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Thirst changes in offspring of hyperreninemic rat dams

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Abstract

The aim of this study was to investigate the changes in thirst dependence on the renin–angiotensin system (RAS), in offspring of hyperreninemic, hyperdipsic, and natriophilic rat dams. Female rats underwent a partial aortic ligature between the renal arteries (PAL) or were sham-operated (SHAM). At 6 days of age, offspring of PAL (O-PAL) and SHAM (O-SHAM) dams were injected with isoproterenol (subcutaneously, 500 μg/kg body weight) or vehicle. Pretreatment with captopril (intraperitoneally, 50 mg/kg) on isoproterenol-induced thirst was also studied. Plasma renin activity in dams and hematocrit and osmolality in pups were measured. O-PAL had a greater water intake than O-SHAM. However, they responded similarly to isoproterenol or isoproterenol with captopril pretreatment. Only minor differences in hematocrit and osmolality were found between O-SHAM and O-PAL rats after isoproterenol or vehicle treatment. Beta-adrenergic or angiotensinergic responsivity seems not to be altered in offspring of hyperrenimic, hyperdipsic, and natriophilic dams. Nevertheless, other thirst responses of offspring may be critically dependent upon uterine conditions.

Keywords: Thirst; Gestation; Development; RAS; Isoproterenol; Captopril; Offspring; Salt; NaCl

1. Introduction

The renin–angiotensin system (RAS) has been suggested to be an important regulator of fetal growth and renal organogenesis. It is up-regulated during renal development and in the perinatal period (Wolf, 2002).

Increasing interest is given to uterine environment effects on development. Barker's hypothesis postulates that numerous diseases in later stages of life (e.g., cardiovascular disease and diabetes) can be programmed during fetal growth (Barker, 1995, 2000). In this context, it is imaginable that development "in utero" under altered conditions (i.e., high renin secretion) might involve permanent and long-term conditioning of the mechanisms underlying body fluid and sodium homeostasis and in particular water and salt intake regulation. Accordingly, abnormal expression of the components of the RAS in the fetal–placental unit have been reported to "programme" in some way later hypertension in offspring (Langley-Evans et al., 1999; Dodic et al., 2001; Moritz et al., 2002).

Partial aortic ligature (PAL) between the renal arteries leads to an increase in thirst and sodium appetite. These changes could be attributed mainly to renin secreted by the left (ischemic) kidney, and to a subsequent increase in circulating levels of angiotensin II (Costales et al., 1984).

The aim of this study was to investigate the possible changes in the response to thirst stimuli, particularly dependent on the renin–angiotensin system, in offspring of dam rats undergoing a PAL procedure.

2. Methods

2.1. Dams

Twenty-three nulliparous female rats (Wistar; 3 months of age and 300 g body weight (BW)) were housed individually in plastic cages in a temperature-controlled

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(21±1 °C) room with a 12:12 h light/dark cycle. Animal care was in accordance with guidelines from the 86/609/EEC Directive and the study received the approval of the Institutional Animal Ethical Committee.

Rats had free access to a standard laboratory diet (containing 0.14% NaCl), tap water, and 2.7% NaCl (liquids available from graduated glass tubes fitted with glass spouts). After 5 days of adaptation to these conditions, under ether anesthesia, animals were subjected to either partial aortic ligature (PAL) or a sham operation (SHAM). The abdominal aorta was approached through an abdominal incision; once the aorta between the renal arteries was cleared, it was partly occluded by tying a silk thread (no. 4/0) around it just below the mesenteric artery with a stylus (0.6 mm) included within the ligature, and then removing the stylus. The abdominal incision was closed in layers. The sham operation was identical except that the aorta was not ligated (Costales et al., 1984). One week after surgery, when polydipsia and natriophilia associated with PAL were well established, a male was introduced into each female cage (PAL and SHAM) for mating.

