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 type1 dynamics in dendrites of Purkinje cells

Kazumi Fukatsu (1), Hiroko Bannai (1), Takafumi Inoue (2), and Katsuhiko Mikoshiba (1, 3). (1: Lab. for Dev. Neurobiol., Brain Sci. Inst., RIKEN; 2: Dept. Life Sci and Med BioSci, Faculty Sci and Eng., Waseda Univ.; 3: Calcium Oscillation Project, ICORP-SORST, JST)

IP₃ receptor type1 (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

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

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

Shimpei Higo (1), Yasushi Hojo (1), Suguru Kawato (1). (1: Dept. Life Sci, Grad. Sch. Art and Sci, Tokyo Univ. Tokyo, Japan)

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

Yasushi Hojo(1),(2), Shimpei Higo(1),(2), Hideo Mukai(1),(2), Shiro Kominami(3), Nobuhiro Harada(4), Seiji Honma(5), Tetsuya Kimoto(1),(2) and Suguru Kawato(1),(2)(1: Dept. of Biophys. and Life Sci., Grad. Sch. of Arts and Sci., The Univ. of Tokyo; 2:CREST, Bioinformatics Project, JST; 3: Fac. of Integrated Arts and Sci., Hiroshima Univ.; 4: Dept. of Biochem., Sch. of Med., Fujita Health Univ.; 5:Teikoku Hormone Research Center)

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 P450aromatase 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

Jun JIANG(1), Takeomi MIZUTANI(1), Hisashi HAGA(1), Suguru KUDOH(2), Takahisa TAGUCHI(3) and Kazushige KAWABATA(1). (1: Division of Biological Sciences, Graduate School of Science, Hokkaido University; 2: Cell Engineering RI, AIST; 3: Neuroscience RI, AIST)

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

Ayumu Kikuchi(1), Suguru Kawato(2) and Yoshihiro Ohta(1). (1:Dept Life Sci & Biotech, Graduate School of Engineering, Tokyo University of Agriculture & Technology; 2:Dept of Biophysics and Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo)

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