SA1

TRP channels in vascular endothelium: TRPV4 in focus

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The TRP cation channel family consists of 6 mammalian subfamilies which comprise ~30 members. Endothelial cells express TRPV4, TRPM4, TRPP2 and several canonical TRPCs. We focus on the functional role of TRPV4 in mouse aorta endothelial cells, MAEC, from wild type TRPV4+/+ mice and from TRPV4 knockout mice TRPV4-/-. TRPV4 integrates a large variety of stimuli ranging from hypotonic cell swelling (HTS), shear stress, temperature, and α-phorbol ligands, to endogenous agonists such as arachidonic acid (AA) and epoxyeicosatrienoic acids. The binding site of the α-phorbol ligands have been identified as a complex binding pocket spanned by TM3 and TM4. TRPV4 is involved endothelium-dependent vasorelaxation, which can be modulated via the cytochrome P450 (CYP) pathway. The loss of TRPV4 in MAEC mice attenuated responses to all TRPV4 activating stimuli. TRPV4-dependent responses can be modulated via CYP enzymes, which metabolize AA to EETs. Upregulation of CYP2C expression by nifedipine in MAEC from TRPV4+/+ mice causes a potentiated response to AA and cell swelling. Sulfaphenazole, an inhibitor of CYP2C9, decreased responses induced by AA and HTS. 1-Adamantyl-3-cyclo-hexylurea (ACU), an inhibitor of the soluble epoxide hydrolase, which converts EETs to dihydroxyeicosatrienoic acids, increased the response induced by AA, HTS and EETs. All these data demonstrate that cytochrome P450-derived EETs modulate the activity of TRPV4 channels in endothelial cells.TRPV4-/- mice have a reduced flow-dependent vasorelaxation indicating that this channels my act as a mechano-sensor. As a model for mechano-sensing, we studied TRPV4 activation by cell swelling will be discussed in more detail. We show that N-terminal binding of Pacsin 3 to TRPV4 inhibits the mechano-activation by cell swelling. A possible mechanism and the underlying structural residues will be discussed.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA2

TRP channels, membrane potential and vasomotor mechanisms in resistance arteries

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Members of the Transient Receptor Potential (TRP) superfamily of cation channels are expressed by vascular smooth muscle cells, endothelial cells, and periarterial nerves. However, the functional significance of these channels in the vasculature is just beginning to be revealed. Members of the canonical TRPC subfamily contribute to calcium influx mechanisms triggered by depletion of vascular calcium stores or following receptor (adrenergic, purinergic) activation in vascular smooth muscle. In cerebral artery myocytes, receptor ligands activate TRPC3 channels. In these cells, receptor-mediated Ca^{2+} entry occurs via direct permeation through the TRPC3 channels and also via voltage-dependent Ca^{2+} channels (VDCC); the smooth muscle VDCC are activated by TRPC3 channel-mediated cation influx which depolarizes the smooth muscle cell membrane (Reading et al. 2005). In systemic arteries, TRPC3 channels in smooth muscle are tonically active and contribute to resting membrane potential. TRPC6 channels appear to be receptor-coupled in the peripheral vasculature. The Ca^{2+}-activated TRP channel, TRPM4, is a member of the melastatin TRP subfamily and is present in vascular cells. TRPM4 plays an essential role in pressure-induced, smooth muscle cell depolarization, and development of myogenic tone *in vitro*. Increased intravascular pressure activates TRPM4 channels, which depolarizes the smooth muscle cells, increases VDCC Ca^{2+} influx and triggers myogenic tone (Earley et al. 2004). Cerebral blood flow measurements *in vivo* indicate that TRPM4 channels contribute to blood flow autoregulation via this myogenic mechanism. Protein Kinase C, which is activated by increased intravascular pressure, enhances TRPM4 activity in vascular smooth muscle cells by increasing the Ca^{2+} sensitivity of the channel. TRP channels of the vanilloid receptor subfamily (TRPV) also play important roles in vasomotor regulation. TRPV4 is present in endothelial and vascular smooth muscle cells and contributes to the endothelial response to vasodilator stimuli as well as the smooth muscle cell response to epoxyeicosatrienoic acids (EETs), which are one type of endothelium-derived hyperpolarizing factor. EETs activate TRPV4 channels. Direct Ca^{2+} entry through the activated TRPV4 channels induces Ca^{2+} release from the sarcoplasmic reticulum in the form of Ca^{2+} sparks; the increased Ca^{2+} spark frequency enhances the activity of BKCa channels, which hyperpolarizes and relaxes smooth muscle cells (Earley et al. 2005). The observations described above indicate that TRP channels play unique and important roles in control of tone in resistance arteries. Ongoing experiments are directed towards further understanding of the mechanisms by which TRP channels in the brain and the peripheral circulation are regulated.

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SA3

Store-operated channels, their molecular identity, mechanism of activation and the role in vascular smooth muscle function

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Store-operated channels (SOC) and Ca^{2+} entry (SOCE) have been described in a wide variety of vascular smooth muscle cells

