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Vascular insulin resistance in prehypertensive rats: Role of PI3-kinase/Akt/eNOS signaling

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ABSTRACT

It is well known that systemic insulin resistance is closely associated with the metabolic syndrome including type 2 diabetes and hypertension. However, it remains unclear whether vascular insulin resistance acts as an early etiologic factor for the development of hypertension. Male spontaneously hypertensive rats (SHRs) aged 5 weeks (young) and 15 weeks (adult) were studied and vascular insulin resistance was assessed as the function of isolated aortic vasodilatory response to insulin *in vitro*. Compared with Wistar–Kyoto (WKY) rats, adult SHRs exhibited significant hypertension with significantly decreased aortic vasodilatation to insulin, whereas young SHRs had normal blood pressure but exhibited similar vascular insulin resistance. Both young and adult SHRs showed significant downregulated expression of PI3-kinase and decreased insulin-stimulated phosphorylations of Akt and eNOS in vascular tissues. Treatment with rosiglitazone (RSG), an insulin sensitizer, for 2 weeks increased vascular PPARγ expression and restored PI3-kinase/Akt/eNOS-mediated signaling pathway only in young SHRs. More importantly, this treatment improved aortic vasodilatory response to insulin in young but not in adult SHRs. In summary, vascular insulin resistance, characterized by the impairment of PI3-kinase/Akt/eNOS-mediated signaling in vascular endothelium, may play important roles in endothelial dysfunction and subsequent development of hypertension in normotensive young SHRs.

1. Introduction

The term metabolic syndrome describes a cluster of cardiovascular risk factors, characterized by insulin resistance, dyslipidemia, and hypertension (Johnson and Weinstock, 2006). The condition afflicts as estimated 15–25% of individuals in industrialized countries (Hauner, 2002). The mechanisms underlying the metabolic syndrome are not fully known; however, insulin resistance appears to modify biochemical responses in a fashion predisposing to metabolic risk factors (Gogia and Agarwal, 2006). It is well-recognized that insulin resistance is closely associated with a series of both metabolic and hemodynamic disorders. In addition, increasing evidence in animals and humans support the existence of reciprocal relationships between insulin resistance and endothelial dysfunction (Koh et al., 2004, 2005, 2006). Reaven and Hoffman (1987) hypothesized that insulin resistance potentially contributes to the pathogenesis of hypertension, but the association between insulin resistance and hypertension-prone normotension remains unknown, and scant relevant evidence to verify this hypothesis exists.

In the vascular endothelium of insulin-resistant animal models, the balance between the vasodilatory and vasoconstrictive properties of insulin is impaired by disruption of insulin-stimulated PI3-kinase signaling (with intact MAPK/ET-1 signaling) (Kobayashi et al., 2004). The spontaneously hypertensive rat (SHRs), a genetic model of hypertension harboring features of the metabolic syndrome, has recently been shown to exhibit metabolic insulin resistance associated with impaired vascular endothelial insulin-stimulated PI3-kinase signaling (Jiang et al., 1999; Kobayashi et al., 2004; Zecchin et al., 2003). However, the relevancy of this defective vascular insulin signaling in the pathogenesis of hypertension remains elusive.

Thiazolidinediones, ligands for peroxisome proliferator-activated receptor γ (PPARγ), improve metabolic actions of insulin in the insulin-sensitive tissues. They also improve endothelial function and reduce blood pressure in SHRs, even in the face of insulin resistance (Potenza

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et al., 2006). Rosiglitazone (RSG), a member of the thiazolidinediones agents, is an insulin sensitizer and is currently an efficacious drug utilized for clinical treatment of type 2 diabetes. It is unclear whether RSG treatment could improve vascular endothelial insulin resistance, and subsequently retard the progress of hypertension.

Therefore, the aims of the present study were to test the hypothesis that vascular insulin resistance in the conduit arteries contributes to the pathogenesis of hypertension in hypertension-prone normotensive rats, and to further investigate the underlying mechanisms, especially the role of PI3-kinase mediated insulin signaling pathway.

2. Materials and methods

2.1. Materials

All chemicals and reagents including bovine insulin were purchased from Sigma (St. Louis, MO) unless indicated otherwise. N^{ω}-nitro-L-arginine methyl ester (L-NAME) was from Calbiochem (San Diego, CA). The antibodies of PI3-kinase p85, Akt, pAkt, eNOS and peNOS were from Cell Signaling Technology (Danvers, MA). The PPARγ antibody was from Santa Cruz (CA). The BCA Protein Assay Kit was from Pierce (Rockford, IL).

