The effect of high glucose concentration and oxidative stress on transient receptor potential vanilloid 4 ion channel-mediated relaxation in rat carotid artery

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Cardiovascular disease is the leading cause of death globally and is greatly exacerbated by diabetes. The elevated glucose levels associated with diabetes are known to induce oxidative stress and consequently impair endothelium-mediated vasorelaxation in large and small arteries. Effective drug targets for treatment of diabetes-induced endothelial dysfunction remain to be identified. Recent studies provide good evidence that the vanilloid 4 member of the transient receptor potential (TRPV4) ion channel family modulate endothelial intracellular calcium concentration (Sonkusare *et al.*, 2012) and this is a regulator of nitric oxide synthase and endothelium-dependant relaxation. In this study the selective TRPV4 agonist GSK101067A (GSK) and the selective antagonist HC067047 (HC) were used to test the hypothesis that TRPV4 mediated vasorelaxation is impaired by glucose- mediated oxidative stress.

Methods: Isometric tension was measured *in vitro* in carotid artery segments collected from adult male Wistar rats, killed humanely according to RMIT University Animal Ethics Committee endorsed procedures. Generation of superoxide by carotid artery was measured using lucigenin-enhanced chemiluminescence. Western blotting was used to determine protein expression of Nox2, eNOS and TRPV4 protein.

Results: Phenylephrine pre-contracted carotid arteries incubated in Krebs solution containing 11 mM glucose-29 mM mannitol relaxed to cumulative concentrations of acetylcholine (ACh 0.01 to 10 μM) and this response was impaired following 2 hour incubation in 40 mM glucose . The sensitivity (pEC_{50}) and maxiumum relaxation (R_{max}) to ACh were both reduced significantly (pEC_{50} mannitol = 7.17 ± 0.14, 40 mM glucose = 6.65 ± 0.15; R_{max} mannitol = 97 ± 4, 40 mM glucose = 78 ± 7%, p<0.05). The antioxidant tempol improved relaxation to ACh following incubation in 40 mM glucose (pEC_{50} 40 mM glucose = 5.93 ± 0.18, 40 mM glucose + tempol = 6.70 ± 0.15; R_{max} 40 mM glucose = 49 ± 8 , 40 mM glucose + tempol = 76 ± 5, P<0.05). Segments of carotid arteries relaxed to GSK (R_{max} = 85 ± 13%), however following nitric oxide synthase inhibition GSK caused contraction (C_{max} = 81 ± 12%). In contrast the relaxation to increasing concentrations of GSK was not affected by 40 mM glucose. Pyrogallol-induced oxidant stress attenuated the response to ACh (pEC_{50} control = 6.45 ± 0.08, 30 μM pyrogallol = 5.67 ± 0.31 and R_{max} control = 83 ± 4, 30 μM pyrogallol R_{max} = 53 ± 7%). Relaxation to cumulative concentrations of GSK was also impaired in arteries exposed to pyrogallol (pEC_{50} control = 6.39 ± 0.11, 30 μM pyrogallol = 6.09 ± 0.07;R_{max} control = 70 ± 4, 30 μM pyrogallol = 45 ± 2%, P<0.05). The 2 hour incubation in 40 mM glucose increased carotid artery superoxide production (11 mM glucose = 260 ± 21, 40 mM glucose = 432 ± 38 counts / mg tissue, P<0.05) when measured by lucigenin-enhanced chemiluminescence and this was attenuated in GSK treated artery segments (40 mM glucose + 1 μM GSK = 299 ± 28 counts / mg tissue). TRPV4 channel antagonism using HC did not reverse attenuation in GSK treated arteries (40 mM glucose + 1 μM GSK + 10 μM HC = 302 ± 42 counts / mg tissue). Western blot analysis showed that protein levels of Nox2, eNOS and TRPV4 were unaffected by these treatments.

Conclusion: TRPV4 mediated endothelium dependant relaxation of rat carotid arteries is nitric oxide (NO) mediated and was impaired by pyrogallol-induced oxidative stress but not by a high concentration of glucose. Measurement of vascular superoxide indicates that GSK can attenuate oxidant stress caused by high glucose, independently of the activation of TRPV4, but the mechanism of that effect remains to be elucidated.

Sonkusare SK, Bonev AD, Ledoux J, Liedtke W, Kotlikoff MI, Heppner TJ, Hill-Eubanks DC & Nelson MT. (2012). Elementary Ca^{2+} signals through endothelial TRPV4 channels regulate vascular function. *Science* **336,** 597-601.