Behavioural Brain Research 278 (2015) 535–541

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Gabaergic and opioid receptors mediate the facilitation of NaCl intake induced by α_2-adrenergic activation in the lateral parabrachial nucleus

C.A.F. Andrade[a,*], L.B. De Oliveira[b], G.M.F. Andrade-Franzé[a], L.A. De Luca Jr[a], Débora S.A. Colombari[a], J.V. Menani[a]

[a] Departament of Physiology and Pathology, School of Dentistry, UNESP, Araraquara-SP, 14801-903, Brazil
[b] Department of Biological Sciences, DECBI-NUPEB, Federal University of Ouro Preto, Ouro Preto, MG, Brazil

HIGHLIGHTS

- α_2-adrenoceptor activation in the LPBN increases sodium intake in fluid-depleted rat.
- Opioidergic receptor blockade partially reduced α_2-adrenoceptor activation effects.
- α_2-adrenoceptor activation effects are partially reduced by $GABA_A$ receptor blockade.
- Opioidergic/GABAergic blockade partially reduced α_2-adrenoceptor activation effects.
- α_2-adrenoceptor activation effects are partially dependent on opioid/$GABA_A$ receptors.

ARTICLE INFO

Article history:
Received 14 July 2014
Received in revised form 1 October 2014
Accepted 6 October 2014
Available online 14 October 2014

Keywords:
Sodium appetite
Adrenergic
GABA
Opioid
Dehydration
Thirst

ABSTRACT

Alpha$_2$-adrenergic, gabaergic or opioidergic activation in the lateral parabrachial nucleus (LPBN) increases sodium intake. In the present study, we investigated the effects of single or combined blockade of opioidergic and gabaergic receptors in the LPBN on the increase of 0.3 M NaCl intake induced by α_2-adrenoceptor activation in the LPBN. Male Holtzman rats (n = 5–9/group) with cannulas implanted bilaterally in the LPBN were treated with the diuretic furosemide (10 mg/kg b wt.) combined with low dose of the angiotensin converting enzyme inhibitor captopril (5 mg/kg b wt.) subcutaneously. Bilateral injections of moxonidine (alpha$_2$-adrenergic/imidazoline receptor agonist, 0.5 nmol) into the LPBN increased furosemide + captopril-induced 0.3 M NaCl intake (25.8 ± 1.4, vs. vehicle: 3.8 ± 1.1 ml/60 min). The opioidergic receptor antagonist naloxone (100 nmol) or the $GABA_A$ receptor antagonist bicuculline (5 nmol) injected into the LPBN partially reduced the increase of 0.3 M NaCl intake produced by LPBN moxonidine (11.8 ± 4.0 and 22.8 ± 4.5, respectively, vs. vehicle + moxonidine: 31.6 ± 4.0 ml/60 min, respectively). Similar to the treatment with each antagonist alone, the combined injections of naloxone (100 nmol) and bicuculline (5 nmol) into the LPBN also partially reduced moxonidine effects on 0.3 M NaCl intake (15.5 ± 6.5 ml/60 min). The $GABA_B$ receptor antagonist saclofen (5 nmol) injected into the LPBN did not change the effects of moxonidine on 0.3 M NaCl intake (24.3 ± 7.8 ml/120 min). These results suggest that the increase of 0.3 M NaCl intake by α_2-adrenergic receptor activation in the LPBN is partially dependent on $GABA_A$ and opioid receptor activation in this area.

1. Introduction

The lateral parabrachial nucleus (LPBN) is a pontine area strongly involved with inhibitory mechanisms that control water and NaCl intake [1–7]. The LPBN is reciprocally connected to forebrain areas implicated in the maintenance of blood pressure and body fluid homeostasis, such as the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala and the median preoptic nucleus. The LPBN is also richly interconnected with medullary regions, which includes the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS), [8–15]. Therefore, the LPBN receives taste and visceral signals that ascend from AP/mNTS en route to forebrain

* Corresponding author. Address: Rua Humaitá 1680, Araraquara–SP, 14801-903, Brasil, Tel.: +55 16 3301 6487; fax: +55 16 3301 6488.
E-mail address: carina.andrade@foar.unesp.br (C.A.F. Andrade).

http://dx.doi.org/10.1016/j.bbr.2014.10.007
0166-4328/

areas involved in the control of fluid and electrolyte balance [5–7,16,17].

Studies that have investigated the involvement of the LPBN in the control of fluid-electrolyte balance demonstrated that different neurotransmitters like serotonin, cholecystokinin, glutamate and corticotrophin releasing factor (CRF) or receptors like α_2-adrenoceptors, GABAergic, opioid or purinergic receptors in the LPBN are involved with the control of sodium intake [1,3,5,18–28]. The activation of the α_2-adrenoceptors with bilateral injections of noradrenaline or moxonidine into the LPBN increases 0.3 M NaCl and water intake induced by the treatment with the diuretic furosemide (FURO) combined with low dose of the angiotensin converting enzyme inhibitor captopril (CAP) injected subcutaneously. Activation of the same receptors in the LPBN has no effect on water and hypertonic NaCl intake of satiated rats [21,25].

The activation of $GABA_A$, $GABA_B$ or opioid receptors with bilateral injections of muscimol, baclofen or β endorphin, respectively, into the LPBN strongly increases 0.3 M NaCl intake by satiated or sodium depleted rats [20,27,29,30]. Therefore, the activation of $GABA_A$, $GABA_B$, opioid receptors in the LPBN deactivates the inhibitory mechanisms, releasing sodium intake if excitatory signals were activated by sodium depletion or not [20,27,29].

