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EARLY FREE ACCESS TO HYPERTONIC NACL SOLUTION INDUCES A LONG-TERM EFFECT ON DRINKING, BRAIN CELL ACTIVITY AND GENE EXPRESSION OF ADULT RAT OFFSPRING

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Abstract—Exposure to an altered osmotic environment during a pre/postnatal period can differentially program the fluid intake and excretion pattern profile in a way that persists until adulthood. However, knowledge about the programing effects on the underlying brain neurochemical circuits of thirst and hydroelectrolyte balance, and its relation with behavioral outputs, is limited. We evaluated whether early voluntary intake of hypertonic NaCl solution may program adult offspring fluid balance, plasma vasopressin, neural activity, and brain vasopressin and angiotensinergic receptor type 1a (AT1a)-receptor gene expression. The manipulation (M) period covered dams from 1 week before conception until offspring turned 1-month-old. The experimental groups were (i) Free access to hypertonic NaCl solution (0.45 M NaCl), food (0.18% NaCl) and water [M-Na]; and (ii) Free access to food and water only [M-Ctrol]. Male offspring (2-month-old) were subjected to iv infusion (0.15 ml/min) of hypertonic (1.5 M NaCl), isotonic (0.15 M NaCl) or sham infusion during 20 min. Cumulative water intake (140 min) and drinking latency to the first lick were recorded from the start of the infusion. Our results indicate that, after systemic sodium overload, the M-Na group had increased water intake, and diminished neuronal activity (Fos-immunoreactivity) in the subfornical organ (SFO) and nucleus of the solitary tract. They also showed reduced relative vasopressin (AVP)-mRNA and AT1a-mRNA expression at the supraoptic nucleus and SFO, respectively. The data indicate that the availability of a rich source of sodium during the pre/postnatal period induces a long-term effect on drinking, neural activity, and brain gene expression implicated in the control of hydroelectrolyte balance.

Key words: pre/postnatal programing, AVP and AT1a mRNA relative gene expression, *c-fos*, subfornical organ, nucleus of the solitary tract, vasopressin.

INTRODUCTION

Development in an altered osmotic environment during a sensitive prenatal and postnatal period can differentially program fluid intake patterns in animals and humans that persist even until adulthood (Mouw et al., 1978; Contreras and Kosten, 1983; Contreras and Ryan, 1990; Nicolaidis et al., 1990; Crystal and Bernstein, 1995; Galaverna et al., 1995; Stein et al., 1996; Leshem, 1998, 2009; Argüelles et al., 2000; Katovich et al., 2001; Wang et al., 2003; Curtis et al., 2004; Perillan et al., 2004; Shirazki et al., 2007; Mecawi et al., 2010; Wu et al., 2011; Zhang et al., 2011). However, knowledge is limited about the effects of prenatal and postnatal programing on underlying brain circuit activity or gene expression mediating the neuroendocrine and behavioral osmoregulatory responses (Roitman et al., 2002; Clark and Bernstein, 2006; McBride et al., 2006; Na et al., 2007).

Our recent results indicate that maternal voluntary ingestion of hypertonic NaCl solution during pregnancy and lactation until one week post-weaning alters the offspring's central osmoregulatory mechanisms in adulthood, modulating water and sodium intake after Furosemide-sodium depletion and also the cell activity of brain nuclei involved in the control of hydroelectrolyte

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Abbreviations: 5-HT, serotonin; ab, antibody; ABP, arterial blood pressure; ANOVA, analyses of variance; AP, area postrema; AT1a, angiotensinergic receptor type 1a; AVP, vasopressin; BNST, bed nucleus of the *stria terminalis*; BW, body weight; CeA, central lateral amygdaloid nucleus; CVOs, circumventricular organs; DAB, diaminobenzidine hydrochloride; DRN, dorsal raphe nucleus; HP, hypertonic sodium solution; ISO, isotonic sodium solution; LPBN, lateral parabrachial nucleus; LT, lamina terminalis; M, manipulation; MnPO, median preoptic nucleus; MnPOd, dorsal subdivisions of MnPO; MnPOv, ventral subdivisions of MnPO; NHS, normal horse serum; NTS, nucleus of the solitary tract; OT, oxytocin; OVLT, organum vasculosum of the lamina terminalis; PaLM, lateral magnocellular subdivision of paraventricular nucleus; PaV, parvocellular ventral subdivision of paraventricular nucleus; PB, phosphate buffer; PD, postnatal day; PVN, paraventricular nucleus; RT, retrotranscription; SFO, subfornical organ; SON, supraoptic nucleus.

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balance (Macchione et al., 2012). As the main effect of this imprinting model was on offspring water balance, altering water intake and vasopressinergic system activity, we now tested the hypothesis that this pre/postnatal availability of a rich source of sodium may program osmoregulatory mechanisms in response to sodium overload.

