Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research 1016 (2004) 33–39

BRAIN RESEARCH

www.elsevier.com/locate/brainres

Research report

The role of carbon monoxide and nitric oxide in hyperosmolality-induced atrial natriuretic peptide release by hypothalamus in vitro

Dayane Aparecida Gomes, Wagner Luiz Reis, Renato Rizo Ventura, Alexandre Giusti-Paiva, Lucila Leico Kagohara Elias, Fernando Queiroz Cunha, Jose Antunes-Rodrigues *

Departamento de Fisiologia, Faculdade de Medicina de Ribeirao Preto, Av. Bandeirantes 3900, Ribeirao Preto, SP 14090-900, Brazil

Accepted 19 April 2004
Available online 15 June 2004

Abstract

We evaluated the participation of the nitrergic and carbon monoxide (CO) systems in the atrial natriuretic peptide (ANP) release induced by osmotic stimulation of the rat anterior and medial basal hypothalamus (BH) fragments in vitro. The increase in the medium osmolality (NaCl, 340 mOsm/kg H_2O) induced an elevated ANP release, which was associated with a decrease in nitric oxide synthase (NOS) activity ($p < 0.001$), nitric oxide (NO) production and nitrate ($p < 0.001$) release into the medium. The NO donors sodium nitroprusside (SNP, 300 μM), S-nitroso-*N*-acetylpenicillamine (SNAP, 300 μM) and 3-morpholinylsydnoneimine chloride (SIN-1, 300 μM) promoted a significant decrease in ANP release in response to hyperosmolality ($p < 0.001$). ANP release observed in the present study did not result from injury to the BH caused by the increase in medium osmolality nor a toxic effect of the NO donors as demonstrated by the ANP release after incubation with KCl (56 mM). Furthermore, hyperosmolality or NO donors did not increase the LDH content in the medium. The hyperosmotic-induced ANP release and reduction of NOS activity were prevented by the heme oxygenase inhibitor, zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG). In conclusion, these results suggest that NO, the production of which is dependent on CO, modulates the osmolality-induced ANP release by BH fragments.

Theme: Endocrine and autonomic regulation
Topic: Osmotic regulation

Keywords: Hyperosmolality; Hypothalamus; ANP; Nitric oxide; Carbon monoxide

1. Introduction

Natriuretic peptides, including atrial natriuretic peptide (ANP), and their receptors are present in several structures of the central nervous system (CNS) [14,20,37,38,45]. In rats, the ANP-immunoreactive neurons are localized to the region extending from the paraventricular nucleus (PVN) rostrally to the subfornical organ (SFO) and ventrally to the organum vasculosum lamina terminalis (OVLT) [22,30,37,43,47], and their axons also project to the median eminence (ME) and neurohypophysis (NH) [31]. In fact, this distribution in the brain suggests an important role for ANP in the regulation of body fluid balance through centrally mediated mechanisms. ANP neurons are activated by acute and chronic changes in body fluid homeostasis. Thus, after volume load or volume depletion there is an increase or a reduction in the ANP concentration in the ME and NH, respectively [31].

Injection of carbachol into the anteroventral portion of the third ventricle (AV3V) produces the expected natriuresis which is accompanied by an increase in plasma ANP concentration and also an increase in the ANP content of the medial basal hypothalamus (MBH), NH and anterior hypophysis (AH), but with no change in the levels of ANP in the lungs or the right or left atrium [4]. The marked elevation of the ANP content of the basal hypothalamus, NH and AH suggested that the natriuresis resulting from this stimulation is brought about, at least in part, by release of ANP from the brain. On the other hand, icv injections of ANP affect thirst and sodium appetite. For example, this peptide can dramatically suppress dehydration- and angiotensin II-induced drinking and salt intake [1,2,12].

* Corresponding author. Tel.: +55-16-6023018; fax: +55-16-6330017.
E-mail address: antunes@fmrp.usp.br (J. Antunes-Rodrigues).

0006-8993/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.brainres.2004.04.047

ANP-immunoreactive neurons from the AV3V region form the major ANP input to the PVN [44] and an up-regulation of ANP receptors in the latter has been observed in response to water deprivation [36]. The AV3V region also has connections with the SFO, which has been shown to contain a high density of ANP terminals and high levels of ANP receptors [32,33]. We have shown that acute extracellular volume expansion (EVE) causes ANP release that is important for the induction of subsequent natriuresis and diuresis, which, in turn, suppress the increase in blood volume [3]. Lesions of the AV3V, NH and ME markedly decrease the basal plasma ANP concentration and natriuresis. EVE induced by intra-atrial injection of hypertonic saline (0.3 M NaCl), isotonic saline or 5% glucose determined a rapid increase in plasma ANP concentration, which was blocked by lesions of the AV3V, ME, NH or by hypophysectomy [3]. In addition, it has been shown that ANP is released under hyperosmolality from a hypothalamic explant system [42]. These results strongly support the hypothesis that brain ANP plays an important role in the mediation of systemic ANP release under basal conditions as well as after volume expansion [27].

