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TRPM7 Current Inactivation: Evidence for Inside-Out Signaling

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electrophysiological approach revealed that zTRPM2 is activated by pollutants, such as hydrogen peroxide (H2O2), nitrite (NO2-) a pollutant in nitrogen cycle and NO which is released from SNAP (s-nitroso-n-acetyl-dl-penicillamine) or SNAC (s-nitroso-n-acetylcysteine). However, temperature, pH conditions and hypoxia are not effect to zTRPM2 channel activity. The activation of zTRPM2 was inhibited by mammalian TRPM2 inhibitor econazole and clotrimazole. These results suggest a functional role of zTRPM2 in the detection of oxidants that are known as pollutants and zTRPM2 inhibitors may assists in protecting fish from the polluted environments.

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Biochemical Characterization of the Interaction of TRPM3 with $G_{\beta\gamma}$ Proteins

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TRPM3 is a member of the melastatin subfamily of transient receptor potential (TRP) channels. It is expressed in various cell types as one of numerous isoforms, many of which form calcium-permeable ion channels. In somatosensory neurons, TRPM3 contributes to sensation of noxious heat and inflammatory hyperalgesia. Furthermore, it is expressed in pancreatic beta cells and has been implicated in the regulation of glucose-induced insulin release. In the eye, mutations in TRPM3 are linked to inherited forms of cataract and glaucoma. Recently, it was shown that binding of $G_{\beta\gamma}$ subunits of $G_{i/o}$ proteins to TRPM3 inhibits the channel. $G_{i/o}$ proteins act downstream of GPCRs like the μ-opioid receptor, whose activation by morphine leads to TRPM3 inhibition. Inhibition of TRPM3 itself might therefore be a new therapeutic principle for a novel class of analgesic drugs. By overexpressing TRPM3 proteins with various deletions and $G_{i/o}$-coupled receptors in HEK293 cells, we screened TRPM3, in order to identify regions that were dispensible for channel function, but necessary for the inhibition by $G_{\beta\gamma}$. In these experiments, TRPM3 was activated with pregnenolone sulfate, the only known endogenous substance to activate these channels. After identifying a stretch of 10 amino acids in the N-terminus of TRPM3, we verified its importance for inhibiting TRPM3 by analyzing single point mutations. To further characterize the binding between $G_{\beta\gamma}$ and TRPM3 *in vitro* with a biochemical approach, we demonstrated that the stretch of 10 amino acids of TRPM3 binds directly to $G_{\beta\gamma}$. This site mediates binding to natively prenylated $G_{\beta\gamma}$, but also to an artificially soluble $G_{\beta\gamma}$ mutant. We conclude that $G_{\beta\gamma}$ acts as an allosteric inhibitor of TRPM3 and its binding site may also serve as a potential target for small molecule allosteric modulators.

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Volatile Anaesthetics Inhibit Thermosensitive TRPM3 Ion Channels

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Volatile anaesthetics (VAs) are the most widely used compounds to maintain general anaesthesia during operation both in human therapeutic interventions and animal experiments. Although the mechanism of their action is not fully understood in details, it is generally believed, that VAs depress central nervous system functions mainly via acting on ion channels of the neuronal membrane, like GABA receptors, NMDA receptors or 2-pore-domain K^+ channels. Recent research also reported their action on nociceptive and thermosensitive TRP channels, TRPV1, TRPA1, and TRPM8. In our current study, we investigated the effect of VAs on TRPM3, a less characterized member of the thermosensitive TRP channels. We investigated the effect of VAs on the activity of TRPM3 both in recombinant and native systems using intracellular Ca^{2+} measurements and patch clamp electrophysiology. All the investigated VAs inhibited the pregnenolone sulphate and CIM0216 induced Ca^{2+} signals and transmembrane currents in a dose dependent way in HEK293T cells overexpressing recombinant TRPM3. Among the applied VAs, halothane was found to be the most effective blocker of TRPM3 ($IC50 \approx 0.5$ mM). We also investigated the effect of VAs on native TRPM3 channels expressed by sensory neurons of the dorsal root ganglia. We found that VAs activated certain sensory neurons independently of TRPM3, but applied in 1 mM, they strongly inhibited the agonist induced TRPM3 activity in a reversible way. Our results further enhance our knowledge about VAs' mechanism of action and might contribute to the development of novel analgesics targeting TRPM3.

