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Prenatal water deprivation alters brain angiotensin system and dipsogenic changes in the offspring

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Abstract

Objective—Central renin–angiotensin system (RAS) plays an important role in regulating body fluid balance. The present study determined the effect of maternal dehydration on brain expression levels of angiotensinogen, angiotensin II receptor subtypes, and dipsogenic responses in offspring.

Methods—Pregnant rats were deprived of water during late gestation. Expressions of brain angiotensinogen, angiotensin II receptors, and dipsogenic responses were determined.

Results—Maternal water deprivation significantly decreased fetal body and brain weight, and body and tail length. Fetal plasma sodium, osmolality, and hematocrit were increased. Both AT₁R and AT₂R protein abundance was significantly increased in the fetal brain, associating with increased mRNA levels of AT_{1a}R and AT₂R. Additionally, angiotensinogen mRNA was increased. In adult offspring, prenatal dehydration resulted in significant increases in AT₁R protein and AT_{1a}R mRNA, as well as angiotensinogen mRNA in the forebrain in both males and females. In contrast, AT₂R mRNA and protein were increased only in males. Prenatal dehydration resulted in a significant increase in intracerebroventricular angiotensin II-induced water intake in male, but not female, offspring.

Conclusion—The results provided new information that antenatal water deprivation induces a reprogramming of brain RAS and Ang II receptor expression patterns and alters the central Ang II-mediated dipsogenic response in offspring in a sex-dependent manner.

Keywords

Prenatal water deprivation; Fetus; Offspring; Central AT₁R and AT₂R

1. Introduction

A number of physiological and pathophysiological conditions during pregnancy, including over sweating, diarrhea, and vomiting, can induce maternal dehydration. Water deprivation (WD) is a potent stimulus to affect body fluid homeostasis, and the excretion of water

causes increased natriuresis and both intracellular and extracellular dehydration (McKinley et al., 1983; Salas et al., 2004). It is well known that the brain has its own local renin–angiotensin system (RAS) that plays a critical role in the regulation of water and salt balance (Fitzsimons, 1998; Paul et al., 2006).

Angiotensin II (Ang II) is the most active peptide in the RAS in the brain *via* its actions on the two major subtypes of Ang II receptors, types 1 (AT₁R) and 2 (AT₂R) (Mao et al., 2009; Morris et al., 1999; Kottenberg-Assenmacher et al., 2008). Numerous studies have demonstrated that central AT₁R confers most classical actions of the peptide such as inducing dipsogenic responses. In rodents, AT₁R is subdivided into two isoforms, AT_{1a}R and AT_{1b}R. AT_{1a}R mediates most of physiological actions of Ang II. Centrally injected Ang II is a potent stimulus of water and salt intake in several species (Deloof et al., 1999; De Luca LA et al., 2002; Gottlieb et al., 2006). While it is clear that the central RAS plays an important role in the body fluid regulation under the condition of dehydration, little is known about the effect of dehydration on the development of the brain RAS and its receptors, as well as on the dipsogenic response mediated by Ang II.

Previous studies have demonstrated that adverse intra-uterine conditions and environmental insults, including malnutrition, hypoxia, and dehydration, may affect the expression patterns of AT₁R and AT₂R in the peripheral organs, tissues, or systems in the fetus in late gestation (Dodic et al., 2001, 2002; Ding et al., 2010). Nonetheless, it remains unknown whether and to what extent maternal water deprivation-induced dehydration during pregnancy may influence the development of AT₁R and AT₂R in the fetal brain, and whether such effect has long-term impact on AT₁R- and/or AT₂R-mediated dipsogenic response in off-spring. Thus, the present study determined the effect of maternal dehydration during late gestation on gene expression patterns of brain AT₁R and AT₂R in the fetus and offspring, and examined the effect of prenatal dehydration on Ang II-mediated dipsogenic response in adult offspring. Given the previous findings of sex difference in fetal programming of adult disease (Maric, 2007; Grigore et al., 2008; Mueller and Bale, 2008; Xiao et al., 2008), the studies were conducted in both male and female offspring.

2. Results

2.1. Fetal growth

Maternal water deprivation did not affect the litter size and the length of gestation. Fetal survival rate was the same between the control and WD groups. Although placental weight and dried placental weight were not significantly changed between the control and WD groups ($P > 0.05$), maternal WD caused a reduction of fetal body weight, brain weight, dried body and brain weight, as well as body and tail length, as compared to the control group (Table 1).

