General and Comparative Endocrinology 157 (2008) 196–202

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Effects of hypertonic stimuli and arginine vasotocin (AVT) on water absorption response in Japanese treefrog, *Hyla japonica*

Sho Maejima [a], Toshiki Yamada [a], Takayuki Hamada [b], Kouhei Matsuda [a], Minoru Uchiyama [a,*]

[a] Department of Biological Science, Graduate School of Science and Engineering, University of Toyama, 3190 Gofuku, Toyama 9308555, Japan
[b] Department of Biology, Faculty of Science, University of Toyama, 3190-Gofuku, Toyama 9308555, Japan

ARTICLE INFO

Article history:
Received 9 March 2008
Revised 9 April 2008
Accepted 28 April 2008
Available online 3 May 2008

Keywords:
Arginine vasotocin
Anurans
Water absorption response
V_1-like receptor
Central nervous system

ABSTRACT

Anuran amphibians do not drink orally but absorb water osmotically through the highly permeable ventral skin. In this cutaneous water absorption, roles of the putative cerebral osmoreceptors and functions of arginine vasotocin (AVT) were examined in the central nervous system of the Japanese treefrog, *Hyla japonica*. Intracerebroventricular (ICV) or intralymphatic sac (ILS) administration of various hypertonic solutions (NaCl, mannitol and urea) significantly extended the residence time in water in a dose-dependent manner, suggesting facilitation of water absorption in frogs. ICV injection of AVT also increased significantly the residence time in a dose-dependent manner. The water absorption effect of AVT was significantly inhibited by pretreatment of ICV OPC-21268, a vasopressin V_1 receptor antagonist. But pre-ICV injection of OPC-31260, a vasopressin V_2 receptor antagonist, did not block the water absorption effect of AVT. Extension of the residence time induced by hyperosmotic NaCl (1000 mOsm) ICV injection was significantly inhibited by pretreatment of ICV OPC-21268. The present results showed that increases of osmotic pressure in plasma and/or cerebrospinal fluid stimulate water absorption response, suggesting that osmoreceptors are certainly present in the central nervous system and AVT may directly stimulate water absorption in the treefrog. It is also suggested that AVT activates cellular mechanisms via V_1-like but not V_2-like receptors in the central nervous system and facilitates water absorption response in the treefrog.

1. Introduction

All vertebrates maintain plasma osmolality and extracellular volume primarily by regulating the drinking or ingestion, as an input, and urinary excretion, as an output, of water and electrolytes. Drinking usually reflects the physiological need or desire for water intake and is regulated by a special sensation of thirst in terrestrial vertebrates. In mammals, it is known that the sensation of thirst is initiated in the brain, especially in the anteroventral part of the third ventricle and the hypothalamus (Fitzsimons, 1998) and drinking behavior is mainly influenced by changes of plasma osmolality and fluid volume (Stricker and Sved, 2000). It has been reported that mammals, birds and reptiles utilize the renin-angiotensin system to induce thirst in response to decreases in the volume of the body fluids (Fitzsimons, 1998; Nishimura, 2001; Takei, 2000). In mammals, arginine vasopressin (AVP) also facilitates water intake or augments the motivational drinking (Camargo et al., 2003; Quadri et al., 1998; Szczepanska-Sadowska, 1996; Szczepanska-Sadowska et al., 1987). AVP or its non-mammalian analog arginine vasotocin (AVT), known as antidiuretic hormone, is a nonapeptide synthesized in the hypothalamus. Two different types of hypothalamic neurons, magnocellular and parvocellular, synthesize AVP or AVT. In mammals, an increase in plasma osmolality and a decrease of arterial pressure are the most potent stimulus of AVP release. When plasma is hyperosmotic, osmosensitive neurons of the circumventricular organs, primarily the organum vasculosum of lamina terminalis and the subfornical organ, activate the hypothalamus to mediate AVP release and cause water reabsorption in the kidney and colon (Burbach et al., 2001; Treschan and Peters, 2006).

Because amphibians have permeable skins, the flux of water across the integument reverses as the animal moves between terrestrial and aquatic environments. Therefore it must be important that terrestrial amphibians have evolved numerous mechanisms to survive in terrestrial environments. It is accepted that amphibians typically do not drink orally but instead absorb water osmotically across their permeable pelvic skin (Bentley, 2002). In anurans, exogenous angiotensin II (Ang II) stimulated the water absorption response (WR) including water seeking and water uptake across the skin (Goldstein et al., 2003; Propper and Johnson,

* Corresponding author. Fax: +81 76 445 6549.
E-mail address: uchiyama@sci.u-toyama.ac.jp (M. Uchiyama).