It is not known if α_2-adrenoceptor, GABAergic and opioidergic mechanisms interact in the LPBN to so potently induce sodium intake. In the present study, we investigated the effect of combined antagonism of opioidergic and/or gabaergic receptor with α_2-adrenoceptor activation in the LPBN on 0.3 M NaCl and water intake induced by FURO + CAP in rats.

2. Material and methods

2.1. Animals

Male Holtzman rats weighing 290 to 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. 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 7:30 AM.

The experimental protocols used in the present study were approved by Ethical Committee for Animal Care and Use from Dentistry School of Araraquara–UNESP, Brazil (protocol no: 06/2006) and followed the recommendations from the National Council for the Control of Animal Experimentation (CONCEA) 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 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. Bilateral stainless steel 23-gauge cannulas were implanted in direction to the LPBN using the following coordinates: 9.4 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 at a point 2 mm above each LPBN. The cannulas were fixed to the cranium using dental acrylic resin and jeweller screws. A 30-gauge metal obturator filled the cannulas between tests. The rats were allowed to recover 6 days before drug injections into the LPBN.

2.3. Injections into the LPBN

Injections into the LPBN were made using 5-μl Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At time of testing, 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 injection, and the rats were placed back into the cage.

2.4. Drugs

Furosemide (FURO, Sigma Chem., St Louis, MO, USA) was administered subcutaneously at 10 mg/kg of body weight. Captopril (CAP, Sigma Chem., St Louis, MO, USA) was administered subcutaneously at 5 mg/kg of body weight [7,21,31]. Moxonidine hydrochloride (a donation of Solvay Pharma, Hannover, Germany) was administered into the LPBN at the dose of 0.5 nmol/0.2 μl [19,21,24,32]. Bicuculline, ($GABA_A$ receptor antagonist, Tocris, Ellisville, MO, USA) was administered into the LPBN at the doses of 1.6 and 5.0 nmol/0.2 μl [27]; saclofen ($GABA_B$ receptor antagonist, Tocris, Ellisville, MO, USA) was administered into the LPBN at the dose of 5.0 nmol/0.2 μl [30] and naloxone hydrochloride (opioidergic receptor antagonist, Sigma Chemicals, St. Louis, MO, USA) was administered into the LPBN at the dose of 100 nmol/0.2 μl) [20].

Moxonidine, naloxone, saclofen and bicuculline were dissolved in a mix of propylene glycol/water 2:1 (vehicle). Vehicle was injected as control into the LPBN. Captopril was dissolved in isotonic saline. Furosemide was dissolved in alkaline saline (pH adjusted to 9.0 with NaOH).

2.5. Ingestive test

Rats were tested in their home cages. Water, 0.3 M NaCl and food were removed and the animals received subcutaneous FURO (10 mg/kg of body wt) + CAP (5 mg/kg of body wt) as described previously [7,21,31]. One hour later, water and 0.3 M NaCl were provided in burettes with 0.1-ml divisions that were fitted with metal drinking spouts. Cumulative water and 0.3 M NaCl intakes were measured at 15, 30, 60, 90, and 120 min (ingestive test). The injections of moxonidine or vehicle 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. The opioidergic and/or GABAergic antagonist, or vehicle, was injected into the LPBN 15 min before the injection of moxonidine or its vehicle.

Each group of rats was submitted to four tests, each test in a different day, at a 48-hour minimum interval. In each test the group was divided in two and each half received a different treatment into the LPBN. The sequence of the treatments into the LPBN in the different tests was randomized. All animals received a total of four treatments into the LPBN: vehicle + vehicle; vehicle + moxonidine; opioidergic and/or GABAergic antagonist + moxonidine; opioidergic and/or GABAergic antagonist + vehicle.

2.6. Histology

The animals received bilateral injections of 2% Evans blue solution (0.2 μl) into the LPBN after the fourth ingestive test. They were then deeply anesthetized with sodium thiopental (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 50-μm sections, stained with cresyl violet, and analyzed by light microscopy to confirm the injection sites into the LPBN.

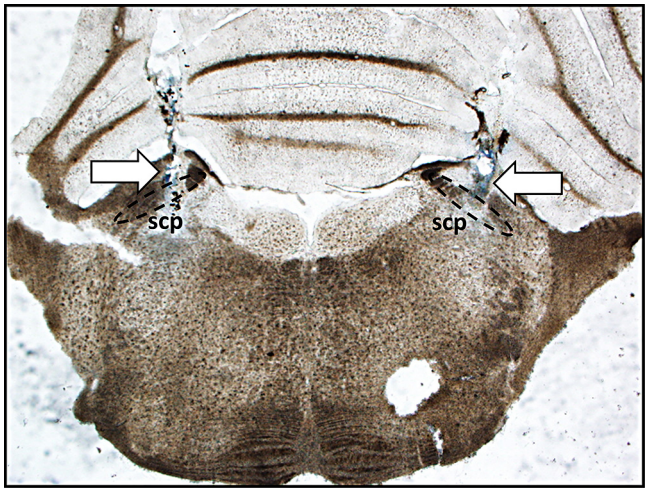

Fig. 1. Photomicrograph of a coronal section of a rat brain showing (arrows) the sites of bilateral injections into the LPBN. scp: superior cerebellar peduncle.

