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p66 Shc Mediates Effect of ET-1 on TRPC Channels Activity and Changes in Intracellular Ca²⁺ in Renal Vascular Smooth Muscle CellsAndrey Sorokin¹, Oleg Palygin², Bradley Miller¹, Alexander Staruschenko²¹Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA, ²Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Hypertension-induced nephropathy is accompanied by impaired renal vascular responsiveness and structural changes, but molecular mechanisms involved remain elusive. Elevation of intracellular Ca²⁺ [Ca²⁺]_i is strongly linked to renal microvascular responses and is crucial for ET-1-induced contraction of smooth muscle cells (SMC). The adaptor protein p66 Shc is overexpressed in renal vascular SMC of hypertensive Dahl S rats. In patch clamp electrophysiology experiments carried out with primary SMCs isolated from renal vessels of Zinc Finger Nuclease-mediated p66 Shc rat knockouts we established that p66 Shc deficiency results in a dramatic increase in TRPC channels activity in response to ET-1. Knockout of p66 Shc resulted in increase of channel activity. Next we showed that ET-1 produced dynamic changes in cytosolic Ca²⁺ concentration in SMCs derived from p66 Shc knockout rats, when compared with SMCs derived from their WT littermates. Fura 2-AM was used to measure changes in the [Ca²⁺]_i before and after administration of 100 nM ET-1. We also tested activation of [Ca²⁺]_i-dependent signaling pathways in renal SMCs isolated either from p66 Shc knockouts or from WT littermates. We have previously shown that ET-1-mediated activation of calcium regulated cytoplasmic tyrosine kinase Pyk2 caused an activation of p38 MAP kinase which is known to contribute to actin remodeling in SMC. Accordingly, we detected increased activation of Pyk2 and p38 MAP kinase in ET-1-treated SMCs isolated from p66 Shc knockout rat. Our data suggest that p66 Shc restrains activity of TRPC channels, which mediate influx of Ca²⁺ in SMC in response to ET-1, contributing to renal vascular dysfunction.

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Contractions to Endogenous and Exogenous Endothelin-1 in Segmental Renal Arteries of the Mouse: Up-Regulation in ObesityOliver Baretella¹, Aimin Xu^{1,2,3}, Paul M. Vanhoutte^{1,3}¹Department of Pharmacology & Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, ²Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, ³Research Centre of Heart, Brain, Hormone & Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Endothelin-1 (ET-1) is implicated in cardiovascular risk factors such as obesity, and the endothelin system is prominent in the kidney. In murine arteries, the contractile profile of the peptide is heterogeneous among different preparations, and the renal vascular bed is largely unexplored. Segmental renal arteries branching from the main renal arteries of age-matched lean and 30 weeks diet-induced obese WT mice were investigated by isometric tension recording in Halpern-Mulvany myographs. Contractions after administration of big endothelin-1 (bET-1) or ET-1 (both 10 pM to 100nM) were determined in the absence and presence of L-NAME, followed by full concentration-response curves to serotonin (5-HT) or the TP receptor agonist U46619. At the highest concentrations of bET-1 contractions were similar in rings of lean and obese mice in the absence of L-NAME. Inhibition of NO synthesis facilitated responses particularly in obese animals ($n=6-9$, $P<0.01$). Exogenous ET-1 contracted potently preparations of all groups starting from 3 nM on; the response to the peptide was augmented by obesity in the absence and presence of L-NAME (each $n=6-10$, $P<0.001$). ECE-activity calculated as the ratio of the responses bET/ET-1 was significantly higher in rings from obese mice in the presence of NO at 10 nM ($n=5-9$, $P<0.01$) and at 30 nM of the peptides in the presence of L-NAME ($n=6-9$, $P<0.05$). Contractions to 5-HT and U46619 were comparable between groups. These experiments demonstrate the high responsiveness of the renal vascular bed to both endogenous and exogenous ET-1, and an increased activity of the endothelin system in obesity, whereas responses to 5-HT₂ and TP receptor activation are unaltered.