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HYPOTHALAMIC VASOPRESSIN SYSTEM REGULATION BY MATERNAL SEPARATION: ITS IMPACT ON ANXIETY IN RATS

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Abstract—Maternal separation (MS) has been used to model the causal relationship between early life stress and the later stress-over-reactivity and affective disorders. Arginine vasopressin (AVP) is among several factors reported to be abnormal. The role of AVP on anxiety is still unclear. In order to further investigate this causal relationship and its possible role in anxiogenesis, male rat pups were separated from their dams for 3 h daily (3hMS) from post-natal day (PND) 2 to PND15. Fos expression in AVP+ neurons in the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON) triggered by 3hMS, and AVP-mRNA expression, were examined at PND10 and PND21 respectively, whereas AVP-mRNA expression, PVN and SON volumes and plasma AVP concentration were assessed in adulthood. Elevated plus maze test (EPM) and Vogel conflict test (VCT) were also performed to evaluate unconditioned and conditioned anxious states at PND70–75. At PND10, a single 3hMS event increased Fos expression in AVP+ neurons fourfold in PVN and six to twelvefold in SON. AVP-mRNA was over-expressed in whole hypothalamus, PVN and SON between 122% and 147% at PND21 and PND63. Volumes of AVP-PVN and AVP-SON measured at PND75 had marked increases as well as AVP plasma concentration at 12 h of water deprivation (WD). MS rats demonstrated a high conditioned anxious state under VCT paradigm whereas no difference was found under EPM. These data demonstrate direct relationships between enhanced AVP neuronal activation and a potentiated vasopressin system, and this latter one with high conditioned anxiety in MS male rats.

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Abbreviations: 3hMS, procedure of maternal separation for 3 h; AFR, animal facility-reared pups; AVP, arginine vasopressin; DEPC, diethyl pyrocarbonate; ELISA, Enzyme linked ImmunoSorbent Assay; EPM, elevated plus maze test; ISH, *in situ* hybridization; mRNA, messenger ribonucleic acid; MS, maternal separation; MS3h, experimental group of maternal separation 3 h (PND2–PND14); PND, post-natal day; PVN, paraventricular nucleus; SON, supraoptic nucleus; VCT, Vogel conflict test; VP, vasopressin; WD, water deprivation.

Key words: maternal separation, arginine vasopressin, paraventricular nucleus, supraoptic nucleus, Vogel conflict test, elevated plus maze.

INTRODUCTION

Seymour Levine's group first reported the effects of early life experience on emotionality and stress-responsiveness in adult rats half a century ago (Levine, 1957; Levine et al., 1957). Following this discovery, rodent maternal separation (MS) models have been widely used to investigate the effects of early postnatal adversity at adulthood. Plotsky, Meaney and others had developed a relatively standardized handling/MS model for manipulating early postnatal interaction between mother rats and their pups (Plotsky and Meaney, 1993; Wigger and Neumann, 1999; Lehmann and Feldon, 2000; Cirulli et al., 2003). The most widely studied paradigm consists of periods of daily separation (usually 3 h) performed from post-natal day (PND) 2 to PND14 (Fumagalli et al., 2007). While it is a generally accepted idea that MS permanently changes the offspring's neuroendocrine and behavioral stress reactivity, the factors that promote the sustained effects of early-life stress have not yet been fully elucidated (Wigger and Neumann, 1999; Lehmann and Feldon, 2000; Cirulli et al., 2003).

It has been shown that protracted periods (3 h or more) of separation from the dam may increase the hypothalamus–pituitary-adrenal axis (HPA) activity in pups, and may also increase the stress reactivity during adulthood (Anisman et al., 1998). Our classical understanding of the HPA axis comprises that the release of corticotropin-releasing factor (CRF) and vasopressin (VP) from the paraventricular nucleus (PVN) of the hypothalamus elicits pituitary adrenocorticotropin hormone (ACTH) secretion, which in turn, provokes release of the adrenal glucocorticoids. In addition to a considerable amount of reports describing CRF-ACTH-glucocorticoids secretion and their receptor abnormalities observed in the MS rodent model (Kuhn and Schanberg, 1998; Kalinichev et al., 2002; Fumagalli et al., 2007; Korosi and Baram, 2009), VP system has been reported to undergo developmental changes from the perinatal period through adulthood and MS was shown to disrupt this age-dependent changes. It is interesting to observe that there is a controversy about levels of AVP in MS rodent models, which were found either increased (Murgatroyd et al., 2009; Veenema and Neumann, 2009), decreased (Desbonnet et al., 2008) or unchanged (Oreland et al., 2010) in the hypothalamus, whereas no information about the possible

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physiological mechanism(s) underlying this abnormality, regulated by neonatal recurrent MS, is available.

Vasopressin (VP, also widely known as antidiuretic hormone, ADH) is nonapeptide synthesized mainly in magnocellular neurons in the supraoptic nucleus (SON) and PVN of the hypothalamus. Its foremost physiological functions are the regulation of water-electrolyte metabolism, hepatic glucose metabolism, and cardiovascular function in adults (Hatton, 1990). However, together with another nonapeptide, oxytocin, VP is an important generator of behavioral diversity (Goodson, 2008) and provides an integrational neural substrate for the dynamic modulation of behaviors by endocrine and sensory stimuli (Goodson and Bass, 2001).

