Department of Basic Medical Sciences
Division of Neuronal Network
神経ネットワーク分野

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Our major research interest is the molecular mechanisms of higher brain functions in mammals such as emotion, and learning and memory. We are especially focusing on the roles of functional molecules localized in synapses, for instance, neurotransmitter receptors, signal transduction molecules and adhesion molecules, in neuronal information processing. We are examining receptor functions, synaptic transmission and plasticity, and their roles in whole animals with electrophysiological, biochemical, molecular genetic and behavioral approaches.

1. NMDA receptor phosphorylation and synaptic plasticity

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In the hippocampus, excitatory synaptic transmission is regulated dynamically depending on the pattern of synaptic activation: high-frequency activation induces long-lasting enhancement of synaptic efficacy referred to as long-term potentiation (LTP), and prolonged lower-frequency activation causes long-term depression (LTD) of synaptic transmission. Excitatory synaptic transmission is mediated by glutamate receptors and the N-methyl-D-aspartate (NMDA) receptor, one of the glutamate receptor subtypes, plays crucial roles in LTP and LTD induction.

Tyrosine phosphorylation of NMDA receptors by Src-family tyrosine kinases such as Fyn is implicated in synaptic plasticity. We identified Fyn-mediated phosphorylation sites on the GluRε2 (NR2B) subunit of NMDA receptors and Tyr1472 was the major phosphorylation site. We then generated rabbit polyclonal antibodies specific to Tyr1472-phosphorylated GluRε2, and showed that Tyr1472 of GluRε2 was indeed phosphorylated in murine brain using the antibodies. Moreover, Tyr1472 phosphorylation grew evident when mice reached the age when hippocampal LTP started to be observed and its magnitude became larger. Finally, Tyr1472 phosphorylation was significantly enhanced after the induction of LTP in the hippocampal CA1 region. These data suggest that Tyr1472 phosphorylation of GluRε2 is important for synaptic plasticity. We are currently examining mutant mice that have a point mutation in this residue (tyrosine → phenylalanine) electrophysiologically and behaviorally.

2. Adhesion molecules and synaptic plasticity

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Adhesion molecules play critical roles in synaptic transmission and plasticity. The HNK-1 carbohy-

drate epitope, a sulfated glucuronic acid at the non-reducing terminus of glycans, is expressed characteristically on a series of cell adhesion molecules and is synthesized through a key enzyme, glucuronyltransferase (GlcAT-P). We generated mice with a targeted deletion of the GlcAT-P gene. The GlcAT-P deficient mice exhibited normal development of gross anatomical features, but the adult mutant mice exhibited reduced long-term potentiation at the Schaffer collateral-CA1 synapses and a defect in spatial memory formation. This is the first evidence that the loss of a single non-reducing terminal carbohydrate residue attenuates higher brain functions.

3. Synaptic plasticity at the mossy fiber-CA3 synapse

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a. Kainate receptor-dependent short-term plasticity of presynaptic Ca^{2+} influx

Transmitter release at the hippocampal mossy fiber (MF)-CA3 synapse exhibits robust use-dependent short-term plasticity with an extremely wide dynamic range. Recent studies revealed that presynaptic kainate receptors (KARs), which specifically localized on the MF axons, mediate unusually large facilitation at this particular synapse in concert with the action of residual Ca^{2+}. However, it is currently unclear how activation of kainate autoreceptors enhances transmitter release in an activity-dependent manner. Using fluorescence recordings of presynaptic Ca^{2+} and voltage in hippocampal slices, we demonstrated that paired-pulse stimulation (with 20-200 ms intervals) resulted in facilitation of Ca^{2+} influx into the MF terminals, as opposed to other synapses, such as the Schaffer collateral-CA1 synapse. These observations deviate from typical residual Ca^{2+} hypothesis of facilitation, assuming an equal amount of Ca^{2+} influx per action potential. Pharmacological experiments reveal that the facilitation of presynaptic Ca^{2+} influx is mediated by activation of KARs. We also found that action potentials of MF axons are followed by prominent afterdepolarization, which is partly mediated by activation of KARs. Notably, the time course of the afterdepolarization approximates to that of the paired-pulse facilitation of Ca^{2+} influx, suggesting that these two processes are closely related to each other. These results suggest that the novel mechanism amplifying presynaptic Ca^{2+} influx may underlie the robust short-term synaptic plasticity at the MF-CA3 synapse in the hippocampus, and this process is mediated by KARs whose activation evokes prominent afterdepolarization of MF axons and thereby enhances action potential-driven Ca^{2+} influx into the presynaptic terminals.

