

Research report

Opioid and α_2 adrenergic mechanisms are activated by GABA agonists in the lateral parabrachial nucleus to induce sodium intake

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ABSTRACT

The activation of GABA, opioid or α_2 adrenergic mechanisms in the lateral parabrachial nucleus (LPBN) facilitates hypertonic NaCl intake in rats. In the present study, we combined opioid or α_2 adrenergic antagonists with GABA agonists into the LPBN in order to investigate if NaCl intake caused by GABAergic activation in normohydrated rats depends on opioid or α_2 -adrenergic mechanisms in this area. Male Holtzman rats with stainless steel cannulas implanted bilaterally in the LPBN were used. Bilateral injections of muscimol or baclofen (GABA_A and GABA_B agonists, respectively, 0.5 nmol/0.2 μ l) into the LPBN induced strong ingestion of 0.3 M NaCl (45.8 ± 7.3 and 21.8 ± 4.8 ml/240 min, respectively) and water intake (22.7 ± 3.4 and 6.6 ± 2.5 ml/240 min, respectively). Naloxone (opioid antagonist, 150 nmol/0.2 μ l) into the LPBN abolished 0.3 M NaCl and water intake to muscimol (2.0 ± 0.6 and 0.9 ± 0.2 ml/240 min, respectively) or baclofen (2.3 ± 1.1 and 0.8 ± 0.4 ml/240 min, respectively). RX 821002 (α_2 adrenoceptor antagonist, 10 nmol/0.2 μ l) into the LPBN reduced 0.3 M NaCl intake induced by the injections of muscimol or baclofen (26.6 ± 8.0 and 10.1 ± 4.9 ml/240 min, respectively). RX 821002 reduced water intake induced by muscimol (7.7 ± 2.9 ml/240 min), not by baclofen. The results suggest that sodium intake caused by gabaergic activation in the LPBN in normohydrated rats is totally dependent on the activation of opioid mechanisms and partially dependent on the activation of α_2 adrenergic mechanisms in the LPBN.

1. Introduction

The lateral parabrachial nucleus (LPBN), located dorsolaterally to the superior cerebellar peduncle, is an important area for the control of fluid-electrolyte balance (Edwards and Johnson, 1991; Callera et al., 1993; Menani et al., 1996; Menani et al., 1998a; Menani et al., 1998b; Menani and Johnson, 1998; Andrade et al., 2004; Callera et al., 2005b; De Castro e Silva et al., 2006; de Oliveira et al., 2007; De Oliveira et al., 2008; De Oliveira et al., 2011). Different neurotransmitters and receptors present in the LPBN are involved in the control of water and NaCl intake. Some neurotransmitters/receptors like serotonin, cholecystokinin, corticotrophin release factor and glutamate in the LPBN activates the inhibitory mechanisms, whereas α_2 -adrenoceptors, opioids and GABA deactivates the inhibitory mechanisms (Edwards and Johnson, 1991; Callera et al., 1993; Menani et al., 1996; Menani et al., 1998a; Menani et al., 1998b; Menani and Johnson, 1998; Andrade et al., 2004; Callera et al., 2005b; De Castro e Silva et al., 2006; de

Oliveira et al., 2007; De Oliveira et al., 2008; De Oliveira et al., 2011; Menani et al., 2014; Andrade et al., 2015; Gasparini et al., 2015b; Pavan et al., 2015).

The activation of α_2 -adrenoceptors with bilateral injections of nor-adrenaline or the α_2 -adrenoceptor agonist moxonidine into the LPBN increases 0.3 M NaCl intake induced by dipsogenic or natriorexigenic stimuli (Andrade et al., 2004; Andrade et al., 2006, 2007; Andrade et al., 2015; Gasparini et al., 2015a; Gasparini et al., 2015b). In addition to increase fluid depletion-induced sodium intake, the activation of GABA or opioid receptors in the LPBN also induces strong ingestion of hypertonic NaCl and water in normohydrated rats (Callera et al., 2005b; de Oliveira et al., 2007; De Oliveira et al., 2008; De Oliveira et al., 2011), suggesting an important role of the LPBN inhibitory mechanisms limiting or restraining sodium intake in different conditions and even in normohydrated animals.

It is not clear why many neurotransmitters/receptors modulate sodium intake in the LPBN, how the neural circuitry is organized in the

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LPBN, if each neurotransmitter/receptor is activated by a specific signal involved in the control of sodium intake or if some of these neurotransmitters/receptors are part of the same pathway. Interaction among different neurotransmitters/receptors in the LPBN for the control of sodium intake is a possibility. Previous results showed that gabaergic or opioid receptor blockade in the LPBN partially inhibited the enhancement of hypertonic NaCl intake produced by moxonidine in rats submitted to acute fluid depletion with furosemide + captopril (Andrade et al., 2015). However, it is not known if α_2 -adrenoceptors or opioid receptors were also recruited by gabaergic activation in the LPBN to release sodium appetite in normohydrated animals.

Considering the similar effects of GABAergic, opioid and α_2 -adrenergic activation in the LPBN on sodium intake in different conditions, in the present study, we investigated if either opioid receptor or α_2 -adrenoceptor blockade affects hypertonic NaCl intake induced by GABA receptor activation in the LPBN in normohydrated rats.

