binding characteristics of cholera toxin subunit B (CTB) to ganglioside GM1 (GM1) incorporated in the self-spreading lipid bilayer was investigated by a single molecule tracking observation. The diffusivity of CTB-GM1 conjugates were significantly dependent both on CTB and GM1 concentrations. The present results yielded direct information on a dynamic change in the binding behavior between CTB and GM1. We have also investigated the effect of nano-gates for the CTB diffusion in order to establish a novel molecular manipulation system.

2P-239 リン脂質の生合成過程に関わる膜蛋白質のリボソーム内合成 Cell-free expression of phospholipid-synthesizing membrane proteins in the interior of liposomes

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Two membrane proteins were synthesized inside liposomes in their functional state. The proteins, sn-glycerol-3-phosphate acyltransferase (GPAT) and lysophosphatidic acid acyltransferase (LPAAT), are part of the phospholipid biosynthetic pathway. Hence, the goal of this study is to design phospholipid-producing liposomes via expressing the GPAT and LPAAT proteins. The synthesized lipids are expected to be incorporated into the liposome lipid bilayer. Consequently, the resulting increase of the liposomal membrane surface stimulates destabilization of the vesicles, and eventually will lead to spontaneous self-division. Such phenomenon can be considered a model of self-reproducing synthetic cells. GPAT and LPAAT were expressed by a reconstructed cell-free translation system encapsulated in liposomes composed of four types of phospholipids. The acyltransferase activities of the internally synthesized GPAT and LPAAT were independently confirmed by the detection of their radioisotope labeled products. Additionally, these reactions were also performed in a semi-coupled manner; i.e., the independently synthesized proteins were mixed to produce phosphatidic acid in consecutive acyltransferase reactions. In summary, the present study shows the possibility of self-reproducible synthetic cells by using a novel methodology that combines of cell-free expression system and liposomes.

2P-240 バクテリオロドプシンのアルカリpHでの光誘起構造変化

Light-induced structural changes of bacteriorhodopsin at alkaline pH

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Bacteriorhodopsin (bR), the sole membrane protein found in the purple membrane of *Halobacterium salinarum*, functions as a light-driven proton pump. The hexagonal P622 crystal of bR allowed us to obtain structural information of several states of bR, including the reaction intermediates K, L and M in the trans photocycle. In this study, we investigated how the lattice constant of the crystal affects the decay kinetics of M. When the crystal was soaked at various concentrations of ammonium sulfate (precipitant) between 1.5 M and 4 M, the lattice constant along the c axis decreased linearly with the precipitant concentration, from 112.8 Å to 112.0 Å. This shrinkage was explained by slight deformations of the BC and EF loops. Interestingly, this shrinkage was accompanied by remarkable elongation of the half life of M, from 15 ms to 300 ms at 297 K. This observation led us to consider that the N intermediate might be trapped if the crystal soaked at a low precipitant concentration and at a high pH was illuminated. After many trials, we were able to collect diffraction data at 2.5 Å resolution from a crystal that was soaked in a solution containing 1.5 M ammonium sulfate and at pH 9.3. The light-induced structural change observed under this condition was found to be nearly identical to that observed upon formation of M at pH 5.2; i.e., the crystal lattice force was still large so that the N intermediate was scarcely accumulated in the illuminated crystal.

2P-241 量子ドット1分子イメージングによる抑制性シナプス制御機構の解明

Regulation of inhibitory synapses revealed by single molecule imaging with quantum dots

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Neurotransmitter receptors dynamically travel between synaptic and extra-synaptic domains by means of lateral diffusion. The regulation of receptor flux in/out synapses and receptor stabilization at synapses are now considered as key determinants for synaptic strength. Single-molecule imaging using fluorescent semiconductor quantum dots (QDs) is a powerful tool to study the diffusion dynamics of molecules on the plasma membrane. Using this technique, we have studied the lateral diffusion dynamics of γ -aminobutyric acid type-A GABA receptors (GABA_ARs), a chloride-selective ligand gated ion channels that mediates fast inhibitory neuronal transmission in the central nervous system. We report here that neuronal activity modified diffusion properties of GABA_ARs in cultured hippocampal neurons. The analysis of the movement of QDs targeted to $\gamma 2$ subunit of GABA_AR revealed that enhanced excitatory activity reduced receptor dwell time at synapses. The diffusion coefficient and the confinement domain size of GABA_AR at synapses increased in parallel with neuronal activity, suggesting that synaptic GABA_ARs are less stabilized when neuronal activity increases. Effects of neuronal activity on GABA_AR diffusion dynamics were

dependent on calcium influx and calcineurin activity that are responsible for GABAergic synaptic plasticity. These results indicate that GABA_AR diffusion dynamics may be directly linked to rapid and plastic modifications of inhibitory synaptic transmission.

