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Decrease in TRPV4 Expression in Vascular Endothelium From STZ Treated Rats is Reversed by Insulin Treatment

Diabetes mellitus (DM) is a pandemic metabolic disorder characterized by chronically elevated blood glucose concentration (hyperglycaemia) that contributes to vascular complications (1). Vascular endothelial dysfunction (VED) is a major complication where vasodilation is compromised rendering the diabetic prone to elevated blood pressure (1). The vanilloid transient receptor potential channel (TRPV) subfamily is comprised of 6 members including TRPV4 which is found in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) (2, 3). As a cation channel, TRPV4 mediates Ca^{2+} influx that plays a major vasodilator role through an endothelium-dependent mechanism and VSMCs hyperpolarization (4, 5). In this study the role of TRPV4 in diabetic VED was investigated in addition to the role of insulin to correct the VED.

Two groups of male Wistar rats (n= 4-5/group) were studied, the first group was injected i.p. with 65mg/kg streptozotocin (STZ) while the second was injected with 20mM citrate buffer (Control). Rats were euthanized after 8-14 days from being injected with STZ and thoracic aortic rings were isolated and suspended in organ baths attached to a force transducer allowing quantitative isometric tension measurement. Arterial rings were pre-contracted with noradrenaline (300nM) followed by TRPV4-agonist, RN-1747 (3nM-3µM) to evoke relaxation dose response curves. Moreover, primary VSMCs and ECs were isolated from rat thoracic aorta and cultured for 7 to 10 days till becoming confluent. The cells were then lysed and studied through Western blotting. TRPV4 protein was probed with rabbit Anti-TRPV4 antibody and visualized through confocal immunocytochemistry (ICC).

Thoracic aortic rings from diabetic rats showed compromised TRPV4-induced relaxation compared to control (Diabetic: 24.3±5.3% max. relaxation **p 0.01 vs control; 62.4±8.7% max. relaxation). Moreover, primary diabetic VSMCs showed significant reduction in expressed TRPV4 when normalised to control VSMCs' TRPV4 (56.2±5.4% vs control 100±8.8%, **p 0.003). The significant decrease in diabetic primary ECs TRPV4 was reversed with insulin when normalised to control ECs' TRPV4 (270mIU/day for 5 days (Diabetic: **p 0.01, 58.9±5.8% vs diabetic treated with insulin: 95.4±5.4% and ***p 0.001 vs control 100±8.8%). Additionally, insulin reversed diabetic ECs reduction in caveolin-1 when normalised to control ECs' caveolin-1 (Diabetic: **p 0.01, 73.9±4.3% vs diabetic treated with insulin: 103.1±3.8% and *p 0.05 vs control 100±7.2%).

These results showed the TRPV4 function is compromised in both aortic endothelial and VSMC in STZ-diabetic rats and is in part due to reduction in channel number. Endothelial TRPV4 reduction was accompanied with caveolin-1 reduction and was shown to be reversed with insulin. Caveolin-1 is a preliminary protein found in caveolae, a cone shaped pocket of proteins and lipids and serves as a docking site for channels and receptors in endothelium (6, 7).

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