

Would right atrial stretch inhibit sodium intake following GABA_A receptor activation in the lateral parabrachial nucleus?



Caroline Gusson Shimoura^a, Silas Pereira Barbosa^b, Jose Vanderlei Menani^b, Juliana Irani Fratucci De Gobbi^{a,*}

^a Department of Physiology, Institute of Bioscience, São Paulo State University (UNESP), 18618-000 Botucatu, SP, Brazil

^b Department of Physiology and Pathology, School of Dentistry, São Paulo State University (UNESP), 14801-903 Araraquara, SP, Brazil

HIGHLIGHTS

- GABA_A into the lateral parabrachial nucleus (LPBN) induces sodium intake.
- Simulating volume overload with right atrial stretch decreases fluid intake.
- Influence of GABA_A into the LPBN associated with right atrial stretch was tested.
- GABA_A into the LPBN abolished the right atrial stretch effect on fluid intake.

ARTICLE INFO

Article history:

Received 3 February 2013

Received in revised form 31 July 2013

Accepted 3 August 2013

Keywords:

Right atrial stretch

Lateral parabrachial nucleus

Sodium intake

GABA_A

Cardiopulmonary volume receptors

Circulating volume

ABSTRACT

The knowledge of the mechanisms underlying circulating volume control may be achieved by stretching a balloon placed at the junction of the superior vena cava–right atrial junction (SVC–RAJ). We investigated whether the inflation of a balloon at the SVC–RAJ inhibits the intake of 0.3 M NaCl induced by GABA_A receptor activation in the lateral parabrachial nucleus (LPBN) in euhydrated and satiated rats. Male Wistar rats (280–300 g) with bilateral stainless steel LPBN cannulae and balloons implanted at the SVC–RAJ were used. Bilateral injections of the GABA_A receptor agonist muscimol (0.5 μmol/0.2l) in the LPBN with deflated balloons increased intake of 0.3 M NaCl (30.1 ± 3.9 vs. saline: 2.2 ± 0.7) ml/210 min, *n* = 8) and water (17.7 ± 1.9 vs. saline: 2.9 ± 0.5 ml/210 min). Conversely, 0.3 M NaCl (27.8 ± 2.1 ml/210 min) and water (22.8 ± 2.3 ml/210 min) intake were not affected in rats with inflated balloons at the SVC–RAJ. The results show that sodium and water intake induced by muscimol injected into the LPBN was not affected by balloon inflation at the SVC–RAJ. We suggest that the blockade of LPBN neuronal activity with muscimol injections impairs inhibitory mechanisms activated by signals from cardiopulmonary volume receptors determined by balloon inflation.

© 2013 The Authors. Published by Elsevier Ireland Ltd. Open access under [CC BY license](http://creativecommons.org/licenses/by/3.0/).

1. Introduction

Knowledge of the mechanisms underlying the control of circulating volume may strengthen understanding of some pathophysiological processes related to edematous states such as heart failure. Cardiopulmonary volume receptors are suggested to regulate circulating volume [8,9,15,17] and may be activated by a distension of a small intravascular balloon placed at the superior vena cava–right atrial junction [SVC–RAJ, 1]. Inflation of a balloon at the SVC–RAJ attenuates water drinking in response to 24 h

water deprivation, subcutaneous injection of isoproterenol, peritoneal dialysis with hyperoncotic colloid, and *ad libitum* water intake without affecting drinking in response to intravenous hypertonic saline in rats [9]. In addition, balloon inflation also reduces sodium intake induced by overnight sodium depletion, peritoneal dialysis with hypertonic colloid, or by daily injections of deoxycorticosterone acetate [DOCA, 17]. Ablation of the ventrolateral parabrachial nucleus (LPBN) abolished the reduction in fluid intake caused by isoproterenol injection following balloon inflation [15]. Furthermore, balloon inflation at the SVC–RAJ increases immunohistochemically detected Fos expression in the LPBN following acute sodium depletion [8]. Conversely, neurons in the LPBN respond to hemorrhage [3,18]. Therefore, the LPBN is a central site involved in control of circulating volume.

The LPBN is strategically positioned in the pons and receives important visceral-related information that ascends from the area postrema/nucleus of the solitary tract (AP/NTS) being reciprocally

* Corresponding author. Tel.: +55 1438800319.