2.2. Blood values

Maternal and fetal blood [Na]⁺, [K]⁺, hematocrit (Hct), and osmolality were significantly increased by maternal WD ($P < 0.05$). Blood pH, pO₂, and pCO₂ were not significantly changed (Table 2). In adult offspring, there was no significant difference in the blood values between the WD and control groups (Table 3).

2.3. Expression of mRNA of angiotensinogen, AT₁R, and AT₂R in the forebrain

Angiotensinogen mRNA abundance in the fetal forebrain was significantly increased by WD during pregnancy (Fig. 1A). The increased angiotensinogen mRNA abundance persisted into the adulthood in both male and female offspring (Fig. 1B and C). Both mRNA and protein of AT_{1a}R, AT_{1b}R, and AT₂R were determined in the fetal forebrain. Maternal WD for 3

days significantly increased mRNA levels of AT_{1a}R and AT₂R (Fig. 2A). In contrast, AT_{1b}R mRNA was not altered. This changed gene expression pattern of Ang II receptors in the forebrain persisted into the male offspring (Fig. 3A). Unlike males, different expression patterns were demonstrated in the forebrain of female offspring with an increase in AT_{1a}R mRNA abundance but no changes in AT_{1b}R or AT₂R mRNA levels in animals of prenatal dehydration, as compared with the control group (Fig. 3B).

2.4. Expression of AT₁R and AT₂R protein in the forebrain

After maternal WD for 3 days, protein levels of both AT₁R and AT₂R in the fetal forebrain (Fig. 2B) were significantly higher than those of the control. In offspring, both AT₁R and AT₂R protein abundance was increased in the forebrain in males of prenatal dehydration (Fig. 4A). Unlike males, protein abundance of AT₁R, but not AT₂R, was significantly increased in the forebrain of female offspring (Fig. 4B).

2.5. The effect of intracerebroventricular angiotensin II on dipsogenic behavior

In both male and female offspring, daily water and salt intake was the same between control and prenatal dehydration animals. However, the stimulated thirst response was increased under the condition of intracerebroventricular (i.c.v.) application of Ang II. In female offspring exposed to prenatal WD, the Ang II-stimulated water intake remained similar to that of the control (Fig. 5A). In contrast, the Ang II-stimulated water intake was significantly increased in male offspring of prenatal dehydration, as compared with that of the control animals (Fig. 6A). Salt intake in response to i.c.v. administration of Ang II was unchanged in both male and female offspring between prenatal WD and control animals (Figs. 5B and 6B).

3. Discussion

The present study demonstrates in a rat model that the dehydration during pregnancy alters brain expression patterns of angiotensinogen and the Ang II receptors in the fetus and offspring, which is accompanied with the changes in the central Ang II-induced dipsogenic behavior in male offspring in a sex-dependent manner.

In the present study, we found that although the placental weight was not changed, the fetal body weight was significantly decreased by maternal water dehydration during gestation days of 18–20 in fetal rats, which is similar to the mid-to-late gestation period in human fetus in the development. Given that the fetus has higher body fluids than the adult (Zhu, 2008), it is possible that the decreased fetal body weight caused by maternal water deprivation is due to a reduction of fetal body fluids. However, the finding that the dried fetal body weight was also significantly decreased would argue against this possibility. Together with the findings of reduced fetal body and tail length, the present results suggest that maternal water deprivation may cause fetal growth restriction. The present study also shows that both wet and dry weights of the fetal brain were significantly reduced, suggesting that maternal dehydration may affect the development of the brain *in utero*.

The finding that maternal blood sodium, Hct, and plasma osmolality were significantly increased by water deprivation during late gestation is consistent with the previous observations in adult animals (McKinley et al., 1983; Makino et al., 1996; De Luca LA et al., 2002). The fetal blood values in response to maternal dehydration were also changed in parallel to those of the mother, suggesting that maternal dehydration during pregnancy causes fetal dehydration *in utero*. Similar findings were obtained in the previous studies in sheep (Ross et al., 1998).