(SMC), and were shown to play an important role in agonist-induced constriction and nitric oxide-induced relaxation in conduit vessels. Molecular identity and mechanism of SOC activation remain highly controversial, making it hard to assess the exact role of SOCE in microvascular function. Recent advancements in the SOCE field identified Orai1 as a new molecular candidate for SOC (Feske et al. 2006; Vig et al. 2006; Yeromin et al. 2006; Luik et al. 2006b), STIM1 as an activator of the SOCE pathway (Liou et al. 2005; Mercer et al. 2006; Wu et al. 2006; Baba et al. 2006; Luik et al. 2006a; Csutora et al. 2007), and Ca^{2+}-independent phospholipase A_2 beta ($iPLA_2\beta$) as a crucial component for signal transduction from the stores to the plasma membrane channels (Smani et al. 2003; Smani et al. 2004; Vanden Abeele et al. 2004; Martinez & Moreno, 2005; Singaravelu et al. 2006; Boittin et al. 2006; Csutora et al. 2006; Csutora et al. 2007). However, alternative models suggest that SOC may be encoded by TRPC1 (Beech, 2005; Liu et al. 2003; Rosado et al. 2002; Ambudkar, 2007), and instead of $iPLA_2\beta$ and a diffusible messenger, conformational coupling of TRPC1 to the IP_3 receptor, STIM1, or other components of the endoplasmic reticulum may be responsible for SOCE activation (Boulay et al. 1999; Rosado & Sage, 2001; Rosado & Sage, 2000; Yuan et al. 2003; Huang et al. 2006; Lopez et al. 2006; Ong et al. 2007). In this review new experimental evidence will be presented on the crucial role of Orai1, STIM1 and $iPLA_2\beta$ in SOCE mechanism and SMC function. A novel unifying model will be introduced that could account for most of the experimental evidence obtained so far by different groups of investigators that was originally used in favor of multiple mutually exclusive SOCE mechanisms. Using new knowledge and experimental tools we will address the questions on what is the role of SOC channels in Ca^{2+} entry and constriction of cerebral, mesenteric and carotid arteries, and how SOC, TRPC1 and voltage-gated L-type Ca^{2+} channels may peacefully play together, and could all be very important for SMC and microvascular function.

This study was supported by the National Institutes of Health (RO1HL54150 and RO1HL71793).

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SA4

TRP channels and lipid second messengers

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Transient receptor potential (TRP) superfamily of cation channels includes about 30 related proteins which are responsive to a large array of physical and chemical stimuli, typically in a polymodal manner. However, one common emerging feature of TRP regulation appears to be their promiscuous interaction with various phospholipids, which often alters the voltage dependence of the channel gating (Nilius et al. 2007). Several members of the TRPV and TRPM sub-families have been well characterised, especially in terms of their interaction with phosphatidylinositol 4,5-bisphosphate (PIP_2), the ubiquitous anionic lipid. Apart from PIP_2, lysophospholipids (LPLs) such as lysophosphatidylcholine (LPC) and lysophosphatidylinositol (LPI) could both activate the cold- and menthol receptor TRPM8. We recently showed that TRPM8 activity reduced in inside-out patches due to PIP_2 depletion could be fully restored and even augmented above the cell-attached level following application of LPC or LPI to the intracellular side of the membrane. Ca^{2+} store depletion resulting in Ca^{2+}-independent $iPLA_2$ activation/LPLs production appears to be the main coupling mechanism in this alternative to cold "chemical" mode of TRPM8 activation (Abeele et al. 2006).

Regulation of members of the "canonical" TRPC subfamily by PIP_2 is much less characterised. Here we focused on one member, TRPC4, which is typically activated by phospholipase C (PLC)-coupled receptors (Plant & Schaefer, 2003). Its two most abundant transcripts, TRPC4α and TRPC4β (the latter lacks 84 amino acids in the cytosolic C terminus), are widely expressed in various smooth muscles (Walker et al. 2001), and in the gastrointestinal tract TRPC4 is an essential component of the cation channel activated by muscarinic receptor stimulation (Lee et al. 2005). Both for the heterologously expressed channels and the native current the signal transduction downstream of PLC activation is still debated. We thus investigated a possible role of PIP_2 in the regulation of TRPC4. Two mouse isoforms, TRPC4α and TRPC4β, were expressed in HEK293 cells and studied with the patch-clamp recording techniques using Cs^+-rich (125 mM) external and internal solutions (internal $[Ca^{2+}]_i$ was clamped at 100 nM using 10 mM BAPTA to avoid Ca^{2+}-dependent channel modulation). Infusion of 200 μM GTPγS via pipette slowly activated cation currents with peak densities at -50 mV of -93.1±34.8 pA/pF and -28.9±11.3 pA/pF for the TRPC4α and TRPC4β, respectively (n=7-11). The currents showed characteristic doubly-rectifying current-voltage relationships. The non-metabolisable PIP_2 analogue diC8-PIP_2 added to the pipette solution (20 μM) strongly suppressed TRPC4α activation (peak current was reduced to -14.7±3.5 pA/pF, n=7; $P<0.05$ by non-paired *t*-test), but had no effect on TRPC4β activation (peak current of -25.4±7.6 pA/pF, n=11). Activation of both TRPC4 isoforms was strongly inhibited by the PLC blocker U73122 (2.5 μM, n=4; U73343 used as a negative control had no effect, n=4) as well as by pre-treatment with pertussis toxin (100 ng/ml for 16-18 hrs) indicating involvement of distinct G proteins. Consistent with these findings, in *in vitro* binding study we showed that PIP_2 binds directly to the 84 amino acid C-terminal domain that is only present in TRPC4α. Since PIP_2 can act as an anchor for various signalling molecules and cytoskeleton we further investigated possible causal relation between PIP_2 effects and cytoskeleton disruption. Treatment of HEK293 cells with cytochalasin D (5 μM for 2 hrs) completely prevented inhibition of TRPC4α by diC8-PIP_2. Finally, we confirmed by Western blotting that both channel variants were expressed in guinea-pig ileal myocytes in which carbachol-induced currents showed essentially similar properties (i.e. diC8-PIP_2 suppressed the current while cytochalasin D treatment accelerated its activation, produced a negative voltage shift of channel gating and abolished the inhibitory effect of diC8-PIP_2).