b. Presynaptic Ca^{2+} entry during mossy fiber LTP

The hippocampal mossy fiber (MF)-CA3 synapse exhibits NMDA receptor-independent long-term potentiation (LTP), which is expressed by presynaptic mechanisms leading to persistent enhancement of transmitter release. Recent studies have identified several molecules that may play an important role in MF-LTP. These include Rab3A, RIM1α, kainate autoreceptor, and hyperpolarization-activated cation channel (I_h). However, the precise cellular expression mechanism remains to be determined because some studies noticed essential roles of release machinery molecules, whereas others suggested modulation of the ionotropic processes affecting Ca^{2+} entry into the presynaptic terminals. Using fluorescence recordings of presynaptic Ca^{2+} in hippocampal slices, we demonstrated that MF-LTP is not accompanied by an increase in presynaptic Ca^{2+} influx during an action potential. Whole-cell recordings from CA3 neurons revealed long-lasting increases in mean frequency, but not mean amplitude, of miniature EPSCs after the high-frequency stimulation of MFs. These data indicate that the presynaptic expression mechanisms responsible for enhanced transmitter release during MF-LTP involve persistent modification of presynaptic molecular targets residing downstream of Ca^{2+} entry.

4. Analysis of muscarinic acetylcholine receptor functions using knockout mice

Minoru Matsui, Yuji Kiyama, Norikazu Katayama, Fumiko Arima, Toru Shinoe[1], Ayako M. Watabe, and Toshiya Manabe

We are investigating the biological function of muscarinic acetylcholine receptors (mAChRs) using mutant mice lacking corresponding genes (mAChR KO mice). These mice have been established by Matsui *et al*. at the Laboratory of Biomedical Genetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo (Prof. Makoto Mark Taketo's Lab.). The mAChRs (M_1, M_2, M_3, M_4 and M_5) belong to a group of seven transmembrane-spanning receptors and are distributed widely in the both central and peripheral nervous systems. We have cloned all five genes for mouse mAChRs (*Chrm1, Chrm2, Chrm3, Chrm4*, and *Chrm5*), and determined their chromosomal locations.

Elucidation of the subtype-specific functions of mAChRs has been a matter of considerable interests, especially because they are suitable targets for pharmacological therapeutics. However, because of poor subtype-selectivity of the available ligands, pharmacological approaches to discriminate their roles

remain inconclusive. The use of mAChR KO mice is an alternative strategy to achieve complete subtype specificity. In order to minimize the concomitant effects reflecting the possible difference in the genetic background, we are backcrossing these mice to two representative inbred strains, C57BL/6J and DBA/2J.

We have previously reported that the M_3 KO mice were retarded in post-weaning growth and devoid of pilocarpine-induced salivation. These mice also showed partial mydriasis, and male-selective urinary retention. We newly reported the phenotype of a mutant mouse line that lacks both M_2 and M_3 receptors (M_2/M_3 double KO mice). These mice were viable in spite of complete lack of cholinergic smooth muscle contraction *in vitro*. Because these mice did not develop intestinal obstruction, the widely-accepted view that acetylcholine is essential for normal gut movements such as peristalsis should be challenged. In the eyes, the M_2/M_3 double KO mice had smaller pupils than those of the M_3 KO mice, which suggests that the signals through M_2 and M_3 counteract to control the pupil size. We also found that M_2 in the smooth muscle mediates inhibition of the relaxant agents that increase cAMP levels.

