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Calcium-sensing receptor stimulation in mesenteric artery endothelial cells

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Introduction: Hypertension is a major risk-factor of cardiovascular disease [1]. As hypertension involves vasoconstriction of peripheral resistance arteries, it is vital to determine the cellular mechanisms that control vascular tone as these are potential therapeutic targets. Calcium-Sensing Receptors (CaSR) are expressed in the vasculature and are reported to regulate vascular tone through nitric oxide (NO) production in endothelial cells (ECs) [2]. However, the intracellular cascade linking CaSR stimulation to NO generation remains unclear. The driver of NO production is the rise in intracellular calcium [3]. EC non-selective calcium channels namely heteromeric transient receptor potential channels, TRPV4/C1, are important for calcium influx [4]. This study examines the potential link between the CaSR and TRPV4/C1 channels.

Method: Single ECs, enzymatically isolated from second-order mesenteric arteries of New Zealand white rabbits, and wild-type (WT) and TRPC1-knockout ($TRPC1^{-/-}$) mice (killed in accordance to the Schedule 1 Animals Scientific Procedure Act, 1986), were incubated with an NO indicator, DAF-FM diacetate. CaSRs were activated by increasing extracellular calcium ($[Ca^{2+}]_o$) from 1-6 mM, and TRPV4 channels were activated with GSK1016790A. Pre-treated ECs with Calhex-231 (CaSR-negative allosteric modulator) was used to confirm CaSR involvement, and L-NAME (NO-synthase inhibitor) was used to verify that increasing fluorescence was due to NO production. To investigate the role of TRPV4 and TRPC1, we used the TRPV4 inhibitor, RN1734, and the externally-acting anti-TRPC1 antibody, T1E3. Each data set had n=3 animals of ≥30 cells. Significance ($p<0.05$) was determined using Student's un-paired t-test to compare two independent EC populations.

Results: In rabbit and WT mice ECs, increasing $[Ca^{2+}]_o$ from 1-6mM induced a 30% increase in fluorescence, which was approximately abolished by 300μM L-NAME, 3μM Calhex-231, and 30μM RN1734, and significantly reduced by T1E3 (1:100). In contrast, in $TRPC1^{-/-}$ ECs, increasing $[Ca^{2+}]_o$ from 1-6mM produced a significantly lower increase in fluorescence of ~15%, which was blocked by Calhex-231, L-NAME, and RN1734, but was not affected by T1E3. Moreover, stimulation of TRPV4 with 10 nM GSK1016790A increased fluorescence by ~40%, which was abolished by L-NAME and RN1732, partially inhibited by T1E3, and unaffected by Calhex-231.

Conclusions: Endothelial CaSR-induced NO production is mediated by TRPV4 and TRPC1. The TRPV4-induced NO production involves TRPC1. This suggests a link between activation of TRPV4/C1 channels in CaSR-induced NO production.

References:

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[2] Weston AH *et al.* (2005). *Circ Res* **97**:391-8.]
[3] Lin *et al.* (2000). *J. Biol. Chem.* **275**:17979-17985 [4] Ma *et al.* (2010). *Thromb Vasc Biol*.**30(4)**:851-8