Recent experiments have indicated that nitric oxide (NO) plays a key role in controlling the physiological release of a number of hypothalamic peptides and classical neurotransmitters: the enzyme NO synthase (NOS) has been detected in several brain areas, including key structures involved in the control of body fluid balance, such as the magnocellular neurons of the SON and PVN [6,11,24,35], NH, SFO [35], median preoptic nucleus (MnPO) and OVLT [6], where NO may operate as a neuromodulator [13].

Carbon monoxide (CO) is also produced by neurons and modulates synaptic activity. CO is generated by heme oxygenase (HO), the enzyme that cleaves heme and produces biliverdin, CO and iron [25]. Heme oxygenase is present in rat hypothalamus, and careful localization studies have identified both isoenzymes HO_1 and HO_2 in the PVN and SON [52]. The heme-HO and L-arginine–NOS pathways interact at several levels and are likely to regulate multiple neuroendocrine functions [8].

We have speculated that NO may be involved in the regulation of ANP secretion in the CNS. In the present study, we investigated the ANP release from basal hypothalamus and NOS activity when incubated in a medium with graded NaCl concentration. We also evaluated the effect of different NO donors on basal and hyperosmolality-induced ANP release and the interaction between endogenous CO and the NO system as a modulator of the hypothalamic response to osmotic stimulation.

2. Materials and methods

2.1. Animals

Adult male Wistar rats, 180–200 g, were housed in individual cages in a temperature-controlled environment (23 ± 2 °C) with a 12:12-h light/dark cycle (lights on 0600 h) with free access to rat chow and tap water. All experiments were performed between 0900 and 1000 h.

2.2. Incubation of rat hypothalamic fragments

After decapitation, the brain was quickly removed and the hypothalamic fragments were immediately dissected out from an area 1.0 mm lateral to the midline at the anterior border of the optic chiasm anteriorly and the anterior border of the mammillary bodies posteriorly, the depth of the fragments was approximately 2.5 mm. Hypothalamic fragments include the region of OVLT, MnPO, supraoptic, paraventricular, suprachiasmatic and arcuate hypothalamic nuclei. The total dissection time was less than 1 min after decapitation. Each hypothalamic fragment was transferred to a well containing 0.5 ml of cold Krebs Ringer bicarbonate buffer (KRBG) (118.46 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.18 mM NaH_2PO_4, 1.18 mM MgSO4, 24.88 mM $NaHCO_3$, pH 7.4, 280 mOsm/kg H_2O) with 1% glucose, and pre-incubated in a Dubnoff shaker (50 cycles/min) in an atmosphere of 95% O_2 and 5% CO_2 at 37 °C, for 60 min. After pre-incubation, the medium was gently aspirated and replaced by KRBG isotonic (280 mOsm/kg H_2O) or hypertonic (340 mOsm/kg H_2O) fresh medium with or without the substances to be tested, before incubating the fragments for a further 30 min. The viability of the fragments was tested by examining the effect of cell depolarization with 56 mM KCl at the end of each experiment. We have also measured lactate dehydrogenase by an automated method (Roche Diagnostic System) to study the tissue viability. The medium obtained from each incubation was kept frozen at − 70 °C until ANP or nitrate determination.

2.3. ANP radioimmunoassay

ANP measurements were performed by radioimmunoassay as previously described by Gutkowska [16]. All samples from the same experiment were measured in duplicate in the same assay. The minimum detectable dose in the assay was 0.8 pg/ml and the intra-assay error was 6%.

2.4. Determination of NOS activity

After the incubation the hypothalamic fragments were homogenized with 0.2 ml 20 mM HEPES (pH 7.4) containing 1.25 mM $CaCl_2$, 1 mM dithiothreitol (DTT) and 100 mM tetrahydrobiopterin (BH4). After homogenization, 1 mM NADPH and 200,000 cpm of [^{14}C]arginine (270 μCi/mmol) were added and incubated for 15 min at 37 °C, then the homogenates were centrifuged at 10,000 × *g* for 10 min at 4 °C. The resulting supernatants were applied to 2 ml columns of Dowex AG WX-8 (Na^+ form) and these were eluted with 3 ml of double-distilled water. [^{14}C]Citrulline was then determined in a beta counter. This method is based on the equimolar production of citrulline and nitric oxide

from arginine, mediated by NOS activity. The results are expressed as citrulline production per mg of tissue.

2.5. Determination of nitrate

The nitrate (NO_3^-, a metabolite of NO) concentration was determined using an NO analyser (Nitric Oxide Analyser, NOA™ 280, Sievers Instruments, Colorado, USA) [18]. Sodium nitrate (Sigma, St. Louis, USA) was used as a standard.

2.6. Determination of protein

The protein content of the basal hypothalamus fragment was determined as previously described by Bradford [7].

2.7. Determination of osmolality

Osmolality was determined by freezing point depression (FisKe Os TM, USA).

