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Research report

Natriorexigenic effect of baclofen is reduced by AT_1 receptor blockade in the lateral parabrachial nucleus

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ABSTRACT

$GABA_A$ and $GABA_B$ receptors activation with agonists muscimol and baclofen, respectively in the lateral parabrachial nucleus (LPBN), induces water and hypertonic NaCl intake in rats. The purpose of this study was to examine the effects of previous injections of losartan (AT_1 angiotensin receptor antagonist) into the LPBN on 0.3 M NaCl and water intake induced by baclofen injected bilaterally in the same area in fluid replete rats and in rats treated with the diuretic furosemide combined with a low dose of the angiotensin-converting enzyme inhibitor captopril injected subcutaneously. Male Wistar rats with stainless steel cannulas implanted bilaterally into the LPBN were used. Bilateral injections of baclofen (0.5 nmol/0.2 μl, $n = 6$) into the LPBN in fluid replete rats induced 0.3 M NaCl intake (22.4 ± 6.5 vs. saline: 0.1 ± 0.1 ml/210 min) and water intake (14.2 ± 4.0 vs. saline: 0.6 ± 0.6 ml/210 min) and pre-treatment of the LPBN with losartan (50 μg/0.2 μl) reduced 0.3 M NaCl intake (7.4 ± 7.0 ml/210 min) and water intake (2.8 ± 2.4 ml/210 min) induced by baclofen. In rats treated with furosemide + captopril, pre-treatment with losartan into the LPBN attenuated the increase in 0.3 M NaCl intake (13.3 ± 3.2 vs. saline + baclofen: 24.3 ± 3.9 ml/180 min) and water intake (4.8 ± 2.1 vs. saline + baclofen: 19.5 ± 6.6 ml/180 min) produced by baclofen. We conclude that baclofen may produce a non-specific blockade of the inhibitory mechanisms of LPBN (deactivation of LPBN inhibitory mechanisms) and this blockade is facilitated by angiotensin II acting on AT_1 receptors in the LPBN, which drives rats to ingest large amounts of water and hypertonic NaCl independent if rats are fluid depleted or normohydrated.

1. Introduction

The lateral parabrachial nucleus (LPBN), a pontine structure that lies dorsal to the superior cerebellar peduncle (SCP) is an important area involved in the control of water and sodium intake [4,13,31]. The LPBN receives afferent projections from the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS), and it sends efferent projections to areas of the forebrain, such as the paraventricular nucleus of the hypothalamus (PVN), the central nucleus of the amygdala (CeA) or the median preoptic nucleus (MnPO) [21–23].

The cardiovascular, neuroendocrine and ingestive effects of ANG II acting centrally are mediated mainly by angiotensin type 1 (AT_1) receptors located in different areas of the central nervous system, such as the LPBN, anterior hypothalamic area (AHA), amygdala and SFO [16,28,38].

It has been reported that ANG II acting on AT_1 receptors may modulate GABAergic synaptic transmission producing opposite effects, depending on whether pre- or post-synaptic AT_1 receptors are activated. Studies showed that ANG II acting on pre-synaptic AT_1 receptors reduces GABA release and decreases the amplitude of evoked GABAergic inhibitory post-synaptic currents (IPSCs) [26,27,40]. In contrast, the amplitude of muscimol-activated $GABA_A$ currents in the median preoptic nucleus was reduced by the treatment with losartan, the nonpeptide antagonist that selectively binds on AT_1 receptors, suggesting a post synaptic action of endogenous ANG II that facilitated the effect of the GABAergic input to the MnPO [20]. In addition, other studies showed that central administration of ANG II stimulates $GABA_B$ receptor expression and augments $GABA_B$ receptor-mediated responses in neuronal cultures from the nucleus tractus solitarii [42,43].

Abbreviations: ANG II, angiotensin II; AHA, anterior hypothalamic area; CRF, corticotropin-releasing hormone; CNS, central nervous system; FURO, furosemide; CAP, captopril; IPSCs, inhibitory post-synaptic currents; LPBN, lateral parabrachial nucleus; SCP, superior cerebellar peduncle; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus of the hypothalamus; CeA, central nucleus of the amygdala; MnPO, median preoptic nucleus.

