1773-Pos
Complex Regulation of TRPV1 by Phosphoinositides
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TRPV1 is a nonselective highly calcium permeable cation channel present in the peripheral nervous system exclusively in polymodal nociceptors. A sensory integrator of several noxious stimuli, TRPV1 plays a crucial role in the development of inflammatory pain and hypersensitivity. Plasmamembrane phosphoinositides are recognized as important regulators of TRPV1 function; the precise nature of their effect is, however, controversial.
Phosphatidylinositol-4,5-bisphosphate (PIP_2) has initially been proposed to tonally inhibit TRPV1 via a C-terminal inhibitory domain. Furthermore, receptor-mediated depletion of PIP_2 was proposed to be involved in sensitization of TRPV1 in response to pro-inflammatory agents. However, in subsequent studies including our own, direct intracellular application of PIP_2 reproducibly potentiated TRPV1 currents rather than inhibiting them. In addition, PIP_2 depletion concurrent with robust TRPV1 activation is an important contributing factor to channel desensitization consistent with the activating effect of PIP_2. We attempt to address this controversy utilizing multiple independent approaches to selectively regulate plasmamembrane PIP_2 levels in heterologous expression systems. Our results show that TRPV1 currents in intact cells elicited by low to moderate, but not high agonist concentrations are potentiated in response to PIP_2 depletion. Conversely, increasing PIP_2 levels inhibits low but not high agonist-induced TRPV1 currents. These effects are reduced or absent in the mutant channel lacking the putative C-terminal inhibitory domain. The inhibitory effect of PIP_2 however was never observed in excised patches even at low agonist concentrations. Our results are consistent with an agonist concentration-dependent dual regulatory effect of PIP_2. The inhibitory effect furthermore appears to be indirect. Such dual effects of PIP_2 have previously been described for voltage-gated calcium channels as well as other TRP channels and raise important questions as to the identity of interacting molecules conferring the inhibitory effect and the physiological relevance of such complex regulation.

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TRPV1 Activation by Allyl Isothiocyanate
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Allyl isothiocyanate (mustard oil, MO) is a highly reactive electrophilic compound known to cause irritation, pain and inflammation. These effects are thus far thought to be mediated by activation of TRPA1, a Transient Receptor Potential (TRP) cation channel expressed in nociceptive neurons. Recent research has shown that TRPV1, the heat and capsaicin receptor, can be also activated by reactive compounds such as allicin and leek and onion extracts. Here, we show that both human and mouse TRPV1 are activated by MO, at concentrations at which TRPA1 undergoes fast desensitization and block. In Ca2+ imaging experiments of intact HEK293 cells, MO induces an increase of the intracellular Ca2+, which was not present when Ca2+ was omitted in the bath solution. Activation of TRPV1 by MO is dose-dependent and is caused by a shift of the voltage dependence of channel activation to more negative potentials, similar to the activation of TRPV1 by capsaicin. Stimulation of TRPV1 by MO can be observed in inside-out patches, indicating a membrane-delimited mechanism of activation. Furthermore, the heat-induced activation of TRPV1 could be sensitized with sub-activating MO concentrations.
Notably, MO was able to stimulate a large population of sensory neurons isolated from Trpa1 KO mice. This population was significantly reduced in Trpa1/Trpv1 double KO mice, indicating the physiological importance of TRPV1 activation by MO. WT, Trpa1 and Trpv1 KO mice displayed significantly stronger aversion to MO than double KO mice in forced drinking and open field exploration assays. The identification of TRPV1 as a novel target of MO is essential for the full understanding of the mechanisms of action of this compound in vivo and prompts to re-evaluate the results of previous research, in which MO was used as specific activator of TRPA1.

1775-Pos
Molecular Determinants of the Activation Gate of the TRPV1 Channel
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The ability of ion channels to transit among different conformations allows them to regulate different types of cellular functions. Transient Receptor Potential Vanilloid 1 (TRPV1)channels participate in several types of physiological responses such as pain detection and inflammation, little is known about how their structural components convert different types of stimuli into channel activity. To localize the activation gate of these channels, we used the substituted cysteine accessibility method (SCAM) and inserted cysteines along the S6 segment of the TRPV1 channel and assessed their accessibility to thiol-modifying agents and silver. Our results show that access to the pore of the TRPV1 is gated by the S6 both in response to capsaicin binding and to increases in temperature, that the pore-forming S6 segments are helical structures and that there are two constrictions in the pore. One located at residue L681 which hampers the access to large molecules and one located at residue Y671 which impedes the entrance of smaller ions a and constitutes the activation gate of these channels. These data have also allowed us to produce a model of this region in the structure of TRPV1 based on functional findings.

1776-Pos
Interactions between Quaternary Ammoniums and the Gate of the TRPV1 Channel
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TRP channels play a fundamental role in neuronal signalling and in the detection of painful stimuli and inflammatory processes. The TRPV1 (vanilloid 1) channel functions as an integrator of noxious chemical and physical signals known to cause pain. Structural and functional information of the pore domain shows that access to the pore is gated by the S6 in response to capsaicin and temperature. Our group recently found the presence of two intracellular constrictions: L681 which obstructs the ion conduction pathway for large molecules and Y671 which obstructs the ion conduction pathway for small permeating molecules and constitutes de activation gate of TRPV1 channels. Quaternary ammoniums (QA) are a family of pore blockers that have been successfully used in structure-function studies. Previous results using QA on TRPV1 show that these compounds block the channel in a state-dependent fashion. Since it has been shown that aromatic residues interact with quaternary ammoniums by direct hydrophobic interactions we decided to test if the actions of tetrabutylammonium (TBA) are mediated by the interaction with the aromatic residue Y671 . Our preliminary results indicate that this residue is not involved in the binding of TBA to TRPV1.

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Sensitization of Vanilloid Receptors TRPV3
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Vanilloid receptors of the transient receptor potential family have functions in thermal sensation and nociception. Among them, TRPV3 is expressed in skin keratinnocytes and has also been implicated in flavor sensation in oral and nasal cavities as well as being a molecular target of some allergens and skin sensitizers. The channel displays a unique property that repeated stimulation results in gradual increases of its activity, a process that is known as sensitization and is observed in both native cells and cell lines. Transient calcium release from internal stores has been thought to underlie the sensitization process through a mechanism involving relief of Ca2+-dependent inhibition of the channel due to calmodulin binding at the distal N-terminal. In support of the hypothesis is the differential effect of the calcium chelators BAPTA and EGTA, where BAPTA, which has a fast buffering kinetics, is able to modulate the sensitization, while EGTA is ineffective. Here we suggest an alternative mechanism for the sensitization process. We distinguish two types of sensitizations; one is reversible and the other irreversible. The irreversible sensitization is intrinsic to the gating of the channel, while the reversible one such as that mediated by BAPTA is attributable to a modulation effect. We show that analogs of BAPTA that apparently lack Ca2+ buffering capability similarly sensitize the channel. We conclude that the sensitization of the channel, including the effects of BAPTA, also involves a membrane-delimited mechanism.

1778-Pos
Ca2+ Inhibition of Cation Conductance through TRPV1 Receptors
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TRPV1 receptors are polymodal cation channels that show a marked permeability to Ca2+. In the present study, we used single channel electrophysiology and whole cell patch clamp photometry to further study the interaction of extracellular Ca2+ ([Ca2+]o) with the recombinant TRPV1 receptor expressed in HEK293 cells. In the presence of and 140 mM [NaCl]o, we observed that