2. Experimental procedures

2.1. Animals

Male Holtzman (a total of 55 rats weighing 290–310 g) rats 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. Room temperature was maintained at $23 \pm 2^\circ\text{C}$, and humidity at $55 \pm 10\%$ on a 12:12 light-dark cycle with light onset at 07:30 AM. 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.

2.2. Cerebral cannulas

Rats were anesthetized with a combination of ketamine (80 mg/kg of body weight, Agener Uniao, Embu-Guacu, SP, Brazil) and xylazine (7 mg/kg of body weight, Agener Uniao, Embu-Guacu, SP, Brazil) sc administered and were 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 in the LPBN using the coordinates 9.3 mm caudal to bregma, 2.2 mm lateral to the midline, and 4.3 mm below the dura mater (Paxinos & Watson, 2004). The tip of the cannulas was positioned at a point 2 mm above each LPBN. Dental acrylic resin and watch screws were used to fix the cannulas to the cranium. Metal obturators (30-gauge) filled the cannulas between tests. After the surgery, the rats received intramuscular injections of the analgesic cetoprophren 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.

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 and the injection cannula (2 mm longer than the guide cannula) was carefully inserted into the guide cannula, and bolus injection was initiated 15 s later. For bilateral injections, the first injection was initially performed in one side, the needle was removed 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 cage.

2.4. Drugs

The drugs injected into the LPBN were muscimol HBr, baclofen, RX 821002 and naloxone hydrochloride purchased from Sigma Chemicals (St Louis, MO, USA). Muscimol (0.5 nmol/0.2 μl) and baclofen (0.5 nmol/0.2 μl) were dissolved in saline and RX 821002 (10 nmol/0.2 μl) and naloxone (50, 100 and 150 nmol/0.2 μl) were dissolved in a mix of propylene glycol/water 2:1 (vehicle).

The doses of muscimol (GABA_A receptor agonist) and baclofen (GABA_B receptor agonist) were based in previous studies that showed the natriorexigenic effect of muscimol and baclofen injected into the LPBN (Callera et al., 2005b; de Oliveira et al., 2007; De Oliveira et al., 2011). The dose of RX 821002 and naloxone was based in previous studies that also tested the effects of these drugs into the LPBN (Andrade et al., 2004; De Oliveira et al., 2008).

2.5. Water and 0.3 M NaCl intake in normohydrated 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 for the rats during the tests. Cumulative intake of 0.3 M NaCl and water was measured at every 30 min during 240 min, starting immediately after bilateral injections of muscimol (0.5 nmol/0.2 μl), baclofen (0.5 nmol/0.2 μl) or saline (0.2 μl) into the LPBN.

To study a possible interaction between opioid and gabaergic mechanisms in the LPBN, normohydrated rats were tested for the effects of the combination of naloxone and muscimol into the LPBN on water and 0.3 M NaCl intake (two-bottle test). Naloxone (50, 100 or 150 nmol/0.2 μl) was injected into the LPBN 20 min before muscimol (0.5 nmol/0.2 μl) into the same area. The doses of 50 and 150 nmol of naloxone combined with muscimol into the LPBN were tested in the same group of rats. In different experimental sessions, these rats received the following 6 combinations of treatments into the LPBN: vehicle + saline, vehicle + muscimol, naloxone 50 + muscimol, naloxone 150 + muscimol, naloxone 50 + saline and naloxone 150 + saline. Naloxone 100 nmol combined with muscimol into the LPBN was tested in a separate group of rats that received the following four combinations of treatments into the LPBN: vehicle + saline, vehicle + muscimol, naloxone 100 + muscimol and naloxone 100 + saline. In each experimental session, the group of rats was divided into two and each half of the group received one of the combinations of treatments described above. The sequence of the treatments in the different tests was randomized and at the end of 4 or 6 tests all the rats received the 4 or 6 combinations of treatments. The combination of naloxone and baclofen into the LPBN was tested in other groups of rats submitted to the same experimental protocol, except that instead of muscimol, it was injected baclofen (0.5 nmol/0.2 μl) into the LPBN.

The results of the doses of 50 and 150 nmol of naloxone were analyzed and presented separately for each dose of naloxone used.

The interaction between α_2 adrenergic and gabaergic mechanisms in the LPBN on 0.3 M NaCl and water intake was tested in normohydrated rats submitted to a protocol similar to that described above for the combination of naloxone 100 nmol combined with muscimol or baclofen into the LPBN, except that RX 821002 (10 nmol/0.2 μl) was injected into the LPBN instead of naloxone.

A recovery period of at least 3 days was allowed between tests.

2.6. Histology

At the end of the experiments, the animals received bilateral injections of 2% Evans blue solution (0.2 μl) into the LPBN. They were then deeply anesthetized with thiopental sodium (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 serial coronal sections, stained with Giemsa, and analyzed by

