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 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, Sayuri Inagaki, 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. We have established an efficient system of mouse breeding and a total of 4,900 pups have been born within this year. Various compound mutant mice (M_1/M_2, M_1/M_3, M_1/M_5, M_2/M_3 and M_2/M_4) are also available.

Our original research articles of this year are as follows. Using a DBA/2J congenic strain, we have revealed that the M_4 receptor is a target of anticholinergic therapy of catalepsy induced by haloperidol (Karasawa et al., 2003). We have collaborated with Dr. Ehlert, UC Irvine, about the roles of M_2 in smooth muscle contractility (Matsui et al., 2003; Griffin et al., 2004). We have collaborated with Dr. Kano, Kanazawa Univ., and discovered that both M_1 and M_3 are responsible for the muscarinic enhancement of retrograde endocannabinoid signaling in the hippocampus (Ohno-Shosaku et al., 2004). We have also collaborated with Dr. Okabe, Kyoto Pharmaceutical Univ., and found that M_3 is essential for normal gastric secretion (Aihara et al., 2003).

Muscarinic receptors 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.

3. Identification and characterization of a novel GTPase activating protein p250GAP

Ayako M. Watabe, Takanobu Nakazawa[2], Tohru Tezuka[2], Tadashi Yamamoto[2], and Toshiya Manabe

N-methyl-D-aspartate (NMDA) receptors regulate structural plasticity by modulating actin organization within dendritic spines. We have reported identification and characterization of p250GAP, a novel GTPase-activating protein for Rho family proteins that interacts with the GluRε2 (NR2B) subunit of NMDA receptors in vivo. The p250GAP mRNA was enriched in brain, with high expression in cortex, corpus striatum, hippocampus, and thalamus. Within neurons, p250GAP was highly concentrated in the postsynaptic density and colocalized with the GluRε2 (NR2B) subunit of NMDA receptors and with postsynaptic density-95. p250GAP promoted GTP hydrolysis of Cdc42 and RhoA in vitro and in vivo. When overexpressed in neuroblastoma cells, p250GAP suppressed the activities of Rho family proteins, which resulted in alteration of neurite outgrowth. Finally, NMDA receptor stimulation led to dephosphorylation and redistribution of p250GAP in hippocampal slices. Together, p250GAP is likely to be involved in NMDA receptor activity-dependent actin reorganization in dendritic spines.

4. Role of Ras signaling in synaptic plasticity

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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 p44/42 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.

5. Modulatory neurotransmitters and synaptic plasticity

Ayako M. Watabe, Fumiko Arima, Shizuka Kobayashi, 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 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.

Publications

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