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Insulin resistance due to chronic salt restriction is corrected by α and β blockade and by L-arginine

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Abstract

Dietary salt restriction is associated with evidence of low insulin sensitivity. The current study was undertaken to investigate whether sympathetic nervous system and L-arginine–nitric oxide pathway activities are linked to insulin resistance in rats under chronic low salt intake. Male Wistar rats were fed a low (LSD) or normal (NSD) salt diet from weaning to adulthood. A euglycemic hyperinsulinemic clamp was performed in 4 sub-groups on each diet: (1) sympathetic nervous system blockade (propranolol and prazosin), (2) vehicle, (3) L-arginine, and (4) D-arginine. Blood pressure, heart rate and metabolic measurements were done before and 45 min after drug infusion and at the end of the clamp. At baseline conditions, body weight, hematocrit, blood glucose, plasma insulin, cholesterol, and triacylglycerols were higher in LSD than in NSD rats. Systolic blood pressure was lower and heart rate was higher in rats on LSD than on NSD. Glucose uptake was lower on LSD compared to NSD. Sympathetic nervous system blockade and L-arginine did, and vehicle and D-arginine did not improve glucose uptake in LSD rats. On NSD there was no effect of any of the infused drugs. A positive correlation between plasma nitrate and nitrite at the end of clamp and glucose uptake was observed in L-arginine — but not in D-arginine-infused LSD rats. These results provide evidence that the sympathetic nervous system and the L-arginine–nitric oxide pathway are involved in the glucose uptake impairment induced by chronic dietary salt restriction.

Keywords: Sodium; Dietary; Insulin resistance; Plasma lipids; Sympathetic nervous system; Nitric oxide; Blood pressure

1. Introduction

It has been observed that high salt intake is associated with elevated blood pressure. This has been corroborated by studies in normotensive [1,2] and hypertensive humans [3] and in laboratory animals [4]. Therefore, salt restriction is recommended for antihypertensive treatment [5,6]. Insulin resistance, glucose intolerance and increased plasma triacylglycerols are some of the side effects of low salt intake in humans and experimental models [7–10]. However, some studies have shown insulin resistance associated with high but not with low salt intake in humans and in rats [11,12].

A lower blood pressure and insulin-independent and-dependent glucose uptake was previously observed in isolated epididymal adipocytes [4] in rats fed, from weaning to adulthood, a low-salt diet (LSD), compared to animals on high salt intake. In a subsequent study, it was found that insulin resistance in LSD rats was reversed by chronic administration of angiotensin I-converting enzyme blocker, but not by angiotensin II type 1 receptor antagonist [8].

It is well known that LSD intake increases sympathetic nervous system activity [13] and decreases tissue perfusion [14]. It

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has further been observed that these two factors are associated with insulin resistance [15–17]. In addition to a decreased circulating volume induced by dietary salt restriction [14], low tissue perfusion may also be due to an impairment of the insulin-induced nitric oxide mediated endothelial-dependent vasodilation (for review see reference [18]).

The present study was planned to find out if inhibition of the sympathetic nervous system and stimulation of the L-arginine–nitric oxide pathway can revert the lower insulin sensitivity induced by LSD.

2. Methods

The Ethics Committee of the University of São Paulo School of Medicine, Brazil, approved all experiments reported herein.

2.1. Animals

Male Wistar rats obtained from the institutional facilities were fed, from weaning, either a low-salt diet (LSD — 0.06% Na, TD 92141 — Harlan Teklad, USA) or a normal-salt diet (NSD — 0.5% Na, TD 92140). The animals were housed in a temperature-controlled environment (25±1 °C), with light cycling (lights on at 6 AM, and lights off at 6 PM) and with free access to chow and tap water.

