to what has been widely shown for other ion channels, by directly interacting with the C-terminus of the channel. These data highlight TRPV1 as a direct molecular target of the pain-producing molecule LPA constituting the first example of LPA binding directly to an ion channel to acutely regulate its function.

1734-Pos Board B504
Activation of TRPV1 by a Recombinant Double-Knot Toxin from a Chinese Bird Spider
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TRPV1 is a tetrameric voltage-sensitive cation channel that is activated by heat and vanilloids. The architecture of TRP channels are thought to be related to Kv channels, with each subunit containing six transmembrane segments. In addition to being activated by capsaicin, TRPV1 is activated by Double-Knot Toxin (DkTx), a protein toxin purified from the Chinese Bird Spider, *Selenocosmia huwena* (Bohlen et al. 2010, Cell 141, 834-35). DkTx is unique in that it contains two Inhibitor Cysteine Knots (ICK) motifs connected by a peptide linker. Although these ICK motifs are related to those found in tarantula toxins that target voltage sensors in Kv channels, DkTx does not appear to interact with classical voltage-activated cation channels. We set out to explore the mechanism of DkTx activation of TRPV1, and began by producing the toxin in *E. Coli* and testing for activity against TRPV1. DkTx was expressed as a fusion with bacterial Ketosteroid Isomerase (KSI), cleaved from KSI using hydroxylamine, and purified using reverse phase HPLC. Reduced DkTx was folded in vitro in a solution containing (GSH/GSSG) and guanidine HCl, and the folding reaction monitored by HPLC. Using this procedure we obtained a predominant species of the toxin that was further purified by reverse phase HPLC. When tested for activity on TRPV1 expressed in *Xenopus laevis* oocytes, DkTx produced robust and slowly reversible activation of the channel when voltage clamped at −60 mV. At a concentration of 2μM, DkTx produced comparable activation to 2 μM capsaicin, suggesting that the apparent affinity of the recombinant toxin is similar to that reported for the native toxin. We are currently working to solve the structure of DkTx using NMR and further investigating it's mechanism of activation.

1735-Pos Board B505
Effect of Cholesterol on the Pore Dilation of TRPV1
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The transient receptor potential vanilloid 1 (TRPV1) ion channel is expressed in nociceptors, where pharmacological modulation of its function may offer a way of easing pain and neurogenic inflammation processes in the human body. The aim of this study was to investigate how depletion of cholesterol from the cell membrane may modulate ion-permeability of the TRPV1 ion channel expressed in CHO-cells. The ion-permeability properties of TRPV1 measured with whole-cell patch clamp on a Chinese hamster ovary (CHO) cell line expressing TRPV1. Sustained capsaicin-induced activation of TRPV1 with N-methyl-D-glucamine (NMDG) as the sole external cation, generated a biphasic current, with a first outward current and a second inward current. Similarly, sustained proton-activation (pH 5.5) of TRPV1 in the absence of external calcium also generated a biphasic current, with a first fast current peak followed by a second larger one. Also, patch clamp recordings of reversal potentials revealed a change of the ion-permeability during prolonged activation of the TRPV1 channel in extremely low extracellular calcium. Our findings show that depletion of cholesterol from the cell membrane inhibited both the second current resulting from sustained agonist-activation, and the change in ion-permeability of the TRPV1 channel. Our results propose a novel mechanism by which cholesterol-depletion may modulate the ion channel function of TRPV1, which may constitute a novel pharmacological based approach for the treatment of pain and neurogenic pain.

