

## **Involvement of cholesterol-rich membrane domains in the arteriolar myogenic response**

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Membrane events underlying arteriolar smooth muscle contraction following an increase in intraluminal pressure (myogenic response) remain unclear. Caveolin proteins, which associate with cholesterol to form membrane invaginations known as caveolae, and cluster intracellular transduction proteins, may act as sites of mechanotransduction in smooth muscle. To investigate this possibility, studies of myogenic and agonist-induced constriction were conducted in cannulated arterioles isolated from cremaster muscle, removed from pentothal anaesthetized (100mg/kg) rats. Diameter and calcium changes were measured using video microscopy and fluorescence imaging. Caveolae were disrupted by removal of membrane cholesterol using  $\beta$ -cyclodextrin (CyD, 10mM, 1hr). As control for non-specific effects of CyD, additional experiments were conducted with a mixture of CyD and free cholesterol. CyD treatment resulted in vasoconstriction from baseline  $48.9 \pm 4.0\%$  (diameter expressed as % of maximum, measured in  $0 \text{ Ca}^{2+}$ , 2mM EGTA buffer) to  $34.5 \pm 4.5\%$  max ( $n=7$ ), and rendered the arterioles largely passive to the acute myogenic stimulus of a 50-120 mmHg luminal pressure step (at 120 mmHg, steady-state arteriole diameter before CyD was  $41.1 \pm 2.1\%$  and after  $69.4 \pm 11.6\%$  max). In separate experiments the initial CyD-induced constriction was associated with an increase in intracellular  $\text{Ca}^{2+}$  as detected by changes in fura-2 fluorescence, while further pressure-induced increases in  $\text{Ca}^{2+}$  were uncoupled from active myogenic contraction following CyD. In contrast to the pressure-induced response, CyD did not alter contractions to either phenylephrine ( $10^{-8}$ - $10^{-5}$ M) or KCl (45-75mM) (for phenylephrine pre CyD:  $\text{Log EC}_{50} -6.8 \pm 0.9$ , post CyD:  $-6.6 \pm 0.1$ ,  $n=3$ ,  $p=0.22$ ). Arterioles exposed to CyD in the presence of cholesterol (0.2mM) continued to elicit an active myogenic response (baseline values were  $48.9 \pm 3.7$  and  $51.3 \pm 2.7$  and after 5 min at 120 mmHg were  $37.3 \pm 2.4\%$  and  $50.3 \pm 2.8\%$  max,  $n=4$ , before and after treatment with CyD and cholesterol) consistent with the inhibitory effect of CyD (alone) being mediated through the removal of membrane cholesterol and not a non-specific effect. Collectively the data are consistent with a specific role for cholesterol-rich membrane domains in arteriolar myogenic (pressure-induced) vasoconstriction.