It has been shown that ligated uterus and protein restriction during pregnancy causes fetal growth retardation (Glazier et al., 1996; Torres et al., 2010), and affects fetal brain functions in rats (Torres et al., 2010). The present study demonstrates that maternal dehydration adversely affects the development of the RAS and Ang II receptors in the fetal forebrain. Ang II is the main active peptide of the RAS and acts at its specific receptors, AT₁ and AT₂ receptors (De Gasparo et al., 2000). Several lines of studies have suggested fundamental differences in the expression patterns of AT₁ and AT₂ receptors between adults and fetuses (Obillard et al., 1995; Shanmugam and Sandberg, 1996; Hu et al., 2004). In the fetal brain, the AT₂ receptor expression was increased to high levels during the late stage of fetal development (Obillard et al., 1995). The present study showed the coexistence of both AT₁R and AT₂R in the fetal forebrain at near term, and demonstrated that the protein abundance of both AT₁R and AT₂R, as well as mRNA levels of AT_{1a}R and AT₂R, was significantly increased by maternal water deprivation. This suggests that the change in AT₁R protein is mainly caused by increased AT_{1a}R, but not AT_{1b}R, mRNA in the fetal brain. These findings suggest that *in utero* development of central AT₁R and AT₂R is vulnerable to environmental insults such as water deprivation, and provide new information that exposure to maternal dehydration at the late fetal developmental stage could reset the expression levels of Ang II receptors in the fetal brain.

A question raised is whether there is a long-term consequence for the change of Ang II receptors in the fetal brain in offspring. To address this question, the present study investigated the central Ang II receptors and drinking behavior in 5-month-old adult offspring rats. A recent study demonstrated the altered salt appetite in offspring exposed to prenatal malnutrition (Wu et al., in press). In the present study, we demonstrated the changes in the expression patterns of Ang II receptors in the forebrain in adult offspring, resulting from prenatal dehydration. In male offspring, the brain expression of both AT₁R and AT₂R was increased, while AT₁R, but not AT₂R, was upregulated in female brains. These findings suggest that water deprivation during pregnancy induces a reprogramming of brain Ang II receptor expression patterns in offspring in a sex-dependent manner. While this finding is consistent with previous studies showing the sex difference in fetal programming of adult disease (Maric, 2007; Grigore et al., 2008; Mueller and Bale, 2008; Xiao et al., 2008), the possible mechanisms of genetic factors or sex hormones in fetal programming of differential Ang II receptor expression patterns in the brain remain to be determined.

The RAS and Ang II receptors in the brain play an important role in the regulation of water and salt intake in adults (Fitzsimons, 1972). Previous studies showed a critical contribution of brain AT₁R and central Ang II in dipsogenic responses *via* the central network in the control of body fluid balance. Several important nuclei located around the ventricular area, including the paraventricular nuclei, subfornical organ, median preoptic nuclei, and supraoptic nuclei, play an important role in water and salt intake in response to i.c.v. Ang II (Fitzsimons, 1998; Davissou et al., 2000; Mao et al., 2007a, b). Those areas around the ventricular system are also rich of Ang II receptors (Fitzsimons, 1998). Intracerebroventricular application of Ang II-induced dipsogenic responses via central AT₁R and AT₂R in the high-salt fed rats (Camara and Osborn, 2001), suggesting both brain AT₁R and AT₂R, are involved in dipsogenic regulations. In the present study, we demonstrated that i.c.v. Ang II increased water intake in the adult offspring exposed to prenatal water deprivation was higher in the male, not female, showing an interesting association with the different alteration of expression of AT₂R in the forebrain in the offspring. Notably, the expression of angiotensinogen, AT_{1a}R, and AT_{1b}R mRNA, as well as AT₁R protein in the brain was the same between the male and female offspring. Recent study demonstrated that high salt intake increased more AT₁R and caused higher pressor responses mediated by Ang II in the male rodents evidenced as sex difference (Schneider et al., 2010). Notably, there is possibility for difference between male and female capability, including renin activity, in

response to dehydration. The behavioral difference following stimulation of i.c.v. Ang II between the male and female offspring was associated with different expression patterns of the central AT₂R mRNA and protein levels. This deserves further studies on mechanisms behind those gender differences.

In conclusion, the present study demonstrates that central expression of fetal AT₁R and AT₂R is vulnerable to the challenge of maternal water deprivation during late pregnancy. Importantly, prenatal water deprivation increases the brain expression of both angiotensinogen and Ang II receptors and the dipsogenic response in male offspring in a sex-dependent manner. These results provide new information in the preventive medicine related to pregnancy outcome and the development of RAS.

