regulated by androgens in prostate. We propose for the first time a physiological role of androgen and its receptor in the induction and stabilization of TRPM7 protein in carcinogenesis. Finally, our studies suggest that TRPM7 may have a new therapeutic application for prostate cancer patients in the future.

1695-Pos Board B425
Properties of the Steroid Binding Site of TRPM3 Channels
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TRPM3 channels are non-selective cation channels that have been implicated in a variety of functions, including the detection of noxious heat and the secretion of insulin. These channels can be activated by the endogenous steroid pregnenolone sulfate and the voltage-gated calcium channel blocker nifedipine, but it is unclear how these compounds interact with the channels.

In electrophysiological and calcium imaging experiments we provide evidence that pregnenolone sulfate and nifedipine bind to distinct binding sites. Furthermore, we show that pregnenolone sulfate needs to bind to a chiral, and thus proteinaceous, binding site in order to activate TRPM3 by utilizing the enantiomer of pregnenolone sulfate. Employing further structural analogs of pregnenolone sulfate, we additionally determined that the binding site of pregnenolone sulfate needs to accommodate a large, negatively charged substituent at the C3 position of the steroid backbone.

By combining these data we devised a strategy to find candidate amino acid residues of TRPM3 important for channel activation, possibly by being part of the pregnenolone sulfate binding site. We systematically mutated positively charged amino acids accessible from the extracellular side (from which pregnenolone sulfate is capable of activating TRPM3). We then evaluated whether mutated channels that displayed a reduced response to pregnenolone sulfate still responded to nifedipine comparably to wild-type channels. We identified one amino acid with these properties, which is predicted to be located at the transmembrane-extracellular domain interface.

These data will lead to the characterization of the steroid binding site responsible for activating TRPM3 channels. Likely, this will help to identify more potent and specific pharmacological tools, with the ultimate goal to manipulate these channels for experimental and therapeutic purposes.

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Single-Cell Na\(^+\) Flux Assay for Measurement of TRPM7 Channel Activity
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TRPM7 is a Ca\(^{2+}\)/Mg\(^{2+}\) permeable ion channel highly expressed in immune cells. Like many other TRP superfamily members, it conducts physiologically important monovalent cations Na\(^{+}\) and K\(^{+}\). Na\(^{+}\) influx through most TRP channels has not been measured directly, however. We set out to quantify Na\(^{+}\) influx through TRPM7 channels by using fluorescence microscopy and SBF1 ratiometric Na\(^{+}\) indicator dye in HEK293 cells expressing mTRPM7. Kv1.3 was co-expressed in order to make the membrane more hyperpolarized.

In the presence of divalent cations TRPM7 current is strongly outwardly rectifying, and in their absence becomes semi-ohmic with a large inward component. In TRPM7-expressing HEK cells, removal of external divalents resulted in consistently steep increases in intracellular Na\(^{+}\) signal detected by SBF1. Spermine blocks monovalent TRPM7 currents with IC\(_{50}\) of 2 \(\mu M\). Accordingly, Na\(^{+}\) flux was reduced by ~50\% when 3 \(\mu M\) spermine was included in the divalent-free buffer. Higher spermine concentrations (10 and 30 \(\mu M\)) did not result in higher conductance. More surprisingly strong inhibition was observed for free Mg\(^{2+}\), which dose-dependently reduced Na\(^{+}\) flux by 31, 62 and 100 \(\mu M\), suggested that Na\(^{+}\) flux occurs through TRPM7 channels. The phenolic compound carvacrol, previously reported to be a blocker of TRPM7 channels, potently suppressed the Na\(^{+}\) signal at 150 \(\mu M\) but not at 30 \(\mu M\). In TRPM7-overexpressing cells, switching from no divalents to a 100 \(\mu M\) Ca\(^{2+}\) containing buffer caused Ca\(^{2+}\) elevation, measured by Fura-2, but it was not significantly different from control untreated HEK cells. Cytoplasmic alkalization, which activates TRPM7 channels, was effective in increasing the Na\(^{+}\) flux through these channels. Na\(^{+}\) flux could also be measured in Jurkat T cells, which express TRPM7 and Kv1.3 channels endogenously. The presented Na\(^{+}\) flux assay will be useful for measurement of TRPM7 channel activity in intact cells.