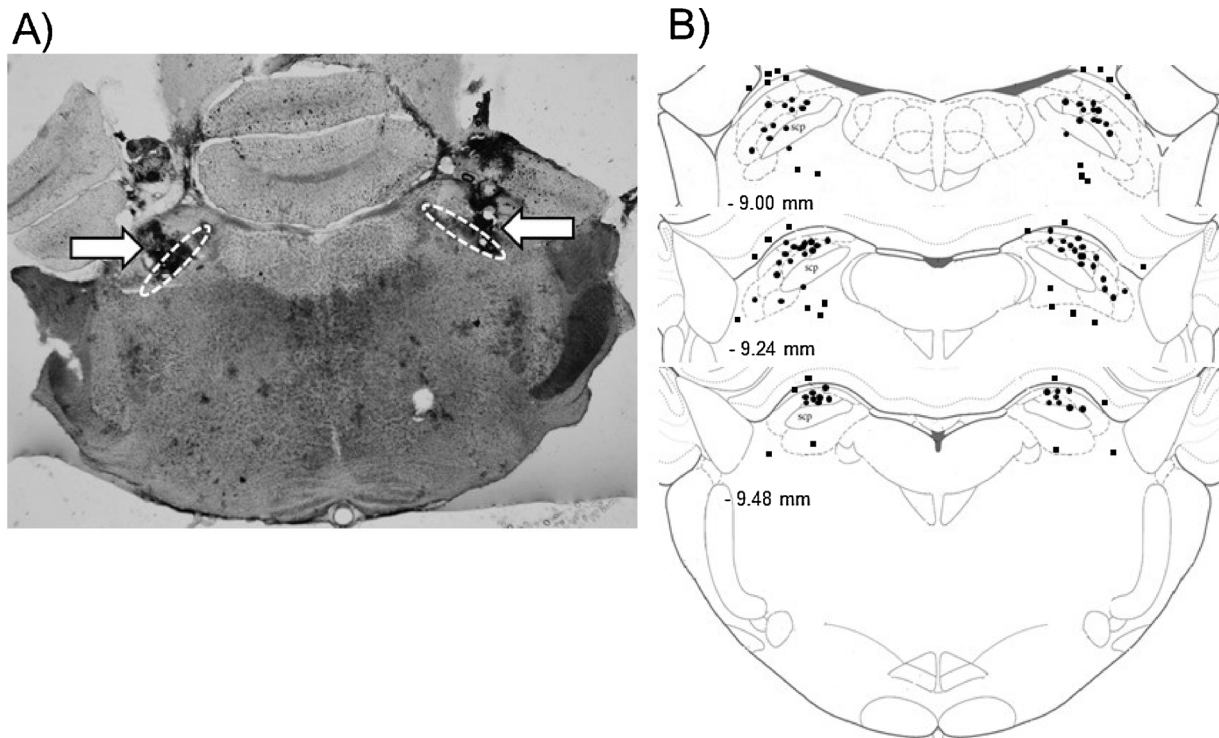


Fig. 1. Photomicrograph showing the sites of injections into the LPBN (arrows). The white dots outline the superior cerebellar peduncle.

light microscopy to confirm the injection sites into the LPBN.

2.7. Statistical analysis

The results are reported as means \pm SEM. One or two way repeated measures ANOVA using treatments and times as factors and Fisher's LSD tests were used for comparison. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Histological analysis

LPBN injections were centered in the central lateral and dorsolateral portions of the LPBN (Fig. 1). Injections reaching the ventral lateral and external lateral portions, Kolliker-Fuse nucleus, as well as brachium (superior cerebellar peduncle) or slightly ventral to this structure, reaching the dorsal portions of the medial parabrachial nucleus (MPBN) uni- or bilaterally were observed in some rats and the results from these rats were included in the analysis.

3.2. Water and 0.3 M NaCl intake in normohydrated rats treated with muscimol or baclofen combined with naloxone into the LPBN

In the different groups of normohydrated rats tested, bilateral injections of muscimol (0.5 nmol/0.2 μ l) into the LPBN induced strong ingestion of 0.3 M NaCl [$F(3;18) = 8.8$, $p < 0.05$] (Fig. 2A); [$F(3;15) = 16.6$, $p < 0.05$] (Fig. 2B); [$F(3;18) = 18.9$, $p < 0.05$] (Fig. 2C) and water [$F(3;18) = 15.6$, $p < 0.05$] (Fig. 2D); [$F(3;15) = 16.6$, $p < 0.05$] (Fig. 2E); [$F(3;18) = 22.3$, $p < 0.05$] (Fig. 2F).

The previous injection of naloxone (150 nmol/0.2 μ l) abolished, whereas naloxone (100 nmol/0.2 μ l) partially reduced the natriorexigenic effect of muscimol during the whole test (Fig. 2B–C). Naloxone (50 nmol/0.2 μ l) reduced muscimol-induced 0.3 M NaCl in the first 120 min of the test (Fig. 2A). Naloxone (150 nmol/0.2 μ l) abolished, whereas naloxone (50 nmol/0.2 μ l) reduced and naloxone (100 nmol)

did not affect water intake in rats treated with muscimol injected into the LPBN (Fig. 2D–F)

Bilateral injections of baclofen (0.5 nmol/0.2 μ l) into the LPBN also induced strong ingestion of 0.3 M NaCl [$F(3;12) = 5.9$, $p < 0.05$] (Fig. 3A); [$F(3;15) = 5.8$, $p < 0.05$] (Fig. 3B); [$F(3;12) = 1.5$, $p < 0.05$] (Fig. 3C) and water as showed by the significant interaction between treatments and times [$F(21;84) = 2.7$, $p < 0.05$] (Fig. 3D); [$F(21;105) = 11.6$, $p < 0.05$] (Fig. 3E) or significant differences between treatments [$F(3;12) = 8.0$, $p < 0.05$] (Fig. 3F), in the different experimental groups of rats tested