E-mail address: jdegobbi@ibb.unesp.br (J.I.F. De Gobbi).

connected with forebrain areas involved in behavioral and autonomic responses [5,12,15]. The NTS is the primary site in the central nervous system that receives important afferents from visceral receptors involved in the control of fluid and electrolyte balance [8]. From the NTS, the signals from visceral receptors might reach the LPBN before ascending to forebrain sites involved in the control of fluid and electrolyte balance.

The participation of the LPBN in the control of fluid intake has been extensively described for a variety of neurotransmitters such as serotonin, cholecystokinin, corticotropin-releasing hormone, glutamate, opioids, noradrenaline, and GABA [1,4,6,7,13,14,16]. The effect of GABA on the LPBN is particularly interesting, and it has been shown that bilateral injections of the GABA_A receptor agonist muscimol in the LPBN result in strong intake of 0.3 M NaCl in fluid-replete rats [4]. However, similar inhibition of the LPBN by activation of GABA_A receptors does not affect sucrose intake, suggesting that its effects on sodium are not a result of the non-specific effect of GABA_A receptor activation on the LPBN [16]. It should be noted that the effects of muscimol injection into the LPBN on sodium intake are not secondary to reductions in blood pressure or increased sodium urinary excretion [4,16].

Considering the importance of cardiopulmonary/volume receptors, their possible action through the LPBN, and the effects of the neuronal deactivation of the LPBN with muscimol on sodium and water intake, we investigated whether the inflation of a balloon at the SVC–RAJ affects 0.3 M NaCl and water intake induced by bilateral injections of muscimol into the LPBN in fluid-replete rats.

2. Methods

2.1. Animals

Male Wistar rats weighing 280–300 g were used. They were housed individually in hanging, stainless steel cages with free access to standard laboratory diet (Labina Purina® Rat Chow), water, and 0.3 M NaCl solution. Temperature was maintained at $23 \pm 2^\circ\text{C}$ and humidity at $54 \pm 10\%$, on a 12:12 light:dark cycle (onset at 6:00 am). All procedures were in accordance with the Animal Care and Use policies of the Institute of Biosciences at Botucatu, Brazil and the Brazilian College of Animal Experimentation (COBEA) policies. All efforts were made to minimize animal discomfort and the number of animals used.

2.2. Drugs

Muscimol HBr (a gabaergic receptor agonist) was purchased from Research Biochemicals International (RBI; Natick, MA). Saline was injected in the LPBN as control.

2.3. Cerebral cannulae

Rats were anesthetized with ketamine (80 mg/kg body weight, Dopalen®, Vetbrands, Jacaréí, SP) combined with xilazine (7 mg/kg body weight, Anasedan®, Vetbrands, Jacaréí, SP) and placed in a Kopf Model 900 stereotaxic instrument (David Kopf Instruments). The skull was leveled between bregma and lambda. Then, 23-gauge stainless steel cannulae were implanted bilaterally into the LPBN at 9.5 mm caudal to bregma, 2.2 mm lateral to the midline, and 4.1 mm below the dura mater [similar to previous studies: 6,7,13,14,16]. The tips of the cannulae were positioned at points 2 mm above each LPBN. The cannulae were fixed to the cranium using dental acrylic resin and jeweler's screws. A 30-gauge metal obturator filled the cannulae between tests. After the surgery, the rats received intramuscular injections of the analgesic ketoprofen 1% (0.03 ml/rat) and a prophylactic dose of the antibiotic penicillin (30,000 IU). Rats

were allowed to recover for four days before the surgery for balloon placement.

2.4. Balloon placement

Inflatable balloons were made of Silastic® tubing. One end of the tube was sealed with silicone glue and allowed to dry. Then, a small portion close to this end was warmed, stretched, and expanded repeatedly by injecting saline into it [according to previous studies: 8, 15]. The rats were anesthetized for balloon placement as described above for implantation of cerebral cannulae. In the rat, the left superior vena cava joins the inferior vena cava, so that placement of the balloon does not interfere with venous return from the head to the heart [9]. Thus, the right jugular vein was exposed where the inflatable balloon was inserted and positioned to terminate at the SVC–RAJ [as described in 9]. The Silastic® tube was tunneled subcutaneously, exteriorized at the nape of the neck, and fixed with suture thread. The animals were allowed three days to recover from surgery before starting the tests. During the tests, balloons were inflated by filling them with isotonic saline (0.1 ml), this inflatable portion produce an expansion of 5 mm of diameter.