Daily testing for the presence of spermatozoids in vaginal smears was deemed as an indication of pregnancy. The maternal water and saline intake was recorded daily, before and after surgery and throughout pregnancy. Thirteen dams are used for the isoproterenol experiment and 10 dams for the isoproterenol+captopril experiment.

All dams gave birth after 21 days. Plasma renin activity (PRA) was determined in dams used for the isoproterenol experiment 6 days after delivery (RIA: DRG DIAGNOSTIC, Sensitivity 0.2 ng, intraassay variance: 12.5%). Dams were sacrificed by decapitation at the end of the test period. Five millilitres of blood were centrifuged immediately at 4 °C and plasma stored at −20 °C until analysis.

2.2. Offspring

Litters, usually 8–12 pups, were kept together until the day of testing (6 days of age). Pups increased their intakes of water and saline in response to isoproterenol at 6 days of age (Wirth and Epstein, 1976). Daily inspection of litters was performed at 17:00 H, taking the date as "day 0" for those animals born in the last 24 h. Different litters were used in different experiments.

The protocol consisted of five steps: 1. Deprivation: 4 h 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 were placed under a 25-W lamp in a plastic box with paper towels, and skin temperature monitored, with a thermistor probe on the skin, was maintained at 33 °C. Pups were weighed to the nearest 0.01 g on a top-loading Mettler balance, before and after deprivation. 2. Nursing: pups were then returned to the dam for 1 h and 45 min and re-weighed. One pup of each sex that had gained the least during the nursing period was excluded, thereby minimizing the effect of hunger on subsequent thirst testing. 3. Challenge: three pups from each litter were injected subcutaneously (s.c.) with isoproterenol (SIGMA, 500 μg/kg) and three with saline solution (control). After the Wirth and Epstein (1976) dose–response study, the largest amount of water consumption was provoked by a 500 μg/kg dose. Volume injected was 1.25 mL/100 g body weight. 4. Testing: immediately after the challenge, the pups were weighed and replaced in the box for the start of a 2-h test period. During testing, skin temperature was maintained at 31±1 °C under the 25-W lamp. The test apparatus consisted of an infusion pump which delivered distilled water at room temperature through a PE-50 tubing spout extending 2 mm beyond the blunted end of a needle. The infusion rate was 0.7 mL/min. For each bout, the pup was grasped between the experimenter's thumb and forefinger on either side of the neck, just firmly enough to stabilize the head. The pup's mouth was opened and with the pump running, the plastic tubing was inserted into the pup's mouth. The suckling was held on this spout for 15s. It can either lick, struggle or remain active. In any case, at the end of this time, the pup is dried and returned to the box. This procedure was repeated every 15 min during the 2 h test period (9×15 sec bouts for each pup). 5: End of testing: pups were reweighed and sacrificed.

Percent weight gain during the test period was calculated as:

$$100 \times \frac{\text{Weight Gain during the test period}}{\text{Body Weight before nursing}}$$

Weight Gain is used as an adequate estimation of water intake in pups (Houpt and Epstein, 1973).

Each pup was decapitated with a razor blade, its blood collected in heparinized capillary tubes and hematocrit values were read and osmolality was determined using a vapor pressure osmometer (Wescor).

2.3. Captopril treatment

Pups from PAL and SHAM dams (10 dams) were injected with the ACE inhibitor (captopril) before testing

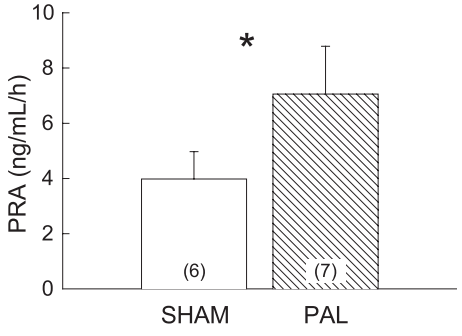

Fig. 1. Plasma renin activity (PRA) in partial aortic ligated (PAL) and Sham-PAL dam rats after delivery. (Bars represent mean±standard error of mean; *n* in brackets, at the bottom of bars; *$p<0.05$).