Injections of naloxone (100 and 150 nmol/0.2 μ l) into the LPBN abolished, whereas naloxone (50 nmol/0.2 μ l) did not affect 0.3 M NaCl intake induced by baclofen injected into the LPBN (Fig. 3). Naloxone (50 nmol/0.2 μ l) increased water intake induced by baclofen injected into the LPBN during the last 30 min of the test (Fig. 3D). Naloxone (100 and 150 nmol/0.2 μ l) abolished water intake (Fig. 3E–F)

3.3. Water and 0.3 M NaCl intake in normohydrated rats treated with muscimol or baclofen combined with RX 821002 into the LPBN

Bilateral injections of muscimol (0.5 nmol/0.2 μ l) into the LPBN induced 0.3 M NaCl intake [$F(3;15) = 8.2$, $p < 0.05$] and water intake as indicated by the significant interaction between treatments and times [$F(21;105) = 8.7$, $p < 0.05$] (Fig. 4). The previous injection of RX 821002 (10 nmol/0.2 μ l) into LPBN reduced 0.3 M NaCl intake in the last 90 min of the test and abolished water intake induced by injections of muscimol into the same area (Fig. 4).

Bilateral injections of baclofen (0.5 nmol/0.2 μ l) into LPBN induced 0.3 M NaCl intake [$F(3;18) = 3.7$, $p < 0.05$] and water intake as indicated by the significant interaction between treatments and times [$F(21;126) = 1.9$, $p < 0.05$] (Fig. 5). The previous injections of RX 821002 (10 nmol/0.2 μ l) into LPBN reduced the natriorexigenic effect of baclofen in the last 30 min of the test, without affecting water intake induced by baclofen (Fig. 5B)

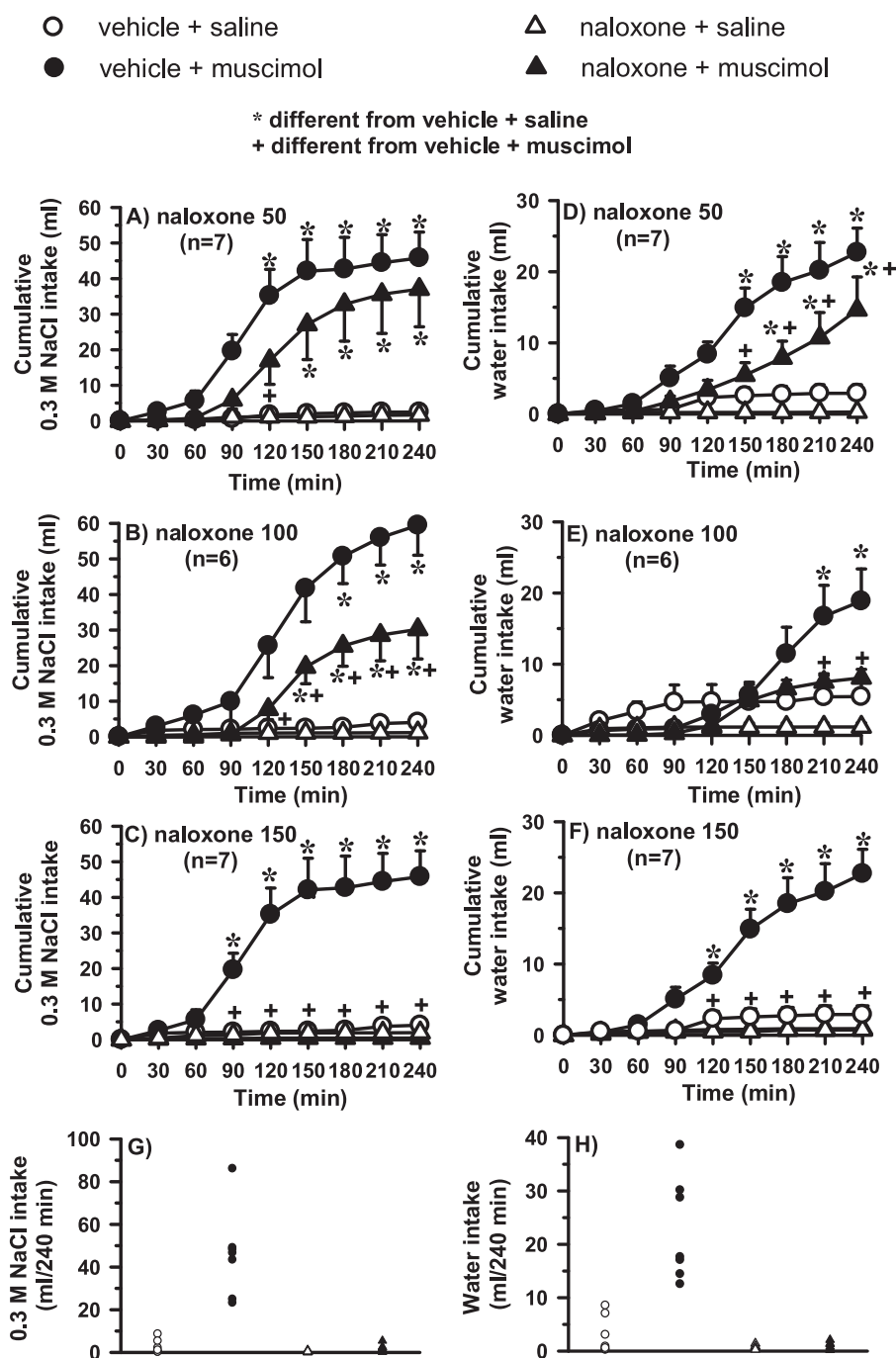


Fig. 2. Cumulative (A–C) 0.3 M NaCl intake and (D–F) water intake in normohydrated rats that received bilateral injections of vehicle or naloxone (50, 100 or 150 nmol/0.2 μ l) combined with muscimol (0.5 nmol/0.2 μ l) or saline into the LPBN. Results are expressed as means \pm SEM, n = number of rats.

3.4. Specificity of the LPBN as the site of action of naloxone or RX 821002 combined to gabaergic activation to produce effects on 0.3 M NaCl intake

Results from rats that received bilateral injections of naloxone or RX 821002 combined with muscimol or baclofen injections outside the LPBN (misplaced injections) were also analyzed to confirm that the effects on water and 0.3 M NaCl intake were due to the action of opioid, α_2 adrenoceptor and GABA ligands specifically in the LPBN.

Bilateral injections of vehicle, naloxone (150 nmol/0.2 μ l) or RX 821002 (10 nmol/0.2 μ l) combined with bilateral injections of muscimol (0.5 nmol/0.2 μ l) or baclofen (0.5 nmol/0.2 μ l) in sites outside the LPBN produced no effect on water or 0.3 M NaCl intake in normohydrated rats (Table 1), except a reduction on water intake in rats

that received naloxone + saline compared to vehicle + baclofen [F (3;15) = 3.6, p < 0.05] (Table 1). Bilateral misplaced injections were ventral (MPBN) or dorsal to LPBN, and part of them were rostral to LPBN.

4. Discussion

The present results show that the blockade of opioid receptors or α_2 adrenoceptors abolished or reduced, respectively, sodium and water intake induced by gabaergic activation in the LPBN in normohydrated rats. The results suggest that sodium and water intake induced by gabaergic activation in the LPBN, in normohydrated rats, depends on the activation of opioid mechanisms and partially dependent on the