2.5. Injections into the LPBN

Bilateral injections into the LPBN were made using 10 μl Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulae. At the time of testing the rats were removed from the home cage, the obturators were removed, and the injection cannulae were introduced into the brain. The animals were hand held as drugs were injected. Injection volume was 0.2 μl per site. After the injections, the obturators were replaced and rats were placed back into their cages.

2.6. Sodium and water intake tests

Fluid-replete rats treated with bilateral injections of saline or muscimol into the LPBN were used to test the effects of balloon inflation on water and sodium intake. The animals were tested in their home cages with continuous free access to food, water, and 0.3 M NaCl. In each test, half the animals received bilateral injections of muscimol and half received saline into the LPBN. Half the rats in each group had their balloons inflated whereas the other half had their balloons kept deflated. Thus, rats were submitted to four procedures: LPBN muscimol + balloon inflated; LPBN muscimol + balloon deflated; LPBN saline + balloon inflated; and LPBN saline + balloon deflated. Balloons were inflated immediately after rats had received injections into the LPBN. Then, rats were returned to their cages where water and 0.3 M NaCl intake started being recorded immediately. The fluids were provided from burettes with 0.1 ml divisions fitted with metal drinking spouts in front of the cages. Water and 0.3 M NaCl intake were measured at 30, 60, 90, 120, 150, 180, and 210 min. Next, the burettes were replaced by 100 ml tubes with 1 ml divisions with water and 0.3 M NaCl for the next 13 h (overnight), while balloons were kept inflated or deflated as they were in the last 210 min of the previous afternoon. The experiments started at 3:00 pm, and at 5:30 pm the burettes were replaced by 100 ml tubes which remained in place until 6:30 am in the following morning when the volume of water and 0.3 M NaCl ingested was measured and balloons were deflated. The long intake period enabled us to study the inhibitory effect of balloon distension on water and 0.3 M NaCl intake in the absence of muscimol action in the LPBN, which was necessary to determine the correct position of the balloon at the SVC–RAJ.

Table 1

Cumulative intake of water and 0.3 M NaCl in rats in which central injections or balloons were misplaced.

Treatment	n	0.3 M NaCl intake (ml) 210 min 13 h	Water intake (ml) 210 min 13 h
Saline + balloon deflated	7	0.2 ± 0.1	1.7 ± 0.3
Saline + balloon inflated	7	0.3 ± 0.1	0.5 ± 0.1
Muscimol + balloon deflated	7	3.1 ± 0.6	2.9 ± 0.4
Muscimol + balloon inflated	7	5.9 ± 1.2	6.6 ± 1.0

Results are expressed as mean ± SEM; n = number of rats.

2.7. Histology and autopsy

At the end of the experiments, the animals received bilateral injections of 2% Evans blue dye (0.2 µl/injection site) into the LPBN. They were then deeply anesthetized with sodium pentobarbital (80 mg/rat, Thiopentax®, Cristália Pharmaceutical and Chemicals Products, Itapira, SP) and perfused transcardially with isotonic 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 in the LPBN.

Before the cardiac perfusion, the position of the balloon at the SVC–RAJ was confirmed. Animals whose balloon was not correctly positioned at the SVC–RAJ were eliminated from the study.

2.8. Statistical analysis

Two-way repeated measures ANOVA was used to analyze data from cumulative ingestion using treatment and time as factors. One-way ANOVA was used to compare the results of overnight intake (13 h intake). Two-way or one-way ANOVA was combined with the Student-Newman-Keuls post-hoc test. Significance level was set at $p < 0.05$. Data are expressed as means ± standard error of the mean.

3. Results

3.1. Histological analysis

The LPBN injection sites were comparable to previous studies [7,13,14] that investigated the effects of injection of serotonergic drugs on NaCl and water intake. The injections were centered in the middle lateral and dorsal lateral portions of the LPBN. Injections reaching the ventral lateral and external lateral portions, as well as the Kölliker-Fuse nucleus, were observed in some rats and the results from these rats were included in the analysis. Results from rats in which injections did not reach the LPBN, or did so only unilaterally, were analyzed separately, and results are shown in Table 1. There were no statistical significance between those results, either for 0.3 M NaCl [$F(3,19) = 1.58$; $p > 0.05$] and water [$F(3,19) = 1.97$; $p > 0.05$] intake. Fig. 1 shows a typical photomicrography of LPBN injection sites in this study.

