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Maternal RAS influence on the ontogeny of thirst

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Abstract

Perillan, C., Costales, M., Vijande, M., and J. Arguelles. Maternal RAS influence on the ontogeny of thirst. Physiol Behav XX (X) 000–000, 2006. The main objective of this study was to investigate the effect of an altered ambiance in utero, on the development of thirst mechanisms in the offspring. Female rats underwent a partial ligature of the aorta (PAL), which induces an intrinsic activation of the renin angiotensin system (RAS), thirst and sodium appetite. A second group of female rats was treated with desoxycorticosterone (DOCA) which depresses the RAS. The offspring of these two groups were tested for their responses to several thirst stimuli at 2, 4 and 6 days of age. The offspring from PAL mothers responded like their controls to cellular dehydration (NaCl hypertonic injection) at 2 days of age, and also did to extracellular dehydration by polyethyleneglycol at 4 days. Nevertheless, they responded more to isoproterenol at 6 days of age in comparison to their control group. The offspring from DOCA treated mothers did not show statistically significant responses (in comparison with vehicle injected pups) to hypertonic NaCl at two days nor to polyethyleneglycol at four days. Water intake at 6 days of age after isoproterenol administration in DOCA was statistically enhanced, but not differently from the response obtained from pseudo-DOCA treated pups. In particular, rats developed in a hypereninemic ambiance (O-PAL) during gestation, responded with higher water intake when treated with a strong RAS and thirst activator (isoproterenol) but responded normally to a more gentle and complex stimulus (PG). Therefore it seems that in utero conditions can determine the chronology and intensity of thirst responses in offspring.

Keywords: Salt; NaCl; Thirst; Gestation; Renin angiotensin system; Development

1. Introduction

Numerous articles over the past years have tried to determine whether some of the most important diseases in adulthood such as hypertension, coronary heart disease and non-insulin dependent diabetes, could be related to the conditions during intrauterine growth and development. This so called, "programming", would be the effect of any stimulus or changes at a critical sensitive period of the development that could affect the structure, physiology or metabolism of the individual in a permanent way, leading to a predisposition to suffer cardiovascular diseases in adult life [1–3]. In humans, low birth weight has been associated with an increased rate of cardiovascular diseases and non-insulin dependent diabetes in adult life [4–6].

Several animal studies have shown other examples of possible in utero programming. There is evidence that alterations in maternal nutrition may have long-term effects in offspring. Arguelles et al. [7] suggested that a high salt environment in utero conditions the mechanisms underlying cardiovascular responsiveness to angiotensin II. Langley and Jackson [8] have described that offspring from rats fed with low protein diets during pregnancy, showed higher blood pressure values. On the contrary, it has also been reported that rats with maternal diet-induced hypertension appear to be insensitive to the hypertensive effects of sodium chloride [9].

Episodes of extracellular dehydration by polyethyleneglycol administration, in pregnant rats, led to an increased salt appetite of adult offspring [10]. The same group confirmed an increased salt appetite in the offspring when acute and repeated hydrosaline losses had occurred during pregnancy after diuretic–natriuretic treatment [11].

Handelman et al. [12,13] have shown that neonatal exposure to vasopressin induced long-term changes in renal responses to the hormone, due to a decrease in renal binding. Arguelles et al. [14] and Perillan et al. [15] have described modifications in rat offspring's ingestive behavior from hypereninemic, hypertensive and natriophilic mothers. In a similar way, Ross and Nijland

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[16] have highlighted the importance of the uterine environment in the development of dipsic and regulatory mechanisms during fetal life.

These findings, all together, indicate that a hydrosaline and hormonal altered environment may modify thirst responses later in life. Water drinking responsiveness to different thirst stimuli has a known sequential activation. Newborn rats already respond to cellular dehydration at 2 days of age, to hypovolemia at 4 days, and to beta-adrenergic activation at 6 days [17].

The aim of the present study is to investigate the effects of an altered maternal hydrosaline and an altered renin-angiotensin system during pregnancy and early lactation, on the development pattern of drinking behavior. This has not been fully studied until now.

2. Material and methods

2.1. Animals

All experiments were performed on Wistar rats (from the vivarium of the Universidad de Oviedo) housed individually in a light and temperature controlled room, (8:00 h lights on, 20:00 h lights off and 21±1 °C). Animal care was in accordance with guidelines from 86/609/EEC Directive and the study had the approval of the Institutional Animal Ethical Committee. They had free access to a standard laboratory diet, tap water and 2.7% NaCl (liquids available from graduated glass tubes fitted with glass spouts) where appropriate.

