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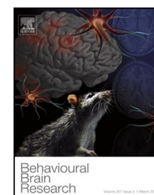

Research report

Lateral parabrachial nucleus and opioid mechanisms of the central nucleus of the amygdala in the control of sodium intake

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HIGHLIGHTS

- CeA facilitatory and LPBN inhibitory mechanisms interact to control NaCl intake.
- Naloxone in the CeA blocks NaCl intake by euhydrated rats with muscimol into the LPBN.
- Naloxone in the CeA blocks NaCl intake by hyperosmotic rats with LPBN moxonidine.
- Opioids in the CeA are important for sodium intake induced by LPBN deactivation.
- Connection between LPBN and CeA mechanisms may depend on opioids action in the CeA.

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ABSTRACT

Facilitatory and inhibitory mechanisms in the central nucleus of the amygdala (CeA) and the lateral parabrachial nucleus (LPBN), respectively, are important for the control of sodium and water intake. Here we investigated the importance of the opioid mechanisms in the CeA for water and 0.3 M NaCl intake in euhydrated or hyperosmotic rats treated with injections of muscimol ($GABA_A$ agonist) or moxonidine (α_2 adrenergic/imidazoline agonist) into the LPBN, respectively. Male Holtzman rats (n = 4–8/group) with stainless steel cannulas implanted bilaterally in the CeA and in the LPBN were used. The ingestion of 0.3 M NaCl and water by euhydrated rats treated with muscimol (0.5 nmol/0.2 μl) into the LPBN (29.4 ± 2.7 and 15.0 ± 2.4 ml/4 h, respectively) was abolished by the previous injections of naloxone (opioid antagonist, 40 μg/0.2 μl) into the CeA (0.7 ± 0.3 and 0.3 ± 0.1 ml/4 h, respectively). The ingestion of 0.3 M NaCl by rats treated with intragastric 2 M NaCl (2 ml/rat) combined with moxonidine (0.5 nmol/0.2 μl) into the LPBN (17.0 ± 3.8 ml/2 h) was also strongly reduced by the previous injections of naloxone into the CeA (3.2 ± 2.5 ml/2 h). Sucrose intake was not affected by naloxone injections into the CeA, which minimized the possibility of non-specific inhibition of ingestive behaviors with this treatment. The present results suggest that opioid mechanisms in the CeA are essential for hypertonic NaCl intake when the LPBN inhibitory mechanisms are deactivated or attenuated with injections of muscimol or moxonidine in this area.

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1. Introduction

The control of sodium appetite is strongly dependent on the action of the facilitatory mechanisms of the central nucleus of the amygdala (CeA) and the inhibitory mechanisms of the lateral parabrachial nucleus (LPBN), two areas suggested have functional interactions for the control of sodium intake [1–6].

The LPBN is reciprocally connected to forebrain areas such as the paraventricular nucleus of the hypothalamus (PVN), central nucleus of amygdala (CeA) and median preoptic nucleus (MnPO), and to medullary regions, like the area postrema (AP) and medial

Abbreviations: ANG II, angiotensin II; CAP, captopril; CeA, central nucleus of amygdala; FURO, furosemide; LPBN, lateral parabrachial nucleus; NTS, nucleus of the solitary tract; s.c., subcutaneously.

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portion of the nucleus of the solitary tract (mNTS) [7–14]. Cells in the LPBN are activated after ingestion of sodium solutions [15–17], suggesting that LPBN cells are activated by visceral or taste signals. Therefore, the LPBN may convey signals that ascend from AP/mNTS to forebrain areas involved in the control of fluid and electrolyte balance.

Different neurotransmitters modulate the activity of the LPBN inhibitory mechanisms. Some neurotransmitters like serotonin, cholecystokinin, corticotrophin-releasing factor (CRF) and glutamate increase the inhibitory action of the LPBN, reducing NaCl intake, whereas others like GABA, opioids, ATP and noradrenaline reduce the LPBN inhibitory action, increasing NaCl intake [5,18–28]. A microdialysis study also showed that the release of serotonin and its metabolite 5-hydroxyindoleacetic acid is reduced in the LPBN in fluid depleted rats and enhanced by 0.3 M NaCl intake [29].

In the CeA, the presence of facilitatory mechanisms for sodium intake was demonstrated by different studies using electrolytic lesions or neuronal blockade with injections of the $GABA_A$ agonist muscimol [1,2,30,31]. Electrolytic lesions or injections of muscimol into the CeA also abolished the ingestion of water and 0.3 M NaCl caused by the deactivation of the LPBN mechanisms, suggesting an interaction between the facilitatory mechanisms of the CeA and the inhibitory mechanisms of the LPBN [3,4,6].

