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High salt exacerbates programmed hypertension in maternal fructose-fed male offspring

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Salt

Abstract *Background and aims:* Consumption of food and drinks containing high fructose (HF), which is associated with hypertension, is increasing steeply. Moreover, increased salt intake significantly increases hypertension risk. We examined whether maternal HF and postnatal high salt (HS) intake had synergistic effects on blood pressure (BP) elevation in adult offspring and determined the underlying mechanisms.
Methods and results: Pregnant Sprague-Dawley rats received regular chow or chow supplemented with 60% fructose during the entire pregnancy and lactation periods. Half of the male offspring received 1% NaCl in drinking water from weaning to 3 months of age. Male offspring were assigned to 4 groups (control, HF, HS, and HF + HS) and were sacrificed at 12 weeks of age. Offspring in HF and HS groups developed hypertension, indicating that HF and HS synergistically increased BP. Postnatal HS intake increased *Ace* expression and decreased *Agtr1b* and *Mas1* expression in the kidneys. Renal mRNA levels of *Ace* and *Agtr1a* were significantly higher in HF + HS group than in control group. Renal levels of Na–K–2Cl cotransporter, type 3 sodium hydrogen exchanger, and Na^+/Cl^- cotransporter were higher in HS and HF + HS groups than in control group.
Conclusion: Postnatal HS intake exacerbated prenatal HF-induced programmed hypertension. HF and HS induced programmed hypertension by differentially inducing renin-angiotensin system and sodium transporters in the kidneys. Better understanding of the effect of the relationship between HF and HS on hypertension development will help prevent hypertension in mothers and children exposed to HF and HS.

Introduction

Hypertension can develop during early life. Maternal and postnatal insults lead to various adult diseases through a process called developmental programming [1]. The developing kidney is particularly vulnerable to insults during early life, which can lead to permanent morphological changes and functional adaptations, i.e., renal programming [2,3]. Several mechanisms are proposed to be involved in renal programming and programmed

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hypertension, including nitric oxide (NO) deficiency, oxidative stress, epigenetic regulation, alteration of renin–angiotensin system (RAS) and sodium transporters, and reduction in nephron numbers [4]. However, it is unclear whether various maternal and postnatal suboptimal conditions involve common genes and pathways that induce programmed hypertension.

Maternal and postnatal insults may independently or synergistically contribute to renal programming and programmed hypertension. Our previous studies showed that maternal high fructose (HF) intake induced renal programming and hypertension in adult male offspring [5,6]. Maternal melatonin therapy decreased HF-induced programmed hypertension, which was associated with increased L-arginine-to-asymmetric dimethylarginine (ADMA, an NO synthase inhibitor) ratio and NO levels in the kidneys.

The change in eating habit of modern society increases significantly the consumption of food and drinks containing HF, thus increasing the prevalence of metabolic syndrome and associated comorbidities, including hypertension [7]. In addition, our diet contains unbalanced nutrients, mainly salts. Increased salt intake in early life significantly increases hypertension risk during adulthood [8]. High salt (HS) intake induces and exacerbates programmed hypertension in the offspring of dams fed a low-protein diet [9,10]. Our previous study showed that HS intake, which increases oxidative stress, aggravates hypertension and kidney damage in spontaneously hypertensive rats [11]. Given that hypertension is a multifactorial disorder, that maternal and postnatal insults may have additive effects on developmental programming, and that HF and HS intake independently causes hypertension, we examined whether maternal HF and postnatal HS intake exerted synergistic effects on blood pressure (BP) elevation in adult offspring.

Methods

Experimental design

This study was approved and was performed according to the Guidelines for Animal Experiments of Chang Gung Memorial Hospital and Chang Gung University. Experimental animals were treated according to the guidelines of the US National Institutes of Health. Virgin Sprague-Dawley (SD) rats (age, 12–16 weeks) obtained from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan) were housed in a facility accredited to the Association for Assessment and Accreditation of Laboratory Animal Care International. The rats were exposed to 12-/12-h light/dark cycle. Male SD rats were caged with female rats until mating was confirmed by examining vaginal plug.

Pregnant SD rats received regular chow or chow supplemented with 60% fructose during the entire pregnancy and lactation periods [5]. Male offspring were assigned to 4 groups (N = 8–10/group): control, HF, HS, and HF + HS. Male offspring in HS group received NaCl (1%) in drinking water from weaning to 3 months of age. NaCl dose used in this study was based on that used in our previous study [11]. BP was measured in conscious rats at 3, 4, 6, 8, 10, and 12 weeks of age by using an indirect tail-cuff method (BP-2000; Visitech Systems, Inc., Apex, NC, USA) [5]. To ensure accuracy and reproducibility, the rats were acclimated to restraint and tail-cuff inflation for 1 week before the experiment. Three stable measurements were obtained and were averaged. The rats were sacrificed at 12 weeks of age. Heparinized blood samples were collected, and the kidneys were harvested and stored at −80 °C.

Detection of L-arginine, L-citrulline, and dimethylarginines by performing HPLC

Plasma L-arginine, L-citrulline, ADMA, and symmetric dimethylarginine (SDMA, a stereoisomer of ADMA) levels were measured using HPLC (HP Series 1100; Agilent Technologies, Inc., Santa Clara, CA, USA) with *o*-phthaldialdehyde/3-mercaptopropionic acid derivatization reagent [12]. Concentrations of L-arginine, L-citrulline, ADMA, and SDMA in the standards were in the range of 1–100, 1–100, 0.5–5, and 0.5–5 μM, respectively.

Quantitative real-time polymerase chain reaction

RNA was extracted using TRIzol reagent, treated with DNase I (Ambion, Austin, TX, USA) to remove DNA contamination, and reverse transcribed using random primers (Invitrogen, Carlsbad, CA, USA). Control RT reactions were performed by omitting RT enzyme. RNA concentration and quality were checked by measuring optical density at 260 and 280 nm. Complementary DNA was synthesized using M-MLV Reverse Transcriptase (Invitrogen). Two-step quantitative real-time polymerase chain reaction (PCR) was performed using QuantiTect SYBR Green PCR Kit (Qiagen, Valencia, CA, USA) and iCycler iQ Multi-Color Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Expression of RAS components, including angiotensinogen (*Agt*), angiotensin-converting enzyme (*Ace*), *Ace2*, angiotensin II type 1 (*Agtr1a*) and 2 receptor (*Agtr1b*), and angiotensin (1–7) MAS receptor (*Mas1*), was analyzed. Expression of 4 sodium transporters, namely, Na–K–2Cl cotransporter (*Slc12a1*), type 3 sodium hydrogen exchanger type 3 (*Slc9a3*), Na^+/Cl^- cotransporter (*Slc12a3*), and Na^+/K^+-ATPase α1 subunit (*Atp1a1*), was analyzed. Serum and glucocorticoid-inducible kinase 1 (*Sgk1*), a downstream signal of glucocorticoid receptor, was analyzed because it is proposed to participate in fetal programming of hypertension by regulating several sodium transporters [13]. The 18S rRNA gene (*Rn18s*) was used as a reference. Sequences of primers used in this study are provided in Supplementary Table 1. Primer efficiency between 1.8 and 2.2 was acceptable. All the samples were examined in duplicate. Comparative threshold cycle (C_T) method was used to quantify relative gene expression. ΔC_T of each sample was calculated by subtracting its average C_T value from the corresponding average value of *Rn18s*. $\Delta\Delta C_T$ was calculated by subtracting the average control ΔC_T value from the average experimental ΔC_T