2P-242 カフェインはウシ毛様体平滑筋細胞のムスカリン応答に関与する低コンダクタンスの非選択性陽イオンチャネルを活性化する

Caffeine activates a small-conductance non-selective cation channel involved in muscarinic responses of bovine ciliary muscle cells

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In bovine ciliary muscle, stimulation of M₃-muscarinic receptor opens two types of non-selective cation channel with different unitary conductances of 35 pS and 100 fS which serve as major pathways for Ca²⁺ entry during the sustained contraction. Here we studied the effects of caffeine, an activator of Ca²⁺ release from intracellular stores, on the intracellular Ca²⁺ concentration ([Ca²⁺]_i) and NSCC currents. We also examined the existence of STIM1, a putative calcium sensor of the endoplasmic reticulum.

Methods Smooth muscle cells enzymatically isolated from the ciliary body of slaughtered oxen were used. The [Ca²⁺]_i was recorded using Fluo-4 as the indicator. For immunofluorescence microscopy secondary antibodies conjugated with AlexaFluor dyes were used.

Results In the normal saline solution containing 2.4 mM Ca²⁺, superfusion of caffeine (20 mM) caused an elevation of [Ca²⁺]_i which showed an initial peak followed by a plateau. In the absence of extracellular Ca²⁺, the initial peak of the caffeine-induced response was only moderately reduced whereas the plateau was abolished. In myocytes under whole-cell voltage clamp at the holding potential of -50 mV, caffeine induced an opening of 100 fS-NSCC whereas it failed to open 35 pS-NSCC. The expression of STIM1 in bovine ciliary muscle was confirmed by RT-PCR and immunofluorescence microscopy.

Conclusion Further study is encouraged to examine the possibility that involvement of signals elicited by depletion of intracellular Ca²⁺ store in activation of 100 fS-NSCC by muscarinic stimulation.

2P-243 レシニフェラトキシンはラット脊髄後角ニューロンのグルタミン酸作動性の自発性興奮性シナプス伝達を促進する

Enhancement by resiniferatoxin of glutamatergic spontaneous excitatory synaptic transmission in rat spinal dorsal horn neurons

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TRPV1 activation enhances glutamatergic spontaneous excitatory transmission in substantia gelatinosa (SG; lamina II) neurons of the spinal dorsal horn. The present study examined how an ultrapotent TRPV1 agonist resiniferatoxin affects the excitatory transmission by applying the whole-cell patch-clamp technique to SG neurons of adult rat spinal cord slices. Bath-applied resiniferatoxin dose-dependently increased the frequency of spontaneous excitatory postsynaptic current (sEPSC) in a range of 0.005 to 2 μ M with a minimal increase in its amplitude. This effect was resistant to Na⁺-channel blocker tetrodotoxin while being inhibited by TRPV1 antagonist capsazepine. In about a half of the neurons tested, such a presynaptic effect was accompanied by an inward current which was resistant to tetrodotoxin while being sensitive to capsazepine and glutamate-receptor antagonists. A repeated application of resiniferatoxin at a time interval of at least 2 hr had no effects on excitatory transmission. Their excitatory effects were occluded by another TRPV1 agonist capsaicin but not TRPA1 agonist allyl isothiocyanate, both of which enhance spontaneous excitatory transmission in a manner similar to that of resiniferatoxin. It is concluded that resiniferatoxin enhances the spontaneous release of L-glutamate from nerve terminals and produces a membrane depolarization by activating TRPV1 in the SG with a slow recovery from desensitization. Resiniferatoxin may serve as a tool to know a physiological role of TRPV1 in the spinal dorsal horn.

2P-244 オピオイドによる蛙坐骨神経の複合活動電位抑制とその化学構造との関連

Relationship between inhibition by opioids of compound action potentials in frog sciatic nerves and their chemical structures

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It is well-known that opioids have a local analgesic action by nerve conduction block, but it has been examined yet which parts of the chemical structures of opioids are related to their inhibitory actions on compound action potentials (CAPs). We investigated inhibition by opioids, tramadol, mono-O-demethyl-tramadol (M1), morphine, codeine, ethylmorphine and dihydrocodeine, of CAPs in frog sciatic nerves by applying air-gap method. All of the opioids at concentrations less than 10 mM reduced the peak amplitude of the CAP in a reversible and dose-dependent manner; this reduction was accompanied by an increase in the half-peak duration of CAP. A potency sequence of the CAP peak amplitude reductions by various opioids was found to be tramadol (IC₅₀ = 2.3 mM) > ethylmorphine (IC₅₀ = 4.6 mM) > codeine > dihydrocodeine $\geq$ morphine > M1 in a manner resistant to opioid-receptor antagonist naloxone. There was no correlation in magnitude between CAP peak amplitude reductions by the opioids and their dissociation constants for μ -, δ - and κ -opioid receptors.