4. Experimental procedures

4.1. Animals and experiments

Fetal experiments were performed on Sprague–Dawley rats housed in a controlled environment of 22–25 °C and a 12-h light/dark cycle (8:00 am–8:00 pm as light period). Pregnant rats were randomly divided into two groups: control group and water-deprived group (water bottle removed during deprived period). In the control group, the pregnant rats had *ad libitum* access to fresh tap water and rat food. In the WD group, the pregnant rats were deprived of water for 3 days (Ji et al., 2007) from day 18 of gestation, while food was provided. Maternal food intake was measured and reported before (Guan et al., 2009). On day 21 of gestation, pregnant rats were anesthetized with sodium pentobarbital (20 mg/kg, i.p.) in the control and WD groups ($n=5-6$ litters, each group), and a small cut was made at the middle of abdomen of rats, fetuses and their brains were removed immediately. Other pregnant animals were allowed to give birth naturally.

For offspring experiments, pregnant rats were given water after 3-day water deprivation as described above. They were allowed to give birth naturally. Pups born to the dams were kept with their mothers until weaning. At weaning, male and female pups were separated. Both male and female offspring were used at 5 months old. All procedures in this study were approved by the Institute Animal Care Service.

4.2. Fetal weight and length

Fetal weight and length were measured (two fetuses from the same dam, mean of the two as $n=1$). Both fetal body length (from crown to rump), tail length were measured. Fetal body, brain, and placenta also were weighted. Then fetal body, brain, and placental tissue were dried in an oven at 65 °C for 24 h to remove water. Dried fetal body, brain, and placenta were measured for dry weight ($n=6-8$, each group).

4.3. Measurement of blood values

Water deprivation was selected at late gestation while fetal blood collection is only possible in rats during that period. Maternal, fetal, and adult offspring blood samples were collected into ice-cold plastic tubes as reported (Mao et al., 2007a, b). Briefly, fetal blood samples (7–8 fetuses from the same dam as $n=1$, and $n=6-8$, each group) were collected directly from the heart using intracardiac puncture, and blood was pooled together in the same litter. Blood samples were obtained from the abdominal aorta in adult offspring (Salas et al., 1995). Blood pH, sodium concentrations, and Hct were determined with a Nova analyzer (Nova Biochemical, Model pHox Plus L, Waltham, MA) at 39 °C base. The remaining blood was centrifuged at 4 °C for 15 min. Then plasma was used for measuring osmolality with an advanced digmatic osmometer (model 3MO, Advanced Instruments, Needham Heights, MA).

4.4. Real-time PCR for angiotensinogen, AT_{1a}R, AT_{1b}R, and AT₂R mRNA

Quantitative real-time PCR analysis was employed for angiotensinogen, and AT_{1a}, AT_{1b}, and AT₂ receptors mRNA in the fetal and offspring forebrain ($n=5-6$, each group). The primers were designed based on the published rat mRNA and genomic sequences in Genbank (Table 4). Purification of total RNA from the forebrain tissue was performed using a protocol (Yen et al., 2002). The tissue was homogenized in TRIZOL (60–100 mg tissue in 1 ml TRIZOL) (Invitrogen, USA). RNA was then immediately isolated, precipitated, washed, and stored in 75% ethanol at -70°C until analysis. RNA was only used if the ratio between spectrophotometer readings (260:280 nm) was between 1.8 and 2.0, denoting minimum contamination from cellular proteins.

Samples were digested with DNase I to degrade any DNA present in the RNA isolation. A reverse transcription and first strand cDNA synthesis was performed using MMLV-RT reverse transcriptase (Invitrogen USA). The first strand cDNA was checked using the housekeeping gene to assess quality of the reverse transcription. First strand synthesis was achieved using 2 μg of total RNA. Real-time PCR (BIO-Rad icycler iQ, USA) was performed in a 96 well plate; 12.5 μl 2 $\times$ SYBRGreen master mix (Takara, Japan), 0.5 μl forward primer, 0.5 μl reverse primer, 2.0 μl cDNA, and 9.5 μl dd water were added for a total volume of 25 μl per well. Amplification conditions involved a pre-incubation at 95°C for 1 min followed by amplification of the target DNA for 45 cycles (95°C for 10 s and 60°C for 20 s), and the fluorescence collection at 60°C . Melting curve analysis was performed at a linear temperature transition rate of 0.5°C/s from 65°C to 95°C with continuous fluorescence acquisition. All experiments were repeated three times for reproducibility. The relative gene expression was normalized to GAPDH and calculated as follows (Kenneth and Schmittgen, 2001): relative gene expression (RGE) = $2^{-(T\Delta\text{Ct} - N\Delta\text{Ct})}$, where T represents the level of experimental groups, N represents the control group whose expression level was calculated, and ΔCt is the difference of threshold cycle (Ct) between the gene of interest and GAPDH.