2.8. Drugs

All drugs used in this study were freshly prepared immediately before use. Sodium nitroprusside (SNP), superoxide dismutase-polyethylene glycol (PEG-SOD), *N*(G)-nitro-L-arginine methyl ester (L-NAME), N^G-monomethyl-L-arginine (L-NMMA), dithiothreitol (DTT), tetrahydrobiopterin (BH4), and hemoglobin were obtained from Sigma. Dowex-AG-WX-8 Na^+ form mesh 200–400 was obtained from Bio Rad. *S*-nitroso-*N*-acetylpenicillamine (SNAP) and 3-morpholinylsydnoneimine chloride (SIN-1) were obtained from Tocris and the zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) from Porphyrin Products.

2.9. Statistical analysis

The results are reported as means ± S.E.M. and were submitted to one-way analysis of variance (ANOVA) followed by Newman–Keuls post hoc test. The level of significance was set at $p < 0.05$.

3. Results

3.1. Effects of osmotic stimulation on ANP release, NOS activity, and nitrate release

The results presented in Fig. 1A show that 30 min after osmotic stimulation (340 mOsm/kg H_2O) there was a significant increase ($p < 0.001$) in ANP release from hypothalamic fragments into the incubation medium, compared to the isotonic stimulation (280 mOsm/kg H_2O). In addition, treatment with 340 mOsm/kg H_2O induced a decrease ($p < 0.001$) in NOS activity (Fig. 1B) and nitrate content (Fig. 1C). Fig. 1D shows that there is an inverse correlation between NOS activity and ANP release in response to osmotic stimulation of hypothalamic tissue.

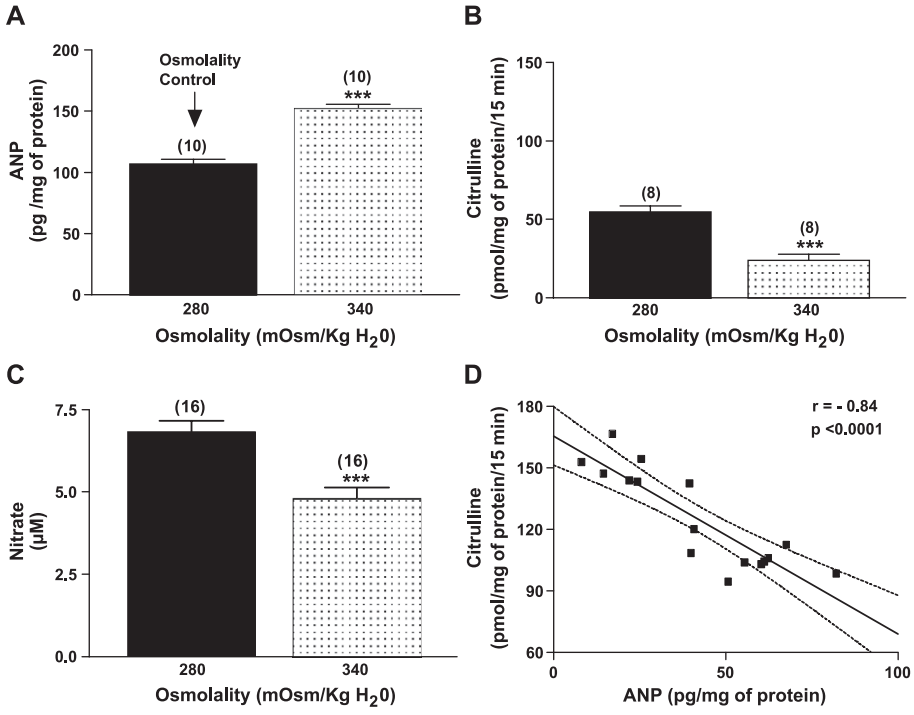

Fig. 1. Effects of osmotic stimulation on ANP release (A), NOS activity (B), and nitrate release (C). Data are reported as mean ± S.E.M. ***$p < 0.001$ (280 versus 340 mOsm/kg H_2O). (D) Correlation between ANP and nitric oxide produced during the incubation of basal hypothalamus in vitro. The number of basal hypothalamus incubated is given in parenthesis.

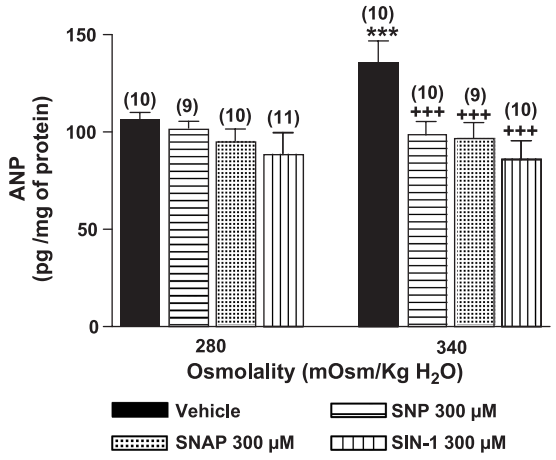

Fig. 2. Effects of nitric oxide donors SNP, SNAP or SIN-1 on ANP release from basal hypothalamus in isotonic and hypertonic medium. Data are reported as mean ± S.E.M. ***$p<0.001$ (280 versus 340 mOsm/kg H_2O), $^{+++}p<0.001$ (SNP, SNAP or SIN-1 versus vehicle). The number of basal hypothalamus incubated is given in parenthesis.