2.7. Statistical analysis

The results are reported as means ± S.E.M. Two-way repeated-measures ANOVA (treatment and time as factors) and Student Newman-Keuls tests were used for comparison. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Histological analysis

Fig. 1 shows the typical sites of bilateral injections into the LPBN. The LPBN injections were centered mainly in the central lateral or dorsal lateral portions of the LPBN [33]. Injections centered in the ventral lateral and external lateral portions, as well as in the Kölliker-Fuse nucleus observed in some rats were also considered as correctly placed in the LPBN. The sites of the injections in the present study were similar to those of previous studies that showed the effects of moxonidine, muscimol or opioids injected into the LPBN on NaCl and water intake [19–21,24,27].

3.2. $GABA_A$ receptor blockade and moxonidine injected into LPBN

Bilateral injections of moxonidine (0.5 nmol/0.2 μl) into the LPBN strongly increased FURO + CAP-induced 0.3 M NaCl intake when compared to control (vehicle + vehicle) injections. Bicuculline (5 nmol/0.2 μl) partially reduced the increase of 0.3 M NaCl induced by moxonidine in FURO + CAP-treated rats during the whole ingestive test. Bicuculline (5.0 nmol/0.2 μl) combined with vehicle did not affect FURO + CAP-induced 0.3 M NaCl intake (Fig. 2A). ANOVA showed significant effect for treatment on NaCl 0.3 M intake [$F(3, 24) = 22.9$; $P < 0.05$], (Fig. 2A).

Moxonidine increased water intake at 120 min of the test (Fig. 2B). Bicuculline (5.0 nmol) reversed the increase of water intake induced by moxonidine in FURO + CAP-treated rats. Bicuculline (5.0 nmol) + moxonidine also reduced water intake until 30 min of the test when compared to control (veh + veh) (Fig. 2B). Bicuculline (5.0 nmol/0.2 μl) combined with vehicle did not affect FURO + CAP-induced water intake (Fig. 2B). ANOVA showed significant interaction between treatment and time [$F(12, 96) = 7.9$; $P < 0.05$] for water intake (Fig. 2B).

In another group of rats, (Table 1) the increase in 0.3 M NaCl intake induced by moxonidine in FURO + CAP-treated rats was partially reduced by bicuculline (1.6 nmol/0.2 μl) until 90 min of the test. FURO + CAP-induced water intake was not modified by injections of moxonidine alone or combined with bicuculline (Table 1). ANOVA showed significant effect for treatment on 0.3 M NaCl intake [$F(3, 12) = 26.7$; $P < 0.001$], but no effect on water intake [$F(3, 12) = 0.8$; $P > 0.05$] (Table 1).

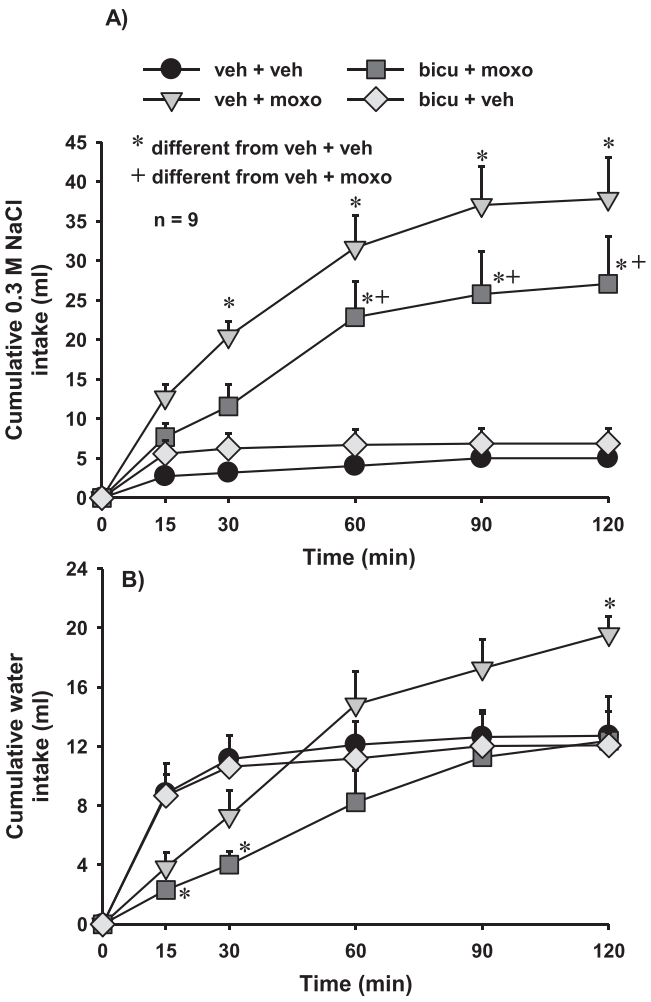

Fig. 2. (A) Cumulative 0.3 M NaCl intake; (B) cumulative water intake induced by s.c. FURO + CAP treatment in rats that received bilateral injections of bicuculline (bicu, 5.0 nmol/0.2 μl) or vehicle (veh, 0.2 μl) combined with moxonidine (moxo, 0.5 nmol/0.2 μl) or vehicle (veh, 0.2 μl) into the LPBN. Results are expressed as means ± SEM; n = number of rats.

3.3. $GABA_B$ receptor blockade and moxonidine injected into LPBN

Saclofen (5.0 nmol/0.2 μl) did not significantly change the increase of FURO + CAP-induced 0.3 M NaCl intake produced by moxonidine (0.5 nmol/0.2 μl). Saclofen combined with vehicle did not change FURO + CAP-induced 0.3 M NaCl intake. ANOVA showed significant effect for treatment on 0.3 M NaCl intake [$F(3, 18) = 9.7$; $P < 0.05$] (Fig. 3A).

