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Importance of the lateral parabrachial nucleus to sodium balance in fluid-depleted rats

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ABSTRACT

The lateral parabrachial nucleus (LPBN) exerts an important inhibitory influence for the control of sodium and water intake. However, the importance of LPBN on renal responses and cardiovascular changes during extracellular dehydration are still unknown. Here we investigated the effects of bilateral injections of moxonidine (alpha$_2$-adrenergic and imidazoline receptor agonist) on renal and cardiovascular changes in fluid-depleted rats. Male Wistar rats (n = 4–8 per group) with bilateral stainless steel guide-cannulas implanted into the LPBN were treated with subcutaneous furosemide (10 mg/kg) + captopril (5 mg/kg) to induce fluid depletion. Forty-five min later vehicle or moxonidine (0.5 nmol/0.2 μl) were bilaterally injected into the LPBN. In fluid-depleted rats, moxonidine produced strong 0.3 M NaCl and water intake without noticeable changes in cardiovascular parameters. Moxonidine did not change sodium excretion (488 ± 135, vs. vehicle: 376 ± 75 μEq/1 h) or urinary volume (2.5 ± 0.7, vs. vehicle: 2.5 ± 0.3 ml/1 h) in fluid-depleted rats without access to fluids for rehydration. However, moxonidine decreased natriuresis (462 ± 127, vs. vehicle: 888 ± 122 μEq/1 h) and diuresis (2.5 ± 0.5, vs. vehicle: 4.5 ± 0.5 ml/1 h) in fluid-depleted rats submitted to i.g. rehydration. These data suggest that alpha$_2$-adrenergic mechanism of the LPBN facilitates sodium/water retention and body fluid volume expansion during extracellular dehydration.

1. Introduction

The lateral parabrachial nucleus (LPBN) integrates and relays taste and visceral signals that ascend from AP/mNTS to the forebrain areas involved in the control of fluid and electrolyte balance (Ciriello et al., 1984; Fulwiler and Saper, 1984; Herbert et al., 1990; Jhamandas et al., 1992; Jhamandas et al., 1996; Krukoff et al., 1993; Lanca and van der Kooy, 1985; Norgren, 1981). This bilateral pontine structure is responsible for important inhibitory mechanisms for the control of water and NaCl intake [for review see Andrade et al., 2014; Johnson and Thunhorst, 2007; Menani et al., 2014].

The alpha$_2$-adrenoceptor/imidazoline receptor agonist moxonidine injected into the LPBN strongly enhances the sodium appetite of acutely sodium-depleted rats, but has no effect on sodium intake of hydrated and satiated rats (Andrade et al., 2004). Bilateral injections of noradrenaline or the specific α_2-adrenoceptor agonist alpha-methylnoradrenaline into the LPBN also increase sodium intake in sodium-depleted rats (Gasparini et al., 2009; Menani et al., 2006). These effects of moxonidine, noradrenaline and alpha-methylnoradrenaline were abolished by previous treatment with RX 821002, an α_2-adrenoceptor antagonist, showing that alpha$_2$-adrenoceptor activation into the LPBN reduces inhibitory mechanisms of the LPBN that limit sodium intake (Andrade et al., 2014).

Although LPBN moxonidine injections failed to induce water or 0.3 M NaCl intake in satiated rats (Andrade et al., 2004), α_2-adrenoceptor activation into the LPBN induces an unexpected strong ingestion of 0.3 M NaCl and reduces diuresis and natriuresis in hyperosmotic rats, enhancing the positive sodium balance (Andrade et al., 2006, 2012).

However, the effects of moxonidine injected into the LPBN on the urinary volume and sodium excretion were evaluated only in hyperosmotic rats, and its effects on renal responses, sodium balance and cardiovascular parameters in fluid-depleted rats during the sodium appetitive test remain unclear. Therefore, in the present study we investigated the importance of the LPBN on renal and cardiovascular responses induced by extracellular dehydration.

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2. Materials and methods

2.1. Animals

Male Wistar rats ($n = 35$) weighing 290–310 g obtained from the Central Animal Facility of the Federal University of Alfenas (Alfenas–MG, Brazil) were used. The animals were housed in individual stainless steel cages with free access to food, water, and 1.8% (0.3 M) NaCl solution. Temperature was maintained at $23 \pm 2\,^{\circ}C$, and humidity was maintained at $55 \pm 10\%$ on a 12:12 light–dark cycle with light onset at 7:30 AM.

All procedures performed in studies involving animals were in accordance with the Ethical Committee for Animal Care and Use from Alfenas Federal University–Unifal-MG, Brazil (protocol no: 289/2010). The experimental protocols followed the recommendations of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23, 1996).

2.2. LPBN 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 stereotaxic instrument (Insight EFF 331, Insight Instruments, Ribeirão Preto, Brazil). The surgery for bilateral LPBN cannula placements is described in earlies studies from our lab (Andrade et al., 2004., 2012, 2015).

