of Pl(4,5)P₂ depletion. These results thus demonstrate that TRPC3/C6/C7 channels are differentially regulated by depletion of Pl(4,5)P₂ and bimodal signal produced by PLC activation (i.e. depletion of PIP₂ and production of DAG) simultaneously controls these channels in a self-limiting manner.

2724-Pos Board B494
Selective Ga, Subunits as Novel Direct Activators of TRPC4 and TRPC5 Channels
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Finally, to check out that the Ga–PLC pathway regulates the TRPC4/5 channels and how these channels are regulated by other Ga proteins is unknown.
Here, we discovered that Ga₂s, subunits, rather than Ga₃s, are the primary and direct activators of TRPC4 and TRPC5. These channels were activated by the stimulation of Mscaraerin receptor 2 that regulated by pertussis toxin sensitive manner in the activation process of channel. The expression of the constitutively active Ga₂s-subunits selectively activates TRPC4 and TRPC5 channels. TRPC4 is activated by several Ga₂s-subunits, most prominently by Ga₃s₂ and Ga₅. The result from these mutants does not suggest the role of Gβγ subunit as a key modulator for TRPC4/5 activation. Finally, to check out that the mechanism of TRPC4 activation by Ga₂s₂, we expressed TRPC4 C-terminus deletion and truncation mutants in HEK293 cells. When the region from 700 to 720 in C-terminal region of TRPC4 channel was deleted, electrophysiological activity did not elicited by Ga₂s₂, QL and infused GTPγS. Also co-IP between TRPC4 and Ga₂s₂ QL was altered by the deleted c-terminal region (700-720). These findings indicate an essential role of Ga₂s proteins as novel activators for TRPC4/5 and reveal the molecular mechanism by which G proteins activate the channels.

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Closely Spatio-Association of TRPC4 with Ga2s in the TRPC4 Activation Process
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Canonical transient receptor potential (TRPC) channels are Ca²⁺-permeable nonselective cation channels that are widely expressed in numerous cell types. Seven different members of TRPC channels are isolated and canonical type of TRP channel family transduces signals of GPCR with various extracellular stimuli. TRPC4 and TRPC5 are closely homologous members of the Canonical Transient Receptor Potential, Canonical (TRPC) channels function as non-selective, Ca²⁺-permeable channels and mediate numerous cellular functions. It is commonly assumed that TRPC channels are activated by stimulation of Ga₂s coupled receptors. However, whether the Ga₂s-PLC pathway regulates the TRPC4/5 channels and how these channels are regulated by other Ga proteins is unknown.

Here, we discovered that Ga₂s, subunits, rather than Ga₃s, are the primary and direct activators of TRPC4 and TRPC5. These channels were activated by the stimulation of Mscaraerin receptor 2 that regulated by pertussis toxin sensitive manner in the activation process of channel. The expression of the constitutively active Ga₂s-subunits selectively activates TRPC4 and TRPC5 channels. TRPC4 is activated by several Ga₂s-subunits, most prominently by Ga₃s₂ and Ga₅. The result from these mutants does not suggest the role of Gβγ subunit as a key modulator for TRPC4/5 activation. Finally, to check out that the mechanism of TRPC4 activation by Ga₂s₂, we expressed TRPC4 C-terminus deletion and truncation mutants in HEK293 cells. When the region from 700 to 720 in C-terminal region of TRPC4 channel was deleted, electrophysiological activity did not elicited by Ga₂s₂, QL and infused GTPγS. Also co-IP between TRPC4 and Ga₂s₂ QL was altered by the deleted c-terminal region (700-720). These findings indicate an essential role of Ga₂s proteins as novel activators for TRPC4/5 and reveal the molecular mechanism by which G proteins activate the channels.

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Chemical Modification of Cysteine Residues Activates TRPC5 Channels
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The crucial cysteine residues can be involved in modulation of protein activity via formation of thiol (Sulfhydryl-·SH) groups. These reactions can take several forms, such as redox events (chemical reduction or oxidation) or S-nitrosylation. TX, USA. The Transient Receptor Potential, Canonical (TRPC) channels function as non-selective, Ca²⁺-permeable channels and mediate numerous cellular functions. It is commonly assumed that TRPC channels are activated by stimulation of Ga₂s coupled receptors. However, whether the Ga₂s-PLC pathway regulates the TRPC4/5 channels and how these channels are regulated by other Ga proteins is unknown.

Here, we discovered that Ga₂s, subunits, rather than Ga₃s, are the primary and direct activators of TRPC4 and TRPC5. These channels were activated by the stimulation of Mscaraerin receptor 2 that regulated by pertussis toxin sensitive manner in the activation process of channel. The expression of the constitutively active Ga₂s-subunits selectively activates TRPC4 and TRPC5 channels. TRPC4 is activated by several Ga₂s-subunits, most prominently by Ga₃s₂ and Ga₅. The result from these mutants does not suggest the role of Gβγ subunit as a key modulator for TRPC4/5 activation. Finally, to check out that the mechanism of TRPC4 activation by Ga₂s₂, we expressed TRPC4 C-terminus deletion and truncation mutants in HEK293 cells. When the region from 700 to 720 in C-terminal region of TRPC4 channel was deleted, electrophysiological activity did not elicited by Ga₂s₂, QL and infused GTPγS. Also co-IP between TRPC4 and Ga₂s₂ QL was altered by the deleted c-terminal region (700-720). These findings indicate an essential role of Ga₂s proteins as novel activators for TRPC4/5 and reveal the molecular mechanism by which G proteins activate the channels.

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Differential Modulation of Detergents on Single Channel Activity of TRPC4/C5
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The effects of detergents on ion channel modulation have been studied before. Specifically, it has been noted that detergents used in the preparation of subunits of heterotrimeric G proteins can differentially modulate channel activity. A detailed analysis of the modulation, however, has not been performed. Here we show that detergents typically used in G protein subunit preparations, in sub-critical micelle concentrations, can affect the ion channel under consideration. TRPC4 and TRPC5 are closely homologous members of the Canonical Transient Receptor Potential family of non-selective cation channels. Activation of TRPC4 and TRPC5 elicits membrane depolarization and intracellular calcium signaling in neurons, vascular endothelium and smooth muscle cells. Both channels have been previously shown to be synergistically regulated by Gαq-coupled receptor pathways and pertussis toxin sensitive Gαi₃-coupled receptor pathways. The zwiterionic detergent CHAPS (3-[3-cholamidopropyl] dimethylammonio)-1-propanesulfonate, 14-50 μM) and nonionic surfactant lubrol (C₁₂E₅, 0.001-0.004% w/v), which are typically used in G protein subunit preparations, were applied to the cytosolic side of inside-out membrane patches excised from HEK293 cells expressing TRPC4 or TRPC5. Although both CHAPS and lubrol caused ~40-60% reduction in Popen under basal non-stimulated conditions, only CHAPS resulted in an increase in frequency of transitions between the open and closed states of the single channel activity. Therefore, the effects of detergents, especially at a single channel resolution, are