Pharmacology, Biochemistry and Behavior 111 (2013) 111–119

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Facilitation of sodium intake by combining noradrenaline into the lateral parabrachial nucleus with prazosin peripherally

S. Gasparini [a], J.M.C. Gomide [a], G.M.F. Andrade-Franzé [a], L.T. Totola [b], L.A. De Luca Jr. [a], D.S.A. Colombari [a], P.M. De Paula [a], T.S. Moreira [b], J.V. Menani [a,*]

[a] Department of Physiology and Pathology, School of Dentistry, São Paulo State University, UNESP, Araraquara, São Paulo, Brazil
[b] Department of Physiology and Biophysics, Institute of Biomedical Science, University of São Paulo-USP, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 22 March 2013
Received in revised form 20 August 2013
Accepted 28 August 2013
Available online 13 September 2013

Keywords:
Noradrenaline
Baro and volume receptors
Parabrachial nucleus
Sodium appetite
Blood pressure

ABSTRACT

Injections of noradrenaline into the lateral parabrachial nucleus (LPBN) increase arterial pressure and 1.8% NaCl intake and decrease water intake in rats treated with the diuretic furosemide (FURO) combined with a low dose of the angiotensin converting enzyme inhibitor captopril (CAP). In the present study, we investigated the influence of the pressor response elicited by noradrenaline injected into the LPBN on FURO + CAP-induced water and 1.8% NaCl intake. Male Holtzman rats with bilateral stainless steel guide-cannulas implanted into LPBN were used. Bilateral injections of noradrenaline (40 nmol/0.2 μl) into the LPBN increased FURO + CAP-induced 1.8% NaCl intake (12.2 ± 3.5, vs., saline: 4.2 ± 0.8 ml/180 min), reduced water intake and strongly increased arterial pressure (50 ± 7, vs. saline: 1 ± 1 mm Hg). The blockade of the α_1 adrenoceptors with the prazosin injected intraperitoneally abolished the pressor response and increased 1.8% NaCl and water intake in rats treated with FURO + CAP combined with noradrenaline injected into the LPBN. The deactivation of baro and perhaps volume receptors due to the cardiovascular effects of prazosin is a mechanism that may facilitate water and NaCl intake in rats treated with FURO + CAP combined with noradrenaline injected into the LPBN. Therefore, the activation of α_2 adrenoceptors with noradrenaline injected into the LPBN, at least in dose tested, may not completely remove the inhibitory signals produced by the activation of the cardiovascular receptors, particularly the signals that result from the extra activation of these receptors with the increase of arterial pressure.

1. Introduction

The lateral parabrachial nucleus (LPBN) is a major site that receives ascending projections from the area postrema and the medial portion of the nucleus of the solitary tract (AP/mNTS), an important area of the hindbrain innervated by afferents from arterial baroreceptors, cardiopulmonary receptors, gustatory receptors and other visceral receptors that influence water and NaCl intake (Herbert et al., 1990; Lança and van der Kooy, 1985; Johnson, 2007; Johnson and Thunhorst, 1997, 2007; Norgren, 1981).

The blockade of serotonin, cholecystokinin (CCK), corticotrophin releasing factor (CRF) or glutamate receptors, or activation of α_2-adrenoceptors in the LPBN increases hypertonic NaCl intake and occasionally also water intake produced by the treatment with the diuretic furosemide (FURO) combined with a low dose of the angiotensin-converting enzyme inhibitor captopril (CAP), both injected subcutaneously (s.c.), suggesting the existence of important inhibitory mechanisms for the control of sodium and water intake in the LPBN (Menani et al., 1996; Menani and Johnson, 1998; De Gobbi et al., 2001; Andrade et al., 2004; De Castro e Silva et al., 2006; De Gobbi et al., 2009; Gasparini et al., 2009).

