The Subfornical Organ, a Specialized Sodium Channel, and the Sensing of Sodium Levels in the Brain

MASAHARU NODA
Division of Molecular Neurobiology, National Institute for Basic Biology, and School of Life Science, Graduate University for Advanced Studies, Okazaki, Japan

Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluid. For Na homeostasis of the body, controls of Na and water intake and excretion are of prime importance. However, though the circumventricular organs (CVOs) are suggested to be involved in body-fluid homeostasis, the system for sensing the Na level within the brain that is responsible for the control of Na- and water-intake behavior has long been an enigma. The authors found that the Na channel is preferentially expressed in the CVOs in the brain and that Na knockout mice ingest saline in excess under dehydrated conditions. Subsequently, the authors demonstrated that Na is an Na-level-sensitive Na channel. When Na cDNA was introduced into the brain of the knockout mice with an adenoviral expression vector, only animals that received a transduction of the Na gene into the subfornical organ (SFO) among the CVOs recovered salt-avoiding behavior under dehydrated conditions. Here, the authors advocate that the SFO is the center of the control of salt-intake behavior in the brain, where the Na-level-sensitive Na channel is involved in sensing the physiological increase in the level of Na in body fluids. NEUROSCIENTIST 12(1):80–91, 2006. DOI: 10.1177/1073858405279683

KEY WORDS Na channel, Circumventricular organs, Subfornical organ, Na homeostasis, Salt-intake behavior

Sodium (Na) is the major ionic component of extracellular fluids, and sodium homeostasis is inseparably linked with body fluid control (Andersson 1978). Thus, the dynamic regulation of Na and water intake is essential to life. When an animal is dehydrated, the Na concentration in body fluids, and accordingly plasma osmolarity, increases by 5% to 10% (Wakerley and others 1978; Nose and others 1992). A line of experimental evidence indicates that an increase of Na in body fluids is detected by a putative Na sensor within the brain at the circumventricular organs (CVOs), that is distinct from the osmosensor (McKinley and others 1978; Denton and others 1996).

CVOs are specialized brain structures, so named because of their proximity to the ventricles of the brain (Hofer 1958). Five CVOs are known in mammals: the subfornical organ (SFO), organum vasculosum of the lamina terminalis (OVLT), median eminence (ME), neurohypophysis (NHP), and area postrema (AP). In addition to the periventricular midline location in the brain, they have common features such as an extensive vasculature lacking a blood-brain barrier, a dense population of a variety of peptidergic factor receptors, and the existence of atypical ependymal cells. Of these CVOs, only three (SFO, OVLT, and AP) include neuronal cell bodies with efferent fibers extending to many other brain regions and are termed the sensory CVOs (Johnson and Gross 1993). Because their neurons are directly exposed to the chemical environment of the circulation, the sensory CVOs have been thought to be the organs sensing various kinds of circulating information including Na concentrations and osmotic pressure in the body fluid (McKinley and others 2003).

The Na x channel—formerly called NaG/SCL11 (in rats), Na2.3 (in mice), and Na2.1 (in humans)—has been classified as a subfamily of voltage-gated sodium channels (Goldin and others 2000). The primary structure of Na2.1, however, markedly differs from that of other voltage-gated sodium channel family members (~50% identify) and includes differences even in the key regions for voltage sensing and inactivation (Fig. 1). Moreover, all attempts to express functional Na channels using heterologous systems had been unsuccessful, and accordingly the functional properties were not clear. The gene encoding Na2.1 is designated SCN7A (Online Mendelian Inheritance in Man number 182392). We generated Na2.1-gene knockout (Na2.1−) mice by insertion of the lacZ

This research was supported by grants from the Ministry of Education, Culture, Sports and Technology of Japan and Japan Science and Technology Agency (CREST). The author thanks Dr. Eiji Watanabe and Dr. Takeshi Y. Hiyama for their significant contributions to this research and to Ms. Akiko Kodama for secretarial assistance.

Address correspondence to: Masaharu Noda, Division of Molecular Neurobiology, National Institute for Basic Biology, 5-1 Higashiyama, Myodaiji-cho, Okazaki 444-8787, Japan (e-mail: madon@nibb.ac.jp).
reporter gene in-frame (Watanabe and others 2000) and recently demonstrated that this atypical sodium channel, NaX, is the specific sodium sensor monitoring sodium levels in body fluids in the brain (Hiyama and others 2002, 2004; Noda and Hiyama 2005).