A group of 80 female rats (250–300 g) was equally divided in four groups as follows:

a) Partial aortic ligated rats (PAL). Partial aortic ligature produces a chronic ischemia on the left kidney and lower aortic territory as it has been described elsewhere [18]. One week after surgery, female rats were mated and pregnancy assessed by daily vaginal smears, with the presence of spermatozoids taken as day 1 of pregnancy.
b) Pseudo PAL rats (sPAL). Sham operated rats were used as control for the PAL group.
c) Deoxicorticosterone treated rats (DOCA). Untouched female rats, were daily injected subcutaneously with 2.5 mg of DOCA (in 2 mL of olive oil) from the first day of pregnancy during 10 days, and 3 mg of DOCA from the 11th to the 17th day of pregnancy [19].
d) Pseudo DOCA group (sDOCA). Female rats were daily injected subcutaneously with 2 ml of olive oil only from day 1 to 17 of pregnancy.

In all four groups daily water intake and 2.7% saline was recorded. Immediately after the end of the intake test period of their pups, the mothers were sacrificed by decapitation and 5 ml of blood collected. Hematocrit was immediately determined and the remaining blood was centrifuged at 4 °C. Plasma osmolality (using a vapor pressure osmometer-WESCOR) was measured. The remaining plasma was stored at −20 °C for renin activity determinations (RIA: DRG DIAGNOSTIC, Sensitivity 0.2 ng, intra-assay variance: 12.5%).

2.2. Intake testing in offspring

The day of birth was designated as “day 0”. The final inspection of litters was at 17:00 h. Litters, usually 8–12 pups, were kept intact until the day of testing. The protocol consisted of five steps: A) Deprivation: four hours prior to testing, the litter size was adjusted to 8 pups (4 females and 4 males). The pups were separated from the dam during the last 2 h and placed under a 25-W lamp. The skin temperature was monitored with a thermistor probe and maintained at 33 °C. Pups were weighed to the nearest 0.01 g, before and after deprivation. B) Nursing: pups were then returned to the dam for 1 h and 45 min and re-weighed. In order to minimizing the effect of hunger on subsequent thirst testing, one pup of each sex that had gained the least during the nursing period was excluded. C) Challenge: three pups from each litter were subcutaneously injected with one of the following thirst stimuli: i) hypertonic saline (1 M): 2.5 mmol/100 g bw, in 2 day-old pups. ii) polyethyleneglycol (PG) (MERCK, wt 35.000): 30%, in 4 day-old pups. iii) isoproterenol (SIGMA): 500 μg/kg, in 6 day-old pups. The other three pups from each litter were subcutaneously injected with 0.15 M NaCl (control). All subcutaneous injections were made over the scapulae at a volume of 1.25 mL/100 g body wt just before testing, but when PG was the challenge, in which the litter was injected 4 h prior. D) Testing: the pups were weighed and replaced in the box after the challenge for a 2-h test period. Skin temperature was still maintained at 31±1 °C under the 25-W lamp. The apparatus for pups thirst testing consisted of an infusion pump delivering room temperature distilled water through a PE-50 plastic spout extending 2 mm beyond the blunted end of a needle. The infusion rate was 0.7 mL/min. Each rat was offered a 15 s bout every 15 min, during two hours (9 bouts for each rat). It can either lick, struggle or remain active. E) End of Testing: at the end of the 2 h test period, each rat was weighed. Finally percent weight gain during the test period was calculated. Weight gain was used as a measure of water ingested, since there was no other source of weight gain (no mother available). According to Wirth and Epstein [17] who first designed this intake test, evaporative loss is assumed to be the same for all challenge and control rats, and there is no spontaneous excretion in the suckling rat.

At the end of the test each pup was decapitated with a razor blade, its blood collected in heparinized capillary tubes. Hematocrit values were read after centrifugation and osmolality was determined as previously described.

2.3. Statistical analysis

The results are presented as means±SEM. Unpaired “*t*” Student test was used where appropriated. Values of $p<0.05$ were deemed as statistically significant.

3. Results

3.1. Mothers

Biological data obtained during pregnancies are shown in Table 1. As previously described, PAL [14] and DOCA [19]