decreased the amplitude of EPSCs in all cases. Regarding the extent of inhibitory effect, there was strong correlation between NA- and DA-induced inhibitory actions. Moreover, an α_2 -AR antagonist RS79948 partly inhibited the DA-induced inhibitory action. These results suggested that 1) the mossy fiber terminals innervating the DCN had heterogeneity in the expression of D2R; 2) DA affected not only D2R but also α_2 -AR at the mossy fiber terminals.

1P-201 固体 NMR 分光法による SWAP-70 PH ドメインの脂質膜界面における構造転移および機能の解析

A solid state NMR study of the structure and function of the SWAP-70 PH domain induced at the membrane interface.

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The pleckstrin homology (PH) domain is a rather small lipid-binding domain consists of 100-120 amino acid residues frequently found in the proteins included in the important cellular functions such as the cellular signal transduction. A core structure of the PH domain is a β -sandwich consists of seven anti-parallel β -strands and a C-terminal α -helix located at one of the splayed corners of the β -sandwich. The unoccupied splayed corner of the β -sandwich includes a specific binding site for phosphoinositide. One of the well-known functions of the PH domain is to mediate membrane localization of the protein through stereoselective recognition of headgroup of phosphoinositides. In this study, we investigated structural alteration the switch associated protein (SWAP)-70 PH domain at the membrane surface by using solid-state NMR spectroscopy. It is reported that SWAP-70 is translocated from the cytoplasm to the plasma membrane as a result of a specific binding of the PH domain to PtdIns(3,4,5)P₃, and subsequently transported from the membrane to the nucleus by Importin- α depend on the nuclear localization signal (NLS) involved in the C-terminal α -helix of the PH domain. In the nucleus, SWAP-70 is reported to contribute to the class-switching of B lymphocyte. The solid-state ¹³C NMR spectra of the membrane associated SWAP-70 PH domain revealed that a conformational transition of the C-terminal α -helix from the α -helical structure to the random coil is induced at the membrane surface. This conformational transition of the C-terminal α -helix would facilitate the recognition of the PH domain by Importin- α and subsequent nuclear localization as a result of a conformational alteration of the NLS from the α -helix to the water-exposed extended structure.

1P-202 サリニバクター-SRI シグナル伝達系を用いた、走光性大腸菌作成に向けた試み

Towards engineering of phototactic *Escherichia coli* by using sensory rhodopsin I and Che proteins from *Salinibacter ruber*

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Microbial organisms utilize light not only as energy sources but also as signals by which rhodopsins work as photoreceptors. Sensory rhodopsin I (SRI) is known as a dual photoreceptor that regulates both negative and positive phototaxis in the archaeon, such as *Halobacterium salinarum* [1]. SRI forms 2:2 complexes with a methyl-accepting transducer protein (HtrI) and transmits light signals to a two-component signal transduction cascade mediated by Che proteins, which controls the flagella motor motion. To understand the signal-relay mechanism, we tried to engineer *E. coli* having the activity of the phototaxis. Recently we characterized the first eubacterial SRI protein from *Salinibacter ruber* and named it SrSRI [3-5]. This is an advantage for the engineering because *E. coli* is eubacterium as well as *S. ruber*. In fact, SRI from *H. salinarum* does not work in *E. coli*. The genome sequence of *S. ruber* also predicts that the bacterium has HtrI gene under the control of the same promoter as SrSRI and one set of Che genes. We cloned these genes and succeeded in expressing them as a recombinant protein in *Escherichia coli* [5]. Interestingly we found that SrHtrI works as a receptor for thermo-taxis in *E. coli*, indicating that SrHtrI transfer the external signal to cytoplasm Che proteins from *E. coli*.

[1] Spudich, J.L. and Bogomolni, R.A. (1984) *Nature* [2] Kitajima-Ihara, T. et al. (2008) *J. Biol. Chem.* [3] Suzuki, D. et al. (2008) *Biochemistry* [4] Suzuki, D. et al. (2009) *J. Mol. Biol.* revised. [5] Sudo, Y. et al. in preparation.

