Neuroscience Letters 472 (2010) 161–165

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Na_x-deficient mice show normal vasopressin response to dehydration

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ARTICLE INFO

Article history:
Received 24 September 2009
Received in revised form
28 December 2009
Accepted 29 January 2010

Keywords:
Vasopressin
Subfornical organ
Supraoptic nucleus
Na^+ homeostasis
Na_x-knockout mice

ABSTRACT

In dehydrated animals, the antidiuretic hormone vasopressin (VP) is released from the nerve terminals of magnocellular neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) into the systemic circulation at the posterior pituitary. Increases in sodium (Na^+)-level and osmolality in body fluids upon dehydration are reportedly sensed by a Na^+-sensor and/or an osmosensor, respectively. However, it is still unknown whether both are involved in the regulation of production and/or release of VP. Na_x is the cerebral Na^+-level sensor and Na_x-knockout mice do not stop ingesting salt even when dehydrated. Here we examined VP production/release in Na_x-knockout mice, and found that they are normal in the VP response to dehydration or intraperitoneal-administration with hypertonic saline. *In situ* hybridization using an intron-specific probe showed that *VP* gene expression in the SON did not differ from wild-type mice when dehydrated. Also, there was no significant difference in the activity of subfornical organ neurons projecting to the SON between the two genotypes when stimulated by water deprivation. Furthermore, Na_x-knockout mice showed a normal response in urine excretion to dehydration. All these results indicate that the information of Na^+-level increase detected by Na_x does not contribute to the control of VP production/release.

Animals show several prominent and effective responses to dehydration. One of the important responses is secretion of the antidiuretic hormone vasopressin (VP), which is synthesized by magnocellular neurons in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus and released at the posterior lobe of the pituitary gland into the blood [2,3]. Increases in plasma osmolality of a tiny percent which are detected by putative cerebral osmoreceptors are reportedly sufficient to induce secretion of VP. Receptors are postulated to be present in the sensory circumventricular organs (CVOs) in the brain, such as the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) [1–3,11,26,27]. The SFO and OVLT, the primary sensing organs of Na^+-level and osmolality in body fluids [3,16], have robust efferent connections directly to the SON and PVN [17,18], thereby systemic osmotic stimuli lead to the regulation of the synthesis and release of VP [2,5,6]. It was reported that lesions of SFO efferent attenuate VP secretion and drinking response to systemic administration of hypertonic solutions [15,16]. It was also demonstrated that lesions within the anteroventral third ventricle region (AV3V, including the SFO) suppressed c-Fos expression in the SON after water deprivation, indicating that the cellular response of SON neurons to osmotic stimulation requires input from the AV3V regions [8,14]. These studies indicate that receptors detecting changes in osmolality or Na^+-level in the SFO play an important role in osmoregulation of VP secretion.

Because Na^+ is a major electrolyte in extracellular fluids and the main determinant of osmolality, cerebral Na^+-specific sensor may have a role in the osmoreception. Thus, the contribution of Na^+-level to the regulation of VP is of great interest, which has been argued long time [3,11]. However, it has been difficult to confirm it experimentally until the molecular entity of Na^+-specific sensor is identified. We recently demonstrated that Na_x is a concentration-sensitive Na channel with a threshold value of approximately 150 mM for extracellular Na^+ [10,19,20]. Na_x is expressed in the posterior pituitary and three CVOs: the SFO, OVLT, and median eminence [28,29]. We revealed that Na_x in the SFO is involved in Na^+-level sensing in body fluids and controls salt-intake behavior by electrophysiological and behavioral analyses of Na_x-knockout (Na_x-KO) mice [9,10,24,28]. Na_x-KO mice showed hyper-activation of neurons in the SFO and OVLT time-dependently after water deprivation compared with wild-type (WT) mice as estimated by Fos-immunoreactivity [28].

We have presumed that Na_x in the SFO makes an important contribution to not only the control of salt-intake behavior but that of VP production/release. Here, Na_x-KO mice allow the distinction between responses to an increase in Na^+-level and osmolality for the first time. In the present study, we conducted experiments to know whether VP production/release and renal Na^+/water excretion are altered in Na_x-KO mice during dehydration.

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doi:10.1016/j.neulet.2010.01.077

All the experiments with animals were carried out according to the guidelines of the National Institute for Basic Biology (Okazaki, Japan). Males of WT and homozygous Na_x-KO mice (backcrossed for more than 10 generations to the C57BL/6J line) were used for experiments. Mice weighing 20–30 g (8–12 weeks old) were individually housed in plastic cages under a constant room temperature (23 °C) and humidity (60%) with a 12-h light/dark cycle.

For measurement of plasma VP-levels, two modes of osmotic stimulus were used: (1) dehydration for 24, 48, or 72 h, and (2) intraperitoneal (ip)-injection of hypertonic saline (HS) (0.8 M NaCl, 0.6 ml/20 g body weight). During the dehydration, mice were provided with food (Rodent Diet CA-1, CLEA Japan). Food and water were removed after ip-administration of salt solutions. Blood samples were collected by decapitation in polypropylene tubes with EDTA after water deprivation or 60 min after ip-injection of saline. The blood plasma was obtained by centrifugation, and VP was extracted with acetone and diethyl ether [21]. VP concentrations were determined by radioimmunoassay (RIA) according to the manufacturer's instructions (AVP RIA, Mitsubishi Chemical Medience). The serum Na^+-concentration and osmolality were determined using blood analyzer (i-STAT, Fuso Pharmaceutical Industries) and osmometer (One-ten, Fiske associates), respectively.

