Effects of advanced glycation end-products (AGE) on Ca^{2+} signalling in vascular endothelial cells and endothelium-dependent responses in rat arteries

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Post-translational modification of proteins in diabetes is believed to contribute to vascular dysfunction and subsequent overt vascular disease. AGE interact with receptors (RAGE) leading to the accumulation of reactive oxygen species (ROS) and activation of kinase-mediated signalling pathways. The aim of this study was to examine acute effects of characterized AGE (*in vitro* modified protein) on Ca^{2+} signalling in bovine aortic endothelial cells (BAEC) and also the effects of AGE on endothelium-dependent dilation of small arteries, with emphasis on the contribution of ROS (Bishara *et al.*, 2002).

AGE were formed by incubating bovine or human serum albumin with various glycating agents for up to 90 days. Ca^{2+} signalling in BAEC was investigated using the fluorescent indicator dye Fura2-AM. Exposure of BAEC to AGE for 5 min caused an elevation in basal [Ca^{2+}] and a concentration-dependent attenuation of intracellular Ca^{2+} release caused by ATP (100 μM), thapsigargin (0.1 μM) and ionomycin (3 μM), but AGE did not affect capacitative Ca^{2+} entry (CCE) induced by these agents. The NAD(P)H oxidase inhibitors apocynin (500 μM) and DPI (1 μM) abolished these effects of AGE on BAECs, as did the IP_3 receptor antagonist xestospongin C (1 μM) and overnight incubation of the BAEC with the PPARγ activator rosiglitazone (10 μM).

Studies on endothelium-dependent vasodilation were performed in rat isolated, pressurized arterioles with myogenic tone. Male Sprague-Dawley rats were anesthetized (sodium thiopentone 100 mg/kg, i.p.), the cremaster muscles removed and segments of the main artery dissected free. Artery segments were cannulated and superfused in a pressure myograph, maintained at an intraluminal pressure of 70 mmHg. AGE introduced intra-luminally inhibited both endothelium-dependent dilation caused by acetylcholine (ACh) and endothelium-independent dilation caused by sodium nitroprusside (SNP) and NS 1619, an activator of the large-conductance, Ca^{2+}-activated K^+ channel (BK_{Ca}), but not the vasodilation caused by adenosine, which is an endothelium-independent vasodilator in this preparation. AGE alone did not cause significant vasodilation or alter arteriolar diameter. Apocynin (500 μM) and the nitric oxide synthase inhibitor L-NAME (100μM) abolished the inhibitory effect of AGE on ACh and SNP-induced vasodilation while apocynin also prevented the inhibitory effect of AGE on NS 1619-induced vasodilation. Iberiotoxin (0.1 μM), an inhibitor of BK_{Ca}, decreased ACh-induced vasodilation of the rat cremaster arteriole and in the presence of iberiotoxin, AGE did not further inhibit ACh-induced vasodilation.

In conclusion, AGE have an acute effect on BAEC to release Ca^{2+} from the intracellular, IP_3-sensitive store. The mechanism involves generation of ROS from NAD(P)H oxidase and activation of the IP_3 receptor, possibly a direct effect of H_2O_2 on the IP_3 receptor. The depletion of the intracellular Ca^{2+} store inhibits subsequent Ca^{2+} signalling by other agents activating the store. AGE did not inhibit CCE induced by other agents and in fact could induce CCE themselves. AGE inhibited the endothelium-dependent dilation caused by acetylcholine and the endothelium-independent, NO-mediated dilation induced by SNP, but not that caused by adenosine, which is an endothelium-independent vasodilator. The fact that these inhibitory effects could be prevented by inhibitors of NAD(P)H oxidase, nitric oxide synthase and BK_{Ca} suggests they may be mediated by the local generation of peroxynitrite from superoxide and NO, acting to inhibit the BK_{Ca}.

Bishara NB, Dunlop ME, Murphy TV, Darby IA, Rajanayagam MAS, Hill MA. (2002) *Journal of Cellular Physiology*, **193(1)**: 80-92.