0016-6480/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2008.04.014

1994; Viborg and Rosenkilde, 2001). It has been reported that AVT increases cutaneous osmotic permeability in semiaquatic and terrestrial amphibians (Alvarado and Johnson, 1966; Dicker and Elliot, 1967; Goldenberg and Warburg, 1977). The expression of mRNAs of both the V_1- and V_2-like receptors has been reported in the various organs and tissues of the frog including the abdominal skin (Acharjee et al., 2004; Kohno et al., 2003). AVT is also known to facilitate water uptake through the ventral skin, not only by increasing cutaneous water permeability, but also by promoting cutaneous blood flow (Malvin, 1993). Tanaka and his colleagues have recently cloned cDNAs encoding several water channel proteins (AQPs) from the osmoregulatory tissues including the abdominal skin in the treefrog, *Hyla japonica*. AVT stimulated expression of some AQPs and facilitated water absorption in the ventral skins (Hasegawa et al., 2003; Tanii et al., 2002). We recently measured plasma aldosterone, Ang II and AVT levels in *Bufo marinus*, and showed that AVT was secreted in response to increases in plasma osmolality and to plasma volume shrinkage, while renin–angiotensin–aldosterone system hormones were stimulated in response to extracellular and plasma volume reduction (Konno et al., 2005). Thus, volumetric and osmometric systems regulated by renin–angiotensin–aldosterone system hormones and AVT are present in the frog. However, little is known concerning the functional coupling of the central nervous system (CNS) and the water uptake in amphibians. Kloas and Hanke (1992) suggested that localization of binding sites for Ang II and atrial natriuretic peptide might act as neurotransmitters and neuromodulators in amphibian CNS by *in vitro* autoradiography. McLeod and Donald (1999) also observed the distribution of both natriuretic peptide-like and AVT-like immunoreactive neurons in the CNS of *Bufo marinus*, and suggested that there might be mutual regulation of these peptides modulating the brain function. Although the action of AVT on body fluid regulation has been recognized in the osmoregulatory organ, central effect of AVT has not been elucidate yet.

The aim of the present study was to assess mechanisms underlying both hyperosmotic stimulation and AVT stimulation of the water absorption response (WR) in the Japanese treefrog, *Hyla japonica*.

2. Materials and methods

2.1. Animals

Adult Japanese treefrogs, *Hyla japonica*, weighing 1.5–2.5 g, were collected from suburbs of Toyama city, Toyama Prefecture, Japan. Female and male frogs were housed in cages and maintained on a 12L:12D cycle at 25–28 °C for at least 1 week before use in the experiment. They were allowed free access to water and fed mealworms twice a week. All animal experiments were conducted in accordance with the University of Toyama's guidelines for the care and use of animals.

2.2. Animal preparations and quantification of water absorption response

In a preliminary experiment, when fully dehydrated frogs lost of 20% of body mass by evaporation were kept in a plastic tank (20 × 30 × 20 cm) with a plastic dish (5 × 5 cm) containing tap water, some frogs could not reach to the water place at all for three and more hours. When fully dehydrated frogs were put in the water dish they continuously stayed there and were restored within about 60–90 min. Then the experiment of WR was planned as follows. Frogs were at first continuously kept in tap water for 24 h in order to secure equally and fully hydrated condition. In the experiment of WR, hydrated frogs were administered test solutions, and were kept in the plastic tank with the water dish. Their behaviors were monitored for 90 min with a videocamcorder (Handycam DCR-HC46, Sony Co., Tokyo, Japan). In order to measure water gain quantitatively, frogs were weighed carefully. Before weighing the urinary bladder was emptied using dull forceps by which the cloaca was extended gently and lower abdomen was pushed for forced urination gently with fingers. Water on the body surface was wiped with a soft tissue paper and the frog was weighted quickly with an autonull-type electronic balance (PM300, Nihon SiberHegner K.K., Tokyo, Japan). Net water gain was calculated from difference of body weight between before and after the treatments of an injection and behaviors recording.

