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$GABA_A$ RECEPTOR ACTIVATION IN THE LATERAL PARABRACHIAL NUCLEUS INDUCES WATER AND HYPERTONIC NaCl INTAKE

J. C. CALLERA,[a] L. B. OLIVEIRA,[b] S. P. BARBOSA,[b] D. S. A. COLOMBARI,[b] L. A. DE LUCA JR.[b] AND J. V. MENANI[b]*

[a]*Department of Basic Science, School of Dentistry, Paulista State University, UNESP, Araçatuba, SP, Brazil*

[b]*Department of Physiology and Pathology, School of Dentistry, UNESP, Rua Humaitá 1680, 14801-903, Araraquara, SP, Brazil*

Abstract—Inhibitory serotonergic and cholecystokinergic mechanisms in the lateral parabrachial nucleus and central GABAergic mechanisms are involved in the regulation of water and NaCl intake. In the present study we investigated if the $GABA_A$ receptors in the lateral parabrachial nucleus are involved in the control of water, NaCl and food intake in rats. Male Holtzman rats with stainless steel cannulas implanted bilaterally into the lateral parabrachial nucleus were used. Bilateral injections of muscimol (0.2 nmol/0.2 μl) into the lateral parabrachial nucleus strongly increased 0.3 M NaCl (20.3±7.2 vs. saline: 2.6±0.9 ml/180 min) without changing water intake induced by the treatment with the diuretic furosemide combined with low dose of the angiotensin converting enzyme inhibitor captopril s.c. In euhydrated and satiated rats, bilateral lateral parabrachial nucleus injections of muscimol (0.2 and 0.5 nmol/0.2 μl) induced 0.3 M NaCl intake (12.1±6.5 and 32.5±7.3 ml/180 min, respectively, vs. saline: 0.4±0.2 ml/180 min) and water intake (5.2±2.0 and 7.6±2.8 ml/180 min, respectively, vs. saline: 0.8±0.4 ml/180 min), but no food intake (2±0.4 g/240 min vs. saline: 1±0.3 g/240 min). Bilateral lateral parabrachial nucleus injections of the $GABA_A$ antagonist bicuculline (1.6 nmol/0.2 μl) abolished the effects of muscimol (0.5 nmol/0.2 μl) on 0.3 M NaCl and water intake. Muscimol (0.5 nmol/0.2 μl) into the lateral parabrachial nucleus also induced a slight ingestion of water (4.2±1.6 ml/240 min vs. saline: 1.1±0.3 ml/240 min) when only water was available, a long lasting (for at least 2 h) increase on mean arterial pressure (14±4 mm Hg, vs. saline: −1±1 mm Hg) and only a tendency to increase urinary volume and Na^+ and K^+ renal excretion. Therefore the activation of $GABA_A$ receptors in the lateral parabrachial nucleus induces strong NaCl intake, a small ingestion of water and pressor responses, without changes on food intake. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: muscimol, sodium appetite, thirst, blood pressure, satiety.

The lateral parabrachial nucleus (LPBN), a pontine structure localized dorsal to the superior cerebellar peduncle (SCP) is an important area involved in the control of water and sodium intake (Ohman and Johnson, 1986, 1995; Menani and Johnson, 1995; Colombari et al., 1996; Menani et al., 1996). The LPBN receives afferent projections from the area postrema (AP) and the medial nucleus of the solitary tract (mNTS) (Norgren, 1981; Herbert et al., 1990) and sends efferent projections to forebrain areas involved in fluid and electrolyte balance such as the paraventricular nucleus of the hypothalamus, central nucleus of amygdala, and median preoptic nucleus (Ciriello et al., 1984; Fulwiler and Saper, 1984; Jhamandas et al., 1992; Krukoff et al., 1993; Jhamandas et al., 1996).