FURO + CAP-induced water intake was not modified by moxonidine alone or combined with saclofen. ANOVA showed no significant effect for treatment [$F(3, 18) = 3.1$; $P > 0.05$] (Fig. 3B).

3.4. Opioid receptor blockade and moxonidine injected into LPBN

Naloxone (100 nmol/0.2 μl) partially reduced the increase of FURO + CAP-induced 0.3 M NaCl intake produced by moxonidine (0.5 nmol/0.2 μl) as indicated by the significant difference between treatments [$F(3, 12) = 10.1$; $P < 0.05$] (Fig. 4A).

Table 1
Cumulative 0.3 M NaCl and water intake induced by s.c. FURO + CAP treatment in rats that received bilateral injections of bicuculline (bicu, 1.6 nmol/0.2 μl) or vehicle (veh, 0.2 μl) combined with moxonidine (moxo, 0.5 nmol/0.2 μl) or vehicle (veh, 0.2 μl) into the LPBN. Results are expressed as means ± SEM; $n = 5$.

Fluid ingested (ml)	Treatment	30 min	60 min	90 min	120 min
0.3 M NaCl	veh + veh	3.5 ± 0.9	3.6 ± 0.9	3.6 ± 0.9	3.6 ± 0.9
	veh + moxo	26.6 ± 2.5[a]	36.6 ± 4.0[a]	41.2 ± 5.7[a]	42.8 ± 6.1[a]
	bicu + moxo	13.5 ± 3.2[b]	22.5 ± 5.4[a,b]	31.2 ± 5.8[a,b]	34.4 ± 5.0[a]
	bicu + veh	2.4 ± 1.0	2.5 ± 1.0	2.6 ± 1.0	2.7 ± 1.0
water	veh + veh	11.7 ± 1.8	12.2 ± 1.7	12.2 ± 1.7	12.2 ± 1.7
	veh + moxo	7.4 ± 2.3	12.4 ± 3.2	20.1 ± 6.1	20.1 ± 6.1
	bicu + moxo	4.6 ± 1.9	11.0 ± 2.4	14.9 ± 3.8	14.6± 4.2
	bicu + veh	8.5 ± 2.0	9.7 ± 2.4	9.8 ± 2.4	9.9 ± 2.4

[a] Different from veh + veh.
[b] Different from veh + moxo.

Naloxone (100 nmol/0.2 μl) combined with vehicle did not change FURO + CAP-induced 0.3 M NaCl intake, (Fig. 4A). FURO + CAP-induced water intake was not modified by moxonidine alone or combined with naloxone [$F(3, 12) = 1.3$; $P > 0.05$], (Fig. 4B).

3.5. Combined opioid and $GABA_A$ receptor blockade and moxonidine injected into LPBN

The previous injections of naloxone (100 nmol/0.2 μl) + bicuculline (5 nmol/0.2 μl) into the LPBN, similar to these treatments alone, partially reduced the increase of FURO + CAP-induced 0.3 M NaCl and water intake produced by the injections of moxonidine (0.5 nmol/0.2 μl) into the LPBN as indicated by the significant differences between treatments for 0.3 M NaCl [$F(3, 12) = 16.1$; $P < 0.001$] and water intake [$F(3, 12) = 9.1$; $P < 0.05$] (Fig. 5). The injections of naloxone (100 nmol/0.2 μl) + bicuculline (5 nmol/0.2 μl) combined with vehicle did not affect FURO + CAP-induced water and 0.3 M NaCl intake (Fig. 5).

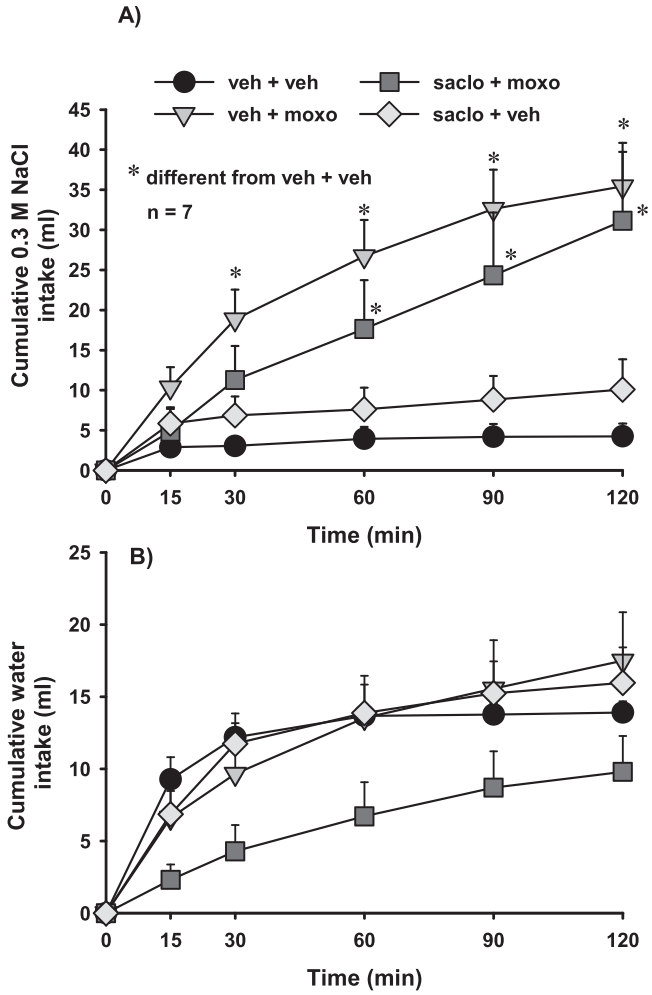

Fig. 3. (A) Cumulative 0.3 M NaCl intake; (B) cumulative water intake induced by s.c. FURO + CAP treatment in rats that received bilateral injections of saclofen (saclo, 5.0 nmol/0.2 μl) or vehicle (veh, 0.2 μl) combined with moxonidine (moxo, 0.5 nmol/0.2 μl) or vehicle (veh, 0.2 μl) into the LPBN. Results are expressed as means ± SEM; n = number of rats.

