Spatial association of TRPC3, IK_{Ca} and myoendothelial gap junctions in rat mesenteric artery

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Sites of endothelial-smooth muscle cell close association (<30 nm) are integral for endothelium-dependent relaxation, and thus for control of blood flow and pressure. In rat mesenteric artery such specialized myoendothelial microdomain signalling sites consist of localized gap junction connexins (Cxs), endoplasmic reticulum (ER) inositol 1,4,5-trisphosphate receptors, and intermediate conductance calcium-activated potassium channels (IK_{Ca}). With previous data, such close spatial associations are consistent with potential for functional interaction. This study identifies a prospective channel responsible for ER calcium refilling at myoendothelial microdomain signalling sites in adult male SD rat mesenteric artery. Specificity of TRPC3 antibody against C amino acids of mouse 822-835 TRPC3 (Alomone ACC-016; batches AN-02, 03, 07; AN-06 was non-specific), was characterized in fresh rat liver and HEK cells stably transfected with TRPC3 mouse cDNA using Western blotting and cell transfection, respectively. PCR amplification and sequencing verified the presence of transfected mouse TRPC3 gene transcript in HEK cells. Western blotting and confocal and ultrastructural immunohistochemistry determined the TRPC3 expression in rat mesenteric artery ($n \geq 3$, for all experiments). Western blotting in liver confirmed antibody specificity with a faint ~98 kDa band that was partially blocked by peptide, and an apparent monoglycosylated band at ~120 kDa, which is recognized as the functional channel (Dietrich *et al.*, 2003); labelling for which was blocked by peptide. Antibody specificity was further confirmed by labelling transfected HEK cells, whilst untransfected cells failed to label. Western blotting confirmed monoglycosylated TRPC3 expression in rat mesenteric artery. Confocal and ultrastructural immunohistochemistry demonstrated TRPC3 localization at myoendothelial microdomains in close spatial association with IK_{Ca} and myoendothelial gap junction Cxs, consistent with potential for functional interaction.

Dietrich A, Mederos y Schnitzler M, Emmel J, Kalwa H, Hofmann T, Gudermann T. (2003) *J .Biol. Chem.* **278**:47842-52.