Bilateral injections of methysergide, a serotonergic receptor antagonist, into the LPBN increase hypertonic NaCl (0.3 or 0.5 M) intake induced by sodium depletion (treatment with the diuretic furosemide+24 h of sodium deficient diet) or induced by the combined treatment with furosemide (FURO) and low dose of the angiotensin converting enzyme inhibitor captopril (CAP) s.c., (Menani et al., 1996, 1998a). Methysergide into the LPBN also increases water intake induced by angiotensin II (ANG II) injected i.c.v. and 0.3 M NaCl intake induced by ANG II injected i.c.v. or into the subfornical organ, s.c. isoproterenol, acute s.c. FURO and s.c. deoxycorticosterone acetate (DOCA) treatment (Menani and Johnson, 1995; Colombari et al., 1996; Menani et al., 1996, 1998b, 2000; De Gobbi et al., 2001). Bilateral injections of proglumide, a cholecystokinin antagonist, into the LPBN also increase ANG II- and FURO+CAP-induced 0.3 M NaCl intake (Menani and Johnson, 1998; De Gobbi et al., 2001). However, methysergide or proglumide injected bilaterally into the LPBN in euhydrated and satiated rats not submitted to any other dipsogenic/natriorexigenic treatment produces no intake of water or NaCl (Menani et al., 1996; De Gobbi et al., 2000).

GABA is an inhibitory neurotransmitter widely distributed in the CNS (Christie and North, 1988; Meeley et al., 1989; Araki et al., 1992). The effects of GABA depend on its action on two pharmacologically distinct receptor subtypes, $GABA_A$ and $GABA_B$ receptors (Bowery et al., 1987; Bowery, 1989). A dense plexus of GABA-immunoreactive varicosities exists in the parabrachial nucleus, and there is a suggestion that neural processing in the parabrachial nucleus is under a strong GABAergic inhibition mediated by different types of GABA receptors in functionally different pathways (Guthmann et al., 1998). GABA, mu-opioid and M_2-muscarinic receptors are present in the rat LPBN neurons and the activation of any of these receptors increases the potassium conductance of the membrane and inhibits the neurons through hyperpolarization (Christie and North, 1988). One study using whole cell recordings in

*Corresponding author. Tel: +55-16-3301-6486; fax: +55-16-3301-6488.
E-mail address: menani@foar.unesp.br (J. V. Menani).

Abbreviations: ANG II, angiotensin II; AP, area postrema; bpm, beats per minute; CAP, captopril; FURO, furosemide; HR, heart rate; LPBN, lateral parabrachial nucleus; MAP, mean arterial pressure; MPBN, medial parabrachial nucleus; NaCl, sodium chloride.

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brain slices of the rat also showed that the majority (62%) of LPBN neurons tested responds to GABA superfusion and this response was partially or completely blocked by the $GABA_A$ antagonist bicuculline (Kobashi and Bradley, 1998).

Peripheral injections of the $GABA_A$ or $GABA_B$ receptor agonist muscimol and baclofen, respectively, reduce water deprivation-induced water intake in rats (Ebenezer et al., 1992; Houston et al., 2002). Muscimol i.c.v. also drastically reduces ANG II-induced water intake and renin-induced water and NaCl intake (Unger et al., 1983; Abe et al., 1988). Muscimol injected into the medial preoptic nucleus also attenuates central ANG II- and intracellular dehydration-induced water intake, while the blockade of $GABA_A$ receptors with bicuculline increases ANG II-induced water intake (Tanaka et al., 2003). On the contrary, muscimol into the median and dorsal raphe nuclei and ventral tegmental area elicits food intake and nonprandial drinking in nondeprived rats (Klitenick and Wirtshafter, 1988, 1989). Muscimol or baclofen injected into the nucleus accumbens also elicits an intense dose-related feeding in satiated rats without altering water intake (Stratford and Kelley, 1997).

Considering the involvement of central GABAergic mechanisms in the regulation of ingestive behavior, the existence of inhibitory mechanisms in the LPBN to control sodium intake, and the evidence that GABA is an important neurotransmitter in the LPBN, in the present study we investigated the effects of the activation of $GABA_A$ receptors in the LPBN on water and 0.3 M NaCl induced by FURO+CAP s.c. or in satiated and euhydrated rats. In addition, the effects of the activation of $GABA_A$ receptors in the LPBN on food intake, arterial pressure and renal excretion were also tested.

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 (Purina Rat Chow, Paulinia, SP, Brazil), water and 0.3 M NaCl solution. Room temperature was maintained at 23±2 °C, and humidity at 55±10% on a 12-h light/dark cycle with light onset at 07:30 AM.

