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Sodium-level-sensitive Sodium Channel and Salt-Intake Behavior

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Mammals feel thirsty or develop an appetite for salt when the correct balance between water and sodium in the body fluid has been disrupted, but little is known about the mechanism in the brain that controls salt homeostasis. It has been postulated that the existence of both an osmoreceptor and a specific sodium receptor is required to accommodate the experimental data (Johnson and Edwards, 1990; Denton *et al.*, 1996). Several candidate osmoreceptors have been reported (Oliet and Bourque, 1993; Wells, 1998; Liedtke *et al.*, 2000); however, a specific sodium receptor has not been identified.

The Na_x channel—formerly called NaG/SCL11 (in rats), $Na_v2.3$ (in mice) and $Na_v2.1$ (in humans)—has been classified as a subfamily of voltage-gated sodium channels (Goldin *et al.*, 2000). The primary structure of Na_x, however, markedly differs from that of other voltage-gated sodium channel family members and includes differences in the key regions for voltage sensing and inactivation. The functional properties of the channel are poorly understood, as all attempts to induce functional expression of Na_x in heterologous systems have failed.

Several years ago, we generated mice in which the *Na*$_x$ gene was knocked-out by insertion of the *lacZ* gene in-frame and found that the Na_x channel is expressed in cells in the circumventricular organs (CVOs) (Watanabe *et al.*, 2000), in particular the subfornical organ (SFO) and organum vasculosum lamina terminalis (OVLT), which are important regions for the control of body fluid ionic balance; for the expression other than the CNS (see Watanabe *et al.*, 2002). Under thirst conditions, *Na*$_x$-deficient mice showed hyperactivity of the neurons in these two areas and ingested excessive salt: Wild-type mice take water and stop salt ingestion under dehydrated condition.

Infusion of a hypertonic Na solution into the cerebral ventricle also induced extensive water intake and aversion to saline (0.3 M

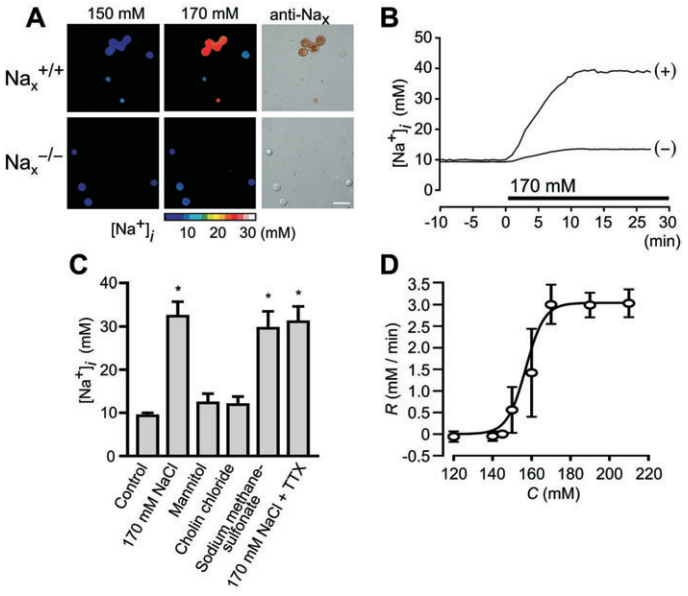

Figure 1 Sodium-concentration sensitivity was lost in SFO neurons in the *Na*$_x$-null mutants. **(A)** Pseudocolor image showing the $[Na^+]_i$ of the cells in the control and high sodium solutions. Scale bar = 50 µm. **(B)** Time-course of $[Na^+]_i$ responses of the cells positive (+) and negative (–) for Na_x expression. Time 0 is the time at which the extracellular fluid was changed. **(C)** The $[Na^+]_i$ response is dependent on $[Na^+]_o$, but not on extracellular $[Cl^-]_o$ or osmotic pressure. Instead of NaCl, 50 mM mannitol, 25 mM choline chloride or 25 mM sodium methanesulfonate was added to the control solution. *$P < 0.001$ by one-tailed Mann–Whitney tests; $n = 85$. **(D)** Relationship between the $[Na^+]_i$ increase rate (R) and $[Na^+]_o$. $R = R_{Max}/1+\exp((C_{1/2} - C)/a)$. The values $R_{Max} = 3.04$ mM/min, $C_{1/2} = 157$ mM and $a = 4.67$ mM were used; $n = 20$. Figure 1A, C and D are from Hiyama *et al.* (2002).

