Neuroscience 208 (2012) 69–78

MOXONIDINE INTO THE LATERAL PARABRACHIAL NUCLEUS REDUCES RENAL AND HORMONAL RESPONSES TO CELL DEHYDRATION

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Abstract—The deactivation of the inhibitory mechanisms with injections of moxonidine (α_2-adrenoceptor/imidazoline receptor agonist) into the lateral parabrachial nucleus (LPBN) increases hypertonic NaCl intake by intra- or extracellular dehydrated rats. In the present study, we investigated the changes in the urinary sodium and volume, sodium balance, and plasma vasopressin and oxytocin in rats treated with intragastric (i.g.) 2 M NaCl load (2 ml/rat) combined with injections of moxonidine into the LPBN. Male Holtzman rats (n=5–12/group) with stainless steel cannulas implanted bilaterally into LPBN were used. Bilateral injections of moxonidine (0.5 nmol/0.2 μl) into the LPBN decreased i.g. 2 M NaCl-induced diuresis (4.6±0.7 vs. vehicle: 7.4±0.6 ml/120 min) and natriuresis (1.65±0.29 vs. vehicle: 2.53±0.17 mEq/120 min), whereas the previous injection of the α_2-adrenoceptor antagonist RX 821002 (10 nmol/0.2 μl) into the LPBN abolished the effects of moxonidine. Moxonidine injected into the LPBN reduced i.g. 2 M NaCl-induced increase in plasma oxytocin and vasopressin (14.6±2.8 and 2.2±0.3 vs. vehicle: 25.7±7 and 4.3±0.7 pg/ml, respectively). Moxonidine injected into the LPBN combined with i.g. 2 M NaCl also increased 0.3 M NaCl intake (7.5±1.7 vs. vehicle: 0.5±0.2 mEq/2 h) and produced positive sodium balance (2.3±1.4 vs. vehicle: -1.2±0.4 mEq/2 h) in rats that had access to water and NaCl. The present results show that LPBN α_2-adrenoceptor activation reduces renal and hormonal responses to intracellular dehydration and increases sodium and water intake, which facilitates sodium retention and body fluid volume expansion.

Key words: osmoreceptor, sodium, oxytocin, natriuresis, cell dehydration, thirst.

The lateral parabrachial nucleus (LPBN), a pontine structure located dorsolaterally to the superior cerebellar peduncle (SCP), is reciprocally connected to forebrain areas, such as the paraventricular nucleus of the hypothalamus, central nucleus of the amygdala, and median preoptic nucleus, and to medullary regions, like the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS) (Norgren, 1981; Ciriello et al., 1984; Lança and van der Kooy, 1985; Herbert et al., 1990; Jhamandas et al., 1992, 1996; Krukoff et al., 1993). Cells in the LPBN are activated after ingestion of sodium solutions by dehydrated rats or by rats that received intragastric (i.g.) load of hypertonic NaCl (Kobashi et al., 1993; Yamamoto et al., 1993; Franchini and Vivas, 1999), suggesting that LPBN cells are activated by visceral or taste signals. Therefore, the LPBN may convey signals that ascend from AP/mNTS to forebrain areas involved in the control of fluid and electrolyte balance.

