LABORATORY OF NEUROCHEMISTRY

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Our major research interest is to understand the physiological role of 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 analyze the function of the gene of interest in detail. We analyze function of neurotransmitter receptor complex by using biochemical analysis of the dystrophin complex on the skeletal muscle membrane.

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 neuron. The dopaminergic system is also implicated in control of emotion, motivation and cognition. Dysfunction of dopaminergic system can result in several neurological and psychiatric disorders such as Parkinson's disease and schizophrenia.

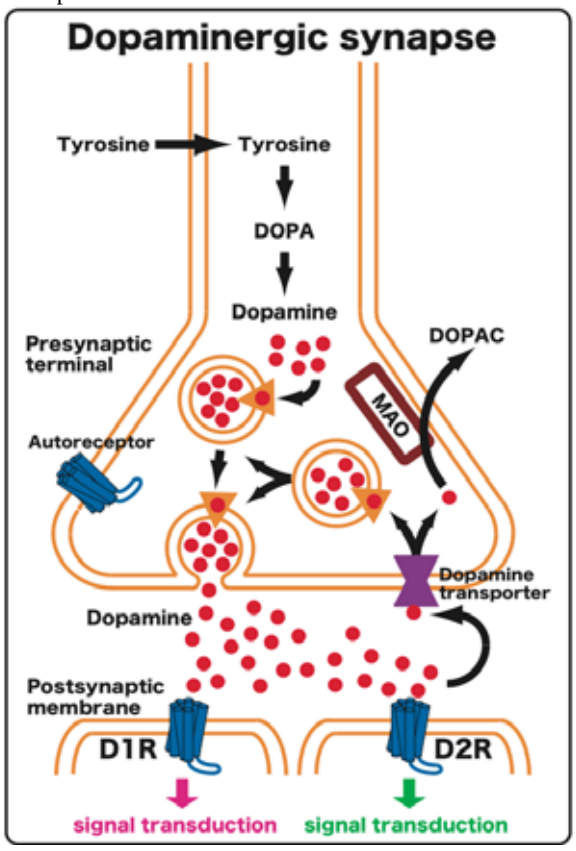

Figure 1. Schematic drawing of dopaminergic synapse

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 role synergistically. D1R has an opposite property to D2R with respect to the intracellular signal transduction.

We have been investigating the involvement of dopaminergic transmission via D1R and D2R in the regulation of locomotion and eating behavior in collaboration with the Laboratory of Director General. 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 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 amount of transgene expression after Dox administration (Figure 2).

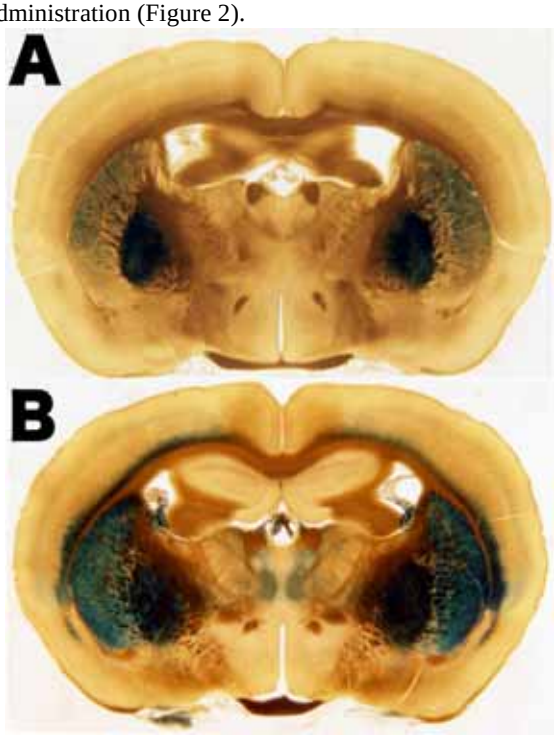

Figure 2. (A) The amount of transgene expression was suppressed in the striatum of the *D1R/D2R* DKO-*D1R* rescued mice by doxycycline (Dox) administration. (B) Before Dox administration the intensive expression of transgene was seen in the striatum. Frontal sections of mouse brains with X-gal staining were shown.

We are attempting to identify areas in which the *D1R* expression was suppressed by Dox administration in order to point out areas responsible for regulation of locomotion and eating/drinking behavior. In addition we are investigating whether or not there is critical period in

development for 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.

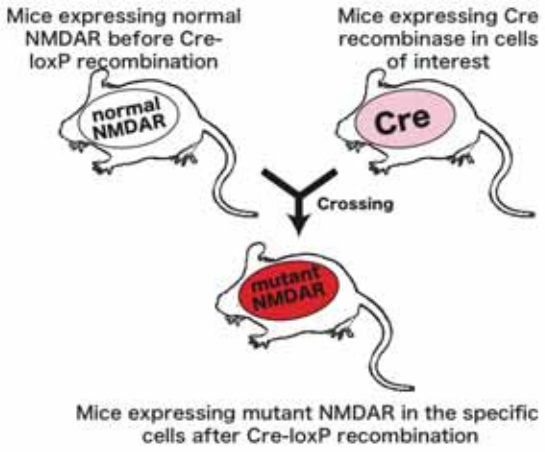

Figure 3. Conditional mutagenesis in mice. First, mutant mice expressing normal NMDAR molecule before Cre-loxP recombination were generated. Second, transgenic mice expressing Cre recombinase in cells of interest were generated. Third, these two mouse lines were crossed to generated mice expressing mutant NMDAR molecule in the cells in which Cre-loxP recombination was executed.

However, the role of Asp595 of the NR2A subunit and the effects of substitution of it with Gln595 on the function of NMDAR *in vivo* remain to be clarified.

We develop conditional mutagenesis method in mice using Cre-loxP recombination (Figure 3). 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. Interestingly, the phenotype of the mice was completely suppressed by administration of NMDAR antagonists. This clearly indicates that the NMDAR activation by the critical amino acid substitution leads to the neurological phenotype.

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

III. The molecular architecture and the physiological role of the sarcoglycan complex (SGC)

Sarcoglycans (SGs) are trans-sarcolemmal glycoproteins that associate together to form SGC and are present in the sarcolemma. SGC, together with dystrophin and the dystroglycan complex, comprises the dystrophin complex, which is considered as 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.

We have generated the KO mice of the other subunit of the dystrophin complex to analyze the physiological role of the subunit.

Publication:

Original paper

Tanaka, T., Watanabe, N., and Sasaoka, T. (2005). Unidirectional subcloning to generate more than 10^9 transformants from 1 microgram of vector DNA. The Nihon University Journal of Medicine, in press.

Review article

Ozawa, E., Mizuno, Y., Hagiwara, Y., Sasaoka, T., and Yoshida, M. (2005). Molecular and cell biology of the sarcoglycan complex. Muscle and Nerve. *32*, 563-576.