Injections of DAMGO (μ-opioid agonist) into the CeA increased 0.3 M NaCl intake and the previous administration of CTAP (μ-opioid antagonist) in the same area reversed these effects [32]. The μ opioid receptor agonists usually produce neuronal hyperpolarization mediated by potassium channel opening, causing inhibition of CeA neurons [33,34]. However, DAMGO in the CeA surprisingly increased 0.3 M NaCl, suggesting that DAMGO increases the activity of CeA facilitatory mechanisms. To produce these effects, DAMGO may remove the action of some inhibitory mechanism like the GABAergic mechanism present in the CeA, similarly to that proposed for nociception in other central areas [35]. The activity of the CeA is essential for the ingestion of water and 0.3 M NaCl caused by the deactivation of the LPBN mechanisms and the increase of sodium intake produced by opioid activation in the CeA is similar to the blockade of LPBN mechanisms [3,4,6,32]. Therefore, it is possible that opioid mechanisms in the CeA are important for the increase of sodium intake produced by the blockade of the LPBN inhibitory mechanisms. The CeA receives enkephalinergic projections from different areas involved in the control of fluid and electrolyte balance like the ventromedial nucleus of the hypothalamus, the parabrachial nucleus and mainly from the bed nucleus of the stria terminalis (BST) and other amygdaloid nuclei [36]. Signals from the LPBN may affect the release of opioid in the CeA by direct connections with the CeA or indirect connections with other areas like other amygdaloid nuclei.

To investigate the importance of the opioid mechanisms in the CeA for sodium intake that occurs after the blockade of the LPBN inhibitory mechanisms, in the present study, 0.3 M NaCl and water intake was tested in euhydrated rats treated with muscimol into the LPBN or hyperosmotic rats treated with moxonidine (α_2 adrenergic/imidazoline receptor agonist) into the LPBN combined with naloxone (opioid receptor antagonist) into the CeA. The previous study showed the importance of opioids for water and 0.3 M NaCl intake induced by water deprivation-partial rehydration or by the treatment with the diuretic furosemide combined with low dose of captopril (angiotensin converting enzyme inhibitor), two protocols that induce sodium intake due to the activation of angiotensinergic mechanisms [32]. In the present study, 0.3 M NaCl and water intake was tested in euhydrated or hyperosmotic rats treated with bilateral injections of muscimol or moxonidine, respectively, into the LPBN. In these conditions, rats ingest strong amount of 0.3 M NaCl, although they have no activation of the angiotensinergic mechanisms [22–24,37–39]. The present results show that opioid mechanisms in the CeA are also important for sodium intake in these conditions.

2. Experimental procedures

2.1. Animals

Adult male Holtzman rats (n = 33) bred in the UNESP facility at School of Dentistry, Araraquara, SP, Brazil, weighing 250–270 g at the beginning of the tests were used. The animals were housed in individual stainless steel cages with free access to normal 0.5–1.0% sodium diet (BioBase rat chow, Brazil), water and 0.3 M NaCl. Rats were maintained at a temperature of 23 ± 2°C, humidity of 55 ± 10% and on a 12-h light/dark cycle with light onset at 7:00 A.M. All the experimental procedures were approved by Ethical Committee in Animal Use (CEUA) from Dentistry School of Araraquara – UNESP (Proc. CEUA nr. 35/2010). 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).

2.2. Brain surgery

Rats were anesthetized with ketamine (80 mg/kg of body weight; Cristalia, Itapira, SP, Brazil) combined with xylazine (7 mg/kg of body weight; Agener União, Embu-Guaçu, SP, Brazil) intraperitoneally, placed in a stereotaxic instrument (Kopf, Tujunga, CA, USA) and had the skull leveled between bregma and lambda. Bilateral stainless steel cannulas (0.6 mm o.d.) were implanted dorsally to the LPBN and CeA using the following coordinates: 9.2 mm caudal to bregma, 2.1 mm lateral to the midline and 4.2 mm below the dura mater for the LPBN and 2.2 mm caudal to bregma, 4.0 mm lateral to the midline and 4.5 mm below the dura mater for the CeA [40]. The tips of the cannulas were positioned 2 mm above the LPBN and the CeA. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. A metal obturator (0.3 mm o.d.) filled the cannulas between tests. At the end of the surgery, the animals received an intramuscular injection of antibiotic (Pentabiótico Veterinário – Pequeno Porte, Fort Dodge Saúde Animal Ltda., 0.2 ml/rat) and a subcutaneous injection of the analgesic Ketoflex (ketoprofen 1%, 0.03 ml/rat). The experimental procedures started five days after the surgery. Rats were tested in their home cages.

2.3. Drugs

Moxonidine hydrochloride (α_2-adrenoceptor/imidazoline agonist, 0.5 nmol/0.2 μl) was dissolved in a mix of propylene glycol and water 2:1 (vehicle). Muscimol HBr ($GABA_A$ receptor agonist, 0.5 nmol/0.2 μl) and naloxone hydrochloride (nonspecific opioid antagonist, 40 μg/0.2 μl) were dissolved in saline. All drugs were purchased from Sigma-Aldrich Chem., St Louis, MO, USA.

The doses of the drugs used were based on previous studies that injected these drugs centrally [6,37,41].

2.4. Injections into the LPBN and CeA

Injections into the LPBN and the CeA were made using 5-μl Hamilton syringes (Hamilton, Reno, NV, USA) connected by polyethylene tubing (PE-10) to injection needles (0.3 mm o.d.). Starting one day after cerebral surgery, rats were handled daily and trained for the procedure of central injections. At time of testing, rats were removed from the cages and restrained by a hand on a table. Obturators were removed and the injection needles (2 mm longer than the guide cannulas) introduced in the brain. Injections into the CeA and LPBN were performed separately in each side. The duration of each injection was about 30 s.

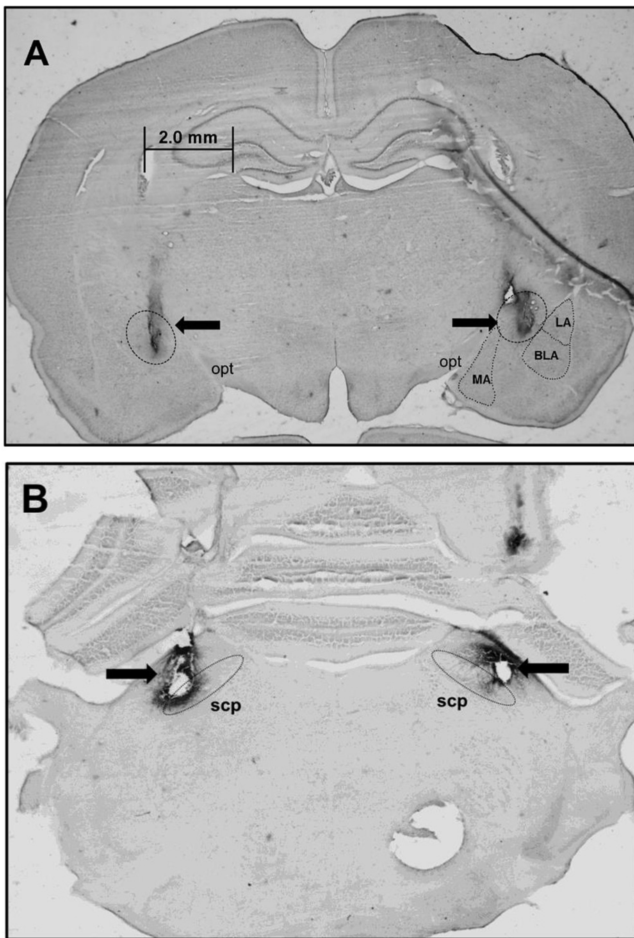

Fig. 1. Photomicrographs of coronal sections of a brain from one rat representative of the group studied showing (arrows) (A) the sites of bilateral injections into the CeA and, (B) the sites of bilateral injections into the LPBN. BLA, basolateral amygdaloid nucleus; LA, lateral amygdaloid nucleus; MA, medial amygdaloid nucleus; OPT, optic tract; SCP, superior cerebellar peduncle.

The injection volume into the LPBN and the CeA was 0.2 μl each site. The obturators were replaced after injections, and the rats placed back into the cages.

2.5. Water and 0.3 M NaCl intake by euhydrated rats treated with bilateral injections of muscimol into the LPBN combined with naloxone into the CeA

Euhydrated rats with cannulas implanted bilaterally in the CeA and the LPBN received bilateral injections of saline or naloxone (40 μg/0.2 μl) into the CeA and ten minutes later received bilateral injections muscimol (0.5 nmol/0.2 μl) or saline into the LPBN. The blockade of the neuronal activity with injections of muscimol into the LPBN causes robust ingestion of 0.3 M NaCl in euhydrated rats that received no additional treatment [4,37–39,42]. Water and 0.3 M NaCl intake was measured every 30 min for 4 h. During this period, rats had no access to food.

For this study, one group of animals was submitted to two tests with an interval of at least 48 h between them. In each test, the animals were divided into two subgroups and each subgroup received the following combinations of treatment: (1) saline into the CeA + muscimol into the LPBN; (2) naloxone into the CeA + muscimol into the LPBN, in a counterbalanced manner in the two tests. Another group of animals was also divided into two subgroups and submitted to two tests with each subgroup receiving one the following combinations of treatment in each test: (1) saline into the CeA + saline into the LPBN; (2) naloxone into the CeA + saline into the LPBN, also in a counterbalanced manner in the two tests.

2.6. Water and 0.3 M NaCl intake by hyperosmotic rats treated with bilateral injections of moxonidine into the LPBN combined with naloxone into the CeA

Rats with cannulas implanted bilaterally in the CeA and the LPBN received an intragastric gavage of 2 M NaCl (2 ml/rat) after removing water, 0.3 M NaCl and food from the animals' cage. This intragastric 2 M NaCl load produces 4% elevation of both plasma osmolality and sodium concentration inducing cell dehydration and thereby fluid intake [43]. Concurrent reduction of plasma renin activity and no alteration in plasma volume indicate that the procedure does not induce extracellular dehydration [43]. Forty-five minutes after the gavage, moxonidine (0.5 nmol/0.2 μl) or vehicle was injected bilaterally into the LPBN. Fifteen minutes later, naloxone (40 μg/0.2 μl) or saline was bilaterally injected into the CeA and immediately the animals had access to both water and 0.3 M NaCl in graduated (0.1 ml divisions) glass burettes. Water and 0.3 M NaCl intake was measured every 30 min for 2 h. For this study, one group of animals was submitted to two tests with an interval of at least 72 h between them. In each test, the animals were divided into two subgroups, and each subgroup received one of the following combinations of treatment: (1) moxonidine into the LPBN + saline into the CeA; (2) moxonidine into the LPBN + naloxone into the CeA, in a counterbalanced manner in the two tests. Another group of animals was also divided into two subgroups and submitted to two tests with each subgroup receiving one the following combinations of treatment: (1) saline into the LPBN + saline into the CeA; (2) saline into the LPBN + naloxone into the CeA, in a counterbalanced manner in the two tests.

2.7. Water and 0.06 M sucrose intake in rats treated with bilateral injections of naloxone into the CeA

To test if injections of naloxone into the CeA produced non specific inhibition of ingestive behaviors, the effects of naloxone injected into the CeA on 0.06 M sucrose intake were also analyzed in a group of rats that was trained to ingest sucrose. These rats received food and water ad libitum and had access to 0.06 M sucrose for 2 h every day during 1 week (i.e. a dessert test) [23]. After this period, naloxone (40 μg/0.2 μl) or saline was injected bilaterally into the CeA 15 min before rats had access to 0.06 M sucrose solution. Cumulative water and 0.06 M sucrose solution intake were measured at each 30 min for 2 h. Half of the group received saline and the other received naloxone into the CeA. This procedure was reversed in a second experimental session performed 3 days later.

2.8. Histology

At the end of ingestive behavior tests, rats received bilateral injections of 2% Evans blue solution (0.2 μl) into the LPBN and the CeA. 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 Giemsa stain and analyzed by light microscopy to confirm the injection sites into the LPBN and the CeA.

2.9. Statistical analysis

The results are reported as means ± S.E.M. Two way analysis of variance (ANOVA) using treatments and times as within-subjects factors followed by Newman-Keuls test was used for comparisons. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Histological analysis

Fig. 1A shows the typical bilateral injection sites into the CeA. The CeA injection sites were located laterally to the tip of the optic tract, above the basomedial amygdaloid nucleus and medial to the lateral amygdaloid nucleus. The sites of the injections in the present study were similar to those of previous studies that showed the effects of lesions or injections of muscimol or opioid drugs in the CeA on NaCl intake [3,4,6].

Fig. 1B shows the typical bilateral injection sites into the LPBN. The LPBN injection sites were centered in the central lateral and dorsal lateral portions of the LPBN [see Ref. [9], for definitions of LPBN subnuclei]. The sites of the injections in the present study were similar to those of previous studies that showed the effects of muscimol or moxonidine injected into the LPBN on NaCl and water intake [3,4,6,23,24,44].

