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Role of cardiovascular nitric oxide system in C-type natriuretic peptide effects

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Abstract

The aims were to evaluate the role of cardiovascular nitric oxide (NO)-system in C-type natriuretic peptide (CNP) actions and to investigate receptor types and signaling pathways involved in this interaction. Wistar rats were infused with saline or CNP. Mean arterial pressure (MAP) and nitrites and nitrates (NOx) excretion were determined. NO synthase (NOS) activity and NOS expression (Western blot) were analyzed in atria, ventricle and aorta. CNP decreased MAP and increased NOx excretion. CNP estimulated NOS activity, inducing no changes on cardiac and vascular endothelial NOS expression. NOS activity induced by CNP was abolished by suramin and calmidazoliumand but it is not modified by anantin.

CNP would interact with NPR-C receptor coupled via G proteins leading to the activation Ca^{2+}-calmodulin dependent endothelial NOS, increasing NO production which would induce the reduction in cardiac myocyte contractility and ANP synthesis and secretion in right atria and the relaxation of vascular smooth muscle.

Keywords: CNP; Nitric oxide; Nitric oxide synthase; Heart; Aorta artery; Natriuretic receptors; NPR-C; Natriuretic peptides; Right atria; Left ventricle; Vascular smooth muscle; Endothelium

Natriuretic peptides and nitric oxide (NO) are two important factors involved in the regulation of hydroelectrolyte balance and vascular tone and they play a fundamental role in cardiovascular homeostasis and disease [1,2].

The natriuretic peptides family involves atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) [3,4]. CNP have been localized in several tissues, including nervous system, endothelial cells and cardiac myocytes [2–4]. This peptide would act in a paracrine manner causing local vasodilation and preventing smooth muscle cell proliferation [3–5].

Additionally, there are three types of natriuretic receptors, NPR-A, NPR-B and NPR-C, which are expressed in cardiac atria and ventricles, as well as in the aorta and peripheral vasculature, kidney, etc. [3,6,7]. NPR-A and NPR-B activation induces cGMP synthesis which mediates cellular responses [7]. In contrast, NPR-C is not linked to a guanylyl cyclase domain and it was earlier proposed as a clearance receptor to remove natriuretic peptides from the circulation [8]. However, a number of studies show that NPR-C is involved in the biological actions of natriuretic peptides, activating Gi-protein and/or inhibiting adenylyl ciclase [9–11].

The cellular effects of CNP are mainly mediated by NPR-B and NPR-C receptors. CNP selectively stimulates NPR-B, while the subtype NPR-A is mainly activated by ANP and BNP. The three natriuretic peptides are able to bind to NPR-C more or less equally [12].

On the other hand, the free radical NO is synthesized by nitric oxide synthase enzyme (NOS), which have three isoforms: neuronal (nNOS), inducible (iNOS) and endothelial

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(eNOS), that are expressed in several tissues, including endothelium, vascular smooth muscle, nephron and heart [13]. nNOS and eNOS are constitute expressed and require Ca^{2+} to maintain the binding of calmodulin to be active. Whereas, iNOS expressed after transcriptional induction and independent of Ca^{2+}-calmodulin complex, induces large amounts of NO compared with the other isoforms [14].

We previously demonstrated that the activation of NO-pathway is involved in ANP-hypotensive effect and that NOS activation would mediate, almost in part, the cardiovascular and renal actions of this peptide [15,16].

In addition, Amin et al. showed that CNP dilates the afferents arterioles via prostaglandin/NO pathway in renal microvessels [17]. This finding was confirmed by other authors who found a close relationship between iNOS expression and CNP in vascular smooth muscle cells [18].

Taken in account the findings suggesting interactions between natriuretic peptides and NO, the aims of this study were to evaluate the involvement of NO-system on CNP cardiovascular actions, and to investigate the receptor as well as the molecular mechanisms of signaling involved in this interaction.

Materials and methods

Animals

Male Wistar rats weighting 250–300 g, from the breeding laboratories of the Facultad de Farmacia y Bioquímica (UBA, Argentina), were used in the experiments, in compliance with the research animal use guidelines of the American Heart Association.

