LABORATORY OF NEUROCHEMISTRY

Associate Professor
SASAOKA, Toshikuni

NIBB Research Fellow SATO, Asako

Our major research interest is to understand the physiological role of the dopaminergic system in animal behavior, particularly locomotion and eating behaviors, 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 modulation of locomotor activity, the regulation of several peptide hormones in the pituitary, 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 the dopaminergic system can result in several neurological and psychiatric disorders, such as Parkinson's disease and schizophrenia.

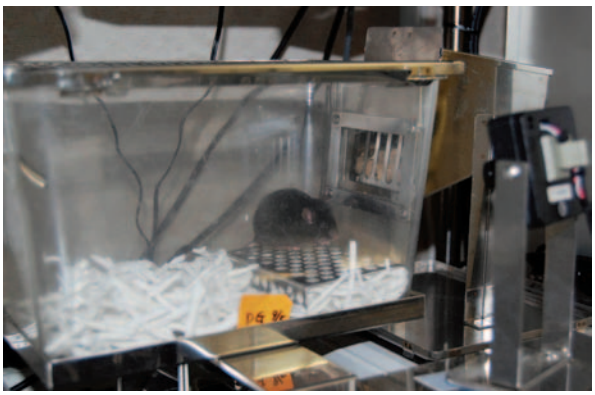

Figure 1. Experimental equipment for measurement of locomotor activity and food/water intake

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

In collaboration with Dr. Motoya Katsuki, Executive Director, National Institute of Natural Sciences, 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 severe impairment in locomotion, no initiation of eating, and died by 4 weeks of age. To investigate the molecular mechanism of motor control 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 decreases in locomotion and food/water intake as well as a decrease in the amount of transgene expression after Dox administration. After the withdrawal of Dox administration, the *D1R/D2R* DKO-*D1R* rescued mice exhibited transient hyperactivity and recovered locomotor activity and food/water intake (Figure 2). We examined the expression levels of D1R protein in the striatum, which is considered to be a major region responsible for control of locomotor activity and containing abundant D1R expression. During the process of recovery of locomotor activity after Dox withdrawal, the transgene D1R expression gradually increased while locomotor activity fluctuated strikingly. These results indicate that the increment of locomotor activity is not simply in proportion to the amount of D1R expression. We are analyzing these results to identify the mechanism of the relationship between the *D1R* expression and altered behavior. In addition, we are also investigating whether or not there is a critical period in development for the regulation of locomotion and eating behavior by dopaminergic transmission.

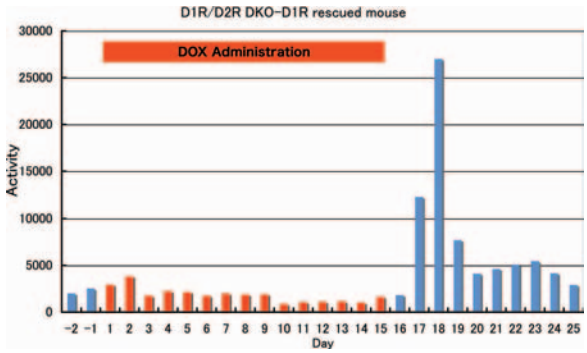

Figure 2. Locomotor activity of *D1R/D2R* DKO-*D1R* rescued mice. Two weeks of Dox administration led to decreased activity followed by transient hyperactivity.

Ⅱ. Developing a novel conditional mutagenesis method in mice

In collaboration with Prof. Yoichi Nabeshima of Kyoto University, we developed a novel mouse developmental biotechnology by introducing an amino acid substitution into a target gene in a spatially and temporally restricted manner.

The goal of the study was 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 (the 595th asparagines, Asp595) of N-methyl-D-aspartate receptor (NMDAR), leading to an aberrant activation of NMDAR. 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 has been suggested that the aberrant activation of NMDAR causes excitotoxicity, leading to neuronal death of various neurological diseases.

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 the 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). We generated the β-SG KO and γ-SG KO mice and found that 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.