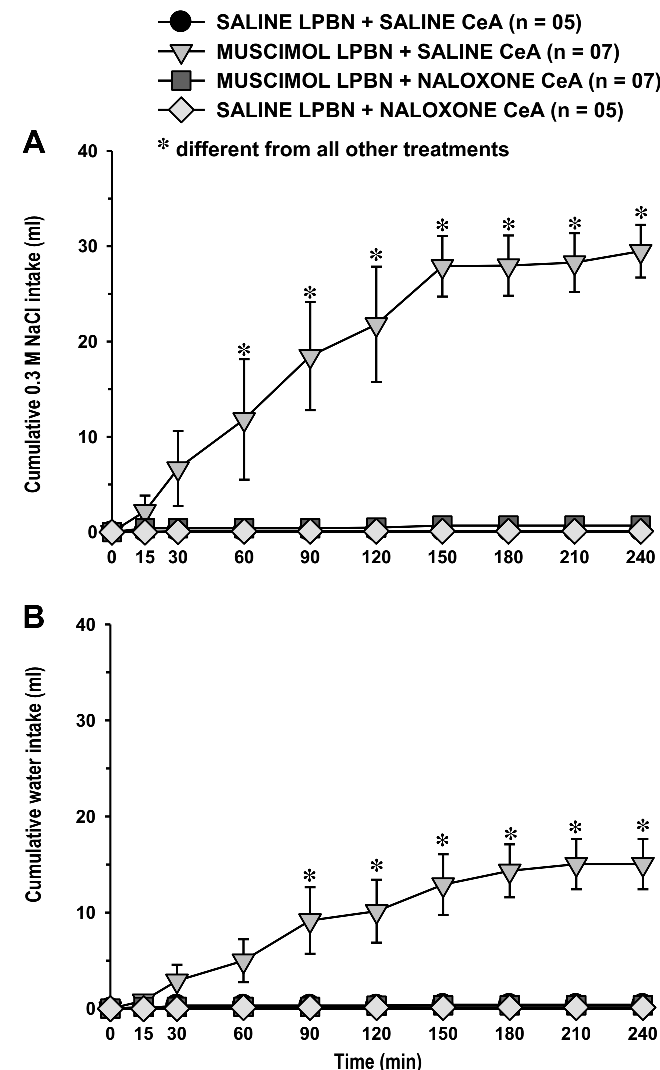

Fig. 2. Cumulative (A) 0.3 M NaCl and (B) water intake by euhydrated rats that received bilateral injections of naloxone (40 μg/0.2 μl) or saline into the CeA combined with bilateral injections of muscimol (0.5 nmol/0.2 μl) or saline into the LPBN. The results are expressed as means ± SEM, n = number of animals.

3.2. Water and 0.3 M NaCl intake by euhydrated rats treated with bilateral injections of muscimol into the LPBN combined with naloxone into the CeA

Rats (n = 7) treated with bilateral injections of muscimol (0.5 nmol/0.2 μl) into the LPBN combined with saline injections into the CeA ingested significant amount of 0.3 M and water (Fig. 2). Bilateral injections of naloxone (40 μg/0.2 μl) into the CeA completely abolished 0.3 M NaCl intake and water intake in rats treated with muscimol (0.5 nmol/0.2 μl) into the LPBN (Fig. 2). Injections of naloxone into the CeA combined with vehicle into the LPBN (n = 5) did not affect 0.3 M NaCl or water intake (Fig. 2).

ANOVA showed significant effect of treatment and time for NaCl [$F(3, 180) = 141.0$; $p < 0.05$] and [$F(8, 180) = 4.4$; $p < 0.05$], respectively, and for water intake [$F(3, 180) = 87.5$; $p < 0.05$] and [$F(8, 180) = 3.2$; $p < 0.05$], respectively. There was also an interaction between treatment and time for NaCl [$F(24, 180) = 4.5$; $p < 0.05$] and water intake [$F(24, 180) = 3.3$; $p < 0.05$] (Fig. 2).

3.3. Water and 0.3 M NaCl intake by hyperosmotic rats treated with bilateral injections of moxonidine into the LPBN combined with naloxone into the CeA

Rats (n = 4) treated with 2 M NaCl ig that received bilateral injections of moxonidine (0.5 nmol/0.2 μl) into the LPBN combined with saline injections into the CeA ingested significant amount of 0.3 M NaCl (Fig. 3A). Bilateral injections of naloxone (40 μg/0.2 μl) into the CeA abolished 0.3 M NaCl intake in rats treated with 2 M NaCl load combined with bilateral injections of moxonidine into the LPBN (Fig. 3A). Bilateral injections of naloxone (40 μg/0.2 μl) into the CeA combined with bilateral injections of vehicle into the LPBN (n = 5) did not change 0.3 M NaCl intake in rats treated with 2 M NaCl load (Fig. 3A). The only difference in water intake was between vehicle + naloxone and moxonidine + saline (Fig. 3B).

ANOVA showed a significant effect of treatment for NaCl [$F(3, 56) = 61.6$; $p < 0.05$] and for water intake [$F(3, 56) = 10.76$; $p < 0.05$]. There was no significant effect of time for NaCl [$F(3, 56) = 0.56$; $p > 0.05$] and water [$F(3, 56) = 0.94$; $p > 0.05$]. ANOVA also showed no interaction between treatment and time for NaCl [$F(9, 56) = 0.23$; $p > 0.05$] and water intake [$F(9, 56) = 0.31$; $p > 0.05$] (Fig. 3).

3.4. Water and 0.06 M sucrose intake in rats treated with bilateral injections of naloxone into the CeA

To test if injections of naloxone into the CeA produced non specific inhibition of ingestive behaviors, the effects of naloxone injected into the CeA on 0.06 M sucrose intake were also analyzed.