4.5. Western-blot analysis for AT₁R and AT₂R immunoreactivity

Forebrain tissue was homogenized ($n=5-6$, each group), and 80 μg of protein quantified by Bradford assay was loaded in each lane for electrophoresis in 0.1% sodium dodecyl sulfate (SDS) and 10% polyacrylamide gel with 4% stacking gel, and then transferred to immobilon polyvinylidene difluoride membranes (Millipore, MA), and then incubated in the primary antibodies overnight at 4°C with the rabbit polyclonal antibody (Santa Cruz Biotech, Santa Cruz, USA) against AT₁R (sc-1173, 1:500) or AT₂R (sc-9040, 1:1000), respectively. Then the membranes were washed and incubated in horseradish peroxidase-conjugated goat anti-rabbit antibody at 1:5000 dilution. The bound antibody was then visualized by using enhanced chemiluminescence (ECL) detection (Amersham Biosci, Piscataway, USA) and exposed to X-ray film. All samples were normalized to the β -actin signal as control. For quantification of AT₁R and AT₂R protein levels reflected by their immunoreactivity, the photographs were digitalized and analyzed using a scanner. The magnitude of the immunosignal was given as a percentage of the internal control. We realized limitation of the technology in concern of specificity of the antibodies (Adams et al., 2008). Thus special attention was paid for exact conditions and procedures used in the Western blot experiments between the control and experimental groups.

4.6. Cannulation of a lateral cerebral ventricle

Under condition of anesthesia using ketamine (75 mg/kg) and xylazine (5 mg/kg), adult offspring rats were implanted with stainless-steel intracranial cannula (23-gauge, length 9.5 mm) at the lateral cerebral ventricle. The stereotaxic coordinates with the surface of the skull horizontal were: 1 mm behind bregma, 1.5 mm from the mid-line and 2.5 mm down from

the dura. At the end of the operation, the cannula was closed off with a stainless steel obturator (30-gauge) of the same length as the cannula. The rats were left for 2–3 days to recover from surgery and were then handled for 10 min each day for 3 days for acclimating the hands of researchers before testing.

4.7. Intracranial injection procedure and dipsogenic responses monitored

All offspring rats were adapted to tap water and 1.8% NaCl solution *ad libitum* for 5–6 days before beginning the behavioral experiments ($n=6-8$, each group). Amount of daily water intake and salt intake as spontaneous consumption of water and salt was measured. On testing day, intracranial injections were carried out on conscious adult offspring rats (12:00 pm–4:00 pm). The obturator was removed and replaced by a stainless-steel injector (30-gauge) connected by polyethylene tubing to a 10- μ l Hamilton syringe. The tip of the injector protruded 1 mm beyond the end of the cannula. The injector end of the injection system contained about 10 μ l of the solution to be injected separately by a small air bubble (0.5 μ l) from the rest of the system, which was filled with water. One microliter of solution was injected over a period of 30 s, the movement of the air bubble acting as a convenient marker of the progress of the injection. The injector was left in place for a further 30 s and was then removed and replaced by the obturator. Finally, the rat was put back in its cage. The rat's drinking response to i.c.v. 0.1 nmol Ang II (Sigma, St. Louis, MO) was measured for 2-h period.

4.8. Data analysis

Statistical analysis was performed with SPSS software (version 13.0). Student's *t*-test and repeated measures of ANOVA followed with Tukey's test were used for analysis between the control and the experimental groups. All data are expressed as mean $\pm$ SEM, and significance was at $P<0.05$.

