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Dorsal raphe nuclei integrate allostatic information evoked by depletion-induced sodium ingestion

Daniel Badauê-Passos Jr. [a,b,*], Andrea Godino [c], Alan Kim Johnson [b], Laura Vivas [c], José Antunes-Rodrigues [a]

[a] Departmento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil
[b] Department of Psychology, University of Iowa, Iowa City, IA, USA
[c] Instituto de Investigación Médica Mercedes y Martın Ferreyra (INIMEC-CONICET), Córdoba, Argentina

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Abstract

Structures of the lamina terminalis (LT) sense and integrate information reflecting the state of body water and sodium content. Output from the LT projects into a neural network that regulates body fluid balance. Serotonin (5-HT) and the dorsal raphe nuclei (DRN) have been implicated in the inhibitory control of salt intake (i.e., sodium appetite). Signals arriving from the LT evoked by fluid depletion-induced sodium ingestion interact with this inhibitory serotonergic system. We investigated the role of neurons along the LT that directly project to the DRN. We analyzed the pattern of immunoreactivity (ir) of LT cells double-labeled for Fos (a marker of neural activity) and Fluorogold (FG; a retrograde tracer) following sodium depletion-induced sodium intake. Seven days after injection of FG into the DRN, sodium appetite was induced by furosemide injection and overnight access to only a low sodium diet (Furo-LSD) and distilled water. Twenty-four hours later, access to 0.3 M NaCl was given to depleted or sham-depleted rats and sodium intake was measured over the following 60 min. Ninety minutes after the termination of the intake test, the animals were perfused and their brains were processed for immunohistochemical detection of Fos and FG. Compared to sham-depleted animals there was a significantly greater number of Fos-/FG-ir double-labeled cells in the subfornical organ, the organum vasculosum of the lamina terminalis and the median preoptic nucleus in rats that ingested NaCl. Projections from the LT cells may contribute to inhibitory mechanisms involving 5-HT neurons in the DRN that limit the intake of sodium and prevent excess volume expansion.

Keywords: Sodium appetite; Sodium satiety; Fos and Fluorogold immunoreactivity; Dorsal raphe nucleus; Subfornical organ; Organum vasculosum of the lamina terminalis; Median preoptic nucleus

Introduction

Body fluid balance requires complex, integrated systems involving neural, endocrine and behavioral components that function to maintain plasma volume and osmolality within a narrow range (Andersson and McCann, 1956; Fitzsimons, 1998; Johnson and Thunhorst, 1997, 2007; McCann et al., 1989). Water and sodium intake and excretion need to be controlled to minimize disturbances of hydromineral homeostasis. In this context, sodium intake constitutes important homeostatic behaviors involved in seeking out and acquiring sodium from the environment. This motivational state and the related behaviors are referred to as salt appetite.

Numerous approaches have been used to induce salt appetite, such as bilateral adrenalectomy (Richter, 1936), subcutaneous colloid treatment (Stricker and Jalowiec, 1970), peritoneal dialysis (Chiaraviglio, 1984) and dietary NaCl deprivation associated with furosemide (Jalowiec, 1974). Among them, sodium depletion induced by furosemide followed by access to a low sodium diet (Furo-LSD) evokes a robust sodium intake followed by satiety for sodium (Jalowiec, 1974). The cerebral structures involved in controlling the excitatory appetitive and inhibitory or satiety phases of sodium intake are likely to be interconnected with one another.

The lamina terminalis (LT) has been demonstrated to play a major role in many aspects of body fluid homeostasis (Denton

* Corresponding author. Department of Psychology, University of Iowa, 11 Seashore Hall E, Iowa City, IA 52242, USA. Fax: +1 319 335 0191.
E-mail address: daniel-passosjunior@uiowa.edu (D. Badauê-Passos).

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et al., 1996; Johnson, 1985; McKinley et al., 1999; Rowland, 1998; Thunhorst et al., 1998). Anatomically, the LT forms the anterior wall of the third ventricle and contains three forebrain structures: the subfornical organ (SFO), the median preoptic nucleus (MnPO) and the organum vasculosum of the lamina terminalis (OVLT) (Johnson and Gross, 1993). The SFO and OVLT lack a blood–brain barrier and contain cells which are sensitive to humoral signals such as changes in plasma and cerebrospinal fluid, sodium concentration (Vivas et al., 1990), osmolality (Sladek and Johnson, 1983) and angiotensin II (ANG II) levels (Ferguson and Bains, 1997; Simpson et al., 1978). Such unique features make the SFO and OVLT key brain regions for sensing the status of the body fluids and electrolytes. Humoral signals are passed on from the SFO and OVLT to other structures in the central nervous system including the MnPO (Lind et al., 1982). In turn, structures of the LT receive inputs from many different brain areas. Collectively the structures of the LT constitute a local neural network processing information that is important for the control of behaviors related to thirst and sodium appetite (Fitts et al., 1990; Johnson and Thunhorst, 1997, 2007; McKinley et al., 1982, 1996; Thunhorst et al., 1990; Weisinger et al., 1990).

