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Research Report

Effects of chronic alcohol consumption and withdrawal on the response of the male and female hypothalamic–pituitary–adrenal axis to acute immune stress

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ARTICLE INFO

Article history:
Accepted 7 January 2012
Available online 25 January 2012

Keywords:
HPA axis
Chronic alcohol consumption
Withdrawal
LPS
Sex steroid

ABSTRACT

The hypothalamic–pituitary–adrenal (HPA) axis plays a central role in the response to stress, and its activity is sexually dimorphic and modulated by sex steroids. Recent work indicates that HPA axis functioning is disturbed by chronic alcohol consumption and subsequent withdrawal in rats of both sexes, but particularly in females. To examine the influence of sex steroid hormones in HPA axis response to acute stress after ingestion of a 20% ethanol solution over 6 months and subsequent withdrawal (2 months), intact males, and estradiol- and oil-injected ovariectomized females received a single intraperitoneal injection of lipopolysaccharide (LPS). Six hours after LPS administration, corticosterone concentrations were increased in all male groups; however, in ethanol-treated rats they remained below those of control and withdrawn rats. mRNA levels of corticotrophin-releasing hormone (CRH) increased, and were identical in all groups after LPS stimulation, whereas those of vasopressin, although increased, remained below control levels. LPS stimulation elevated corticosterone concentrations in all oil-injected female groups, but did not alter those of estradiol-injected females. In oil- and estradiol-injected ethanol-treated females, CRH mRNA levels did not change in response to LPS stimulation, whereas those of vasopressin increased, but stayed below control levels. In withdrawn oil- and estradiol-injected females, CRH and vasopressin gene expression increased, but did not reach control levels. These data show that prolonged alcohol consumption produces long-lasting, possibly irreversible, changes in the neuroendocrine system that regulates the production of corticosteroids, and that these consequences are more profound in females, particularly when estrogen levels are low.

1. Introduction

It is widely known, from both human research and studies with experimental animals, that the acute exposure to ethanol leads to a transient, but powerful activation of the main components of the hypothalamic–pituitary–adrenal (HPA) axis (for a review, see Madeira and Paula-Barbosa, 1999). Specifically, a single alcohol administration increases the blood levels of adrenocorticotrophic hormone (ACTH) and corticosterone, and enhances the synthesis of corticotrophin-releasing hormone (CRH) and vasopressin (VP) by neurons of the medial parvocellular division of the hypothalamic para-

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doi:10.1016/j.brainres.2012.01.013

ventricular nucleus (PVNmp), the central site that regulates the activity of the HPA axis (Whitnall, 1993). There are also data indicating that more prolonged exposures to ethanol lead to the development of tolerance in laboratory animals. In particular, it has been shown that when the exposure is maintained for a few days or weeks, the plasma concentrations of corticosterone are lower than those detected after acute exposures, and the mRNA levels of CRH are either increased or unchanged, and those of VP are decreased (reviewed in Madeira and Paula-Barbosa, 1999). In addition, studies using experimental models of alcohol consumption over months have shown that corticosterone concentrations return to normal levels, but the synthesis, and consequent expression, of CRH and VP in the PVNmp is reduced (Silva et al., 2002, 2009). In theory, these changes might merely reflect the adaptation of the HPA axis to alcohol exposure (Ogilvie et al., 1998; Spencer and McEwen, 1990). However, the observation that withdrawal does not abrogate the effects of chronic alcohol consumption on the number of CRH- and VP-producing neurons in the PVNmp (Silva et al., 2002, 2009) suggests that the down-regulation of these neuropeptides might actually result from ethanol effects on cellular function.

