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Original article

Propranolol treatment lowers blood pressure, reduces vascular inflammatory markers and improves endothelial function in obese mice

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ABSTRACT

Obesity-associated hypertension is accompanied by a number of cardiovascular risk factors including vascular insulin resistance (IR) and higher sympathetic nervous activity. Therefore, autonomic blockade was demonstrated to reverse hypertension, endothelial dysfunction and IR in obese individuals. We hypothesized that β-AR blockade with propranolol would restore endothelial function and vascular insulin signaling in obesity, associated with an anti-inflammatory effect. Body weight, systolic blood pressure (SBP), plasma biochemical parameters and aortic endothelial function were analyzed in mice fed standard diet (control group) or a high fat diet (HFD) that were treated with vehicle (water) or propranolol (10 mg/kg/day) for 8 weeks. Propranolol treatment did not modify obesogenic effect of HFD feeding. However, propranolol was effective in preventing the rise in SBP, the hyperinsulinemia and the impaired endothelium-dependent relaxation to acetylcholine and to insulin in obese mice. Protective effect of propranolol administration in endothelial function was associated with increased nitric oxide (NO) production and phosphorylation of Akt (Ser473) and eNOS (Ser1177), but with reduced phospho-IRS-1(Ser307) and phospho-ERK1/2 (Thr202/Tyr204). In addition, β-blocker propranolol prevented the NF-kB nuclear translocation and the increase in phospho-IκB-α (Ser32) and in interleukin(IL)-6 expression in aorta of obese mice, without significant changes in either aortic reactive oxygen species production or in circulating IL-6 and TNF-α levels. In β_2-AR knockout mice, despite increasing body weight and visceral fat, HFD did not increase SBP and showed a partial improvement of endothelial function, revealing a role of β_2-AR in cardiovascular effects of obesity. In conclusion, our results suggest that β-AR blockade with propranolol is effective to prevent the endothelial dysfunction, vascular IR and pro-inflammatory state displayed in HFD-induced obesity, independent of changes in body weight.

1. Introduction

Obesity is one of the most challenging public health issues and represents a major epidemic worldwide [1]. Increased body mass index (>30Kg m^{-2}) is a well-established risk factor for insulin resistance (IR), type 2 diabetes, and cardiovascular diseases (CVD) such as hypertension and atherosclerosis. Importantly, endothelial dysfunction is an early marker of the onset of obesity-associated IR and CVD [2,3].

Sympathetic activation is also a hallmark of obesity and IR, mainly driven by hyperleptinemia and hyperinsulinemia [4,5]. Elevated sympathetic nervous activity is positively related to endothelial and renal dysfunction, as well as with excessive left ventricular mass in obese individuals [6], supporting a role for sympathetic nervous system in the obesity-associated cardiovascular injury. Accordingly, autonomic blockade improved insulin sensitivity [7], reversed endothelial function, as well as lowered total peripheral resistance and blood pressure [8] in obese subjects. However, the mechanisms associated with this cardiovascular protection induced by autonomic blockade are still unknown.

Insulin at physiological levels stimulates vasodilation via endothelium-derived nitric oxide (NO) production [9]. This vasodilator effect is mediated by tyrosine-phosphorylated insulin receptor substrate-1 (IRS-1), with subsequent phosphatidylinositol 3-kinase (PI3 K) activation and protein kinase B (Akt) phosphory-

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lation, that leads to endothelium NO synthase (eNOS) activation [10,11]. However, the NO-dependent insulin vasodilator signaling pathway is impaired by hyperinsulinemia and IR, favoring the activation of mitogen-activated protein kinases (MAPK) in vascular tissue [12,13]. MAPKs-induced serine phosphorylation of IRS-1 leads to IR, vasoconstriction, expression of pro-inflammatory and atherogenic factors [14–16].

Although β-adrenoceptor (β-AR) agonists are known to induce vasodilation, persistent β-AR stimulation induces MAPK ERK1/2 activation in vascular cells [17–19] and increases the expression of pro-inflammatory cytokines in the aorta [20]. This pro-inflammatory effect was associated with activation and higher expression of the nuclear factor-κB (NF-κB), and reduced expression of its inhibitor IκB-α. Enhanced NF-κB activity and reduced NO levels in aortic tissue was also reported in high-fat (HF) diet fed mice, leading to increased local expression of pro-inflammatory factors such as ICAM-1, VCAM-1 and interleukin (IL)-6 [21]. In addition, blocking NF-κB activity restores aortic endothelial dysfunction in obese hyperinsulinemic mice [22]. These observations raised the possibility that enhanced β-AR overstimulation is involved in the endothelial dysfunction and inflammation in obesity and IR. In this regard, although the non-specific β-blockade with propranolol enhanced the body weight and free fat acids of HF diet-induced obese rats, it significantly reduced circulating C-reactive protein levels [23]. In addition, propranolol treatment improved glycemic control in diabetic rats, associated with an anti-inflammatory effect in skeletal muscle [24]. Considering that there are no specific pharmacological treatment options available for the vascular complications of obesity and IR [25], the aim of the present study was to evaluate the effect of the β-AR antagonist propranolol in the endothelial function, insulin signaling, NO production and pro-inflammatory markers of HF diet-induced obese mice.