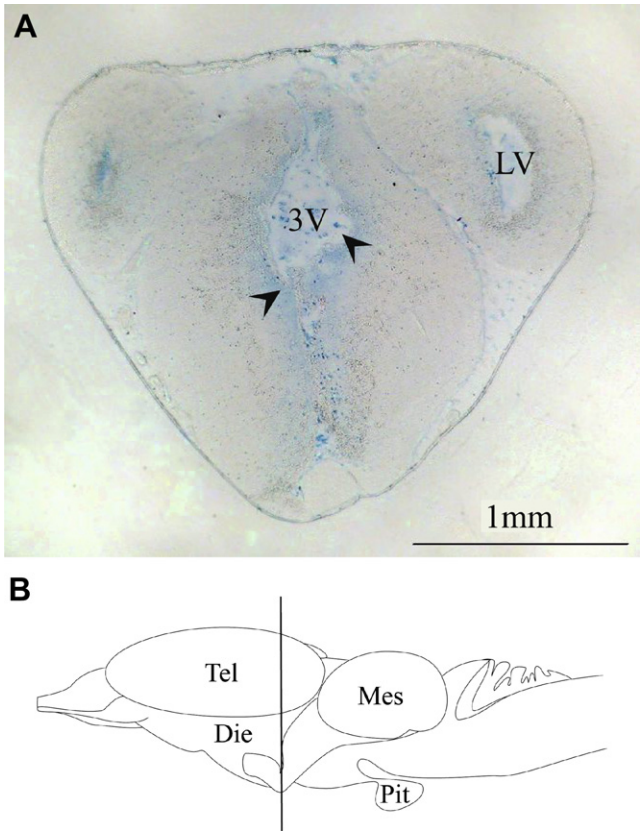

Fig. 1. (A) Brain coronal section showing the third ventricle of *Hyla japonica*. Accuracy of the injection site was confirmed after the experiment by examining whether Evans blue dye was present in the ventricle (arrowheads in figure). 3V, third ventricle; LV, lateral ventricle. Scale bar = 1 mm. (B) The schema of the treefrog brain. The site of the coronal section is shown by the vertical line. Tel, telencephalon; Mes, mesencephalon; Die, diencephalon; Pit, pituitary.

2.3. ICV or ILS injection of hypertonic solutions

In the experiment, NaCl (125, 150 and 500 mM), urea (250, 300 and 1000 mM) or mannitol (250, 300 and 1000 mM) solutions were injected into the third ventricle or the dorsal lymphatic sac to raise plasma and/or cerebrospinal fluid osmolality. Each concentration of the solutes is corresponding to 250, 300 and 1000 mOsm/l, respectively. All test solutes were dissolved in deionized water, and osmotic pressure of test solutions was measured with an osmometer (OM-6020, Arkray Inc., Kyoto, Japan). For intracerebroventricular (ICV) administration of test solutions, a small part of the parietal bone was carefully removed using a surgical blade under anesthesia with 0.1% MS-222 (3-aminobenzoic acid ethyl ester, Sigma, St. Louis, MO, USA). Under a dissecting microscope, 0.2 μl/g body weight (BW) of test solution was injected into the third ventricle of the brain by using a manipulator (IM-3, Narishige Scientific Lab., Tokyo, Japan) and a 10 μl Hamilton syringe (Hamilton Company, Reno, NV, USA). Injections were performed at midline and 0.8 mm depth from the surface of the diencephalon. After termination of the experiment, Evans blue dye was used to confirm whether the solution was accurately administered into the third ventricle. Frozen section of the forebrain area was made and staining of Evans blue dye was observed in the third ventricle and circumventricular tissues by light microscopy (Fig. 1). The success of each injection was checked in about one-tenth of tested animals and was confirmed that more than 90% was accurately injected into the third ventricle of animals. Five microliters per gram BW of test solution was injected into intralymphatic sac (ILS). Frogs in the control group were injected with the same volume of Ringer's solution. The Ringer's solution contained (in mM): NaCl, 111; $CaCl_2$, 2.70; KCl, 3.35; $NaHCO_3$, 2.38; glucose, 5.50; pH 7.4; osmolality 230 mOsm/l. A hydrated frog administered ICV injection of the test solution or Ringer's solution was firstly kept in the water dish after a 15 min of recovery period, and the WR was recorded using the camcorder. In the ILS injection group, same method of recording was conducted after the injection.

2.4. ICV or ILS injection of AVT and AVT receptor antagonists

In the first set of experiments, fully hydrated frogs were treated with AVT (ICV: 5, 10, or 20 pmol/g BW; ILS: 100, 500 or 1000 pmol/g BW) dissolved in Ringer's solution or Ringer's solution alone, which served as the control group. The ways of ICV and ILS administrations and recording of the WR were remarked above in