1697-Pos Board B427
Properties of the C-Terminus of the YVC1P TRP Channel
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Transient receptor potential (TRP) channels often serve as environmental sensors. In the budding yeast Saccharomyces cerevisiae, the YVC1p TRP channel releases vacuolar calcium into the cytoplasm in response to hyperosmotic shock. While YVC1p has the 6TM architecture of other TRP channels, it has a comparatively long C-terminal segment that contains both a DDDD motif and a putative coiled-coil domain. The precise mechanism of gating in this channel remains unknown, but the DDDD motif is clearly involved in Ca\(^{2+}\)-dependent activation and the coiled-coil, positioned at the extreme C-terminus, could also play an important role.

In this study we examine the biochemical and functional properties of the Yvc1p C-terminus. We are applying a genetic approach to confirm the cellular location of the C-terminus within the context of full-length protein. Using a panel of constructs with varying length linkers, we are exploring the functional importance of DDDD and coiled-coil motif spacing. Finally, the role of the putative coiled-coil is being examined by establishing the oligomeric state of the isolated C-terminus using gel filtration and site-specific mutagenesis of the predicted coiled-coil interface.


1698-Pos Board B428
Pl(4,5)P2 Positively Regulates Transient Receptor Potential Vanilloid 1 Channel Activation in Planar Lipid Bilayers
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Transient Receptor Potential Vanilloid 1 (TRPV1) channels are non-selective cation channels activated by heat, low pH, capsaicin, and a variety of endogenous mediators of nociception. TRPV1 channel activity has long been controversial. To understand this regulation we aimed to incorporate the purified TRPV1 protein in planar lipid bilayers. The TRPV1 channel protein was purified from the TRPV1-HEK stable cell line by immunoprecipitation. Further, the channel was incorporated in planar lipid bilayers consisting of a mixture of 1-palmitoyl-2-oleoyl-glycerol-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-glycerol-3-phosphoethanolamine (POPE) (3:1, v/v), and examined for the single channel activity at different conditions. The results showed that TRPV1 channel could not open without any stimuli, and it could not be activated by capsaicin alone as well. However, addition of 2.5 \(\mu M\) of phosphatidylinositol-4,5-bisphosphate C-8 (Pl(4,5)P2) resulted in burst openings of TRPV1, 5 \(\mu M\) Pl(4,5)P2 induced further increase of channel opening. Moreover, the incorporated TRPV1 channels demonstrated different open probability and gating modes for current flowing in outward and inward directions. Outward current exhibited mean slope conductance values of 98.4 ± 1.8 pS, and inward currents were observed with conductance level of 48.4 ± 2.4 pS in the presence of 2.5 \(\mu M\) Pl(4,5)P2 and 2 \(\mu M\) capsaicin, which is consistent with previous reports (Hui et al., 2003; Raisinghani et al., 2005). These results indicate that Pl(4,5)P2 is required for capsaicin activation of TRPV1 channels.

Reference

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The Cation Channel TRPV4 Regulates Epithelial Barrier Responses to Lipopolysaccharide
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TRPV4 is a calcium-permeable non-selective cation channel belonging to the vanilloid subfamily of Transient Receptor Potential proteins. This channel has a rather ubiquitous expression in epithelial cells (skin, airways, endothelium and urothelium), and it is known to be sensitive to a wide variety of physical and chemical stimuli, including heat, arachidonic acid metabolites and synthetic \(\alpha\)-phorbol derivatives. We have found that lipopolysaccharide (LPS), an outer membrane component of Gram-negative bacteria, activates TRPV4 in HEK293T cells, independently of the renowned TLR4 pathway. Interestingly, conical LPS from E.coli induced larger TRPV4 response than...