LABORATORY OF NEUROPHYSIOLOGY

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When the correct balance between water and sodium level in the body fluid has been broken, terrestrial animals feel water and salt appetite or satiety, and these perceptions subsequently induce the animal behaviors referred to as ingestion or aversion. Our research is focused on understanding the molecular and neural mechanisms underlying the animal behaviors essential to homeostasis of the body fluid.

To explain the properly regulated animal behaviors, neurobiologists have postulated the existence of both osmoreceptors and specific sodium receptors in the brain. However, the molecular entities of these receptors have not been found for a long time. In 2000, by using the gene-targeting technology, we first clarified that Na_x sodium channel is a probable candidate for the specific sodium receptor in the brain.

Na_x had long been classified as a subfamily of voltage-gated sodium channels (NaChs) that serve to generate action potentials in electrically excitable cells such as neuronal and muscle cells. Compared to the other NaChs, however, Na_x has unique amino acid sequences in the regions, which are known to be involved in voltage-dependent activation and inactivation, suggesting that it must have specific functional properties.

To clarify the functional role of Na_x channel, *Na_x*-gene deficient mice were generated by gene-targeting technique and the physiological phenotypes have been examined. Behavioral studies suggested that the Na_x channel plays an important role in the central sensing of body-fluid sodium level and regulation of salt intake behavior. Na_x-deficient mice ingested hypertonic sodium chloride solution in excess in comparison with wild type-mice. LacZ reporter gene knocked into *Na_x*-gene locus revealed that *Na_x* gene is expressed in the circumventricular organs, which are the specialized central organs involved in sensing of sodium concentration and osmosity in the body fluids.

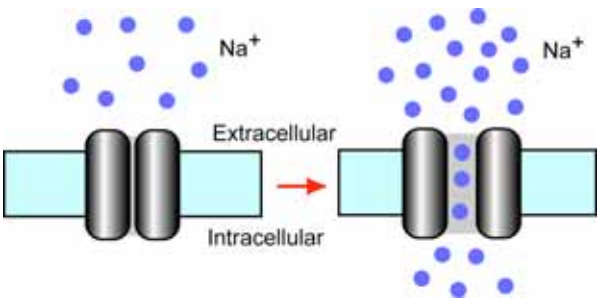

Figure 1. Na_x is a sodium channel sensitive to extracellular sodium level. When the extracellular sodium concentration increases, Na_x channel opens the gate pore and generates the sodium ion influx into the cells. This view was hypothesized by ion-imaging studies.

In 2002, sodium ion imaging and electrophysiological studies using cultured cells derived from the subfornical organs demonstrated that Na_x channel is an extracellular sodium-level sensitive sodium channel (Figure 1). Further, we found that Na_x channel is expressed in non-myelinating Schwann cells and alveolar type II cells in addition to the cells in the circumventricular organs. Na_x channel is thus likely to be involved in reception of sodium-level in the body fluids at the circumventricular organs and sodium absorption in the visceral nervous system and in the lung.

In 2003, we found in collaboration with Prof. Yamamoto's group at Osaka University that the peripheral nervous system has only subtle effects on the higher preference for sodium chloride as observed in the mutant mice. The results suggest that the mutant phenotype is mainly due to the lack of Na_x channel in the central nervous system.

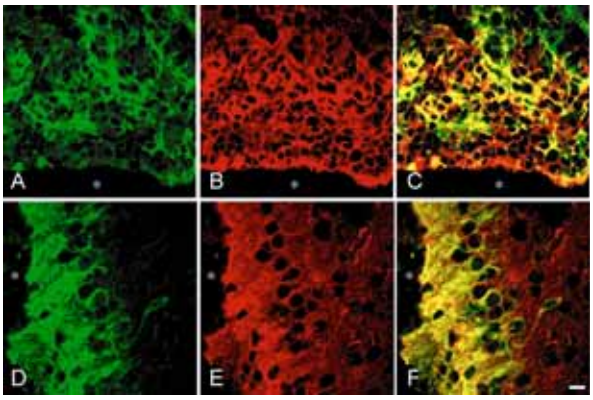

Figure 2. The Na_x channel is co-localized with a glia-specific glutamate transporter GLAST in the SFO and OVLT. Coronal tissue sections of the SFO (*A-C*) and OVLT (*D-F*) were double-stained with anti-Na_x (*A, D*) and GLAST (*B, E*) antibodies. Right panels (*C, F*) are merged images of the left (*A, D*) and middle (*B, E*) panels. Asterisks indicate the ventricles. A large number of round GLAST- and Na_x-negative black holes represent neuronal cell bodies. Scale bar: 10 μm.

In 2004, we developed an automatic measurement equipment for intake volume of drinking solutions. Using this equipment, we showed that the subfornical organ is the principal site for the control of salt-intake behavior, where the Na_x channel is the sodium-level sensor. Infusion of a hypertonic sodium solution into the cerebral ventricle induced extensive water intake and aversion to saline in wild-type animals but not in the knockout mice. Importantly, the aversion to salt was not induced by the infusion of a hyperosmotic mannitol solution with physiological sodium concentration in either genotype of mice. When Na_x cDNA was introduced into the brain of the knockout mice with an adenoviral expression vector, only animals which received a transduction of the *Na_x* gene into the subfornical organ among the circumventricular organs recovered salt-avoiding behavior under dehydrated conditions.

