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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. The molecular entities of these receptors, however, were not discovered for a long time. In 2000, by using gene-targeting technology, we first clarified that $Na_x$ sodium channel is a probable candidate for the specific sodium receptor in the brain.

A line of studies using $Na_x$-gene deficient mice showed that 1) $Na_x$-deficient mice ingested hypertonic sodium chloride solution in excess in comparison with wild type-mice; 2) $Na_x$ gene is expressed in the circumventricular organs, which are the specialized central organs involved in the sensing of sodium concentration and osmolality in the body fluids; 3) $Na_x$ channel is an extracellular sodium-level sensitive sodium channel (Figure 1); and 4) 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 suggest 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.

Recent immuno-electron-microscopic 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.

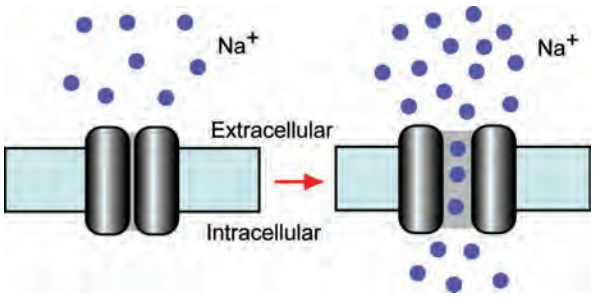


Figure 1. $Na_x$ is a sodium channel sensitive to extracellular sodium level. When the extracellular sodium concentration increases, $Na_x$ channel opens the gate pore and generates the sodium ion influx into the cells. This view was hypothesized by ion-imaging studies.

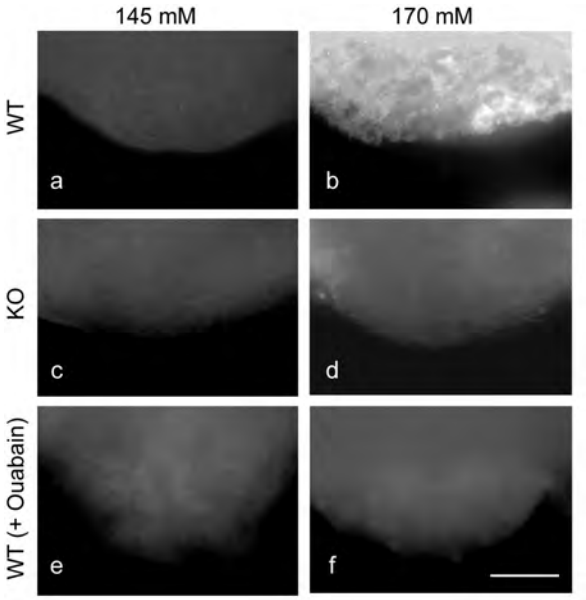


Figure 2. Imaging analysis of the uptake of glucose in the subfornical organ using a fluorescent glucose derivative 2-NBDG. The subfornical organ was isolated from wild-type (WT; a, b, e, and f) and Nax-knockout (KO; c and d) mice, and incubated with 2-NBDG in the 145 mM (a, c, and e) or 170 mM (b, d, and f) sodium solution. In some experiments, the extracellular solutions contained 1 mM ouabain that is a potent blocker of $Na^+/K^+$-ATPase (e and f). Scale bars: 50 mm. For details see the following paper.

In 2007, we showed direct interaction between $Na_x$ channels and subunits of $Na^+/K^+$-ATPase, which brings about sodium-dependent activation of the metabolic state of the glial cells (Figure 2). Metabolic enhancement leading to extensive lactate production was observed in the subfornical organ of wild-type mice, but not in the Nax-knockout mice. Furthermore, lactate, as well as Na, stimulated the activity of GABAergic neurons in the subfornical organ. These results suggest that the information ofn a physiological increase of the sodium level in body fluids sensed by $Na_x$ in glial cells is transmitted to neurons by lactate as a mediator to regulate neural activities of the subfornical organ. These findings suggest that the neuron-glia complex plays a key role in sodium sensing in the circumventricular organs.

### Publication List

**〔Original paper〕**

● Shimizu, H., Watanabe, E., Hiyama, T.Y., Nagakura, A., Fujikawa, A., Okado, H., Yanagawa, Y., Obata, K., and Noda, M. (2007). Glial $Na_x$ channels control lactate signaling to neurons for brain [$Na^+$] sensing. Neuron *54*, 59-72.