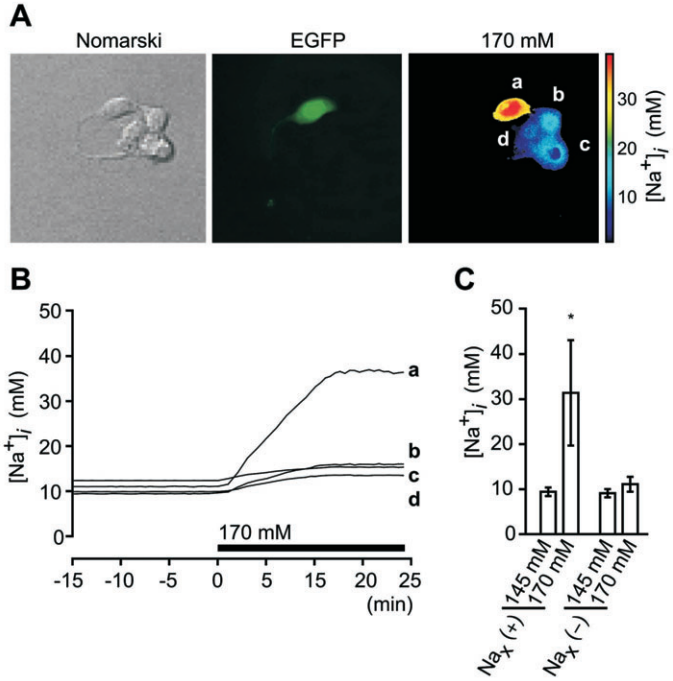

Figure 2 *Na*$_x$ cDNA transfection conferred $[Na^+]_o$ sensitivity on *Na*$_x$-deficient SFO cells from the knockout mice. **(A)** Nomarski (left), EGFP fluorescence (middle) and pseudocolor image showing $[Na^+]_i$ increase in 170 mM NaCl solution (right). Only cell (a) was transfected with *Na*$_x$ and *egfp* expression vectors. **(B)** Time-course of $[Na^+]_i$ responses of the cells shown in (A). **(C)** Comparison of the response of cells; transfectant with *Na*$_x$ expression vector (left) and nontransfectant (right). *$P < 0.001$ by one-tailed Mann–Whitney tests; $n = 20$.

NaCl) in wild-type animals, but not in the knockout mice (unpublished data): The *Na_x*-deficient mice did not show aversion to saline. Importantly, the aversion to salt was not induced by infusion of a hyperosmotic mannitol solution with physiological Na concentration in both genotypes of mice (unpublished data).

These findings led us to propose that Na_x is involved in the sodium-level sensing mechanism in the brain. We verified this possibility by imaging analysis of changes in the intracellular sodium-ion concentration $[Na^+]_i$ when the extracellular sodium-ion concentration $[Na^+]_o$ was raised stepwise from the normal amount (Hiyama *et al.*, 2002). When $[Na^+]_o$ was increased from the control amount of 145 mM (physiological level) to 170 mM by bath application, the $[Na^+]_i$ of some cells dissociated from the SFO of wild-type mice showed a pronounced increase (Figure 1A,B). Importantly, all the responsive cells were Na_x-immunoreactive. These neurons responded to the rise in $[Na^+]_o$, but not to the rise in osmolarity or $[Cl^-]_o$ (Figure 1C). Tetrodotoxin (TTX) at 1 μM did not antagonize the response (Figure 1C). $[Na^+]_o$ at the half-maximal ($C_{1/2}$) was 157 mM (Figure 1D). When *Na_x* cDNA was introduced into the dissociated SFO cells derived from *Na_x*-deficient mice, $[Na^+]_i$ response similar to that in wild-type cells appeared (Figure 2). Thus, Na_x is a newly identified type of sodium channel that is sensitive to an increase in the extracellular sodium concentration.