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References

- Adams JM, McCarthy JJ, Stocker SD. Excess dietary salt alters angiotensinergic regulation of neurons in the rostral ventrolateral medulla. *Hypertension*. 2008; 52 (5):932–937. [PubMed: 18779436]
- Camara AKS, Osborn J. Central AT1 and AT2 receptors mediate chronic intracerebroventricular angiotensin II-induced drinking in rats fed high sodium chloride diet. from weaning. *Acta Physiol Scand*. 2001; 171:195–201. [PubMed: 11350280]
- De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology: XXIII. The angiotensin II receptors. *Pharmacol Rev*. 2000; 52:415–472. [PubMed: 10977869]
- Deloof S, De Seze C, Montel V, Chatelain A. Effects of water deprivation on atrial natriuretic peptide secretion and density of binding sites in adrenal glands and kidneys of maternal and fetal rats in late gestation. *Eur J Endocrinol*. 1999; 141:160–168. [PubMed: 10427160]
- De Luca LAJ, Xu ZC, Schoorlemmer GHM, Thunhorst RL, Beltz TG, Menani JV. Water deprivation-induced sodium appetite: humoral and cardiovascular mediators and immediate early genes. *Am J Physiol Regulatory Integrative Comp Physiol*. 2002; 282:R552–R559.
- Ding Y, Lv JX, Zhang HY, Wang AQ, Zhu LY, Zhu H, Mao CP, Xu ZC. High salt diet during pregnancy and angiotensin-related cardiac changes. *J Hypertension*. 2010; 28:1290–1297.
- Dodic M, Baird R, Hantzis V. Organs/systems potentially involved in one model of programmed hypertension in sheep. *Clin Exp Pharmacol Physiol*. 2001; 28:952–956. [PubMed: 11703404]
- Dodic M, Abouantou T, O'Connor A. Programming effects of short prenatal exposure to dexamethasone in sheep. *Hypertension*. 2002; 40:729–734. [PubMed: 12411469]

- Davisson RL, Oliverio MI, Coffman TM, Sigmund CD. Divergent functions of angiotensin II receptor isoforms in the brain. *J Clin Invest*. 2000; 106:103–106. [PubMed: 10880053]
- Fitzsimons JT. Thirst. *Physiol Rev*. 1972; 52:468–561. [PubMed: 4336576]
- Fitzsimons JT. Angiotensin, thirst, and sodium appetite. *Physiol Rev*. 1998; 78:583–686. [PubMed: 9674690]
- Glazier JD, Sibley CP, Carter AM. Effect of fetal growth restriction on system A amino acid transporter activity in the maternal facing plasma membrane of rat syncytiotrophoblast. *Pediatr Res*. 1996; 40 (2):325–329. [PubMed: 8827785]
- Gottlieb HB, Ji LL, Jones H, Penny ML, Fleming T, Cunningham JT. Differential effects of water and saline intake on water deprivation-induced c-Fos staining in the rat. *Am J Physiol Regul Integr Comp Physiol*. 2006; 290:R1251–R1261. [PubMed: 16306162]
- Grigore D, Ojeda NB, Alexander BT. Sex differences in the fetal programming of hypertension. *GendMed*. 2008; 5:S121–S132.
- Guan JC, Mao CP, Geng CS, Xu FC, Zhu LY, Wang AQ, Xu ZC. Prenatal dehydration affected renin–angiotensin system associated with angiotensin-increased blood pressure in young offspring. *Hypertens Res*. 2009; 32:1104–1111. [PubMed: 19779489]
- Hu F, Morrissey P, Yao JM, Xu ZC. Development of AT1 and AT2 receptors in the ovine fetal brain. *Dev Brain Res*. 2004; 150:51–61. [PubMed: 15126038]
- Ji LL, Gottlieb HB, Penny ML. Differential effects of water deprivation and rehydration on Fos and FosB/ Δ FosB staining in the rat brainstem. *Exp Neurol*. 2007; 203 (2):445–456. [PubMed: 17027755]
- Kenneth JL, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*. 2001; 25:402–408. [PubMed: 11846609]
- Kottenberg-Assenmacher E, Massoudy P, Jakob H, Philipp T, Peters J. Chronic AT₁-receptor blockade does not alter cerebral oxygen supply/demand ratio during cardiopulmonary bypass in hypertensive patients. *Acta Anaesthesiol Scand*. 2008; 52 (1):73–80. [PubMed: 17976222]
- Makino Y, Minamino N, Kakishita E, Kangawa K, Matsuo H. Natriuretic peptides in water-deprived and in salt-loaded rats. *Peptides*. 1996; 17:1031–1039. [PubMed: 8899824]
- Mao CP, Guan JC, Yuan X, Miao LY, Zhu H, Chen LQ, Xu ZC. Got pure blood in fetal rats? *Pediatr Hematol Oncol*. 2007a; 24:457–460. [PubMed: 17710664]
- Mao CP, Lv JX, Zhu H, Zhou Y, Chen RZ, Feng X, Xu ZC. Fetal functional capabilities in response to maternal hypertonicity associated with altered central and peripheral angiotensinogen mRNA in rats. *Peptides*. 2007b; 28:1178–1184. [PubMed: 17513012]
- Mao CP, Shi LJ, Xu FC, Zhang LB, Xu ZC. Development of fetal brain renin angiotensin system and hypertension programmed in fetal origins. *Prog Neurobiol*. 2009; 87:252–263. [PubMed: 19428956]
- Maric C. Mechanisms of fetal programming of adult hypertension: role of sex hormones. *Hypertension*. 2007; 50:605–606. [PubMed: 17724274]
- McKinley MJ, Denton DA, Nelson JF, Weisinger RS. Dehydration induces sodium depletion in rats, rabbits, and sheep. *Am J Physiol Regul Integr Comp Physiol*. 1983; 45:R287–R292.
- Morris M, Li P, Callahan MF, Oliverio MI, Coffman TM, Bosch SM, Diz DI. Neuroendocrine effects of dehydration in mice lacking the angiotensin AT1a receptor. *Hypertension*. 1999; 33:482–486. [PubMed: 9931152]
- Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. *J Neurosci*. 2008; 28:9055–9065. [PubMed: 18768700]
- Obillard JE, Page WV, Mathews MS, Schutte BC, Nuyt AM, Segar JL. Differential gene expression and regulation of renal angiotensin II receptor subtypes (AT₁ and AT₂) during fetal life in sheep. *Pediatr Res*. 1995; 38:896–904. [PubMed: 8618791]
- Paul M, Mehr AP, Kreutz R. Physiology of local renin–angiotensin systems. *Physiol Rev*. 2006; 86:747–803. [PubMed: 16816138]
- Ross MG, Sherman DJ, Ervin MG, Castro R, Humme J. Maternal dehydration rehydration: fetal plasma and urinary responses. *Am J Physiol*. 1998; 255:E674–E679. [PubMed: 3189537]

