Physiology & Behavior 105 (2012) 1035–1044

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Availability of a rich source of sodium during the perinatal period programs the fluid balance restoration pattern in adult offspring

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ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form 10 November 2011
Accepted 11 November 2011

Keywords:
Perinatal manipulation
Hypertonic sodium exposure
Fos-ir cells
Vasopressin
Furosemide–sodium depletion

ABSTRACT

Osmoregulatory mechanisms can be vulnerable to electrolyte and/or endocrine environmental changes during the perinatal period, differentially programming the developing offspring and affecting them even in adulthood. The aim of this study was to evaluate whether availability of hypertonic sodium solution during the perinatal period may induce a differential programming in adult offspring osmoregulatory mechanisms. With this aim, we studied water and sodium intake after Furosemide–sodium depletion in adult offspring exposed to hypertonic sodium solution from 1 week before mating until postnatal day 28 of the offspring, used as a perinatal manipulation model [PM-Na group]. In these animals, we also identified the cell population groups in brain nuclei activated by Furosemide–sodium depletion treatment, analyzing the spatial patterns of Fos and Fos–vasopressin immunoreactivity.

In sodium depleted rats, sodium and water intake were significantly lower in the PM-Na group vs. animals without access to hypertonic sodium solution [PM-Ctrol group]. Interestingly, when comparing the volumes consumed of both solutions in each PM group, our data show the expected significant differences between both solutions ingested in the PM-Ctrol group, which makes an isotonic cocktail; however, in the PM-Na group there were no significant differences in the volumes of both solutions consumed after Furosemide–sodium depletion, and therefore the sodium concentration of total fluid ingested by this group was significantly higher than that in the PM-Ctrol group.

With regard to brain Fos immunoreactivity, we observed that Furosemide–sodium depletion in the PM-Na group induced a higher number of activated cells in the subfornical organ, ventral subdivision of the paraventricular nucleus and vasopressinergic neurons of the supraoptic nucleus than in the PM-Ctrol animals. Moreover, along the brainstem, we found a decreased number of sodium depletion-activated cells within the nucleus of the solitary tract of the PM-Na group.

Our data indicate that early sodium availability induces a long-term effect on fluid drinking and on the cell activity of brain nuclei involved in the control of hydromineral balance. These results also suggest that availability of a rich source of sodium during the perinatal period may provoke a larger anticipatory response in the offspring, activating the vasopressinergic system and reducing thirst after water and sodium depletion, as a result of central osmosensitive mechanism alterations.

1. Introduction

Environmental changes during critical periods of intrauterine life and/or the early postnatal stage (perinatal period) have a strong impact, even in the long term, differentially programming the systems in developing individuals. The process by which early insults at critical stages of development have irreversible, permanent and long-term effects in tissue structure and function is known as intrauterine programming and may result in adult disease originated *in utero*, and this is often called the 'developmental origins of adult disease' hypothesis [1–3]. At present, a great amount of research is being carried out to evaluate how the adult phenotype is a consequence of environmental signals operating on genes during perinatal development. Permanent changes in the homeostatic regulation of these systems could lead to increased risk factors for certain increasingly prevalent diseases in our society, such as hypertension, non-insulin dependent diabetes, impaired glucose tolerance and obesity [2,4–9].

Hydroelectrolytic homeostatic systems are not exempt from the effects of perinatal programming and many studies indicate that, during sensitive periods of ontogeny, different perinatal stimuli such as dehydration, hypernatremia, sodium overload, sodium depletion or

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doi:10.1016/j.physbeh.2011.11.015

angiotensin converting enzyme (ACE) inhibitors, induce an imprinting on osmoregulatory mechanisms, altering their endocrine and behavioral regulatory responses [10–16]. Perinatal experiences of the stimuli of sodium overload (water deprivation, dietary sodium overload) or sodium depletion (diuretics administration, repeated vomiting during the first trimester of pregnancy, etc.) alter salt preference, sodium appetite and/or fluid intake of the offspring in adult life [11,13,17–22]. Despite the varying results, all these studies provide increasing evidence that exposure to altered osmotic environments during ontogeny can program subsequent adult systems governing thirst and sodium appetite and, if persistent through adulthood, these alterations may have adverse clinical effects.

On the other hand, it is well known that an increased sodium intake, like that of many other mineral and nutrient requirements, occurs during pregnancy, [23–26]. Barelare and Ritcher (1938) [27] for example, demonstrated for the first time that, when pregnant dams have access to a rich sodium source, the total sodium intake increases between 60–98% compared with pregnant rats who have access to water only. Taking this into account, the present study sought to determine if voluntary access to hypertonic sodium chloride solution during the perinatal period may be sufficient to produce lasting changes in osmoregulatory mechanisms in adult offspring.

