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.

On the other hand, the CAP peak amplitude reductions produced by morphine, codeine and ethylmorphine and also by tramadol and M1 were related to their chemical structures in such that this extent enhanced with an increase in the number of -CH₂ in a benzene ring. It is suggested that the substituted groups of -OH bound to the benzene ring of morphine, codeine and ethylmorphine and also of tramadol and M1 may play an important role in producing nerve conduction block.

2P-245 プルキンエ細胞樹状突起における IP₃ 受容体タイプ 1 の動態解析 IP₃ receptor type 1 dynamics in dendrites of Purkinje cells

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IP₃ receptor type 1 (IP₃R1) is an intracellular Ca²⁺ release channel that plays crucial roles in neuronal functions, and Ca²⁺ release from IP₃R1 is strictly regulated spatially to satisfy physiological functions. IP₃R1 dynamics on the endoplasmic reticulum (ER) membrane and its regulation are considered to be important for the spatial regulation of Ca²⁺ release, however, they still remain unknown. Since IP₃R1 is crucial for synaptic plasticity in cerebellar Purkinje cells, we analyzed IP₃R1 dynamics in Purkinje cell dendrites using cerebellar slice culture expressing GFP-tagged IP₃R1. We observed the movement by the fluorescence recovery after photobleaching (FRAP). IP₃R1 showed lateral diffusion with an effective diffusion constant of ~0.27 μm²/sec, which is slower than other ER protein such as sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA). We also studied whether actin cytoskeleton was related to the regulation of ER protein diffusion. From pharmacological studies, we showed that IP₃R1 diffusion was dependent on actin filaments, however, SERCA was not. We have previously reported that IP₃R1 diffusion in hippocampal neurons was regulated by actin filaments and 4.1N, which serves as a linker protein between IP₃R1 and actin filaments. In Purkinje cells, 4.1N expression level is much less, however, IP₃R1 diffusion was regulated by actin filaments. These results suggest that there may be a new regulatory mechanism of IP₃R1 diffusion by actin filaments and another linker protein other than 4.1N in Purkinje cell dendrites.

2P-246 男性ホルモンの海馬樹状突起スパインに及ぼす急性効果

Androgens rapidly increased the density of dendritic spines in the hippocampus

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The hippocampus is a center for learning and memory. Dendritic spines are postsynaptic structures. It has been reported that the application of testosterone and dihydrotestosterone have anti-anxiolytic effects. Therefore, androgens modulation of the morphological changes of hippocampal spines has been attracting much attention. Androgens are known to change the synaptic plasticity of hippocampal neurons by modulating gene transcription, but its genomic actions are very slow. We demonstrated androgens rapidly affect the number and morphology of dendritic spines in rat dorsal hippocampus. Hippocampal slices were incubated with testosterone or dihydrotestosterone for another 2 hours. We observed that these androgens increased spine density by approximately 1.3 times, especially for thin-type spine, which have small head and functional membrane molecules. After washing out these androgens, the number of spines returned to control level. Because these changes of spine morphology and density are very rapid, and transcription inhibitor have no effect, it is suggested that those processes are mediated not by gene transcription but by membrane associated androgen receptor responses and its downstream signal transducers. We have uncovered that MAP kinase superfamily, such as ERK1/2 and p38 were involved in this signaling.

2P-247 女性ホルモンによる海馬神経スパインの形態変化

Dynamics of synapses modulated by estradiol in hippocampus

Rei Sato, Yusuke Hatanaka and Suguru Kawato (Dept Life Sciences, Graduate School of Arts and Sciences., Univ of Tokyo)

Single neuron has nearly 10,000 spines along about 40 dendrites. Spines are postsynaptic structures and they store memories in the hippocampus. We demonstrate that sex steroids are synthesized not only in the gonads but also locally in the brain hippocampus, and that these steroids rapidly modulate neuronal plasticity via synaptic receptors. The depletion of estrogen causes impairment of learning and memory and increase in the risk of Alzheimer disease. We demonstrated that dendritic spines were rapidly modulated by estradiol application, by means of single spine analysis of Lucifer-Yellow injected neurons in adult male hippocampal slices. 3D images were obtained from confocal microscopic measurements. Following a 2h treatment with estradiol in the stratum radiatum of CA1 region, the treated dendrites had significantly more spines at 1 nM estradiol (1.35 spines/um) than control dendrites at 0 nM estradiol (0.94 spines/um). To determine the pathway of estrogen signaling, we examined the effect of various inhibitors. The antagonist of estrogen receptor alpha and the inhibitor of ERK MAP kinase both suppressed the effect of estradiol. The channel blocker of NMDA receptor also suppressed the estradiol effect. These results indicate that estrogen acts via ER alpha and drives the ERK MAP kinase pathway, and that the cascade needs the basic calcium concentration via the NMDA receptor.