3.2. Effects of SNP, SNAP and SIN-1 on ANP release

Incubation of hypothalamic fragments with 340 mOsm/kg H_2O medium and NO donors resulted in a significant inhibition ($p<0.001$) of ANP secretion (Fig. 2). By contrast, NO donors had no significant effects on ANP release when the tissue was incubated with isotonic medium (Fig. 2). The inhibitory effect of SNP on ANP release was prevented by hemoglobin, which is described as an NO scavenger (Fig. 3).

To determine the possible participation of superoxide production during the hypothalamic fragments incubation in a medium supplemented with SIN-1 (that releases nitric oxide and superoxide simultaneously [19]), we investigated the effect of PEG-SOD, a membrane-permeable superoxide scavenger enzyme, on ANP release induced by hyperosmolality. The addition of PEG-SOD (10 U/ml) did not prevent the inhibitory effect of SIN-1 (300 µM) on ANP release (Fig. 3).

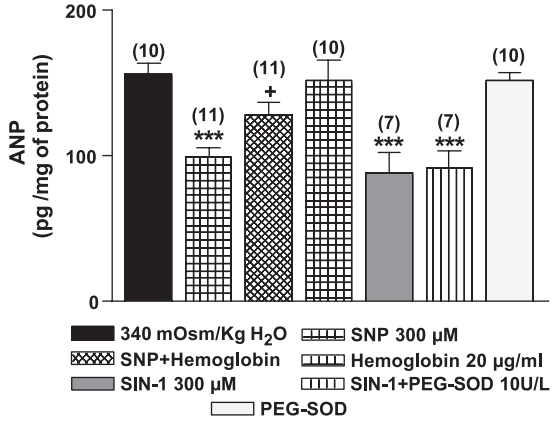

Fig. 3. Effect of SNP associated with hemoglobin and SIN-1 associated with PEG-SOD on ANP release from basal hypothalamus in hypertonic medium. Data are reported as mean ± S.E.M. ***$p<0.001$ versus 340 mOsm/kg H_2O; $^{+}p<0.05$ SNP versus SNP + hemoglobin. The number of basal hypothalamus incubated is given in parenthesis.

3.3. Effects of L-NAME and L-NMMA on ANP release

To confirm the inhibitory influence of NO on ANP release induced by hyperosmolality, the fragments were incubated with different doses of L-NAME as well as L-NMMA. However, neither L-NAME nor L-NMMA altered the basal release of ANP or that occurring in response to conditions of hyperosmolality (Fig. 4).

3.4. Effects of ZnDPBG on ANP release

To verify the influence of CO on ANP release induced by hyperosmolality, the fragments were incubated with different doses of ZnDPBG. This heme oxygenase inhibitor induced a dose-dependent decrease of ANP release ($p<0.001$) in response to the osmotic stimulation (Fig. 5A). In addition, this effect was associated with an increase ($p<0.001$) in the NOS activity (Fig. 5B).

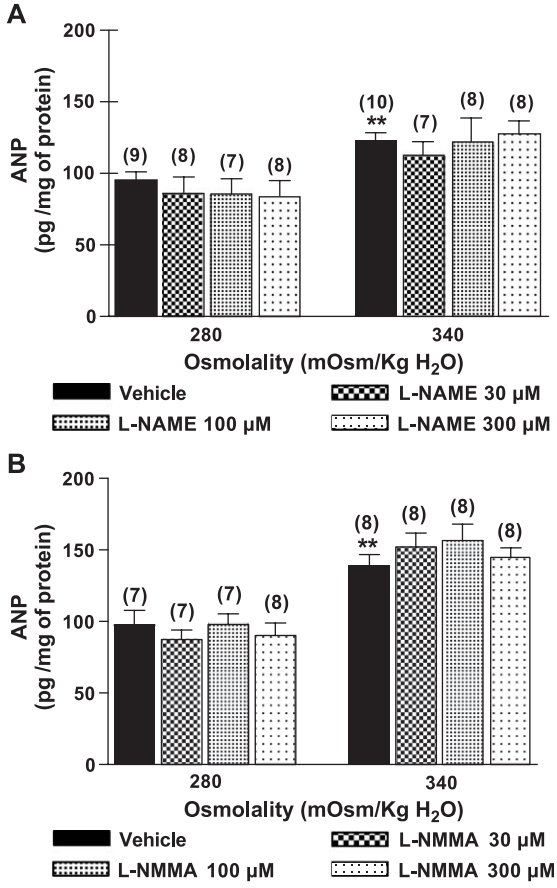

Fig. 4. Effects of L-NAME (A) and L-NMMA (B) on ANP release induced by isotonic and hypertonic medium from basal hypothalamic in vitro. Data are reported as mean ± S.E.M. **$p<0.01$ (280 versus 340 mOsm/kg H_2O). The number of basal hypothalamus incubated is given in parenthesis.