Modulation of salt and water intake involves interactions between the LT and inhibitory hindbrain systems. Serotonin (5-HT) has been implicated in the inhibitory control of salt and water intake. Particularly relevant are the serotonergic neurons with their soma in the dorsal raphe nucleus (DRN) and 5-HT terminals within the lateral parabrachial nucleus (LPBN) (Castro et al., 2002a,b, 2003; Cavalcante-Lima et al., 2005b,a; Colombari et al., 1996; Lima et al., 2004; Menani et al., 1996, 1998a,b, 2000; Menani and Johnson, 1995; Olivares et al., 2003; Tanaka et al., 1998, 2001, 2003, 2004). Previous studies have shown that the activity of serotonergic cells within the DRN is affected by body sodium status (Franchini et al., 2002). Fos expression used as a marker of neuronal activity in 5-HT DRN cells was decreased when the animals were sodium depleted and increased when the animals were either in balance or were in the process of restoring sodium homeostasis by ingesting a 0.3-M solution of NaCl. These results are consistent with the concept that there is a tonic inhibition of sodium appetite exerted on the LPBN by serotonergic cells originating in the DRN. Such an inhibitory influence would be likely to be reduced in a state of fluid deficiency and increased when the animals ingest water and NaCl to restore hydromineral balance. Such a mechanism would be important in preventing excess NaCl consumption and reduce the possibility of excess extracellular fluid.

Lind (1986) has anatomically demonstrated a neural angiotensinergic connection originating in the SFO and projecting to the DRN. ANG II injected via the carotid artery or into the SFO enhances the electrical activity of SFO neurons that project to the DRN (Tanaka et al., 1998, 2003). A recent microdialysis study (Tanaka et al., 2003) indicates that ANG II activation of SFO neurons projecting to the DRN results in inhibition of neurons in this nucleus to effect a reduction in local 5-HT release. These results suggest that SFO neurons projecting to the DRN may be involved in monitoring circulating levels of ANG II and carrying such information to the DRN. A comparable projection originating in the MnPO and terminating in the DRN may play a similar role (Zardetto-Smith and Johnson, 1995). Thus, LT structures may act to inform the DRN of sodium status or sodium consumption and/or volume expansion by a descending neural pathway.

Recent data obtained by Reis and his colleagues have implicated the DRN as an inhibitory structure for the control of water and sodium appetite. Rats subjected to electrolytic and excitotoxic lesions of the DRN show increased *ad libitum* (need free) sodium intake and enhanced saline consumption after various manipulations that stimulate sodium appetite (Cavalcante-Lima et al., 2005a,b; Olivares et al., 2003). These researchers suggest that DRN may tonically inhibit sodium intake.

The aim of the present work was to investigate the role of the connection between the LT and DRN. In these studies we tested whether neurons located in the LT projecting to the DRN are activated in the course of sodium satiety. To identify LT neurons projecting to the DRN that are activated in this process, we used Fos immunocytochemistry in combination with the immunodetection of the retrograde tracer, Fluorogold (FG), injected into the DRN. We analyzed the Fos and FG immunoreactivity (Fos-/FG-ir) double-labeled neurons along the LT nuclei after sodium ingestion induced by Furo-LSD treatment. We also determined sodium intake, plasma volume, plasma sodium and osmolality before and after the stimulation of sodium intake.

Materials and methods

Animals

Male, Wistar-derived rats from the colony of the Instituto de Investigación Médica Mercedes y Martin Ferreyra (INIMEC, Córdoba, Argentina) were used for immunohistochemical studies. Behavioral and physiological experiments (plasma volume, osmolality and serum sodium concentration) were carried out in male, Wistar-derived rats obtained from the Central Animal Facility of Ribeirão Preto Campus (University of São Paulo). All animals (270–300 g) were housed individually in hanging wire cages for at least 1 week before the beginning of the experiments and had free access to food, water and 0.3 M NaCl, except as noted. Room lights were on for 14 h/day, and the temperature was controlled at 23 °C.

All experimental protocols were approved by the appropriate animal care and use committees of both Institutions, and the guidelines of the International Public Health Service Guide for the Care and Use of Laboratory Animals were followed.

Sodium appetite studies

Sodium appetite was stimulated by an acute treatment with furosemide (Astra, Westborough, MA, USA) in combination with a sodium-deficient diet (ICN, Costa Mesa, CA, USA). Animals of the experimental group were injected subcutaneously with furosemide (20 mg/kg in isotonic saline vehicle). Immediately after treatment, experimental animals were placed in clean individual