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LATERAL PARABRACHIAL NUCLEUS AND CENTRAL AMYGDALA IN THE CONTROL OF SODIUM INTAKE

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Abstract—The lateral parabrachial nucleus (LPBN) and the central nucleus of the amygdala (CeA) are important areas for the control of sodium appetite. In the present study we investigated the effects of bilateral lesions of the CeA on the facilitation of water and 0.3 M NaCl intake produced by the blockade of serotonergic mechanisms or activation of α_2-adrenoceptors with bilateral injections of methysergide or moxonidine, respectively, into the LPBN. Male Holtzman rats (n=5–8) with bilateral sham or electrolytic lesions of the CeA (2 mA; 10 s) and stainless steel cannulas implanted bilaterally in the LPBN were used. In sham rats treated with the diuretic furosemide (10 mg/kg b.w.) combined with the angiotensin converting enzyme inhibitor captopril (5 mg/kg b.w) subcutaneously, bilateral injections of moxonidine (0.5 nmol) or methysergide (4 μg) into the LPBN increased 0.3 M NaCl intake (29.8±5.1 and 19.5±3.7 ml/2 h, respectively, versus vehicle: 8.3±1.4 ml/2 h) and water intake (17.9±3.7 and 23.3±2.8 ml/2 h, respectively, versus vehicle: 11.5±1.6 ml/2 h). Lesions of the CeA (5–18 days) abolished the increase in 0.3 M NaCl and water intake produced by bilateral injections of moxonidine (10.3±2.8 and 6.8±2.3 ml/2 h, respectively) and reduced the increase produced by methysergide (13.6±2.5 and 14.5±3.2 ml/2 h, respectively) into the LPBN. The present results show that the increase in water and 0.3 M NaCl intake produced by serotonergic blockade and α_2-adrenergic activation in the LPBN depends on the integrity of the CeA, suggesting that facilitatory mechanisms present in the CeA are essential for the increase of water and hypertonic NaCl intake produced by the blockade of the inhibitory mechanisms of the LPBN.

Key words: sodium appetite, parabrachial nucleus, amygdala, thirst, angiotensin II, serotonin.

Important inhibitory mechanisms for the control of water and NaCl intake have been demonstrated in the lateral parabrachial nucleus (LPBN), a pontine structure that lies dorsolaterally to the superior cerebellar peduncle (Edwards and Johnson, 1991; Menani and Johnson, 1995; Colombari et al., 1996; Menani et al., 1996, 1998a,b, 2000). Electrolytic or neurotoxic lesions (with ibotenic acid injections) in the LPBN increase water intake induced by central or peripheral administration of angiotensin II (ANG II) or the β-adrenergic agonist isoproterenol in rats (Ohman and Johnson, 1986, 1989; Johnson and Edwards, 1990; Edwards and Johnson, 1991). Bilateral injections of methysergide, a serotonergic receptors antagonist, into the LPBN strongly increase 0.3 M NaCl intake induced by ANG II administered i.c.v. or into the subfornical organ (SFO) or by the combined treatment with the diuretic furosemide (FURO) and the angiotensin converting enzyme inhibitor captopril (CAP), both injected s.c. (Menani et al., 1996; Colombari et al., 1996). In addition, injections of DOI (5-$HT_{2A/2C}$ receptors agonist) into the LPBN reduce NaCl intake induced by FURO+CAP treatment (Menani et al., 1996). Similar to methysergide, moxonidine (α_2-adrenoceptor and imidazoline receptor agonist) injected bilaterally into the LPBN also strongly increases FURO+CAP-induced sodium intake, an effect completely abolished by RX 821002 (an α_2-adrenoceptor antagonist) injected into the LPBN, suggesting that moxonidine increases hypertonic NaCl intake by acting on α_2-adrenoceptors (Andrade et al., 2004).

