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BACLOFEN INTO THE LATERAL PARABRACHIAL NUCLEUS INDUCES HYPERTONIC SODIUM CHLORIDE AND SUCROSE INTAKE IN RATS

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Abstract—$GABA_A$ and $GABA_B$ receptors are present in the lateral parabrachial nucleus (LPBN), a pontine area involved with inhibitory mechanisms related to the control of sodium appetite. Activation of $GABA_A$ receptors in the LPBN induces strong ingestion of 0.3 M sodium chloride (NaCl) in normonatremic and euhydrated rats. In the present study, we investigated the effects of the $GABA_B$ receptor agonist baclofen, injected alone or combined with $GABA_A$ or $GABA_B$ receptor antagonists into the LPBN on 0.3 M NaCl, water, 0.06 M sucrose and food intake in normonatremic and euhydrated rats. Male Holtzman rats with stainless steel cannulas implanted bilaterally in the LPBN were used. In normonatremic and euhydrated rats, bilateral injections of baclofen (0.5 nmol/0.2 μl) into the LPBN induced 0.3 M NaCl (24.0±3.1 vs. saline: 2.0±0.8 ml/240 min) and water intake (10.6±1.4 vs. saline: 3.5±0.7 ml/240 min) in a two-bottle test. Injections of $GABA_B$ receptor antagonists CGP 35348 (50 nmol/0.2 μl) or 2-hydroxysaclofen (5 nmol/0.2 μl) or $GABA_A$ receptor antagonist bicuculline (1.6 nmol/0.2 μl) into the LPBN reduced 0.3 M NaCl (14.1±4.7 ml/240 min; 9.97±2.5 ml/210 min; 8.8±5.9 ml/240 min, respectively) and water intake induced by baclofen injected into the LPBN. Baclofen (0.5 nmol/0.2 μl) injected into the LPBN also induced 0.06 M sucrose intake (21.8±5.9 vs. saline: 5.0±2.6 ml/180 min). Urinary volume and sodium excretion had a tendency to decrease after baclofen injection into the LPBN, whereas arterial pressure and food intake were not affected. The results show that baclofen injected into the LPBN, in normonatremic and euhydrated rats, produces a natriorexigenic effect dependent on $GABA_A$ and $GABA_B$ receptor activation. The natriorexigenic effect is not secondary to alterations in blood pressure or sodium urinary excretion. In addition, baclofen injected into the LPBN also induces 0.06 M sucrose intake.

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Abbreviations: ANG II, angiotensin II; CCK, cholecystokinin; CRF, corticotropin-releasing hormone; HR, heart rate; K^+, potassium; LPBN, lateral parabrachial nucleus; MAP, mean arterial pressure; MPBN, medial parabrachial nucleus; NAc, nucleus accumbens; NaCl, sodium chloride; Na^+, sodium; PB, parabrachial nucleus; sc, subcutaneous.

Key words: sodium appetite, sucrose intake, water intake, GABA receptors, urinary excretion, blood pressure.

The parabrachial complex (PB) is an important pontine area involved in the control of ingestive behavior receiving visceral and gustatory signals (Rogers et al., 1979; Hermann and Rogers, 1985; Yamamoto et al., 1994; Baird et al., 2001). Main inhibitory mechanisms involved in the control of water and especially sodium intake are present in the lateral parabrachial nucleus (LPBN), the portion of the PB located dorsolaterally to the superior cerebellar peduncle (Ohman and Johnson, 1989; Menani and Johnson, 1995, 1998; Menani et al., 1996; Andrade et al., 2004; Callera et al., 2005; De Oliveira et al., 2007, 2008; De Gobbi et al., 2009).

The LPBN sends projections to forebrain areas involved in the control of fluid-electrolyte balance, like specific hypothalamic nuclei (ventromedial, dorsomedial, paraventricular and supra optic) and amygdala and receives projections from the area postrema and the medial portion of the nucleus of the tractus solitary (Ciriello et al., 1984; Shapiro and Miselis, 1985; Herbert et al., 1990; Krukoff et al., 1993; Jhamandas et al., 1996). Signals from arterial baroreceptors, cardiopulmonary volume receptors and other visceral receptors like taste receptors reach the nucleus of the tractus solitary before ascending to LPBN that in turn may send these signals to forebrain areas involved in the control of fluid-electrolyte balance (Johnson and Thunhorst, 1997).

Bilateral injections of the serotonergic antagonist methysergide into LPBN increased water and hypertonic NaCl intake induced by different treatments, such as central angiotensin II (ANG II) administration, 24 h of sodium depletion produced by the subcutaneous (sc) treatment with the diuretic/natriuretic furosemide combined with 24 h of sodium deficient diet, treatment with the combination of sc furosemide+low dose of captopril (angiotensin converting enzyme blocker), s.c. deoxycorticosterone (Menani et al., 1996, 1998; De Gobbi et al., 2000). Conversely, bilateral injections of the serotonergic agonist DOI (2,5-dimethoxy-4-iodoamphetamine) reduced sodium and water intake induced by furosemide+captopril. These results suggest that an inhibitory serotonergic mechanism involved in the control of water and sodium intake is present in the LPBN. Besides serotonin (5-HT), other neurotransmitters in the LPBN like cholecystokinin (CCK), corticotropin-releasing hormone (CRF), glutamate, opioids, GABA and noradrenaline may modulate the inhibitory mechanisms in the LPBN affecting sodium and water intake (Menani et al., 1996; Menani and Johnson, 1998; Andrade

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et al., 2004; Callera et al., 2005; De Castro e Silva et al., 2006; De Oliveira et al., 2007, 2008; De Gobbi et al., 2009; Gasparini et al., 2009). Voluntary intake of sucrose induced c-fos protein expression in medial and lateral portion of PB and activation of 5-HT_{1B} receptors in the LPBN also reduces food intake, whereas administration of midazolam (benzodiazepine) into LPBN in satiated rats increased food and 0.09 M sucrose intake (Streefland et al., 1996; Higgs and Cooper, 1996; Lee et al., 1998; Simansky and Nicklous, 2002), suggesting that LPBN is also important in the control of food and sucrose intake.