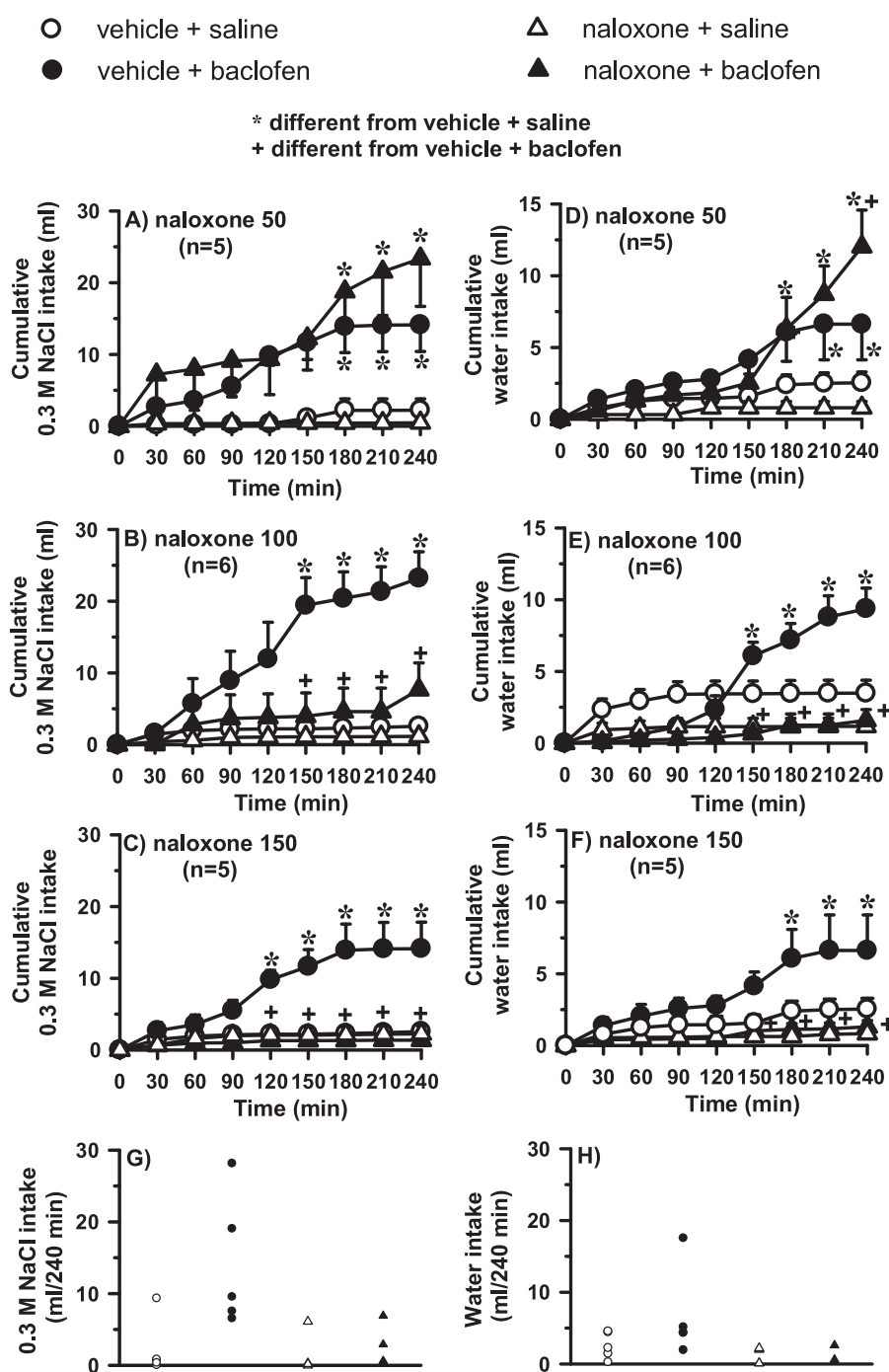


Fig. 3. Cumulative (A–C) 0.3 M NaCl intake and (D–F) water intake in normohydrated rats that received bilateral injections of vehicle or naloxone (50, 100 or 150 nmol/0.2 μ l) combined with baclofen (0.5 nmol/0.2 μ l) or saline into the LPBN. Results are expressed as means \pm SEM, n = number of rats.

activation of α_2 adrenergic mechanisms in the LPBN. The specificity of the LPBN as the site in which gabaergic activation induces 0.3 M NaCl and water intake was confirmed by the absence of effects of muscimol or baclofen injections in sites outside the LPBN. The dose of RX 821002 (10 nmol) that partially reduced the effects of muscimol is a dose that completely abolished the effects of moxonidine injected into the LPBN (Andrade et al., 2004).

Muscimol injections into the LPBN in normohydrated rats usually induce only a small ingestion of water, whereas baclofen induces no water intake when only water is available (one bottle test) (Callera et al., 2005a). Therefore, the strong ingestion of water produced by the injections of muscimol or baclofen into the LPBN when water and 0.3 M NaCl are simultaneously available (two bottle test) is probably a

consequence of the increased plasma osmolarity due to excessive ingestion of hypertonic NaCl (Callera et al., 2005b; De Oliveira et al., 2011). Thus, the reason for the reduced ingestion of water combining opioid or α_2 adrenoceptor blockade with muscimol into the LPBN is probably the reduced ingestion of hypertonic NaCl.

Opioid mechanisms may modulate GABA release and vice-versa (Bergevin et al., 2002; Kawamata et al., 2002; Hjelmstad and Fields, 2003). The importance of the interaction between opioid and GABAergic mechanisms in the control of food intake was previously investigated. Baclofen, muscimol or morphine injected into the ventral tegmental area (VTA) or the nucleus accumbens (NAc) induces food intake and the previous administration of naltrexone (opioid antagonist) reduces food intake induced by baclofen in the VTA or by

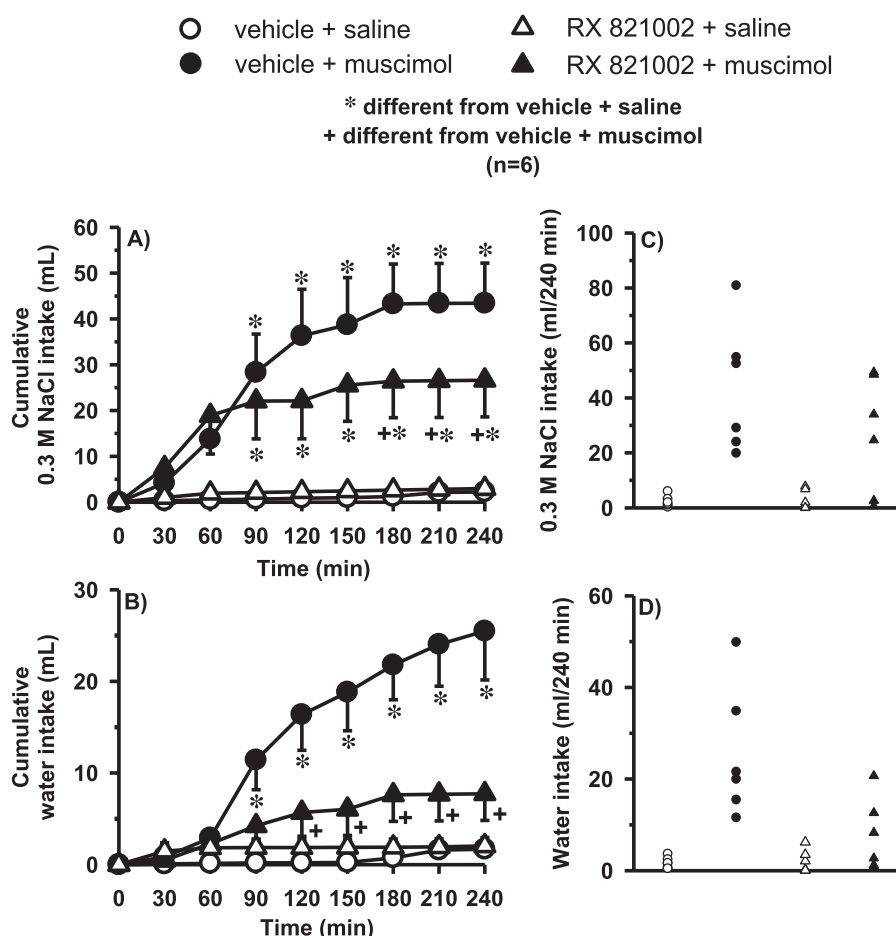


Fig. 4. Cumulative (A) 0.3 M NaCl intake and (B) water intake in normohydrated rats that received bilateral injections of vehicle or RX 821002 (10 nmol/0.2 μ l) combined with muscimol (0.5 nmol/0.2 μ l) or saline into the LPBN. Results are expressed as means \pm SEM, n = number of rats.

muscimol into the NAc (Mucha and Iversen, 1986; Znamensky et al., 2001; Echo et al., 2002; Khaimova et al., 2004). Food intake elicited by gabaergic activation in the VTA or NAc is reduced or enhanced depending on the type of opioid receptor antagonist used (Khaimova et al., 2004). Therefore, the activation of gabaergic mechanisms in the VTA and NAc may depend on the release of opioids to stimulate food intake, similar to which is shown by the present results for sodium intake control in the LPBN.