2.2. Experimental protocols

All procedures involving animals were approved by the local authorities for animal research. 5-week-old (young) and 15-week-old (adult) male spontaneous hypertensive rats (SHR/NHsd, haplotype RT1k) and age-matched normotensive Wistar–Kyoto (WKY) control rats were randomized into two groups and treated daily by gavage with vehicle (distilled water) or rosiglitazone (RSG) (3 mg/kg/day). Systolic blood pressure was measured in all animals in conscious state using an noninvasive tail cuff method (Letica 5100; PanLab, Barcelona, Spain) as previously described(Bunag, 1973). Reported systolic blood pressure values are the average of three sequential blood pressure measurements with less than 10 mmHg variations. After two weeks of treatment, the animals were anesthetized through the intraperitoneal administration of 20% urethane. Vena cava blood was drawn and immediately centrifuged to determine serum insulin levels. Aortae from the heart to the iliac bifurcation were removed and placed in ice-cold Krebs buffer consisting of (in mM): NaCl 118, KCl 4.8, $CaCl_2 \cdot 2H_2O$ 2.5, $MgCl_2 \cdot 6H_2O$ 2.5, $NaH_2PO_4 \cdot 2H_2O$ 1.2, $NaHCO_3$ 8.5, and Glucose$\cdot H_2O$ 11. Aortae were cleaned of adherent tissue for immediate organ chamber experiments or frozen in liquid nitrogen for other assays.

2.3. Organ chamber experiments

Organ chamber experiments were performed as previously described (Li et al., 2007). Briefly, the aorta was cut into rings of 2 mm in length. In some segments, the endothelium was mechanically removed by pulling silk suture through the vessel. The rings were mounted onto hooks, suspended in organ chambers filled with Krebs buffer and aerated with 95% O_2/5% CO_2 at 37 °C, and connected to force transducers (WPI, Sarasota, FL) to record changes via a Maclab data acquisition system. After 30 min equilibration at an optimal preload of 9.8 mN, the rings were then pre-contracted with noradrenaline (0.1 nM). Once a stable contraction was achieved, the rings were exposed to cumulative concentrations of insulin (1, 3, 10, 30, 60, 120 U/L), which was about 10–1000 folds of maximal physiological concentration and was comparable with that used in some other in vitro studies in which the vasorelaxation effect of insulin was investigated (Hasdai et al., 1998a; Ma et al., 2006). To assess the influence of nitric oxide (NO, a vasodilator) on insulin-induced vasorelaxation, part of the rings was pre-treated with N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.5 mM), an inhibitor of NO synthase (NOS), 20 min before insulin stimulation. After determination of endothelial function, the aortic segments were subjected to immunoblotting measurement.

2.4. Glucose and insulin determinations

Serum concentrations of insulin were measured by RIA test kit (Peninsula Laboratories). Plasma glucose concentrations were determined with a diagnostic glucometer (SureStep, LifeScan).

2.5. Immunoblotting

The aortic segments were pulverized and solubilized in lysis buffer. Protein quantification with the BCA protein assay kit (Pierce) was performed to ensure equal loading amount in each lane, that is, 80 μg protein. β-actin was chosen as a loading control to further assure the same volume for all the samples. Proteins were separated by electrophoresis on a 14% SDS-polyacrylamide gel, and electrophoretically transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). After blocking with 5% skim milk powder, the membrane was incubated with an antibody against PPARγ, PI3-kinase p85, Akt, pAkt, eNOS or peNOS overnight at 4 °C, followed by incubation with HRP-conjugated IgG antibody (Cell Signaling) for 1 h at 37 °C. The blots were developed with an enhanced chemiluminescense detection kit (Pierce Biotechnology, Rockford, IL). The immunoblotting was visualized with ChemiDocXRS (Bio-Rad Laboratory, Hercules, CA) and the blot densities were analyzed with LabImage software.

2.6. Statistical analysis

All values in the text, table, and figures are presented as mean ± S.E.M. The data of vasodilatation with L-NAME pretreatment and Akt and eNOS phosphorylation were analyzed with factorial design ANOVA. All other comparisons were made using the two-way ANOVA. When analysis of variance revealed significant differences, Bonferroni correction for post hoc *t*-tests was used to correct for multiple comparisons. Probabilities of <0.05 were considered to be statistically significant. All of the statistical tests were performed with the GraphPad Prism software version 5.0 (GraphPad Software, Inc., San Diego, CA).

3. Results

3.1. The impact of RSG on physiological parameters in SHRs

The effects of RSG on body weight, hemodynamic, and biochemical parameters in SHRs are shown in Table 1. As expected, adult 15-week SHRs exhibited higher systolic blood pressure compared with age-matched WKY rats. Adult SHRs were overweight, hyperinsulinemic, but normoglycemic when compared to their WKY counterparts. Young 5-week SHRs had no significant difference in body weight, systolic blood pressure, serum glucose or insulin concentration when compared to their WKY counterparts. After two weeks the body weight of SHRs treated with vehicle was significantly increased compared with the animals before treatment, while the fasting insulin levels were only mildly increased and there was no statistical difference between the pre- and post-treatment. Two weeks of RSG treatment did not alter body weight or fasting glucose levels in young or adult SHRs in comparison to vehicle treatment, but RSG-treated rats did manifest significantly decreased fasting insulin and systolic blood pressure measurements. In addition, RSG treatment has been shown to lead to cardiac hypertrophy, however, in the present study heart weights in RSG-treated animals had no difference with those in