Reproductive Toxicology 71 (2017) 150–158

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Prenatal caffeine exposure induced high susceptibility to metabolic syndrome in adult female offspring rats and its underlying mechanisms

Lin-guo Pei[a,c], Chao Yuan[a], Yi-tian Guo[a], Hao Kou[a,b], Li-ping Xia[a,b], Li Zhang[a], You-e Yan[a], Dan Xu[a,b], Hui Wang[a,b,*]

[a] Department of Pharmacology, Basic Medical School of Wuhan University, Wuhan, China
[b] Hubei Provincial Key Laboratory of Developmentally Originated Disease, Wuhan, China
[c] Basic Medical College of Nanyang Medical University, Nanyang 473061, China

ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form 2 June 2017
Accepted 13 June 2017
Available online 15 June 2017

Keywords:
Prenatal caffeine exposure
Metabolic syndrome
Catch-up growth
Insulin resistance

ABSTRACT

Our previous studies have demonstrated that prenatal caffeine exposure (PCE) induced an intrauterine programming of hypothalamic–pituitary–adrenal axis (HPAA)-associated neuroendocrine metabolism in 3-month-old offspring rats. In this study, we aimed to confirm this programming disorder and high susceptibility to metabolic syndrome (MS) in 10-month-old female PCE offspring with postnatal catch-up growth. We found that PCE female offspring rats showed decreased bodyweight but a higher rate of weight gain after birth. Moreover, in the offspring, basal hyperinsulinemia and insulin resistance were observed before unpredictable chronic stress (UCS), but serum total cholesterol (TCH) levels and triglyceride/high-density lipoprotein–cholesterol (TG/HDL-C), TCH/HDL-C and low-density lipoprotein–cholesterol/HDL-C (LDL-C/HDL-C) ratio changes were increased after UCS, accompanied by morphological damage of the related tissues. These results suggested that PCE adult female offspring rats were highly susceptible to MS, which is related to HPAA-associated neuroendocrine-metabolic programming disorder.

1. Introduction

Metabolic syndrome (MS) represents a cluster of metabolic risk factors, which can be defined as the presence of three or more metabolic disorders such as central obesity, hypertension and dyslipidaemia, with either increased triglyceride (TG) levels or decreased high-density lipoprotein-cholesterol (HDL-C) levels, and glucose intolerance [1,2]. Insulin resistance (IR) is a key event underlying the occurrence of MS [3]. Epidemiological and animal studies [4–6] have shown that an adverse intrauterine environment contribute to a high susceptibility to MS. Barker et al. also suggested that an increased incidence of MS in adulthood was associated with low birth weight, which may be related to the theory of Developmental Origins of Adult Disease [7–9]. These above statements suggest that MS has a developmental origin. Intrauterine programming alterations [10–12] could be used to explain the relationships between an adverse environment during pregnancy and foetal growth and development [13]. A series of reports have indicated that an adverse intrauterine environment resulted in foetal over-exposure to maternal glucocorticoid, which may be the primary initiating factor for intrauterine programming alterations [14,15]. An adverse intrauterine environment could cause the abnormal development of foetal hypothalamic–pituitary–adrenal axis (HPAA) and other endocrine axes as well as changes in peripheral tissue metabolism.

Caffeine is a xanthine alkaloid, and its global consumption has been increasing yearly, including widespread use in pregnant women [16]. Studies have shown that prenatal caffeine exposure (PCE) could cause a variety of adverse pregnancy outcomes, including intrauterine growth retardation (IUGR) [17,18]. In our previous reports [19–21], PCE induced an intrauterine programming of HPAA-associated neuroendocrine metabolism in 3-month-old offspring rats; this was associated with intrauterine maternal glucocorticoid over-exposure caused by the downregu-

Abbreviations: MS, metabolic syndrome; IR, insulin resistance; PCE, prenatal caffeine exposure; HPAA, hypothalamic-pituitary-adrenal axis; ACTH, adrenocorticotropic-releasing hormone; CORT, corticosterone; TG, triglyceride; TCH, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; GD, gestational day; PW, postnatal week; UCS, unpredictable chronic stress; OGTT, oral glucose tolerance test.

* Corresponding author at: Department of Pharmacology, Basic Medical School of Wuhan University, Wuhan 430071, China.
E-mail address: wanghui19@whu.edu.cn (H. Wang).

http://dx.doi.org/10.1016/j.reprotox.2017.06.045
0890-6238/

lated expression of placental 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2). High circulating glucocorticoid levels could inhibit foetal HPAA development and alter glucose and lipid metabolic function in multiple foetal tissues. These effects may be sustained after birth and manifested as low basal activity and high sensitivity to chronic stress of HPAA, accompanied by glucocorticoid-dependent glucose and lipid metabolic changes. Moreover, this HPAA-associated neuroendocrine-metabolic programming by PCE was observed in the second generation [22]. However, it remains unclear whether PCE could induce susceptibility to MS in the adult offspring or whether it was related to the HPAA-associated neuroendocrine-metabolic programming disorder.

