LABORATORY OF NEUROCHEMISTRY

Associate Professor: *SASAOKA, Toshikuni*

Our major research interest is to understand the physiological role of the dopaminergic system in animal behavior, especially locomotion and eating behavior, using genetically altered mice, both transgenic and gene knockout mice. In addition, we have developed a novel method of conditional mutagenesis in mice in order to substitute the amino acid sequence of the target gene in particular cells. We analyze the physiological roles of the components of the dystrophin complex on the skeletal muscle membrane using genetically modified mice.

I. Role of dopaminergic transmission in locomotion and eating behavior

The dopaminergic system is implicated in the regulation of the several peptide hormones in the pituitary, the modulation of locomotor activity, the modulation of synaptic plasticity and the development of neurons. The dopaminergic system is also implicated in the control of emotion, motivation and cognition. Dysfunction of dopaminergic system can result in several neurological and psychiatric disorders such as Parkinsonís disease and schizophrenia.

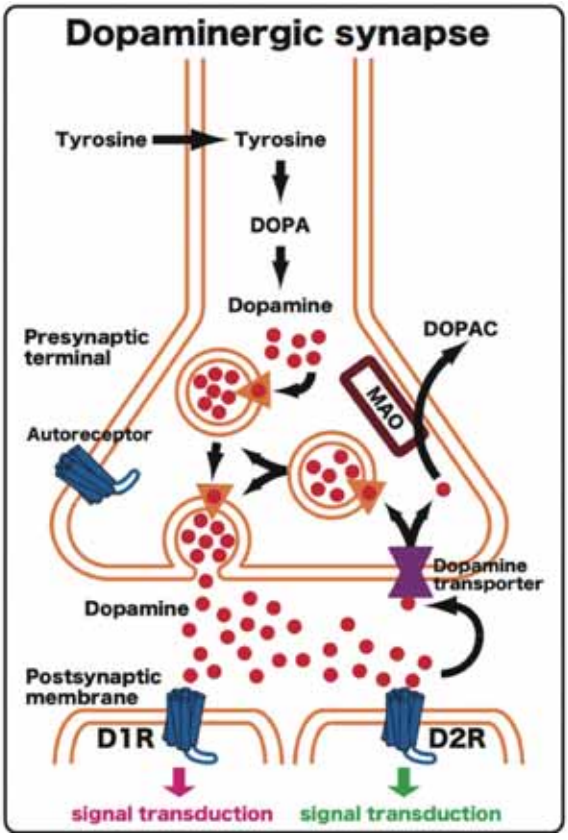

Figure 1. Schematic drawing of dopaminergic synapse

In mammals five subtypes of dopamine receptor (D1R, D2R, D3R, D4R and D5R) are identified and divided into two subgroups referred to as D1-like (D1R, D5R) and D2-like (D2R, D3R and D4R) receptors on the basis of their gene structure and their pharmacological and transductional properties. D1R and D2R are most abundantly and widely expressed in the brain and often play a synergistic role. D1R has an opposite property to D2R with respect to the intracellular signal transduction.

In collaboration with the Laboratory of Director General we have been investigating the involvement of dopaminergic transmission via D1R and D2R in the regulation of locomotion and eating behavior. We generated *D1R*/*D2R* double knockout (DKO) mice by crossing *D1R* knockout (KO) with *D2R* KO mice, and observed that *D1R*/*D2R* DKO mice exhibited impairment in locomotion and eating behavior and died prematurely. To investigate the molecular mechanism of regulation in locomotion and eating behavior, we generated transgenic mice harboring tetracycline-regulated expression of the *D1R* gene on the *D1R*/*D2R* DKO background. Several transgenic mouse lines successfully rescued lethal phenotype of the *D1R*/*D2R* DKO mice and showed doxycycline (Dox) controllable expression of transgenic *D1R* gene (named as *D1R*/*D2R* DKO-*D1R* rescued mice). The *D1R*/*D2R* DKO-*D1R* rescued mice exhibited decrease in locomotion and food/water intake as well as decrease in the amount of transgene expression after Dox administration. We are analyzing these results to identify the mechanism of the relationship between the *D1R* expression and altered behavior. In addition we are investigating whether or not there is a critical period in development for the regulation of locomotion and eating behavior by dopaminergic transmission.

II. Developing a novel conditional mutagenesis method in mice

In order to overcome the limitations of the conventional mouse molecular genetic approach in the functional analysis of target genes, we substituted one critical amino acid residue of N-methyl-D-aspartate receptor (NMDAR), leading to NMDAR activation. The NMDARs are widely expressed in the nervous system, fundamental to excitatory neurotransmission, and play a number of important roles at different brain loci and time points. The NMDARs act as a coincidence detector and are not only important for neuronal differentiation, migration, and survival, but are also critical for activity dependent synapse formation. It is suggested that the aberrant activation of NMDAR causes excitotoxicity, leading to neuronal death in various neurological diseases.

That the Ca^{2+} permeability through NMDAR is blocked by magnesium (Mg^{2+}) in a voltage-dependent manner indicates an essential role of NMDAR as a coincidence detector. Functional NMDARs consist of NMDAR1 (NR1) subunit and at least one subunit of NMDAR2A-2D (NR2A-NR2D). It has been shown that the NR1/NR2A complex expressed in cultured cell is highly sensitive to the voltage-dependent Mg^{2+} block and that the substitution of asparagine (Asp595) by glutamine (Gln595) in the second transmembrane domain of the NR2A subunit results in a reduction of the Mg^{2+} block of the NR1/NR2A complex.

