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Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat

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Abstract

Growing evidences suggest a role of oxidative stress in hypertension and cardiac hypertrophy. The fructose (60%)-fed rat represents a model of metabolic syndrome, associating insulin resistance and high blood pressure. In this model, hypertension, cardiac and vessels hypertrophy and markers of oxidative stress were determined. In addition, the production of reactive oxygen species (ROS) was evaluated at different times after the initiation of fructose-enriched diet in aorta, heart and polymorphonuclear cells. High fructose feeding was associated with an early (1-week) increase in ROS production by aorta, heart and circulatory polymorphonuclear cells, in association with enhanced markers of oxidative stress. Vascular and cardiac hypertrophy was also rapidly observed, while the rise in blood pressure was significant only after 3 weeks. In summary, our study suggests that the production of reactive oxygen species can be a key-event in the initiation and development of cardiovascular complications associated with insulin resistance.

Keywords: Oxidative stress; NADPH oxidase; Insulin-resistance; Cardiovascular remodeling; Hypertension; Fructose-fed rat

1. Introduction

There are considerable evidences suggesting that insulin resistance is associated with the development of cardiovascular diseases including atherosclerosis and hypertension [1,2]. Recently, the role of oxidative stress in the development of atherosclerosis in the insulin resistance syndrome (IRS) has been evoked [3]. Major components of IRS (insulin resistance, hypertension, dyslipidemia) generate oxidative stress in response to an overproduction of superoxide anion by the activation of NADPH oxidase. Recently, Inoguchi et al. [4] showed that free fatty acids (FFA) and elevated concentrations of glucose, were able to activate the NADPH oxidase from vascular (endothelial and smooth muscle) cells in a PKc-dependant mechanism. Similarly, Advanced Glycation End products (AGEs) resulting from interactions between glycation and oxidation, induced free radicals production through receptor-dependant NADPH oxidase activation [5]. Hypertension per se, is also associated to NADPH oxidase activation. Many studies on animal models of hypertension including angiotensin II-induced hypertension [6,7], DOCA-Salt hypertensive rat [8], spontaneous hypertensive rat (SHR) [9] and clinical studies [10] have shown an overproduction of reactive oxygen species. Moreover, the inhibition of NADPH by apocynin or the inactivation of superoxide anion by scavengers (tempol) lowers elevated blood pressure [8,11].

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High-fructose feeding promotes insulin resistance by alterations of enzymes that regulate hepatic carbohydrates metabolism, including decreased glucokinase and increased glucose-6-phosphatase activities. In many ways, fructose-fed rats display the metabolic changes observed in the human polymetabolic syndrome, including dyslipidemia characterized by enhanced plasma triglyceride levels [12] and moderate hypertension [13]. Despite the lack of obesity, enlarged adipose tissue associated with increased adipocyte size, which are in part prevented by metformin, has been recently reported suggesting fat redistribution [14,15]. In addition, oxidative stress parameters have been observed in this model including defects in enzymatic antioxidant mechanism, increase in TBARS concentrations [16] and aortic NADPH oxidase activation [17]. However, the link between oxidative stress and cardiovascular complications has not been documented in this model.

This study was designed to evaluate the involvement of tissue and systemic reactive oxygen species (ROS) production in the development of insulin resistance-induced cardiovascular complications in the fructose fed rats. Our data indicate that high fructose-induced insulin resistance is associated with an overproduction of superoxide anion by leukocytes, aorta and heart, and that high fructose feeding promotes cardiovascular alterations such as moderate hypertension, cardiac and vascular hypertrophy.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats (8-week-old) were provided by Charles River (l′Arbresle, France). They were housed (three animals/per cage) and allowed free access to water and food. Rats were randomly divided into two experimental groups, a control group (C, $n = 36$) receiving a regular chow and a high fructose fed group (HF, $n = 36$) receiving a chow supplemented with fructose (60%) during 42 days. Six animals of each groups were sacrified with sodium pentobarbital (60 mg/kg body weight, i.p.) at different times (D0, D7, D14, D21, D28 and D42).

2.2. Metabolic parameters and cardiovascular morphology

Food and water intake were recorded every day and weight of animals was recorded twice a week. Tail-cuff pressure was evaluated once a week using a Letica/Panlab (Barcelona, Spain) apparatus. At the end of the treatment period, blood was collected on heparin coated tubes and the thoracic aorta was immediately removed, cleaned of adherent fat, washed in an ice-cold KREBS buffer and kept at 4 °C until the measurement of superoxide anion production. The heart was removed and weighed for the calculation of heart to body weight ratio and left ventricle was used for the detection of superoxide anion production.

Plasma and buffy coat were obtained by low speed centrifugation. Buffy coat was immediately used to obtain polymorphonuclear leukocytes (PMNL) and plasma samples were stored at −20 °C until analysis. All procedures were designed in accordance with French law and institutional guidelines for the care and use of laboratory animals.

2.3. Histomorphometric study of arterial mesenteric system

Histomorphometric analysis of mesenteric arterial system was performed as previously described [18]. Briefly, the mesenteric vascular tree was collected by dissecting the superior mesenteric artery and its branches until their penetration into jejunum, immerged for 6 h in Bouin solution and kept in 70% ethanol until processed. Tissue samples were cut cross-way to the general direction of vessels, at the distal portion of jejunal arteries resulting from the division of the cranial mesenteric artery and embedded in paraplast (Histomed Standard, Labo Moderne, France); 3-μm-thick transverse sections, obtained at ≈5 mm from the distal end of the arteries, were stained with PAS for histomorphometric analysis in order to visualize elastic laminae.

Morphometric measurements were performed using the axioHOME® system (Carl Zeiss, Oberkochen, Germany). Briefly, the system consists of an IBM-PC compatible computer using the 2.04 version of the Zeiss-Alcatel TITN Answare software (Meylan, France) and a light microscope in which a built-in high resolution image is superimposed on the optical image of the specimen. Five to thirteen arteries (vessels showing several elastic laminae) per animal were quantified, for a total of 69–90 vessels per group. For each artery examined, the following parameters were measured: total vessel (T), media + lumen (ML) and lumen (L) areas. Adventitia (A) and media (M) areas were calculated as (T–ML) and (ML–L), respectively, assuming that intima area was negligible in the conditions of quantification. Media/lumen ratio was calculated for each sample as M/L.

2.4. Biochemical analysis and markers of oxidative stress

Plasma cholesterol and triglycerides were measured by an enzymatic method. Insulin was measured using a radioimmunoassay kit from Linco (St. Charles, MO). Plasma concentration of thiobarbituric acid reactive substances (TBARS, moles/L), taken as an index of lipid peroxidation, was estimated by fluorimetry [19]. Plasma concentration of advanced oxidation protein products (AOPP), considered as an index of protein oxidation, was measured by spectrophotometry (340 nm) using chloramine T and expressed as micromoles per liter of chloramine T equivalents [20,21].

Plasma concentration of Advanced Glycation End products (AGEs) was determined on 25-fold diluted (water)