the plasma membrane (PM) and endolysosomal membranes. Although wild-type TRPML1 localized exclusively in LEL and were barely detectable in the PM, the GOF mutants were not restricted to LEL compartments, and most significantly, exhibited significant surface expression. As a Ca^{2+}-permeable channel, the constitutive Ca^{2+} permeability due to Pro substitutions may allow TRPML1 proteins traffic to the PM via Ca^{2+}-dependent lysosomal exocytosis, resulting in the surface expression and whole cell currents of TRPML1. Consistent with the hypothesis, surface staining of lysosome-associated membrane protein-1 (Lamp-1) was dramatically increased in cells expressing GOF TRPML1 channels. Interestingly, the extent of the lysosomal exocytosis appeared to be correlated with the degree of channel gain-of-function of TRPML1 mutants. Our results suggest that upon unidentified cellular stimulations, TRPML1 mediates intralysosomal Ca^{2+} release to trigger lysosomal exocytosis. Currently we are investigating whether inhibiting exocytosis could reduce surface expression of GOF TRPML1 mutants, and whether stimulating exocytosis could enhance surface expression of TRPML1.

1768-Pos

TRPM8 Near-Membrane Dynamics and Channel Stabilization after Stimulation

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TRPM8 is a non-selective cation channel expressed on a subset of peripheral neurones, and is the molecular machine that allow us to detect cold signals from our surroundings. Some members of the TRP channel family changes their cellular distribution in response to agonist stimulation. Here, we will describe membrane/near-membrane dynamics of TRPM8-GFP containing particles in both, HEK-293T and F-11 transfected cells. 2D and 3D trajectories together with the velocity of individual protein containing vesicles were obtained by Total Internal Reflexion of Fluorescence Microscopy (TIRFM) and single particle tracking (SPT), and analyzed by plotting of the mean-square displacement against time. Four characteristic types of motion were observed: (a) stationary; (b) simple Brownian diffusion; (c) directed diffusion; and (d) confined diffusion, in which particles undergoing Brownian diffusion are confined within a limited area. Our data suggests that TRPM8, when inserted into the plasma membrane, is confined into small domains of about 3 um in diameter, in which receptor molecules resides in the time scale of 2-8 s. In the absence of stimuli TRPM8 vesicles constantly move along a network that cover the plasma membrane, periodically stopping, most often just briefly. Stimulation halted this hop-diffusion probably by stabilizing TRPM8 channels, as a result, release from plasma membrane became significantly slower. This slow release of TRPM8 determined the overall increase of available receptors.

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1769-Pos

Ligand Stoichiometry of TRPM8

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Temperature-sensitive TRP channels (thermoTRPs) play a key role in somatosensory thermosensation. Several thermoTRPs, including the heat-activated TRPV1 and the cold-activated TRPM8, are not only sensitive to changes in temperature but also to ligands that evoke a thermal sensation, such as the 'hot pepper' compound capsaicin (TRPV1) and the cooling agent menthol (TRPM8). Recent data indicate that the binding site of these lipophilic compounds is located in the so-called 'sensor domain' made up of transmembrane domains S1-S4. For example, mutating S4 arginine at position 842 in TRPM8 to histidine (mutant R842H) produces a channel with unaltered cold and voltage sensitivity, but with a ~100-fold reduction in menthol affinity. Given that TRP channels are tetramers and that the sensor domains are spatially apart, a functional TRPM8 channel can potentially bind four menthol molecules. However, the contribution of each individual menthol binding event to channel gating is unknown. To address this question, we made vectors encoding tandem tetramers with all possible combinations of WT and R842H subunits. Expression of these vectors in HEK293 cells exclusively produced protein of the expected tetrameric molecular weight, and whole-cell recordings further confirmed that the tandem linkage of subunits did not significantly affect channel gating. This approach will further be used to determine the exact stoichiometry of menthol-induced TRPM8 gating.