- Salas SP, Altermatt F, Campos M, Giacaman A, Rosso P. Effects of long-term nitric oxide synthesis inhibition on plasma volume expansion and fetal growth in the pregnant rat. *Hypertension*. 1995; 26:1019–1023. [PubMed: 7498960]
- Salas SP, Giacaman A, Vio CP. Renal and hormonal effects of water deprivation in late-term pregnant rats. *Hypertension*. 2004; 44:334–339. [PubMed: 15277406]
- Schneider MP, Wach PF, Durley MK, Pollock JS, Pollock DM. Sex differences in acute ANG II-mediated hemodynamic responses in mice. *Am J Physiol*. 2010; 299 (3):R899–R906.
- Shanmugam S, Sandberg K. Ontogeny of angiotensin receptors. *Cell Biol Inter*. 1996; 20:169–176.
- Torres N, Bautista CJ, Tovar AR, Ordáz G, Rodríguez-Cruz M, Ortiz V, Granados O, Nathanielsz PW, Larrea F, Zambrano E. Protein restriction during pregnancy affects maternal liver lipid metabolism and fetal brain lipid composition in the rat. *Am J Physiol Endocrinol Metab*. 2010; 298 (2):E270–E277. [PubMed: 19920218]
- Wu L, Mao C, Liu Y, Shi A, Xu F, Zhang L, Xu Z. Altered dipsogenic responses and expression of angiotensin receptors in the offspring exposed to prenatal high sucrose. *Peptides*. 2010 (Article in Press). 10.1016/j.peptides.2010.10.012
- Xiao DL, Xu ZC, Huang XH, Longo LD, Yang SM, Zhang LB. Prenatal gender-related nicotine exposure increases blood pressure response to angiotensin II in adult offspring. *Hypertension*. 2008; 51:1239–1247. [PubMed: 18259024]
- Zhu, DN. Fetal urinary system development, fetal body fluid regulation. In: Xu, ZC., editor. *Fetal Developmental Physiology*. High Education Press; Beijing: 2008. p. 170-192.
- Yen K, Linda L, Jennifer M, Dominick D. Differential expression of nicotine acetylcholine receptor subunits in fetal and neonatal mouse thymus. *J Neuroimmunol*. 2002; 130:140–154. [PubMed: 12225896]

Fig. 1. The effect of maternal water deprivation during late pregnancy on expression of angiotensinogen mRNA in the forebrain. A. Fetus; B. male offspring; C. female offspring. Water deprivation (WD) group compared with control group (Con), * $P < 0.05$, ** $P < 0.01$.

Fig. 2.
The effect of maternal water deprivation during late pregnancy on expression of Ang II receptor in the fetal forebrain. A. mRNA levels; B. protein levels. Water deprivation (WD) group compared with control group (Con). ** $P < 0.01$.

