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 the whole animal 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 Tyr 1472 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. Analysis of muscarinic acetylcholine receptor functions using knockout mice

Minoru Matsui, Shinji Kusakawa, Yuji Kiyama, Hideki Miwa, Toru Shinoe, Naoki Hirahara, Sayuri Inagaki, Naoko Numata, Shiho Sato, 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 Laboratory of Biomedical Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo (Prof. Makoto M. Taketo 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 both the central and peripheral nervous systems. Elucidation of the subtype-specific functions of mAChRs has been a matter of considerable interest, 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 have backcrossed most of these mutant lines to the two representative inbred strains, C57BL/6J and DBA/2J, for more than 10 generations. Various compound mutant mice (M_1/M_2, M_1/M_3, M_1/M_4, M_1/M_5, M_2/M_3, M_2/M_4, and M_3/M_5) are also available.

We are investigating the significance of each subtype, employing molecular biology, electrophysiology, and behavioral experiments. During this year, we published eight original research articles (see the Publications section) and made nine presentations at scientific meetings.

3. Intracellular signaling in synaptic plasticity

Ayako M. Watabe, Sheng-Tian Li, Norikazu Katayama, Hideki Miwa, Saknan Bongsebandhu-phubhakdi, Thomas J. O'Dell[3], and Toshiya Manabe: [3]Department of Physiology, School of Medicine, University of California, Los Angeles, USA

The small GTPase Ras as well as Ras regulators and effectors are associated with NMDA-type glutamate receptors in the postsynaptic density of excitatory synapses. Although the role of Ras in NMDA receptor-mediated signaling has not been well characterized, several findings indicate that Ras signaling pathways have an important role in NMDA receptor-dependent forms of synaptic plasticity, such as long-term potentiation (LTP). For instance, mice with mutations affecting H-Ras or SynGAP (a synaptic Ras-GTPase-activating protein) have alterations in hippocampal LTP. Moreover, pharmacological inhibition of the Ras effectors phosphatidylinositol 3-kinase (PI3-kinase) and the p 44/42 MAPK (mitogen-activated protein kinase) pathway disrupts LTP. Although Ras-activated signaling pathways are clearly involved in LTP, the molecular details of how these pathways contribute to an enhancement of synaptic strength remain unclear. We therefore examined the role of PI3-kinase and ERK in LTP at excitatory synapses in the CA1 region of the mouse hippocampus. Consistent with the notion that PI3-kinase links NMDA receptors to the ERK pathway, PI3-kinase inhibitors significantly reduced both NMDA and high-frequency stimulation-induced increases in ERK2 phosphorylation. We found, however, that PI3-kinase inhibitors suppress LTP under conditions in which blocking ERK activation with MEK (MAP kinase kinase) inhibitors has no effect. Thus, although PI3-kinase contributes to NMDA receptor-mediated ERK activation, our results demonstrate that the induction of LTP is also dependent on PI3-kinase signaling through ERK-independent pathways.

We are currently extending these studies and have started new projects on a few signaling molecules.

4. Modulatory neurotransmitters and synaptic plasticity

Ayako M. Watabe, Shizuka Kobayashi, Hiroyuki Kato, Michiko Nakamura, Takayuki Morimoto, Akiko Moro, Thomas J. O'Dell[3], 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 modula-

tory 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.

We are currently extending these studies and have started new projects.

5. Age-dependent modulation of hippocampal LTP and spatial learning

a. Age-dependent enhancement of hippocampal LTP and impairment of spatial learning through the ROCK pathway in protein tyrosine phosphatase receptor type Z (Ptprz)-deficient mice

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Although protein tyrosine phosphatases are abundantly expressed in the brain, their roles in synaptic plasticity have not been well elucidated. In this study, we have examined the physiological functions of Ptprz, which is a receptor-type protein tyrosine phosphatase predominantly expressed in the brain as a chondroitin sulfate proteoglycan. We have examined phenotypes of mutant mice deficient in *Ptprz*, using electrophysiological, pharmacological and behavioral approaches. Mutant mice exhibit enhanced LTP in the CA1 region of hippocampal slices and impaired spatial learning abilities in an age-dependent manner: young adult (less than 10 weeks old) mutant mice show normal LTP and learning abilities in Morris water maze task, whereas adult (more than 13 weeks old) mutant mice exhibit enhanced LTP and impairment in the task. The enhanced LTP is specifically canceled out by the ROCK inhibitor Y-27632. These findings suggest that the lack of *Ptprz* leads to aberrant activation of ROCK, and resultantly to enhanced LTP in the slice and learning impairments in the animal.