2P-248 ラット海馬におけるニューロステロイド合成系の解析

Neurosteroids synthesis in the rat hippocampus

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Hippocampus is one of the main target of sex steroids (androgens and estrogens) and stress steroids (corticosteroids). It is widely accepted that these steroid hormones reach the brain via blood circulation and modulate efficiency of learning and synaptic plasticity. However, recent studies suggest the possibility that these steroids are synthesized in the rat brain as well as in peripheral endocrine organs. In peripheral organs, steroid hormones and their precursors are synthesized by series of steroidogenic enzymes, but the presence and the activity of those enzymes in the rat brain are still unclear. To prove the presence of these steroid hormones synthesis pathway, we examined the expression level of mRNA of those enzymes in adult male rat brain by means of the semi-quantitative RT-PCR. We also examined histological localization of these steroidogenic enzymes using in situ hybridization.

2P-249 海馬神経細胞シナプスにおけるステロイドの合成と局所分泌

Steroid synthesis and synaptocrinology in the hippocampal synapses

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The endogenous synthesis of androgens and estrogens is elucidated for the hippocampus of adult and neonatal male rats. By means of HPLC, the rate of conversion (per 5h) from 3H-testosterone (T) to ³H-dihydrotestosterone (DHT) was much more rapid than from ³H-T to ³H-17β-estradiol (E2). DHT (androgen) was immediately converted into inactive form, 3α, 5α-androstanediol (androstanediol) whereas E2 (estrogen) was stably present with little further conversion. The production of T (the activity of 3β-HSD) was very small. The concentration of DHT, E2 and T in the hippocampus was determined. E2 concentration was much higher than that in plasma which was enough to change the density of dendritic spines in CA1 and CA3 region (Mukai et al., *J. Neurochem.* 2007). Immunohistochemistry and immunogold electron microscopy were performed in order to reveal the localization of the brain steroidogenic enzymes. These steroidogenic enzymes were localized in the principal neurons (pyramidal cells and granule cells) of hippocampus. Furthermore, P45017α and P450aromataase were localized in pre- and post synaptic compartments in the principal neurons. These results suggest that brain steroids were synthesized principal neurons of hippocampus and modulate synaptic transmission rapidly and locally.

2P-250 走査型プローブ顕微鏡による生きた海馬神経細胞シナプスの3次元形態観察

Visualization of three-dimensional synaptic morphology of living hippocampal neurons by Scanning Probe Microscope

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Dynamic changes of synaptic morphology play important roles in neuronal function. For example, in order to control neurotransmission among neurons, synapses change morphologically to separate pre- and post-ones. Recently it is proposed that some kinds of proteins are involved in the change. However, the mechanisms inducing the change are not well understood. We think a clue to understanding the mechanism is to observe detailed dynamic morphology of dendrite of living nerve cells.

We constructed a system to observe detailed morphology of synapses in living hippocampal neurons. Primary hippocampal neurons were harvested on a glass Petri dish coated with polyethylenimine, and cultured for some days to construct synapses among neurons. After the cultivation, morphology of neurons was measured using tapping-mode scanning probe microscopy under a physiological condition.

We observed some synapse-like structures formed on the dendrites. The synapse-like structures had size of about 2.5 μm in the x-y direction and of about 600 nm in the z direction. Time lapse observation showed that the synapse-like structures changed morphologically in some 10 minutes. We also observed that there were some granular domains with a diameter of 5 μm on dendrites. The domains appeared and disappeared within 10 minutes. We speculate that both the morphological changes in the synapse-like structures and the granular domains could be important for synaptic functions such as neurotransmission or synapse formation.

2P-251 単一シナプトソームを用いたシナプス小胞分極化の蛍光イメージング

Fluorescence imaging of synaptic vesicles' polarization in single synaptosomes

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Accumulation of neurotransmitters in synaptic vesicles is the important step for release of neurotransmitters from presynapses. The accumulation is driven by the membrane potential and electrochemical proton gradient formed by v-type ATPase. The mitochondrial role on the supply of ATP to v-type ATPase, however, remains controversial. In this study, to understand the role of mitochondria on