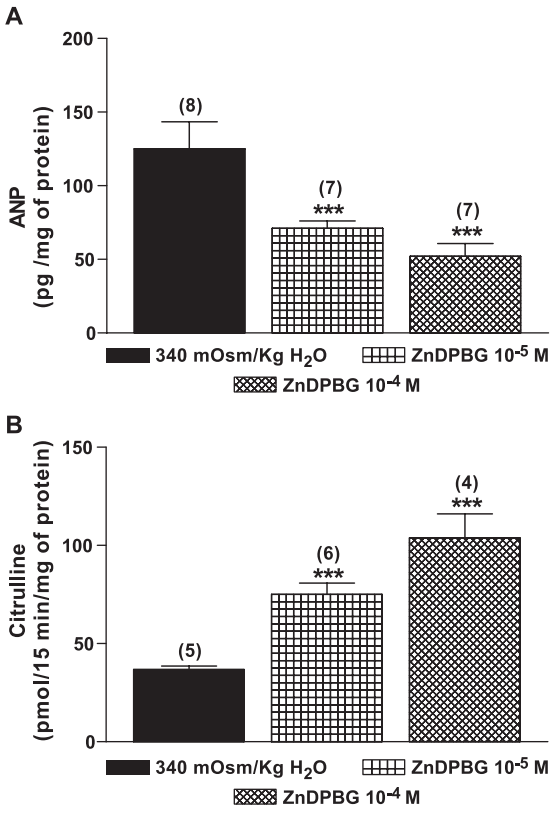

Fig. 5. Effect of heme oxygenase inhibitor, ZnDPBG on ANP release (A) and NOS activity (B) from basal hypothalamic incubated in hypertonic medium. Data are reported as mean ± S.E.M. ***$p < 0.001$ versus 340 mOsm/kg H_2O. The number of basal hypothalamus incubated is given in parenthesis.

3.5. Tissue viability

The tissue viability was verified by studying the effect of KCl (56 mM) on ANP release from basal hypothalamus at the end of each experiment. The data show a significant increase ($p < 0.05$) in ANP release induced by 56 mM KCl compared to values obtained before cell depolarization either in isosmotic (162.7 ± 12 versus 100.0 ± 6.3 pg/mg protein) or hypertonic media (195.3 ± 20.5 versus 141.0 ± 2.8 pg/mg protein). Lactate dehydrogenase levels were similar during the isosmotic (329.8 ± 34.28 U/l) and the hypertonic incubation (336.1 ± 31.9 U/l).

4. Discussion

The present data show that incubation of hypothalamic fragments with medium containing increasing concentrations of sodium chloride (280 and 340 mOsm/kg H_2O) results in an increase of ANP release associated with a reduction in the activity of the nitrergic system. In addition, we demonstrated that heme oxygenase activity modulates ANP secretion as well as NO production.

Previous work has shown that ANP release from hypothalamic fragments is stimulated by Na^+, choline chloride and glucose in a dose–response pattern. The ANP release was more sensitive to changes in the concentration of Na^+ than to those of choline chloride and glucose. Therefore, it can be proposed that Na^+ is the candidate that controls ANP release from brain tissue and, also, that ANP in the hypothalamus and/or OVLT may play a role in the physiological regulation of sodium ion and water balance in the central nervous system [42].

It has been reported previously that NOS activity in structures of the CNS, such as the SFO, MnPO, OVLT, PVN and SON, is altered in response to changes in the osmolality of the extracellular fluid. There is growing evidence that NO functions as a local modulator of hypothalamus in hyperosmolality conditions [46,51]. However, there are conflicting data concerning the role of NO, as observed by the reports showing an increase [24,50] or decrease [9] of NOS mRNA expression in response to osmotic stimulation. In addition, the role of NO on the AVP and OT release induced by hyperosmolality has been contradictory [23,46,51]. Thus, we investigated the participation of the nitrergic system in the modulation of the ANP release in response to osmotic stimulation. We found that ANP release was significantly elevated with increased osmolality and this result was associated with a reduction in the NOS activity and nitrate content. The use of the NO donors, SNP, SNAP and SIN-1, significantly decreased the hyperosmolality-induced ANP release to the incubation medium. Taken together, these data suggest a participation of NO in the modulation of the ANP release in response to osmotic stimulation.

To confirm the inhibitory influence of NO, the fragments were incubated with different doses of the NOS inhibitors, L-NAME or L-NMMA. However, L-NAME and L-NMMA had no effect on ANP release in basal conditions nor in response to hyperosmolality. We believe that NOS is already inactive in the presence of 340 mOsm/kg H_2O and, therefore, no further enzyme blockade would occur with L-NAME or L-NMMA. In fact, L-arginine, a NOS substrate, did not change ANP release induced by hyperosmolality (data not shown). Furthermore, in the isosmotic condition the release of ANP may not be subject to modulation by the L-arginine/NOS/NO pathway. This suggestion is reinforced by the observation that NO donors at the same doses that reduced hypertonic-induced ANP release had no effect on ANP release in the isosmotic condition.

At first sight, these results appear to be contradictory. However, it has been suggested that the nitrergic system can have different effects under basal or stimulated conditions. Turnbull et al. [49] have shown that L-NAME increases baseline adrenocorticotropin hormone (ACTH) levels, while the same drug blunts the ACTH response to electrofootshock. Furthermore, using a different experimental approach, Haley et al. [17] showed that the intraplantar or systemic administration of L-NAME induces a dose-dependent antinociception in the formalin test in rats, while it had no effect in saline-injected control animals.

As stated in the Introduction to the present work, there is compelling evidence for an interaction between the NO and CO systems. This aspect was analyzed in the present study through inhibition of heme oxygenase activity under hypertonic stimulation. The data demonstrate a clear, dose-related decrease in ANP release with a concomitant increase in citrulline production in response to the osmotic stimulation in the presence of ZnDPBG. These results indicate an interaction between CO and NO systems and ANP secretion, suggesting that NOS activity is negatively regulated by CO.

Osmotic stimuli are known to activate the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors with a consequent increase in intracellular calcium [29], promoting NOS and HO_2 activation [5,34]. However, in the present study, we observed a decrease in citrulline production under osmotic stimulation. This leads us to speculate that, since carbon monoxide possesses affinity for the heme domain of NOS [39,40,41,48], it can inhibit the activity of the enzyme. Indeed, CO has been reported to inhibit NOS activity in other in vitro studies [15,21,26,28,53].

The possibility that ANP release in response to hyperosmolality could be due to leakage from ANP neurons injured by high osmotic pressure, or by NO donors, was excluded by the demonstration of peptide release in response to 56 mM KCl. However, the effect of the NO donors on ANP levels could have resulted from the toxicity of products generated by the action of nitric oxide donors. Previous studies have reported that NO rapidly reacts with superoxide to form peroxynitrite, a powerful oxidant [10]. However, in the present study, the cytotoxic effect of peroxynitrite was excluded by the experiment employing SIN-1 with PEG-SOD, in which there was a similar decrease in ANP release compared to the treatment with SIN-1 alone. Furthermore, tissue viability was also confirmed by the unchanged lactate dehydrogenase levels in the incubation medium.

In conclusion, the present data indicate the participation of the NOS/NO and HO/CO systems in the regulation of hypothalamic ANP secretion in response to hyperosmolality.

Acknowledgements

This work was supported by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP), Conselho Nacional de Desenvolvimento Cientifico (CNPq) and Programa de Apoio a Nucleos de Excelencia (PRONEX). We are grateful to Dr. Cuttle for the revision of the English text. We thank Marina Holanda and Maria Valci Aparecida dos Santos Silva for technical assistance.

References

[1] J. Antunes-Rodrigues, S.M. McCann, L.C. Rogers, W.K. Samson, Atrial natriuretic factor inhibits dehydration- and angiotensin II-induced water intake in the conscious, unrestrained rat, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 8720–8723.

[2] J. Antunes-Rodrigues, S.M. McCann, W.K. Samson, Central administration of atrial natriuretic factor inhibits saline preference in the rat, Endocrinology 118 (1986) 1726–1728.

[3] J. Antunes-Rodrigues, M.J. Ramalho, L.C. Reis, J.V. Menani, M.Q. Turrin, J. Gutkowska, S.M. McCann, Lesions of the hypothalamus and pituitary inhibit volume-expansion-induced release of atrial natriuretic peptide, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 2956–2960.

[4] S. Baldissera, J.W. Menani, L.F. dos Santos, A.L. Favaretto, J. Gutkowska, M.Q. Turrin, S.M. McCann, J. Antunes-Rodrigues, Role of the hypothalamus in the control of atrial natriuretic peptide release, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 9621–9625.

[5] D. Boehning, S.H. Snyder, Novel neural modulators, Annu. Rev. Neurosci. 26 (2003) 105–131.

[6] G.K. Bhat, V.B. Mahesh, C.A. Lamar, L. Ping, K. Aguan, D.W. Brann, Histochemical localization of nitric oxide neurons in the hypothalamus: association with gonadotropin-releasing hormone neurons and co-localization with *N*-methyl-D-aspartate receptors, Neuroendocrinology 62 (1995) 187–197.

[7] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 7 (1976) 248–254.

[8] D.W. Brann, G.K. Bhat, C.A. Lamar, V.B. Mahesh, Gaseous transmitters and neuroendocrine regulation, Neuroendocrinology 65 (1997) 385–395.

[9] V.M. Campese, P. Mozayeni, S. Ye, M. Gumbard, High salt intake inhibits nitric oxide synthase expression and aggravates hypertension in rats with chronic renal failure, J. Nephrol. 15 (2002) 407–413.

[10] V. Darley-Usmar, B. Halliwell, Blood radicals: reactive nitrogen species, reactive oxygen species, transition metal ions, and the vascular system, Pharm. Res. 13 (1996) 649–662.

[11] T.M. Dawson, D.S. Bredt, M. Fotuhi, P.M. Hwang, S.H. Snyder, Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 7797–7801.

[12] C.R. Franci, G.P. Kozlowski, S.M. McCann, Water intake in rats subjected to hypothalamic immunoneutralization of angiotensin II, atrial natriuretic peptide, vasopressin, or oxytocin, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 2952–2956.

[13] J. Garthwaite, S.L. Charles, R. Chess-Williams, Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain, Nature 336 (1988) 385–388.