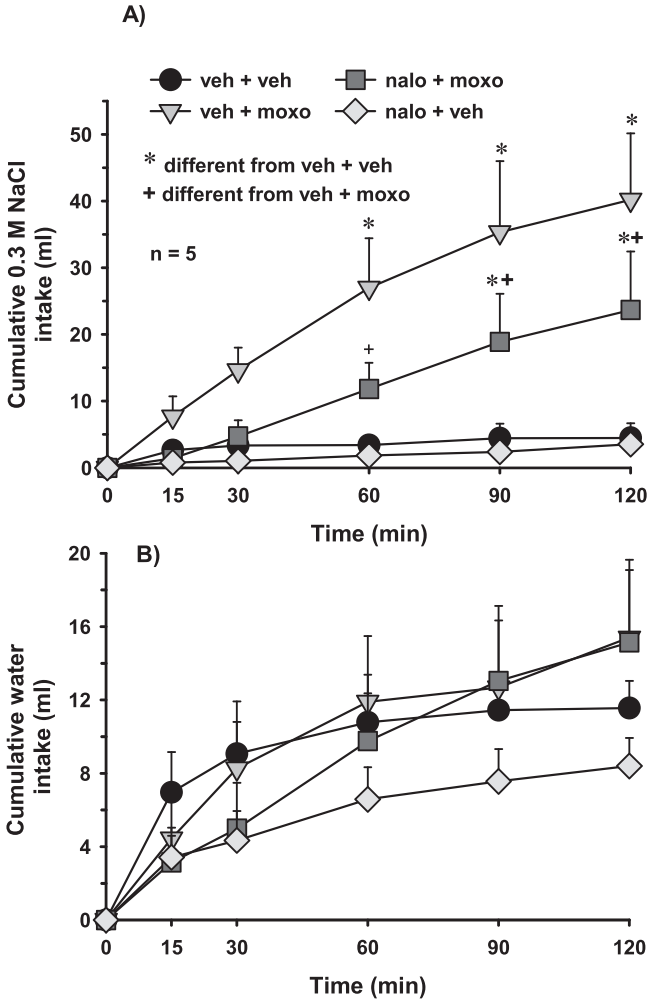

Fig. 4. (A) Cumulative 0.3 M NaCl intake; (B) cumulative water intake induced by s.c. FURO + CAP treatment in rats that received bilateral injections of naloxone (nalo, 100 nmol/0.2 μl) or vehicle (veh, 0.2 μl) combined with moxonidine (moxo, 0.5 nmol/0.2 μl) or vehicle (veh, 0.2 μl) into the LPBN. Results are expressed as means ± SE; n = number of rats.

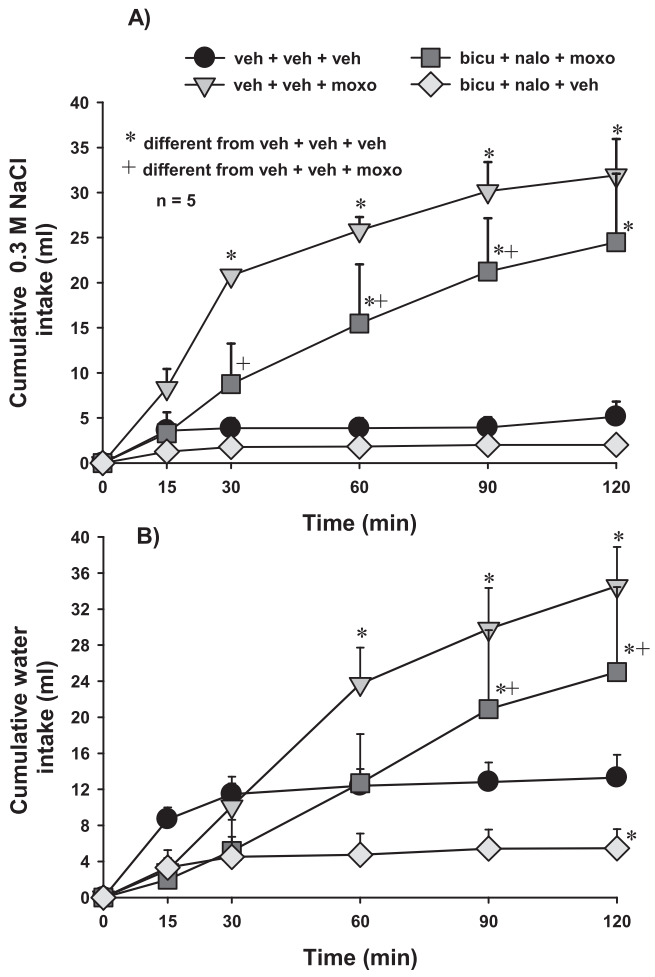

Fig. 5. (A) Cumulative 0.3 M NaCl intake; (B) cumulative water intake induced by s.c. FURO + CAP treatment in rats that received bilateral injections of bicuculline (bicu, 5.0 nmol/0.2 μl) + naloxone (nalo, 100 nmol/0.2 μl) or vehicle (veh, 0.4 μl) combined with moxonidine (moxo, 0.5 nmol/0.2 μl) or vehicle (veh, 0.2 μl) into the LPBN. Results are expressed as means ± SE; *n* = number of rats.