1770-Pos

Exploring Structural Relationships between TRP and Kv Channels

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Transient Receptor Potential (TRP) ion channels are homotetrameric, non-selective cation channels that are expressed in diverse cell types in eukaryotes, ranging from yeast to humans. TRP channels have been implicated in many physiological roles such as thermosensation, mechanosensation, chemesthesis, hearing, and sensing pain. Indeed, TRP channels are gated by a plethora of stimuli. For example, TRPV1 is activated by heat, voltage, and small molecules such as capsaicin, and TRPM8 is activated by cold, voltage and cooling agents such as menthol. The molecular mechanisms underlying this polymodal gating of TRP channels are poorly understood. In contrast, the mechanism of voltage-activation of the voltage-gated potassium (Kv) channels is well understood. The architectural similarity of TRP channels and Kv channels, in addition to several mutagenesis-based reports published on TRP channels, raise the possibility that the mechanisms of voltage gating of these two families of ion channels may have common features. To address this hypothesis, we have created a series of chimeras between the Kv channel, Kv2.1, and two TRP channels, TRPV1 and TRPM8, and studied them using electrophysiological techniques. Replacing the critical S3b-S4 paddle motif of Kv2.1 with analogous regions of TRPV1 or TRPM8, results in channels that activate in response to membrane depolarization. In both instances, the slopes of voltage-activation relations were decreased in the chimeras as compared to wild-type Kv2.1.These results are consistent with the hypothesis that TRP channels contain a structural motif related to the paddle motifs in Kv channels, and that this motif can sense voltage in the context of a Kv channel. We are currently testing whether this motif can sense voltage in TRP channels and exploring structural relationships between these two families of channels.

1771-Pos

TRPM3 Expression and Function in Vascular Smooth Muscle

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The Transient Receptor Potential (TRP) channels form a diverse superfamily of cation channels. Currently there are few well characterised, selective, physiological regulators of TRP channels, which can impede the discovery of native channel functions. TRPM3, a member of the melastatin subfamily of TRP channels, is reported to form constitutively active Ca^{2+}-permeable cation channels activated by sphingolipids, hypotonic shock, and the neurosteroid pregnenolone sulphate. Expression is predominantly in brain and kidney, although expression has recently been reported in pancreatic β-cells. Here we report TRPM3 expression and function in vascular smooth muscle cells. Anti-TRPM3 blocking antibody and short-interfering RNA targeted to TRPM3 were used to confirm the involvement of TRPM3 in the pregnenolone sulphate elicited Ca^{2+} influx in cultured human vascular smooth muscle cells. Although pregnenolone sulphate is a useful pharmacological and potentially therapeutic agent, the physiological significance in the vasculature is unknown. We therefore screened for novel modulators, and found that cholesterol inhibited both pregnenolone sulphate-induced and constitutive TRPM3 activity. The data suggest TRPM3 is a novel calcium-entry channel of vascular smooth muscle cells.

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1772-Pos

Clotrimazole Potentiates TRPM3 Responses to Pregnenolone Sulfate

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Clotrimazole (CLT) is an antifungal compound commonly used in over-the-counter medications for the topical treatment of fungal infections of the skin, vagina, and mouth. CLT exerts its antifungal actions by inhibiting P450-dependent enzymes. TRPM3a2 (1), a splice variant of TRPM3, is rapidly and reversibly activated by pregnenolone sulfate (PS) and nifedipine (1). Here, we demonstrate that CLT strongly potentiates the response of TRPM3a2 to pregnenolone sulfate (PS) stimulation. Direct application of CLT to TRPM3a2 has no effect, however preapplication of CLT followed by PS stimulation strongly potentiates the TRPM3a2 response. The potentiation by CLT is reversible, repetitive and independent of external calcium. At CLT concentrations above 1 mM, the intensity of potentiation does not dependent CLT dose but rather on PS concentration. The response to PS after priming with CLT was relatively slow, suggesting a modulation in the signaling cascade rather than a direct effect. We conclude that CLT potentiates the TRPM3a2 response in an indirect manner, possibly by preventing further metabolization of pregnenolone sulfate by inhibiting P450-dependent enzymes.

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