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SEX-DEPENDENT EFFECTS OF MATERNAL SEPARATION ON PLASMA CORTICOSTERONE AND BRAIN MONOAMINES IN RESPONSE TO CHRONIC ETHANOL ADMINISTRATION

S. E. KAWAKAMI, I. M. H. QUADROS,* R. B. MACHADO AND D. SUCHECKI

Department of Psychobiology, Universidade Federal de São Paulo, Brazil

Abstract—Prolonged and repeated periods of maternal separation produce behavioral phenotype of increased vulnerability to neuropsychiatric disorders and drug abuse. Most of the changes in behavior, corticosterone (CORT) and monoamine levels induced by long maternal separation (LMS) are observed after a challenge, but not in basal conditions. LMS increases ethanol-induced locomotor response and self-administration, possibly due to changes in CORT release and/or monoamine concentrations. This study examined the effects of LMS in association with chronic ethanol treatment on plasma CORT and brain monoamine concentrations in male and female Swiss mice, which were kept undisturbed (animal facility rearing – AFR) or separated from their mothers for 3 h/day, from 2 to 14 days of age (LMS). As adults, one set of male and female mice received no drug treatment to assess the effect of LMS per se. Another set of animals received saline injections for 20 days and one ethanol injection (2.2 g/kg, i.p.) on day 21 (acute) or ethanol for 21 days (chronic). Locomotor activity, plasma CORT levels and monoamines in the frontal cortex, striatum and hippocampus of AFR and LMS mice were evaluated in non-treated, acute and chronic ethanol-treated animals. In non-treated mice, no differences were found in CORT or locomotor activity, with small changes in monoamines content. In LMS females, chronic ethanol increased dopamine and serotonin concentrations in the frontal cortex, relative to acute ethanol LMS and to chronic ethanol-treated AFR groups ($p < 0.05$). In LMS males, chronic ethanol increased hippocampal noradrenaline, dopamine, serotonin and metabolites when compared to respective AFR controls, as well as acute LMS. Moreover, chronic ethanol treatment resulted in higher CORT concentrations in LMS than in AFR males. Overall, these results indicate that LMS mice were more susceptible to the effects of chronic ethanol administration on CORT and brain monoamine concentrations, and that these effects were sex-dependent.

Key words: early life stress, brain monoamines, corticosterone, behavioral sensitization, alcohol, sex differences.

INTRODUCTION

The interaction between genetic and environmental factors has been claimed to determine the phenotypical plasticity that will ultimately lead to resilience or vulnerability to stress-induced disorders (Caldji et al., 1998; Plotsky et al., 1998; Macri et al., 2011; Franklin et al., 2012). Repeated separations of pups from their mothers for 3 to 6 h during the first two weeks of life (long maternal separation, LMS) produce long-term alterations in behaviors, endocrine and neurotransmitter systems thought to be involved with depression (Lee et al., 2007; Aisa et al., 2008), anxiety (Wigger and Neumann, 1999; Kalinichev et al., 2002; Romeo et al., 2003; Lee et al., 2007) and drug abuse (Roman and Nylander, 2005; Moffett et al., 2007; Faturi et al., 2010).

LMS augments or facilitates consumption of psychostimulants (Moffett et al., 2007), morphine (Vazquez et al., 2005) and ethanol (Huot et al., 2001; Ploj et al., 2003; Roman and Nylander, 2005; Cruz et al., 2008). However, escalated ethanol intake was only observed in males, but not in female rats (Roman et al., 2004; Gustafsson et al., 2005), suggesting sex-dependent consequences of LMS on ethanol taking behavior (Roman and Nylander, 2005). LMS may also facilitate neuroadaptations induced by chronic drug administration, such as the potentiation of locomotor stimulant effects (i.e. behavioral sensitization) promoted by repeated cocaine administration (Kikusui et al., 2005). In a previous study, we reported that female mice submitted to LMS show faster development of a sensitized locomotor response to chronic ethanol (Kawakami et al., 2007). LMS may contribute to increased sensitivity to the stimulant and rewarding effects of drugs by facilitating drug-induced neuroadaptations in brain reward pathways (Meaney et al., 2002).

One possible mediator of the increased drug-induced behavioral sensitization is the elevated activity of the hypothalamic–pituitary–adrenal (HPA) axis, since corticosterone (CORT) involvement in this process has

*Corresponding author. Address: R. Napoleão de Barros, 925, São Paulo SP 04024-002, Brazil. Tel: +55-11-21490159; fax: +55-11-55725092.
E-mail address: isabel.quadros@unifesp.br (I. M. H. Quadros).

Abbreviations: 5-HIAA, 5-hydroxyindolacetic acid; 5-HT, 5-hydroxytryptamine; AFR, animal facility rearing; ANOVA, analysis of variance; CORT, corticosterone; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediamine tetraacetic acid; EPM, elevated plus-maze; EtOH, ethanol; HPA, hypothalamic–pituitary–adrenal; HPLC, high-performance liquid chromatography; HVA, homovanilic acid; LMS, long maternal separation; N.Acc., nucleus accumbens; NE, noradrenaline; PND, post-natal day; SAL, saline.

