Nutrition, Metabolism & Cardiovascular Diseases (2011) 21, 808–816

available at www.sciencedirect.com

ScienceDirect

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

Nutrition, Metabolism & Cardiovascular Diseases

Obesity induced by neonatal treatment with monosodium glutamate impairs microvascular reactivity in adult rats: Role of NO and prostanoids

N.S. Lobato [a], F.P. Filgueira [a], E.H. Akamine [a], A.P.C. Davel [b], L.V. Rossoni [b], R.C. Tostes [a], M.H.C. Carvalho [a], Z.B. Fortes [a,*]

[a] Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo 05508-900, Brazil
[b] Department of Physiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

Received 3 August 2009; received in revised form 5 February 2010; accepted 8 February 2010

KEYWORDS
Obesity;
Monosodium glutamate;
Mesenteric arteriolar bed;
Nitric oxide;
Prostanoids;
Reactive oxygen species

Abstract *Background and aim:* given that obesity is an independent risk factor for the development of cardiovascular diseases we decided to investigate the mechanisms involved in microvascular dysfunction using a monosodium glutamate (MSG)-induced model of obesity, which allows us to work on both normotensive and normoglycemic conditions.
Methods and results: Male offspring of Wistar rats received MSG from the second to the sixth day after birth. Sixteen-week-old MSG rats displayed higher Lee index, fat accumulation, dyslipidemia and insulin resistance, with no alteration in glycemia and blood pressure. The effect of norepinephrine (NE), which was increased in MSG rats, was potentiated by L-nitro arginine methyl ester (L-NAME) or tetraethylammonium (TEA) and was reversed by indomethacin and NS-398. Sensitivity to acetylcholine (ACh), which was reduced in MSG rats, was further impaired by L-NAME or TEA, and was corrected by indomethacin, NS-398 and tetrahydrobiopterin (BH4). MSG rats displayed increased endothelium-independent relaxation to sodium nitroprusside. A reduced prostacyclin/tromboxane ratio was found in the mesenteric beds of MSG rats. Mesenteric arterioles of MSG rats also displayed reduced nitric oxide (NO) production along with increased reactive oxygen species (ROS) generation; these were corrected by BH4 and either L-NAME or superoxide dismutase, respectively. The protein expression of eNOS and cyclooxygenase (COX)-2 was increased in mesenteric arterioles from MSG rats.
Conclusion: Obesity/insulin resistance has a detrimental impact on vascular function. Reduced NO bioavailability and increased ROS generation from uncoupled eNOS and imbalanced release of COX products from COX-2 play a critical role in the development of these vascular alterations

* Corresponding author. Tel./fax: +55 11 3091 7317.
E-mail address: zbfortes@icb.usp.br (Z.B. Fortes).

0939-4753/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.numecd.2010.02.006

Introduction

The prevalence of obesity is rising to epidemic proportions not only in industrialized nations but also in developing nations [1,2]. Obesity is an independent risk factor for hypertension, coronary artery disease, stroke, and type 2 diabetes [3]. Endothelial dysfunction, which presents as an altered ability of the endothelium to maintain vascular homeostasis through the release of endothelium-derived relaxing factors (EDRFs) and endothelium-derived contracting factors (EDCFs), is present in obesity and may be crucial for the increased cardiovascular risk associated with this condition [4,5].

The monosodium glutamate (MSG)-induced obese rat is a model associated with insulin resistance and dyslipidemia that may occur without the presence of hypertension or type 2 diabetes, depending on the age at which the animals are studied [6–8]. The administration of MSG to newborn rats results in distinctive lesions in hypothalamic arcuate nucleus (ARC) neurons. The neuronal loss impairs insulin and leptin signaling and impacts energy balance as well as pituitary and adrenal activity. In contrast to other models of obesity, MSG-treated rats are characterized by increased plasma levels of corticosterone as well as increased lipogenesis and reduced lipolysis in the adipose tissue, despite their normophagia [9–13].