2. Materials and methods

2.1. Animals

This study was approved (protocol number: 3773-1) by the Ethics Committee on Animal Use of the University of Campinas (CEUA-UNICAMP, Campinas-SP, Brazil) and conformed to the guidelines for ethical principles in the care and use of animals adopted by the Brazilian Society of Laboratory Animal Science (SBCAL/COBEA). Mice were maintained at 22 ± 2 °C on a 12 h light–dark cycle with free access to food and water.

C57BL/6J male mice were obtained from the breeding colony at UNICAMP. Male mice were used in the present study based on previous report of an increase sympathetic contribution to blood pressure in male obese mice compared to females [26]. The mice (2-month old) were randomly assigned to receive for 8 weeks a chow diet (C group) or a high fat diet (HF group, 37% fat) concomitantly without or with propranolol (C + P and HF + P groups, 10 mg/kg/day propranolol available in drinking water). Diets were purchased from Prag Soluções Biociências® (Jaú, SP, Brazil) and the HF diet composition [27] has been previously described to induce obesity and IR in mice [28,29]. A cohort of male FVB mouse strain lacking β_2-adrenoceptor (β_2KO) and their FVB wild type (WT) were also used in this study [30]. WT and β_2KO mice at 2 months of age were fed with chow or HF diet for 8 weeks. Body weight was evaluated weekly and then propranolol (Sigma-Aldrich, Saint Louis, MO, USA) dose was adjusted in the C + P and HF + P groups for body mass and water intake measurement.

2.2. Blood pressure measurement

Systolic blood pressure (SBP) was determined weekly by tail cuff plethysmography (LE5001 Pressure meter, Panlab, Harvard Apparatus, Barcelona, Spain). Mice were initially trained twice daily with 10 tail cuff inflations for at least 3 days. SBP measurement was considered successful when mouse did not move and a clear initial pulse could be identified. The SBP weekly values were obtained from the average of 10 sequential measures from each animal in the week [31].

2.3. General plasma biochemical parameters

After 12 h fasting, animals were killed by decapitation and blood samples were collected. Glycemia was verified using a glucometer (Accu-Chek Advantage, Roche Diagnostics). Plasma total cholesterol (CHOL) and triglycerides (TG) levels were measured using specific commercial kits (Chod-Pap, Roche Diagnostic GmbH, Mannheim, Germany), according to the manufacturer's instruction. Insulinemia were measured by radioimmunoassay, as previously reported [29]. Plasma adiponectin and leptin concentration were determined using standard commercial kits (R&D Systems, MN, EUA), according to the manufacturer's instruction.

2.4. Vascular reactivity

Thoracic aortas were cut into segments (2 mm in length), free of adipose tissue, and then mounted in an isolated tissue chamber containing Krebs-Henseleit solution (in mM: 118 NaCl, 4.7 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$-$2H_2O$, 1.2 KH_2PO_4, 1.2 $MgSO_4$-$7H_2O$, 11 glucose, and 0.01 EDTA) gassed with 95% O_2 and 5% CO_2. The rings were maintained at a resting tension of 0.5 g at 37 °C, pH = 7.4, as previously reported [32]. After a 60 min equilibration period, the aortic rings were exposed to 125 mM KCl to assess the maximal tension. Relaxation concentration-response curves to acetylcholine (1 nmol/L – 10 μmol/L, Sigma-Aldrich), insulin (0.01–3 nmol/L; Humulin R, Eli & Lilly Company, Indianopolis, IN, USA), NO-donor sodium nitroprusside (0.01 nmol/L – 1 μmol/L, Sigma-Aldrich) or isoproterenol (β-AR agonist; 0.1 nmol/L – 3 μmol/L; Sigma-Aldrich) were generated in aortic rings contracted with U46619 (Enzo Life Sciences, Farmingdale, NY, USA) until 50–70% of maximum contraction with 125 mM KCl was reached. Some aortic rings were incubated for 30 min with the nonselective NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME, 100 μM, Sigma-Aldrich) before the relaxation response curves to acetylcholine were generated. Nonlinear regression analysis to determine the maximal response (R_{MAX}) and the agonist concentration needed to achieve 50% maximal response (-Log EC_{50}) were calculated using GraphPad Prism software (GraphPad Software, CA, USA).

2.5. Western blotting

Total protein extracts were obtained from isolated thoracic aortas homogenized in cold RIPA lysis buffer (Merck Millipore, Billerica, MA, USA) containing phenylmethylsulfonyl fluoride (1 mM PMSF), Na_3VO_4 (1 mM) and a protease inhibitor cocktail (2 μL/mL, Sigma-Aldrich). For nuclear and cytoplasmic protein extracts, aortas were pulverized in liquid nitrogen and the fragments were incubated in ice-cold HEPES buffer [10 mM HEPES (pH = 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 0.5 mM PMSF and 2 μL/mL PIC] for 10 min. Next, samples were centrifuged at 4 °C for 10 min at 850*g*. Supernatants were discarded, and pellets were suspended in ice-cold HEPES buffer containing 0.1% Triton-X for 10 min. Samples were centrifuged and the resulting supernatants represent the cytoplasmic fraction. Pellets were resuspended in ice-cold buffer containing 20 mM HEPES (pH = 7.9), 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 0.5 mM PMSF and 2 μL/mL PIC, and then centrifuged (16,000*g*, 15 min). The supernatants represent the nuclear protein extract. Proteins were quantified using a BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA)