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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.

Table 1
Ingestion of water and 0.3 M NaCl by fluid replete rats or FURO + CAP-treated rats that received saline or losartan combined with saline or baclofen in sites outside the LPBN (misplaced injections).

Fluid replete rats ($n = 8$)	0.3 M NaCl intake (ml/3 h)	Water intake (ml/3 h)
Saline + saline	0.1 ± 0.1	0.2 ± 0.1
Saline + baclofen	2.6 ± 1.5	2.2 ± 1.4
Losartan + baclofen	2.3 ± 1.8	2.3 ± 1.5
Losartan + saline	0.5 ± 0.4	1.9 ± 1.6
FURO + CAP-treated rats ($n = 9$)	**0.3 M NaCl intake (ml/3 h)**	**Water intake (ml/3 h)**
Saline + saline	6.7 ± 4.9	10.5 ± 2.0
Saline + baclofen	3.6 ± 1.6	6.9 ± 3.5
Losartan + baclofen	6.2 ± 2.9	6.3 ± 3.3
Losartan + saline	5.5 ± 2.3	8.2 ± 1.6

Values are means ± S.E.M. n = Number of rats. Losartan (50 μg/0.2 μl); baclofen (0.5 nmol/0.2 μl).

3. Results

3.1. Histological analysis

Fig. 1 is a photomicrograph of a transverse section of the brainstem of one rat, representative of the groups tested, showing the typical bilateral injection sites in the LPBN. The injections were centered in the central lateral and dorsal lateral portions of the LPBN (see Ref. [17] for definitions of LPBN subnuclei). In some rats, LPBN injections reached the ventral lateral and external lateral portions, as well as the Kölliker–Fuse nucleus. The sites of injections were similar to those in previous studies that showed the effects of LPBN injections of methysergide, moxonidine, muscimol or baclofen on water and 0.3 M NaCl intake [2,8,13]. In some rats, injections spread to the brachium (superior cerebellar peduncle), or slightly ventral to this structure, reaching the dorsal portions of the medial parabrachial nucleus (MPBN) uni-or bilaterally. There was no difference in the effects if the injections were restricted to the LPBN or if they spread to the ventral structures described above.

Rats in which injections did not reach the LPBN (misplaced injections) were excluded from the analyses of data presented in Figs. 2 and 3 and the values of their intakes are presented in Table 1. Bilateral injections of baclofen or losartan alone or of baclofen combined with losartan in sites outside the LPBN did not affect water and 0.3 M NaCl intake in fluid replete rats or FURO + CAP-treated rats. ANOVA showed no significant differences between treatments for 0.3 M NaCl intake [$F(3,21) = 1.0$; $P > 0.05$] or water intake [$F(3,21) = 0.8$; $P > 0.05$] in fluid replete rats or between treatments for 0.3 M NaCl intake [$F(3,24) = 0.6$; $P > 0.05$] or water intake [$F(3,24) = 3.3$; $P > 0.05$] in FURO + CAP-treated rats that received injections in sites outside the LPBN.

Misplaced injections were ventral (MPBN), dorsal or rostral to the LPBN. Some rats had unilateral injections partially into the LPBN.

3.2. Effects of combined injections of losartan and baclofen into the LPBN on water and 0.3 M NaCl intake in fluid replete rats

ANOVA showed significant differences between treatments and time for 0.3 M NaCl intake [$F(18,90) = 4.1$; $P < 0.001$] and water intake [$F(18,90) = 5.1$; $P < 0.001$] in fluid replete rats that received injections of saline or losartan combined with injections of saline or baclofen into the LPBN (Fig. 2).

In two-bottle tests, bilateral injections of baclofen (0.5 nmol/0.2 μl at each site, $n = 6$) into the LPBN in fluid replete rats induced 0.3 M NaCl intake (22.4 ± 6.5 ml/210 min, vs. saline + saline: 0.1 ± 0.1 ml/210 min, Fig. 2A and B) and water intake (14.2 ± 4.0 ml/210 min, vs. saline + saline: 0.6 ± 0.6 ml/210 min, Fig. 2C and D). Previous injections of the AT_1 receptor antagonist losartan (50 μg/0.2 μl each site) into the LPBN reduced the effects of baclofen (0.5 nmol/0.2 μl) injected in the same area on 0.3 M NaCl intake (7.4 ± 7.0 ml/210 min, Fig. 2A and B) and water intake (2.8 ± 2.4 ml/210 min, Fig. 2C and D).

