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Maternal low-protein diet induces changes in the cardiovascular autonomic modulation in male rat offspring

M.A.V. Barros [a], J.L. De Brito Alves [a], V.O. Nogueira [a], A.G. Wanderley [b], J.H. Costa-Silva [a,*]

[a] Department of Physical Education and Sport Sciences, Academic Center of Vitoria (CAV), Federal University of Pernambuco, 55608-680, Brazil
[b] Department of Physiology and Pharmacology, Federal University of Pernambuco, Brazil

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Abstract *Background and aims:* Maternal undernutrition induces development of the arterial hypertension. We investigated the effects of a maternal low-protein diet on cardiovascular autonomic control in the offspring.
Methods and results: Male Wistar rats were divided into two groups according to the diets of their mothers during gestation and lactation: the control (normal protein, NP, 17% casein; $n = 14$) and low-protein (LP, 8% casein; $n = 14$) groups. Direct measurements of arterial pressure (AP) were recorded from wakeful 90-day-old male offspring. The LP offspring presented higher mean AP than did the NP rats (NP: 93 ± 4 vs. LP: 113 ± 2 mmHg; $p < 0.05$), whereas the heart rate (HR) was similar in the two groups. In the spectral analysis, the LP group showed higher power at low (NP: 1.98 ± 0.25 vs. LP: 3.7 ± 0.3 $mmHg^2$; $p < 0.05$) and high (NP: 1.28 ± 0.18 vs. LP: 2.13 ± 0.42 $mmHg^2$; $p < 0.05$) frequencies of systolic arterial pressure (SAP). In the pulse interval, the LP group presented an increase in the LF/HF ratio (NP: 0.32 vs. LP: 0.56; $p < 0.05$). After propranolol (4 mg/kg, intravenous (iv)), the bradycardia was higher in the LP group (NP: −36 ± 8 vs. LP: −94 ± 12 bpm; $p < 0.05$), after methylatropine (2 mg/kg, iv), the tachycardia was similar to NP group. After administration of the ganglionic blocker (hexamethonium; 25 mg/kg, iv), the LP animals showed larger delta variation in the AP (NP: −33.7 ± 5 vs. LP: −53.6 ± 4 mmHg; $p < 0.05$).
Conclusion: The rats subjected to protein malnutrition presented an increase in the cardiovascular sympathetic tone, which contributed to the elevated AP observed in these animals.

Introduction

Epidemiological and experimental studies have shown that malnutrition that occurs during gestation and lactation is an important predisposing factor for the development of non-communicable diseases of the offspring in adulthood [1–4]. In rats, studies have shown that the consumption of a low-protein (LP) diet during the pre- or postnatal periods induces changes in cardiovascular function and predisposes the offspring to the development of hypertension [5,6].

Although there are several studies showing the relationship between malnutrition in early life and hypertension in adulthood, the mechanisms involved in this process are not yet well understood. We recently demonstrated that protein restriction during gestation and

* Corresponding author. Núcleo de Educação Física e Ciências do Esporte – CAV – UFPE, Rua Alta do Reservatório, S/N, Bela Vista, Vitória de Santo Antão, PE, CEP 55608-680, Brazil. Tel./fax: +55 81 35233351.
E-mail addresses: joao.hcsilva@ufpe.br, joaohenriq@hotmail.com (J. H. Costa-Silva).

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lactation leads to increased arterial pressure (AP) after 90 days of life in the offspring, and this increase might be due to changes in the respiratory system during early life. [2] The role of cardiovascular autonomic modulation was not considered or studied in that work, but it is has been described as an important factor in the development of other hypertension models [7,8].

AP control is a complex mixture of hormonal, neural and intrinsic factors, which all act on different time scales and with different means of control. The action of these factors on the cardiovascular system promotes spontaneous oscillations, named a high-frequency (HF) oscillation related to respiration and associated with cardiac vagal modulation and a low-frequency (LF) oscillation related to cardiac and vasomotor sympathetic modulation of the heart and blood vessels [9,10]. Experimental studies have shown that spontaneously hypertensive rats (SHR) exhibited an increase in the LF band of the AP and a high LF/HF ratio, which could contribute to sympathetic overactivity and the development of hypertension [11].