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A dense plexus of GABA-immunoreactive varicosities exists in the parabrachial nucleus [19]. It was also already shown the presence of $GABA_A$ and $GABA_B$ receptors in the LPBN [3,5].

The blockade of LPBN neurons with bilateral injections of the $GABA_A$ and $GABA_B$ agonists muscimol and baclofen, respectively induces ingestion of hypertonic NaCl and water in fluid replete rats [4,13]. In addition, injections of muscimol into the LPBN increases FURO + CAP- and 24 h of sodium depletion-induced sodium intake, suggesting that a GABAergic mechanism present in LPBN is involved in the control of sodium intake [4,10,13].

A recent study [8], showed that the blockade of AT_1 receptor antagonist with bilateral injection of losartan into the LPBN reduced 0.3 M NaCl and water intake induced by $GABA_A$ receptor activation with muscimol injected into the same area, suggesting that the deactivation of LPBN inhibitory mechanisms by muscimol is facilitated by angiotensin II acting on AT_1 receptors in the LPBN.

Considering the effects of activation of $GABA_B$ receptors in the LPBN on hypertonic NaCl and water intake, the results of previous studies showing that ANG II augments $GABA_B$ receptor-mediated responses, AT_1 receptor activation may modulate the action of the GABAergic mechanisms and injection of losartan into the LPBN reduced sodium intake induced by muscimol injected into the same area in rats, in the present study we investigated the effects of injections of losartan into the LPBN on water and hypertonic NaCl intake induced by the activation of $GABA_B$ receptors by baclofen injections into the LPBN in fluid replete or FURO + CAP-treated rats.

2. Material and methods

2.1. Animals

Male Wistar 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 solution. The positions of the bottles containing water and 0.3 M NaCl were rotated daily to avoid place preference. Room temperature was maintained at 23 ± 2 °C and humidity was maintained at 55 ± 10% on a 12:12 light–dark cycle with light onset at 07:30 am.

The procedures were approved by the Institutional Ethical Committee for Animal Care from the School of Dentistry, UNESP, Araçatuba, Brazil (Proc. CEEA no. 986/2007) and followed the recommendations from the Brazilian College of Animal Experimentation (COBEA) and the American National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 80-23, 1996, USA).

All efforts were made to minimize animal discomfort and the number of animals used.

2.2. Cerebral cannulas

Rats were anesthetized with subcutaneous (sc) ketamine (80 mg/kg of body weight, Cristália, Brazil) combined with xylazine (7 mg/kg of body weight, Agener, Brazil) and placed in a stereotaxic instrument (Kopf, USA). The skull was leveled between bregma and lambda. Stainless steel guide-cannulas (12 mm × 0.6 mm o.d) were implanted bilaterally into the LPBN using the following coordinates: 9.2 mm caudal to bregma, 2.2 mm lateral to the midline, and 3.8 mm below the dura mater [36]. 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. 30-Gauge metal obturators filled the cannulas between tests. After the surgery, the rats received intramuscular injections of the analgesic cetoprophen 1% (0.03 ml) and a prophylactic dose of the antibiotic penicillin (30,000 IU). Rats were allowed to recover for 5 days before starting ingestion tests and during this period they had free access to standard sodium diet, water and 0.3 M NaCl solution.

2.3. 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, the injection cannula (2 mm longer than the guide cannula) was carefully inserted into the guide cannula. For bilateral injections, the first injection was performed on one side, the needle was removed and repositioned on the contra lateral side, and then the second injection made. Therefore injections were made ~1 min apart. The injection volume into the LPBN was 0.2 μl on each site. The obturators were replaced after the injections, and the rats were placed back into their cages.

2.4. Drugs

Furosemide (FURO) (Sigma–Aldrich, Saint Louis, MO, USA) was dissolved in alkaline saline (pH adjusted to 9.0) and administered sc at the dose of 10 mg/kg of body weight (bw). Captopril (CAP) (Sigma–Aldrich, Saint Louis, MO, USA), was dissolved in 0.15 M NaCl and administered sc at the dose of 5 mg/kg of bw.