2.3. Injections into the LPBN

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 cannulas (2 mm longer than the guide cannulas) were introduced into the brain. The injection volume into the LPBN was 0.2 μl each site. The obturators were replaced after injection, and the rats were placed back into the cage.

2.4. Drugs

Furosemide (FURO, Sigma Chem., St Louis, MO, USA) was administered subcutaneously at 10 mg/kg of body weight. Captopril (CAP, Sigma Chem., St Louis, MO, USA) was administered subcutaneously at 5 mg/kg of body weight. Moxonidine hydrochloride (Sigma Chem., St Louis, MO, USA) was administered into the LPBN at a dose of 0.5 nmol/0.2 μl. Moxonidine was dissolved in a mix of propylene glycol/water 2:1 (vehicle). Vehicle was injected as control into the LPBN. Captopril was dissolved in isotonic saline. Furosemide was dissolved in alkaline saline (pH adjusted to 9.0 with NaOH).

2.5. Urine analyses

Rats were housed in metabolic cages. Spontaneously eliminated urine was collected in 0.1-ml graduated polypropylene tubes, and urinary volume was measured. The concentration of sodium and potassium in the urine was measured by a sodium and potassium sensitive electrode analyzer (Cobas B121, Roche, Rotkreuz, Switzerland). The amount of sodium and potassium in the urine was determined by the product of urine volume times the concentration of each ion in the urine. Sodium and water balance were calculated as the difference between the amount ingested (during the intake test or administered as gavage) and the amount excreted.

2.6. Arterial pressure recording

Under ketamine (80 mg/kg of body weight) combined with xylazine (7 mg/kg of body weight) anesthesia, polyethylene tubing (PE-10 connected to a PE-50) was inserted into the abdominal aorta through the femoral artery on the day before the experiments. The tubing was tunneled s.c. and exposed on the back of the rat. Rats were tested in their home cages. The mean arterial pressure (MAP) was recorded in the unanesthetized freely moving rats using an amplifier coupled to a computerized acquisition system (MP100; Biopac System Inc., CA, USA).

2.7. Experiment 1: moxonidine injection into the LPBN: urinary sodium excretion and urinary volume in fluid-depleted rats

The main objective of this experiment was to verify the effects of alpha$_2$-adrenergic activation in the LPBN on sodium excretion in fluid-depleted rats. Experiment 1 also provided behavioral parameters to guide Experiment 2.

Rats ($n = 6$) were habituated to metabolic cages at least one day prior to the experiments. During the experiment, water and sodium were removed and the animals were injected with furosemide (FURO) + captopril (CAP) treatment as described previously (Andrade et al., 2004; Fitts and Masson, 1989; Menani et al., 1996). Immediately after FURO + CAP treatment, a 0.1-ml graduated polypropylene tube was attached to each metabolic cage to allow urine sample collections. The first urine sample was collected 1 h after FURO + CAP treatment. The next samples were collected each 30 min. Moxonidine or vehicle were bilaterally injected into the LPBN 45 min after FURO + CAP treatment. Rats were tested under two conditions: with and without free access to water and 0.3 M NaCl during urine collection. Water and 0.3 M NaCl were available 1 h after FURO + CAP, provided from burettes with 0.1-ml divisions that were fitted with metal drinking spouts.

This group of rats was tested four times. In order to avoid any effect on sodium and water intake due to sensitization after multiple FURO + CAP treatments (Pereira et al., 2010), we controlled the experiments dividing the experimental groups and assigning the different treatments in a randomized way. In each test, the group was divided in two subgroups that received different treatments (vehicle or moxonidine into the LPBN combined with or without free access to water and 0.3 M NaCl). The sequence of treatments in each rat in different tests was randomized and by the end of the tests, each rat had received all the treatments.

Sodium and water balance was calculated as the difference between the amount ingested ad libitum and the amount excreted.

2.8. Experiment 2: moxonidine injection into the LPBN: sodium balance in fluid-depleted rats after i.g. rehydration

The main objective of this experiment was to verify the effects of alpha$_2$-adrenergic activation in the LPBN on sodium excretion and sodium balance in fluid-depleted rats after rehydration.

Rats ($n = 7$) were trained to the gavage procedure daily during at least 5 days. The training was performed once a day. The rat was held carefully and injected into the stomach with 1 ml of water through PE-200 polyethylene tubing from a 5-ml syringe. Rats were also habituated to metabolic cages at least one day prior to the experiments.

During the experiment, water and sodium were removed and the animals were injected with furosemide (FURO) + captopril (CAP). Immediately after FURO + CAP treatment, a 0.1-ml graduated polypropylene tube was attached to each metabolic cage to allow urine sample collections. After 45 min, moxonidine or vehicle were bilaterally injected into the LPBN.