Previous studies showed that injection of RX 821002 abolished the increase of 0.3 M NaCl produced by moxonidine or noradrenaline injected into the LPBN in rats treated with sc furosemide + captopril or by moxonidine injected into the LPBN in cell dehydrated rats (Andrade et al., 2004; Andrade et al., 2006), suggesting that activation of α_2 adrenoceptors removes LPBN inhibitory mechanisms increasing sodium intake. The present results suggest that the activation of gabaergic mechanisms depends also on α_2 adrenoceptor activation to release sodium intake. Different types of interaction between adrenergic and gabaergic mechanisms have been proposed (Zarrindast et al., 2001; Han et al., 2002; Tanaka et al., 2002; Sakamaki et al., 2003). Although the activation of α_2 adrenoceptors located in the gabaergic neurons may reduce the spontaneous inhibitory synaptic current (Han et al., 2002), the modulation of noradrenaline release by gabaergic activation was described in the median preoptic nucleus and subfornical organ (SFO) (Tanaka et al., 2002; Sakamaki et al., 2003). In addition, GABA_B receptors are located in post-synaptic neurons that receives noradrenergic input (Suzdak and Gianutsos, 1985) and baclofen effects on memory retention was reduced by α_2 adrenoceptor blockade (Zarrindast et al., 2001).

Neurons containing pre-proenkephalin (opioid peptide precursor),

endomorphin-2 and α_2 adrenergic binding sites are present in the parabrachial nucleus (Harlan et al., 1987; Pierce and Wessendorf, 2000; Greco et al., 2008; Lü et al., 2009). The parabrachial nucleus receives and sends opioid projections (Chen et al., 2004; Greco et al., 2008; Lü et al., 2009) and receives catecholaminergic projections from the hindbrain, locus coeruleus and paraventricular nucleus of the hypothalamus (Herbert, 1995; Sevigny et al., 2012). Opioid, gabaergic and α_2 adrenergic mechanisms investigated in the present study are suggested to reduce the action of the inhibitory mechanisms by acting directly in the LPBN output neurons or indirectly by reducing the release of serotonin, cholecystokinin, corticotrophin release factor, glutamate or other neurotransmitters (Menani et al., 2014). The present results suggest that gabaergic activation releases opioids in the LPBN to produce its effects on sodium intake. In addition, the present results suggest that part of the effects of gabaergic activation also depends on the release of noradrenaline and its actions on α_2 adrenoceptors. In turn, the effects of α_2 adrenoceptor activation may also depend on opioid release (Andrade et al., 2015), which suggests that directly or indirectly, through α_2 adrenoceptor activation, gabaergic activation releases opioids in the LPBN, which acts as the final pathway that deactivates LPBN inhibitory mechanisms, increasing sodium intake.

The release of neurotransmitters that modulate LPBN inhibitory mechanisms are controlled by signals from peripheral high and low pressure baroreceptors, cardiopulmonary volume receptors, taste receptors or other signals that may reach the LPBN through the area postrema/nucleus tractus solitarius (Toth et al., 1987; Thunhorst et al., 1994; Johnson and Thunhorst, 1997; Shimoura et al., 2013). From the LPBN, signals may ascend to integrative areas that also receive facilitatory signals from forebrain areas involved in the control of sodium