2.2. Experimental design

At 12 weeks of age, carotid and jugular catheters were inserted under anesthesia (sodium pentobarbital — 50 mg/kg, ip) and exteriorized at the back of the neck, filled with heparinized saline (150 U/mL), and sealed with stainless steel plugs. Four to five days later, after 6 h fasting, blood pressure and heart rate were determined, and baseline blood samples were collected for glucose, insulin (only in vehicle and sympathetic nervous system blockade groups), cholesterol and triacylglycerols measurements. Then, in each experimental group, a bolus injection of saline, prazosin and propranolol, D-arginine, or L-arginine was given. D-arginine was used as a control since it is not a substrate for nitric oxide synthase and has the same biophysical properties as L-arginine. Forty-five minutes later, after blood pressure and heart rate determination, a euglycemic hyperinsulinemic clamp was started in awake and unrestrained animals. At the end of the clamp, blood pressure and heart rate were determined, and blood samples for the same measurements were collected again.

2.3. Experimental groups

Each of the dietary groups was subdivided into: (1) sympathetic nervous system blockade ($n=6$), (2) vehicle infusion ($n=6$), (3) L-arginine infusion ($n=6$), and (4) D-arginine infusion ($n=6$).

Prazosin, an alpha 1 antagonist, (1 mg/kg body weight, iv — Sigma, USA) and propranolol, a β-blocker (5 mg/kg body weight, ip — Sigma, USA), were used for sympathetic nervous system inhibition. For L-arginine–nitric oxide pathway stimulation, a bolus infusion of L-arginine (300 mg/kg body weight, iv — Ajinomoto, Brazil) was given. For control, D-arginine (300 mg/kg body weight, iv — Sigma, USA) was infused.

2.4. Euglycemic hyperinsulinemic clamp

The clamp was performed in conscious and unrestrained animals as described by DeFronzo and co-workers [19] and modified by Prada et al. [8]. A continuous regular insulin infusion (2.23 pmol kg^{-1} min^{-1} — Actrapid®, Novo Nordisk A/S Bagsvaerd, Denmark) was carried out through the jugular line, over 2 h. Insulin was diluted in 26.61 μmol/L bovine serum albumin solution. To maintain euglycemia similar to the basaline levels, a glucose infusion (1.11 mmol/L D-glucose) through the jugular vein was started 5 min after the beginning of the insulin infusion and was corrected every 5 min based on a servo-control negative feedback principle [19]. Samples (25 μL) for blood glucose determinations were taken through the carotid artery every 5 min. At 75, 90, 105 and 120 min of the clamp, 600 μL blood samples were taken for insulin determination. Blood samples for insulin measurements were centrifuged immediately and the plasma was stored at −20 °C until assayed. Blood cells were re-suspended in saline and re-infused into the animal. During the last 30 min of the clamp, steady-state blood glucose and plasma insulin were attained as the glucose infusion rate equals glucose uptake in all body tissues and is therefore a measure of tissue sensitivity to exogenous insulin. Experiments in which the coefficient of variation (standard deviation/mean) of blood glucose and/or plasma insulin during the steady-state exceeded 10% were excluded. The hematocrit was determined at the beginning and at the end of all experiments.

2.5. Blood pressure and heart rate

Blood pressure and heart rate were measured in conscious animals through the carotid artery catheter attached to a pressure transducer connected to a computerized system. Records of carotid artery blood pressure and heart rate were performed at baseline and at the end of the clamp. Blood pressure and heart rate measurements were recorded during two or three minutes. The mean of all values obtained during each determination was considered for calculations.

2.6. Analytical methods

Blood samples were collected and immediately centrifuged, and plasma was stored at −20 °C until assayed. Plasma nitrate and nitrite are nitric oxide metabolites and indicative of its production. Plasma nitrate and nitrite were determined by the Griess reaction [20], after treating the samples with nitrate reductase. Blood glucose concentration was measured with a glucometer (Advantage, Boehringer Mannheim). Insulin, total cholesterol and triacylglycerols were measured using commercial radioimmunoassay kits (Diagnostic Products Corporation, USA, and Roche Diagnostics Corporation, Indianapolis, USA, respectively).