The ingestion of 0.3 M NaCl and water after bilateral injections of baclofen into the LPBN in replete rats was significantly different from those after saline injected into the LPBN (control) from 150 min to the end of the test (210 min) and the pre-treatment with losartan injected into the LPBN reduced the ingestion of water in the same period (Fig. 2C and D). Pre-treatment with losartan reduced the ingestion of 0.3 M NaCl induced by baclofen from 180 to 210 min of the test (Fig. 2A and B). Losartan injected alone into the LPBN did not affect water or 0.3 M NaCl intake.

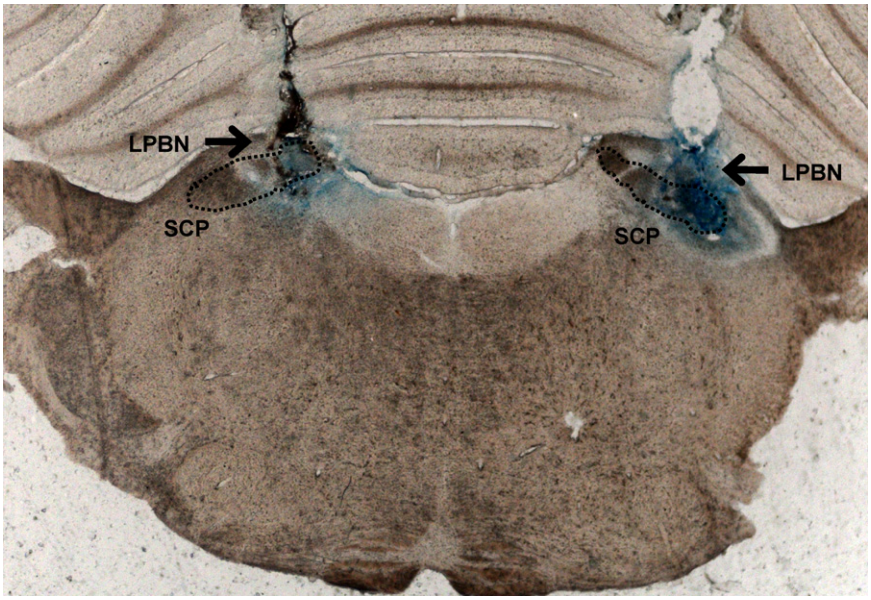

Fig. 1. Photomicrograph of a brain slice from one rat representative of the groups studied showing site of injection into the lateral parabrachial nucleus (LPBN, arrows). Dots outline the superior cerebellar peduncles (SCP).