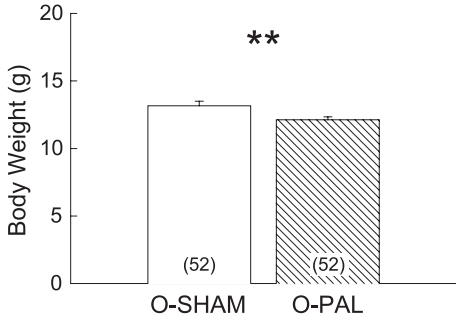

Fig. 2. Body weight of partial aortic ligated dams' offspring (O-PAL) and Sham-partial aortic ligated dams' offspring (O-SHAM) when tested for isoproterenol thirst challenge at six days of age. (Bars represent mean ±standard error of mean; *n* in brackets, at the bottom of bars; **$p<0.01$).

with isoproterenol. Captopril (Bristol–Myers Squibb) was injected intraperitoneally (i.p., 50 mg/kg dissolved in distilled water; volume injected 1 mL/100 g BW), before the nursing period.

2.4. Statistical analysis

The results are presented as means±standard error of mean (S.E.M.). Student's unpaired "*t*" and two-way ANOVA tests were used where appropriate. Values of $p<0.05$ were deemed statistically significant.

3. Results

3.1. Dams

High salt and water intakes were found during gestation and lactation in the PAL group (data not shown) as expected (Argüelles et al., 2000). Plasma renin activity was higher in PAL dams after delivery than in SHAM dams (Fig. 1),

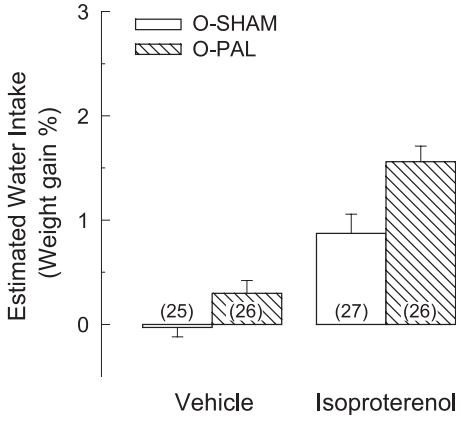

Fig. 3. Estimated water intake (body weight gain %) by offspring from partial aortic ligated dams (O-PAL) and Sham-partial aortic ligated dams (O-SHAM) in a 2-h test drinking period after an isoproterenol challenge (s.c., 500 μg/kg BW) or vehicle. (Bars represent mean±standard error of mean; *n* in brackets, at the bottom of bars. Two-way ANOVA; O-PAL vs. O-SHAM: F1=12.34; $p<0.001$; Vehicle vs. isoproterenol: F1=55.17, $p<0.001$; interaction: F1,100=1.45, $p=0.24$).

Table 1
Blood parameters in pups groups

	Hematocrit (%)		Osmolality (mmol/kg)	
	Vehicle	Isoproterenol	Vehicle	Isoproterenol
O-SHAM	32.31±0.60 (26)*	30.20±0.91 (25)	286.32±1.36 (22)	287.86±2.05 (22)
O-PAL	30.11±0.77 (27)	31.00±0.62 (19)	287.80±3.22 (25)	286.61±2.92 (23)

(n) (* $p<0.05$, O-SHAM vs. O-PAL, Vehicle).
Data represents mean±standard error of mean.

demonstrating the long-term effect of this surgical procedure on the RAS in pregnant rats.

The surgical procedure initially provoked body weight losses as previously reported (Costales et al., 1984). Neither differences between control and ligated maternal body weight at the end of the testing period nor in their litter sizes were found.

3.2. Offspring

Pups from PAL dams presented lower body weight than pups from SHAM dams ($p<0.01$) when tested for isoproterenol thirst challenge at 6 days of age (Fig. 2).