3.2. Effects of balloon inflation on water and 0.3 M NaCl intake on fluid-replete rats treated with muscimol into the LPBN

Bilateral injections of muscimol (0.5 µmol/0.2 µl) into the LPBN of fluid-replete rats induced higher 0.3 M NaCl (35.1 ± 3.9 vs. saline: 2.2 ± 0.7 ml/210 min, $n = 8$) [$F(3,21) = 16.13$; $p < 0.01$] and water intake (17.7 ± 1.9 vs. saline: 2.9 ± 0.5 ml/210 min) [$F(3,21) = 14.02$; $p < 0.01$]. Balloon inflation did not affect 0.3 M NaCl intake induced by muscimol injections into the LPBN (27.8 ± 2.1 ml/210 min), whereas water intake increased slightly in rats with inflated balloons (22.8 ± 2.3 ml/210 min; Fig. 2) [treatment and time as factor $F(18,126) = 11.78$; $p < 0.01$].

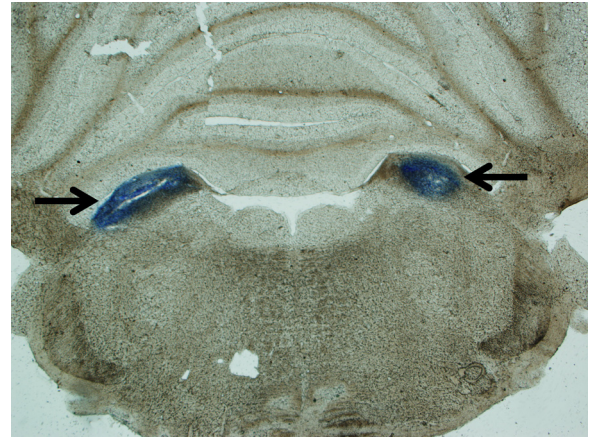


Fig. 1. Photomicrograph showing the injection site into the lateral parabrachial nucleus (lateral parabrachial nucleus, arrows).

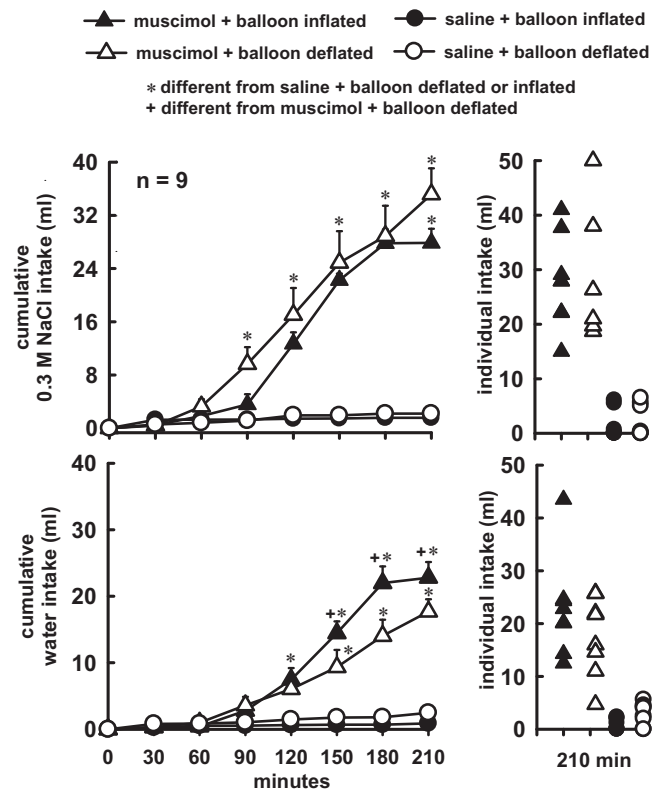


Fig. 2. Cumulative 0.3 M NaCl (top) and water (bottom) intake in rats treated with bilateral injections of saline or muscimol (0.5 µmol/0.2 µl) into the lateral parabrachial nucleus combined with balloon inflation/no inflation at the superior vena cava–right atrial junction. Results are expressed as mean ± SEM. *Different from saline treatment with balloon inflated or deflated; +different from muscimol + balloon deflated, $n = 8$.

Table 2

Cumulative 0.3 M NaCl and water intake following 13 h of bilateral injection of saline or muscimol (0.5 μ mol/0.2 μ l) into the lateral parabrachial nucleus of euhydrated and satiated rats combined with balloon inflation/deflation at the superior vena cava–right atrial junction.

