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Altered dipsogenic responses and expression of angiotensin receptors in the offspring exposed to prenatal high sucrose

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Abstract

The present study determined water and salt intake as well as expression of AT₁ and AT₂ receptors in the brain and kidney in the adult offspring rats prenatally exposed to high sucrose. Following the exposure during pregnancy, water intake and salt intake at baseline levels were not changed in the adult offspring. However, after 24 hour water deprivation, consumption of water and salt was significantly increased compared to that of the control. Plasma sodium and osmolality levels remained the same between the offspring in the control and the exposed groups, while hematocrit was higher in the offspring exposed to prenatal high sucrose immediately following water deprivation. Density of renal AT₁ receptor protein was the same between the control and the exposed group, while AT₂ receptor protein in the kidney was significantly increased in the offspring exposed to prenatal high sucrose in association of thicker basal membrane of glomerular. In the forebrain, both AT₁ and AT₂ receptor levels were significantly increased in the offspring with history of prenatal high sucrose. In addition, water deprivation induced more c-fos expression in the central dipsogenic areas, including the paraventricular and supraoptic nuclei in the offspring exposed to prenatal high sucrose. The results suggested that prenatal high intake of sucrose may affect development of pathways in regulation of dipsogenic behavior in face of dehydration, which was associated with altered expression of AT₁ or/and AT₂ receptors in the kidney and brain.

Keywords

high sucrose; fetus; offspring; RAS; dipsogenic responses

1. Introduction

Increasingly evidence has shown that adverse factors or environmental insults (e.g., hypoxia, malnutrition, smoking, etc.) during prenatal life could “program” long-term problems on health in later life [16,22]. Recently, others’ and our studies have demonstrated that prenatal

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exposure to hypoxia, nicotine, and other stressors could induce alterations in cardiovascular regulations and body fluid homeostasis [12,23,24]. It is known that over consumption of sugar during pregnancy may cause metabolic or cardiovascular problems [10]. For pregnant women with diabetes, their babies may face cardiovascular risk [3], suggesting sugar consumption and metabolism in the body during pregnancy may play a role in the fetal development and postnatal health.

Accumulated data demonstrated that multiple environmental insults during pregnancy could affect the development of the renin-angiotensin system (RAS) and its receptors, which could influence health or diseases after birth [11,21,33,36]. For example, glucocorticoids could cause acute and long-term effects on renal angiotensin (Ang) II receptor subtypes [25]; Hypoxia during pregnancy could alter the development of AT₁ and AT₂ receptors (AT₁R and AT₂R) in the fetal kidney, and such alterations may persist after birth [20]; High intake of salt diet during pregnancy could influence expression of AT₁R and AT₂R in the cardiac tissue, which was linked to increase of cardiovascular risks [7]. Therefore, in study of long-term influence of malnutrition during pregnancy, this study determined the impact of over use of sucrose during pregnancy on development of Ang II receptors in the kidney and brain in association with dipsogenic behavior.

Behavioral, hormonal, and neural routes are three major ways in the control of body fluid balance [8]. In behavioral regulation, water intake and salt intake can be defined as dipsogenic responses. One of main mechanisms behind stimulated water intake and salt appetite is via the RAS [31]. Angiotensin II can act as a powerful dipsogenic signal to induce thirst and sodium appetite. This peptide can cause an immediate water intake followed by a slower increase of NaCl intake [8,36]. Physiological actions of Ang II, especially dipsogenic stimulation, are expressed via its subtypes of receptors, mainly AT₁R in the brain and other organs [27]. In the central nervous system, the anteroventral third ventricular (AV3V region), including the subfornical organ (SFO), and the hypothalamus are distributed with both AT₁R and AT₂R [15]. Numerous reports have demonstrated those areas in the brain, together with the kidney, play a critical role in the control of water and salt intake [19], as well as body fluid homeostasis [4].

Notably, the previous studies reported that insulin, an important hormone in the control of sugar levels in the body, stimulated water intake in human [32], and intake of sucrose affected thirst responses [28], suggesting that over intake of sugar and abnormal metabolism of this carbohydrate in the body may influence thirst and drinking behavior. However, it is unknown whether over consumption of sweeten food and beverages during pregnancy may have effects on the development of dipsogenic behavior. Therefore, the present study also determined water and salt intake in the adult offspring rats exposed to prenatal high sucrose.

