the mobility of Rh*-G_i upon subsequent addition of GTP. Hence, we concluded that Rh and G_i in SPB were active. At the same time, we also observed some immobile Rh molecules in SPB, which could interfere with the analysis of raftophilicity. We discuss on the reconstitution strategies of Rh to establish the means to measure the raftophilicity in micropatterned *I*_o domains.

3PT162 カリウムチャネル KcsA の開閉によるリン脂質の flip-flop 誘起能の評価

Induction of phospholipid flip-flop by KcsA channel gating

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Regulation of phospholipid flip-flop is important for homeostasis of biological membranes. Flip-flop of phospholipids in the endoplasmic reticulum (ER) is very fast, compared to that in other membranes. However the flippases that govern the fast flip-flop in the ER have not been identified, and the mechanism by which the flip-flop is induced is still unknown. It has been reported that KcsA induces flip-flop of phospholipids. KcsA is a pH-dependent potassium channel, activated by acidic pH. Conformational change of transmembrane region during channel gating was recently observed at a single channel level. We therefore hypothesized that the fluctuation of transmembrane region influences flip-flop of phospholipids.

Dithionite reduction assay was used to measure flip of C₆-NBD-phosphatidylcholine (C₆-NBD-PC) in proteoliposomes, composed of PC and KcsA. KcsA was found to induce flip of phospholipids at acidic pH, while less affected at neutral pH. To confirm whether the fluctuation of transmembrane region of KcsA induce flip, we measured flip of C₆-NBD-PC at acidic pH in the presence of tetrabutylammonium, which locks KcsA in the open state. The results showed that KcsA mediates flip of phospholipids even in the open-locked state. These results suggest that KcsA enhances flip of phospholipids not by the fluctuation of transmembrane region, but by the open structure or the change of charged state of amino acids in transmembrane region.

3PT163 人工細胞膜アレイを用いた全自動イオンチャネル計測システム

Automated Drug Screening System for Ion Channel Proteins using Artificial Cell Membranes

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This paper describes an automated drug screening system for Ca²⁺-activated K⁺ channel (BK channel). BK channels are pharmacological targets for the treatment of stroke. We have been developing multiple lipid bilayer membranes (BLMs) system for reconstituting the membrane proteins. In this study, we are succeeded in simultaneous reconstitution of BK channels in the BLMs array using an injection robot, and the channel inhibition behavior was examined using K⁺ channel blocker. Our system can be applied to a high-throughput screening (HTS) system for drug discovery. Membrane proteins account for approximately 25% of the polypeptides encoded within the human genome. Of these, ion channels are an important class of proteins that mediate ion flow across cellular membranes and which, among other things, are responsible for the excitability of neurons. In the drug discovery, the high-throughput screenings of the ion channels are highly desirable. We have been developing the lipid bilayer membrane array for the HTS of the ion channels using MEMS technology. BK channels are essential for the regulation of several key physiological processes including smooth muscle tone and neuronal excitability. We have therefore attempted to reconstitute BK channel within the artificial lipid bilayers. In this study, BK channels were automatically reconstituted in the BLMs array device by using an injection robot, and also the channel activity was examined using its inhibitor.

3PT164 KcsA カリウムチャネル活性に影響を及ぼすリン脂質作用部位の同定 Identification of the interaction site with the effective phospholipids for the KcsA potassium channel activity

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Membrane lipids are known to affect the function and activity of ion channels. Specific lipid molecules are indispensable for some ion channels to become active. How the specific lipids regulate the ion channel activity is essential to

understand the structure-function relationship of ion channels in the membrane environment. For the KcsA potassium channel, anionic phospholipids such as phosphatidylglycerol (PG) have been thought to play an essential role in its activity as the KcsA channel exhibits quite low open probability in the absence of these lipids. Our previous study revealed by means of single-channel current recordings in the asymmetric lipid bilayer that the KcsA channel requires PG molecules in the inner leaflet to exhibit high open probability. In this study we tried to identify the interaction site with PG within the KcsA molecule for further understanding of lipid-mediated regulation of the KcsA channel activity. Potential positive charges such as Arg, Lys and His were expected to be candidates for the interaction site with PG. We found that mutation of several sites out of 24 potential positive charges in the monomeric KcsA molecule were sensitive to the PG effect on the KcsA channel activity. Mechanism underlie PG-mediated regulation of the KcsA channel opening will be discussed.

3PT165 Kv1.2 チャネルでの選択的イオン透過

Selective Ion Permeation through the Kv1.2 Channel

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Ion selectivity is a crucial function for ion channels. So far, it has been considered the K⁺ channels discriminate for K⁺ over Na⁺ more than a thousand-fold due to the destabilized binding of Na⁺ at the selectivity filter. However, several recent researches showed that Na⁺ can enter into the selectivity filter and permeate: the x-ray crystallographic structure of the K⁺ channel with Na⁺ was resolved, where Na⁺ ions form six-coordination structure in the selectivity filter. Its structure was then supported by theoretical calculations. The electrophysiological measurement also suggested that a certain amount of Na⁺ ions can permeate through the K⁺ channel by punch-through mechanism. Thus, there still exists controversial arguments concerning the ion selectivity. Here we observed the efflux of ion in the mixed ion solution, 200 mM KCl and 200 mM NaCl, through the Kv1.2 channel by the molecular dynamics simulation. It is found that K⁺ ions permeate at rates at about five times higher than Na⁺ ions, which is approximately the same ratio the punch-through mechanism reported. We also found that once ions, regardless of ion species, enter into the central cavity, they cannot return to the cytoplasmic side solution. This means the selectivity is caused by the free energy barrier at the entrance of the channel. Therefore, the selectivity is a part of ion permeation issues rather than the stability of ions in the channel; kinetics, instead of thermodynamics, determines the ion selectivity.

3PT166 Controlling an ion channel's voltage sensing domain without voltage

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Voltage gating - the opening and closing of ion channels on response to changes in membrane potential - is fundamental to signal transduction in living organisms. Voltage gating is achieved by four voltage sensing domains (VSDs) that surround the ion-conducting pore of the channel protein. In these VSDs, conserved positively charged residues located in the S4 transmembrane helix move according to the transmembrane electrical field, and the resulting conformational change is transmitted to the channel pore. Up to date, however, direct structural information on the deactivated, closed state of a channel has remained elusive. Consequently, the extent and direction of the VSD movement remain controversial, with both small translations and large-scale movements reported in the literature depending on the technique used in the investigation. Here, we attempt the manipulation of voltage sensor conformation using engineered probes as a substitute for voltage to shed light on the transitions involved in voltage gating.

3PT167 ガラス針による人工平面膜へのイオンチャネルの再構成

Reconstitution of ion channel into lipid bilayer using glass needle

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Ion channels are membrane proteins that regulate cell functions by controlling ion flow through cell membrane. To elucidate the molecule mechanism of the regulation for the ion flow, it is necessary to investigate the relationship between the conformation and function and interaction with some factors. To aim this task, ion channel is kept suppressing lateral diffusion in the membrane for