Sodium concentrations in the plasma and CSF increase by 5–10% during thirst conditions (Nose *et al.*, 1992). The sensitivity and threshold of Na_x channels to $[Na^+]_o$ is in this range of physiological change. The CVOs including SFO and OVLT are regions where the blood–brain barrier is missing, enabling cells to directly monitor body fluid conditions. When *Na_x* cDNA was introduced into the brain of the knockout mice with adenoviral expression vector, animals that received transduction of the *Na_x* gene into the SFO among the CVOs regained the salt-avoiding behavior under dehydrated conditions (unpublished data). This indicates that Na_x channel in the SFO is essential and sufficient for the control of salt-intake behavior. Taken together, we advocate that SFO is the principal site for the control of salt-intake behavior, where Na_x channel functions as the Na-level sensor.

References

Denton, D.A., McKinley, M.J. and **Weisinger, R.S.** (1996) *Hypothalamic integration of body fluid regulation.* Proc. Natl Acad. Sci. USA, 93, 7397–7404.

Goldin, A.L., Barchi, R.L., Caldwell, J.H., Hofmann, F., Howe, J. R., Hunter, J.C., Kallen, R.G., Mandel, G., Meisler, M.H., Netter, Y.B., Noda, M., Tamkun, M.M., Waxman, S.G., Wood, J.N. and **Catterall, W.A.** (2000) *Nomenclature of voltage-gated sodium channels.* Neuron, 28, 365–368.

Hiyama, T.Y., Watanabe, E., Ono, K., Inenaga, K., Tamkun, M.M., Yoshida, S. and **Noda, M.** (2002) *Na_x channel involved in CNS sodium-level sensing.* Nat. Neurosci., 5, 511–512.

Johnson, A.K. and **Edwards, G.L.** (1990) *The neuroendocrinology of thirst:* afferent *signaling and mechanisms of central integration.* Curr. Top. Neuroendocrinol., 10, 149–190.

Liedtke, W., Choe, Y., Martí-Renom, M.A., Bell, A.M., Denis, C.S., Sali, A., Hudspeth, A.J., Friedman, J.M. and **Heller, S.** (2000) *Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor.* Cell, 103, 525–535.

Nose, H., Doi, Y., Usui, S., Kubota, T., Fujimoto, M. and **Morimoto, T.** (1992) *Continuous measurement of Na concentration in CSF during gastric water infusion in dehydrated rats.* J. Appl. Physiol., 73, 1419–1424.

Oliet, S.H.R. and **Bourque, C.W.** (1993) *Mechanosensitive channels transduce osmosensitivity in supraoptic neurons*. Nature, 364, 341–343.

Watanabe, E., Fujikawa, A., Matsunaga, H., Yasoshima, Y., Sako, N., Yamamoto, T., Saegusa, C. and **Noda, M.** (2000) *Na_x2/NaG channel is involved in control of salt intake behavior in the central nervous system.* J. Neurosci., 20, 7743–7751.

Watanabe, E., Hiyama, T.Y., Kodama, R. and **Noda, M.** (2002) *Na_x sodium channel is expressed in non-myelinating Schwann cells and alveolar type II cells in mice*. Neurosci. Lett., 330, 109–113.

Wells, T. (1998) *Vesicular osmometers, vasopression secretion and aquaporin-4: a new mechanism for osmoreception?* Mol. Cell. Endocrinol., 136, 103–107.