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Changes in taste reactivity to intra-oral hypertonic NaCl after lateral parabrachial injections of an α_2-adrenergic receptor agonist

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ABSTRACT

Bilateral injections of moxonidine, an α_2-adrenoceptor and imidazoline receptor agonist, into the lateral parabrachial nuclei (LPBN) enhance sodium appetite induced by extracellular dehydration. In the present study, we examined whether LPBN moxonidine treatments change taste reactivity to hypertonic NaCl solution administered into the mouth by intra-oral (IO) cannula. Male Holtzman rats prepared with IO and bilateral LPBN cannulas received subcutaneous injections of furosemide (FURO; 10 mg/kg) and captopril (CAP; 5 mg/kg) to induce hypovolemia with mild hypotension and an accompanying salt appetite and thirst before testing the taste reactivity to oral infusions of 0.3 M NaCl (1.0 ml/min). In the first experiment 45 min after subcutaneous injections of FURO + CAP or vehicle, moxonidine was bilaterally injected into the LPBN, and then 15 min later both bodily and oral–facial ingestive and rejection responses to 0.3 M NaCl delivered through the IO cannula were assessed. Both LPBN vehicle and moxonidine treated rats showed increased ingestive and decreased rejection responses to the IO hypertonic solution. The IO 0.3 M NaCl infusion-evoked ingestive and rejection taste related behaviors were comparable in the LPBN vehicle- vs. the LPBN moxonidine-injected groups. In a second experiment, rats received the same FURO + CAP treatments and LPBN injections. However, beginning 15 min after the LPBN injections, they were given access to water and 0.3 M NaCl and were allowed to consume the fluids for most of the next 60 min with the free access intake being interrupted only for a few minutes at 15, 30 and 60 min after the fluids became available. During each of these three brief periods, a taste reactivity test was conducted. On the three taste reactivity tests rats that received LPBN vehicle injections showed progressive declines in ingestive responses and gradual increases in rejection responses. However, in contrast to the LPBN vehicle treated rats, animals receiving bilateral injections of LPBN moxonidine maintained a high number of ingestive responses and a low number of rejection responses throughout the test period even in spite of evidencing substantial water and 0.3 M NaCl consumption during the periods of free access. The results suggest that after α_2-adrenoceptor agonist delivery to the LPBN the acceptance of 0.3 M NaCl is sustained and the negative attributes of the solution are minimized. The maintained positive rewarding qualities of 0.3 M NaCl are likely to account for why LPBN moxonidine treated rats show such a remarkable salt appetite when assayed by the volume of hypertonic 0.3 M NaCl consumed.

1. Introduction

Sodium chloride (NaCl) is an important constituent of the extracellular fluid compartment and the major determinant of plasma osmolality and extracellular fluid volume. The acquisition and ingestion of water and of salty substances are necessary behavioral responses for an animal to recover from deficits in body fluids. Sodium deficiency results in behaviors collectively known as sodium appetite (a.k.a. salt appetite) and which can be operationally defined by an

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increased ingestion of sodium solutions of concentrations which are normally avoided [1–3].

In the hindbrain, important inhibitory mechanisms for the control of water and NaCl intake have been demonstrated in the lateral parabrachial nucleus (LPBN) [4–10]. The LPBN, a pontine structure that lies dorsolateral to the superior cerebellar peduncle, is reciprocally connected to forebrain areas that have been implicated in the maintenance of blood pressure and body fluid homeostasis, such as the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala and the median preoptic nucleus. The LPBN is also richly interconnected with medullary regions, which include the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS), [11–18]. Cells in the LPBN are activated after ingestion of sodium solutions by dehydrated rats or in rats that received intragastric loads of hypertonic NaCl [19–21], suggesting that the LPBN might receive inhibitory visceral or taste signals. Therefore, the LPBN may integrate and relay taste and visceral signals that ascend from AP/mNTS en route to forebrain areas involved in the control of fluid and electrolyte balance [7–9,22,23].

The inhibitory mechanisms of the LPBN are modulated by different neurotransmitters like serotonin, cholecystokinin, glutamate, corticotrophin releasing factor, opioids and noradrenaline [7–10,24–32]. Activation of α_2-adrenoceptors with bilateral LPBN injections of moxonidine (α_2-adrenoceptor/imidazoline receptor agonist) or noradrenaline strongly enhances 0.3 M NaCl intake induced by subcutaneous treatment with the diuretic furosemide (FURO) when combined with a low dose of the antihypertensive drug, captopril (CAP) [24,32,33]. This suggests that activation of α_2-adrenoceptors in the LPBN may reduce the effects of inhibitory mechanisms that limit sodium intake [24,32,33]. The effects of α_2-adrenoceptor agonist treatment of the LPBN on sodium intake are not due to a non-specific facilitation of all ingestive behaviors, because sucrose solution intake is not affected by bilateral LPBN injections of moxonidine [33].