An understanding of the alterations associated with MSG-induced obesity is of great relevance because the ARC is among the principal sites that regulate energy homeostasis [14]. Although the endocrine, metabolic, and autonomic aspects of MSG-induced obesity have been extensively studied, the association between MSG and the development of vascular alterations is less understood. Therefore, we decided to investigate the mechanisms involved in alterations of vascular reactivity using this obesity model, which allows evaluation under normotensive and normoglycemic conditions [6–8]. The role of nitric oxide (NO), reactive oxygen species (ROS), endothelium-derived hyperpolarizing factor (EDHF), and cyclooxygenase (COX) pathways were investigated.

Methods

Animals

The investigation was approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of Sao Paulo (Protocol *n*°007/04) and conforms to the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996). Male Wistar rats received subcutaneous injections of MSG [4.0 g/kg body weight] dissolved in 0.9% NaCl or an equivalent volume of vehicle from the second to the sixth day after birth. Breeding conditions were followed as previously described [15].

At 16 weeks of age, the rats were weighed and placed in metabolic cages to measure water and food consumption. Blood samples were taken from the descending aorta after food deprivation (5 h) under sodium thiopental anesthesia (50 mg/kg, Cristália, Brazil). Glucose levels and lipid profiles were assessed spectrophotometrically (Celm, Brazil). Insulin levels were determined by radioimmunoassay (Linco, USA). The Homeostasis Model Assessment (HOMA-IR), an index of insulin resistance, was calculated using this equation: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5 [16]. Lee's obesity index was calculated as follows: body weight$^{1/3}$(g)/nasal–anal length(cm) × 100. White adipose tissue and lean mass were weighed.

The blood pressure (BP) was measured in unanesthetized animals by an indirect tail-cuff method (PowerLab 4/S, ADInstruments, Australia).

Intravenous insulin tolerance test

Tail blood samples were collected before and 4, 8, 12 and 16 min after an intravenous injection of regular insulin (0.75 U/kg b.w., Biobras, Brazil). The constant rate for blood glucose disappearance during the test (*k*ITT) was calculated based on the linear regression of the neperian logarithm of glucose concentrations.

Vascular reactivity in the perfused mesenteric arteriolar bed

The perfused mesenteric arteriolar bed (MAB) was prepared as previously described [17]. Under anesthesia, the abdominal cavity was opened and a polyethylene cannula was inserted into the superior mesenteric artery. The whole MAB was cut close to the intestinal border, transferred to

Table 1 Anthropometric and several metabolic characteristics in sixteen-week-old control and MSG-damaged Wistar rats.

Parameter	Control	MSG
Body weight (g)	386.6 ± 8.3	333.8 ± 7.8[a]
Lee index, (×100)	29.05 ± 0.22	30.43 ± 0.24[a]
Retroperitonial WAT, (g/100 g)	0.97 ± 0.07	2.81 ± 0.11[a]
Periepididymal WAT, (g/100 g)	1.16 ± 0.10	2.62 ± 0.15[a]
Soleus muscle, (g/100 g)	0.033 ± 0.003	0.036 ± 0.002
Extensor digitorum longus muscle, (g/100 g)	0.030 ± 0.002	0.032 ± 0.001
Food intake (g/100 g/day)	5.16 ± 0.31	5.24 ± 0.35
Water intake (mL/100 g/day)	8.3 ± 0.1	8.1 ± 0.2
Total Colesterol, (mg/dL)	71.6 ± 4.3	64.4 ± 3.2
Triacylglycerols, (mg/dL)	48.8 ± 11.6	123.8 ± 18.3[a]
LDL-cholesterol, (mg/dL)	24.3 ± 3.7	38.2 ± 5.8[a]
Glucose, (mg/dL)	116.0 ± 3.8	115.8 ± 3.0
Insulin, (ng/mL)	2.1 ± 0.33	3.5 ± 0.34[a]
*k*ITT, (%/min)	4.2 ± 0.20	2.8 ± 0.30[a]
HOMA-IR index	13.8 ± 2.3	25.5 ± 2.8[a]
Blood pressure, (mmHg)	113.2 ± 1.5	108.0 ± 2.7

Values are mean ± SEM; WAT, white adipose tissue; LDL, low density lipoprotein; *k*ITT, constant rate for blood glucose disappearance; HOMA-IR, homeostasis model assessment-insulin resistance.

[a] $P < 0.05$ vs. control. $N = 10–12$/group.