LABORATORY OF BIOLOGICAL DIVERSITY

SAKUTA Group

Assistant Professor: *SAKUTA, Hiraki*
Technical Assistant: *ISOSHIMA, Yoshiko*
KODAMA, Akiko

The homeostatic osmoregulation of body fluids (such as plasma and cerebrospinal fluid (CSF)) is vital to life. This is because substantial changes in cell volumes due to hypertonicity or hypotonicity cause irreversible damage to organs and lead to lethal neurological trauma. Water deprivation (loss of water from the body) elevates the concentration of Na^+ ($[Na^+]$) and osmolality in body fluids. Animals exhibit prominent and effective responses to water deprivation, including behavioral responses, such as inducing water intake and avoiding sodium (Na), along with vasopressin-induced reductions in urine volumes. The aim of our research group is to reveal the brain systems for body-fluid homeostasis.

I. Thirst control by Na_x and TRPV4

$[Na^+]$ is the main factor influencing osmolality *in vivo*, and is continuously monitored in the brain to be maintained within a physiological range. We have shown that Na_x, which structurally resembles voltage-gated sodium channels ($Na_v1.1$–1.9), is the brain $[Na^+]$ sensor to detect increases in $[Na^+]$ in body fluids. Na_x is preferentially expressed in specific glial cells of sensory circumventricular organs (sCVOs) including the subfornical organ (SFO) and organum vasculosum lamina terminalis (OVLT). We have found that Na_x signals in these brain regions deficient in a blood-brain barrier are involved in the control of salt intake.

We recently demonstrated that Na_x signals are also involved in the control of water intake behavior. Our pharmacological experiments suggested that Na_x signals led to the activation of neurons bearing TRPV4 by using epoxyeicosatrienoic acids (EETs) as gliotransmitters to stimulate water intake. This year, we performed selective lesions of individual sCVOs in wild-type (WT) mice and the site-directed rescue of *Na$_x$* expression in *Na$_x$* knockout (*Na$_x$*-KO) mice. These experiments revealed that the Na_x channel in the OVLT functions as a $[Na^+]$ sensor for the control of water intake behavior. Direct measurements of 5,6-EET and 8,9-EET in the OVLT revealed that EET levels were indeed increased two-fold by water deprivation for two days in WT, but not *Na$_x$*-KO mice. This indicates that EETs were Na_x-dependently produced in the OVLT in response to increases in $[Na^+]$ in body fluids. More importantly, the ICV injection of 5,6-EET at the same level was effective in inducing water intake.

The signaling mechanisms in the OVLT for water-intake induction by increases in $[Na^+]$ in body fluids are presented in Figure 1. When $[Na^+]$ in plasma and CSF increases, Na_x channels in glial cells in the OVLT are activated, leading to the synthesis of EETs in Na_x-positive glial cells. EETs released from Na_x-positive glial cells function as gliotransmitters to activate neurons bearing TRPV4 channels in the OVLT, which are involved in the stimulation of water-intake behavior.

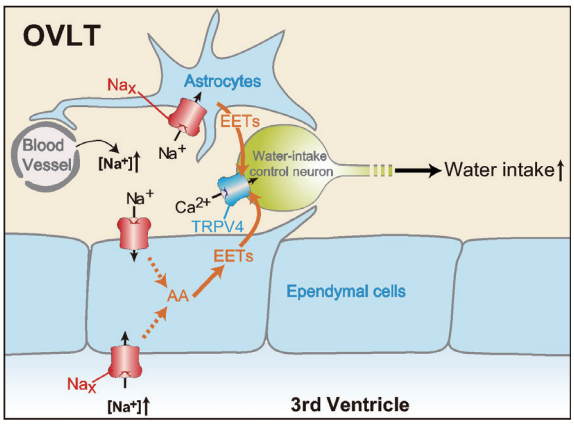

Figure 1. Proposed mechanisms for water intake induction by increases in $[Na^+]$ in body fluids via Na_x activation in the OVLT. AA, arachidonic acid.

II. Identification of novel sensors involved in water intake control

Water intake by *Na$_x$*-KO mice after the ICV injection of hypertonic NaCl solution was small, but still approximately half that by WT mice and, noteworthily, significantly higher than that by *Na$_x$*-KO and WT mice after the ICV injection of an equimolar hypertonic sorbitol solution. These findings suggest the existence of another unknown $[Na^+]$ sensor and osmosensor. In order to identify the novel sensors involved in water intake control, we performed RNA-seq analysis of OVLT and identified several candidates. We are now examining the functional roles of these candidates in water intake.

Publication List:

〔Original papers〕

- Nomura, K., Hiyama, T.Y., Sakuta, H., Matsuda, T., Lin, C.-H., Kobayashi, K., Kobayashi, K., Kuwaki, T., Takahashi, K., Matsui, S., and Noda, M. (2019). $[Na^+]$ increases in body fluids sensed by central Na_x induce sympathetically mediated blood pressure elevations via H^+-dependent activation of ASIC1a. Neuron *101*, 60-75. doi: 10.1016/j.neuron.2018.11.017
- Winkelman, B.H.J., Howlett, M.H.C., Hölzel, M.-B., Joling, C., Fransen, K.H., Pangeni, G., Kamermans, S., Sakuta, H., Noda, M., Simonsz, H.J., McCall, M.A., De Zeeuw, C.I., and Kamermans, M. (2019). Nystagmus in patients with congenital stationary night blindness (CSNB) originates from synchronously firing retinal ganglion cells. PLoS Biol. *17*, e3000174. doi: 10.1371/journal.pbio.3000174

〔Original paper (E-publication ahead of print)〕

- Sakuta, H., Lin, C.-H., Yamada, M., Kita, Y., Tokuoka, S.M., Shimizu, T., and Noda, M. Na_x-positive glial cells in the organum vasculosum laminae terminalis produce epoxyeicosatrienoic acids to induce water intake in response to increases in $[Na^+]$ in body fluids. Neurosci. Res. 2019 May 28. doi: 10.1016/j.neures.2019.05.006