Studies about the involvement of the LPBN in the control of fluid-electrolyte balance have investigated mainly the inhibitory mechanisms for water and NaCl intake and different neurotransmitters like serotonin, cholecystokinin, glutamate, and corticotrophin releasing factor (CRF) or receptors like α_2-adrenoceptors and GABAergic, opioid or purinergic receptors in the LPBN have been shown to be involved with these mechanisms (Menani and Johnson, 1995; Colombari et al., 1996; Menani et al., 1996, 1998a,b, 2000; De Gobbi et al., 2000, 2009; Andrade et al., 2004; Callera et al., 2005; De Castro e Silva et al., 2005; Andrade et al., 2006; De Oliveira et al., 2008; Gasparini et al., 2009; Andrade-Franzé et al., 2010; Menezes et al., 2011). Particularly interesting are the effects of the α_2-adrenoceptor activation with moxonidine (α_2-adrenoceptor/imidazoline receptor agonist) or noradrenaline injected into the LPBN (Andrade et al., 2004, 2006; Gasparini et al., 2009). Although α_2-adrenoceptor activation in the forebrain inhibits water and 0.3 M NaCl intake (de Oliveira et al., 2003; Sugawara et al., 1999; Menani et al., 1999), the activation of α_2-adrenoceptors with bilateral injections of noradrenaline or moxonidine into the LPBN similar to the blockade of serotonin in the LPBN strongly increases 0.3 M NaCl and water intake induced by the treatment with the diuretic furosemide (FURO) combined with low dose of the angiotensin converting enzyme inhibitor captopril (CAP) injected subcutaneously. This suggests that forebrain and hindbrain (or more specifically LPBN) α_2-adrenergic mechanisms have opposite roles on water and sodium intake (Andrade et al., 2004; Gasparini et al., 2009). Although moxonidine injected into the LPBN in satiated rats produces no effect on water or 0.3 M NaCl intake (Andrade et al., 2004), α_2-adrenoceptor activation with moxonidine injected into the LPBN in rats treated with i.g. 2 M NaCl (2 ml/rat) induces strong ingestion of 0.3 M NaCl (Andrade et al., 2006).

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Abbreviations: ANP, atrial natriuretic peptide; AP, area postrema; AVP, arginine-vasopressin; CAP, captopril; CRF, corticotrophin releasing factor; DOI, 2.5-dimetoxi-4-iodoamphetamine hydrobromide; EDTA, Ethylenediaminetetraacetic acid; FURO, furosemide; i.g., intragastric; LPBN, lateral parabrachial nucleus; mNTS, medial portion of the nucleus of the solitary tract; OT, oxytocin; SCP, superior cerebellar peduncle.

0306-4522/12 $36.00
doi:10.1016/j.neuroscience.2012.02.013

Besides the involvement in the control of water and sodium intake, at least one study suggested that the LPBN mechanisms are also important for the control of renal excretion. This study showed that bilateral injections of the serotonergic receptor antagonist methysergide into the LPBN reduce the diuresis, natriuresis, and kaliuresis and also the increase in plasma levels of atrial natriuretic peptide (ANP) and oxytocin (OT) produced by isotonic blood volume expansion, whereas activation of the serotonergic 5-$HT_{2A/2C}$ receptors with bilateral injections of 2.5-dimetoxi-4-iodoamphetamine hydrobromide (DOI) into the LPBN produced opposite effects in the same responses (Margatho et al., 2007). Therefore, LPBN serotonergic mechanisms simultaneously inhibit sodium and water intake and facilitate renal and hormonal responses increasing sodium and water excretion, all of them responses to protect against blood volume expansion.

The previous study (Margatho et al., 2007) showed that serotonergic LPBN mechanisms are strongly involved with the renal and hormonal responses to isotonic blood volume expansion; however, no study has investigated if the LPBN mechanisms are involved with the renal or hormonal responses to increased plasma osmolality. Previous studies have shown that moxonidine injected into the lateral ventricle induces diuresis and natriuresis (Penner and Smyth, 1994a,b; Menani et al., 1999, 2006; El-Ayoubi et al., 2005; Andrade et al., 2009), and moxonidine injected into the LPBN increases hypertonic NaCl intake in rats treated with i.g. 2 M NaCl (Andrade et al., 2006). Therefore, in the present study, we investigated the effects of bilateral injections of moxonidine into the LPBN on the diuresis, natriuresis, water and sodium balance, and plasma ANP, arginine-vasopressin (AVP), and OT in cell-dehydrated rats by i.g. 2 M NaCl load. In addition, the effects of the blockade of α_2-adrenoceptors with RX 821002 injected into the LPBN on the responses to moxonidine injected into the same area were also investigated. The i.g. 2 M NaCl produces 4% increase in plasma sodium and osmolality, reduces plasma renin activity by half without changing blood volume or arterial pressure (Pereira et al., 2002; Blanch et al., 2007).