Many studies have demonstrated developmental plasticity changes in the osmosensitive mechanisms that alter, for example, the osmotic threshold for vasopressin (AVP) release or water drinking in offspring exposed to an increase in maternal hypernatremia (Ramirez et al., 2002; Desai et al., 2003; Ross et al., 2005) or maternal high sodium diet (Contreras and Kosten, 1983; Curtis et al., 2004). However, it is important to note that, in these studies, the animals were subjected to obligatory high sodium intake, while the present report investigates the effect of early hypertonic NaCl consumption as free choice, eliminating the stress provoked by dehydration-induced anorexia and possible chronic renal alterations (Balbi et al., 2008; Machado et al., 2008; Sánchez et al., 2008; Mecawi et al., 2010).

The aim of the present study was to evaluate whether early access to hypertonic NaCl intake may program adult offspring fluid balance, neural activity, and brain AVP and angiotensinergic receptor type 1a (AT1a)-receptor gene expression. Hence, the behavioral profile of water intake, the brain activity of specific areas implicated in the control of salt and water homeostasis, as well as AVP-mRNA and AT1a-mRNA relative gene expression in the supraoptic nucleus (SON) and subfornical organ (SFO), respectively, were analyzed after hypertonic NaCl infusion in adult offspring. Urinary excretion parameters and plasma AVP concentration were also analyzed.

EXPERIMENTAL PROCEDURES

Animals

Thirty Wistar-derived female rats, born and reared in the vivarium of the Instituto Ferreyra (INIMEC-CONICET-UNC, Córdoba, Argentina), weighing 220–250 g, 70–75 days old and non-littermates, were individually housed in standard holding chambers (40 × 40 × 70 cm). Room lights were on for 12 h/day, beginning at 08:00 am, and temperature was controlled at 23 °C ± 1. Animal handling and experimental procedures were approved by the Animal Care and Use Committee of our institute, and the National Institutes of Health (NIH) Guidelines were followed.

The protocol was executed according to Macchione et al. (2012), briefly summarized as follows: 7 days before mating, female rats were randomly divided in two groups to receive the appropriate manipulation (M): one group without manipulation [M-Ctrol group] had free access to tap water and standard commercial diet (Cargill Inc. Argentina, containing approx. 0.18% NaCl) and the other group, in addition to tap water and commercial diet, had voluntary access to a hypertonic NaCl solution (0.45 M NaCl) [M-Na group]. After a week of adaptation, one couple per cage was placed for mating in the same standard holding chamber until found sperm-positive, maintaining the hypertonic NaCl solution access in the M-Na group. When pregnancy was confirmed (1–5 days), males were removed. Pregnant rats were maintained in the same holding chamber.

Within 24 h after birth, litters were culled to nine pups, retaining both males and females in each litter. Litters with fewer than six pups were not included. Dams continued to receive their respective conditions of manipulation until pups were weaned at postnatal days 21–22 (PD21–22). After weaning, only male pups continued to the experiments, and these received the same conditions as their dam until reaching 1 month old (PD28). As the pups probably started drinking at about PD18, they therefore had approximately 2 weeks access to high salt solution. From then on, males of both experimental conditions were kept in standard conditions of water and food until 2 months of age (PD60). To avoid litter-specific effects, no more than three males per litter were used for the same condition. As in our previous study (Macchione et al., 2012), we decided to analyze only the males' intake in the present study since we were aware of the sexual dimorphism of thirst and sodium appetite (Stricker et al., 1991; Chow et al., 1992; Dadam et al., 2014), and also how estrogen level changes influence female fluid intake (Dalmasso et al., 2011).

Experiment 1.1: Water intake induced by iv infusion of hypertonic NaCl solution in adult offspring. Adult males (⩾PD60, 11–15 animals/experimental group) from both groups were anaesthetised and were implanted with intravenous (iv) cannulas via the femoral vein, as detailed in Section 'Femoral vein cannulation'. Once the animals recovered from anesthesia, they were placed in individual metabolic cages with *ad libitum* access to commercial food and distilled water. Body weight at surgery (BW-1), percentage of weight lost as a result of surgery (% BW lost) as well as overnight water intake and latency at test were recorded. Furthermore, the state of health and hydration were determined and the opening of the catheter was checked with a small infusion (∼0.1 ml) of isotonic saline. Subsequently, the animals were connected to an infusion pump (SyringePump, NE1800) in their individual cages and the iv infusion was started 5 min later at a rate of 0.15 ml/min. Animals were infused with a hypertonic (1.5 M NaCl) or isotonic (0.15 M NaCl) NaCl solution for 20 min (Ho et al., 2007). Latency to drink and water intake were evaluated during the 20-min infusion and 120 min thereafter (140 min). Another infusion control group was included that received the same protocol (surgery, fluid access and handling) but with sham infusions (Sham group).

Experiment 1.2: Urinary excretion in response to iv infusion of hypertonic NaCl solution in adult offspring. In the same animals used in Experiment 1.1, the urine excreted was collected during the infusion period and also during the water intake test (140 total minutes). Samples were immediately centrifuged (4 °C, 3000 rpm, 20 min) and 1 ml of the supernatant was collected and