2. Method and material

2.1. Experimental Animals

Pregnant Sprague-Dawley rats were housed in a controlled environment of 22-25°C and a 12-hour light/dark cycle. They were randomly divided into two groups. Control group (C) was provided with fresh tap water and standard rat food. High sucrose (HS) group were given 20% sucrose solution and standard rat food from day 1 of gestation to day 21. After delivery, both groups were given fresh tap water and standard rat food for 5 months. Male and female pups were separated at weaning. All procedures were approved by the Institute of Animal Care Committee.

2.2 Fetal experiments

On gestational day 21, pregnant rats randomly selected (n=8, each group) were anesthetized with sodium pentobarbital (50mg/kg, i.p.). A small cut was made at the middle of abdomen of the maternal rat. Fetuses were removed immediately and their body weight was measured. Maternal and fetal blood samples were collected from the heart by intracardiac puncture. Fetal blood was pooled in fetuses from the same dam. All blood samples were collected in chilled heparinized syringes. Blood Na⁺ and K⁺ concentrations were determined with a Nova analyzer (Nova Biochemical, Model pHoxPlus L, Waltham, MA) at 39°C base. The remained blood samples were centrifuged at 12,000 rpm/min for 10 min. 20 ul plasma was used for measurement of osmolality.

2.3. Offspring experiments

Other pregnant rats were allowed to give birth. Newborns were kept with their mothers until weaning. Five months after birth, male offspring were weighted and used for experiments. All animals were adapted to tap water and 1.8% NaCl solution *ad libitum* for at least 5 days before beginning of behavioral experiments. Daily water and salt (1.8% NaCl solution) intake as spontaneous consumption of water and salt were measured.

2.4. Determination of water deprivation-induced water intake and salt appetite

After 5-day adaptation to 1.8% NaCl solution, group C and group HS (n=8) were deprived of water for 24 h while food was provided. Immediately following water deprivation, all offspring were tested for their drinking of water and 1.8% NaCl solution during the first 2 h and 24 h.

2.5. Measurement of blood values

Blood samples of offsprings were collected before water deprivation (WD) as a baseline level, and immediately after 24 h of water deprivation. Blood samples were collected into iced tubes containing lithium heparin. Blood pH, sodium concentrations, hemoglobin (Hb), glucose (Glu), and hematocrit (Hct) were determined with a Nova analyzer at 39°C base. The remaining blood was centrifuged at 4°C for 15 min. Then plasma (20 ul) was used for measuring osmolality with an advanced digmatic osmometer (model 3MO, AD Instruments, Needlham Heights, MA).

2.6. Determination of AT₁R and AT₂R expression in the kidney and brain of the offspring

Following experiments above, all animals (group C and group HS) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) The kidney and brain from the adult offspring were collected and homogenized, and 80 µg of proteins quantified by Bradford assay were loaded each lane for electrophoresis in 0.1% sodium dodecyl sulfate (SDS) and 10% polyacrylamide gel with 4% stacking gel, and then transferred to immobilon P polyvinylidene difluoride membranes (Millipore, MA). Immunoblotting was performed by incubating each membrane in blocking buffer (5% bovine serum albumin in PBS plus 0.1% [v/v] Tween) for 1 hour at room temperature, and then incubated in the primary antibodies overnight at 4°C with the rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) against AT₁R (1:500) or AT₂R (1:1,000), respectively. Then the membranes were washed for 10 minutes three times in TBS-T and incubated at room temperature 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 Biosciences, Piscataway, NJ, USA) and exposure to x-ray film. All samples were normalized to the β-actin signal as a internal control.

For quantification of AT₁R and AT₂R protein levels, photographs were digitalized and analyzed using a scanner. The magnitude of the immunosignal was given as a percentage of the internal control.

2.7. Renal histology

At the end of 24 h water deprivation, the animals were anesthetized and perfused with 0.1 M phosphatebuffered saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. The perfusion period was about 6–8 min. The kidney was removed immediately following perfusion. Postfixation was performed in the same PFA solution for 24 hours and embedded in paraffin with 20um successive section. The sections were stained with hematoxylin & eosin (HE) followed by gradient alcohol dehydration from 70% to 100%. The histological analysis was performed with light microscope by professional histological professionals at Histology Laboratory in a blinded manner.