Table 1
Water and 0.06 M sucrose intake by rats that received injections of naloxone or saline bilaterally into the CeA.

Treatment	30 min	60 min	90 min	120 min
		0.06 M sucrose (ml)		
Saline	4.2 ± 1.3	4.2 ± 1.3	4.2 ± 1.3	4.2 ± 1.3
Naloxone	2.9 ± 1	3.3 ± 1	3.3 ± 1	3.4 ± 1
		Water intake (ml)		
Saline	0.7 ± 0.7	0.7 ± 0.7	0.7 ± 0.7	0.7 ± 0.7
Naloxone	0.8 ± 0.5	0.8 ± 0.5	0.8 ± 0.5	0.8 ± 0.5

The results are expressed as means ± SEM, n = 8. Naloxone (40 μg/0.2 μl).

In rats trained to ingest 0.06 M sucrose (n = 8), bilateral injections of naloxone (40 μg/0.2 μl) into the CeA produced no change on 0.06 M sucrose intake or water intake (Table 1).

ANOVA showed no significant effect of treatment for 0.06 M sucrose [$F (1, 7) = 1.10$; $p > 0.05$] and water intake [$F (1, 7) = 0.02$; $p > 0.05$]. ANOVA also showed no significant effect of time [$F (3, 21) = 1.1$; $p > 0.05$] or interaction between treatment and time for 0.06 M sucrose intake [$F (3, 21) = 1.1$; $p > 0.05$] (Table 1).

3.5. Water and 0.3 M NaCl intake by rats that received injections of naloxone outside of the CeA combined with muscimol injected into the LPBN

To demonstrate the anatomical specificity CeA for the effects of naloxone, the results from rats (n = 4) that received injections outside the CeA (misplaced injections) were also analyzed. Injections outside the CeA reached the basomedial amygdaloid nucleus, the basolateral amygdaloid nucleus, the medial amygdaloid nucleus or the external globus pallidus.

In rats treated with muscimol (0.5 nmol/0.2 μl) into the LPBN, injections of naloxone (40 μg/0.2 μl) outside the CeA did not significantly reduce 0.3 M NaCl (22.4 ± 9.1, vs. saline + muscimol: 30.3 ± 4.8 ml/4 h, n = 4), [$F (1, 3) = 2.4$; $p > 0.05$]. This ingestion of 0.3 M NaCl of rats that received naloxone outside the CeA is strikingly different from the ingestion of rats treated with naloxone bilaterally into the CeA (0.7 ± 0.3 ml/4 h, Fig. 2), [$F (3, 18) = 13.8$; $p < 0.05$].

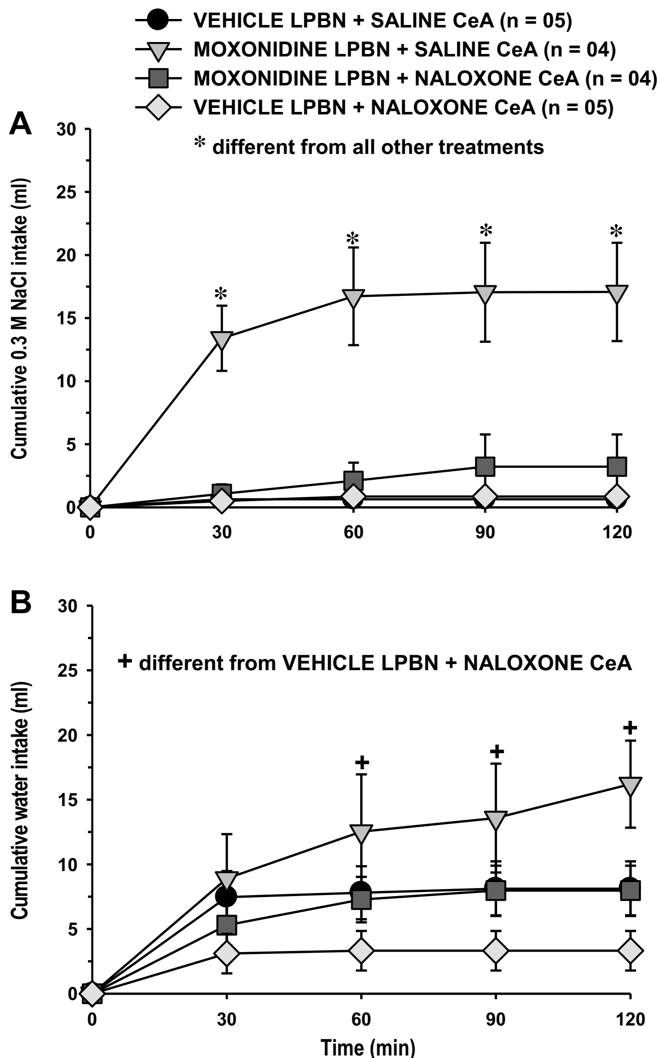

Fig. 3. Cumulative (A) 0.3 M NaCl and (B) water intake in rats with intracellular dehydration that received bilateral injections of moxonidine (0.5 nmol/0.2 μl) or vehicle into the LPBN combined with bilateral injections of naloxone (40 μg/0.2 μl) or saline into the CeA. The results are expressed as means ± SEM, n = number of animals.