Treatment	n	0.3 M NaCl (ml) 13 h	Water (ml) 13 h
Saline + balloon deflated	8	12.4 \pm 4.3	31.8 \pm 2.4
Saline + balloon inflated	8	6.4 \pm 3.4	21.5 \pm 3.1 ^a
Muscimol + balloon deflated	8	12.0 \pm 2.9	28.1 \pm 3.1
Muscimol + balloon inflated	8	10.0 \pm 3.8	16.6 \pm 3.5 ^a

Results are expressed as mean \pm SEM. n = number of rats.

^a Different from saline + balloon deflated.

To confirm that the balloons were correctly positioned, they remained inflated until the next morning (13 h later) when water and 0.3 M NaCl intake was measured again. Balloon inflation was effective in decreasing water intake in muscimol [$F(1,14)=4.85$; $p<0.05$] or saline treated rats [$F(1,14)=6.13$; $p<0.05$], Table 2. The rats that did the balloon misplaced (above or below the SVC–RAJ) are presented in Table 1, together with the LPBN misplaced injections.

4. Discussion

The inflation of a balloon at the SVC–RAJ produced no changes in 0.3 M NaCl and water intake induced by injections of muscimol into the LPBN of fluid-replete rats. The information from cardiac volume/mechanoreceptors might reach the LPBN, modifying the secretion of neurotransmitters that control fluid intake. Moreover, in our study, the reduction of water during the overnight period in rats with inflated balloons confirms the accuracy of balloon position. Concern sodium intake balloon inflation was not reduced, only slightly, maybe because muscimol into the LPBN might produce some lasting effect during part of the overnight period that impairs fully sodium inhibition. In addition, repeated injections of muscimol into the LPBN, since there were two injections of muscimol in each site of the LPBN, might produce some alterations in activity of the neurotransmitters in this site promoting some sort of sodium intake release. The results also confirm previous studies that have shown that neuronal blockade with bilateral injections of muscimol into the LPBN induces strong ingestion of hypertonic NaCl and water in fluid-replete rats. Other studies have also shown that the inflation of a balloon at the SVC–RAJ reduces sodium and/or water intake for a variety of protocols [8,9,17]. In fact, the participation of the LPBN as part of the central pathways involved in volume regulation has been previously reported. For instance, bilateral electrolytic lesions of the LPBN abolished the inhibition of subcutaneous isoproterenol-induced water intake by the distension of a balloon at the SVC–RAJ [15].

Balloon effects might be not only neurally mediated, but also humoral. Inflation of a balloon at the SVC–RAJ does not influence renin release or plasma oxytocin concentrations [10], increases plasma atrial natriuretic peptide [ANP, 11], and lowers plasma vasopressin concentrations in hydrated and water-deprived rats [2]. Moreover, balloon inflation at the SVC–RAJ in rats submitted to a protocol that acutely challenged fluid intake reduced *c-fos* levels in the organum vasculosum of the lamina terminalis and subfornical organ and increased *c-fos* in the LPBN and caudal ventrolateral medulla when compared to the deflated condition [8]. The higher *c-fos* levels at the LPBN may reflect an increase in the levels of a neurotransmitter that inhibits sodium and water intake, eventually altering humoral secretion. In this sense, at the 90 min there was a tenuous delay in sodium intake in the rats that received LPBN muscimol injections and had balloons inflated

compared with those with deflated balloons. Then, some inhibitory signal is still activated, other than only the signals from the LPBN. The pharmacological stimulation of GABA in the LPBN produces an imperfection of the central mechanisms that control circulating volume information. Our results reinforce the notion that the LPBN is an important central site involved in volume regulation. This pharmacological stimulation turns the animal unable to detect that the intake of sodium and water produces further increase in circulating volume. There was a slightly increase in water intake when balloon inflation was combined with muscimol into the LPBN. May be that was a shift from sodium preference to water intake. Although muscimol produces a huge sodium intake preference, the balloon informs the brain an overload situation trying to reduce the intake. Drinking ultimately is less dangerous than hypertonic sodium solution intake that lastly will induce more drinking to dilute the hypertonic sodium solution. This endeavor may be due some circulating hormone that might help to decrease circulating volume.

In conclusion, balloon inflation at the SVC–RAJ did not change sodium and water intake induced by muscimol injections into the LPBN. The central processing of volume information when blocked produces drink of great amount of sodium and water intake. Therefore, the cardiopulmonary volume receptors signals seem to convey to LPBN.

