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 a variety of brain regions, excitatory synaptic transmission is regulated dynamically depending on the pattern of synaptic activation: high-frequency activation induces long-lasting enhancement of the 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 the 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, 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 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. The achievement of this year includes elucidation of mAChR functions in intestinal ACh release, smooth muscle contraction/relaxation, gastric acid secretion and hippocampal synaptic plasticity (see the publication list for details).

3. Neuromodulators and synaptic plasticity

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Neuronal leucine-rich repeat proteins (NLRRs) are type I transmembrane proteins and expressed in neuronal tissues, but their function remains unknown. We identified and characterized a new member of the NLRR family, NLRR 4. In order to elucidate its roles in the central nervous system, we generated NLRR4-deficient (NLRR4(−/−)) mice and found that they showed impaired memory retention. In hippocampus-dependent learning tasks, NLRR4(−/−) mice were able to learn and maintain the memories for one day but unable to retain the memories for four days after learning. In contrast, in a hippocampus-independent task, NLRR4 (−/−) mice were able to retain the memory normally for at least seven days. These results suggest that NLRR4 plays a key role in hippocampus-dependent long-lasting memory.

We are currently examining many other neuromodulators that localize in central synapses, including intracellular signal transduction molecules and adhesion molecules.

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

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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 protein tyrosine phosphatase receptor type Z (Ptprz), which is 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 Rho-associated kinase (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.

5. Dynamics of the actin cytoskeleton in dendritic spines: roles in morphological regulation and synaptic plasticity

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Dendritic spines of pyramidal cells in the mature brain receive excitatory inputs. Each spine provides a postsynaptic biochemical compartment. Since Santiago Ramon y Cajal discovered dendritic spines of neurons more than 100 years ago, it has been a long-lasting question whether shapes of spines are related to their function. Recent advanced techniques of imaging GFP-tagged proteins reveal that spine shapes are unexpectedly dynamic, responding to glutamate stimulation. The actin cytoskeleton predominates in spines, and regulates their morphological plasticity and the anchoring of certain postsynaptic molecules. Numerous studies suggest that actin remodeling is a key to understand the molecular mechanism underlying activity-dependent morphological changes. This project aims to elucidate a role of reorganization of the spinous actin cytoskeleton in synaptic functions.

Stability and mechanical property of actin filaments are generally regulated by their side-binding proteins. Drebrin, one of the actin side-binding proteins, is highly enriched in dendritic spines of mature brains. Using immunoelectron microscopy and a newly developed antibody against drebrin A, we have shown that drebrin A, a neuron-specific isoform of drebrin, localizes in sites of prospective excitatory synapses in the immature brain. We have also found that 25% of dendritic spines contain no drebrin. Since Alzheimer's disease shows major loss of drebrin in the dendritic spine and since down-regulation of the drebrin-A isoform caused by antisense oligonucleotides induces cognitive deficits, we hypothesize that the drebrin content in a dendritic spine is closely related to its synaptic function. It has been immunohistochemically shown that down-regulation of the drebrin-A isoform caused by antisense oligonucleotides in developing cultured hippocampal neurons prevents spine formation and PSD-95 accumulation in dendritic spines. We are now interested in a role of drebrin in trafficking of glutamate receptors during synaptogenesis.

We have reported that intense stimulation with glutamate induces the translocation of drebrin from dendritic spines to their parent dendrites. The translocation of drebrin might be a cause of actin reorganization associated with synaptic activity. Further immunohistochemical and DiI-labeling studies on the effects of glutamate on spine shapes are now progressing. The ionic mechanisms underlying the drebrin translocation have also been examined using glutamate receptor antagonists and Ca^{2+} channel blockers. We are now investigating the ATPase-dependent mechanism of the drebrin translocation. We have just started to examine the effects of ATPase inhibition on synaptic plasticity such as LTP at excitatory synapses in the CA1 region of the rat hippocampus.

6. Spatial and temporal patterns of the signal propagation in hippocampal neuronal circuits: gating mechanisms in the dentate gyrus and the CA2 region in the hippocampal network

Yuko Sekino, Shizuka Kobayashi, Akihiro Fukushima, Akiko Moro, Makoto Ito[7], Kenji Doya[7], Toshiya Manabe, and Tomoaki Shirao[6]: [7]IRP, OIST, JST

