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High sucrose intake during gestation increases angiotensin II type 1 receptor-mediated vascular contractility associated with epigenetic alterations in aged offspring rats

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ABSTRACT

Accruing evidence have confirmed that the fetal programming in response to adverse environmental in utero factors plays essential roles in the pathogenesis of hypertension in later life. High sugar intake has been accepted worldwide in everyday life diet and becomes the critical public health issue. Our previous studies indicated that intake of high sucrose (HS) during pregnancy could change the vascular reactivity and dipsogenic behavior closely associated with abnormal renin-angiotensin system (RAS), to increase the risk of hypertension in adult offspring. In the present study, we tested the hypothesis that maternal HS intake in pregnancy may further deteriorate the Ang II-induced cardiovascular responses in the aged offspring. HS intake was provided to pregnant rats throughout the gestation. Blood pressure (BP) in conscious state and vascular contractility in vitro were measured in 22-month-old aged offspring rats. In addition, mRNA and protein expressions and epigenetic changes of Ang II type 1 receptor (AT_1R) gene in blood vessels were determined with the methods of real-time RT-PCR, Western blotting, and Chromatin Immunoprecipitation Assay (CHIP). Results showed that, in the aged offspring, maternal HS intake during gestation would cause the elevation of basal BP which could be diminished by losartan. Although the circulatory Ang II was not changed, levels of local Ang II were significantly increased in blood vessels. In addition, prenatal HS exposure would significantly enhance the AT_1R-mediated vasoconstrictions in both aorta and mesenteric arteries of the aged offspring. Moreover, in the aged offspring of prenatal HS exposure, mRNA and protein expressions of AT_1R gene in both large and small blood vessels were significantly increased, which should be closely associated with the changes of epigenetic mechanisms such as histone modifications. Collectively, we proposed that maternal HS intake during gestation would cause abnormal BP responses mediated via the enhancement of vascular RAS, together with the increased expression of AT_1R gene related to the its epigenetic changes, which would actually lead to the overt phenotype of hypertension in the aged offspring.

1. Introduction

Since the development of modern food industry, the over-processed food with high energy and high sugar has been accepted worldwide in everyday life diet. As a consequence, over intake of sugar during pregnancy has become the critical public health issue [9,16,50]. Indeed, growing epidemiologic evidence suggested that maternal consumption of high sugar not only may cause metabolic or cardiovascular problems in pregnant women, but also could exert various effects on fetal development and postnatal health [6,41]. Our previous studies demonstrated that intake of high sucrose (HS) during pregnancy could change the vessel tone associated with the altered reactivity of certain ion channels, as well as the dipsogenic behavior tightly related to the body fluid homeostasis in adult offspring [25,44,45]. Accordingly, although the basal blood pressure (BP) was not elevated, we proposed that prenatal HS exposure might lead to the increased risk of hypertension in adult offspring. However, it still lacks information that whether the altered cardiovascular responses induced by prenatal HS expo-

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sure would exacerbate over a long period of time, and even develop to the overt abnormal cardiovascular phenomena (e.g. basal BP) in the aged offspring approximately equivalent to two-thirds of the life span (i.e. more than 20 months in rodents).

Renin-angiotensin system (RAS), which serves either the systemic endocrine or local paracrine/autocrine functions, plays both beneficial and detrimental effects on cardiovascular and body fluid homeostasis [12]. The most important bioactive peptide of RAS, angiotensin II (Ang II), exerts crucial roles in regulating cardiovascular system such as vascular tone in physiological status, as well as in the pathogenesis of hypertension and other cardiovascular dysfunctions, mediating by targeting virtually all organs/tissues, including heart, vasculature, kidney and brain [28,31]. Moreover, our and other researchers' studies implicated that enhancement of Ang II sensitivity for regulating vascular contractility plays fundamental roles in the increased risk of hypertension in adult offspring induced by various prenatal insults such as nicotine and hypoxia [5,17]. It is widely believed that the majority of the physiological as well as the pathological roles by Ang II for controlling vascular contractility is largely mediated via its interaction with the G-protein coupled receptor termed as Ang II type 1 receptor (AT_1R) [13]. In fact, experimental studies indicated that inhibition of AT_1R in early postnatal life after exposure to the maternal malnutrition during pregnancy could prevent the development of hypertension in adult offspring [14]. Our previous research revealed that prenatal HS exposure could cause the prominent increase of vasoconstriction in response to Ang II mediated by AT_1R in the adult offspring [25]. However, whether and to what extent the AT_1R-mediated vascular contractility would be programmed in the aged offspring exposure to maternal HS intake during gestation is still little concerned.

