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Research report

Long-term effects of repeated maternal separation and ethanol intake on HPA axis responsiveness in adult rats

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ABSTRACT

It has been shown that early life manipulations produce behavioral, neural, and hormonal effects. The long term consequences of repeated maternal separation (RMS) plus cold stress and ethanol intake were evaluated during adolescence and adult rats on hypothalamic-pituitary-adrenal (HPA) axis in male adult Wistar rats. RMS+ cold stress was applied from postnatal day (PD) 2 in which the pups were separated from their mothers and exposed to cold stress (4 °C) 1 h per day for 20 days; controls remained with their mothers. Then they were exposed to either voluntary ethanol (6%) or dextrose (1%) intake for 7 days: PD22-29 and PD59-66. Half of the animals were sacrificed, while the others were exposed to acute stress (AS) for 2 h and then they were killed. RMS+ cold stress: a) increased voluntary ethanol intake in adolescent and adult rats; b) reduced protein expression (Western measurements) in corticotropin-releasing hormone (CRH) in hypothalamus (Hyp) and mineralocorticoid receptor (MR) in hippocampus (Hic) while increased glucocorticoid receptor (GR) in Hic; c) decreased plasmatic levels of adrenocorticotropic hormone (ACTH) and increased corticosterone (COR) levels in HPA axis, d) adult rats exposure a new AS incremented ACTH and COR levels. However, this modification did not alter the HPA axis capacity to respond to a new type of stressor. These results demonstrate the consequences of early life stress on the vulnerability of ethanol consumption and HPA axis responsiveness to a stressor in adult rats.

1. Introduction

It has been shown that early life stress exposure produces severe and lasting consequences on growth and maturity (Monti et al., 2005; Crews et al., 2007; McCormick and Mathews, 2010). The hypothalamic-pituitary-adrenal (HPA) axis is the main physiological stress response system, a complex molecular pathway including feedback regulatory interactions between the hypothalamus (Hyp), the pituitary, and the adrenal glands (Manian et al., 2014). Its activation starts with the secretion of corticotropin-releasing hormone (CRH) from the hypothalamic paraventricular nucleus (PVN), which later promotes the release of adrenocorticotropic hormone (ACTH) from the pituitary which provokes, in turn, the release of glucocorticoids (GCs) from the adrenal cortex. The GCs binds to two types of receptors, mineralocorticoid receptor (MR) and glucocorticoid receptor (GR), thus regulating transcription and repression of genes that lead to adaptive changes and to the HPA axis negative feedback (Sawchenko, 1987; Jacobson and Sapolsky, 1991; Sapolsky et al., 2000; Charmandari et al., 2005; Deppermann et al., 2014). Both MR and GR are part of the nuclear hormone superfamily of ligand activated transcription factors (Datson et al., 2001; Huang et al., 2010). However, excessive or chronic stress may lead to persistent maladaptation of neuronal circuits and may promote the development of psychiatric disorders, such as mood or anxiety disorders (Smith and Vale, 2006; Wada and Breuner, 2008; Lupien et al., 2009; Popoli et al., 2012), that often emerge in adolescence (Kessler et al., 2001).

Alcoholism is a debilitating disorder for the individual and very costly for society (Rehm et al., 2009). The impact of stress on alcohol use and the risk of alcohol use disorders (AUDs) depends on

Abbreviations: ACTH, adrenocorticotropic hormone; AS, acute stress; AUDs, alcohol use disorders; CNS, central nervous system; COR, corticosterone; CRH, corticotropin-releasing hormone; GC, glucocorticoid; GR, glucocorticoid receptor; Hic, hippocampus; HPA, Hypothalamic-pituitary-adrenal axis; Hyp, hypotlalamus; MR, mineralocorticoid receptor; MS, maternal separation; PD, postnatal day; PVN, paraventricular nucleus; RMS, repeated maternal separation.

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various factors such as genetic predisposition, early stress, individual consumption patterns, environmental influences, gender, duration and severity of the stress experienced (Roman and Nylander, 2005; Anacker and Ryabinin, 2010; Keyes et al., 2012; Melchior et al., 2014). The positive reinforcing effects of alcohol are accepted as important motivating factors in alcohol-drinking behavior in the early stages of alcohol use and abuse. Conversely, alcohol's negative reinforcing effects may contribute to alcohol-drinking behavior at this stage in people who suffer psychiatric disorders and use alcohol to self-medicate from these disorders (Fidler et al., 2006; Gilpin and Koob, 2008).

Previous studies investigated the effects of maternal separation (MS) (Zimmerberg and Shartrand, 1993) and found that rat pups were maternally separated at cold temperature, during 6 h from PD2 to PD15 were not maintained the temperature of nest, grew slower, developmentally delayed and were less active in an open-field test. Hofer (1973) showed that 2-week-old rat pups were exhibited a reduced levels of locomotor and exploratory behavior on cold environmental conditions.

On the other hand, Acosta et al. (1993) investigated the function of GABAergic system in definite areas of rat brain after acute and chronic cold stress and showed that the most affected mechanism was the neuronal uptake of GABA which thus appear to be a sensitive marker involve specifically by cold stress. Also, they concluded that the effect GABAA receptors implicated in the stress-induced modifications on endogenous GABA levels and locomotor activity (Acosta and Rubio, 1994). This may be generalized effect of the low temperature since a reduction in the turnover of dopamine (Dunn and File, 1983) and noradrenaline (Stone, 1970) have also been induced by cold, but not by warm stress.