The amygdala is part of the basic cerebral circuit involved in the control of sodium and water intake (Covian et al., 1975). The amygdala (or amygdaloid complex), located on tip of temporal lobe, has reciprocal connections with hindbrain areas such as nucleus of the solitary tract (NTS) and the LPBN that receive taste and visceral signals (Norgren, 1995). Bilateral electrolytic lesions of the central nucleus of amygdala (CeA) abolish daily ingestion of 0.5 M NaCl and sodium appetite induced by the mineralocorticoid deoxycorticosterone (DOCA) or the α_2-adrenoceptor antagonist yohimbine s.c., i.c.v. injections of renin or by 24 h of sodium depletion in rats treated with furosemide (Galaverna et al., 1992; Zardetto-Smith et al., 1994). However, water intake induced by s.c. ANG II or by cellular dehydration is not affected by lesions in the CeA, reinforcing the concept that lesions of the CeA reduce specifically sodium appetite (Zardetto-Smith et al., 1994).

The LPBN is reciprocally connected to forebrain areas such as the paraventricular nucleus of the hypothalamus, central nucleus of amygdala and median preoptic nucleus, and to medullary regions, like the area postrema (AP) and medial portion of the nucleus of the solitary tract (mNTS), (Norgren, 1981; Ciriello et al., 1984; Fulwiler and Saper, 1984; Lança and van der Kooy, 1985; Herbert et al., 1990; Krukoff et al., 1993; Jhamandas et al., 1992, 1996). Therefore, the LPBN may convey signals that ascend from AP/

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Abbreviations: Ang II, angiotensin II; AP, area postrema; AV3V, anteroventral third ventricle region; b.w., body weight; CAP, captopril; CeA, central nucleus of amygdala; DOCA, deoxycorticosterone; FURO, furosemide; LPBN, lateral parabrachial nucleus; METHY, methysergide; MnPO, median preoptic nucleus; mNTS, medial nucleus of the solitary tract; MOXO, moxonidine; OVLT, organum vasculosum lamina terminalis; PVN, paraventricular nucleus of the hypothalamus; sal, saline; SFO, subfornical organ; SON, supraoptic nucleus; veh, vehicle.

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mNTS to forebrain areas involved in the control of fluid and electrolyte balance. Retrograde and anterograde neural marker and immunocytochemistry techniques have demonstrated the existence of GABAergic monosynaptic connections between the CeA and the LPBN (Jia et al., 2005). Connections among amygdala, LPBN and NTS are suggested to be part of a neural circuitry related to the modulation of salt intake that includes aldosterone-sensitive neurons (HSD2 neurons) that co-express the mineralocorticoid receptor and the enzyme 11 β-hydroxysteroid dehydrogenase type 2 (HSD2). The HSD2 neurons are activated by sodium depletion and deactivated by the ingestion of sodium (Geerling and Loewy, 2006, 2007).

Therefore, considering the importance of the LPBN and CeA for the control of sodium intake and the anatomical connections between the two structures, in the present study we investigated the effects of bilateral lesions of the CeA on the facilitation of water and 0.3 M NaCl intake produced by the blockade of serotonergic mechanisms or activation of α_2-adrenoceptors in the LPBN.

EXPERIMENTAL PROCEDURES

Animals

Male Holtzman rats weighing 250–270 g at the beginning of the tests were used. The animals were housed in individual stainless steel cages with free access to normal sodium diet (Guabi Rat Chow, Paulinia, SP, Brazil), water and 0.3 M NaCl solution. Rats were maintained on room temperature of 23±2 °C, humidity of 55±10% and on a 12-h light/dark cycle with light onset at 7:00 AM. All the experimental procedures were approved by Ethical Committee in Animal Experimentation (CEEA) from Dentistry School of Araraquara—UNESP (Proc. CEEA nr. 07/2007). The experimental protocols followed the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23, 1996). Efforts were made to reduce animal discomfort and the number of animals used.

Surgery

Rats were anesthetized with ketamine (80 mg/kg of body weight; Cristalia, Itapira, SP, Brazil) combined with xylazine (7 mg/kg of body weight; Agener União, Embu-Guaçu, SP, Brazil), placed in a stereotaxic instrument (Kopf, Tujunga, CA, USA) and had the skull leveled between bregma and lambda.

For bilateral lesions of the CeA, a stainless steel electrode (0.5 mm of diameter) insulated, except 0.5 mm at the tip, was inserted into the brain 2.1, 2.4 and 2.6 mm caudal to bregma, 3.8 mm from the midline, and 6.3 mm below the dura mater (Paxinos and Watson, 1997). Electrolytic lesions were performed bilaterally by passing a cathodal current (2 mA for 10 s each point) in the three stereotaxic points cited above for each side. A clip attached to the tail was used as the indifferent electrode. Sham-lesioned rats were submitted to the same surgical procedures and had the electrode placed along the same coordinates, except that no current was passed.