3.6. *Moxonidine injected into regions outside the LPBN (misplaced injections)*

Results from rats with misplaced injections (injections of drugs outside the LPBN) are shown in Table 2. Moxonidine injected outside the LPBN did not change FURO + CAP-induced 0.3 M NaCl and water intake. The only significant effect was a reduction of FURO + CAP-induced water intake by the injections of naloxone + vehicle into the LPBN.

Table 2
Water and 0.3 M NaCl intake induced by s.c. FURO + CAP treatment in rats that received injections of bicuculline (bicu, 1.6 nmol/0.2 μl), naloxone (nalo, 100 nmol/0.2 μl) or vehicle (veh, 0.2 μl) combined with moxonidine (moxo, 0.5 nmol/0.2 μl) or vehicle (veh, 0.2 μl) outside the LPBN (misplaced injections).

Treatment	(ml/120 min)	
	0.3 NaCl intake	Water intake
(*n* = 3)		
veh + veh	3.7 ± 2.0	14.3 ± 1.4
veh + moxo	12.0 ± 3.7	19.1 ± 1.4
bicu + moxo	15.4 ± 4.4	18.9 ± 3.7
bicu + veh	7.7 ± 3.3	14.2 ± 1.2
(*n* = 3)		
veh + veh	2.7 ± 0.5	14.8 ± 1.0
veh + moxo	5.5 ± 2.6	16.7 ± 1.1
nalo + moxo	4.2 ± 1.6	11.6 ± 1.3
nalo + veh	3.0 ± 1.6	8.7 ± 0.7[a]

[a] Different from veh + veh.
Values are mean ± S.E.M. *n* = number of rats

4. Discussion

The present results show that naloxone or bicuculline injected into the LPBN partially reduced the increase in 0.3 M NaCl intake produced by injections of moxonidine into the LPBN. Combined treatment with naloxone and bicuculline into the LPBN had a similar effect. Naloxone or bicuculline injected alone into the LPBN did not affect FURO + CAP-induced 0.3 M NaCl intake. The specificity of the LPBN as the site of action for α_2-adrenoceptor activation was confirmed by the absence of effects of moxonidine when injected outside the LPBN. The results suggest that the increase in 0.3 M NaCl intake by α_2-adrenoceptor activation in the LPBN partially depends on $GABA_A$ and opioid receptor activation in this area. The effects of the selective blockade of $GABA_A$ or opioid receptors were similar to those produced by simultaneous blockade of these receptors, which suggests that both receptors are part of the same pathway.

Bicuculline into the LPBN at the doses of 1.6 and 5 nmol similarly reduced FURO + CAP-induced 0.3 M NaCl intake in rats treated with moxonidine in the same area. Previous studies showed that the treatment with bicuculline (1.6 nmol) into the LPBN abolished the effects of the $GABA_A$ agonist muscimol (0.5 nmol) injected into the LPBN on 0.3 M NaCl and water intake in fluid replete rats, in FURO + CAP-treated rats or 24 h sodium depleted rats [27,29]. The injections of saclofen (5 nmol) into the LPBN reduced 0.3 M NaCl intake induced by baclofen ($GABA_B$ receptor agonist) injected into the LPBN in satiated rats [30] and naloxone (100 nmol) injected into the LPBN abolished the ingestion of 0.3 M NaCl induced by injections of β-endorphin into the LPBN in satiated rats [20]. Therefore, the doses of the antagonists used in the present study are effective in blocking the gabaergic or opioid receptors in the LPBN.

The results also confirm at least in part the hypothesis that GABA and opioid receptors interact with multiple neurotransmitters independently from the hydration status of the animal [34]. Bicuculline and naloxone abolish 0.3 M NaCl and water intake induced respectively by muscimol and β-endorphin injected into the LPBN of satiated, fluid replete, rats [20,27,29]. Moxonidine, however, has no effect on sodium or water intake when injected into the LPBN of satiated rats [21]. It has been hypothesized that the threshold to increase hypertonic sodium intake when GABA and opioid neurotransmission are activated is much lower than that required to increase NaCl intake when, for example, α_2-adrenoceptors are activated [34]. Dehydration or thirst-related signals decrease such threshold in the LPBN allowing the activation of α_2-adrenoceptors to recruit GABA and opioid mechanisms and so increase hypertonic NaCl intake.

Recruitment of GABA and opioid mechanisms in response to α_2-adrenoceptor activation may also overcome the inhibition of sodium intake produced by serotonergic activation in the LPBN. In a previous study, moxonidine injected into the LPBN completely abolished the inhibition of water and NaCl intake produced by the activation of the LPBN serotonergic mechanism [35]. This suggests that α_2-adrenoceptors and serotonergic receptors are located in the same post synaptic neurons in the LPBN with each receptor producing opposite effects on the activity of these neurons [35].

Blockade of opioidergic and/or gabaergic receptors of the LPBN in the present study partially reduced the increase of 0.3 M NaCl intake caused by the activation of α_2-adrenoceptors in the LPBN, suggesting that the activation of α_2-adrenoceptors may release GABA and opioids in the LPBN. The remaining intake after blocking gabaergic and opioid mechanisms in the LPBN is probably due to the modification of additional parallel mechanisms by the

activation of α_2-adrenoceptors. The additional mechanisms affected by the activation of α_2-adrenoceptors might be the blockade of serotonin release in the LPBN as previously proposed [35]. However, it is still not possible to exclude interactions of α_2-adrenoceptor mechanisms with other neurotransmitters/mechanisms (e.g. CCK, CRF, or glutamate) also known to reduce the inhibition of sodium intake [18,26,34,36]. Future studies may address this possibility.