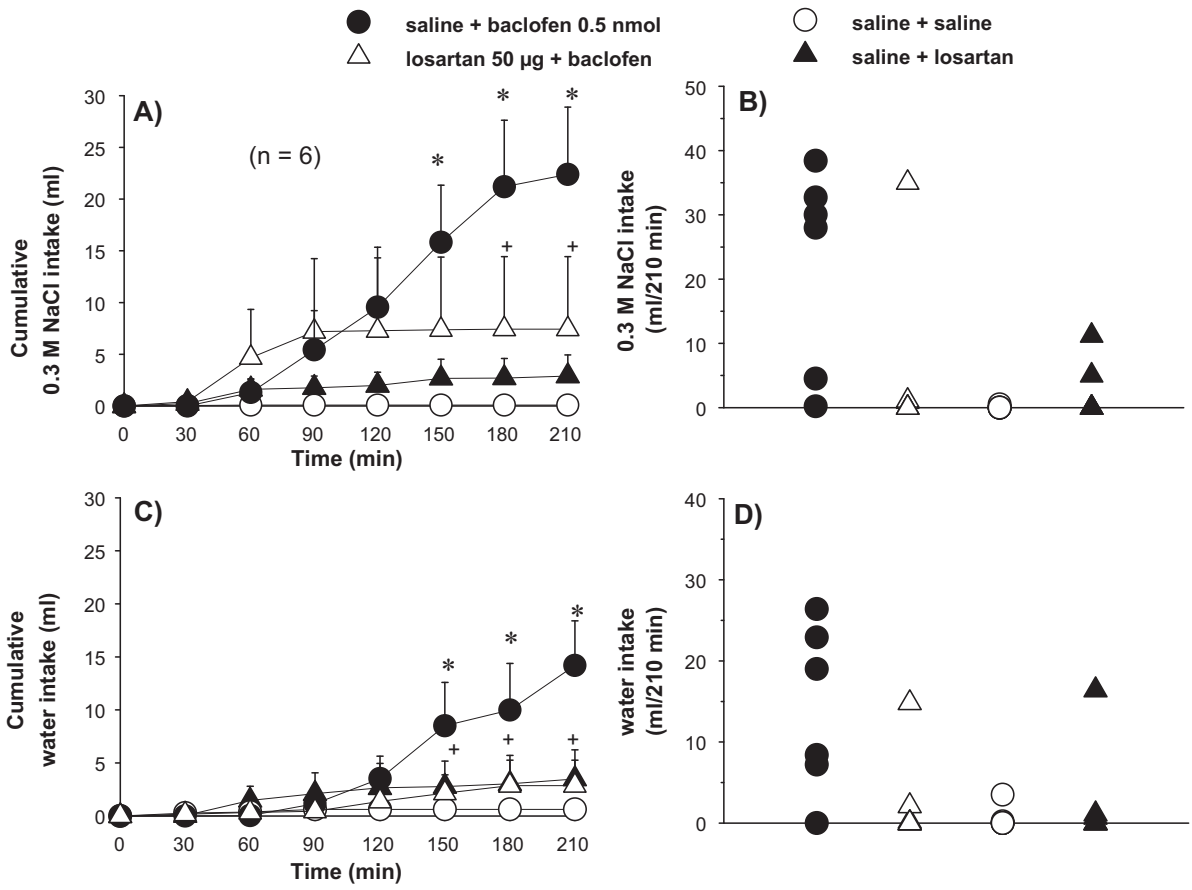

Fig. 2. (A) Cumulative 0.3 M NaCl intake; (B) individual 0.3 M NaCl intakes (210 min); (C) cumulative water intake; (D) individual water intakes (210 min) by fluid replete rats treated with losartan (50 µg/0.2 µl) or saline combined with baclofen (0.5 nmol/0.2 µl) or saline bilaterally into the LPBN. (A and C) Values are means ± S.E.M. *n* = Number of rats. *Different from saline + saline; +different from saline + baclofen 0.5 nmol.

3.3. Effects of combined injections of losartan and baclofen into the LPBN on FURO + CAP-induced 0.3 M NaCl and water intake

ANOVA showed significant interactions between treatments and times for 0.3 M NaCl intake [$F(18,90) = 7.5$; $P < 0.001$] and for water intake [$F(18,90) = 3.2$; $P < 0.001$] induced by FURO + CAP in rats that received injections of saline or losartan combined with injections of saline or baclofen into the LPBN (Fig. 3).

Bilateral injections of baclofen (0.5 nmol/0.2 µl at each site, $n = 6$) into the LPBN increased FURO + CAP-induced 0.3 M NaCl intake from 120 min to the end of the test (24.8 ± 3.7 ml/210 min, vs. saline + saline: 3.9 ± 2.3 ml/210 min) (Fig. 3A and B) and increased FURO + CAP-induced water intake from 180 min to the end of the test (21.1 ± 6.2 ml/210 min, vs. saline + saline: 10.9 ± 2.5 ml/210 min) (Fig. 3C and D). Losartan (50 µg/0.2 µl at each site) injected into the LPBN reduced the effects of baclofen on 0.3 M NaCl intake from 120 to 180 min of the test (13.3 ± 3.2 ml/180 min, Fig. 3A and B) and reduced the effects of baclofen on water intake from 150 min to the end of the test (5.1 ± 2.2 ml/210 min, Fig. 3C). Losartan injected alone into the LPBN produced no change in FURO + CAP-induced 0.3 M NaCl and water intake compared to the treatment with saline (Fig. 3A and C).

4. Discussion

Similar to previous study [13], the present study shows that bilateral injections of baclofen ($GABA_B$ receptor agonist) into the LPBN induce water and hypertonic NaCl ingestion in fluid replete rats. The present study also show that baclofen injected into the LPBN increase 0.3 M NaCl and water intake in FURO + CAP-treated rats. The involvement of $GABA_B$ receptors of the LPBN in the control of NaCl intake is supported by a previous study showing that the $GABA_B$ receptor antagonist (CGP 35348 or 2-hydroxysaclofen) injected into the LPBN was able to reduce the natriorexigenic effect of baclofen injected into the LPBN in fluid replete rats, which suggests that the activation of $GABA_B$ receptor is necessary for the natriorexigenic effect of baclofen [13]. The present results extend the conclusions of the previous study by showing that pretreatment of the LPBN with bilateral injections of the nonpeptide AT_1 receptor antagonist losartan reduce water and 0.3 M NaCl intake caused by baclofen injected into the same site in fluid replete rats, as well as the increase in water and 0.3 M NaCl produced by baclofen injected bilaterally into the LPBN in FURO + CAP-treated rats. Injections of losartan alone into the LPBN did not change water or 0.3 M NaCl intake by untreated rats or FURO + CAP-treated rats. Results from rats with misplaced injections confirm that baclofen effects on water and 0.3 M NaCl intake are specific to the LPBN. The results also suggest that angiotensinergic mechanisms in the LPBN are essential for the dipsogenic and natriorexigenic responses induced by the blockade of LPBN neurons with baclofen in fluid replete rats or FURO + CAP-treated rats.

Pretreatment with losartan into the LPBN reduced baclofen effects on water and NaCl intake by fluid replete or FURO + CAP-treated rats. Therefore, if endogenous GABA release in the LPBN was important for FURO + CAP-induced water and sodium intake, similar effects would be expected when losartan alone was injected in FURO + CAP-treated rats. However, injections of losartan alone did not modify FURO + CAP-induced water or NaCl intake, suggesting that GABA release or its interaction with activated AT_1 receptors

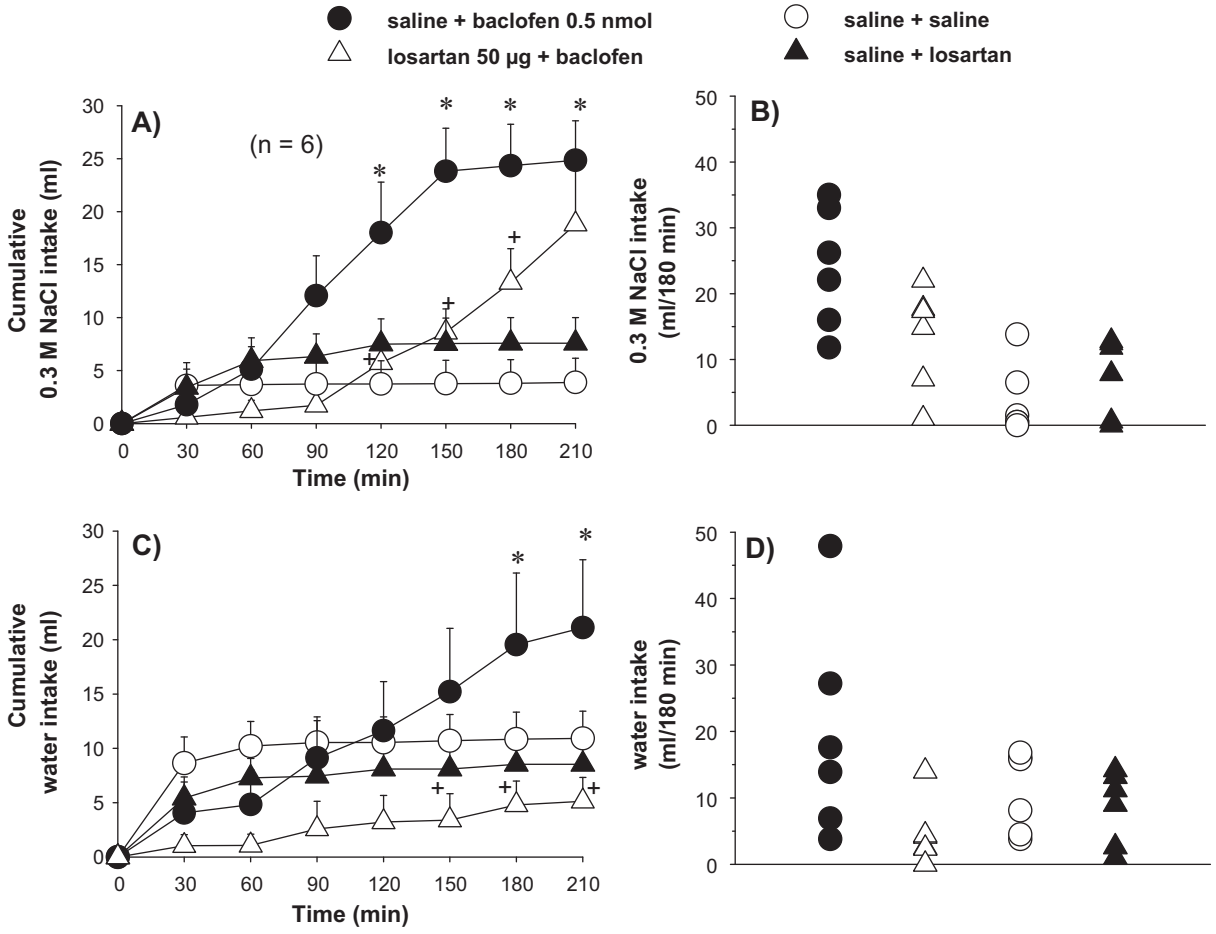

Fig. 3. (A) Cumulative 0.3 M NaCl intake; (B) individual 0.3 M NaCl intakes (180 min); (C) cumulative water intake; (D) individual water intakes (180 min) by FURO + CAP-treated rats that received losartan (50 μg/0.2 μl) or saline combined with baclofen (0.5 nmol/0.2 μl) or saline bilaterally into the LPBN. (A and C) Values are means ± S.E.M. *n* = number of rats. *Different from saline + saline. +Different from saline + baclofen 0.5 nmol.

in the LPBN is not essential for sodium or water intake induced by FURO + CAP. Perhaps any reduction of GABA effects by losartan was compensated for by changes in the release of other neurotransmitters in the LPBN like serotonin, CCK or opioids that also modulate water and sodium intake [9,11,30,31].

AT_1 receptors and ANG II terminals are present in the LPBN [25,29]. The present results suggest that ANG II acting on AT_1 receptors in the LPBN is necessary for the full effects of baclofen injected into the LPBN on water and NaCl intake. The treatment with FURO + CAP increases ANG II centrally [39]. However, it is possible that activation of AT_1 receptors by the baseline levels of ANG II in fluid replete rats is sufficient to facilitate the increase in water and sodium intake produced by baclofen in the LPBN. On the other hand, although there is no evidence that injections of baclofen into the LPBN increase ANG II levels, with the present results do not allow us to exclude the possibility of an increase in central or peripheral levels of ANG II due to baclofen injections into the LPBN. The ingestion of sodium after baclofen injections into the LPBN takes at least 2 h to start, which is a time enough for changes in the levels of ANG II that acting in the LPBN may intensify the effects of baclofen on LPBN neurons, a step necessary for the release of sodium intake.

The cardiovascular, neuroendocrine and ingestive effects of ANG II acting centrally are mediated mainly by AT_1 receptors [16,28,38]. For example, ingestion of water and NaCl is suggested to depend on the action of circulating ANG II on circumventricular organs like the SFO and OVLT [24,35]. At the same time, AT_1 receptors have a role in mediating an enhanced sodium intake produced by blockade of LPBN inhibitory mechanisms with injections of the serotonergic antagonist methysergide [7,32]. More specifically, injections of methysergide into the LPBN combined with treatments that increase ANG II centrally or peripherally, such as FURO + CAP sc, isoproterenol or acute (1 h previous) treatment with FURO, also produce robust ingestion of 0.3 M NaCl [31,33]. Whereas treatment with FURO + CAP alone induces significant ingestion of NaCl, sc treatments with isoproterenol or acute furosemide do not produce significant ingestion of NaCl, despite increases in ANG II signaling, unless LPBN inhibitory mechanisms are deactivated. Therefore, sodium intake does not always increase even with increased levels of ANG II. However, if the LPBN inhibitory mechanisms are deactivated, then ANG II-induced sodium and water intake is strongly facilitated.

