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SODIUM INTAKE COMBINING CHOLINERGIC ACTIVATION AND NORADRENALINE INTO THE LATERAL PARABRACHIAL NUCLEUS

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Abstract—The administration of cholinergic agonists like pilocarpine intraperitoneally (i.p.) or carbachol intracerebroventricularly (i.c.v.) induces water, but non significant hypertonic NaCl intake. These treatments also produce pressor responses, which may inhibit sodium intake. Noradrenaline (NOR) acting on α_2-adrenoceptors in the lateral parabrachial nucleus (LPBN) deactivates inhibitory mechanisms increasing fluid depletion-induced sodium intake. In the present study, we investigated: (1) water and 1.8% NaCl intake in rats treated with pilocarpine i.p. or carbachol i.c.v. combined with NOR into the LPBN; (2) if inhibitory signals from cardiovascular receptors are blocked by NOR in the LPBN. Male Holtzman rats with stainless steel guide-cannulas implanted in the lateral ventricle and bilaterally in the LPBN were used. Bilateral injections of NOR (80 nmol/0.2 μl) into the LPBN decreased water intake (0.8 ± 0.3, vs. saline (SAL): 2.9 ± 0.3 ml/180 min) induced by pilocarpine (1 mg/kg of body weight) i.p., without changing 1.8% NaCl intake (0.8 ± 2.4, vs. SAL: 0.5 ± 0.3 ml/180 min). Prazosin (1 mg/kg of body weight) i.p. blocked pressor responses and increased water and 1.8% NaCl intake (6.3 ± 1.7 and 14.7 ± 3.5 ml/180 min, respectively) in rats treated with pilocarpine combined with NOR into the LPBN. Prazosin i.p. also increased 1.8% NaCl intake in rats treated with carbachol i.c.v combined with NOR into the LPBN. The results suggest that different signals inhibit sodium intake in rats treated with cholinergic agonists, among them those produced by increases of arterial pressure that are not efficiently deactivated by NOR acting in the LPBN. © 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: α_2 adrenoceptors, baro and volume receptors, parabrachial nucleus, sodium appetite, blood pressure.

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Abbreviations: 5-HT, serotonin; ACE, angiotensin-converting enzyme; ANG II, angiotensin II; ANOVA, analysis of variance; CAP, captopril; FURO, furosemide; HR, heart rate; i.c.v., intracerebroventricularly; i.p., intraperitoneal; LPBN, lateral parabrachial nucleus; MAP, Mean arterial pressure; NOR, noradrenaline; SAL, saline; sc, subcutaneous.

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INTRODUCTION

Important inhibitory mechanisms for the control of sodium and water intake are present in the lateral parabrachial nucleus (LPBN) (De Gobbi et al., 2009; Gasparini et al., 2009; De Oliveira et al., 2011; Menezes et al., 2011; Menani et al., 2014). The LPBN receives ascending projections from the medial portion of the nucleus of the solitary tract (mNTS), the site of the first synapse of the afferents from arterial baroreceptors, cardiopulmonary receptors, gustatory receptors and other visceral receptors that influence water and NaCl intake (Norgren, 1981; Lanca and van der Kooy, 1985; Herbert et al., 1990; Johnson and Thunhorst, 1997, 2007). These signals may modulate the activity of LPBN inhibitory mechanisms by releasing different neurotransmitters like serotonin, cholecystokinin (CCK), corticotrophin-releasing factor (CRF) and glutamate which are those that increase the inhibitory action, whereas others like GABA, opioids, ATP and noradrenaline (NOR) reduce the inhibitory action (De Gobbi et al., 2009; Gasparini et al., 2009; De Oliveira et al., 2011; Menezes et al., 2011; Menani et al., 2014). The deactivation of the inhibitory mechanisms by changing the activity of specific neurotransmitters/receptors in the LPBN increases hypertonic NaCl and/or water intake induced by different dipsogenic or natriorexigenic stimuli like angiotensin II (ANG II), sodium depletion, water deprivation, central cholinergic activation or even osmoreceptor activation (Menani and Johnson, 1995, 1998; Menani et al., 1996b, 2002; De Luca et al., 2003; Andrade et al., 2004, 2006; De Gobbi et al., 2009; Almeida et al., 2011).