The study used two groups of pregnant rats, both fed *ad libitum*, one with access to water, and the other with access to water and also a hypertonic sodium chloride solution (0.45 M NaCl), in order to evaluate whether availability of hypertonic sodium solution during the perinatal period may induce a differential programming of central osmoregulatory mechanisms involved in offspring fluid intake control. For this purpose, we investigated the water and sodium intake induced by Furosemide–sodium depletion treatment in adult offspring. We also analyzed the pattern of cell activity, as shown by Fos-immunoreactivity, within vasopressinergic hypothalamic nuclei and along other brain neuron groups involved in fluid balance regulation.

2. Materials and methods

2.1. Animals

Twenty Wistar-derived female rats, born and reared in the vivarium of the Instituto Ferreyra (INIMEC-CONICET, Córdoba, Argentina), were used in the experiments. Animals 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. Animal handling and experimental procedures were approved by the appropriate animal care and use committee of our institute, and the National Institutes of Health (NIH) Guidelines were followed.

As shown in Fig. 1, seven days before mating, female rats were randomly divided in two groups to receive the corresponding perinatal manipulation (PM): one group had free access to tap water and standard commercial diet (Cargill Inc. Argentina, containing approx. 0.18% NaCl) [PM-Ctrol group] and the other group, in addition to tap water and commercial diet, had voluntary access to a hypertonic sodium chloride solution (0.45 M NaCl) [PM-Na group]. After a week of adaptation, one couple per cage was placed for mating in the same standard holding chamber until a sperm-positive test was obtained, maintaining the hypertonic sodium chloride solution access in the PM-Na group. When the pregnancy was confirmed (1 to 5 days), males were withdrawn and pregnant rats were maintained in the same holding chamber. These experimental conditions were maintained throughout pregnancy. 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 perinatal conditions until pups were weaned at postnatal days 21–22 (PD21–22). At the weaning, dams and their female pups were sacrificed for subsequent plasma parameter analysis. Only male pups continued the experiments, and these received the same conditions as their dam until reaching a month of life (PD28). From then on, males of both experimental conditions were kept in standard conditions of water and food until 2 months of age (PD60–70). No more than 3 males/dam were used for the same condition in each experiment. As we were aware of the sexual dimorphism of sodium appetite [28,29] and also how estrogen level changes influence female fluid intake [30], we decided to analyze only the males' intake in the present study.

2.1.1. Experiment #1.a: Maternal water and hypertonic sodium chloride solution (0.45 M) intake

From the week of adaptation and throughout pregnancy and lactation, the maternal water intake of both PM groups was recorded daily and averaged weekly. The same procedure was also performed with the hypertonic sodium chloride solution intake of PM-Na dams. Data are shown as average of fluid consumed per dam (water, sodium and total fluid ingestion) during the following periods: adaptation week; first week of pregnancy and total pregnancy period; and first, second and third lactation weeks. Dams' intake during the days needed for pregnancy confirmation was not taken into account to calculate the volume drunk in any period. The number of pregnancy days, litter size born and percentage of male offspring were also recorded.

2.1.2. Experiment #1.b: Plasma sodium concentration, osmolality and protein assays in dams and female pups

On the weaning day, plasma of dams and female pups was collected by decapitation. Trunk blood was collected in plastic tubes containing EDTA (final concentration 2 mg/ml blood) and immediately centrifuged at 4 °C for 20 min at 3.000 g. Then plasma was removed and kept at −20 °C until determination (note that pooled blood was not necessary for the pup measurements). Plasma sodium concentration [Na^+],was determined using an Ion Selective Electrode (Hitachi Modular P+ISE. Roche 8 Diagnostic). Plasma osmolality was analyzed by vapor pressure osmometry (VAPRO 5520) and plasma volume was indirectly inferred by the plasma protein concentration, measured in an

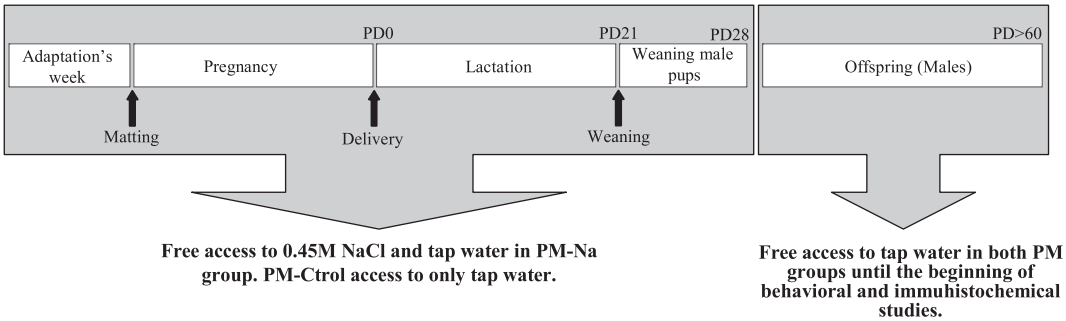

Fig. 1. Schematic diagram showing the conditions to which female rats and offspring were subjected from adaptation week until the beginning of behavioral and brain immunohistochemical studies. Note that from PD28 until PD60, males of both PM groups were kept in standard conditions.