VEGF-A inhibits PAR2-mediated endothelium-dependent relaxation

Protease activated receptor 2 (PAR2) stimulation by SLIGRL mediates an increase in intracellular Ca^{2+} in endothelial cells (ECs), which opens small and intermediate calcium activated K^+ channels (SK_{Ca}, IK_{Ca}), causing hyperpolarization[1]. This hyperpolarization propagates to the vascular smooth muscle cells to evoke vasodilation. In pathological conditions, the local concentration of vascular endothelial growth factor A (VEGF-A) is elevated to 1-10nM[2,3], and is known to have detrimental effects on endothelial function. Here, we investigated whether luminal perfusion of VEGF-A attenuates vasodilation in resistance arteries.

The mRNA expression of VEGFR1, VEGFR2 and its co-receptor neuropilin-1 (NRP-1) were examined in 3rd order mesenteric arteries and intact 'EC tubes' isolated from wildtype C57BL/6 mice. The effect of VEGF-A on SLIGRL-mediated vasodilation was investigated using pressure myography. For EC Ca^{2+} measurements, arteries from transgenic mice expressing the Ca^{2+} sensor GCaMP2 under the control of endogenous connexin40 were used. Statistical analysis was conducted by t-test or ANOVA as appropriate. $P<0.05$ was considered significant.

PCR identified VEGFR1, NRP-1 and a relatively low level of VEGFR2 expression in both intact mouse mesenteric arteries and in EC tubes. At a high (pathological) concentration of 2nM VEGF-A, dilation to bath addition of 3μM SLIGRL was inhibited (to 32.5±7.3%, n=7) compared with no luminal perfusion (89.9±1.3%, n=13), buffer perfusion (vehicle, 87.0±3.8%, n=6) and a more physiological concentration of VEGF-A (2pM) (89.3±3.9%, n=6). Furthermore, luminal perfusion of 2nM VEGF-A switched EC Ca^{2+} activity in response to 3μM SLIGRL from propagating waves to discrete local events, compared with the perfusion control (23.7±5.9% to 0% waves; 76.3±5.9% to 100% local, n=3). The nitric oxide synthase (NOS) inhibitor, L-NAME, did not affect SLIGRL-mediated vasodilation whether in the presence or absence of VEGF-A. NS309, which positively modulates the opening of EC SK_{Ca} and IK_{Ca} channels, to cause vasodilation, was unaffected by VEGF-A.

We have demonstrated that VEGF-A inhibits SLIGRL-mediated EC Ca^{2+} signalling and vasodilation. Furthermore, a lack of effect by NOS inhibition suggests endothelium-dependent hyperpolarization is the predominant pathway influenced by VEGF-A. VEGF-A does not appear to target or inhibit IK_{Ca} and SK_{Ca} directly, supporting an action to reduce Ca^{2+} signals in the endothelium.

1. McGuire JJ *et al*. (2002). *Br J Pharmacol* 135: 155-69
2. Guo L *et al*. (2014). *Diabetes Technol Ther*. 16: 224-34
3. Dirix LY *et al*. (1997). *Br J Cancer*. 76: 238-43