2.8. Immunohistochemistry experiments

Postfixation was performed in the 4% paraformaldehyde solution for 12 h, after which the brain was placed in 20% sucrose in 0.01 M phosphate overnight. Twenty-five micrometer coronal sections were cut through the fetal forebrain on a Leica cryostat. The tissue sections were incubated on a gentle shaker overnight at 4°C in the primary antibody (1:15,000; Santa Cruz Biotechnology, Santa Cruz, CA). The sections were further incubated in goat anti-rabbit serum (1:500) for 1 h and then processed using the Vectastain ABC kit (Vector Labs, Burlingame, CA). The sections were then treated with 1 mg/ml diaminobenzidine tetrahydrochloride. All sections were placed on slides, dehydrated in alcohol, and then coverslipped.

2.9. Statistical analysis

Statistical analysis was performed with SPSS software. Two-way ANOVA was used to determine differences between the control and HS groups followed by Tukey's post hoc test. All data were expressed as mean $\pm$ SEM, and significance was at $p < 0.05$.

3. Results

3.1. Body weight

The fetal dry and fresh body weights were significantly increased ($p < 0.05$) in group HS compared with that of the control. However, there was no significant difference in body weight between group HS and group C in the offspring at 5 month of old (Table 1).

3.2. Water intake and salt appetite

Spontaneous water intake and salt intake were not significantly different between the control group and group HS (Fig. 1a). However, after 24-hour water deprivation, salt and water intake were significantly increased in the offspring prenatally exposed to high sucrose (Fig. 1b).

3.3. Blood values

There was no significant change in fetal blood sodium and potassium concentrations. Plasma osmolality in group HS was significantly increased compared with that of the control group (Table 2, 3). There was no significant difference in fetal arterial blood pH, hemoglobin, hematocrit, glucose, and lactic acid between group HS and control group. Maternal hemoglobin, hematocrit, sodium, and potassium levels were the same in both groups, while plasma osmolality and glucose were higher in group HS (Table 3).

In offspring experiments, after 24 h water deprivation, both blood sodium concentrations and plasma osmolality were significantly elevated, and the hematocrit were also increased in both the offspring from group HS and the control, while other blood values such as potassium concentrations were not changed (Fig. 2, Table 4). There was no significant difference in blood sodium concentrations and plasma osmolality between the experimental and control offspring following water deprivation. However, blood hematocrit in the offspring of group HS was significantly higher than that of the control after 24h water deprivation (Fig. 2c).

3.4. Renal histological analysis

Histological analysis showed that the basal membrane of glomerular in the kidney of group HS is relatively thicker compared with that of the control (Fig. 3). In addition, more intercapillary cells were observed in the offspring kidney of group HS (Fig. 3).

3.5. Immunohistochemistry

After water deprivation, Fos immunoreactivity positive cells in the subfornical organ (SFO), supraoptic nuclei (SON), and paraventricular nuclei (PVN) in the forebrain were significantly increased in the offspring exposed to high sucrose before birth compared to the control (Fig. 4).

3.6. AT₁R and AT₂R expression in the kidney and brain of the offspring

The western blot analysis showed that density of renal AT₁R protein in the offspring of group HS was the same as that of the control. However, AT₂R protein levels in the kidney was significantly increased in group HS (Fig. 5b).

Protein levels of both AT₁R and AT₂R were significantly increased in the forebrain in the offspring of group HS (Fig 5a).

4. Discussion

The present study showed that dipsogenic responses in both water and salt intake stimulated by dehydration were altered following prenatal exposure to high sugar; and demonstrated that in the offspring exposed to high sugar before birth, their neural activity in the central putative areas in the control of dipsogenic responses was increased in association of enhanced expression of the Ang II receptors in the brain in response to water deprivation. In addition, expression of the renal Ang II receptors was changed accompanied with histological alterations.

Since “Barker theory” was first introduced [2], accumulated evidence has demonstrated that prenatal insults may produce long-term impact on health after birth, and behavioral and physiological phenotypes could be “programmed” in fetal origins [18]. Recently, the development of the RAS in normal and abnormal patterns before birth has attracted great attention in link of adult diseases and health conditions [26]. Our studies showed alterations of expression of the Ang II receptors associated with remodeled salt appetite in the offspring exposed to environmental insults such as hypoxia or nicotine during prenatal stages [37]. The present study demonstrated that dehydration during pregnancy not only increased maternal plasma osmolality, but also fetal osmolality at late term. Hypertonicity is well known a strong stimulation to the RAS. Although spontaneous drinking behavior and salt appetite were the same as that of the control in the offspring exposed to prenatal high sugar, the stimulated dipsogenic response, in both water and salt intake, was significantly increased immediately following 24 h water deprivation, indicating dipsogenic regulatory mechanisms or pathways may be altered in the offspring exposed to adverse factors during fetal life.

