**Epac-mediated Vasorelaxation: Epac Increases Spontaneous Transient Outward Current (STOC) In Mesenteric Artery Smooth Muscle Via Activation Of CAMKII**

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Relaxation of vascular smooth muscle, which increases blood vessel diameter, is often mediated through vasodilator-induced elevations of intracellular cyclic AMP (cAMP) [1]. We have recently shown that exchange protein directly activated by cAMP (Epac), a major cAMP effector, induces smooth muscle relaxation by increasing the frequency of localised Ca2+ release from ryanodine-sensitive Ca2+ release channels (RyRs) located on regions of the peripheral sarcoplasmic reticulum in close proximity to the plasma membrane [2]. These subsurface Ca2+ sparks activate large conductance Ca2+-activated K+ (BKCa) channels in the plasma membrane, evoking spontaneous transient outward currents (STOCs) that hyperpolarize the cell and reduce voltage-dependent Ca2+ entry. Here we aimed to explore the signalling mechanism by which activation of Epac increases spark/STOC activity.

In whole-cell recordings from single, freshly isolated rat mesenteric artery smooth muscle cells (RMASMCs), application of the calcium/calmodulin-dependent kinase 2 (CAMKII) inhibitor KN-93 (500nM) reversed the increase in STOC frequency and amplitude induced by application of the selective Epac activator 8-pCPT-2`-*O*-Me-cAMP-AM (8-pCPT-AM; 5M; p<0.05, n=5). This effect was not mimicked by application of KN-92 (500nM), an inactive analogue of KN-93 (n=3). Inclusion in the pipette-filling solution of autocamtide-2-inhibitory peptide (1M), a highly selective inhibitor of CAMKII, blocked the ability of 8-pCPT-AM to increase STOC frequency/amplitude (n=4). Additionally, in immunoblots using phospho-specific antibodies, 8-pCPT-AM (5M) induced phosphorylation of CAMKII at position Thr286, an autophosphorylation site that indicates CAMKII activation. These data suggest that activation of Epac induces downstream activation of CAMKII and we next assessed the potential mechanism.

Inhibition of protein kinase C (PKC), a CAMKII activator in cardiomyocytes, with bisindolylmaleimide IX (250nM) had no effect on 8-pCPT-AM-induced changes in STOC frequency/amplitude (n=3). 8-pCPT-AM, however, was unable to increase STOC activity in RMASMCs pre-incubated in 2-aminoethoxydiphenyl borate (2-APB; 100M), an inhibitor of IP3 receptors. Application of 2-APB following activation of STOCs with 8-pCPT-AM had a biphasic effect on STOC activity, causing an initial rapid increase in frequency followed by a decline to levels significantly below those measured in 8-pCPT-AM alone (p<0.05, n=6). These data suggest that Epac-induced CAMKII activation is independent of PKC and may be triggered by Ca2+ release from intracellular stores via IP3 receptors. It should be noted that application of 2-APB alone caused a significant transient increase in basal STOC frequency (p<0.01, n=4), which may indicate a constant Ca2+ leak from the stores via IP3 receptors which, when blocked, alters store Ca2+ load and RyR activity.

In conclusion, our data suggest that activation of Epac increases spark/STOC frequency in RMASMCs via the activation of CAMKII. CAMKII may phosphorylate downstream targets involved in regulating store load and/or RyR activity.

1. Morgado M et al (2012) Cell. Mol. Life Sci. 69:247

2. Roberts OLl, et al (2013) *J. Physiol* 591:5107-5123