LABORATORY OF NEUROPHYSIOLOGY

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When the correct balance between water and sodium levels in the body fluid has been disrupted, 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 long eluded discovery. 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 the 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 using a 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 the sensing of sodium concentration and osmosity in the body fluids.

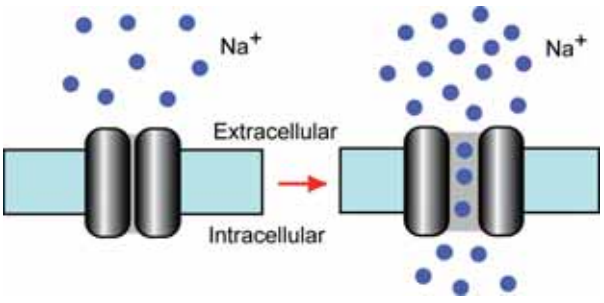

Figure 1. Na_x is a sodium channel sensitive to extracellular sodium level. When the extracellular sodium concentration increases, Na_x channel opens the gate pore and generates the sodium ion influx into the cells. This view was hypothesized by ion-imaging studies.

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 the 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. Yamamotoís 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.

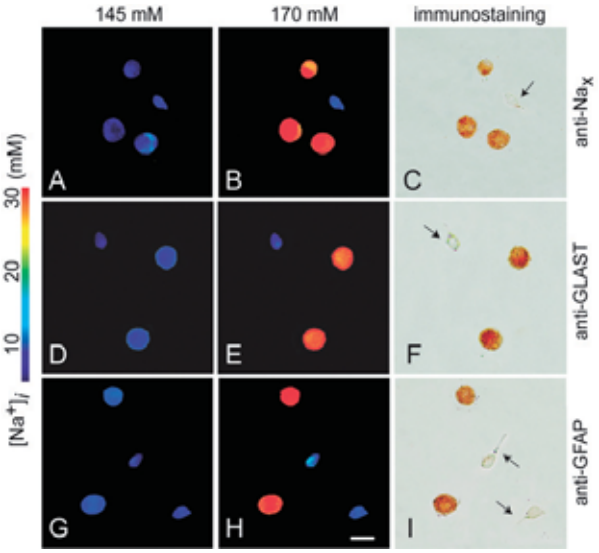

Figure 2. Glial cells isolated from the SFO express Nax channel and show sensitivity to the extracellular sodium level. Sodium imaging study using the dissociated SFO cells. Pseudocolor images of the intracellular sodium concentration ([Na+]i) of SFO cells in the control solution (the extracellular sodium concentration = 145 mM, A , D and G) and in the high sodium solution (170 mM, B, E and H). A, D, G and B, E, H are images 5 min before and 20 min after stimulation with the hypertonic 170 mM [Na+] solution, respectively. After sodium-image recordings, cells were fixed and stained with anti-Na_x (C), anti-GLAST (F) or anti-GFAP (I) antibodies. All the sodium-sensitive cells are immunopositive for Na_x, GLAST and GFAP. Arrows in C, F and I indicate small neurons bearing short neurites, which are all insensitive to the extracellular sodium increase. Scale bar: 20 μm.

In 2004, we developed 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 those animals that 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 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 and immuno-electronmicroscopic 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 the ion-imaging method (Figure 2). In addition, we found that the Na_x-expressing glial cells enveloped multiple kinds of neurons including GABAergic interneurons in the organs (Figure 3). 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 are 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.

In 2006, we tried to construct functional expression systems of Na_x sodium channel using various heterologous cell lines. Attempts to express functional Na_x channels in cultured cell lines have been long unsuccessful. We developed glial cell lines in which the expression of Na_x channel is inducible under the control of the tetracycline responsive element. Several experiments using the cell lines showed that cellular signals via Na_x channels are coupled with a metabolic cascade of the glial cells, suggesting that the information of physiological increase of the sodium level in body fluids sensed by Na_x channel in glial cells is transmitted to neurons by the altered metabolic state of the glial cells.

In addition, we are studying the involvement of Na_x sodium channel in the regulation of hormone release, using a 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 with useful information on the physiological function of Na_x channel.

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 recent years, we newly demonstrated that the primary subcellular locus sensing sodium-level is perineuronal glial processes. This finding suggests that the neuron-glia complex plays a key role in sodium sensing in the circumventricular organs.

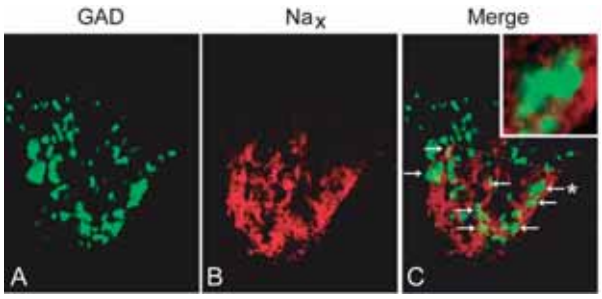

Figure 3. Nax-positive glial cells associate with multiple neurochemical circuitries of the subfornical organ. GFP fluorescence of GAD (A), Texas-Red fluorescence of Nax (B), and merged images (C). Tissue sections derived from GAD67-GFP mice were stained with anti-Na_x antibody and visualized with Texas-Red. Tissue sections 50 μm thick were penetrated with a detergent to enhance Na_x-signals. White arrows in C indicate GAD67-positive neurons enveloped with Na_x-positive glial cells. The area indicated by a white arrow with an asterisk is magnified in the inset of C. Dashed line in C indicates the boundary between the fornix and SFO. Scale bars: 50 μm.

Publication List:

Original paper

Watanabe, E., Hiyama, T.Y., Shimizu, H., Kodama, R., Hayashi, N., Miyata, S., Yanagawa, Y., Obata, K., and Noda, M. (2006). Sodium-level-sensitive sodium channel Nax is expressed in glial laminate processes in the sensory circumventricular organs. Am. J. Physiol. (Regul. Integr. Comp. Physiol.), *290*, R568-R576.