Moxonidine produced consistent preferential increase in induced 0.3 M NaCl intake as expected from previous work [21]. The increased preference for NaCl intake versus water intake was likely reinforced by the hypertonicity of the ingested NaCl solution. As shown in a previous study [32], increase in blood osmolarity facilitates sodium intake in rats treated with moxonidine in the LPBN. Bicuculline (5 nmol) alone or bicuculline combined with naloxone in the LPBN reduced FURO + CAP-induced water intake, independently from changes in NaCl intake, suggesting that $GABA_A$ receptor activation in the LPBN facilitates FURO + CAP-induced water intake.

The release of neurotransmitters that modulate LPBN inhibitory mechanisms are controlled by signals from peripheral high and low pressure baroreceptors, taste receptors or other signals that reach the LPBN through the AP/NTS [5–7,16,17]. From the LPBN, the signals may reach integrative areas that also receive facilitatory signals from forebrain areas involved in the control of sodium and water intake like, SFO, OVLT and other hypothalamic areas [37]. As a consequence of the activation of the inhibitory mechanisms, fluid depleted rats that ingest water and hypertonic NaCl show reduced ingestive reactions and increased rejection responses to an intraoral infusion of 0.3 M NaCl [19]. However, a previous study demonstrated that rats treated with moxonidine into the LPBN continue to show enhanced ingestive reactions and reduced rejection responses to an intraoral infusion of 0.3 M NaCl even after consuming large volumes of 0.3 M NaCl and water [19]. These results suggest that moxonidine in the LPBN possibly reduces some type of inhibitory signals produced as a consequence of the ingestion of NaCl and water. The inhibitory signals affected by moxonidine acting in the LPBN might be the signals from taste receptors or other visceral receptors that reach the LPBN.

In conclusion, the present and previous studies [19,34,35,38] suggest that moxonidine acting in α_2-adrenoceptors in the LPBN increases the release of GABA and opioids and blocks serotonin action, reducing the activity of the inhibitory mechanisms, which might enhance ingestive reactions and reduces rejection responses caused by the ingestion of 0.3 M NaCl and water, increasing the intake.

Acknowledgments

The authors thank Silas P. Barbosa, Reginaldo C. Queiroz and Silvia Fóglia for expert technical assistance, Silvana A. D. Malavolta for secretarial assistance, Ana V. de Oliveira for animal care and also PROPE–UNESP.

This research was supported by Brazilian public funding from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP - 12/01955-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - 478960/2013-1).