"Catch-up growth" of bodyweight after birth has been proposed in several nutrition-sufficient conditions, including high-fat diet (HFD), which is becoming increasingly common in many countries. Additionally, high sensitivity of the HPAA to external stimuli could also aggravate glucose and lipid metabolic disorders and accelerate the occurrence of adult MS [23]. Some studies from other laboratories have indicated that the influence of maternal glucocorticoid on HPAA of female offspring may be greater than that of male offspring [24,25]. Therefore, HFD, stress stimuli and gender are important inducible factors of MS. In the present study, a post-weaning HFD as an over-nutrition condition was administered to both control and PCE groups to simulate catch-up growth, and unpredictable chronic stress (UCS) was applied to induce high glucocorticoid levels to confirm the dependent relationship between glucocorticoid and glucose and lipid metabolism. We aimed to confirm the HPAA-associated neuroendocrine metabolic programming disorder and high susceptibility to MS in 10-month-old PCE female offspring rats with postnatal catch-up growth. This study provided an experimental basis for explaining the development and progression of foetal originated MS.

2. Materials and methods

2.1. Main reagents

Caffeine (CAS # 58-08-2) was purchased from Sigma-Aldrich Corporation (St Louis, MO, USA). The rat adrenocorticotropic-releasing hormone (ACTH) kit was purchased from the Beijing North Institute of Biological Technology (Beijing, China). The rat corticosterone (CORT) kit was purchased from R & D Systems, Inc. (Minneapolis, MN, USA). The insulin enzyme-linked immunosorbent assay (ELISA) kit was purchased from Mercodia AB (Uppsala, Sweden). The glucose oxidase assay kit was purchased from Shanghai Ming-dian Biological Engineering Co., Ltd (Shanghai, China). TG and total cholesterol (TCH) quantification kits were purchased from Sangon Biotech Co., Ltd (Shanghai, China). HDL-C and low-density lipoprotein-cholesterol (LDL-C) assay kits were purchased from Zhejiang Chuangye Biological Co., Ltd (Zhejiang, China).

2.2. Animal procedures

Specific-pathogen-free (SPF) Wistar rats (females weighing 200–240 g and males weighing 260–300 g) were purchased from the Experimental Animal Centre of the Hubei Academy of Preventive Medical Sciences (Certificate of Conformity: No. 2008-0005, Hubei province, China).

Animal experiments were performed in the Centre for Animal Experiment of Wuhan University (Wuhan, China), which has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). The Committee on the Ethics of Animal Experiments of the Wuhan University School of Medicine approved the protocol (permit number: 14016). All animal experimental procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Chinese Animal Welfare Committee. The animals were housed in metal cages with wire-mesh floors in an air-conditioned room under standard conditions (room temperature: 18–22 °C; humidity: 40–60%; light cycle: 12 h light-dark cycle; 10–15 air changes per hour) and allowed free access to rat chow and tap water.

Time schedule of animal experiment was shown in Fig. 1. All rats were acclimated for one week before experimentation, and two female rats were placed together with one male rat overnight in a cage for mating. The appearance of sperm in vaginal smears confirmed mating, and the day of mating was taken as gestational day (GD) 0. Pregnant females were randomly divided into the control and PCE groups with each group containing 8 rats. Starting from GD11, the PCE group was given a daily gavage administration of 120 mg/kg of caffeine [21] until the day that the rats went into labour. The control group was administered an equal volume of distilled water.

The day of litter delivery was designated postnatal week (PW) 0, and litters with 8–14 pups were included in the experiment (the number of male and female pups was more than or equal to 4). To ensure adequate and relatively equal intake of nutrients during the suckling period, we randomly reduced the amount of pups to 8 per litter (4 male pups and 4 female pups). Beginning at PW1, the pups were weighed every 4 weeks. The rate of bodyweight gain was calculated using the formula: the rate of bodyweight gain (%) = (PW_{χ} bodyweight − PW_1 bodyweight) × 100/PW_1 bodyweight. After weaning at PW4, one female rat was randomly selected from each litter in the control and PCE groups (8 female pups from the control group and 8 female pups from the PCE group). The female rats in the control and PCE groups were fed an HFD [26] containing 88.0% corn flour, 11.5% lard and 0.5% cholesterol until PW24, which provided 18.9% kcal from protein, 61.7% kcal from carbohydrates and 19.4% kcal from fat. The female rats were then given a normal diet until PW40, which was purchased from the Hubei Medical and Scientific Academy and provided 21% kcal from protein, 68.5% kcal from carbohydrates and 10.5% kcal from fat.

The offspring rats in the control and PCE groups were exposed to a two-week UCS [26] from PW38 to PW40. One week before UCS and 24 h after UCS, the rats were subjected to blood collection and oral glucose tolerance test (OGTT) [26]. The animals were fasted overnight, and then the blood samples were drawn from caudal vein from 8:30 a.m. to 10:30 a.m. due to the circadian rhythm of HPA axis [26–28], in order to detect the levels of ACTH, CORT, glucose, insulin, TG, TCH, LDL-C and HDL-C. The insulin resistance index (IRI) was calculated according to the following formula [29,30]: IRI = fasting serum insulin level × fasting serum glucose level/22.5. On the third day after the last exposure to UCS, the rats were sacrificed by isoflurane anaesthesia. Adrenal gland (right side), pancreatic and liver tissues were rapidly removed, fixed with paraformaldehyde (n = 5) and subjected to haematoxylin-eosin (HE) staining.

2.3. OGTT

OGTT was conducted as described previously [26]. Rats were fasted overnight (12 h) and then given a glucose solution (2 g/kg) by gavage administration. Blood samples were collected from the caudal veins prior to (0 min) and at 30, 60 and 120 min after the gavage administration of the glucose solution. Serum glucose levels were examined at each time point. A serum glucose curve was constructed, and the area under the curve (AUC) was calculated. To avoid individual differences in fasting (0 min) serum glucose levels, the glucose level of a rat at each OGTT time point was expressed as a percentage relative to its glucose level at 0 min. Glucose levels