In the present study, we investigated long-term effects of the maternal low-protein diet on cardiovascular autonomic modulation of the adult male offspring. To understand the importance of the autonomic nervous system in the maintenance of AP, we hypothesised that rats from dams subjected to protein undernutrition during gestation and lactation could exhibit sympathetic overactivity, which could lead to the development of arterial hypertension in this experimental model.

Methods

Animals and diets

Wistar rats were used and all experimental protocols were approved by the Ethical Committee of the Federal University of Pernambuco, Brazil. Two groups were formed based on dietary manipulations: mothers fed a 17% casein diet (normal-protein (NP) group, $n = 6$) and mothers fed an 8% casein isoenergetic diet (LP group, $n = 6$) ad libitum. During the suckling period, the mothers continued to be provided with the experimental diet of either 8% or 17% of casein. The diets were made according to the American Institute of Nutrition [2]. At weaning, the male offspring received a standard diet (Labina; Purina Agriband Sao Paulo, Brazil) and water ad libitum until they were 90 days old. The experimental groups were formed with two or three rats from each mother (NP offspring, $n = 14$; LP offspring, $n = 14$).

Measurement of arterial pressure and heart rate

Male Wistar rats at 90 days were anaesthetised with ketamine (80 mg/kg) and xylazine (10 mg/kg) to insert femoral artery and vein catheters. After 18–24 h, the AP and heart rate (HR) were recorded in conscious animals by appropriate system (LabChart 7 Pro; ADInstruments, Bella Vista, NSW, Australia). Each animal was placed in the recording chamber for a period of acclimatisation (approximately 60 min). The pulsatile AP was recorded for 60 min under basal conditions, and the values of the mean arterial pressure (MAP) and HR were calculated by selection of the 10 min of this period.

Spectral and symbolic analyses of cardiovascular variability

The cardiovascular autonomic evaluation was performed using the frequency domain analysis of the systolic arterial pressure (SAP) and pulse interval (PI) by an appropriate software program (CardioSeries-v.2.4; www.danielpenteado.com). The spectra were integrated in the LF (0.2–0.75 Hz) and the HF bands (0.75–3 Hz). To assess the sympathovagal index, the LF/HF ratio of the variability was calculated. Moreover, the symbolic analysis was used, a non-linear method based on the conversion of the series into a sequence of symbols [12].

Evaluation of sympathovagal tonus and intrinsic HR

Methylatropine (2 mg/kg, iv) and propranolol (4 mg/kg, iv) were used to evaluate the parasympathetic and sympathetic tonus on HR, respectively. First, methylatropine was injected and the HR recorded during the next 15 min, then, the propranolol was injected, and the HR was recorded over 15 min. On the next day, this sequence was reverse. Only the first value of each sequence was considered. After each sequence, the intrinsic HR (iHR) was calculated [13].

Evaluation of sympathetic tonus on the vascular system

The sympathetic vascular tone was evaluated by an intravenous injection of hexamethonium (25 mg/kg, Sigma, St Louis, MO, USA) [14] and calculated by difference between the MAP after the blocker and the baseline MAP.

Spontaneous baroreflex sensitivity

In this study, the spontaneous baroreflex sensitivity was calculated through sequence method by computer software CardioSeries (v.2.4) [15].

Statistical analysis

The results were expressed as the mean ± the standard error of the mean and compared using Student's unpaired *t*-test, except for LF/HF ratio that was expressed as median and compared using the Mann–Whitney test. These tests were performed after analysis of data distribution (Kolmogorov–Smirnov and Shapiro–Wilk test). The comparisons were performed using GraphPad Prism software (GraphPad, v.5), and differences were considered significant at $p < 0.05$.