[14] R. Gibson, G.M. Wildey, S. Manaker, C.C. Glembotski, Autoradiographic localization and characterization of atrial natriuretic peptide binding sites in the rat central nervous system and adrenal gland, J. Neurosci. 6 (1986) 2004–2011.

[15] J.M. Griscavage, J.M. Fukuto, Y. Komori, L.J. Ignarro, Nitric oxide inhibits neuronal nitric oxide synthase by interacting with the heme prosthetic group, role of tetrahydrobiopterin in modulating the inhibitory action of nitric oxide, J. Biol. Chem. 269 (1994) 21644–21649.

[16] J. Gutkowska, G. Thibault, P. Januszewicz, M. Cantin, J. Genest, Direct radioimmunoassay of atrial natriuretic factor, Biochem. Biophys. Res. Commun. 31 (1984) 593–601.

[17] J.E. Haley, A.H. Dickenson, M. Schachter, Electrophysiological evidence for a role of nitric oxide in prolonged chemical nociception in the rat, Neuropharmacology 31 (1992) 251–258.

[18] V. Hampl, C.L. Walters, S.L. Archer, Determination of nitric oxide by the chemiluminescence reaction with ozone, in: M. Feelisch, J.S. Stamler (Eds.), Methods in Nitric Oxide Research, Wiley, New York, 1996, pp. 309–318.

[19] P. Holm, H. Kankaanranta, T. Metsä-Ketelä, E. Moilanen, Radical releasing properties of nitric oxide donors GEA 3162, SIN-1 and S-nitroso-*N*-acetylpenicillamine, Eur. J. Pharmacol. 346 (1998) 97–102.

[20] H. Imura, K. Nakao, H. Itoh, The natriuretic peptide system in the brain: implications in the central control of cardiovascular