To measure the heteronuclear (hn)-RNA expression of *VP*, *in situ* hybridization was performed using a digoxigenin (DIG)-labeled RNA probe for an intronic sequence of the *VP* gene. The template was a 764-bp fragment of intron 1 of the mouse *VP* gene amplified by polymerase chain reaction (PCR) (primers: AVP-F2, GCTAGGAGAGAGGGAAATGTTATCT; AVP-R2, GCATCTAGATAGCTCACTCGTTTTC). Mice were perfused with 4% paraformaldehyde. Frozen brain sections (16-μm thick) were prepared with a cryostat at −25 °C and mounted onto silanized glass slides for *in situ* hybridization as described [24]. DIG-positive neurons in the SON were detected with alkaline phosphatase-conjugated anti-DIG antibody using nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt.

To retrogradely label SFO neurons projecting to the SON, mice were anesthetized and placed in a stereotaxic frame (Narishige). Injection of retrograde tracer, cholera toxin subunit B conjugated with Alexa Fluor-555 (0.5 mg/ml, 0.1 μl; CTb-Alexa 555; Molecular Probes), was made unilaterally into the SON [22] with a glass micropipette (tip diameter, ~30 μm). Six days after the injection, mice were deprived of water for 48 h with food. After dehydration, mice were perfused with 20% formalin, and the brains were sliced coronally (50 μm) using a vibratome. Fos-immunostaining was performed as described previously [28].

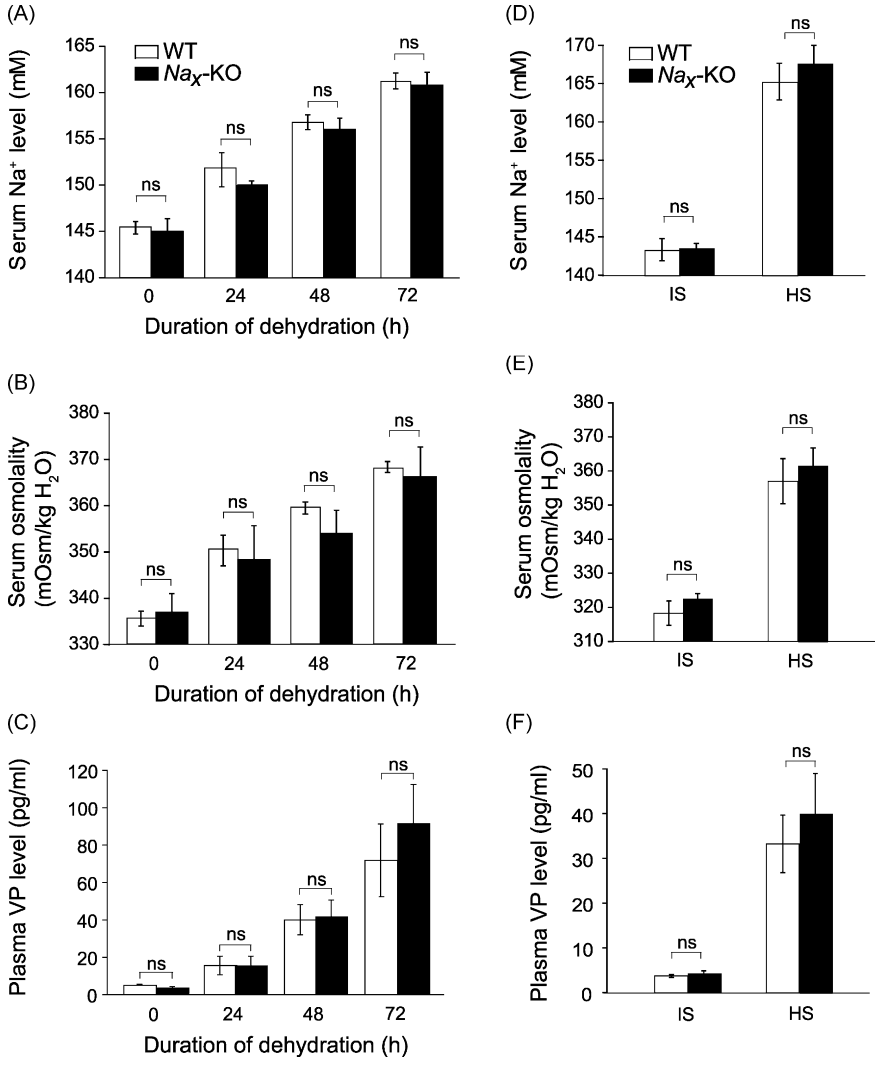

Fig. 1. Na^+, osmolality, and VP-levels in blood are normal in Na_x-KO mice after dehydration and after administration of hypertonic saline. (A) Na^+-level, (B) osmolality, and (C) plasma VP-level of WT and Na_x-KO mice under control (0 h) and dehydrated (24, 48, and 72 h) conditions. Data represent means ± SEMs (n = 8 per group for A and B; n = 5 per group for C). (D) Na^+-level, (E) osmolality, and (F) plasma VP-level of WT and Na_x-KO mice 60 min after ip-injection of isotonic (IS, 0.15 M NaCl) or hypertonic saline (HS, 0.8 M NaCl). Data represent means ± SEMs (each group, n = 7). ns, not significant.