A dense group of immunoreactive varicosities for GABA was described in PB complex/Kolliker fuse nucleus, suggesting that the neuronal process of this area is under an important GABAergic influence, particularly the gustatory and visceral portion of PB (Christie and North, 1988; Araki et al., 1992; Kobashi and Bradley, 1998). GABA is an inhibitory neurotransmitter widely spread in the central nervous system that binds to two subtypes of receptors: $GABA_A$ and $GABA_B$ receptors (Bowery et al., 1987; Araki et al., 1992). The post-synaptic effect of GABA is mediated mainly by $GABA_A$ receptors (bicuculline-sensitive) linked to chloride channels (Bormann, 1988). $GABA_B$ receptors are located mainly on pre-synaptic terminals and belong to G protein family causing membrane hyperpolarization and, therefore, promoting inhibition of neurotransmitter release due to the inhibition of calcium voltage sensitive channels or increased potassium conductance (Bormann, 1988; Zhang and Mifflin, 1998). Both $GABA_A$ and $GABA_B$ receptors are present in LPBN (Christie and North, 1988; Araki et al., 1992) and a previous study showed that muscimol ($GABA_A$ receptor agonist) injected into LPBN induces a strong ingestion of hypertonic NaCl in normonatremic and euhydrated rats (Callera et al., 2005).

Considering the importance of LPBN in the control of sodium and water intake, the existence of $GABA_A$ and $GABA_B$ receptors in the LPBN and the strong ingestion of 0.3 M NaCl intake produced by muscimol injected into the LPBN, in the present study we investigated the effects of the $GABA_B$ receptor agonist baclofen injected alone or combined with $GABA_A$ or $GABA_B$ receptor antagonists into the LPBN on 0.3 M NaCl and water intake in normonatremic and euhydrated rats. In addition, possible changes on food and sucrose intake and on cardiovascular and renal responses to baclofen injected into the LPBN were also investigated.

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, Paulinia, SP, Brazil), water and 0.3 M NaCl (and to 0.06 M sucrose solution when the ingestion of sucrose was tested). Room temperature was maintained at 23±2 °C, and humidity at 55±10% on a 12:12 light-dark cycle with light onset at 7:00 AM. All tests were performed from 8:00 AM to 1:00 PM. The Ethical Committee for Animal Care and Use from Dentistry School of Araraquara—UNESP approved the experimental protocols used in the present study. 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). All efforts were made to minimize animal discomfort and the number of animals used.

Cerebral cannulas

Rats were anesthetized with s.c. ketamine (80 mg/kg of body weight, Agener Uniao, Embu-Guacu, SP, Brazil) combined with xylazine (7 mg/kg of body weight, Agener Uniao, Embu-Guacu, SP, Brazil) and placed in a stereotaxic instrument (Kopf, Tujunga, CA, USA). The skull was leveled between bregma and lambda. Stainless steel 23-gauge cannulas were implanted bilaterally into the LPBN using the following coordinates: 9.4 mm caudal to bregma, 2.2 mm lateral to the midline, and 4.3 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. Stainless steel obturators (30-gauge) filled the cannulas, except during injections. After the surgery, the rats received i.m. injections of the analgesic cetoprophen 1% (0.03 ml/rat) and a prophylactic dose of the antibiotic penicillin (30,000 IU). Rats were allowed to recover for 5 days before starting the tests.

Injections into the LPBN

Bilateral injections into the LPBN were made using 5-μl Hamilton syringe connected by polyethylene tubing (PE-10) to 30-gauge injection cannula (2 mm longer than the guide cannula) that was carefully inserted into the guide cannula 15 s before starting manual injection. For bilateral injections, the first injection was initially performed in one side, the injection cannula was removed and repositioned in the contra-lateral 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. After the injections, rats were placed back into their cage.

Drugs

The drugs injected into the LPBN were baclofen and 2-hydroxysaclofen purchased from RBI-Sigma Chemicals (St Louis, MO, USA); p-(3-aminopropyl)-p-diethoxymethyl-phosphonic acid (CGP 35348) and bicuculline purchased from Tocris (Ellisville, MO, USA). Baclofen, 2-hydroxysaclofen and CGP 35348 were dissolved in saline and bicuculline was dissolved in a mix of propylene glycol/water 2:1 (vehicle).

Saline or vehicle (propylene glycol/water) was injected in the LPBN as control.

Water and 0.3 M NaCl intake by satiated, normonatremic and euhydrated rats

After the cerebral surgery, the rats had free access to water, 0.3 M NaCl and food for at least 5 days (period of recovery). 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. Food was not available for the rats during the tests. Cumulative intake of 0.3 M NaCl and water was measured at every 30 min during 210 or 240 min, starting 15 min after bilateral injections of baclofen (0.5 nmol/0.2 μl) or saline (0.2 μl) into the LPBN. A recovery period of at least 3 days was allowed between tests.

In one group of normonatremic and euhydrated rats, only the effects of baclofen injected into the LPBN on water and 0.3 M NaCl intakes (two-bottle test) were tested. The rats were submitted to two tests. In each test, the group of rats was divided in two. In the first test half of the group received saline and the other half received baclofen (0.5 nmol/0.2 μl) into the LPBN. In the next test, the rats received the same treatments in a counterbalanced design.

In another group of normonatremic and euhydrated rats, the effects of the combination of CGP 35348 ($GABA_B$ receptor antag-