The inflation of a balloon placed at the superior vena cava-right atrial junction reduces 1.8% NaCl intake induced by FURO + CAP treatment and increases c-fos protein in the LPBN, suggesting that the LPBN might be a site that receives signals from cardiovascular receptors that inhibit sodium intake (De Gobbi et al., 2008). The changes in the activities of these cardiovascular receptors caused by reductions or increases in the arterial pressure might facilitate or inhibit, respectively, water and sodium intake (Thunhorst and Johnson, 1994; Johnson and Thunhorst, 1997, 2007; Johnson, 2007; De Gobbi et al., 2008). However, the injections of noradrenaline into the LPBN facilitate FURO + CAP-induced sodium intake, in spite of the

Abbreviations: ACE, angiotensin-converting enzyme; ANG II, angiotensin II; ANOVA, analysis of variance; AP, area postrema; CAP, captopril; DOI, 2,5-dimetoxy-4-iodoamphetamine hydrobromide; FURO, furosemide; HR, heart rate; 5-HT, serotonin; i.c.v., intracerebroventricularly; i.p., intraperitoneal; LPBN, lateral parabrachial nucleus; mNTS, medial portion of the nucleus of the solitary tract; NOR, noradrenaline; NTS, nucleus of the solitary tract; SAL, saline; s.c., subcutaneous; SCP, superior cerebellar peduncle; SFO, subfornical organ.

* Corresponding author at: Department of Physiology and Pathology, School of Dentistry, Sao Paulo State University, UNESP, Rua Humaitá, 1680, Araraquara, 14801 903, SP, Brazil. Tel.: +55 16 3301 6486; fax: +55 16 3301 6488.
E-mail address: menani@foar.unesp.br (J.V. Menani).

0091-3057/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pbb.2013.08.017

strong increase of arterial pressure produced by these injections (Gasparini et al., 2009).

The facilitation of FURO + CAP-induced 1.8% NaCl intake produced by the injections of noradrenaline into the LPBN similar to the facilitation produced by moxonidine (α_2-adrenoceptor and imidazoline agonist) into the LPBN depends on the activation of the α_2-adrenoceptors in the LPBN (Andrade et al., 2004; Gasparini et al., 2009). The only difference is that the activation of the α_2-adrenoceptors with the injections of moxonidine into the LPBN does not modify arterial pressure (Andrade et al., 2004). This may explain why noradrenaline is less effective than moxonidine injected into the LPBN to increase FURO + CAP-induced sodium intake and also differently from moxonidine, noradrenaline reduces FURO + CAP-induced water intake.

In the present study, we investigated the influence of the pressor response produced by noradrenaline on water and 1.8% NaCl intake by rats treated with s.c. FURO + CAP and the possible peripheral mechanisms (sympathetic activation and/or vasopressin secretion) that might be activated by the injections of noradrenaline injected into the LPBN to produce pressor responses. For this, we tested the effects of the blockade of the pressor response to noradrenaline injected into the LPBN with intraperitoneal (i.p.) injection of prazosin (specific α_1 adrenoceptor antagonist) on FURO + CAP-induced water and 1.8% NaCl intake and the effects of the intravenous (i.v.) injection of a vasopressin antagonist combined with hexamethonium (ganglionic blocker) on the pressor and bradycardic responses to noradrenaline injected into the LPBN in unanesthetized rats. Additionally, we also evaluated the cardiovascular and sympathetic responses to noradrenaline injected into the LPBN in anesthetized rats.

2. Material and methods

2.1. Animals

Male Holtzman rats (for tests in unanesthetized animals) or Wistar rats (for tests in anesthetized animals) weighing 280 to 320 g were used. The animals were housed individually in stainless steel cages in a room with controlled temperature (23 ± 2 °C) and humidity (55 ± 10%). The lights were on from 7:00 am to 7:00 pm. Guabi rat chow (Paulínia, SP, Brazil), tap water and 1.8% NaCl were available ad libitum. Animals' use was in accordance with the guidelines approved by the Animal Experimentation Ethics Committee of the Dentistry School of Araraquara — UNESP and Institute of Biomedical Science at the University of São Paulo (ICB/USP). All efforts were made to minimize animal discomfort and the number of animals used.

2.2. Brain surgery

The animals were anesthetized with ketamine (80 mg/kg of body weight i.p.) and xylazine (7 mg/kg of body weight i.p.) and placed in a Kopf stereotaxic instrument. The skull was positioned to have the bregma and lambda at the same horizontal level. Stainless steel cannulas (12 × 0.6 mm o.d.) were implanted bilaterally in the LPBN (coordinates: 9.4 mm caudal to bregma, 2.1 mm lateral to midline and 4.1 mm below the dura mater). The tips of the cannulas were positioned at a point 2 mm above the LPBN. Besides the LPBN cannulas, one group of rats, received also stainless steel cannulas implanted into the lateral ventricle (LV, coordinates: 0.3 mm caudal to bregma, 1.5 mm lateral to midline and 3.5 mm below the dura mater). The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. At the end of the surgery, the animals received an intramuscular injection of penicillin (30,000 IU) and an s.c. injection of the analgesic Ketoflex (ketoprofen 1%, 0.03 ml/rat).