b. Age-dependent enhancement of hippocampal long-term potentiation in knock-in mice expressing human apolipoprotein E4 instead of mouse apolipoprotein E

Hiroyuki W. Kitamura[6], Hiroki Hamanaka[6], Koji Wada[6], Chiharu Yamazaki[6], Nobuyuki Nukina[6], Masahiko Watanabe[7], Shinobu C. Fujita[8], and Toshiya Manabe: [6]Laboratory for Neurodegeneration Signal, RIKEN Brain Science Institute, [7]Department of Anatomy, Hokkaido University School of Medicine, and [8]Mitsubishi Kagaku Institute of Life Sciences

Human apolipoprotein E (apoE) comprises three isoforms, apoE2, apoE3 and apoE4, and apoE4 has been reported as a risk factor of Alzheimer's disease (AD). One of the clinical symptoms of AD is disorder of memory that has been suggested to be related with synaptic plasticity such as long-term potentiation (LTP). Here, we show the enhancement of hippocampal LTP at younger age in knock-in mice lacking mouse apoE, but instead expressing human apoE4. The enhancement of LTP in apoE4 knock-in mice is age dependent, and it disappears in adult apoE4 knock-in mice. In apoE3 knock-in mice LTP is unaltered, thus human apoE4, but not apoE3, specifically modulates synaptic plasticity at younger age. Since basal synaptic transmission and distribution of glutamate receptors, as well as presynaptic functions, are intact in apoE4 knock-in mice, postsynaptic functional modification of LTP through lipid homeostasis is suggested. ApoE4 knock-in mice would be a useful animal model of human apoE4 carriers, and our finding that LTP is enhanced in younger apoE4 knock-in mice is in accord with the previous report showing higher intelligence in young human apoE4 carriers.

6. Neurogenesis in the nervous system

Yoko Tabata[9], Yasuo Ouchi[9], Haruyuki Kamiya[1], Ken-ichi Arai[9], Sumiko Watanabe[9], Sheng-Tian Li, Noriko Takemura, Fumiko Arima, Ayako M. Watabe, and Toshiya Manabe: [9]Division of Molecular and Developmental Biology, Department of Basic Medical Sciences

With the goal of generating retinal cells from mouse embryonic stem (ES) cells by exogenous gene transfer, we introduced the RX/rax transcription factor, which is expressed in immature retinal cells, into feeder-free mouse ES cells, CCE. CCE cells expressing RX/rax as well as EGFP (CCE-RX/E cells) proliferated and remained in the undifferentiated state in the presence of leukemia inhibitory factor (LIF), as did parental ES cells. We made use of mouse embryo retinal explant cultures to address the differentiation ability of grafted ES cells. Dissociated embryo bodies were treated with retinoic acid for use as donor cells and co-cultured with retina explants for 2 weeks. In contrast to the parental CCE cells, which could not migrate into host retinal cultures, CCE-RX/E cells migrated

into the host retina and extended their process-like structures between the host retinal cells. Most of the grafted CCE-RX/E cells became located in the ganglion cell and inner plexiform layers and expressed ganglion and horizontal cell markers. Furthermore, these grafted cells had electrophysiological properties expected of ganglion cells. Our data thus suggest that subpopulations of retinal neurons can be generated in retinal explant cultures from the grafted mouse ES cell expressing ectopically the transcription factor Rx/rax.

We are currently extending these studies and have started new projects on adult neurogenesis.

7. The role of the AP-3 clathrin adaptor in the release of neurotransmitters

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AP-3 is a member of the adaptor protein (AP) complex family that regulates the vesicular transport of cargo proteins in the secretory and endocytic pathways. There are two isoforms of AP-3: the ubiquitously expressed AP-3A and the neuron-specific AP-3B. Although the physiological role of AP-3A has recently been elucidated, that of AP-3B remains unsolved. To address this question, we generated mice lacking μ3B, a subunit of AP-3B. $\mu3B^{-/-}$ mice suffered from spontaneous epileptic seizures. Morphological abnormalities were observed at synapses in these mice. Biochemical studies demonstrated the impairment of γ-aminobutyric acid (GABA) release because of, at least in part, the reduction of vesicular GABA transporter in $\mu3B^{-/-}$ mice. This facilitated the induction of long-term potentiation in the hippocampus and the abnormal propagation of neuronal excitability via the temporoammonic pathway. Thus, AP-3B plays a critical role in the normal formation and function of a subset of synaptic vesicles. This work adds a new aspect to the pathogenesis of epilepsy.

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