Caveolin-3 is a muscle-specific membrane protein, a component of the dystrophin complex, and serves as a scaffold for various molecules. Its gene mutations cause limb-girdle muscular dystrophy (LGMD1C or caveolinopathy) with mild clinical symptoms. In collaboration with Dr. Yasuko Hagiwara of Musashino University, Tokyo, we 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 gene expression profiles of approximately 8, 000 genes in the skeletal muscle of *Cav3-/-* mice using DNA microarray technique. We found that the gene of *osteoponin* (*OPN*), a versatile regulator of inflammation and tissue repair, was significantly down-regulated. This is in contrast to *mdx* mice showing a markedly up-regulated *OPN* gene in their skeletal muscles. Recently, *OPN* has been reported to be important in the pathogenesis of muscular dystrophy. We examined whether up-regulated *OPN* gene expression in *mdx* muscles is altered by the deficiency of caveolin-3. To this end, we developed caveolin-3 and dystrophin double-deficient mice. The levels of *OPN* mRNA and osteopontin in the double-deficient mice clearly decreased compared with those in *mdx* mice. We showed that although the level of *OPN* mRNA expressed in the double-deficient skeletal muscles was lower than that in *mdx* skeletal muscles, macrophage infiltration and muscle regeneration occurred similarly in the double-deficient and *mdx* skeletal muscles. There may still be other, undiscovered factors that are involved in macrophage infiltration and muscle regeneration.

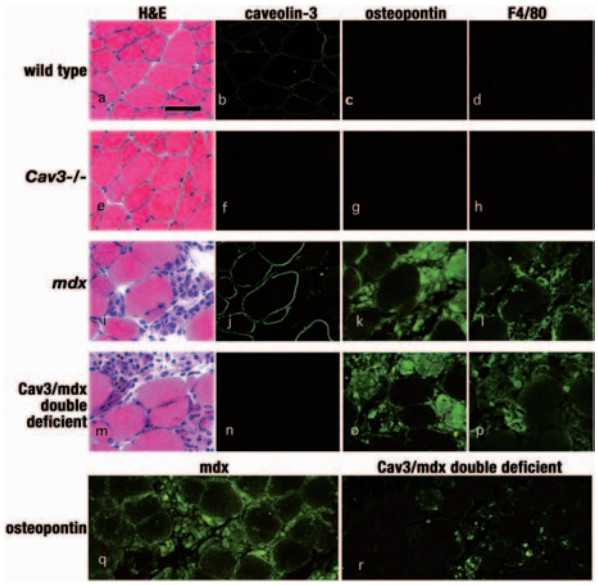

Figure 3. Immunohistochemical analysis of skeletal muscles from mice at 1 month of age. Cross sections were stained with H&E (a, e, i and m) and antibodies against caveolin-3 (b, f, j and n), osteopontin (c, g, k, o, q and r) and F4/80 (d, h, l and p). Note that the surrounding areas of F4/80-positive macrophages in *mdx* and double-deficient mice (l and p) are strongly stained by the anti-osteopontin antibody (k and o). In less infiltrated or noninfiltrated area, the sarcolemma of *mdx* muscle fibers is more strongly stained than that of the double-deficient muscle fibers by the anti-osteopontin antibody (q and r). Bar; 50 μm.

Publication List

〔Original papers〕

- Hagiwara, Y., Fujita, M., Imamura, M., Noguchi, S., and Sasaoka, T. (2006*). Caveolin-3 deficiency decreases the gene expression level of osteopontin in mdx mouse skeletal muscle. Acta Myol. *25*, 53-61.
- Kawasaki, K., Watabe, T., Sase, H., Hirashima, M., Koide, H., Morishita, Y., Yuki, K., Sasaoka, T., Suda, T., Katsuki, M., Miyazono, K., and Miyazawa, K. (2008). Ras signaling directs endothelial specification of VEGFR2+ vascular progenitor cells. J. Cell Biol. *181*, 131-141.

* a paper published after the publication of the previous issue of the Annual Report