Immediately after sham or electrolytic lesions, bilateral stainless steel cannulas (0.6 mm o.d.) were implanted in direction to the LPBN using the following coordinates: 9.2 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 2 mm above each LPBN. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. A metal obturator (0.3 mm o.d.) filled the cannulas between tests. At the end of the surgery, the animals received an i.m. injection of penicillin (Pentabiótico Veterinário—Pequeno Porte, Fort Dodge Saúde Animal Ltda., 0.2 ml/rat) and a s.c. injection of the analgesic Ketoflex (ketoprofen 1%, 0.03 ml/rat, Mundo Animal, São Paulo, SP, Brazil).

Injections into the LPBN

Injections into the LPBN were made using 5 μl Hamilton syringes (Hamilton, Reno, NV, USA) connected by polyethylene tubing (PE-10) to injection cannulas (0.3 mm o.d.). Starting one day after cerebral surgery, rats were handled daily and trained for the procedure of central injections. At time of testing, rats were removed from the cages and restrained by a hand on a table, obturators were removed and the injection cannulas (2 mm longer than the guide cannulas) were introduced in the brain. The injection volume into the LPBN was 0.2 μl each site. The obturators were replaced after injections, and the rats were placed back into the cage.

Drugs

Moxonidine hydrochloride, α_2-adrenoceptor/imidazoline agonist, (donation from Solvay Pharma, Hannover, Germany) was dissolved in a mix of propylene glycol and water 2:1 (vehicle) and administered into the LPBN at the dose of 0.5 nmol/0.2 μl. Methysergide maleate, a non-selective serotonergic receptor antagonist, (Research Biochemicals International, Natick, MA, USA) was also dissolved in the same vehicle and administered into the LPBN at the dose of 4 μg/0.2 μl. Furosemide (Sigma Chem., St. Louis, MO, USA) was dissolved in alkaline saline (pH adjusted to 9.0 with NaOH) and administered s.c. at 10 mg/kg of body weight. Captopril (Sigma Chem., St. Louis, MO, USA), angiotensin converting enzyme inhibitor, was dissolved in saline and administered s.c. at 5 mg/kg of body weight.

Daily ingestion of water and 0.3 M NaCl and body weight

Water and 0.3 M NaCl intake of each animal was recorded daily using 100 ml capacity-polypropylene bottles with 1 ml-divisions. Recordings started 5 days before sham or electrolytic lesions of the CeA and continued until the end of tests (18 days). On the day of induced water and sodium intake tests, the recording of daily intake started immediately after the end of the test.

Body weight was measured 5 days before the cerebral surgery, on the day of the surgery and 5, 8, 15 and 18 days after the surgery.

Water and 0.3 M NaCl intake induced by injections of FURO+CAP

Satiated sham and CeA-lesioned rats received FURO (10 mg/kg of body weight)+CAP (5 mg/kg of body weight) s.c. and were returned to their home cages in the absence of food, water and 0.3 M NaCl solution. Forty five min after FURO+CAP, rats received bilateral injections into the LPBN and 15 min later had access to water and 0.3 M NaCl. Cumulative water and 0.3 M NaCl intake was measured at 15, 30, 60, 90, and 120 min starting 1 h after FURO+CAP treatment.

Five days after sham or CeA lesions, FURO+CAP-induced water and 0.3 M NaCl intake was tested in rats treated with bilateral injections of moxonidine (0.5 nmol/0.2 μl) or vehicle into the LPBN. For the test half of sham and half of CeA-lesioned rats received moxonidine into the LPBN and the other half received vehicle into the LPBN. The same treatment was repeated in the same rats in a counterbalanced design on the 8th day of lesions.

On the 15th and 18th day of sham or CeA lesions, FURO+CAP-induced water and 0.3 M NaCl intake was tested again in the same rats using the same protocol described above, except that methysergide (4 μg/0.2 μl) instead of moxonidine was injected into the LPBN.