After the surgery, rats were allowed to recover for one week before starting water and NaCl intake tests or arterial pressure recordings.

2.3. Arterial pressure and heart rate recordings in unanesthetized animals

The mean arterial pressure and HR were recorded in unanesthetized rats. Under ketamine anesthesia (100 mg/kg of body weight i.p.) and xylazine (7 mg/kg of body weight i.p.), a 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. At the same time, in some rats, a polyethylene tubing was also inserted in the femoral vein for drug administration. Both tubings were tunneled subcutaneously and exposed on the back of the rat to allow access in unrestrained, freely moving rats. To record pulsatile arterial pressure (PAP), MAP and HR, the arterial catheter was connected to a Statham Gold (P23 Db) pressure transducer connected with an ETH-200 amplifier (CB Sciences) and to a PowerLab data acquisition systems (ADInstruments).

2.4. Sympathetic nerve activity recording

The rats were deeply anesthetized with halothane (5% in 100% oxygen in inspired air) for general surgical procedures, such as: a) tracheostomy for artificial ventilation; b) femoral artery and vein catheterization for arterial pressure measurement and administration of fluids and drugs, respectively; c) intracerebral injection by removal of the occipital bone and retracting the underlying dura mater membrane for insertion of a pipette into the medulla oblongata via a dorsal transcerebellar approach; d) splanchnic sympathetic nerve isolation for subsequent nerve activity monitoring. The level of anesthesia was checked by a flexor reflex to the animal's paw pinching.

Splanchnic sympathetic nerve activity (sSNA) was recorded as previously described (Totola et al., 2013). Briefly, the right splanchnic nerve was isolated via a retroperitoneal approach, and the segment distal to the suprarenal ganglion was placed on a pair of Teflon-coated silver wires that had been bared at the tip (250 μm bare diameter; A-M Systems, www.a-msystems.com). The nerves and wires were embedded in adhesive material (Kwik-Cast Sealant, WPI, USP), and the wound was closed around the exiting recording wires.

Upon completion of the surgical procedures, halothane was replaced by urethane (1.2 g/kg of body weight) slowly administered intravenously (i.v.). All rats were artificially ventilated with 100% oxygen throughout the experiment. The rectal temperature was maintained at 37 °C and the end tidal-CO_2 was monitored throughout the experiment with a capnometer (CWE, Inc., Ardmore, PA, USA) that was calibrated twice per experiment against a calibrated CO_2/N_2 mix. The adequacy of the anesthesia was monitored during a 20 minute stabilization period by testing for the absence of withdrawal response and the lack of arterial pressure change to firm toe pinch. After these criteria were satisfied, the muscle relaxant pancuronium was administered at the initial dose of 1 mg/kg i.v. and the adequacy of anesthesia was thereafter gauged solely by the lack of increase in arterial pressure to firm toe pinch. Approximately hourly supplements of one-third of the initial dose of urethane were needed to satisfy these criteria during the course of the recording period (2–3 h).

As previously described (Wenker et al., 2013; Totola et al., 2013), mean arterial pressure (MAP), sSNA and end-expiratory CO_2 ($etCO_2$) were digitized with a micro1401 (Cambridge Electronic Design), stored on a computer, and processed off-line with version 6 of Spike 2 software (Cambridge Electronic Design). Integrated splanchnic nerve activities ($\int$SNA) were obtained after the rectification and smoothing ($\tau = 2$ s) of the original signal, which was acquired with a 30–300 Hz bandpass filter. $\int$SNA was normalized within animals by assigning a value of 100 to resting SNA and a value of 0 to the minimum value recorded either during the administration of a dose of phenylephrine that saturated the baroreflex (5 μg/kg, i.v.) or after ganglionic blockade (hexamethonium; 10 mg/kg, i.v.).