1736-Pos Board B506
Probing TRPV1 Structure with Limited Proteolysis
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The transient receptor potential (TRP) family of ion channels is a large class of ion channels that is involved in various biological functions. These ion channels are activated by a wide range of stimuli but the general membrane topology and permeability to cations are common features.
This study focuses mainly on one of the members, TRPV1, which responds to noxious heat and ligands like protons and capsaicin. This ion channel is situated in nociceptive sensory neurons and thereby upon activation; TRPV1 causes a sensation of pain. TRPV1 has been the subject of intense research due to this involvement in pain reception although much is left to be discovered regarding its detailed structure and the regulatory mechanisms during desensitization and tachyphylaxis.
This study utilizes the principle of limited proteolysis as a way to attain information about surface exposed regions of TRPV1. Since flexible, exposed regions of a protein are more prone to proteolysis, information regarding flexible and dense regions of a protein can be obtained if proteolysis is performed at different time scales. Exposed regions often correlates with ligand binding sites making limited proteolysis a usable tool for fast screening of possible binding sites.
The study is performed by immobilizing membrane vesicles containing the protein in a flow cell, subsequently exposing them to trypsin and finally analyzing eluted peptides with mass spectrometry. A protocol using sequential digestions rather than standard batch digestions proved to yield higher sequence coverage.

1737-Pos Board B507
Phosphoinositide Regulation of TRPV1
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TRPV1 is a nonselective calcium permeable cation channel present in polymodal nociceptors that plays a crucial role in the development of inflammatory pain and thermal hypersensitivity. Plasmamembrane phosphoinositides are recognized as important regulators of TRPV1; the precise nature of their effect is, however, controversial. We and others have shown that phosphatidyl-inositol-(4,5)-bisphosphate [$PI(4,5)P_2$] as well as other phosphoinositides activate TRPV1 in excised patches. Calcium influx via TRPV1 channels activates PLC and results in a robust depletion of both phosphatidyl-inositol-(4)- phosphate [PI(4)P] and $PI(4,5)P_2$ in expression systems. Hydrolysis of $PI(4,5)P_2$ under these circumstances is now accepted to be an important contributor to channel desensitization. However, the involvement of PI(4)P in the process remains an issue of debate. In addition, preceding data corroborated by our own observations suggest an additional indirect inhibitory effect of $PI(4,5)P_2$, but not other phosphoinositides. In the present work we show that potentiation of whole-cell TRPV1 currents by bradykinin is partially impaired by dialyzing diC_8-$PI(4,5)P_2$ through the patch pipette in both expression systems and native cells (DRG neurons), supporting the notion that in addition to the well documented role of PKC-mediated phosphorylation in TRPV1 channel sensitization, $PI(4,5)P_2$ depletion may also be involved in this phenomenon. Using mouse DRG neurons we confirm that in addition to $PI(4,5)P_2$, PI(4)P is also an important activator of TRPV1. Both lipids are simultaneously depleted in response to TRPV1-activation, while dialysis of both lipids via the patch pipette reduced desensitization of TRPV1-positive neurons. In contrast Bradykinin receptor activation differentially regulated $PI(4,5)P_2$ and PI(4)P abundance in DRG neurons, resulting in isolated $PI(4,5)P_2$ depletion. These observations suggest important differences in phosphoinositides handling during calcium-activated and receptor-induced PLC activation, respectively, and may partially explain the differential TRPV1 regulation under these conditions.

1738-Pos Board B508
Biophysical Characterization of TRPV2 Ion Channel
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TRPV2 is a member of the superfamily of the Transient Receptor Potential (TRP) ion channels. These channels are assembled into homotetramers and allow cations across the membrane in response to physico-chemical stimuli such as heat, pressure, osmotic changes, etc. TRPV2 is an orphan receptor, since no specific endogenous ligand has been identified yet. To better understand the role of TRPV2 and to go further into its function, sequence analysis of orthologs for TRPV2 has been performed in order to define common and differential architectural regions. Preliminary biophysical characterization such as thermal stability, and secondary structure composition analysis has been carried out on the different TRPV2 orthologs to identify key structural points in the TRPV2 topology.

1739-Pos Board B509
Pore and Gating Properties of TRPM3 Isoforms
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TRPM3 has recently been identified as a nociceptor channel in sensory neurons, where it mediates pain responses to noxious heat and the neurosteroid pregnenolone sulphate (PS). Several TRPM3 splice isoforms have been identified, including splice variants with differences in the pore region exhibiting concommitant differences in permeability properties. We analysed and compared the heat- and PS-sensitivities of different TRPM3 isoforms, as well as their sensitivity to potential gating modulators. Our results provide insight into the structural determinants for TRPM3 gating, and provide novel tools for studying the role of this channel *in vivo*.