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been suggested (Piazza and Le Moal, 1998). LMS increases CORT response to a variety of stressors (Ladd et al., 2000; Kalinichev et al., 2002; Plotsky et al., 2005), including ethanol administration (Kawakami et al., 2007). However, it does not modify the basal activity of the HPA axis (Plotsky and Meaney, 1993; Wigger and Neumann, 1999; Marin and Planeta, 2004; Parfitt et al., 2004; Tiba et al., 2004; Plotsky et al., 2005; Roman et al., 2006; Arborelius and Eklund, 2007). Increased levels of CORT may facilitate the stimulant and reinforcing effects of ethanol by increasing the release of dopamine (DA) in the nucleus accumbens (N.Acc.) (Rouge-Pont et al., 1998; Barrot et al., 2000). Besides DA in N.Acc., other neurotransmissors, as noradrenaline (NE) and serotonin (5-HT), and other brain structures, as the frontal cortex, striatum and hippocampus, can be involved with LMS-related behavioral consequences. There are some reports that LMS alters basal monoamine content, with higher basal DA concentrations in the striatum (Matthews et al., 2001) and its metabolite, homovanilic acid (HVA), concentrations in the N.Acc. (Arborelius and Eklund, 2007) and lower basal hippocampal 5-HT concentration (Lee et al., 2007). These effects on serotonin transmission can be associated with depressive-like phenotypes of LMS and with higher ethanol intake shown by LMS animals, since this effect on ethanol intake can be reversed with chronic antidepressant treatment (Huot et al., 2001). Few reports were found about the effect of LMS in drug-induced neuroadaptations. While there is no effect of LMS per se on basal levels of dopamine D1 and D2 receptors binding, LMS associated with ethanol consumption produced lower dopamine receptor (D1) binding in the N.Acc. compared to control group (Ploj et al., 2003). These modifications on monoamine neurotransmission induced by LMS can modify chronic ethanol-induced endocrine and monoamine response.

Acute administration of ethanol (EtOH) increases plasma CORT levels (Rivier, 1996) and affects monoamine concentrations in different brain regions, including elevation of dopamine (DA) release in the N.Acc. (Di Chiara and Imperato, 1988) and reduction of 5-HT levels in the medial prefrontal cortex (Boone et al., 1997). Chronically, it induces tolerance of the HPA axis stress response to ethanol, with a decrement of CORT release (Lee and Rivier, 1997; Rivier and Lee, 2001). Chronic EtOH also decreases NE and DA levels and increases 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), levels in the striatum and hippocampus (Vasconcelos et al., 2004), but decreases this neurotransmitter in the cerebral cortex (Uzbay et al., 2000). Thus, the consequences of chronic ethanol treatment are very complex, and the outcomes depend on the neurotransmitter (and metabolites) and brain region studied, and the characteristics of ethanol exposure.

Both LMS and ethanol trigger chronic functional changes in overlapping neuroendocrine and neurobiological mechanisms. The present study assessed the impact of LMS on chronic ethanol-induced changes in brain monoamines and plasma CORT concentrations. Brain regions selected for this study included the hippocampus, the striatum (including the nucleus accumbens) and the frontal cortex (including prefrontal cortex), all of which are important terminal regions innervated by monoaminergic fibers, and known to be involved with emotion regulation and drug abuse. We hypothesized that mice with a history of LMS would present increased sensitivity to ethanol-induced CORT secretion and to neuroadaptations produced by repeated ethanol treatment.

EXPERIMENTAL PROCEDURES

Subjects

Swiss mice from the Department of Psychobiology, UNIFESP, Brazil, were mated by placing two females with one male for 10 days, after which, the male was removed from the cage and females were housed individually. Five days after individual housing, cages were daily inspected for the presence of pups. The day of birth was designated post-natal day (PND) 0. On PND 1, the litters were culled to five males and five females. Animals were maintained in a controlled 12-h light–dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.) and temperature (23 ± 2 °C). Food and water were provided *ad libitum* throughout the entire study. The protocols were approved by the Ethics Committee in Research of Universidade Federal de São Paulo (CEP# 521/07) and the experiments were carried out in accordance with NIH guidelines for use and care of animals.

Maternal separation

From PND 2 to 14, pups were subjected to daily maternal separation for 3 h/day (LMS) or were not separated until weaning on PND 22 (animal facility rearing – AFR) (total of 18 litters). Whole litters in the LMS group were removed from the nest and placed in separate cages on a heating pad set at 33 °C, in a separate room to prevent mothers from hearing ultra-sound vocalizations, as those could attenuate the effects of separation (Ziabreva et al., 2003), at approximately 12:00 h; the mothers remained in the home-cage and, at the end of the allotted period, litter and mother were reunited in the home-cage. First, the mother was removed from the cage, the litter was returned and then the mother was reintroduced in the home-cage. Twice a week during the separation period, half of the old bedding material was mixed with clean material. AFR litters were handled during cage cleaning (three times a week, as in the animal facility routine). Weaning took place on PND 22 and animals were housed by gender, from 2 to 3 litters of the same rearing group in large plastic cages (44 cm × 34 cm × 16 cm) (10–15 animals). Adult male and female mice were used (approximately at PND 90), in a total number of 119 mice, distributed in 29 AFR females, 31 LMS females, 31 AFR males and 28 LMS males.