TRPV1 and TRPV4 in the Cardiovascular System at Baseline and in Inflammation

Claire Sand, Andrew Grant, Manasi Nandi. King’s College London, London, UK

Transient receptor potential vanilloid receptors TRPV1 and TRPV4 are non-selective, highly Ca\(^{2+}\)-permeable cation channels. While traditionally studied in the contexts of nociception and osmosensation respectively, recent evidence suggests that both channels are expressed in vascular tissues and may play a role in the regulation of vascular function. Given that TRPV1 knockout mice exhibit enhanced cardiovascular dysfunction in murine models of sepsis (Fernandes et al., 2012), and both TRPV1 and TRPV4 are sensitized or directly activated by a number of components of the inflammatory milieu, we hypothesized that they may play an important vasoregulatory role in the setting of sepsis.

We characterized ion channel expression in mouse aorta, mouse skin endothelioma cells (sEnd1), and bovine aortic endothelial cells (bAEC). Using RT-PCR we demonstrated TRPV1 and TRPV4 mRNA expression in all vascular samples.

In order to characterize ion channel functionality in bAEC, we used the ratiometric fluorescent Ca\(^{2+}\) dye Fura-2/AM, and selective TRPV1 and TRPV4 agonists capsaicin and GSK1016790A, respectively. bAEC were pretreated with lipopolysaccharide (LPS; 100ng/ml, 24h), tumor necrosis factor \(\alpha\) (TNF\(\alpha\); 30ng/ml, 24h) or culture medium.

A fluorescent ratio change of 0.1 or more was deemed responsive to agonist stimulation (Alexander et al., 2012), and only cells responding to 1\(\mu\)M ATP were included in the analysis. 80% of bAEC (75-120 cells) responded robustly TRPV4 activation with 30nM GSK1016790A (mean ratio change 0.30 ± 0.02; 84% of mean ratio change to 1\(\mu\)M ATP). Surprisingly, LPS treatment reduced the number of bAEC responding to 30nM GSK1016790A to 20% of cells (75-120 cells), though the mean response size was unchanged (0.29 ± 0.03). TNF\(\alpha\) treatment reduced both the number of responding cells to 50% (75-150 cells) and the mean response size (0.17 ± 0.02). Although the marked reduction in response to GSK1016790A did not reach statistical significance in either treatment group, this likely reflects the small sample size (2-3 coverslips) and is expected to prove biologically real with further repetitions.

In contrast to GSK1016790A, bAEC did not respond to capsaicin below 10\(\mu\)M. At 10\(\mu\)M 18% of 175 cells were found to respond, with a small ratio change of 0.18 ± 0.01. With 100\(\mu\)M, a significant increase in the number of responding cells was observed (47% of 225 cells, p<0.01, compared with 1\(\mu\)M capsaicin and p<0.05, compared with 10\(\mu\)M capsaicin, 1-way ANOVA + Bonferroni post-hoc test), and average response size was increased (albeit non-significantly) to 0.21 ± 0.01. There was concern, however, that at such high concentrations capsaicin could induce membrane perturbations and future experiments using selective antagonists will be required to establish the specificity of this response.

Although we found evidence of vascular TRPV1 mRNA expression, neither Western immunoblotting nor Ca\(^{2+}\) imaging provided conclusive evidence of functional protein expression. TRPV4, on the other hand, was found to be both present and functional in different types of vascular cells, and its activity appeared to be altered under inflammatory conditions, although further repetitions will be required to determine statistical significance.
Based on preliminary results, we consider TRPV4 to be a potential target in the vasodysregulation that occurs during sepsis, and will carry out future in vivo experiments to determine its role in that process.