The interaction between offspring condition (SHAM or PAL) and challenge (vehicle or isoproterenol) on dipsic response was not significant (two-way ANOVA: $F=1.45$, $df=1.100$, $p=0.24$). On the contrary, the main effects of each of these factors on drinking were statistically significant (offspring condition: $F=12.34$, $df=1$, $p<0.001$); dipsogenic challenge: $F=55.72$, $df=1$, $p<0.001$, Fig. 3).

Minor differences in blood parameters, hematocrit, and osmolality were found between O-SHAM and O-PAL rats at the end of the 2-h period of intake testing after isoproterenol

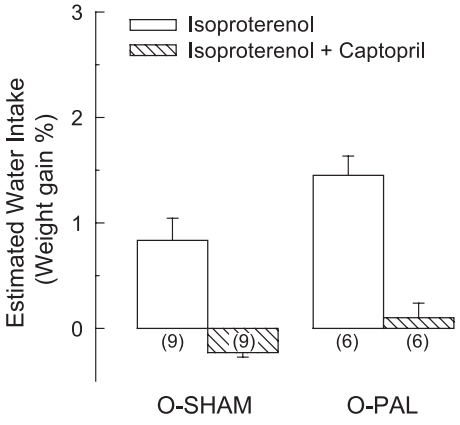

Fig. 4. Estimated water intake (body weight gain %) by offspring from partial aortic ligated dams' (O-PAL) and Sham-partial aortic ligated dams' (O-SHAM) in a 2-h test drinking period after captopril (i.p., 50 mg/kg BW) or vehicle, and isoproterenol (s.c., 500 μg/kg BW). (Bars represent mean±standard error of mean; *n* in brackets, at the bottom of bars. Two-way ANOVA; O-PAL vs. O-SHAM, F1=10.66, $p<0.011$; isoproterenol vs. isoproterenol+captopril, F1=37.27, $p<0.001$; interaction F1,26=2.03, $p=0.17$).

or vehicle treatment. Only the differences in the hematocrit values of O-SHAM vs. O-PAL pups resulted statistically significant values ($p<0.05$; Table 1).

The interaction between offspring condition (SHAM or PAL) and challenge (isoproterenol or isoproterenol+captopril) on dipsic response was not significant (two-way ANOVA: $F=2.03$; $df=1.26$, $p=0.17$). However, the main effects of each of these factors on drinking did result statistically significant values (offspring condition: $F=10.66$, $df=1$, $p<0.01$; dipsogenic challenge: $F=37.27$, $df=1$, $p<0.0001$; Fig. 4).

4. Discussion

The development of dipsogenic and additional regulatory mechanisms for the control of ingestive behavior occur during the fetal and early stages of life and may be susceptible to changes in the pregnancy environment (Ross and Nijland, 1998). Responsiveness to thirst challenges is precocious since this is present before the animal freely ingests water as a separate substance. The dipsic response to the β-adrenergic agonist, isoproterenol, begins at 6 days of age in the rat (Wirth and Epstein, 1976). Studies of brain c-*fos* activation after isoproterenol injection carried out by Oldfield and McKinley (1994) have shown that isoproterenol activates several brain areas involved in the regulation of water intake, cardiovascular control, and neuroendocrine pathways. Fos reactivity in the "lamina terminalis" is abolished by prior treatment with losartan or captopril, indicating that this region is activated by blood-borne angiotensin II (Oldfield and McKinley, 1994).

We studied the effect of a maternal ligation of the abdominal aorta, which produces an activation of the renin angiotensin system on the early water intake of descendents, including that produced by isoproterenol, a partially angiotensin-dependent stimulus (Fitzsimons, 1998).

In our experiment, isoproterenol treatment induced a significant increase in the estimated water intake of 6-day-old pups in full accordance to the sequence described by Wirth and Epstein (1976), independently of their condition (O-PAL or O-SHAM).