Experimental design

Protocol 1

Effects of CNP infusion on mean arterial pressure and NO-system. Rats, anaesthetized with urethane (1 g kg^{-1} bodyweight, i.p.), were cannulated with polyethylene catheters in femoral vein and artery, and urinary bladder, for drug administration, mean arterial pressure (MAP) recording, and urine collection, respectively.

After surgery, an infusion of isotonic NaCl at a rate of 0.05 ml/min was started and maintained for 40 min, to allow stabilization of haemodynamic and renal parameters. Then, one group of animals continued with this saline infusion, and other group was injected with a bolus of CNP (10 μg kg^{-1}) and then received a CNP infusion (1 μg kg^{-1} min^{-1}), over 1 h.

MAP was recorded and urine samples were collected over the 60 min of the experimental infusion period.

Urinary concentration of nitrites and nitrates (NOx), end products derived from NO metabolism, was determined as described Verdon et al. [19].

At the end of the experimental period animals were sacrificed by decapitation, right atria, left ventricle and aorta artery were removed in order to determine NOS activity and expression.

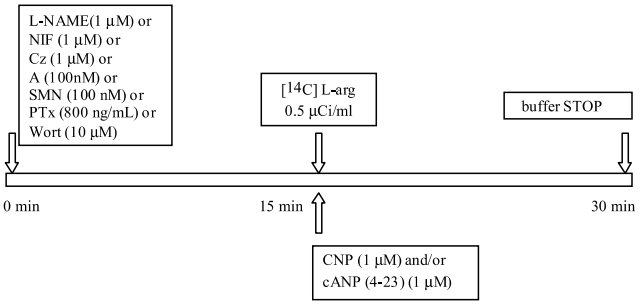
Histochemical determination of NADPH diaphorase activity. Tissues were processed using the NADPH-diaphorase (NADPH-d) histochemical method described by Vincent and Kimura [20]. The NADPH-d-stained cells were photography and measured, optical density (OD), by using a computerized image analyzer.

Determination of NOS activity. Tissues NOS activity was determined using [U-^{14}C]arginine as substrate as previously described [12,17]. The amount of [^{14}C]L-citrulline obtained was determined with a liquid scintillation counter. Nitric oxide production (measured as pmol of [^{14}C]citrulline) was expressed in pmol/g wet weight min.

Western blot analysis. Samples of different tissues containing equal amounts of protein (0.10 mg protein/lane) were separated by electrophoresis in 7.5% SDS–polyacrylamide gels and transferred to a nitrocellulose membrane and then incubated with rabbit polyclonal anti-NOS antibodies (1/500 dilution) and a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1/5000 dilution). Samples were revealed by chemiluminescence using the ECL reagent for 2–4 min. Quantification of the bands was performed by digital image analysis using a Hewlett–Packard scanner and Totallab analyzer software.

Protocol 2

Receptor type and signaling cascade involved in CNP and NOS interaction. Wistar rats were sacrificed by decapitation and NOS activity was measured in the aorta artery, right atria and left ventricle, using [U-^{14}C]arginine as substrate. Tissues slices (2–3 mm thick) were incubated 30 min at 37 °C as was described in protocol 1, but during the incubation period, agonists (15 min after incubation was started) and/or antagonists (at the beginning of the 30 min incubation time) were added according to the following protocol:



L-Nitro arginine metyl ester (L-NAME), NOS inhibitor; cANP(4-23): NPR-C receptor selective agonist; nifedipine (NIF), Ca^{2+} L-type channel blocker; calmidazolium (Cz), calmodulin antagonist; anantin (A), NPR-A antagonist; suramin (SMN), G protein inhibitor; Pertussis toxin (PTx), $Gi_{1\text{-}2}$ proteins inhibitor; Wortmannin (Wort), the structurally and mechanistically dissimilar inhibitor of PI3-K/Akt pathway.

Statistical analysis. All values are expressed as means ± SEM. The program Prism was used for statistical analysis. Data were analyzed using Student's *t* test and one-way analysis of variance (ANOVA) followed by a Bonferroni multiple comparisons post hoc test. p value <0.01 was considered significant difference.