In addition to methysergide, the blockade of other neurotransmitters in the LPBN like CCK, glutamate, or the activation of α_2 adrenoceptors with noradrenaline or moxonidine, deactivate LPBN inhibitory mechanisms and increase sodium and water intake induced by the treatment with FURO + CAP [1,9,30,31]. The blockade of these neurotransmitters or activation of α_2 adrenoceptors in the LPBN produces no sodium or water intake in fluid replete rats that might suggest that sodium intake easily arises only when facilitatory mechanism are activated and inhibitory mechanisms are simultaneously deactivated. However, in contrast to the blockade of the other neurotransmitters or α_2 adrenoceptor activation, either opioid (β endorphin) or GABAergic (muscimol and baclofen) activation of the LPBN induces robust ingestion of water and 0.3 M NaCl in fluid replete rats, suggesting that the deactivation of LPBN

inhibitory mechanisms alone is sufficient to disrupt satiety and to drive rats to ingest hypertonic NaCl [4,10,11,13]. The present results also show an increased sodium intake 2 h after baclofen into the LPBN in FURO + CAP-treated rats. The effects of baclofen into the LPBN on sodium intake are not secondary to decreases in blood pressure or an increase on sodium urinary excretion [13].

The ingestion of hypertonic NaCl solutions increases the activity of LPBN neurons, suggesting that the LPBN can be activated by taste and/or visceral stimuli [15,41]. Signals from volume, taste and other visceral receptors that may participate in the control of water and sodium intake reach the AP/mNTS before ascending to the LPBN that, in turn, sends projections to forebrain areas involved in the control of fluid and electrolyte balance, such as the SFO, MnPO, PVN and amygdala [6,22,35].

AT_1 receptors are present in different areas of the brain, including the LPBN [14,28]. Acting on AT_1 receptors, ANG II may modulate GABAergic neurotransmission producing opposite effects if pre- or post-synaptic AT_1 receptors are activated. It has been suggested that ANG II acting on pre-synaptic AT_1 receptors reduces GABA release and decreases the amplitude of evoked GABAergic IPSCs [26,27,40]. In contrast, it was shown that ANG II acting on post-synaptic AT_1 receptors increases IPSCs in sodium-sensitive neurons in the MnPO [20]. In addition, in a recent study [8] showed that the blockade of AT_1 receptors with injection of losartan into the LPBN reduced 0.3 M NaCl and water intake induced by muscimol injected into the same area, suggesting that the deactivation of LPBN inhibitory mechanisms by $GABA_A$ receptor activation is facilitated by angiotensin II acting on AT_1 receptors in the LPBN.

Similar to previous study [8], the present results show that the blockade of AT_1 receptors by the injection of losartan into the LPBN also reduces hypertonic NaCl and water intake stimulated by the activation of LPBN $GABA_B$ receptors with baclofen injected in the same area in fluid replete or in FURO + CAP-treated rats. We suppose that baclofen may produce a non-specific blockade of the inhibitory mechanisms (deactivation of LPBN inhibitory mechanisms), i.e., baclofen might block all the signals that reach the LPBN and in this condition sodium intake is released independent if animals are fluid depleted or normohydrated. More studies are necessary to investigate which signals that reach the LPBN are involved in the control of sodium intake and if different neurotransmitters in the LPBN are related to different signals.

The present results suggest that ANG II acting on post-synaptic AT_1 receptors in the LPBN facilitates the effects of $GABA_B$ activation with baclofen via a mechanism similar to that described in the MnPO [20]. Therefore, the results of present study suggest that interactions of angiotensinergic and GABAergic mechanisms in the LPBN are important to stimulate water and sodium intake. In other words, the action of ANG II on AT_1 receptors in the LPBN is important for the inhibition of LPBN neurons, thereby facilitating water and hypertonic NaCl intake produced by activation of $GABA_A$ and $GABA_B$ receptors in the LPBN.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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