Cerebral cannulas

Rats were anesthetized with s.c. ketamine (80 mg/kg of body weight) combined with xylazine (7 mg/kg of body weight) and placed in a Kopf stereotaxic instrument. The skull was leveled between bregma and lambda. Stainless steel 23-gauge cannulas were implanted bilaterally into the LPBN using the following coordinates: 9.5 mm caudal to bregma, 2.2 mm lateral to the midline, and 3.9 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 watch screws. A 30-gauge metal obturators filled the cannulas between tests. After the surgery, the rats were allowed to recover for 5 days before starting ingestion tests. The procedures followed the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996, USA) and were approved by the Animal Experimentation Ethics Committee of the School of Dentistry, UNESP, Araraquara, SP, Brazil. All efforts were made to minimize animal discomfort and the number of animals used.

Injections into the LPBN

Bilateral injections into the LPBN were made using 5-μl Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At the time of testing, obturators were removed and the injection cannula (2 mm longer than the guide cannula) was carefully inserted into the guide cannula, and manual injection was initiated 15 s later. For bilateral injections, the first injection was initially performed in one side, the needle was withdrawn and repositioned in the contralateral side, and then the second injection was made. Therefore injections were made ~1 min apart. The injection volume into the LPBN was 0.2 μl in each site. The obturators were replaced after the injections, and the rats were placed back into their cages.

Drugs

FURO (Sigma Chem., St. Louis, MO, USA) was administered s.c. at 10 mg/kg of body weight. CAP (Sigma Chem.) was also administered s.c. at 5 mg/kg of body weight. The drugs injected into the LPBN were muscimol HBr purchased from Research Biochemicals Internationals (RBI, Natick, MA, USA) and bicuculline purchased from Tocris (Ellisville, MO, USA). Muscimol HBr was dissolved in saline and bicuculline was dissolved in a mix of propylene glycol/water 2:1 (vehicle).

Water, 0.3 M NaCl and food intake

The rats were tested in their home cages. Water and 0.3 M NaCl were provided from burettes with 0.1-ml divisions that were fitted with metal drinking spouts. During the tests, a pre-weighed amount of regular chow pellets was available only when food intake was investigated. All the chow spillage under the cages was recovered at every measurement to calculate food intake.

In one group of rats water and 0.3 M NaCl intake were induced by the treatment with s.c. FURO (10 mg/kg of body weight)+CAP (5 mg/kg of body wt) as described previously (Fitts and Masson, 1989; Menani et al., 1996). Rats received s.c. FURO+CAP treatment and were returned to their home cages in the absence of water and 0.3 M NaCl solution. One hour later, water and NaCl, but no food, were available for animals and cumulative water and 0.3 M NaCl intakes were measured at every 30 min during 180 min. The bilateral injections of muscimol (0.2 nmol/0.2 μl) or saline into the LPBN were performed 45 min after FURO+CAP treatment or 15 min before the rats had access to water and 0.3 M NaCl.

In three groups of euhydrated and satiated rats that received no pre-treatment, the effects of muscimol into the LPBN were tested in three different experimental conditions: only water available (one-bottle test); water and 0.3 M NaCl available (two-bottle test); water, 0.3 M NaCl and food pellets available (two-bottle+food test). Cumulative water, 0.3 M NaCl and food intakes were measured at every 30 min during 180 or 240 min, starting 15 min after bilateral injections of muscimol or saline into the LPBN. The group of rats that had only water available (one-bottle test) received bilateral injections of muscimol (0.5 nmol/0.2 μl) and saline into the LPBN. The group of rats that had water and 0.3 M NaCl, no food, simultaneously available (two-bottle test) received bilateral injections of saline, muscimol (0.2 nmol/0.2 μl) and muscimol (0.5 nmol/0.2 μl) into the LPBN. The group of rats that had water, 0.3 M NaCl and chow pellets simultaneously available (two-bottle+food test) received bilateral injections of muscimol (0.5 nmol/0.2 μl) and saline into the LPBN.

In two different groups of euhydrated and satiated rats that had water and 0.3 M NaCl, no food, simultaneously available (two-bottle test), the effects of the combination of bicuculline and muscimol