In the CNS, the four CVOs, SFO, OVLT, ME, and NHP, are distinctively positive for NaX expression (Watanabe and others 2000). NaX−/− mice ingest excessive salt under dehydrated conditions, whereas wild-type mice avoid ingesting salt. NaX−/− mice show hyperneural activity, as estimated by immunoreactivity for Fos, in the SFO and OVLT, compared with wild-type mice after water deprivation (Watanabe and others 2000). The salt-aversive behavior occurs on direct infusion of a hypertonic sodium solution into the cerebral ventricle in wild-type mice but not in NaX−/− mice (Hiyama and others 2004). The behavioral phenotype of NaX−/− mice is completely recovered by site-directed transfer of the NaX gene into the SFO (Hiyama and others 2004). The NaX channel is a concentration-sensitive sodium channel with a threshold value of approximately 150 mM for extracellular sodium ion [Na+]o (Hiyama and others 2002). It is thus probable that the NaX channel is the brain sodium-level sensor that was postulated to be present in the CVOs and involved in water/salt-intake regulation.

Expression of NaX Gene

The null mutant animals (NaX−/−) were healthy, fertile, and apparently normal (Watanabe and others 2000). The genotypic analysis of offspring obtained from breeding heterozygous animals (NaX+/-) showed an approximately Mendelian ratio between wild-type (NaX+/-; 29.5%), heterozygous mutant (NaX+/-; 48.2%), and homozygous mutant (NaX−/−; 22.3%) animals. This suggests that embryonic development and body functions are not significantly impaired in either heterozygous or homozygous mutant animals. We surveyed the lacZ expression throughout the CNS using fixed brains of NaX+/- and NaX−/− mice (Fig. 2). Interestingly, clusters of lacZ expression were limited to specific loci in the CNS: the four CVOs; the medial preoptic, anterior, and dorsomedial parts of the hypothalamic area; the dorsomedial part of the interpeduncular nucleus; the medial part of the median raphe; the mesencephalic nucleus of V; and the medial habenular nucleus. Relatively weak lacZ expression was detected in the cerebral cortex in layer IV of the lateral area (from the most anterior portion to the end of the posterior portion of the cortex) and the basolateral amygdala.
In the peripheral tissue, Na expression was detected in the dorsal root ganglia (DRG), nonmyelinating Schwann cells in the peripheral nervous system, and alveolar type II cells in the lung at the perinatal stage (Watanabe and others 2000). It was also reported that Na shows an acute and transient expression in the perinatal uterine smooth muscle (Felipe and others 1994; Knittle and others 1996). In fact, myometrium of the pregnant uterus at days 18 and 20 was significantly positive for lacZ. However, noteworthy, pups were delivered normally in Na–/– mice.

Abnormal Increase of Fos-Immunoreactivity in the SFO and OVLT

Analysis of the lacZ expression clearly demonstrated that Na is expressed in the four CVOs and several minor nuclei in the CNS. Because the expression of Na thus varied in terms of not only tissue distribution but also cell type, it was difficult to obtain a unified view of channel function or property simply from the distribution. However, the four CVOs were suggestive of a role for Na in body-fluid homeostasis (Andersson 1978; Johnson and Gross 1993; Ferguson and Bains 1996; Bourque and Oliet 1997; Fitzsimons 1998; Johnson and others 1999). If the Na channel functions in the sensory circuits for body-fluid conditions, it was expected that the activity in these organs would be affected in the Na mutant mice. Therefore, we examined the effects of water deprivation on the central expression of a nuclear protein, Fos, the product of the c-fos proto-oncogene (Watanabe and others 2000), as a marker of changes in neural activity (Ueta and others 1995; Chae and Heideman 1998; Morien and others 1999).

The median preoptic nucleus (MnPO), supraoptic nucleus (SON), paraventricular nucleus (PVN), SFO, and OVLT are known to be the sites where c-fos expression is up-regulated in animals under thirst and acute salt-appetite conditions (Rawland 1998). Figure 3 shows the time course of changes in Fos-immunopositive cell density in the five regions of the brain under water-satiated and dehydrated conditions. In the water-satiated condition, Fos-immunopositive cells were not detected in any region examined in Na+/– and Na–/– mice (Fig. 3A).
However, at 12, 24, and 48 hours after water deprivation, numbers of cells with Fos-immunopositive nuclei were remarkably increased in these regions in both mice. Of note, in the SFO and OVLT, approximately twofold increases in Fos-immunopositive nuclei were observed in Na\textsuperscript{+/-} mice as compared with Na\textsuperscript{+/-} mice. In the SON, PVN, and MnPO, on the other hand, the rates of increase in Fos-immunopositive cells were comparable between the two genotypes (Fig. 3B). Of note is that the SFO and OVLT belong to the sensory CVOs.