Notably, emerging studies have revealed that epigenetic mechanisms, including DNA methylation, posttranslational histone modifications and non-coding RNAs, which serve as the biological processes able to change gene expression in the heritable manner without altering the DNA sequence, function as the essential biological underpinnings to explicitly reprogram the expression of certain molecules in the offspring exposure to various abnormal stimuli during prenatal period [43]. In fact, accruing evidence indicated that epigenetic variations of certain RAS genes in the offspring has emerged as the candidate to increase the risk of hypertension induced by abnormal early life environmental factors [2]. Particularly, previous studies showed that prenatal nicotine exposure could prominently change the DNA methylation in the promoter region of AT_1R gene, which was closely associated with the increased vascular reactivity in the adult offspring [46]. As such, whether the altered AT_1R-mediated vascular contractility might be related to the changes of epigenetic mechanisms in the aged offspring exposure to maternal HS intake in pregnancy has to be further elucidated.

Together, in this study, using the experimental animal model of pregnant SD rats, we designed to test the hypothesis that maternal HS intake during gestation may cause the epigenetic changes of AT_1R gene, leading to enhance the down-stream vasoconstriction signaling, so as to elevate BP in the aged offspring. The specific aim of the present study was to detect whether and to what extent prenatal HS exposure may exert impacts on (1) basal BP and systemic/local Ang II levels; (2) AT_1R-mediated vascular contractile function; (3) mRNA and protein expressions of vascular AT_1R gene; and (4) epigenetic modifications in promoter region of vascular AT_1R gene in the aged offspring rats. As such, the cardiovascular consequences in later life but not only the risk of hypertension in the offspring induced by prenatal HS exposure would be tentatively evaluated. Additionally, the molecular underpinnings of altered vascular AT_1R gene expression affecting fetal-programmed hypertension would be preliminarily investigated so as to provide the potential target to prevent or correct the disturbed cardiovascular homeostasis in the offspring.

2. Materials and methods

2.1. Experimental animals and drug administration

Pregnant Sprague-Dawley (SD) rats (five-month-old) weighing 200–250 g were supplied by the Laboratory Animal Research Center of Soochow University. They were maintained under standard conditions for at least one week, and individually housed in plastic cages in an animal room under temperature-controlled environment of 22–25 °C with a 12 h light/dark cycle and access to commercial pellets and water ad libitum. Animals were randomly assigned into two groups, and the following experiments were performed in a double-blind manner. Control group (Con) 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 to day 21 of gestation. After delivery, male pups of both groups were selected at weaning and were given fresh tap water and standard rat food for 22 months as the aged offspring rats and for 5 months as the adult offspring rats. All procedures were approved by Soochow University Animal Ethics Committee and conformed to the Guide for the Care and Use of Laboratory Animals.

2.2. Measurement of blood biochemical indexes

At 22 months, the aged offspring rats were sacrificed by using 3% sodium pentobarbital (50 mg/kg). And then, 2 ml blood samples were collected via the abdominal aorta into iced tubes containing lithium heparin as the anticoagulant. Arterial blood pH, hematocrit (Hct), concentrations of glucose and lactate, and concentrations of sodium and potassium were determined using the Nova eleven-electrode analyzer (Nova Biomedical, Waltham, MA). The remaining blood was centrifuged at $2000 \times g$ for 10 min to remove the erythrocytes, leukocytes, and platelets, and the plasma (20 μl) was used for measuring osmolality by advanced diagnostic Osmometer (Model MO, Advanced Instruments, Needham Heights, MA). Assay and data processing were handled in a blinded manner.

2.3. Measurement for levels of Ang II in plasma and vessel tissues

At 22 months, the aged offspring rats were sacrificed using 3% sodium pentobarbital (50 mg/kg). And then, 2 ml blood samples were collected via the abdominal aorta into iced tubes containing EDTA as the anticoagulant. The plasma was prepared by centrifugation at $2000 \times g$ for 10 min to remove the erythrocytes, leukocytes, and platelets. The plasma was aliquoted and frozen at −80 °C until needed. Meanwhile, the tissues of aorta and mesenteric arteries of the aged offspring rat were collected into chilled plastic tubes containing EDTA-Na_2, 8-hydroxyguinoline and dimercaptopropanol. Following boiling in the water of 100 °C for 10 min, the tissue homogenate of vessels was then undergone by centrifugation at $2200 \times g$ for 10 min to obtain the supernatant. The supernatant was aliquoted and frozen at −80 °C until needed. By the commercially available kits (SINO-UK Inst Bio Tech., Beijing), the levels of Ang II in plasma and tissues of aorta and mesenteric arteries were detected by the routine radioimmunoassay, which had sensitivity 0.01 ng/ml for Angiotensin II (Ang II). The intra- and inter-assay coefficients of variation were 10.5-12.0%. Assay and data processing were handled in a blinded manner.