Review

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Central regulation of body-fluid homeostasis

Masaharu Noda[1,2] and Hiraki Sakuta[1,2]

[1] Division of Molecular Neurobiology, National Institute for Basic Biology, 5-1 Higashiyama, Myodaiji-cho, Okazaki 444-8787, Aichi, Japan
[2] School of Life Science, The Graduate University for Advanced Studies, 5-1 Higashiyama, Myodaiji-cho, Okazaki 444-8787, Aichi, Japan

Body-fluid homeostasis is essential to life, and the concentration of Na^+ ($[Na^+]$) and osmolality in plasma and the cerebrospinal fluid (CSF) are continuously monitored in the brain. To maintain a physiological level of Na/osmolality in body fluids, the control of Na and water intake and excretion are of prime importance. Two independent sensing systems for $[Na^+]$ and osmolality in circumventricular organs (CVOs) have long been postulated to be involved in the monitoring of body-fluid conditions. In the past decade, several molecules were reported as promising candidates for these sensors – Na_x for the $[Na^+]$ sensor and transient receptor potential (TRP) channels for the osmosensor. This review presents a summary of developments in these areas over recent years.

Introduction

Mammals have a set of homeostatic mechanisms that work together to maintain body-fluid osmolality at approximately 300 mOsm/kg largely through the intake or excretion of water and salt [1,2]. This homeostatic osmoregulation is vital because changes in cell volume caused by severe hypertonicity or hypotonicity can lead to irreversible damage to organs and cause lethal neurological trauma [3–5]. Sodium (Na) is a major electrolyte in extracellular fluids and the main determinant of osmolality. When animals are dehydrated, $[Na^+]$ in body fluids increases, together with osmolality.

Animals exhibit several prominent and effective responses to dehydration; for example, behavioral responses such as water intake and Na aversion, and the control of kidney functions for water retention. Renal water retention is mediated by vasopressin (VP), which is synthesized by magnocellular neurons in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus, and are released into the blood at the posterior lobe of the pituitary gland [6,7]. Increases in plasma osmolality of a few percent are sufficient to induce the secretion of VP [8].

Corresponding author: Noda, M. (madon@nibb.ac.jp).

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Earlier studies demonstrated that intracarotid infusions of hypertonic solutions of NaCl or sucrose, but not urea, resulted in antidiuresis [9–12]. Because urea is permeable to cell membranes, these findings suggested that cellular dehydration (cell shrinking) stimulated by hyperosmolality is required for VP release. McKinley *et al.* reported that an injection of hypertonic NaCl solution into the 3rd ventricle of conscious sheep caused greater antidiuretic and drinking responses than that of equiosmolar hypertonic sucrose solution [13,14]. Thus, both a Na-level sensor and osmosensor have been proposed to be present in the brain (see Figure IA in Box 1). Furthermore, NaCl, sucrose, and urea hardly permeate across the blood–brain barrier. Therefore, these findings also suggest that the sensing cells are located at brain regions devoid of a blood–brain barrier, such as sensory circumventricular organs (sCVOs) [14,15].

This was supported by immunohistochemical studies on water-deprived animals, in that the expression of activity-dependent immediate-early genes such as c-Fos was increased in the two sCVOs, the subfornical organ (SFO), and organum vasculosum of the lamina terminalis (OVLT), in addition to the median preoptic nucleus (MnPO), SON, and PVN [16,17] (see Figure IB in Box 1). Neurons in the SFO, MnPO, and OVLT project to the SON [16,18] and the PVN [19] directly or indirectly. An injection of hypertonic solutions into the 3rd ventricle was shown to provoke VP secretion and the drinking response [20,21]. The SFO, MnPO, and OVLT are located in the dorsal to ventral front wall of the 3rd ventricle (the lamina terminalis), and lesions in this area attenuated these responses to the systemic administration of hypertonic solutions [22–25]. Furthermore, functional magnetic resonance imaging (MRI) studies recently revealed that the anteroventral 3rd ventricle (A3V) region was activated during hypertonicity in animals [26] and humans [27]. These studies suggested that sensors for body-fluid conditions are present in these brain regions. In the past decade the identification of these sensors has made significant progress in our understanding of the processes for central regulation of body-fluid homeostasis.

We review here the Na-level sensor, identified by ourselves, and osmosensors, identified by others, in the brain. Na_x (also known as SCN7A; sodium channel, voltage-gated, type VII; also NaG) channels specifically expressed in glial cells in the sCVOs sense changes in $[Na^+]$ in plasma

and CSF, and the signal is transmitted from the glial cells to neurons via lactate in the SFO. The transient receptor potential (TRP) channels, including TRPV1 and TRPV4, expressed in neurons in the sCVOs are suggested to sense cell volume changes associated with the shift of extracellular osmolality. The signals sensed by these sensors in the sCVOs appear to assume vital roles in the control of salt/water intake and VP release.

Na-level sensor in the brain

As the major electrolyte of the extracellular fluid, Na plays a fundamental role in maintaining the volume and composition of every fluid compartment in the body [28]. Therefore, the amount of Na in body fluids must be tightly regulated to ensure the optimal performance of numerous physiological processes based on the ion concentration across cell membranes, including neuronal excitability, substance transport, glomerular filtration, and renal excretion of aqueous waste, and the control of extracellular volume and blood pressure [29]. Thus, Na is a key component of many mammalian physiological functions. Disorders associated with Na homeostasis induce severe conditions, such as exaggerated vascular reactivity, hypertension, or circulatory collapse [30].