Finally, mAChRs are supposed to be important in various brain functions. These include learning and memory, drug addiction, sleep and respiratory control, and striatal function. We are investigating the role of each subtype in these aspects, employing molecular biology, electrophysiology, and behavioral experiments. Our mice are now regarded as invaluable resources and we are organizing many collaborative programs.

5. Role of Ras/MAP kinase signaling in synaptic plasticity

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Small guanine nucleotide-binding protein Ras and mitogen-activated protein kinase (MAPK) signaling cascade has been suggested to play a regulatory role in the induction of LTP. We therefore examined the change in synaptic transmission and plasticity in genetically manipulated mice that carry no SynGAP, a GTPase-activating protein known to interact with PSD-95 and negatively regulate MAPK signaling. The mutant mice showed a reduced level of LTP at all examined protocols and showed deficits in a hippocampus-dependent spatial learning test, which can be overcome by excess training. The molecular mechanisms underlying this LTP and learning impairment are still unclear, and the studies to identify the altered MAPK pathways in the mutant mice are now in progress.

6. Modulatory neurotransmitters and synaptic plasticity

Ayako M. Watabe, Hideki Miwa, Fumiko Arima, Thomas J. O'Dell[6], and Toshiya Manabe

Several signaling mechanisms that are crucial for the induction of LTP by theta frequency (5 Hz) trains of synaptic stimulation are altered in aged animals. Thus, to determine whether the induction of LTP by theta frequency stimulation is particularly sensitive to changes in synaptic function that occur in aged animals, we compared the effects of three different trains of synaptic stimulation pulses delivered at 5 Hz (theta pulse stimulation, TPS) on synaptic strength in the hippocampal CA1 region of aged and young mice. In addition, we investigated whether the modulation of TPS-induced LTP by β-adrenergic and cholinergic receptor activation showed deficits with aging. Our results indicated that TPS-induced LTP was not diminished in the aged hippocampus but showed pronounced dependence on L-type calcium channels that was not seen in slices from young animals. In addition, we observed that the enhancement of TPS-induced LTP by co-activation of β-adrenergic and cholinergic receptors was significantly reduced in slices obtained from aged animals. Since TPS-induced LTP was not altered in aged mice, our results suggest that deficits in modulatory pathways that regulate activity-dependent forms of synaptic plasticity may contribute to memory impairments in older animals. The molecular and biochemical mechanisms underlying this alteration in aged animals are currently under investigation.

7. Mechanisms of bidirectional synaptic modification

Ayako M. Watabe, Fumiko Arima, Norikazu Katayama, Hideki Miwa, Noriko Kumazawa, and Toshiya Manabe

Activity-dependent modification of synaptic strength plays a key role in neural development and some forms of neuronal plasticity. While much focus has been on the LTP mechanisms, not much is known for the molecular mechanisms of LTD, long-lasting suppression of synaptic strength. Recently, it has been reported that activation of the metabotropic glutamate receptor (mGluR) with the group I mGluR agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) induces LTD in the CA1 region of the hippocampus. We investigated potential roles of pre- and postsynaptic processes in the DHPG-induced LTD. DHPG-induced LTD was completely blocked when GDP-βS was delivered into postsynaptic cells, strongly suggesting that DHPG depresses synaptic

transmission through a postsynaptic, G protein-mediated signaling pathway. On the other hand, the effect of DHPG was strongly modulated by experimental manipulations that altered presynaptic calcium influx. Also, enhancing calcium influx by prolonging action potential duration with bath applications of the potassium channel blocker 4-AP strongly reduced the effect of DHPG. Furthermore, while inhibiting both pre- and postsynaptic potassium channels with bath-applied 4-AP blocked the effects of DHPG, inhibition of postsynaptic potassium channels alone with intracellular cesium and TEA had no effect on the ability of DHPG to inhibit synaptic transmission. These results suggest that activation of postsynaptic mGluRs suppresses transmission at excitatory synapses onto CA1 pyramidal cells through presynaptic effects on transmitter release. Further physiological roles of mGluRs in synaptic transmission and activity-dependent modification of synaptic transmission are currently under investigation.