Losartan potassium and (±)-Baclofen (Sigma–Aldrich, Saint Louis, MO, USA) were dissolved in 0.15 M NaCl. The dose of baclofen used in the present study was the same as that used in previous studies that investigated the effects of baclofen injected into the LPBN on water and 0.3 M NaCl intakes [12,13]. This dose of baclofen produces a long-lasting action (at least for 3 h) when injected into the LPBN [13]. The dose of losartan was based on previous studies that have tested the effects of central or LPBN injections of losartan on water and 0.3 M NaCl intake induced by ANG II or muscimol [8,18,34]. The dose of losartan used is effective for at least 2 h [8,34].

2.5. Water and 0.3 M NaCl intake by fluid replete rats

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 during the tests. Measurements were taken at 30-min intervals for 210 min, starting 10 min after bilateral injections of baclofen (0.5 nmol/0.2 μl) or saline (0.2 μl) into the LPBN.

Fluid replete rats that received no pre-treatment ($n = 14$), were tested for the effects of the combination of losartan and baclofen injections into the LPBN on water and 0.3 M NaCl intake. Losartan (50 μg/0.2 μl) was injected into the LPBN 10 min before baclofen (0.5 nmol/0.2 μl). These rats were submitted to four tests and received the following combinations of treatments into the LPBN: saline + saline, saline + baclofen, losartan + baclofen and losartan + saline. In each test, the group of rats was divided in two and half of the group received one of the combination of treatments listed above, while the remaining animals received another combination of treatments into the LPBN. The sequence of the treatments was randomized for each rat so that, at the end of testing, rats had received all four treatments. All tests began between 13:00 pm and 15:00 pm. A recovery period of at least 2 days was allowed between tests.

2.6. Water and 0.3 M NaCl intake by FURO + CAP-treated rats

Another group of rats ($n = 15$) was used to test water and 0.3 M NaCl intake induced by treatment with FURO + CAP sc. On the day of the experiment, food, water and 0.3 M NaCl were removed and the cages were rinsed with water. Rats received sc injections of the diuretic FURO (10 mg/kg bw) plus CAP (5 mg/kg bw) as described previously [8,30,39]. One hour after FURO + CAP treatment, burettes with water and 0.3 M NaCl solution were returned and measurements were taken at 30-min intervals for 210 min (sodium appetite test). Ten minutes before access to water and 0.3 M NaCl, rats received bilateral injections of baclofen (0.5 nmol/0.2 μl) or saline into the LPBN. Bilateral injections of losartan (50 μg/0.2 μl) or saline into the LPBN were performed 10 min before the injections of baclofen or saline into the LPBN. In each experimental session, the group of rats was divided in two and each half of the group received one of the four treatments in the LPBN: saline + saline, saline + baclofen, losartan + baclofen and losartan + saline. The sequence of the treatments was in a randomized order so that at the end of testing, rats had received all four treatments. A recovery period of at least 3 days was allowed between experimental sessions. All tests began between 13:00 pm and 15:00 pm.

The order of treatments was randomized because repeated FURO + CAP injections enhances stimulated and spontaneous NaCl intake [37].

2.7. Histology

At the end of the experiments, the animals received bilateral injections of 2% Evans blue dye solution (0.2 μl/injection site) into the LPBN. They were then deeply anesthetized with sodium thiopental (CRISTALIA, Itapira, SP, Brazil, 80 mg/kg of body weight) and perfused transcardially with saline followed by 10% formalin. The brains were removed, fixed in 10% formalin, frozen, cut in 60 μm sections, stained with Giemsa, and analyzed by light microscopy to confirm the specificity of the LPBN as the site of injections of baclofen that produce the effects on water and sodium intake.

2.8. Statistical analysis

The results are reported as means ± S.E.M. Water and 0.3 M NaCl intake were analyzed by two-way analysis of variance (ANOVA) with repeated measures for both factors (treatments and times), followed by Newman–Keuls post hoc test. Differences were considered significant at $P < 0.05$. The software used to analyze the data was SigmaStat for Windows, version 2.03 from SPSS Inc.