A taste reactivity test determining the frequency of ingestive and rejection behavioral reactions or fixed action patterns in response to intra-orally delivered solutions was originally developed by Grill and Norgren[34]. This method assesses the occurrences of species-typical affective behavioral reactions [such as ingestive-related tongue protrusions or negative (rejection) gapes] in response to oral stimulation [34,35]. Lesions placed within either the NTS, parabrachial nucleus (PBN), or the parvocellular ventral posteromedial thalamic nucleus (VPMpc) disrupt the shift in taste reactivity observed in intact animals after sodium deficiency [36]. Lesions placed in the NTS and PBN, but not the VPMpc, also block increases in home-cage intake observed in intact, sodium deficient rats [36].

Since α_2-adrenoceptor activation with the administration of moxonidine into the LPBN greatly increases NaCl intake in free access intake tests, the present studies tested whether LPBN α_2-adrenoceptor stimulation modifies taste reactivity responses to 0.3 M NaCl in rats with an experimentally-induced sodium appetite. The results of the experiments indicate that before animals ingest 0.3 M NaCl and water, LPBN moxonidine treatment does not increase or decrease the number of ingestive or rejection behaviors in comparison to those seen in LPBN vehicle treated rats. However, the findings do demonstrate that unlike LPBN vehicle treated animals showing decreased ingestive and increased rejection responses over the course of restoring body sodium and water, rats receiving LPBN moxonidine maintain a high level ingestive responses and a low number of rejection responses throughout a period of fluid repletion.

2. Material and methods

2.1. Animals

Male Holtzman rats weighing 290 to 310 g were housed in individual stainless steel cages with free access to normal sodium (0.5–1.0%) diet (Guabi Rat Chow, Paulínia, SP, Brazil), water and 0.3 M NaCl solution. Temperature was maintained at 23 ± 2 °C, and humidity was maintained at 55 ± 10% on a 12:12 light–dark cycle with light onset at 7:30 AM. The Ethical Committee for Animal Care and Use from the Dentistry School of Araraquara — UNESP approved the experimental protocols used in the present study (protocol 06/2006). The experimental protocols also followed the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23, 1996).

2.2. Cerebral and IO cannulas

Rats were anesthetized with ketamine (80 mg/kg of body weight) combined with xylazine (7 mg/kg of body weight) and placed in a Kopf stereotaxic instrument. The skull was leveled between bregma and lambda. Stainless steel 23-gauge cannulas were implanted bilaterally above the LPBN using the following coordinates: 9.4 mm caudal to bregma, 2.1 mm lateral to the midline, and 4.2 mm below the dura mater, according to Paxinos and Watson [37]. The tips of the cannulas were positioned at a point 2 mm above each LPBN. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws, and 20-gauge metal obturators were used to fill the cannulas between tests. Immediately after the implantation of LPBN cannulas, all animals were also implanted with chronic IO cannulas. Each oral cannula (heat-flared PE 50 tubing) entered the mouth just lateral to the first maxillary molar. The tubing was tunneled subcutaneously to ascend lateral to the skull, and posterior to the nape of neck where the free end was exteriorized. The IO cannulas do not interfere with the normal eating behavior of the animal and allow the direct infusion of solutions into the mouth. The rats were allowed to recover for 6 days before drug injections were made into the LPBN.

2.3. Injections into the LPBN

Bilateral injections into the LPBN were made using 5-μl Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At time of testing, obturators were removed and the injection needle (2 mm longer than the guide cannulas) was introduced in the brain. All the injections into the LPBN were 0.2 μl for each site and performed over a period of 1 min, with 1 additional min allowed to elapse before the injection needle was removed from the guide cannula to avoid reflux. The movement of an air bubble inside the polyethylene tubing connected to the syringe confirmed drug flow. The obturators were replaced after injection, and the rats were placed back into the cage.

2.4. Drugs

Moxonidine hydrochloride (0.5 nmol/0.2 μl) (Solvay Pharma, Hannover, Germany) dissolved in a mix of propylene glycol and water 2:1 (vehicle) was injected into the LPBN. Vehicle was injected as control.

The natriuretic/diuretic drug FURO (10 mg/ml; Sigma Chem., St Louis, MO, USA) was dissolved in alkaline saline (0.9% NaCl, pH was adjusted to 9.0 with NaOH) and administered s.c. at the dose of 10 mg/kg of body weight. The angiotensin converting enzyme inhibitor CAP (5 mg/ml; Sigma Chem., St. Louis, MO, USA) was dissolved in saline (0.9% NaCl) and administered s.c. at the dose of 5 mg/kg of body weight. The pH 9.0 saline solution was used as the vehicle control for FURO and normal 0.9% saline as the vehicle control for CAP.

2.5. Taste reactivity test

Prior to the testing period, rats with LPBN and IO cannulas were each given a 3-day habituation period during which they were exposed to the taste reactivity chamber for 10 min, followed by a 1 ml