

Acute effects of insulin on the endothelium-dependent dilation of rat cremaster muscle arteries

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Introduction: Obesity and type II diabetes are hyperinsulinemic conditions that increase the risk of cardiovascular diseases such as atherosclerosis and hypertension. Endothelial dysfunction characterizes these states. However, the underlying mechanisms for cardiovascular and endothelial dysfunction are not well understood. Recent studies have suggested hyperinsulinemia and insulin resistance alter vascular endothelial cell function, possibly contributing to the vascular complications of obesity. (De Boer *et al.*, 2012). Recent observations in our laboratory (Howitt *et al.*, 2012) demonstrated that dietary obesity increased endothelial nitric oxide synthase (NOS) activity, while decreasing the activity of the large-conductance, Ca^{2+} -dependent K^+ channels (BK_{Ca}) in the rat cremaster muscle artery. It was observed that obesity caused an increase in plasma insulin in this model; therefore, it is possible that the obesity-induced changes in the cremaster muscle artery function could have been produced by insulin.

Hypothesis: Insulin mediates the effects of diet-induced obesity on endothelium-dependent vasodilation in the rat cremaster muscle artery.

Method: Six-week-old male Sprague-Dawley rats were anesthetized (Sodium pentathol, 100 mg/kg, i.p.) and the cremaster muscles removed. First-order arteries were dissected free. In pressure myography studies, arteries were cannulated and maintained at 70mmHg/34°C. Internal diameter was measured using video microscopy. Vessels were incubated with or without insulin (100 nM) on the intra-luminal side for 2 h. For biochemical assays, including immunohistochemistry, whole vessels were incubated with or without insulin (100 nM) for 2 h at 34°C, then snap-frozen in liquid nitrogen.

Results: In isolated, pressurized cremaster muscle arteries with myogenic tone (diameter $52.5 \pm 2.9\%$ of maximum, $n=14$), the endothelium-dependent vasodilator acetylcholine (ACh) caused a concentration-dependent dilation of arteries ($98.1 \pm 5.1\%$, $n=8$). This dilation was significantly reduced by the combination of the NOS and guanylate cyclase inhibitors L-NAME (100 μM) and ODQ (10 μM) respectively ($86.4 \pm 2.8\%$) and further addition of the BK_{Ca} blocker TEA (1 mM; $69.9 \pm 5.5\%$). Pre-incubation of the vessels with insulin (100 nM) did not alter ACh-induced vasodilation, but greatly reduced the ability of L-NAME, ODQ and TEA to inhibit the dilation ($86.9 \pm 4.9\%$, $n=5$). Further addition of the intermediate-conductance K_{Ca} (IK_{Ca}) inhibitor TRAM-34 (1 μM) blocked ACh-induced vasodilation of the insulin treated vessels. The effects of insulin were unaltered by PD 98,059 (10 μM), a MAP-kinase inhibitor. Insulin also inhibited vasodilation caused by the BK_{Ca} activator NS1619 and the NO donor sodium nitroprusside. Immunohistochemistry studies showed IK_{Ca} and low levels of $\text{BK}_{\text{Ca}}-\alpha$ subunit were present in the smooth muscle of control and insulin-treated cremaster muscle arterioles, with IK_{Ca} also being present at a comparatively low level in the endothelium.

Conclusion: Acute 2 h treatment with insulin reduced the contribution of NO and BK_{Ca} to endothelium-dependent dilation of the rat cremaster muscle artery, but increased the role of IK_{Ca} . These effects of insulin on BK_{Ca} are similar to those that were observed in diet-induced obesity (Howitt *et al.*, 2012)

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Howitt L, Grayson TH, Morris MJ, Sandow SL, Murphy TV. (2012) *American Journal of Physiology Heart and Circulatory Physiology* **302**: H2464-76.