Water deprivation induces both extracellular and intracellular dehydration [17]. Extracellular and intracellular dehydration could elicit water intake and salt appetite in rodents (also in human) via stimulation of hypovolemic receptors and osmoreceptors (either peripheral or central) [13]. We measured blood values in the offspring, and did not find any significant difference in plasma osmolality, electrolyte concentrations, and hematocrit between the control and experimental groups during baseline periods. It is reasonable that blood sodium and osmolality levels as well as hematocrit were increased in both groups immediately following water deprivation [35]. Although no significant difference in osmolality and sodium concentrations was found between the control and the offspring exposed to prenatal high sugar, blood hematocrit was significantly higher in the offspring of group HS than that of the control, indicating that loss of extracellular volume could be more in group HS than that in the control in response to mixed dehydration produced by water deprivation. Notably, although basal levels of hematocrit were the same between the two groups, their responses to dipsogenic stimulation, water deprivation in this case, was different. This also indicates that loss of extracellular fluids may be a cause. Questions such as why more extracellular volume was reduced in group HS warrant further investigation. Among various possibilities, an alteration in renal functions could be one of directions for future studies.

In the present study, the basal membrane of glomerular became thicker in the offspring exposed to high sugar during fetal stages. Furthermore, histological analysis of intercapillary cells revealed a hyperplasia phenomenon in the kidney in group HS. Those subtle ultra-structure changes in the kidney may not lead to significant changes of renal functions at rest status in water and sodium reabsorption as well as renal excretion [14]. However, in face of challenges like dehydration, renal regulations in body fluid balance may fail working properly. As a consequence, additional loss of extracellular volume was possible.

Among multiple mechanisms in response to dehydration, the pathways via increased osmolality and decreased blood volume are two major routes in body fluid regulations [30]. In the present study, water deprivation-elevated plasma osmolality and sodium levels remained the same between the experimental and control offspring, suggesting that the difference of increased water and salt intake following water deprivation between the control and experimental groups was not mainly caused by osmotic pathways. It is known that hypovolumic stimulation, for instance, loss of extracellular volume or acute hemorrhage, can induce significant dipsogenic responses [9]. There are volume receptors and pathways in both peripheral and central sides [5]. The present study focused on central neural activation in the putative areas linked to the control of body fluid balance. Mapping with c-fos as a marker of cellular activity in the brain has been well accepted [29]. We found that water deprivation-induced Fos-immunoreactivity in the SFO, SON, and PVN was significantly higher in the offspring exposed to prenatal high sugar. The previous studies demonstrated that acute hemorrhage also induced c-fos expression in the SFO, SON, and PVN [1]. Therefore, the data from the behavioral, biochemical, and neural analysis in the present study suggest that the pathways or mechanisms in the control of extracellular volume may be affected in the offspring exposed to prenatal high sugar.

An immediate question raised is how the adverse factor during pregnancy impact on dipsogenic behavior in association with alterations in renal structures and brain functions. We realized a serial of studies required in addressing such questions. In the present study, we determined expression of the receptors in the RAS in the kidney and brain. The RAS plays an important role in body fluid homeostasis [34]. Overall, renal AT₁R is critical in retaining body fluids, while AT₂R was recently suggested to counteract with AT₁R in facilitating renal excretion [6]. Central AT₁R has been repeatedly demonstrated as a major route in Ang II-mediated dipsogenic regulations [38]. In the present study, although renal

AT₁R remained unchanged, AT₂R expression in the kidney was significantly enhanced in the offspring of group HS. In the brain, both AT₁R and AT₂R were increased in the offspring exposed to prenatal high sugar. Whether changes of renal AT₂R leading to ultra-structural alterations in the kidney, and whether changes of expression of both AT₁R and AT₂R in the forebrain were the cause of altered neural activation and dipsogenic responses deserves further investigation.