Fig. 3. The effect of maternal water deprivation during late pregnancy on expression of Ang II receptor mRNA in the adult offspring forebrain. A. Male; B. female. ** $P < 0.01$.

Fig. 4. The effect of maternal water deprivation during late pregnancy on expression of Ang II receptor protein in the adult offspring forebrain. A. Male; B. female. * $P < 0.05$, ** $P < 0.01$.

Fig. 5. Water and salt intake by i.c.v. Ang II in the adult female offspring exposed to prenatal dehydration. A. Water intake; B. salt intake.

Fig. 6.

Water and salt intake by i.c.v. Ang II in the adult male offspring exposed to prenatal dehydration. A. Water intake; B. salt intake. ** $P < 0.05$.

Table 1

The effect of water deprivation on fetal growth.

	Body weight (g)	Dried body weight (g)	Body length (mm)	Brain weight (g)	Dried brain weight (g)	Placental weight (g)	Dried placental weight (g)	Tail length (mm)
Con	3.93±0.13	0.64±0.06	4.18±0.11	0.16±0.06	0.0203±0.0001	0.47±0.02	0.083±0.005	1.44±0.02
WD	2.58±0.12*	0.41±0.03*	3.80±0.07*	0.14±0.04*	0.0178±0.0001*	0.44±0.03	0.081±0.005	1.26±0.02*

Con: control; WD: water deprivation. Values are mean ± SEM. WD group vs. Con group.

* $P<0.01$.

Table 2

The effect of water deprivation on maternal and fetal arterial blood values.

	pH	PCO ₂ (mmHg)	PO ₂ (mmHg)	Hct (%)	[Na ⁺] (mmol/l)	[K ⁺] (mmol/l)	Osm (mOsm/kg)
Maternal	Con	7.39±0.02	35.80±1.87	108.18±6.64	35.33±0.60	128.60±0.61	3.90±0.09
	WD	7.40±0.01	35.79±2.67	110.93±5.07	41.25±0.86*	139.43±1.44*	5.07±0.20*
Fetal	Con	7.39±0.03	51.84±2.28	50.30±2.22	31.27±0.87	124.50±0.76	8.52±0.25
	WD	7.38±0.02	49.76±1.15	53.28±2.02	34.58±0.81#	129.75±1.26*	11.09±0.32*

Con: control; WD: water deprivation. Values are mean ± SEM. WD group vs. Con group.

$P<0.05$;

* $P<0.01$.

Table 3

The effect of water deprivation on arterial values in the adult offspring.

		pH	PCO ₂ (mmHg)	PO ₂ (mmHg)	Hct (%)	[Na ⁺] (mmol/l)	[K ⁺] (mmol/l)	Osm (mOsm/kg)
Male	Con	7.40±0.006	36.78±0.46	106.67±3.19	35.56±1.03	136.20±1.99	4.26±0.08	302.01±3.96
	WD	7.41±0.009	37.48±1.63	107.62±2.22	36.10±0.84	137.05±2.56	4.39±0.08	303.63±4.51
Female	Con	7.39±0.010	36.67±0.67	106.97±2.90	35.67±1.60	138.23±1.62	4.35±0.15	300.33±4.36
	WD	7.40±0.006	37.63±0.68	107.75±2.63	35.81±1.71	137.81±1.81	4.27±0.12	305.82±4.03

Con: control; WD: water deprivation. Values are mean ± SEM.

Table 4

Relative gene primers sequence.

Target gene		Primer sequence	Genbank number	Production size (bp)
GAPDH	Forward primer	5'-GCAAGTTCAACGGCACAG-3'	NM_017008	140
	Reverse primer	5'-TACAGCACCTCAGATGACCG-3'		
Angiotensinogen	Forward primer	5'-CAGAAGCAAGTCCACAGAT-3'	NM_134432	135
	Reverse primer	5'-GTAGTAGAGGAGATGAAAGG-3'		
AT _{1a} R	Forward primer	5'-AACCTCTGTCTACGGC-3'	NM_030985	194
	Reverse primer	5'-ACCTGTCACCTCA-3'		
AT _{1b} R	Forward primer	5'-CTGGGCATTATCCGTGAC-3'	NM_031009	223
	Reverse primer	5'-AAGGCGGTAGGAAAGAG-3'		
AT ₂ R	Forward primer	5'-TGATAATCTCAACGCAACTG-3'	NM_012494	221
	Reverse primer	5'-AAAGGAGTAAGTCAGCCACA-3'		