Moreover, our results demonstrated that the offspring of ligated dams are thirstier: these showed an enhanced water intake when injected with either isotonic saline or isoproterenol. Therefore, pups whose early development occurred in an altered uterine environment after PAL appear to be different in their spontaneous and experimentally induced (isoproterenol) ingestive behavior. The possibility that the O-PAL pups are hungrier and thirstier because of their significantly lower body weight, could not be excluded. The alternative hypothesis, i.e., that they weigh less because they are less hungry, is rejected since these pups weigh less at birth as it has been elsewhere demonstrated by Argüelles et al. (2000).

Leenen and Stricker (1974) revealed that the effect of isoproterenol on water intake is correlated with its ability to induce renin secretion although the hypotensive effects of β-adrenergic stimulation are also known to be responsible, in part, for the dipsic responses (Retting et al., 1981). The almost complete abolition of isoproterenol-induced drinking by captopril in O-PAL and O-SHAM pups confirms its dependence on angiotensin II formation regardless of the maternal condition.

Hematocrit values in isoproterenol-injected pups at the end of the testing period were similar to vehicle-injected pups although they drank significantly more. A plausible explanation for this could be that adrenergic stimulation produces a significant increase in hematocrit (Ojiri et al., 1993). All groups of pups studied showed similar values of osmolality at the end of the testing period. The interpretation of this finding depends on whether or not the suckling rats were able to urinate by themselves during the 2-h treatment period. That possibility has not been purposely investigated in our study. If there was no urinary excretion, a difference in final plasma osmolality between the highest and lowest drinking groups should be found. One possible explanation of this disparity might be that the water was excreted into the bladder and the bladder was not voided; however, pups this age have difficulty concentrating or diluting urine. At least in adult rats, it is known that isoproterenol makes rats anuric because of the large drop in blood pressure as demonstrated by Lehr et al. (1967).

Overall, significant blood parameters after intake testing, in pups, do not change in a clinically considerable manner. Wirth and Epstein (1976) have already suggested that isoproterenol does not act through blood volume depletion or cell dehydration to induce thirst in pups. On the other hand, the nature of the dipsic response in view of the unaltered response to isoproterenol plus captopril seems to indicate that this phenomenon could be RAS-independent. The possibility that a synergy derived of discrete activation of multiple systems together could trigger the dipsic response remains open, without excluding unknown factors. More research needs to be done to clarify this behavioral response.

The hyperreninemic uterine environment maintained during pregnancy and lactation, confirmed by our results (Fig. 1), along with the obvious hemodynamic and other changes induced by PAL, clearly affected the dipsic response of pups. In accordance with Barker's hypothesis, this could lead to long-term changes in these animals. Supporting this, Argüelles et al. (2000) have already reported that O-PAL adult rats presented higher salt preference and increased blood pressure in response to angiotensin II. On the other hand, low birth weight in humans (also present in O-PAL pups) has been considered as a predisposing factor for hypertension and cardiovascular disease in adult life (Godfrey and Barker, 2000).

The hyperreninemic environment could be linked to lower PAL-O body weight. Thus, reduced weight has been reported in fetuses from hypertensive transgenic dams with

elevated plasma prorenin levels (Caragounis et al., 2000). Similarly, spontaneously hypertensive rat (SHR) fetuses also exhibited a lower body weight (Erkadius et al., 1996).

It has already been demonstrated that angiotensin II plays an important role in renal organogenesis (Wolf, 2002) even determining the development of adult disease (Moritz et al., 2002). Dodic et al. (2001) showed that high blood pressure could be programmed in the offspring of animals in which the intrauterine environment has been perturbed at some stage.

Our results are in agreement with the main concept of the hypothesis of Barker (1995, 2000) which considers that early programming could eventually determine a long-term conditioning of physiological mechanisms, in this case related to the control of water intake.

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