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G-Protein Beta-Gamma Subunits Inhibit the Heat-Sensitive TRPM3 Ion Channels

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Transient Receptor Potential Melastatin 3 (TRPM3) is a heat-activated non-selective, Ca^{2+} permeable cation channel also stimulated by chemical agents such as pregnenolone sulphate and CIM0216. Here we show that activation of Gi-coupled cell surface receptors inhibits TRPM3 currents in a mammalian expression system, which was alleviated by co-expression of proteins that bind βγ subunits of heterotrimeric G-proteins (Gβγ). Co-expression of Gβ1γ2, Gβ3γ2, Gβ4γ2, but not Gβ5γ2 or constitutively active mutants of Gαo or Gαi, inhibited pregnenolone sulphate-induced TRPM3 currents. Purified Gβγ proteins applied to excised inside out patches also inhibited TRPM3 activity, indicating a direct effect. Baclofen, somatostatin, and DAMGO, agonists of Gi coupled receptors, inhibited Ca^{2+} signals induced by pregnenolone sulphate and CIM0216 in dorsal root ganglion (DRG) neurons. The GABAB receptor agonist baclofen also inhibited CIM0216-induced currents in DRG neurons, and nocifensive responses elicited by this TRPM3 agonist *in vivo*. Our data show that Gβγ inhibits TRPM3 channels upon Gi-coupled receptor activation.

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TRPM7 Current Inactivation: Evidence for Inside-Out Signaling

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TRPM7 channels conduct metal cations such as Na^+, K^+, Ca^{2+} and Mg^{2+}. In the presence of external Ca^{2+}/Mg^{2+} TRPM7 has a steeply outwardly rectifying current-voltage (I-V) relation. In the absence of Ca^{2+}/Mg^{2+} the IV becomes semi-linear. This has been explained by the removal of pore blockade by divalent cations (e.g. $Ca^{2+}{}_o/Mg^{2+}{}_o$). TRPM7 channels are inhibited by cytoplasmic Mg^{2+} in a voltage-independent manner, primarily by a reversible reduction in the overall number of conducting channels. Here, we have examined the consequences of external Ca^{2+} removal and reintroduction on TRPM7 current kinetics. In whole-cell patch clamp with low internal Mg^{2+}, we rapidly and repeatedly exchanged 2 mM Ca^{2+} with divalent cation free (DVF) Na^+ or Cs^+ containing solutions to compare the time dependent changes in current. This maneuver resulted in a declining current in DVF following Ca^{2+} exposure, which we call *inactivation*. This was followed by a slowly rising current when $Ca^{2+}{}_o$ was reintroduced. The decay of monovalent current occurred over 1-4 minutes and was monoexponential in most cases. Internal Mg^{2+} and spermine, partially inhibiting TRPM7, revealed more pronounced decay/potentiation cycles than seen with Mg^{2+}-free internal solutions. Inactivation was observed with both Na^+ and Cs^+ as charge carriers and extracellular Mg^{2+} could substitute for the potentiating Ca^{2+} effect. Current decay in DVF was distinct from TRPM7 rundown, which we previously documented in patch-clamp recordings. Inactivation persisted in perforated-patch recordings, which allow the maintenance of physiological Mg^{2+} concentrations and prevent rundown. We have also characterized TRPM7 point mutations that abolish Ca^{2+} potentiation/inactivation. TRPM7 current behavior in response to switches between divalent-containing and divalent-free solutions resembled the Ca^{2+} potentiation/depotentiation described for calcium release activated calcium (CRAC) channels. We conclude that TRPM7 response to *extracellular* ions depends on *intracellular* Mg^{2+}/spermine concentrations, presenting a novel case of inside-out signaling.

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PIP_2 Depletion Contributes to Inhibition of TRPM8 Activity by G_Q Protein Coupled Receptors

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Transient Receptor Potential Melastatin 8 (TRPM8) channels detect cold temperatures (< 26°C). They are activated by cold, depolarization, and chemical agents such as menthol, icilin, and WS12. Phosphatidylinositol 4, 5-bisphosphate (PIP_2), a minor component of the cell membrane, is required for TRPM8 activity. Our lab found earlier that intracellular dialysis of PIP_2 through the whole cell patch pipette inhibits desensitization of TRPM8. Activation of Gαq-protein coupled receptors stimulate phospholipase C (PLC), which decreases cellular PIP_2 levels. A recent study proposed that TRPM8 activity is inhibited by G-protein coupled receptors via direct binding to Gαq to the channel. In the presence of PIP_2, the inhibition of TRPM8 by activated Gαq was prominent in excised patches, but it is not known if a decrease in PIP_2 plays a role in TRPM8 inhibition in a cellular context. We hypothesize that PIP_2 depletion contributes to inhibition of TRPM8 by Gαq-protein coupled receptors. We