Na_x is a Na-level-sensitive Na channel

Na_x is an atypical Na channel whose structure is relatively divergent (~50% identical) from the other members of voltage-gated Na channel family [31] (Figure 1A). Na_x is specifically expressed in CVOs in the brain; the SFO, OVLT, median eminence (ME), and posterior pituitary in mice [32] (Figure 1B). Recently, Nehmé *et al.* reported that Na_x is also expressed in the MnPO in rats, which differed from that in mice [33]. In addition to CVOs, Na_x is expressed in some peripheral regions including dorsal root ganglion (DRG) neurons, the myometrium of the pregnant uterus, nonmyelinating Schwann cells, and alveolar type II cells in the lung [32,34].

When the extracellular Na^+ concentration ($[Na^+]_o$) was increased from the physiological level of 145 mM to 170 mM by bath application, the intracellular Na^+ concentration ($[Na^+]_i$) of some cells dissociated from the SFO and OVLT of wild type (WT) mice showed a marked increase [35,36] (Figure 1C). Importantly, all of the responsive cells were Na_x-immunoreactive cells. These Na-sensitive cells were also immunopositive for glia-specific glutamate transporter (GLAST) and glial fibrillary acidic protein (GFAP) (Figure 1D), which indicated that Na_x-bearing cells are glial cells [37]. Double-immunostaining and immunoelectron microscopic analyses revealed that Na_x was exclusively localized to perineuronal lamellate processes extending from ependymal cells and astrocytes in the SFO and OVLT [37].

The activation threshold of Na_x for $[Na^+]_o$ *in vitro* was shown to be ~150 mM and the half-maximal response concentration ($C_{½}$) was ~157 mM [35,36] (Figure 1E).

Box 1. Brain functions for body-fluid homeostasis

Conditions in body fluids (plasma and CSF) are considered to be monitored by two distinct sensory systems for the level of Na and osmolality in the brain. Na/water intake and Na/water reabsorption in the kidney appear to be controlled based on the information supplied by these sensors (Figure IA). Sensory circumventricular organs (sCVOs) in the brain, such as the SFO and OVLT, are characterized by the presence of neuronal cell bodies and extensive networks of fenestrated capillaries that lack a blood–brain barrier. Their ventricular side is partitioned by an ependymal cell layer from the 3rd ventricle. Sensors had been long considered to be expressed in neurons in the sCVOs.

Information supplied by the sensors is transmitted to several brain nuclei, leading to the regulation of Na/water intake and renal functions. For example, neurons relevant to salt intake regulation in the SFO presumably extend efferents to the bed nucleus of the stria terminalis (BST) and central nucleus of the amygdala (CeA) [84] (Figure IB; blue pathways). Some neurons responsible for renal regulation directly or indirectly extend efferents to the magnocellular neurons in the SON and PVN [16,18,19] (Figure IB; red pathways). The activation of these magnocellular neurons causes the release VP at the posterior lobe of the pituitary gland (PP) into the bloodstream to reduce renal water excretion (Figure IB; green pathways). The central regulation of renal Na excretion has been envisaged [85,86], but their details have not been elucidated.

The Na level sensor has been revealed to be Na_x, which populates the perineuronal processes of astrocytes and ependymal cells in the SFO and OVLT [37] (Figure IC). When animals are dehydrated, Na levels in the extracellular fluids ($[Na^+]_o$) in these organs increase. Na_x channels then open and the Na^+ influx elevates $[Na^+]_i$ in these glial cells [87]. In the SFO, ET-3 is expressed at a certain low level even under hydrated conditions, but is markedly increased by dehydration [45]. ET-3 enhances the sensitivity of Na_x channels to $[Na^+]_o$, and Na_x appears to detect an increase in Na levels in the physiological range (133–145 mM) [45]. Na_x activation leads to the activation of Na^+/K^+-ATPase to pump out Na^+ by consuming ATP in these cells. The glial cells enhance glucose uptake and activate anaerobic glycolysis to produce ATP to fuel Na^+/K^+-ATPase. Lactate, the end product of anaerobic glycolysis, is released and supplied to neurons including GABAergic neurons through the glial processes enveloping them [37,48]. Lactate then stimulates the activity of GABAergic neurons as a gliotransmitter [48]. Inhibitory GABAergic neurons presumably regulate the neuronal activities involved in the control of salt intake behavior. Consistent with these findings, *Na_x*-KO mice showed significantly higher neural activity in the SFO and OVLT after water deprivation than that with WT mice, as estimated from c-Fos immunoreactivity [32]. The possibility that Na_x signal is also involved in the control of water intake still remains.

By contrast, osmosensing mechanisms have not yet been fully elucidated though several candidates for osmosensors including TRP channels have been proposed (Figure ID). When animals are dehydrated, osmolality in plasma and CSF increases. An increase in plasma osmolality enhances the extracellular osmolality in the OVLT and SFO: an increase in the CSF osmolality also leads to an increase in the extracellular osmolality in these organs by transcellular water transport through AQPs (green) in the ependymal cells. Water outflow through AQPs (red) causes cellular shrinkage [49] and triggers the opening of TRP channels in osmosensory neurons. The influx of Ca^{2+} through TRP channels causes depolarization of the neurons, which presumably leads to water intake and VP release [57,58].

sCVOs are highly populated with the receptors of vasoactive peptides including angiotensin II (Ang II) and endothelins [28,45,47]. The renin–angiotensin system is activated in dehydrated or Na-depleted animals, and Ang II promotes both Na/water intake [28,88] and reabsorption of water and Na via VP and aldosterone, respectively [2,6,89]. This suggests that Ang II signal has crosstalk with the signaling pathways from the body-fluid sensors downstream. As for the salt intake behavior, the effect of Ang II in the SFO is opposite to that of Na_x activation, which leads to the suppression of salt intake [45].