Results

Effects of CNP infusion on MAP and NO-system

Rats treated with CNP showed lower values of MAP compared with NaCl-treated ones at the end of experimental time (CNP = 81 ± 2 vs. NaCl = 96 ± 4, $p < 0.01$).

The decrease of MAP is associated to the rise of NOx excretion induced by CNP (CNP = 5.7 ± 0.5 vs. NaCl = 1.6 ± 0.2, $p < 0.01$).

Table 1 shows NADPH-d activity in endothelium and smooth muscle cells of aorta artery, right atria and left ventricle in both groups of animals. Vascular staining was more intense in all tissues from CNP-treated rats compared with NaCl (Fig. 1).

NOS activity determined with [^{14}C]L-arginine in aorta artery and heart from NaCl and CNP-treated animals appears in Table 2. Cardiac and vascular tissues obtained from the animals infused with CNP showed an increased

Table 1
Effects of CNP infusion on NADPH-d activity in aorta artery and heart

	OD	
	NaCl	CNP
Aorta artery		
Endothelium	0.197 ± 0.005	0.232 ± 0.011*
Smooth muscle	0.276 ± 0.009	0.304 ± 0.006*
Heart		
Right atria	0.255 ± 0.004	0.303 ± 0.008*
Left ventricle	0.280 ± 0.005	0.301 ± 0.003*

Values represent the means ± SE.
* $p < 0.01$ vs. NaCl. Data were analyzed by ANOVA, followed by Bonferroni's test.

activity of NOS compared with NaCl infused ones (Table 2).

nNOS and iNOS isoforms were undetectable in all studied tissues in both groups when Western blot analysis were performed. CNP did not modify the eNOS protein abundance indicating that the expression of cardiac and vascular eNOS is not affected by this peptide (Fig. 2).

Receptor type and signaling cascade involved in CNP and NOS interaction

Concentration-dependent stimulation of NOS activity in atria, ventricle and aorta artery by CNP and cANP is shown in Fig. 3. The lower concentration of each peptide that induced the maximum effect on NOS was used in the experimental protocol.

Table 2
Changes in NOS activity induced by CNP infusion in heart and aorta artery

	NOS activity (pmol/g tissue min)	
	NaCl	CNP
Aorta artery	225.6 ± 1.3	260.2 ± 4.5*
Heart		
Right atria	240.8 ± 2.8	281.6 ± 7.4*
Left ventricle	181.6 ± 1.7	225.6 ± 4.9*

Values represent the means ± SEM.
* $p < 0.01$ vs. NaCl. Data were analyzed by ANOVA followed by a Bonferroni test.

Table 3 shows changes in NOS activity induced by CNP and cANP(4-23) in aorta artery, right atria and left ventricle. CNP and cANP(4-23) increased NOS activity in all studied tissues. NOS activity stimulation induced by both peptides was blunted when L-NAME was previously added verifying that measured activity was specific from NOS.

NPR-A receptor antagonist, anantin, did not modified NOS activity induced by CNP and cANP indicating that this receptor would not be involved in this mechanism (Fig. 4).

The increase of NOS activity induced by CNP and cANP(4-23) was abolished by nifedipine and calmidazolium in heart and aorta artery, implying a Ca^{2+}-calmodulin dependent NOS pathway (Fig. 4).

The inhibition of G proteins with, suramin or pertussis toxin, or the inhibition of PI3-kinase/Akt pathway with wortmannin had no effect on basal NOS activity in neither