These results clearly show that the subfornical organ is the center of the control of salt-intake behavior in the brain, where the sodium-level-sensitive Na_x channel is involved in sensing the physiological increase in the sodium level of body fluids.

In this year of 2005, in order to understand how the circumventricular organ translates extracellular sodium-level sensed by Na_x channel to the neural activities, we identified subcellular localization of Na_x channel in the organs. Double immunostaining (Figure 2) and immuno-electronmicroscopic (Figure 3) studies clearly showed that Na_x channel was exclusively localized to perineuronal lamellar processes extended from astrocytes and tanycytes in the organs. Importantly, glial cells derived from the organs were capable of sensing extracellular sodium-level, as analysed by ion-imaging method. In addition, we found that the Na_x-expressing glial cells enveloped multiple kinds of neurons including GABAergic interneurons in the organs. Finally, in the organs, neuronal population activated by water deprivation was different from GABAergic interneurons, as monitored by Fos immunoreactivity. Together with previous observation that the organs of *Na_x* knockout mice is hyperactive under water deprivation, these results indicate that the glial Na_x channel senses increased sodium-level in the body fluid and controls the neuronal activity through glial cells.

Since we first reported aberrant behaviors found in *Na_x* knockout mice, a series of our studies have clarified that Na_x channel is a sodium-level sensitive sodium channel playing an essential role in the sodium-sensing of the circumventricular organs and in the control of salt-intake regulation. These works identified the molecular entity of the brain sodium sensor, which has long been hypothesized as one of the important physiological issues. In this year, we newly demonstrated that the primary subcellular locus sensing sodium-level is perineuronal glial processes. This finding suggests that neuron-glia complex plays a key role on the sodium sensing in the circumventricular organs.

Currently, we are now trying to construct functional expression systems of Na_x sodium channel using various heterologous cell lines. The heterologous expression system will provide us useful information on the channel characters. Furthermore, we are studying the involvement of Na_x sodium channel in the regulation of hormone release, using neurohypophyseal vasopressin system. The posterior pituitary is one of simple model systems for research of Na_x sodium channel, since there are only two kinds of cellular components, the nerve terminals releasing neurohypophyseal hormones and glial cells expressing Na_x sodium channel. The model system will also provide us useful information on the physiological function of Na_x channel.

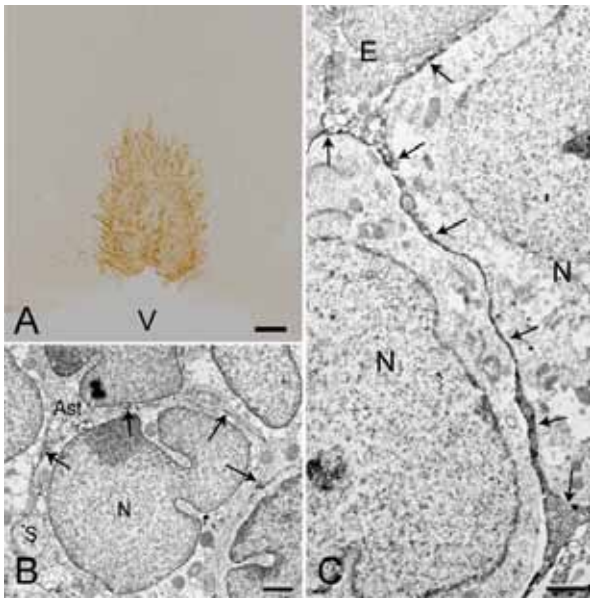

Figure 3. The Na_x channel is localized to glial processes enveloping neurons in the OVLT. *A*, A coronal tissue section of the OVLT stained with anti-Na_x antibody. Fiber-like structures radiating out from the midline and ventricle were immunopositive. *B*, *C*, Immunoelectron microscopy using anti-Na_x antibody. In *B*, the core region of the OVLT is shown. Neurons and their processes are surrounded by immunopositive thin processes of astrocytes. In *C*, a ventricular region in the OVLT is shown. Ventricular side towards the upper side. Neurons are covered by extremely thin immunopositive processes of ependymal cells. Arrows in *B* and *C* indicate immunopositive signals. V, ventricle; N, neuron; S, synapse; E, ependymal cell; Ast, astrocyte. Scale bars: 50 μm for *A*, and 1 μm for *B* and *C*.

Publication List:

Original papers

Niisato, K., Fujikawa, A., Komai, S., Shintani, T., Watanabe, E., Sakaguchi, G., Katsuura, G., Manabe, T., and Noda, M. (2005). Age-dependent enhancement of hippocampal LTP and impairment of spatial learning through the ROCK pathway in protein tyrosine phosphatase receptor type Z-deficient mice. J. Neurosci. *25*, 1081-1088.

Watanabe, E., Hiyama, T.Y., Shimizu, H., Kodama, R., Hayashi, N., Miyata, S., Yanagawa, Y., Obata, K., and Noda, M. (2005). Sodium-level-sensitive sodium channel Nax is expressed in glial laminate processes in the sensory circumventricular organs. Am. J. Physiol., in press.