During ontogenesis, VP contributes to the regulation of proliferation and morphogenesis of the target cells and organs (brain, pituitary, kidney and liver) (Boer, 1987). VP system is known to be activated around birth when VP contributes to the establishment of a new equilibrium in the body fluids and the adaptation of the fetuses to the stress of the labor (Oosterbaan et al., 1985). Following birth, VP induces a redistribution of the blood flow via the cardiovascular system in order to increase blood volume in the vital organs and those responsible for stress reaction (brain, pituitary gland, heart, adrenals), while reducing the blood flow in other peripheral organs (Pohjavuori and Fyhrquist, 1980). Afterward, the physiological role of VP extends to the regulation of the cardiovascular system, water re-absorption in kidney (Dlouha et al., 1982; Siga and Horster, 1991) and glucogenolysis in liver (Ostrowski et al., 1993). Although the physiological mechanism(s) underlying the observed modification of vasopressin system by MS is currently unknown, a recent surprising report from Makara's group demonstrated that AVP was the predominant secretagogue during the perinatal period in a maternal deprivation model using VP producing (AVP+/−) and deficient (AVP−/−) Brattleboro rat pups. Both maternal deprivation and ether inhalation induced remarkable ACTH elevation only in AVP+/− pups, supporting the role of VP in HPA axis regulation. However, corticosterone (CORT) elevations were even more pronounced in AVP−/− pups, suggesting the possibility of an ACTH-independent CORT-secretion regulation (Makara et al., 2008).

AVP's promoting role on anxiogenesis is also not widely accepted yet, although there have been several reports in the literature suggesting that AVP system is critically involved in anxiogenesis (Landgraf and Wigger, 2002; Zhang et al., 2010). On the other hand, the role of MS on anxiogenesis is currently a matter of debate. Several studies have shown that MS promotes an increase in anxiety-like behavior in different anxiety tests (Huot et al., 2002; Wigger et al., 2004; Aisa et al., 2007) while others have found no differences (de Jongh et al., 2005; Slotten et al., 2006; Hulshof et al., 2011; Lajud et al., 2011).

Therefore, the specific aims of the present study were, in the first place, to investigate whether the 3hMS paradigm was capable to modify the neuronal activation of the magnocellular AVP system using the immediate early gene product Fos as a marker of AVP neuron activation and plasticity (Pirnik and Kiss, 2005) and, in the second place, to evaluate both short- and long-term effects of MS on AVP messenger RNA (mRNA) expression by using *in situ* hybridization (ISH) with AVP riboprobe and quantitative analysis at PND21 and PND63. It is worth mentioning that due to a discrepancy in the literature regarding the AVP-mRNA expression evaluated in adulthood of MS offspring (Desbonnet et al., 2008; Murgatroyd et al., 2009; Veenema and Neumann, 2009; Oreland et al., 2010), and the lack of evidences in the neonatal periods, we used AVP-riboprobe-ISH method, which allows observing much stronger hybridization signals due to its greater sensitivity and better signal-to-noise ratios (Herman et al., 1991; Marks et al., 1992; Grino and Zamora, 1998; Young et al., 2006). Moreover, riboprobes form more stable hybrids than oligo-probes. Further, the volumes of the magnocellular regions expressing vasopressin (AVP-SON and AVP-PVN) were morphometrically and quantitatively characterized at PND75. Finally, the hypothesis that the persistent enhancement of hypothalamic magnocellular vasopressin system should generate in rats a high anxious state, when the AVP system was selectively up-regulated, was assessed using the Vogel conflict test (VCT) for conditioned anxiety and comparing with elevated plus maze (EPM) test for unconditioned anxiety. Plasma AVP concentration during water deprivation (WD) was also measured.

EXPERIMENTAL PROCEDURES

Animals and MS procedure

Wistar rats reared from the local animal facility were used in this study. All animal procedures were approved by the local bioethical and research committees, with the approval ID 138-2009, in accordance with the principles exposed in the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

MS (3 h daily, 3hMS) procedure was performed according to Veenema et al. previously described (Veenema et al., 2006). Briefly, female and male adult rats were mated for 2 days. During the last week of the gestation, female rats were single-housed in standard rat Plexiglas cages and maintained under standard laboratory conditions with 12:12 light–dark cycle (light on 0700), temperature maintained at 22 ± 2 °C, food and water *ad libitum*. On the day after parturition, PND2, each litter was culled to 7–8 pups, in which 5–6 were males. During the period from PND2–PND15, the pups were separated daily between 900 h and 1200 h from their dams. Pups were removed and transferred by hands previously coated with fine bedding-powder from the same cage of each litter. They were moved to an adjacent room and placed individually into a small box filled with bedding, and then put into a humid incubator with temperature maintained at 29 ± 1 °C. After the 3 h separation period, the pups were returned to the home-cage followed by reunion with their respective dam. Non-separated litters (animal facility-reared pups, AFR) were left undisturbed except for changes of the bedding twice a week and served as control groups for this study.

Experimental design

Four experiments were performed in 2 postnatal stages, neonatal and young adulthood. Experiment 1 evaluated the immediate