While certain patterns of synaptic stimulation can change synaptic strength, the degree and/or direction of the synaptic modification itself can strongly depend on the previous history of the synaptic activation. This effect of the history of synaptic activity on synaptic plasticity, or plasticity of synaptic plasticity (*metaplasticity*) has been implicated from the theoretical point of view in neuronal network development, but its physiological and biochemical mechanisms are still unclear. To elucidate molecular and cellular mechanisms underlying metaplasticity, we are examining what kinds of transmitter receptors and signaling cascades are involved in metaplasticity.

Publications

Yamamoto, S., Oka, S., Inoue, M., Shimuta, M., Manabe, T., Takahashi, H., Miyamoto, M., Asano, M., Sakagami, J., Sudo, K., Iwakura, Y., Ono, K. and Kawasaki, T. Mice deficient in nervous system-specific carbohydrate epitope HNK-1 exhibit impaired synaptic plasticity and spatial learning. J. Biol. Chem. 277:27227-27231, 2002.

Manabe, T. Does BDNF have pre- or postsynaptic targets? Science 295:1651-1652, 2002.

Kamiya, H., Ozawa, S. and Manabe, T. Kainate receptor-dependent short-term plasticity of presynaptic Ca^{2+} influx at the hippocampal mossy fiber synapses. J. Neurosci. 22:9237-9243, 2002.

Kamiya, H., Umeda, K., Ozawa, S. and Manabe, T. Presynaptic Ca^{2+} entry is unchanged during hippocampal mossy fiber long-term potentiation. J. Neurosci. 22:10524-10528, 2002.

Matsui, M., Motomura, D., Fujikawa, T., Jiang, J., Takahashi, S., Manabe, T. and Taketo, M. M. Mice Lacking M_2 and M_3 Muscarinic acetylcholine receptors are devoid of cholinergic smooth muscle contractions but still viable. J. Neurosci. 22:10627-10632, 2002.

Komiyama, N. H., Watabe, A. M., Carlisle, H. J., Porter, K., Charlesworth, P., Monti, J., Strathdee, D. J. C., O'Carroll, C. M., Martin, S. J., Morris, R. G. M., O'Dell, T. J. and Grant, S. G. N. SynGAP regulates ERK/MAPK signaling, synaptic plasticity and learning in the complex with postsynaptic density 95 and NMDA receptor. J. Neurosci. 22:9721-9732, 2002.

Watabe, A. M., Carlisle, H. J. and O'Dell, T. J. Postsynaptic induction and presynaptic expression of group 1 mGluR-dependent long-term depression in the hippocampal CA1 region. J. Neurophysiol. 87:1395-1407, 2002.

Matsui, M., Griffin, M. T., Shehnaz, D., Taketo M. M. and Ehlert F. J. Increased relaxant action of forskolin and isoproterenol against muscarinic agonist-induced contractions in smooth muscle from M_2 receptor knockout mice. J. Pharmacol. Exp. Ther. (in press)

Watabe, A. M. and O'Dell T. J. Age-related changes in theta frequency stimulation-induced long-term potentiation. Neurobiol. Aging (in press)

松井稔 , 舩田正彦．ムスカリン性受容体サブタイプと疾患－最近の展開－．精神保健研究．48:43-51, 2002.

神谷温之、真鍋俊也．高次脳機能　－学習・記憶と海馬シナプス伝達の可塑性－．総合リハビリテーション．30: 915-919, 2002.

真鍋俊也(分担執筆)．海馬におけるシナプス可塑性．脳の発生・分化・可塑性．御子柴克彦、清水孝雄編集．共立出版．166-177, 2002.

神谷温之、真鍋俊也 (分担執筆)．神経の可塑性と記憶･学習．分子生物学イラストレイテッド(改訂第2版)．田村隆明、山本雅編集．319-323, 2002.