4. Discussion

The present results show that bilateral injections of naloxone into the CeA abolished 0.3 M NaCl and water intake in euhydrated rats treated with muscimol into the LPBN and in hyperosmotic rats that received bilateral injections of moxonidine into the LPBN, suggesting that opioid mechanisms in the CeA are important for sodium intake that arises when the activity of LPBN inhibitory mechanisms is reduced or blocked by the injections of moxonidine or muscimol into this area.

The ingestion of water when the animals simultaneously ingest hypertonic NaCl and water may be due to the increase in plasma osmolarity and osmoreceptor activation caused by excessive hypertonic NaCl intake. Therefore, reduction in water consumption as that produced by the treatment with naloxone may also be a consequence of the reduced 0.3 M NaCl intake. Tests in which only water is available for the animals to ingest are necessary for a definitive conclusion about possible effects on water intake. In spite of the suggestion that CeA is activated after sucrose ingestion [45], the present results show that the treatment with naloxone into the CeA produced no change of sucrose intake, which minimized the possibility of non-specific inhibition of ingestive behaviors with the injection of naloxone into the CeA in the condition of the present study.

Electrolytic lesions of the CeA or neuronal deactivation with injections of muscimol into the CeA strongly reduces fluid depletion-induced sodium intake, suggesting that important facilitatory mechanisms for sodium intake are present in the CeA [1,2,31]. Electrolytic lesions of the CeA or injections of muscimol into the CeA also abolished NaCl and water intake in euhydrated rats treated with bilateral injections of muscimol into the LPBN and the increase in 0.3 M NaCl produced by bilateral injections of moxonidine into the LPBN in animals pretreated with furosemide + captopril s.c., suggesting that the activation of facilitatory mechanisms in the CeA is an essential step for sodium intake when LPBN inhibitory mechanisms are deactivated [4,6]. Sodium intake in rats treated with intragastric 2 M NaCl combined with moxonidine into the LPBN or by muscimol into the LPBN in euhydrated rats may depend on cholinergic and angiotensinergic mechanisms in the forebrain or more specifically in the subfornical organ (SFO) [38,39,42,46]. Signals produced by ANG II and hyperosmolarity acting in the SFO probably reach the CeA and activate the facilitatory mechanisms for sodium intake that are counterbalanced by the action of the LPBN inhibitory mechanisms [5].

A previous study testing the effects of injections of opioid agonist and antagonist (DAMGO and CTAP, respectively) into the CeA demonstrated that the activation of opioid mechanisms in the CeA increases sodium intake induced by water deprivation-partial rehydration or sc furosemide + captopril, tests in which sodium intake is induced by the activation of angiotensinergic mechanisms [32]. The present results show that the increase of sodium intake caused by the deactivation of the LPBN inhibitory mechanisms in euhydrated or hyperosmotic rats is abolished by the blockade of opioid receptors in the CeA, which extends the conclusion of the previous study [32], suggesting that the activity of opioid mechanisms in the CeA is also necessary for sodium intake in these conditions.