Finally, the present study showed fetal weight was increased accompanied with maternal intake of high sugar during pregnancy (maternal blood glucose levels were increased too). However, provided with standard water and food after birth, body weight of the offspring between the experimental and control groups was the same, indicating postnatal care may be able to reverse some adverse influence from the prenatal treatment. In spite of this, the changes in regional AT₁R or AT₂R expression as well as renal histological alterations seemed permanent. As long-term influence, such changes may cause subtle mechanistic alterations when the individuals were challenged with specific stressor. In light of this, the present study provided new and interested information that over consuming of sweeten diet during pregnancy may alter functions of the regulatory systems in the offspring in body fluid homeostasis, and such changes may be linked to a remodeled RAS receptor system.

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Figure 1.

Daily baseline water and salt intake (a), and stimulated water and salt intake after 24h water deprivation (b) in male adult offspring (Fig 1b: Amount of fluid intake during 2 hours period immediately following 24 hours of water deprivation). C, Control; HS, Exposed to prenatal high sucrose. HS vs C group, *, $p < 0.05$, **, $p < 0.01$

Figure 2. Plasma osmolality(a), [Na]⁺(b), and Hct (c) levels after 24h water deprivation. C, Control, HS, Exposed to prenatal high sucrose; Baseline vs WD 24h, *, p < 0.05; HS vs Control group, #, p < 0.05

Figure 3.

The histological photos of the kidney in the adult offspring. In the offspring exposed to prenatal high sucrose (right panel), the basement membrane of the glomerulus is thickened (filled arrow) than that of the control (left panel) (hollow arrow). The glomerulus mesenchyma (filled arrow head) appeared a hyperplasia pattern (mesenchymal cell number increased) compared with that (hollow arrow head) of the control.

Figure 4.

Fos positive immunostained cells in the subfornical organ (top), supraoptic nucleus (middle), and paraventricular nucleus (bottom) in the control (left panel of photos) and the offspring exposed to prenatal high sucrose (right panel of photos) after 24h water deprivation. C, Control; HS, Exposed to prenatal high sucrose. HS vs C group, **, $p < 0.01$

Figure 5.

AT₁R and AT₂R protein expression in the forebrain (left panel) and the kidney (right panel) in the adult offspring. Con, Control; HS, Exposed to prenatal high sucrose. *, p<0.01

Table 1

Body weight of fetus (E21) and adult offspring

Group		Control(C)	High Sucrose(HS)
Body Weight(g)			
Fetal	Dry weight	3.82±0.70	4.26±0.15*
	Fresh weight	5.74±0.21	6.61±0.20*
Offspring Weight		494.60±16.31	497.80±17.18

HS vs C group,

*
 $p < 0.05$

Table 2

Arterial values of fetus (E21)

	pH	Hematocrit (%)	Hemoglobin (g/dL)	[Na] ⁺ (mmol/L)	[K] ⁺ (mmol/L)	Glucose (mmol/L)	Lactate Acid (mmol/L)	Osmolality (mosm/kg)
C	7.38±0.02	33.11±1.39	10.64±0.44	123±1.31	8.90±0.50	2.51±0.17	12.64±1.03	282.75±2.17
HS	7.40±0.04	32.30±1.66	10.49±0.52	124±0.71	8.48±0.60	3.00±0.19	11.30±0.85	298.11±4.66*

C, Control; HS, High Sucrose. HS vs C group,

*
p < 0.05

Table 3

Arterial values of pregnant rats

	Hematocrit %	Hemoglobin (g/dL)	[Na] ⁺ (mmol/L)	[K] ⁺ (mmol/L)	Glucose (mmol/L)	Osmolality (mosm/kg)
C	37.30±2.04	12.43±0.68	129±0.61	3.90±0.93	6.68±0.30	285.75±2.13
HS	35.40±0.69	11.82±0.21	129±0.91	4.17±0.13	8.00±0.39 [*]	299.89±4.42 [*]

C, Control; HS, High Sucrose. HS vs C group,

^{*}
p < 0.05

Table 4

Arterial values at baseline and after WD in the adult offspring

Group	pH	Hemoglobin (g/dL)	[K] ⁺ (mmol/L)	Glucose (mmol/L)	Lactate Acid (mmol/L)
Baseline					
C	7.41±0.01	13.53±0.70	4.62±0.33	7.87±0.93	3.20±0.84
HS	7.41±0.01	12.78±0.43	4.21±0.14	7.75±0.93	3.04±0.30
WD					
C	7.38±0.02	13.82±0.22	4.53±0.21	8.66±0.87	3.61±0.55
HS	7.38±0.01	13.50±0.35	4.32±0.10	8.20±0.68	3.65±0.50

WD, water deprivation for 24 hours; C, Control; HS, High Sucrose.