References

[1] Colombari DS, Menani JV, Johnson AK. Forebrain angiotensin type 1 receptors and parabrachial serotonin in the control of NaCl and water intake. Am J Physiol 1996;271:R1470–6.
[2] Edwards GL, Johnson AK. Enhanced drinking after excitotoxic lesions of the parabrachial nucleus in the rat. Am J Physiol 1991;261:R1039–44.
[3] Menani JV, Johnson AK. Lateral parabrachial serotonergic mechanisms: angiotensin-induced pressor and drinking responses. Am J Physiol 1995;269: R1044–9.
[4] Menani JV, De Luca Jr LA, Thunhorst RL, Johnson AK. Hindbrain serotonin and the rapid induction of sodium appetite. Am J Physiol 2000;279: R126–31.
[5] Menani JV, Colombari DS, Beltz TG, Thunhorst RL, Johnson AK. Salt appetite: interaction of forebrain angiotensinergic and hindbrain serotonergic mechanisms. Brain Res 1998;801:29–35.
[6] Menani JV, De Luca Jr LA, Johnson AK. Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by sodium depletion. Am J Physiol 1998;274:R555–60.
[7] Menani JV, Thunhorst RL, Johnson AK. Lateral parabrachial nucleus and serotonergic mechanisms in the control of salt appetite in rats. Am J Physiol 1996;270:R162–8.
[8] Ciriello J, Lawrence D, Pittman QJ. Electrophysiological identification of neurons in the parabrachial nucleus projecting directly to the hypothalamus in the rat. Brain Res 1984;322:388–92.
[9] Fulwiler CE, Saper CB. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. Brain Res 1984;7:259.
[10] Herbert H, Moga MM, Saper CB. Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. J Comp Neurol 1990;293:540–80.
[11] Jhamandas JH, Petrov T, Harris KH, Vu T, Krukoff TL. Parabrachial nucleus projection to the amygdala in the rat. Electrophysiological and anatomical observations. Brain Res Bull 1996;39:115–26.
[12] Krukoff TL, Harris KH, Jhamandas JH. Efferent projections from the parabrachial nucleus demonstrated with the anterograde tracer *Phaseolus vulgaris* leucoagglutinin. Brain Res Bull 1993;30:163–72.
[13] Jhamandas JH, Harris KH, Petrov T, Krukoff TL. Characterization of the parabrachial nucleus input to the hypothalamic paraventricular nucleus in the rat. J Neuroendocrinol 1992;4:461–71.
[14] Lança AJ, van der Kooy D. A serotonin-containing pathway from the area postrema to the parabrachial nucleus in the rat. Neuroscience 1985;14:1117–26.
[15] Norgren R. The central organization of the gustatory and visceral systems in the nucleus of the solitary tract. In: Katsuki Y, Norgren R, Sato M, editors. Brain Mechanisms of Sensation. New York: Wiley; 1981. p. 143–60.
[16] Johnson AK, Thunhorst RL. The neuroendocrinology, neurochemistry and molecular biology of thirst and salt appetite. In: Lajtha A, Laustein J, editors. Handbook of Neurochemistry and Molecular Neurobiology: Behavioral Neurochemistry, Neuroendocrinology and Molecular Neurobiology. New York: Springer; 2007. p. 641–87.
[17] Johnson AK, Thunhorst RL. The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. Front Neuroendocrinol 1997;18:292–353.
[18] De Castro e Silva E, Fregoneze JB, Johnson AK. Corticotropin-releasing hormone in the lateral parabrachial nucleus inhibits sodium appetite in rats. Am J Physiol Regul Integr Comp Physiol 2006;290:R1136–41.
[19] Andrade CA, Andrade-Franze GM, De Luca Jr LA, Johnson AK, Menani JV. Changes in taste reactivity to intra-oral hypertonic NaCl after lateral parabrachial injections of an alpha2-adrenergic receptor agonist. Physiol Behav 2011;104: 702–8.
[20] De Oliveira LB, De Luca Jr LA, Menani JV. Opioid activation in the lateral parabrachial nucleus induces hypertonic sodium intake. Neuroscience 2008;155:350–8.
[21] Andrade CA, Barbosa SP, De Luca Jr LA, Menani JV. Activation of alpha2-adrenergic receptors into the lateral parabrachial nucleus enhances NaCl intake in rats. Neuroscience 2004;129:25–34.
[22] De Gobbi JIF, De Luca Jr LA, Johnson AK, Menani JV. Interaction of serotonin and cholecystokinin in the lateral parabrachial nucleus to control sodium intake. Am J Physiol 2001;280:R1301–7.
[23] Menani JV, De Luca Jr LA, Johnson AK. Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by sodium depletion. Am J Physiol 1998;274:R555–60.
[24] Andrade CA, Margatho LO, Andrade-Franze GM, De Luca Jr LA, Antunes-Rodrigues J, Menani JV. Moxonidine into the lateral parabrachial nucleus reduces renal and hormonal responses to cell dehydration. Neuroscience 2012;208:69–78.
[25] Gasparini S, de Luca Jr LA, Colombari DS, de Paula PM, Barbosa SP, Menani JV. Adrenergic mechanisms of the Kolliker-Fuse/A7 area on the control of water and sodium intake. Neuroscience 2009;164:370–9.
[26] De Gobbi JI, Beltz TG, Johnson RF, Menani JV, Thunhorst RL, Johnson AK. Non-NMDA receptors in the lateral parabrachial nucleus modulate sodium appetite. Brain Res 2009;1301:44–51.
[27] Callera JC, Oliveira LB, Barbosa SP, Colombari DSA, De Luca Jr LA, Menani JV. $GABA_A$ receptor activation in the lateral parabrachial nucleus induces water and hypertonic NaCl intake. Neuroscience 2005;134:725–35.
[28] Menezes MF, Barbosa SP, De Andrade CA, Menani JV, De Paula PM. Purinergic mechanisms of lateral parabrachial nucleus facilitate sodium depletion-induced NaCl intake. Brain Res 2011;1372:49–58.
[29] de Oliveira LB, Callera JC, De Luca Jr LA, Colombari DS, Menani JV. GABAergic mechanisms of the lateral parabrachial nucleus on sodium appetite. Brain Res Bull 2007;73:238–47.
[30] De Oliveira LB, Kimura EH, Callera JC, De Luca Jr LA, Colombari DS, Menani JV. Baclofen into the lateral parabrachial nucleus induces hypertonic sodium chloride and sucrose intake in rats. Neuroscience 2011;183:160–70.
[31] Fitts DA, Masson DB. Forebrain sites of action for drinking and salt appetite to angiotensin or captopril. Behav Neurosci 1989;103:865–72.

[32] Andrade CA, De Luca Jr LA, Colombari DS, Menani JV. Alpha2-adrenergic activation in the lateral parabrachial nucleus induces NaCl intake under conditions of systemic hyperosmolarity. Neuroscience 2006;142:21–8.
[33] Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 5th ed San Diego: CA: Academic Press; 2004.
[34] Menani JV, De Luca Jr LA, Johnson AK. Role of the lateral parabrachial nucleus in the control of sodium appetite. Am J Physiol Regul Integr Comp Physiol 2014;306:R201–10.
[35] Menani JV, Oliveira LB, Andrade CAF, Sugawara AM, De Luca Jr LA. Role of central α-2-adrenergic/imidazoline receptors in the control of thirst, sodium appetite and renal excretion. In: Chen FJ, editor. New Trends in Brain Research. Nova Science Publishers, Inc; 2006. p. 95–126.
[36] Menani JV, Johnson AK. Cholecystokinin actions in the parabrachial nucleus: effects on thirst and salt appetite. Am J Physiol 1998;275:R1431–7.
[37] Menani JV, Vieira AA, Colombari DSA, De Paula PM, Colombari E, De Luca LA. Preoptic-Periventricular Integrative Mechanisms Involved in Behavior, Fluid-Electrolyte Balance, and Pressor Responses. In: De Luca LA, Menani JV, Johnson AK, editors. Neurobiology of Body Fluid Homeostasis: Transduction and Integration. Boca Raton (FL); 2014.
[38] Andrade CA, Andrade-Franze GM, De Paula PM, De Luca Jr LA, Menani JV. Role of alpha2-adrenoceptors in the lateral parabrachial nucleus in the control of body fluid homeostasis. Braz J Med Biol Res 2014;47:11–8.