**Na\textsuperscript{+} Knockout Mice Do Not Stop Ingesting Salt When Dehydrated**

To analyze the daily pattern of water and salt intake in a free-moving state, we developed a drinking behavior monitoring system for mice (Fig. 4A). Using this system, we measured the amount drunk by individual mice (Hiyama and others 2004). Then the preference ratios, defined as the volume ratio of saline intake divided by saline plus pure water intake in 12 hours, were calculated (Fig. 4B). When fully satiated with water, both the Na\textsuperscript{+/-} and Na\textsuperscript{+/-} mice showed a progressive aversion at concentrations above 0.3 M. The preference-aversion curves for a series of NaCl solutions were nearly identical between the two genotypes.

After dehydration for two days, however, the behavioral difference between the two genotypes became apparent. The preference-aversion curve of Na\textsuperscript{+/-} mice shifted to lower concentrations, that is, aversion to saline became more sensitive (Fig. 4B). Here, the threshold for the concentration of salt that is judged to be harmful to the animal is thought to change from 0.3 to 0.15 M. Accordingly, dehydrated Na\textsuperscript{+/-} mice exhibited an aversion to saline at concentrations over 0.15 M, more than the physiological level. In contrast, the curve of Na\textsuperscript{+/-} mice was not changed at all by the dehydration. Thus, the Na\textsuperscript{+/-} mice appear to be deficient in this ability to reset the threshold in the brain that is required when dehydrated. The difference between the two genotypes was greatest at 0.3 M in the dehydrated condition. We decided to use 0.3 M NaCl in the subsequent preference assays.

Using the same system, we monitored the time course of intake of pure water and 0.3 M NaCl independently. After dehydration, animals of both genotypes rushed to drink fluids in large amounts, and subsequently the drinking rate decreased gradually (Fig. 4C). When dehydrated, Na\textsuperscript{+/-} mice preferred pure water and avoided the hypertonic saline, and as a result, the preference ratio for 0.3 M NaCl was markedly reduced. In contrast, Na\textsuperscript{+/-} mice took both pure water and 0.3 M NaCl equally, and the preference ratio for 0.3 M NaCl was not changed by dehydration (Fig. 4D). Despite this difference, the time-course profiles of the total volume drunk (the total amount of pure water plus 0.3 M NaCl) were not significantly different between the two genotypes not only before but also after the water deprivation (Fig. 4E).

**Na\textsuperscript{-}, Knockout Mice Showed Abnormal Salt-Intake Behavior during Microinjection of Hypertonic NaCl into the Cerebral Ventricle**

In dehydrated animals, the Na concentration of body fluids is obviously inclined to rise all over the body. In this context, it is important to examine the effects of the direct stimulation of CVOs with hypertonic Na solutions from the cerebral ventricle on drinking behavior in the two-bottle test (Fig. 7A). When the isotonic saline (0.145 M NaCl) was continuously infused into the ventricle, both genotypes took 0.3 M NaCl and water equivalently (Fig. 7C), indicating that neither the operation nor injec-
tion itself affected drinking behavior. When the hypertonic saline (0.5 M NaCl) was infused into the cerebral ventricle, however, \( \text{Na}^{+/-} \) animals clearly avoided the salt solution (Fig. 7C). In contrast to the \( \text{Na}^{+/-} \) mice, the \( \text{Na}^{+/-} \) mice did not show such an aversion to the salt solution (Fig. 7C). The time-course of the drinking behavior is shown in Figure 7B: Notably, the initial prompt water-drinking response in \( \text{Na}^{+/-} \) mice was not observed in \( \text{Na}^{+/-} \) mice.

On the other hand, when both genotypes were infused with a hypertonic mannitol solution in isotonic saline (0.145 M NaCl + 0.71 M mannitol, the osmotic pressure being approximately equivalent to 0.5 M NaCl), there was no significant difference in the preference ratios at about 0.5 between the two genotypes (Fig. 7B, C). Importantly, the total intake volume was increased equally in both genotypes when mice were infused with the hypertonic saline solution and hypertonic mannitol solution (Fig. 7D), suggesting that the increase in total intake is regulated by the osmolarity. These results indicate that Na\(_i\) is involved in sensing an increase in the level of Na in the CSF and is specifically relevant to the control of Na intake. On the other hand, the control of the total intake is independent of Na\(_i\).