The fact that early-life environmental factors can interfere with development of HPA axis function is of importance (Heim et al., 2004; Ladd et al., 2005; Jahng, 2011; Manian et al., 2014) with regard to the use of MS as an experimental model to evaluate consequences of early-life impact on vulnerability to alcohol use disorders (AUD) (Keyes et al., 2012) and on addictive behavior (McEwen, 2006; Moffett et al., 2007; Cruz et al., 2008). There is a close interrelationship between stress and ethanol consumption (Prendergast and Little, 2007; Clarke et al., 2008; Miczek et al., 2008; Pautassi et al., 2010; Becker et al., 2011)

We hypothesized that the RMS+ cold stress increase ethanol intake modulating the HPA axis and can affect subsequent brain function during adulthood. The present study investigated whether RMS+ cold stress in combination with voluntary ethanol intake in early adolescence and adults rats induces long-term neurochemical and molecular alterations in adult animals. Hormone concentrations in plasma HPA axis, expression levels of CRH, GR, MR were measured by Western blot analysis in Hyp and Hic adult compared to normal animals. Finally study the susceptibility of the HPA activity induced by exposure to an AS in adulthood in plasma levels of ACTH and COR.

2. Results

Although the repeated treatment, no differences in the weight or other physical parameters (color, abundance of hair) were observed between RMS+ cold stress with control groups in adolescent and adult rats. The general condition of the rats was controlled every week. They showed an overall "good condition", the skin observed was a good quality appearance. The posture of the experimental animals had no visible modifications.

2.1. Average body weight

Body weights of rats were not significantly different amongst experimental groups or throughout the course of the experiment. (Two-way ANOVA, $F_{3,25} = 0.3477$, $p = 0.7911$). In the first intake, the weight gained at day 29: C + Dex: 167.6 ± 12.89; C + Et: 169.1 ± 22.85; St + Dex: 175.0 ± 12.64; St + Et: 167.8 ± 20.22 g body rat (n = 7). In the second intake the weight gained at day 66: C + Dex: 389.7 ± 32.19; C + Et: 385.2 ± 30.15; St + Dex: 405.0 ± 32.64; St + Et: 390.7 ± 28.23 g body (n = 7). The total alcohol consumed during the course of the experiment was: Control group: 42.95 ± 10.65 ml ethanol/kg body rat (t = 4.0357 df = 13, p = 0.0005 vs Stressed group: 114.2 ± 28.80 ml ethanol/kg body

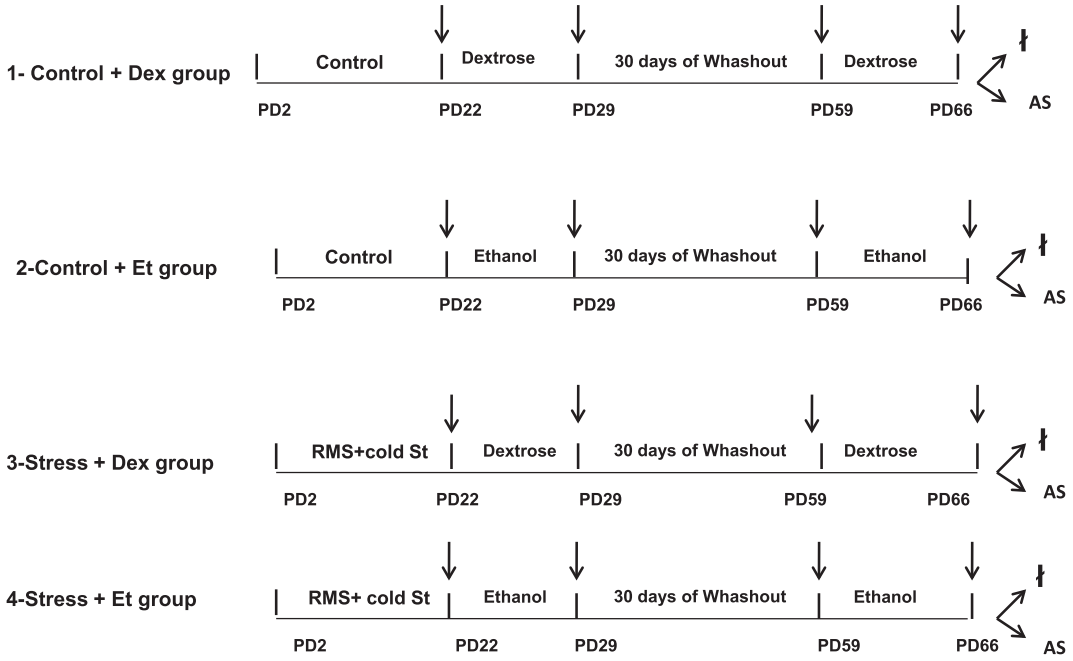

Fig. 1. Schematic representation of experimental design. RMS+ cold stress or not disturbance (Control) from postnatal day (PD) 2. Fist intake was from PD 22–29 (adolescent rats) and second intake was from PD59-66 (adult rats) given to drink either water and dextrose: 1% solution (g/100 ml) or water and ethanol: 6% solution (g/100 ml).