and neuroendocrine functions, Front. Neuroendocrinol. 13 (1992) 217–249.
[21] T. Ingi, G.V. Ronnett, Direct demonstration of a physiological role for carbon monoxide in olfactory receptor neurons, J. Neurosci. 15 (1995) 8214–8222.
[22] D.M. Jacobowitz, G. Skofitsch, H.R. Keiser, R.L. Eskay, N. Zamir, Zamir, Zamir, Evidence for the existence of atrial natriuretic factor-containing neurons in the rat brain, Neuroendocrinology 40 (1985) 92–94.
[23] M. Kadekaro, H. Liu, M.L. Terrell, S. Gestl, V. Bui, J.Y. Summy-Long, Role of NO on vasopressin and oxytocin release and blood pressure responses during osmotic stimulation in rats, Am. J. Physiol. 273 (1997) R1024–R1030.
[24] K. Kadowaki, J. Kishimoto, G. Leng, P.C. Emson, Up-regulation of nitric oxide synthase (NOS) gene expression together with NOS activity in the rat hypothalamo-hypophysial system after chronic salt loading: evidence of a neuromodulatory role of nitric oxide in arginine vasopressin and oxytocin secretion, Endocrinology 134 (1994) 1011–1017.
[25] G.S. Marks, J.F. Brien, K. Nakatsu, B.E. McLaughlin, Does carbon monoxide have a physiological function? Trends Pharmacol. Sci. 12 (1991) 185–188.
[26] M.A. Marletta, Nitric oxide synthase structure and mechanism, J. Biol. Chem. 268 (1993) 12231–12234.
[27] S.M. McCann, J. Gutkowska, C.R. Franci, A.L. Favaretto, J. Antunes-Rodrigues, Hypothalamic control of water and salt intake and excretion, Braz. J. Med. Biol. Res. 27 (1994) 865–884.
[28] K. McMillan, D.S. Bredt, D.J. Hirsch, S.H. Snyder, J.E. Clark, B.S. Masters, Cloned, expressed rat cerebellar nitric oxide synthase contains stoichiometric amounts of heme, which binds carbon monoxide, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 11141–11145.
[29] R.B. Meeker, Metabotropic and NMDA glutamate receptor interactions with osmotic stimuli in supraoptic neurons, Pharmacol. Biochem. Behav. 73 (2002) 475–484.
[30] N. Morii, K. Nakao, A. Sugawara, M. Sakamoto, M. Suda, M. Shimokura, Y. Kiso, M. Kihara, Y. Yamori, H. Imura, Occurrence of atrial natriuretic polypeptide in brain, Biochem. Biophys. Res. Commun. 15 (1985) 413–419.
[31] M. Palkovits, R.L. Eskay, F.A. Antoni, Atrial natriuretic peptide in the median eminence is of paraventricular nucleus origin, Neuroendocrinology 46 (1987) 542–544.
[32] R. Quirion, M. Dalpe, A. De Lean, J. Gutkowska, M. Cantin, J. Genest, Atrial natriuretic factor (ANF) binding sites in brain and related structures, Peptides 5 (1984) 1167–1672.
[33] R. Quirion, M. Dalpe, T.V. Dam, Characterization and distribution of receptors for the atrial natriuretic peptides in mammalian brain, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 174–178.
[34] W. Riedel, Role of nitric oxide in the control of the hypothalamic–pituitary–adrenocortical axis, Z. Rheumatol. 59 (2000) 36–42.
[35] J. Rodrigo, D.R. Springall, O. Uttenthal, M.L. Bentura, F. Abadia-Molina, V. Riveros-Moreno, R. Martinez-Murillo, J.M. Polak, S. Moncada, Localization of nitric oxide synthase in the adult rat brain, Philos. Trans. R. Soc. Lond., B Biol. Sci. 29 (1994) 175–221.
[36] J.M. Saavedra, Regulation of atrial natriuretic peptide receptors in the rat brain, Cell. Mol. Neurobiol. 7 (1987) 151–173.
[37] C.B. Saper, D.G. Standaert, M.G. Currie, D. Schwartz, D.M. Geller, P. Needleman, Atriopeptin-immunoreactive neurons in the brain: presence in cardiovascular regulatory areas, Science 227 (1985) 1047–1049.
[38] C.B. Saper, K.M. Hurley, M.M. Moga, H.R. Holmes, S.A. Adams, K.M. Leahy, P. Needleman, Brain natriuretic peptides: differential localization of a new family of neuropeptides, Neurosci. Lett. 96 (1989) 29–34.
[39] H. Sato, S. Nomura, I. Sagami, O. Ito, S. Daff, T. Shimizu, CO binding studies of nitric oxide synthase: effects of the substrate, inhibitors and tetrahydrobiopterin, FEBS Lett. 430 (1998) 377–380.
[40] J.S. Scheele, V.G. Kharitonov, P. Martásek, L.J. Roman, V.S. Sharma, B.S.S. Masters, D. Madge, Kinetics of CO ligation with nitric-oxide synthase by flash photolysis and stopped-flow spectrophotometry, J. Biol. Chem. 272 (1997) 12523–12528.
[41] J.S. Scheele, E. Bruner, T. Zemojtel, P. Martásek, L.J. Roman, B.S.S. Masters, V.S. Sharma, D. Madge, Kinetics of CO and NO ligation with the Cys(331) → Ala mutant of neuronal nitric-oxide synthase, J. Biol. Chem. 276 (2001) 4733–4736.
[42] T. Shibasaki, M. Naruse, K. Naruse, N. Yamauchi, Y.S. Kim, A. Masuda, T. Imaki, H. Demura, N. Ling, T. Inagami, K. Shizume, Effect of sodium ion on atrial natriuretic factor release from rat hypothalamic fragments, Life Sci. 42 (1988) 1173–1180.
[43] G. Skofitsch, D.M. Jacobowitz, R.L. Eskay, N. Zamir, Distribution of atrial natriuretic factor-like immunoreactive neurons in the rat brain, Neuroscience 16 (1985) 917–948.
[44] D.G. Standaert, C.B. Saper, Origin of the atriopeptin-like immunoreactive innervation of the paraventricular nucleus of the hypothalamus, J. Neurosci. 8 (1988) 1940–1950.
[45] D.G. Standaert, P. Needleman, C.B. Saper, Organization of atriopeptin-like immunoreactive neurons in the central nervous system of the rat, J. Comp. Neurol. 15 (1986) 315–341.
[46] J.Y. Summy-Long, V. Bui, S. Mantz, E. Koehler, J. Weisz, M. Kadekaro, Central inhibition of nitric oxide synthase preferentially augments release of oxytocin during dehydration, Neurosci. Lett. 152 (1993) 190–193.
[47] I. Tanaka, K.S. Misono, T. Inagami, Atrial natriuretic factor in rat hypothalamus, atria and plasma: determination by specific radioimmunoassay, Biochem. Biophys. Res. Commun. 30 (1984) 663–668.
[48] C. Tetreau, M. Tourbez, A. Gorren, B. Mayer, D. Lavalette, Dynamics of carbon monoxide binding with neuronal nitric oxide synthase, Biochemistry 38 (1999) 7210–7218.
[49] A.V. Turnbull, C.K. Kim, S. Lee, C.L. Rivier, Influence of carbon monoxide, and its interaction with nitric oxide, on the adrenocorticotropin hormone response of the normal rat to a physico-emotional stress, J. Neuroendocrinol. 10 (1998) 793–802.
[50] Y. Ueta, A. Levy, H.S. Chowdrey, S.L. Lightman, Water deprivation in the rat induces nitric oxide synthase (NOS) gene expression in the hypothalamic paraventricular and supraoptic nuclei, Neurosci. Res. 23 (1995) 317–319.
[51] R.R. Ventura, D.A. Gomes, W.L. Reis, L.L. Elias, M. Castro, M.M. Valenca, E.C. Carnio, V. Rettori, S.M. McCann, J. Antunes-Rodrigues, Nitrergic modulation of vasopressin, oxytocin and atrial natriuretic peptide secretion in response to sodium intake and hypertonic blood volume expansion, Braz. J. Med. Biol. Res. 35 (2002) 1101–1109.
[52] S.R. Vincent, S. Das, M.D. Maines, Brain heme oxygenase isoenzymes and nitric oxide synthase are co-localized in select neurons, Neuroscience 63 (1994) 223–231.
[53] K.A. White, M.A. Marletta, Nitric oxide synthase is a cytochrome P-450 type hemoprotein, Biochemistry 28 (1992) 6627–6631.