When the hypertonic Na solution (0.5 M NaCl) was infused into the cerebral ventricle, \( \text{Na}^{+/-} \) mice took a large quantity of water immediately in response to the infusion and then stopped drinking once within 1 hour (Fig. 7B); \( \text{Na}^{+/-} \) mice did not show such a response, suggesting that Na\(_i\) is involved in this water intake by sensing a rise in the concentration of Na in the CSF. When the hypertonic mannitol solution was infused, neither genotype of mice showed this prompt and robust water-drinking behavior (Fig. 7B), in contrast to the dehydrated mice (Fig. 5B).
This indicates that the increase in osmolarity in the CSF is not so effective as to immediately induce thirst in animals, as compared with the increase in Na.

The pause in drinking mentioned above is considered a feedback suppression by neural inputs to the brain from the oropharynx and/or hepatic portal vein (Stricker and Sved 2000). However, the mice subsequently began to drink water again, but not the salt solution, during the experiment. Such a delayed phase of water intake, which was not observed in the dehydrated animals (Fig. 5B), was probably induced by the continuous infusion of the hypertonic saline. Due to the persistent infusion of the hypertonic saline, the Na concentration in the CSF presumably kept rising and was not compensated merely by a robust water intake.

**Abnormal Salt-Intake Behavior of Nax-Knockout Mice Was Rescued by Transduction of the Nax Gene into the SFO**

To specify the brain locus from which the difference in Na- and water-intake behavior in the two genotypes originates, the Nax gene was locally introduced into the brain of Nax−/− mice with an adenoviral expression vector carrying Nax, under the control of the CMV promoter. An adenoviral expression vector encoding the egfp gene was coinjected to identify the infected site after the experiments. One week after the injection, the mice were subjected to the behavioral tests to examine whether the salt-aversion behavior was restored. When the preference ratio for 0.3 M NaCl was lower than 0.2 in the preference test under dehydrated conditions, we concluded that the salt-aversion behavior was completely restored. Among 102 knockout mice that received the Nax gene together with the egfp gene, 6 showed the aversion to salt similar to Nax+/+ mice when dehydrated. Here, the intake of 0.3M NaCl was decreased, and that of water was increased by the Nax gene transduction, the total intake again being constant. Then, the infected regions in these mice were analyzed. Of note is that all of the six mice that were conferred with the salt-aversion behavior (a preference ratio of 0.5–0.2) showed a weak infection in the SFO along with the other main loci of infection (data not shown). The expression...
Fig. 5. Sodium-concentration sensitivity was lost in subfornical organ cells in the Nax-knockout mice. 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 Nax 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 U tests; n = 85. D, Relationship between the [Na+]i increase rate (R) and [Na+]o. R = R_max / (1 + exp ((C_o/2 – C)/a). The values R_max = 3.04 mM/min, C_o/2 = 157 mM, and a = 4.67 mM were used; n = 20. E, Whole-cell current responses of dorsal root ganglia (DRG) neurons to an increase of [Na+]o, from 145 to 170 mM (bar). From Noda and Hiyama (2005): (Fig. 5A, C, D, and E are from Hiyama and others 2002).

The current amplitude of Na can be estimated from the ion imaging data as follows: The maximum [Na+]i increase rate (R) in DRG neurons was 2.0 x 10^{-12} M/min (see Hiyama and others 2002, Fig. 1). Because the average radius of DRG neurons was approximately 10 μm, cell volume V is estimated to be 2.1 x 10^{-12} liters when the cell shape was postulated to be hemispheric. Accordingly, the number of Na+ ions, n, passing through the plasma membrane of a single cell during one second can be described by n = RN_i V 60 = 4.2 x 10^{10}, where N_i is the Avogadro constant. Therefore, the Na current (I_Na) is estimated to be 6.7 pA because I_{Na} = n e = 6.7 x 10^{-12} (A), where e is elementary charge (1.6 x 10^{-19} C). This current amplitude is consistent with the inward current with an average amplitude of 8.4 pA obtained from DRG neurons using a patch-clamp technique.
of the Na$_v$ channel in the SFO thus appeared to be essential and sufficient for the control of salt-intake behavior.

