Defining the roles of Ca^{2+} entry in endothelin-1 and thromboxane A_2 receptor mediated vascular contractile responses

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Introduction: Ca^{2+} is an important mediator of vascular contractility, which can enter the cytosol through voltage-gated L- and T-channels and intracellular SR Ca^{2+} release. Upon depletion of the SR Ca^{2+} store, Ca^{2+}-release activated Ca^{2+} (CRAC) channels, which are composed of plasma membrane bound Orai1 and SR-bound STIM1, form functional channels, allowing store refilling. Although endothelin-1 (ET-1) and thromboxane A_2 are both potent vasoconstrictors implicated in various vascular disease states, they mediate Ca^{2+} entry and vasoconstriction through uniquely different mechanisms.

Aim and Method: To identify the role of IP3 receptors, CRAC-, L- and T-channels in ET-1 and thromboxane A_2-mediated vasoconstriction. Using an *in vitro* rat artery model, functional vascular myography coupled with patch clamp analysis were used to identify the activation and inhibition of Ca^{2+} entry pathways mediated by agonists and pharmacological inhibitors of ion channels, respectively.

Results: Brief sequestration of extracellular Ca^{2+} using EGTA (5mM) revealed that approximately 20% ET-1-mediated vasoconstriction involved IP3-mediated SR Ca^{2+} ($p<0.05$; n=4). Following SR Ca^{2+} depletion using cyclopiazonic acid (10mM) (a SERCA pump inhibitor) and 2-aminoethyl diphenyl borate (100mM) (which is known to block IP3 receptors, CRAC channels and potentially non-selective cation channels), vascular contractility was abrogated ($P<0.05$; n=4), indicating a role for both IP3 receptors and CRAC channels. Blocking extracellular Ca^{2+} entry using combined L-/T-channel blockers, mibefradil (1mM) ($p<0.05$; n=7) and efonidipine (0.021mM) ($p<0.05$; n=13) attenuated approximately 65% ET-1-mediated vasoconstriction in the microvasculature (Ball *et al,*, 2009). Patch clamp analysis of I_{CRAC} has revealed that in addition to blocking L- and T-channels, both mibefradil and efonidipine also inhibited CRAC channels. In contrast, thromboxane A_2-mediated vasoconstriction only involved Ca^{2+} entry through L-channels and RhoA-Rho kinase Ca^{2+}-independent sensitization and does not involve IP3 receptors, T or CRAC channels.

Conclusion: In the microvasculature, ET-1 mediates Ca^{2+} entry *via* L, T, IP3 receptors and CRAC channels. In contrast to the traditional L-type Ca^{2+} channel blockers, the more recently developed combined L-/T-channel blockers may provide additional benefit through blockade of CRAC channels, which may effectively enable clinical modulation of SR Ca^{2+} release.

Ball CJ, Wilson DP, Turner SP, Saint DA, Beltrame JF. (2009) Heterogeneity of L- and T-channels in the vasculature: Rationale for the efficacy of combined L- and T-blockade. *Hypertension* **53:** 654-660.