The lamellar hypothesis in the hippocampus is based on physiological data showing that stimulation of the entorhinal cortex activates only a limited number of CA1 cells arranged in a direction along the alvear fibers of the hippocampus. A simple tri-synaptic circuit (DG-CA 3-CA1), which is based on classical anatomical observations with Golgi staining, is consistent with the lamellar hypothesis. However, this hypothesis has been criticized because recent anatomical work has revealed that there is wider distribution of axons along the longitudinal axis of the hippocampus than expected in the simple tri-synaptic concept, and that there are much richer connections among hippocampal subfields (DG, CA3, CA2, and CA1). The discrepancy between results of physiological and anatomical experiments may be due to the inhibitory mechanisms that suppress signal propagation beyond lamellar organization. To examine whether such an inhibitory mechanism is present between lamellae in the rat hippocampus, hippocampal slices were prepared transversely (at a right angle to the long axis), and obliquely (along the alvear fibers). The mossy fiber stimulation evoked population spikes of CA1 neurons in the oblique slices, but not in the transverse slices. These data are consistent with the tri-synaptic circuit classically proposed in the lamellar hypothesis. We found that an adenosine A_1 receptor antagonist, 8-cyclopentyltheo-phylline (8-CPT), produced population spikes in CA1 neurons in the transverse slices. These data indicate that endogenous activity of adenosine A_1 receptors is involved in the inhibition of signal propa-

gation from CA3 to CA1 beyond lamellar organization. We have started to analyze spatial and temporal patterns of the signal propagation from CA3 to CA1 evoked by the mossy fiber stimulation in oblique and transverse slices using a newly developed low-noise CMOS sensor. We have immunohistochemically shown that adenosine A_1 receptors are highly expressed in the CA2 region. Optical recording using a voltage-sensitive dye would enclose whether CA2 neurons are activated by the application of 8-CPT and whether the activation of CA2 neurons is the source of the CA1 activity.

We are currently interested in a role of the supramammillary nucleus (SuM) of the hypothalamic nucleus in the hippocampal function, because the SuM neurons send dense fibers directly to the dentate gyrus and the CA2 region. We have previously shown that intrasupramammillary injection of the $GABA_A$ receptor agonist muscimol prevents the generation of seizure discharges in the rat hippocampus of a kainic acid-induced epileptic model. Our findings suggest that inputs from the SuM to the hippocampus gate the signal flow from the entorhinal cortex to the hippocampus. We have started a new project on signal propagation from the entorhinal cortex to the dentate gyrus using the horizontal slice preparation in which the connection between the two brain regions is preserved.

We hypothesize that the SuM controls the hippocampal memory function. We have tried to trace the fiber tracts from the SuM to the hippocampus with a tracer injection. Since the CA2 region is in the position which controls longitudinal signal propagation in the hippocampal formation, it is important to assess when and how CA2 neurons are activated in vivo. We have analyzed the number of Fos-immunopositive neurons (FN) in the SuM and the hippocampus of the rats that had been placed in an open field. Further, we have analyzed effects of SuM lesions on the increase of FN in the CA2 region. The CA2 region was identified by the absence of the mossy fibers. We are preparing a paper on these results.

7. Regulation of Adenosine A_1 Receptor Expression

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Pertussis toxin ADP-ribosylates G_i- and G_o-transducing proteins and functionally uncouples adenosine A_1 receptor (A_1AR) from its effectors. We hypothesized that this loss in receptor coupling could lead to de novo A_1AR synthesis by the cell in a futile attempt to re-establish normal receptor function. To test this hypothesis, we used hamster ductus deferens tumor (DDT1 MF-2) cells, a cell culture model for studying A_1AR, and showed that pertussis toxin (100 ng/ml) produced a time-dependent loss in A_1AR-Gi interaction and abolished A_1AR activation of extracellular signal regulated kinase (ERK)1/2. Interestingly, pertussis toxin increased the expression of A_1AR, as measured by real time PCR, immunocytochemistry and [^{3}H]-cyclopentyl-1,3-dipropylxanthine (DPCPX) binding, suggesting a compensatory response to Gi protein inactivation. DDT1 MF-2 cells exposed to pertussis toxin demonstrated activation of nuclear factor (NF)-κB within 30 min of exposure, a time point which preceded the loss of function of the A_1 AR. Inhibition of NF-κB attenuated the increase in A_1AR induced by pertussis toxin. Cells exposed to B-oligomer subunit of pertussis toxin, devoid of significant ADP ribosyltransferase activity, showed increased A_1AR protein expression, preceded by activation of NF-κB. B-oligomer increased intracellular Ca^{2+} in DDT1 MF-2 cells. Chelation of intracellular Ca^{2+} with 1,2-bis (2-aminophenoxy) ethane-N,N,N′, N′-tetraacetic acid tetra(acetoxymethyl)ester (BAPTA) or inhibition of protein kinase C (PKC) with bisindolylmaleimide hydrochloride (BIM), reduced the activation of NF-κB and [^{3}H] DPCPX binding. We conclude that pertussis toxin promotes de novo A_1AR synthesis by activating NF-κB through an ADP ribosylation-independent mechanism involving intracellular Ca^{2+} release and PKC activation.

Publications

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