, at intervals of 10 min, solutions of promethazine (10 mg/kg body weight, subcutaneous; Laboratórios Vitória, Amadora, Portugal), followed by xylazine (2.6 mg/kg body weight, intramuscular; Sigma) and, finally, ketamine (50 mg/kg body weight, intramuscular; Merial Portuguesa, Rio de Mouro, Portugal). Then, they were placed on a stereotaxic apparatus with bregma and lambda in the same horizontal plane. After a midline skin incision, the calvaria were exposed. For i.c.v. delivery of NGF, permanent stainless steel cannulae (Alzet brain infusion kit) were stereotaxically placed in the right lateral ventricle, 1.1 mm posterior to the bregma, 1.7 mm lateral to the midline, and 4.0 mm below the surface of the skull (Paxinos and Watson, 1998). The cannulae were connected to methylene blue (0.01%; Sigma) filled Alzet osmotic minipumps (model 2002; Alza Corporation, Palo Alto, CA, USA) via sterile coiled polyethylene tubing (PE-60; Intramedic, Becton Dickinson, Sparks, MD, USA). This tubing was filled with air–oil spacer at the pump end and with NGF (150 μg diluted in 150 μl of vehicle). Osmotic minipumps were pre-tested to confirm their delivery rate, and implanted subcutaneously in the neck. Skin incisions were closed with surgical stitches and treated with local antiseptic. After surgery, rats were individually housed and maintained in a warm place until waking up. Postoperative care consisted of subcutaneous injections of 0.9% saline (2 ml), during the 48 h after surgery, to prevent dehydration and weight loss. Twelve days after the beginning of NGF infusion (i.e., at 10 months of age), rats were killed. The mean volume of NGF injected per rat was 127.1 ± 6.9 μl, corresponding to an average daily NGF dose of 10.6 ± 0.6 μg per animal. The mean flow rate of the pumps was 0.44 ± 0.02 μl/h.