We then prepared knockout mice ($n = 6$) in which the adenoviral vector encoding the egfp gene was singly injected into the SFO. Their preference for saline was unchanged in the dehydrated condition, notwithstanding that the EGFP was successfully expressed in the SFO (Fig. 8 B, C; EGFP (SFO)). This control experiment indicates that the operation and introduction into the SFO of the adenoviral vectors themselves did not affect the salt-intake behavior.

The behavioral data of six mice that had received the Na$_v$ and egfp genes in the OVLT showed that their behavior was not rescued (Fig. 8 B, C; Na$_v$ + EGFP (OVLT)). Furthermore, the behavior of another group of six mice who had received both genes at loci other than the SFO

---

**Fig. 6.** Na$_v$ cDNA transfection conferred [Na$^+$], sensitivity on Na$_v$-deficient subfornical organ cells from Na$_v$–/– mice. A, Nomarski (left), EGFP fluorescence (middle), and pseudocolor image showing the increase in [Na$^+$] in a 170 mM NaCl solution (right). Only cell a was transfected with Na$_v$ and egfp expression vectors. B, Time-course of [Na$^+$] responses of the cells shown in A. C, Comparison of the response of cells; transfectant with the Na$_v$ expression vector (left) and nontransfectant (right). [Na$^+$] was measured 5 minutes after [Na$^+$] leveled off. *P < 0.001 by one-tailed Mann-Whitney U tests; n = 20. From Noda and Hiyama (2005).
and OVLT was not effected either (Fig. 8 B,C; Na\textsubscript{+}-EGFP (Others)). When the Na\textsubscript{+}-adenoviral vector was injected into the ventricle, a small number of ependymal cells were infected; however, this transduction of Na\textsubscript{+} was not effective in the behavioral rescue of the knockout mice.

**Na\textsubscript{+} in the SFO But Not OVLT Is the Primary Sodium-Level Sensing Site**

When Na\textsubscript{+} cDNA was introduced into the brain of the knockout mice with the adenoviral expression vector, only animals that received a transduction of the Na\textsubscript{+} gene into the SFO among the CVOs regained the salt-avoiding behavior under dehydrated conditions. This strongly indicates that Na\textsubscript{+} in the SFO is the primary site of sodium-level sensing and for the control of salt-intake behavior. It is known that the SFO has efferents to integrative and effector motor regions in the brain, including the amygdaloid nucleus (Johnson and others 1999; McKinley and others 2003). These neural pathways would be directly responsible for the control of the Na- and water-intake behavior. On the other hand, transduction of the Na\textsubscript{+} gene into the OVLT could not rescue the abnormal salt-intake behavior. There exist efferent and afferent projections between the SFO and OVLT (McKinley and others 2003), and both regions are selectively hyperactivated in the knockout mice under dehydrated conditions (Watanabe and others 2000). Despite close similarities, there must be functional differences between the two sensory loci. Many neurons in the dorsal cap of the OVLT (and to a lesser extent in the SFO) send efferent projections to the SON (McKinley and others 1994). The neurons in the OVLT are considered to drive the neurons in the SON to secrete vasopressin in response to dehydration.

**Na-Level Sensing and Osmosensing in the Brain**

For body-fluid homeostasis, neuroendocrine and behavioral regulatory systems must respond to fluctuations in the Na and water balance in body fluids. As dehydration is reflected in the loss of free water, an osmotic increase is usually accompanied by proportional rises in the level of Na in the plasma and CSF. For this reason, the existence and necessity of an Na-specific receptor distinct from osmoreceptors were long controversial (Johnson and Edwards 1990). In this study, we injected a hypertonic mannitol solution in physiological saline to investigate the effect of hyperosmolarity independent of the Na level on Na and water intake and found that the response to hyperosmolarity is normal in the knockout mice, suggesting the existence of an osmosensing system(s) distinct from the Na-level sensing system (Hiyama and others 2004). The vanilloid receptor-related channel (VR-OAC/TRPV4) in the OVLT and SFO, activated osmotically, has been reported as a candidate for the vertebrate osmoreceptor (Liedtke and others 2003). It is well recognized that circulating angiotensin II is a stimulator of salt and water intake. Intracerebroventricular infusion of losartan, an angiotensin II type I receptor...
(AT1) antagonist, has been reported to reduce salt and water intake in several species including mice (Blair-West and others 1994). AT1 is preferentially expressed in the SFO and OVLT (McKinley and others 2003), and angiotensin II induces c-fos expression at these loci (Denton and others 1996). Intravenous injection of angiotensin II induced both Na and water intake (Buggy and Fisher 1974). Our results showed that the aversion to Na under dehydrated conditions was impaired in the knockout mice but the total intake of water and saline did not differ between the two genotypes, suggesting that the abnormal behavior in the knockout mice is not directly related to the angiotensin system. This view is supported by a report that different populations of neurons are excited by hypertonic saline and angiotensin II, in both the SFO and OVLT (Denton and others 1996). The total intake of water and saline is speculated to be regulated through distinct mechanisms by signals from osmoreceptors and angiotensin receptors, but salt intake in dehydrated animals is specifically controlled by the Na signal.