Acknowledgements

We thank Antonio Carlos Barros Tardivo, Hélio Kushima, and Lilian Morceli for technical assistance. This research was supported by public funding from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 07/53963-0, 09/50949-1).

References

- [1] C.A. Andrade, S.P. Barbosa, L.A. De Luca Jr., J.V. Menani, Activation of alpha2-adrenergic receptors into the lateral parabrachial nucleus enhances NaCl intake in rats, *Neuroscience* 129 (2004) 25–34.
- [2] M. Benedetti, R. Rorato, M. Castro, B.H. Machado, J. Antunes-Rodrigues, L.L. Elias, Water deprivation increases Fos expression in hypothalamic corticotropin-releasing factor neurons induced by right atrial distension in awake rats, *Am. J. Physiol.* 295 (2008) R1706–R1712.
- [3] M.L. Blair, R.L. Jaworski, A. Want, D.T. Piekut, Parabrachial nucleus modulates cardiovascular responses to blood loss, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280 (2001) R1141–R1148.
- [4] J.C. Callera, L.B. Oliveira, S.P. Barbosa, D.S. Colombari, L.A. De Luca Jr., J.V. Menani, GABA(A) receptor activation in the lateral parabrachial nucleus induces water and hypertonic NaCl intake, *Neuroscience* 134 (2005) 725–735.
- [5] J. Ciriello, D. Lawrence, Q.J. Pittman, Electrophysiological identification of neurons in the parabrachial nucleus projecting directly to the hypothalamus in the rat, *Brain Res.* 322 (1984) 388–392.
- [6] E. De Castro e Silva, J.B. Fregoneze, A.K. Johnson, Corticotropin-releasing hormone in the lateral parabrachial nucleus inhibits sodium appetite in rats, *Am. J. Physiol.* 290 (2006) R1136–R1141.
- [7] J.I.F. De Gobbi, T.G. Beltz, R.F. Johnson, J.V. Menani, R.L. Thunhorst, A.K. Johnson, Non-NMDA receptors in the lateral parabrachial nucleus modulate sodium appetite, *Brain Res.* 1301 (2009) 44–51.
- [8] J.I.F. De Gobbi, J.V. Menani, T.G. Beltz, R.F. Johnson, R.L. Thunhorst, A.K. Johnson, Right atrial stretch alters fore- and hind-brain expression of *c-fos* and inhibits the rapid onset of salt appetite, *J. Physiol.* 586 (2008) 3719–3729.
- [9] S. Kaufman, Role of right atrial receptors in the control of drinking in the rat, *J. Physiol.* 349 (1984) 389–396.
- [10] S. Kaufman, Influence of right atrial stretch on plasma renin activity in the conscious rat, *Can. J. Physiol. Pharmacol.* 65 (1987) 257–259.
- [11] S. Kaufman, Renal and hormonal responses to prolonged atrial stretch, *Am. J. Physiol.* 258 (1990) R1286–R1290.
- [12] T. Krukoff, K.H. Harris, J.H. Jhamandas, Efferent projections from the parabrachial nucleus demonstrated with the anterograde tracer *Phaseolus vulgaris* leucoagglutinin, *Brain Res. Bull.* 30 (1993) 163–172.
- [13] J.V. Menani, A.K. Johnson, Cholecystokinin actions in the parabrachial nucleus: effects on thirst and salt appetite, *Am. J. Physiol.* 275 (1998) R1431–R1437.
- [14] J.V. Menani, R.L. Thunhorst, A.K. Johnson, Lateral parabrachial nucleus and serotonergic mechanisms in the control of salt appetite in rats, *Am. J. Physiol.* 270 (1996) R162–R168.

- [15] L.E. Ohman, A.K. Johnson, Role of lateral parabrachial nucleus in the inhibition of water intake produced by right atrial stretch, *Brain Res.* 695 (1995) 275–278.
- [16] L.B. de Oliveira, J.C. Callera, L.A. De Luca Jr., D.S. Colombari, J.V. Menani, GABAergic mechanisms of the lateral parabrachial nucleus on sodium appetite, *Brain Res. Bull.* 73 (2007) 238–247.
- [17] E. Toth, J. Stelfox, S. Kaufman, Cardiac control of salt appetite, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 252 (1987) R925–R999.
- [18] D.G. Ward, Neurons in the parabrachial nuclei respond to hemorrhage, *Brain Res.* 491 (1989) 80–92.