We develop conditional mutagenesis method in mice using Cre-loxP recombination. By our method, we accomplished conditional substitution of the amino acid in mice and our mutant mice exhibited aberrant NMDAR activation and a neurological phenotype, similar to that of mouse models of neurological disorders. This clearly indicates that the NMDAR activation by the critical amino acid substitution leads to the neurological phenotype.

Our method is vastly applicable to the functional analysis of any desired gene and should contribute to studies on the structural and functional relationships of relevant genes.

III. Analysis of roles of the sarcoglycan complex, dystroglycan complex and caveolin-3

Sarcoglycans (SGs) are trans-sarcolemmal glycoproteins that associate together to form sarcoglycan complex (SGC) and are present in the sarcolemma. SGC, together with dystrophin and the dystroglycan complex, comprises the dystrophin complex, which is considered to be the mechanical link between the basement membrane and the intracellular cytoskeleton for protecting the sarcolemma from mechanical stress during muscle contraction. Each of four SG subunits (α-, β-, γ- and δ-SG) is responsible for four respective forms of SG-deficient muscular dystrophy, sarcoglycanopathy (SGP). All of the SGs and sarcospan are absent in the sarcolemma in any form of SGP, suggesting that the SGC is not assembled if a single subunit of the SGC is absent.

To analyze the function of the SGC, we generated the β-SG KO and γ-SG KO mice. These KO mice developed progressive muscular dystrophy and all SGs and sarcospan were absent in the sarcolemma. The dystrophin complex isolated from the SG-deficient skeletal muscles was biochemically unstable. This indicates that SGC and sarcospan play an important role in stabilizing the dystrophin complex connecting the basement membrane and the cytoskeleton.

Dystroglycan (DG) is a transmembrane glycoprotein complex which plays an important role by connecting the intracellular cytoskeleton and the extracelluler matrix. DG is expressed as an 895 amino acid precursor and cleaved between amino acid residues 653 and 654 to generate α- and β-DG subunits. In collaboration with Dr. Torahiko Tanaka of Nihon University School of Medicine, Tokyo, in order to clarify the mechanisms involved in DG cleavage, we performed mutation analyses to determine which amino acid residues and which regions of DG are critical for cleavage. We transfected HEK 293 cells with wild-type and various mutant DGs, and confirmed the DG cleavage. We found that deletions in the upstream (within residues 550 to 645) and downstream (within residues 660 to 722) regions of the cleavage site abolished the cleavage. In contrast, deletion in the more upstream region (520-550) or downstream region (723-742) did not affect the cleavage. Because the critical regions comprise the epitopes for the association of α- (550-585) and β-DG (691-719), DG cleavage seems to be linked with the subunit association. By coexpression of α- and β-DG (not as a precursor), we confirmed the association of α- and β-DG subunits and found that deletions in the N-terminal region of β-DG deteriorate the subunit association. Furthermore, by alanine scanning around the cleavage site, we found that a mutation Trp659Ala completely abolished the cleavage as Ser654Ala did. We suggest that a relatively large portion of precursor DG (residues 550 to 722) forms a specific tertiary structure, in which the α and β-DG domains interact and the cleavage site becomes accessible.

Caveolin-3 is an integral membrane protein and a component of the dystrophin complex that serves as a scaffold of various molecules and is expressed in striated muscles. Its gene mutations cause limb-girdle muscular dystropy (LGMD1C or caveolinopathy) with mild clinical symptoms. In collaboration with Dr. Yasuko Hagiwara of the National Institute of Neuroscience, NCNP, Tokyo, we previously reported that caveolin-3 deficiency causes muscle degeneration and a decrease in sarcolemmal caveolae in *caveolin-3* gene-knockout (*Cav3*-/-) mice. To examine the pathogenic pathways and identify new or modifying factors involved in caveolinopathy, we examined the expression patterns of approximately 8,000 genes in the skeletal muscle of *Cav3*-/- mice using an oligonucleotide array. This data was compared to data from wild-type mice. The fold-ratio analysis suggested that approximately 400 genes, including the unknown genes examined, differ in expression levels. We focused on the *osteopontin* (*OPN*) gene that was under-expressed to the level of expression of the *Cav-3* gene. To examine the mechanism under-expression of *OPN*, we generated *dystrophin* and *Cav-3* double-deficient mice. Although *OPN* was overexpressed in the *dystrophin*-deficient *mdx* mice, simultaneous *Cav-3* deficiency resulted in a decrease in the *OPN* gene expression.

Publication List:

Original papers

Imai, F., Hirai, S., Akimoto, K., Koyama, H., Miyata, T., Ogawa, M., Noguchi, S., Sasaoka, T., Noda, T., and Shigeo, S. (2006). Inactivation of aPKC lambda results in the loss of adherens junctions in neuroepithelial cells without affecting neurogenesis in mouse neocortex. Development *133*, 1735-1744.

Ishii, Y., Oya, T., Lianshun, Z., Gao, Z., Kawaguchi, M., Sabit, H., Matsushima, T., Tokunaga, A., Ishizawa, S., Hori, E., Nabeshima, Y., Sasaoka T., Fujimori, T., Mori, H., and Sasahara, M. (2006). Mouse brains deficient in neuronal PDGF receptor-beta develop normally but are vulnerable to injury. J. Neurochemistry *98*, 588-600.