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Symposium: TRP and the other channels

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Chair: Anuwat Dinudom

A TR(I)P through the world of epithelial calcium and magnesium channels

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Ca^{2+} and Mg^{2+} are of great physiological importance by their intervention in many enzymatic systems and their function in neural excitability, muscle contraction, blood coagulation, bone formation, hormone secretion and cell adhesion. The human body is equipped with an efficient negative feedback system counteracting variations of the Ca^{2+} and Mg^{2+} balance. This system encompasses parathyroid glands, bone, intestine and kidneys. These divalents are maintained within a narrow range by the small intestine and kidney which both increase their fractional (re)absorption under conditions of deprivation. If depletion continues, the bone store assists to maintain appropriate serum concentrations by exchanging part of its content with the extracellular fluid. After years of research, rapid progress has recently been made in identification and characterization of the Ca^{2+} and Mg^{2+} transport proteins contributing to the delicate balance of divalent cations. Expression cloning approaches in combination with knockout mice models and genetic studies in families with a disturbed Mg^{2+} balance revealed novel Ca^{2+} and Mg^{2+} gatekeeper proteins that belong to the super family of the transient receptor potential (TRP) channels. These epithelial Ca^{2+} (TRPV5 and TRPV6) and Mg^{2+} channels (TRPM6 and TRPM7) form prime targets for hormonal control of the active Ca^{2+} and Mg^{2+} flux from the urine space or intestinal lumen to the blood compartment. The function of these distinctive epithelial Ca^{2+} and Mg^{2+} channels is believed to be relevant to various (patho)physiological situations.

Conformational variability of the glycine receptor M2 domain in response to activation by different agonists

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Glycine receptor chloride channels (GlyRs) mediate inhibitory neurotransmission in the central nervous system. They are members of the pentameric cys-loop ligand-gated ion channel family. Individual subunits are each composed of a large N-terminal extracellular ligand-binding domain and 4 transmembrane α-helices (M1-M4). The pore-lining M2 domains are kinked radially inwards to form a central constriction at the membrane midpoint. Current models of cys-loop receptor activation consider only structural changes associated with transitions from the resting closed to the agonist-induced open states. Little attention has been given to the possibility that different agonists and pharmacological modulators may promote different structural conformations in the pore region. Our aim was to compare the conformational changes induced by agonists, antagonists and allosteric modulators by covalently labelling two residues (αR19′C, L22′C) near the extracellular M2 boundary with a sulfhydryl-reactive fluorophore and simultaneously measuring current and fluorescence changes.

Xenopus laevis frogs were anaesthetised in 1g/l ethyl-m-aminobenzoate according to procedures approved by the University of Queensland Animal Ethics Committee. Stage VI oocytes were then removed and injected with 10 ng of wildtype or mutant α1 GlyR mRNA into the cytosol and incubated for 3-10 days at 18°C. For labelling, oocytes were placed into ice-cold ND96 saline solution containing 10μM sulforhodamine methanethiosulfonate for 25s. Oocytes were then washed and stored in ND96 for up to 6 hrs before recording. For recording, oocytes were placed on the stage of an inverted fluorescence microscope. Fluorescence signals were recorded by a photodiode and membrane currents were recorded using conventional two-electrode voltage-clamp. For spectral analysis a MicroSpec 2150i (Acton Research Corporation, Acton, MA) coupled to an ORCA-ER CCD camera (Hamamatsu, Hamamatsu City, Japan) replaced the photodiode detection system.

During glycine-induced activation of the homomeric α1R19′C GlyR, fluorescence of the label attached to αR19′C increased by ~20% and the emission peak shifted to lower wavelengths, consistent with a more hydrophobic fluorophore environment. In contrast, ivermectin activated the receptors without producing a fluorescence change. Although taurine and β-alanine were weak partial agonists at the α1R19′C GlyR, they induced large fluorescence changes. Propofol, which drastically enhanced these currents, did not induce a glycine-like blue-shift in the spectral emission peak. The inhibitors, strychnine and picrotoxin, elicited fluorescence and current changes as expected for a competitive antagonist and an open channel blocker, respectively. Glycine and taurine (or β-alanine) also produced an increase and a decrease, respectively, in the fluorescence of a label attached to the nearby L22′C residue.

Together, these results lead us to conclude that different agonists activate the GlyR by producing different conformational changes to the external region of the M2 domain. Thus, the top of M2 seems to display a conformational mobility which is not necessarily coupled to movements of the channel gate.

Molecular components of store-operated Ca^{2+} entry in liver cells

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To maintain Ca^{2+} homeostasis and to deliver Ca^{2+} to the right place at the right time living cells use a complex array of Ca^{2+} pumps, transporters and ion channels. It has been generally accepted that in most animal cells replenishment of Ca^{2+} lost due to the activity of plasma membrane Ca^{2+}-ATPase is mediated through a store-operated Ca^{2+} entry (SOCE) mechanism. According to this theory, release of Ca^{2+} from the intracellular Ca^{2+} stores caused by hormone binding to G-protein- or tyrosine kinase- coupled receptors activates store-operated Ca^{2+} channels (SOCs) in the plasma membrane. Recent evidence suggests that the Ca^{2+} release activated Ca^{2+} channel expressed in hematopoietic cells, which is the best characterised SOC, is composed of Orai1 polypeptides that interact with STIM1 (Stromal interaction molecule 1), an EF hand containing, Ca^{2+}-binding polypeptide which senses the decrease in Ca^{2+} in the ER. Depletion of intracellular Ca^{2+} stores causes STIM1 to accumulate in areas of junctional ER located in close proximity to the plasma membrane, while Orai1 accumulates in the areas of plasma membrane apposed to STIM1 puncta. Utilizing siRNA mediated knockdown we have shown that STIM1 and Orai1 proteins are major components of SOCE in liver cells. However, there is evidence that several other proteins are also involved. Knockdown of PLC-γ1 in H4-IIE cells substantially decrease the amplitude of I_{SOC} initiated by either IP_3 or thapsigargin. No interaction between PLC-γ1 and STIM1 is detected in immunoprecipitation experiments. This suggests that PLC-γ1 is required to couple ER Ca^{2+} release to the activation of SOCs independently of any PLC-γ1-mediated generation of IP_3 and independently of a direct interaction between PLC-γ1 and STIM1. The likelihood that there are additional to STIM1 and Orai1 proteins that are involved in SOCE is supported by our recent results of the ectopic expression of STIM1 and Orai1 in liver cells. We have found that some biophysical properties of the Orai1/STIM1 current vary significantly between transfected cells and depend on the levels of expression of STIM1 and Orai1.