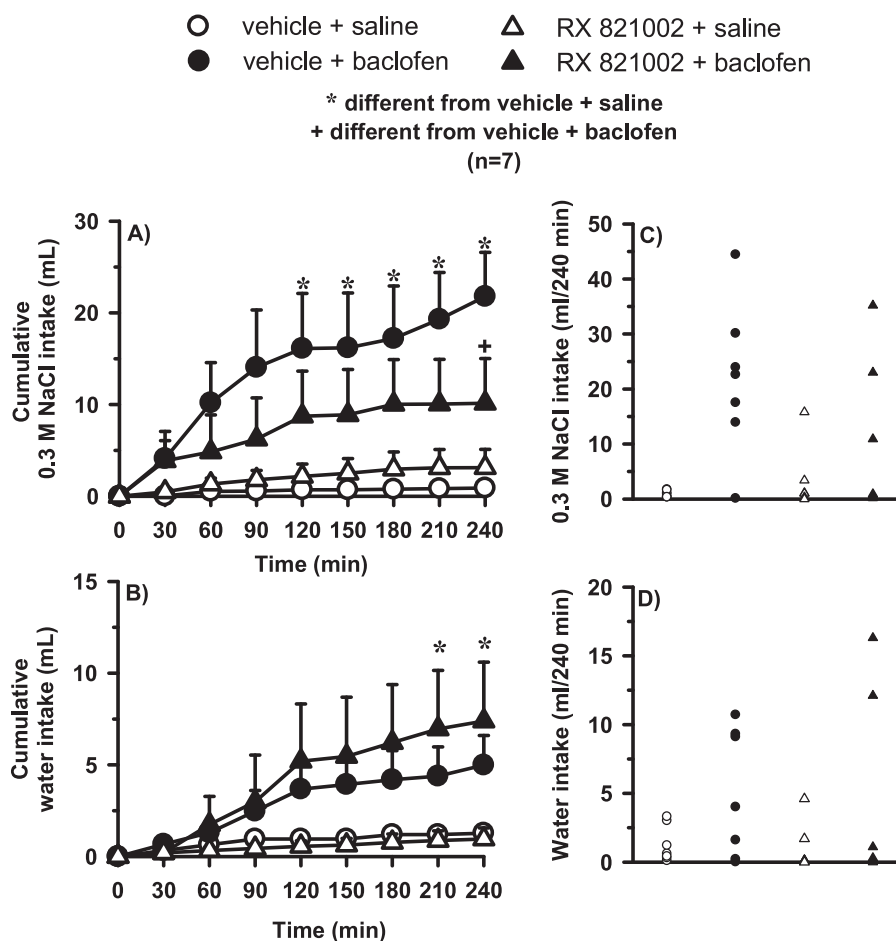


Fig. 5. Cumulative (A) 0.3 M NaCl intake and (B) water intake in normohydrated rats that received bilateral injections of vehicle or RX 821002 (10 nmol/0.2 μ l) combined with baclofen (0.5 nmol/0.2 μ l) or saline into the LPBN. Results are expressed as means \pm SEM, n = number of rats.

Table 1

Water and 0.3 M NaCl intake by normohydrated rats that received bilateral injections of naloxone or RX 821002 combined with muscimol or baclofen in sites outside the LPBN (misplaced injections).

Treatment	Water intake (ml/240 min)	0.3 M NaCl intake (ml/240 min)
<i>(n = 2)</i>		
vehicle + saline	2.9 \pm 2.0	0.7 \pm 0.5
vehicle + muscimol	2.4 \pm 1.4	4.9 \pm 2.8
naloxone + saline	0.2 \pm 0.1	0.5 \pm 0.3
naloxone + muscimol	1.8 \pm 1.1	3.9 \pm 2.6
<i>(n = 7)</i>		
vehicle + saline	1.5 \pm 0.6	0.9 \pm 0.3
vehicle + muscimol	3.9 \pm 1.8	2.0 \pm 0.8
RX 821002 + saline	1.9 \pm 0.6	1.3 \pm 0.5
RX 821002 + muscimol	3.8 \pm 0.6	3.0 \pm 1.0
<i>(n = 6)</i>		
vehicle + saline	1.0 \pm 0.5	0.3 \pm 0.1
vehicle + baclofen	2.7 \pm 1.0	3.5 \pm 1.9
naloxone + saline	0.3 \pm 0.1 ⁺	0.2 \pm 0.1
naloxone + baclofen	1.2 \pm 0.6	2.4 \pm 2.0
<i>(n = 3)</i>		
vehicle + vehicle	0.7 \pm 0.3	1.0 \pm 0.3
vehicle + baclofen	4.9 \pm 2.4	4.4 \pm 3.5
RX 821002 + saline	1.3 \pm 0.5	0.5 \pm 0.3
RX 821002 + baclofen	5.1 \pm 0.4	4.5 \pm 0.4

Results are expressed as means \pm SEM. n = number of rats. + Different from vehicle + baclofen. Naloxone (150 nmol/0.2 μ l), RX 821002 (10 nmol/0.2 μ l), muscimol or baclofen (0.5 nmol/0.2 μ l).

and water intake like, SFO, organum vasculosum of lamina terminalis and other hypothalamic areas (Menani et al., 2014). The ingestion of NaCl induced by muscimol injected into the LPBN is abolished by the blockade of angiotensinergic or cholinergic mechanisms in the SFO, suggesting that the activity of these mechanisms in the forebrain and particularly in the SFO is necessary for NaCl and water intake caused by muscimol injection into the LPBN in normohydrated rats (Asnar et al., 2013; Roncari et al., 2014). Although rats are normohydrated and not stimulated to ingest water or sodium, the blockade of LPBN inhibitory mechanisms with the injections of muscimol or baclofen disrupts satiety and drives rats to ingest hypertonic NaCl, a behavior that also involves signals produced by baseline levels of angiotensin II and/or acetylcholine acting in the SFO (Roncari et al., 2014).

Injections of β endorphin into the LPBN similar to gabaergic activation induce sodium intake in normohydrated rats, whereas the blockade of opioid receptors with naloxone, but not bicuculline (GABA_A receptor antagonist) injected into the LPBN reduced sodium depletion-induced sodium intake (de Oliveira et al., 2007; De Oliveira et al., 2008). Therefore, the present and previous studies (De Oliveira et al., 2008; Asnar et al., 2013; Roncari et al., 2014) suggest that gabaergic stimulation activates opioid mechanisms in the LPBN deactivating the inhibitory mechanisms, which allows the facilitatory mechanisms of the forebrain activated by baseline levels of angiotensin II and/or acetylcholine to drive rats to ingest sodium.

Conflict of interest

I declare that there is no conflict of interest.

Acknowledgments

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