INCREASED P22PHOX EXPRESSION AND REDUCED SOD ACTIVITY ARE INVOLVED IN EARLY IMPAIRMENT OF INSULIN-INDUCED VASODILATION ON RESISTANCE VESSELS OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

GAGLIARDI Sara, POTENZA Maria A., MARASCIULO Flora L., MONTAGNANI Monica

Department of Pharmacology and Human Physiology - Medical School - University of Bari, 70124 Bari, ITALY

Physiological nitric oxide (NO)/superoxide ($O_2^-$) balance is crucial for endothelial integrity. An increased production of reactive oxygen species (ROS) participates in the pathogenesis of vascular complications of diabetes, obesity and hypertension. We have shown that a selective defect in insulin-stimulated NO production precedes overt endothelial dysfunction in 12-wk old SHR, a genetic model of hypertension with features of the Metabolic Syndrome (1). To evaluate whether impaired vascular effects of insulin may depend on reduced bioavailability of NO or impaired $O_2^-$ scavenging, we studied physiological parameters, vascular reactivity, and expression/function of major components of oxidative pathways in 12 to 20-wk old SHR and age-matched WKY rats. Consistent with previous results, in mesenteric vascular beds (MVB) isolated from 12-wk SHR pre-constricted with noradrenaline (NA, 3 $\mu$M), vasodilator responses to insulin (1-100 nM/4 min perfusion) were significantly reduced (vs WKY; p<0.005), whereas vasodilation to ACh (0.1-100 nM) was preserved. WB analysis did not show significant changes in protein expression of CuZn- and Mn- isoforms of superoxide dismutase (SOD) in MVB and aortas homogenates of 12-wk old SHR. However, a significant reduction in vascular SOD activity was measured by ELISA assay (vs WKY, p<0.05). In addition, an increased expression of the NAD(P)H oxidase subunit p22phox was detected by immunohistochemistry in aortas of 12-wk SHR (vs WKY, p<0.05). 20-wk SHR had comparable high blood pressure, but were more hyperinsulinemic (p<0.001) and more insulin resistant (p<0.001) than 12-wk SHR. A significant increase in levels of 8-iso-PGF2$\alpha$, a lipid peroxidation marker, was measured by ELISA in aorta homogenates from 20-wk SHR (vs WKY, p<0.05). In MVB from 20-wk SHR, vasodilation to ACh was impaired and vasodilation to insulin was further reduced (vs 12-wk SHR; p<0.05). Pretreatment of MVB with exogenous SOD (100 UI/20 m) or the NAD(P)H oxidase inhibitor apocynin (100 $\mu$M/20 m) restored insulin-mediated relaxation to values observed in age-matched WKY. Under these conditions, vasodilation to insulin was significantly inhibited by the eNOS antagonist L-NNAME (100 $\mu$M/20 m) (p<0.001 vs respective control). These results suggest that overexpression of p22phox and reduced scavenger activity of SOD precede the onset of “classical” endothelial dysfunction (defined as reduced vasorelaxation to ACh) and may participate in selective insulin-impaired vasodilation in MVB of 12-wk old SHR. Increased insulin resistance and hyperinsulinemia may further reduce NO bioavailability overtime and contribute to endothelial dysfunction to both ACh and insulin in 20-wk old SHR rats.