To explore this possibility, we have tested the hypothesis that rats submitted to prolonged alcohol consumption and to subsequent withdrawal are unable to mount an adequate response of the HPA axis to stressors. It is well known that acute stress activates the HPA axis by increasing the synthesis of CRH and VP in the parvocellular neurons of the PVN and the release of ACTH by the pituitary, leading to the production of glucocorticoids (Harbuz and Lightman, 1992; Itoi et al., 2004; Kovács and Sawchenko, 1996; Ma et al., 1997; Tsigos and Chrousos, 2002). The endotoxin lipopolysaccharide (LPS) is a systemic stressor that induces the production of endogenous cytokines, which, in turn, stimulate the synthesis and the release of CRH and VP into the hypophysial portal blood, with subsequent pituitary-adrenal activation and increased glucocorticoid secretion (Beishuizen and Thijs, 2003; Rivest et al., 1995). In the present experiment, we have administered a single intraperitoneal injection of LPS to rats consuming alcohol for 6 months and to rats withdrawn from chronic alcohol consumption for 2 months, and measured the impact of the immune stimulation on the mRNA levels of CRH and VP in the PVNmp and on the circulating levels of corticosterone.

There is ample evidence that the activity of the HPA axis is sexually dimorphic in basal conditions and after stimulation, with females producing more corticosterone and having more CRH and VP neurons in the PVNmp than males (Figueiredo et al., 2007; Kudielka and Kirschbaum, 2005; Silva et al., 2009). The vulnerability of the HPA axis to excess alcohol and to ethanol withdrawal also differs between the sexes. Females secrete more corticosterone than males in response to acute and short-term alcohol exposures (Ogilvie and Rivier, 1997) and, unlike males, females have reduced circulating levels of corticosterone after withdrawal from chronic alcohol consumption together with a further depression in CRH and VP expression by PVNmp neurons (Silva et al., 2009). There are also studies showing that, in rodents, the HPA response to endotoxin and to cytokines is enhanced by gonadectomy and attenuated by estradiol and testosterone replacement (Kudielka and Kirschbaum, 2005; Seale et al., 2004a, 2004b; Spinedi et al., 1992). Therefore, in this study we have analyzed in parallel the influence of LPS on the functioning of the HPA axis during chronic alcohol consumption and after withdrawal in males and in females. To examine the degree to which the sexual dimorphism of the HPA axis response to LPS is influenced by the estrous cycle, and specifically whether circulating estrogens are responsible for the sexually dimorphic response, females were ovariectomized and injected 2 weeks later, before LPS administration, with either estradiol benzoate or vehicle.

2. Results

2.1. *Ethanol consumption and blood alcohol levels*

The variations in the amount of liquid diet ingested over the period of alcohol consumption were very similar to those described in an earlier study (Silva et al., 2009): the volume of fluid ingested by ethanol-treated rats decreased to about half the amount of water drunk by controls during the first month of alcohol consumption and, then, remained relatively steady. The mean daily ethanol consumption over the entire experimental period, expressed in g/day/ kg bw, was significantly higher ($p<0.001$) in females (10.9±0.04) than in males (8.2±0.02).

Two hours after light onset, blood alcohol concentrations were 25±0.3 mg/dl in males and 23±0.4 mg/dl in females; 2 h after light offset, they were 76±0.4 mg/dl in males and 70±0.4 mg/dl in females.

2.2. *Estrous cycle pattern*

Chronic alcohol consumption only slightly changed the estrous cycle pattern until the fourth month of alcohol consumption, at which time most females exhibited regular, but elongated cycles, relative to control females. After 5 months of alcohol consumption, only 33% of the females showed estrous cycles similar to those of controls. The differences included elongation of metestrous and diestrous phases, leading to an increased time interval between proestrous surges. In withdrawn females, the occurrence of regular cycles was reduced, with some females being in persistent estrous (13%) and others completely acyclic (25%). However, when present, the cycles were also elongated (63%).

2.3. *Uterine and relative adrenal weights*

Uterine weights, expressed in g, were 0.42±0.09 in oil-injected rats and 1.29±0.29 in EB-injected rats. These differences were statistically significant ($p<0.001$).

The relative adrenal gland weights and the results of the respective statistical analyses are shown in Table 1. The relative weight of adrenal glands was higher in females than in males in all groups studied. There were no significant differences in adrenal weights between oil- and EB-injected rats in all groups studied, except between unstressed withdrawn groups, in which adrenal glands were heavier in oil- than in EB-injected rats. In unstressed rats, adrenal glands were significantly heavier in ethanol-treated groups than in the respective controls and, after withdrawal, they returned to control levels in all groups,