EXPERIMENTAL PROCEDURES

Animals

Male Holtzman rats weighing 290–310 g were used. The animals were housed in individual stainless steel cages with free access to normal sodium diet (Guabi Rat Chow, Paulínia, SP, Brazil), water, and 0.3 M NaCl. Temperature was maintained at 23±2 °C and humidity at 55±10% on a 12-h light-dark cycle with light onset at 7:30 AM. The Ethical Committee for Animal Care and Use from Dentistry School of Araraquara—UNESP approved the experimental protocols used in the present study (CEEA-FOAr/UNESP, protocol 06/2006). The experimental protocols were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). All efforts were made to minimize animal discomfort and the number of animals used.

Cerebral cannulas

Rats were anesthetized with ketamine (80 mg/kg of body weight, Agener União, Embu-Guaçu, SP, Brazil) combined with xylazine (7 mg/kg of body weight, Agener União, Embu-Guaçu, SP, Brazil) and placed in a Kopf stereotaxic instrument (Kopf, Tujunga, CA, USA). The skull was leveled between bregma and lambda. Bilateral stainless steel 23-gauge cannulas were implanted in direction to 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. 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. A 30-gauge metal obturator filled the cannulas between tests. The rats were allowed to recover 6 days before drug injections into the LPBN. Starting one day after cerebral surgery, rats were handled daily and trained for the procedures of the tests.

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 in the guide cannulas. 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.

Drugs

Moxonidine hydrochloride (donation from Solvay Pharma, Hannover, Germany), was administered into the LPBN at the doses of 0.5 nmol/0.2 μl. Moxonidine was dissolved in a mix of propylene glycol and water 2:1 (vehicle). RX 821002 hydrochloride (2-methoxyidazoxan, 10 nmol/0.2 μl, Research Biomedical International, RBI, Natick, MA, USA), was dissolved in saline. Vehicle or saline was injected as control. The doses of moxonidine and RX 821002 were chosen based on previous studies in which the effects of these drugs injected into the LPBN on water and NaCl intake were tested (Andrade et al., 2004, 2006, 2007).

Urine analysis

Rats with bilateral stainless steel cannulas implanted into the LPBN were housed in metabolic cages with only water available for 14–16 h before the tests.

One group of rats received an i.g. load of 0.15 M or of 2 M NaCl (2 ml/rat) 15 min after bilateral injections of moxonidine (0.5 nmol/0.2 μl) or vehicle into the LPBN and urine was collected at each 30 min in the next 3 h, starting immediately after the i.g. load. This group of rats was submitted to four tests. In each test the group was divided in two subgroups that received different treatments (i.g. 0.15 or 2 M NaCl combined with vehicle or moxonidine into the LPBN). The sequence of treatments in each rat in different tests was randomized and at the end of tests each rat received all the treatments.

Another group of rats received an i.g. load of 2 M NaCl (2 ml/rat) 45 min before bilateral injections of moxonidine (0.5 nmol/0.2 μl) or vehicle into the LPBN, and urine was collected at each 30 min in the next 2 h, starting 15 min after the injections of moxonidine or vehicle into the LPBN. This group of rats also received bilateral injections of RX 821002 (10 nmol/0.2 μl) or vehicle into the LPBN, 15 min before moxonidine or vehicle injections. This group of rats was also submitted to four tests. In each test the group was divided in two subgroups that received different combinations of treatments into the LPBN. The sequence of the combinations of treatments into the LPBN in each rat in different tests was randomized, and at the end of the tests each rat received all the combinations of treatments into the LPBN.

Urine samples were 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 an ion-specific electrode (Nova Biomedical Analyzer, model Nova 1)