Sensing Osmolarity in the Supraoptic Nucleus

The cephalic receptors were first hypothesized to be osmoreceptors in light of Verney’s elegant demonstration of an antidiuretic hormone, vasopressin (Verney 1947). The integrity of the neural connection linking the SON, OVLT, and MnPO is necessary for SON neurons to respond to osmotic stimulation (Honda and others 1990). In contrast, the magnocellular neurosecretory cells in the SON appear to exhibit an intrinsic osmosensitivity (Leng 1980). The increase in firing rates observed in vasopressin-secreting cells after direct application of a hypertonic saline is thought to be related to a reduction in cell volume (Oliet and Bourque 1993). Na is not expressed in SON, and therefore magnocellular neurons are negative for Na. On the other hand, glial cells in the SON and PVN have a striking morphological appearance with long astrocytic processes radiating between the magnocellular neurons. The glial-specific distribution of the water channels aquaporin-4 (AQP4) in SON (Nielsen and others 1997) implies that it may be glial cells, rather than neurons, that exhibit intrinsic osmosensitivity. Thus, glial cells might function as the primary “vesicular osmometer,” conveying information on the state of water homeostasis to the neural elements by transcellular water movement.

Concluding Remarks

Sodium concentrations in the plasma and CSF increase by 5% to 10% during thirsty conditions (Wakerley and others 1978; Nose and others 1992). In our dehydration experiments for 2 days, the sodium concentration in the blood increased from 148.2 ± 3.3 (in mM; n = 8) to...
176.3 ± 12.2 in wild-type mice, and from 146.1 ± 2.7 to 172.3 ± 9.0 in the knockout mice. The sensitivity and threshold of Na⁺ channels to [Na⁺] are in this range of physiological change. The CVOs including the SFO and OVLT are regions where the blood-brain barrier is missing, enabling cells to directly monitor body fluid conditions. When Na⁺ cDNA was introduced into the brain of the knockout mice with an adenosinergic expression vector, animals that received transduction of the Na⁺ gene into the SFO among the CVOs regained the salt-avoiding behavior under dehydrated conditions. This indicates that the Na⁺ 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⁺ channels function as the Na⁺-level sensor.

We recently found that the Na⁺ channel is specifically expressed in perineuronal glial processes enveloping neural populations in the SFO and OVLT and that these Na⁺-positive glial cells are sensitive to [Na⁺] increase (Watanabe and others 2005). These findings indicate that the Na⁺-expressing glial cells are the primary site of sodium-level sensing. It is known that adenosinergic vectors have a much higher affinity for glial cells than neurons in the CNS (lino and others 2001). On injection into the SFO, a larger proportion of glial cells expressed the transgene (Sinnayah and others 2002). Therefore, the rescue of the normal salt-intake behavior by introduction of Na⁺ cDNA into the SFO of Na⁺−/− mice with an adenoviral vector is presumably attributable to Na⁺ expression induced in glial cells in the SFO.

Glial cells have long been considered to be inert partners of neurons in the central nervous system. However, it is becoming evident that glial cells are intimately involved in neuronal signaling (Newman and Volterra 2004). Our studies on knockout mice demonstrate that the Na⁺ channel exerts inhibitory influences on neuronal activities in the SFO and OVLT, as judged from immunoreactivity for Fos during dehydration (Watanabe and others 2000). Studies to address how the Na⁺ channel in glial cells regulates neural activities in these two sensory CVOs are important next. In addition, to know the functional differences in the sensory CVOs, further investigation based on the identification of cellular species and neural networks would be essential.

References