1P-203 蛙坐骨神経の複合活動電位に及ぼすビスフェノールAの抑制作用 Inhibitory action of bisphenol A on compound action potentials in frog sciatic nerves

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Bisphenol A (BPA) is widely used as a monomer to produce polycarbonate plastics while being one of endocrine-disrupting chemicals owing to its toxicological actions on humans. Although BPA is well-known to have an estrogenic action, it has not been fully examined yet how BPA affects the nervous system. We investigated the effect of BPA on fast-conducting and Na⁺-channel

blocker tetrodotoxin (1 μ M)-sensitive compound action potentials (CAPs) recorded from the frog sciatic nerve by using the air-gap method. Treatment of the sciatic nerve with BPA (0.5 mM) for 20 min resulted in a decrease in CAP peak amplitude to about 40% of control. The CAP amplitude did not recover to control in nerves returned to drug-free solution for up to 1 hr, and remained to be about 65% of control. This reduction of CAP peak amplitude produced by BPA was dose-dependent, and a half-maximal inhibitory concentration (IC₅₀) value for BPA was 0.31 mM (Hill coefficient: 1.5). When compared with the actions of local anesthetics on CAPs, the efficacy of BPA in inhibiting CAPs was larger than those of procaine, lidocaine and cocaine (IC₅₀ = 2.3, 0.74 and 0.80 mM, respectively), smaller than that of tetracaine (0.013 mM) and similar to that of ropivacaine (0.34 mM). These results indicate that BPA has an ability to inhibit nerve conduction with an efficacy comparable to those of local anesthetics.

1P-204 ラット脊髄後角における TRPA1 チャネルの局所麻酔薬によるシナプス前性の活性化

Presynaptic activation by local anesthetics of TRPA1 channels in the rat spinal dorsal horn

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Although local anesthetics are recently reported to activate TRPV1 channels which are cloned and expressed in dorsal root ganglion neurons, this has not been examined yet in the central nervous system. We investigated the effects of lidocaine, procaine, ropivacaine and tetracaine on glutamatergic spontaneous excitatory synaptic transmission in substantia gelatinosa (SG; lamina II of Rexed) neurons of adult rat spinal cord slices by using the whole-cell patch-clamp technique. Bath-applied lidocaine dose-dependently and reversibly increased the frequency but not the amplitude of spontaneous excitatory postsynaptic current (EPSC) recorded at -70 mV in SG neurons. Lidocaine activity was unaffected by the voltage-gated Na⁺-channel blocker tetrodotoxin and the TRPV1 antagonist capsazepine, but was inhibited by the non-selective TRP antagonist ruthenium red. In the same neuron, the TRPA1 agonist allyl isothiocyanate and lidocaine both increased spontaneous EPSC frequency. Like lidocaine, tetracaine also increased spontaneous EPSC frequency in SG neurons. In contrast, procaine and ropivacaine did not increase spontaneous EPSC frequency. It is concluded that some kinds of local anesthetics activate TRPA1 in the SG to increase the spontaneous release of L-glutamate from nerve terminals.

1P-205 フルボキサミンの光異性化の理論的研究

Theoretical study of photoisomerization of fluvoxamine

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Fluvoxamine, a selective serotonin re-uptake inhibitor (SSRI), is used as antidepressant/anxiolytic. Recently, photoisomerization of fluvoxamine has been reported to occur from trans to cis isomer, with respect to the C=N double bond in its structure. Two chains of fluvoxamine implies the existence of many conformations, since there are 8 single bonds in the chains excepting two terminal single bonds with methyl group. The purpose of the present study is to disclose the photoisomerization paths and their activation energies by using a MNDO-AM1 molecular orbital method. The trans and cis isomers of fluvoxamine are constructed by twisting those 8 single bonds by the amount of 0, 120 or 240 degrees, and a total of 663 trans and 1796 cis isomers are obtained, excepting the isomers including steric hindrance. Their optimized structures and molecular vibrations are calculated to find that 191 trans and 1091 cis isomers among those isomers are in their ground states. The transition states of these ground states and then saddle point of the trans-cis isomerization of fluvoxamine are calculated, and our preliminary result shows that the activation energy of the trans-cis isomerization path of fluvoxamine is 2.9 eV (420 nm).

1P-206 モルモット盲腸筋層間神経叢培養グリア細胞の受容体、イオンチャネル及び膜電位に対する神経伝達物質の作用

Neurotransmitters act on receptors, ion channels, and membrane potentials in cultured glial cells from the myenteric plexus of the guinea-pig cecum

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The electrophysiological properties and the role of the glial cells obtained from an explant culture of the myenteric plexus of the guinea-pig cecum were studied using the patch-clamp technique. The myenteric glial cells were identified using fluorescent antibodies, anti-S100 and anti-GFAP. A polygonal shaped-cell was used for the patch-clamp recording. The cell membrane of the glial cells located in the cecum myenteric plexus had high- and low-conductance Cl⁻ channels which were insensitive to Ca²⁺ increases. Adrenalin or forskolin application evoked the