Central cholinergic activation is a stimulus that usually induces thirst, but no significant hypertonic NaCl intake. Inhibitory signals for sodium and water intake arise from arterial baroreceptors or cardiopulmonary receptors that are activated by increases in arterial pressure or body fluid volume (Johnson and Thunhorst, 1997; De Gobbi et al., 2008). Therefore, a reason for the absence of hypertonic NaCl intake in rats treated with central cholinergic agonists might be the inhibitory signals produced in response to the increases in arterial pressure caused by these treatments (Trendelenburg, 1961; Hoffman et al., 1977; Imai et al., 1989; Thunhorst and Johnson, 1994; Johnson and Thunhorst, 1997; Takakura et al., 2003, 2005, 2011; Borella et al., 2008). Reducing the action of the inhibitory signals with bilateral injections of methysergide (serotonergic receptor antagonist) into the LPBN results in significant ingestion of hypertonic NaCl in rats

treated with the cholinergic agonist carbachol intracerebroventricularly (i.c.v.) (Menani et al., 2002). However, it is not clear which signals modulate the LPBN inhibitory mechanisms and particularly which neurotransmitters are released by one specific signal, like the signal produced by increases in arterial pressure.

Few studies in the literature have suggested that central cholinergic mechanisms may facilitate sodium intake (Menani et al., 2002; Asnar et al., 2013; Roncari et al., 2014) and all of them suggest that facilitatory signals produced by central cholinergic receptor activation are strongly inhibited by LPBN inhibitory mechanisms. As commented above, the signals that activate LPBN inhibitory mechanisms may arise from the activation of arterial baroreceptors by increases in arterial pressure. Injections of NOR into the LPBN increase arterial pressure and, in spite of this, strongly increase 1.8% NaCl in rats treated with the diuretic furosemide (FURO) combined with a low dose of the angiotensin converting enzyme (ACE) blocker captopril (CAP) injected subcutaneously (sc) (Gasparini et al., 2009, 2013), which suggests that this treatment in the LPBN might affect signals from cardiovascular receptors. Therefore, in the present study, we investigated water and 1.8% NaCl intake in rats treated with the cholinergic agonists carbachol i.c.v. or pilocarpine intraperitoneally (i.p.) combined with NOR injected into the LPBN and investigated if signals from cardiovascular receptors are blocked by NOR acting in the LPBN.

EXPERIMENTAL PROCEDURES

Animals

Male Holtzman rats weighing 280–320 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%). 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*. Experimental procedures were approved by Ethics Committee in Animal Use (CEUA) from the Dentistry School of Araraquara – UNESP. All efforts were made to minimize animal discomfort and the number of animals used.

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. Bregma and lambda were positioned 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 bellow the dura mater). The tips of the cannulas were positioned at a point 2 mm above the LPBN. Besides the LPBN cannulas, two groups of rats received also a stainless steel cannula implanted into the lateral ventricle (LV, coordinates: 0.3 mm caudal to bregma, 1.5 mm lateral to midline and 3.5 mm bellow 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 a sc 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.

Drugs

All drugs were purchased from Sigma Chemical Co, St. Louis, MO, USA. NOR (norepinephrine bitartrate, 80 n mol/0.2 μl) was injected into LPBN. Prazosin hydrochloride (1 mg/kg of body weight) and pilocapine hydrochloride (1 mg/kg of body weight) were injected i.p. Carbachol hydrochloride (4 nmol/1 μl) was injected into the LV.

Drugs were dissolved in isotonic saline (SAL). SAL was injected into LPBN or i.p. as control treatment.

The doses of the drugs used were based on previous studies that injected these drugs central or peripherally (Camargo et al., 1984, 2009; Menani et al., 1998, 2000; Andrade et al., 2004; Borella et al., 2008).

Central injections

The central injections were made using 5 μl Hamilton syringes connected by polyethylene tubing (PE-10) to injector cannulas (0.3 mm o.d.). The injector cannulas were 2 mm longer than the guide cannulas. The volume of injection into the LPBN was 0.2 μl in each site and into the LV was 1 μl.

Water and 1.8% NaCl intake tests

Rats were tested in their home cage. For the tests, water and 1.8% NaCl were provided from burettes with 0.1 ml divisions that were fitted with metal drinking spouts. Water and 1.8% NaCl intake measurements started immediately after the injections of pilocarpine i.p. or carbachol i.c.v. and the cumulative intake was recorded for 3 h.

A recovery period of at least 2 days was allowed between tests. During the tests rats had no access to food.

Arterial pressure and heart rate (HR) recordings

Mean arterial pressure (MAP) and HR were recorded in unanesthetized rats. Under ketamine (80 mg/kg of body weight i.p.) and xylazine (7 mg/kg of body weight i.p.) anesthesia, 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. 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, MAP and HR, the arterial catheter was connected to a Stathan Gold (P23 Db) pressure transducer connected to an ETH-200 amplifier (CB Sciences) and to a PowerLab data acquisition system (ADInstruments).