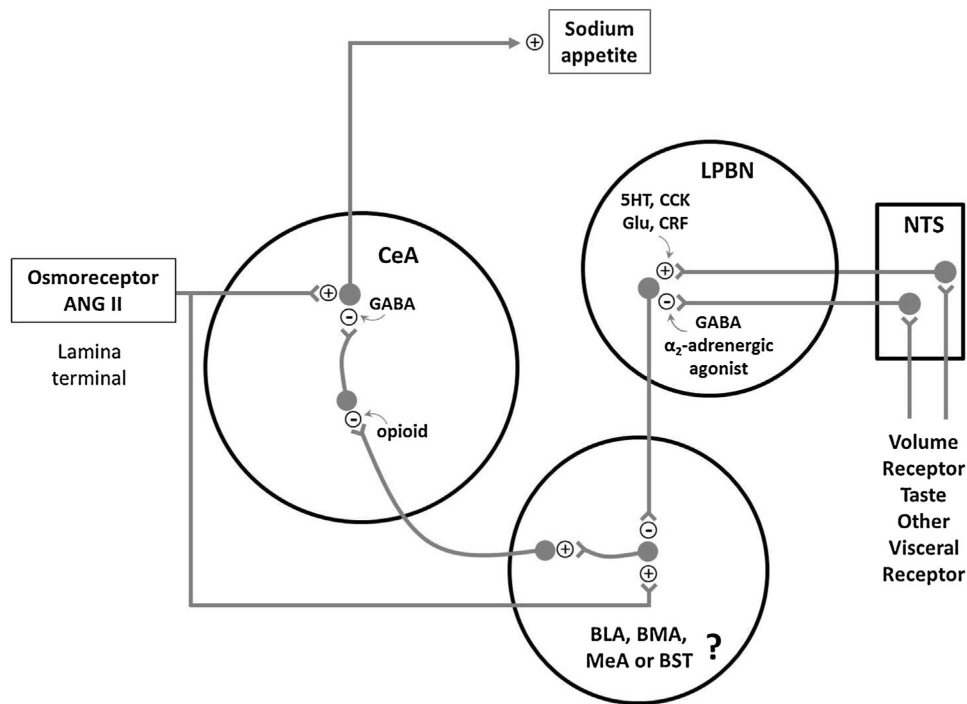

Fig. 4. Schematic diagram showing the possible interaction between the LPBN and the CeA opioid mechanisms for the control of sodium intake. +, excitation; −inhibition; 5HT, serotonin; CCK, cholecystokinin; glu, glutamate; ANG II, angiotensin II; CRF, corticotrophin releasing factor; BLA, basolateral amygdaloid nucleus; BMA, basomedial amygdaloid nucleus; MeA, medial amygdaloid nucleus; BST, bed nucleus of the stria terminalis; CeA, central nucleus of amygdala; LPBN, lateral parabrachial nucleus; NTS, nucleus of the solitary tract.

Opioid peptides and receptors are present in the CeA [36,47,48]. The majority of the CeA neurons are inhibited by μ opioid receptor agonists, which induce hyperpolarization mediated by potassium channel opening [33,34]. Opioid receptor activation in the CeA facilitates sodium intake [32] and present results unlike the neuronal inhibition produced by the injections of the GABAergic agonist muscimol into the CeA that abolishes sodium intake, probably removing the action of facilitatory mechanisms similar to electrolytic lesions of the CeA [6,31]. Therefore, GABAergic mechanisms may reduce the action of the facilitatory mechanisms of the CeA, while the opioid mechanisms produce opposite effects.

One hypothesis is that opioid receptor activation inhibits GABAergic neurons in the CeA driving rats to ingest sodium, a mechanism that might be modulated by the LPBN. Fig. 4 is a schematic model that illustrates a possible interaction between the inhibitory mechanisms of the LPBN and the facilitatory mechanisms of the CeA. The LPBN receives signals from baroreceptors, volume receptors, gustatory receptors or other visceral receptors that are important for the control of sodium and water intake. These signals reach the AP/mNTS before ascending to the LPBN that send inhibitory signals to forebrain areas involved in the control of fluid and electrolyte balance, like the CeA [5,7,8,10–14]. Signals that act in the CeA stimulating sodium appetite may arise from forebrain areas involved in the control of sodium and water intake, particularly in the lamina terminalis [5,49–52]. Recent studies have also shown that GABAergic mechanisms in the CeA inhibit sodium intake, whereas opioid mechanisms facilitate sodium intake induced by ANG II [31,32]. To explain how two inhibitory neurotransmitters in the CeA produce opposite effects on sodium intake, in Fig. 4 it is proposed that the activation of opioid mechanisms in the CeA may reduce the activity of GABAergic neurons in the CeA, as proposed for nociception in other central areas [35]. The CeA receives enkephalinergic afferents from the ventromedial nucleus of the hypothalamus, the parabrachial nucleus and mainly from the BST and other amygdaloid nuclei [36]. The present results show that opioid mechanisms in the CeA are important for the increase of sodium intake produced by the blockade of LPBN mechanisms, suggesting a possible interaction between LPBN and the opioid action in the CeA. The connection between the LPBN and the CeA may involve multi-synaptic pathways and possible a third area like the BST or other amygdaloid nuclei as shown in Fig. 4. The suggestion is that LPBN may inhibit the neurons of the BST or other amygdaloid nuclei that project and release opioid in the CeA, reducing the action of the signals from the lamina teminalis in these neurons. The mechanisms proposed in Fig. 4 is a tentative to explain the functional interaction between LPBN and CeA as suggested by the present data. More studies are necessary to confirm the mechanisms proposed in Fig. 4.

In summary, opioid mechanisms in the CeA are essential for sodium intake when the inhibitory mechanisms are deactivated by muscimol or moxonidine injected into the LPBN in euhydrated or hyperosmotic rats, respectively, which suggests that opioid mechanisms in the CeA are important for the increased activity of the facilitatory mechanisms of the CeA when the inhibitory action of the LPBN is reduced or removed.

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