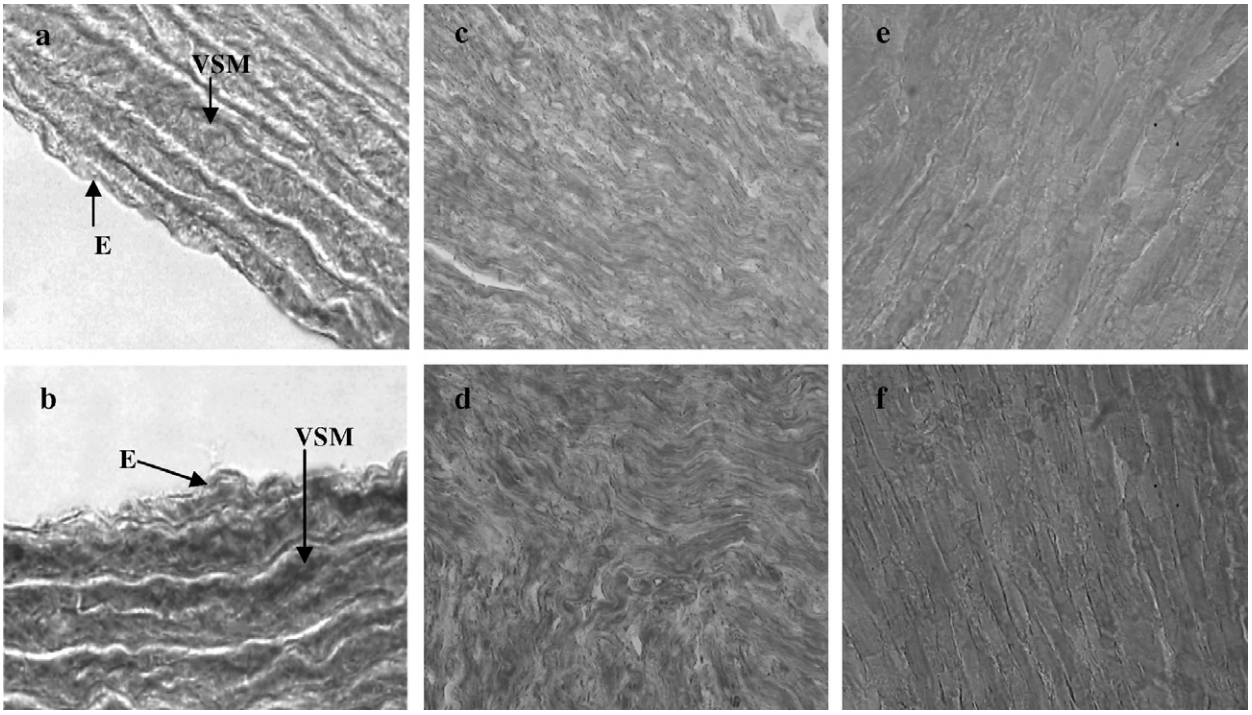


Fig. 1. Photomicrographs of the aorta artery NADPH-d (+) from NaCl (a) and CNP (b), right atria from NaCl (c) and CNP (d) and left ventricle from NaCl (e) and CNP (f). Note the intensity of the tissues from the CNP treated animals compared with NaCl. All images appear with the same magnification. Scale bar = 30 μm.

of the studied tissues (data not shown). The uncouple NPR-C receptor/G protein complexes, with suramin, abolished NOS activity elicited by CNP or cANP(4-23) in all tissues. Pertussis toxin, an inhibitor of Gi_{1-2} proteins, partially reduced NOS stimulation via these peptides, both in heart and aorta artery. The inhibition of PI3-kinasa/Akt pathway, with wortmannin, did not affect NOS activity exhibited by all tissues in response to the studied peptides (Fig. 4).

In order to discard a possible role of NPR-B, which bind CNP with high affinity, we verify whether the addition of anantin + suramin modify the increased NOS activity via CNP. This activation was abolished when we combined NPR-A antagonist and G protein inhibitor (Fig. 4).

Discussion

This study demonstrated, at the first time, that NO-system activation would be involved in cardiovascular effects induced by the C-type natriuretic peptide.

The published results concerning the effects of CNP on blood pressure are controversial. Some studies showed a vasorrelaxation while other authors found no effect of CNP. In this study we observed CNP induced a decrease of MAP in normotensive rats. Our results are in accordance with Clavell et al., that showed CNP administration diminishes MAP in dogs [21]. In contrast, other studies showed that CNP infusions induce a small increment in cGMP and had no significant hemodynamic actions in humans [22].

In addition, our findings showed that the decrease in MAP observed with CNP was associated with an increase on the excretion of NO metabolites, indicating that CNP would enhance NO systemic production. These findings led us to evaluate NOS activity and expression changes in response to CNP infusion. Then, we demonstrated that CNP induced an increase of NOS activity in atria, ventricle and aorta with the subsequent augment of NO, indicating

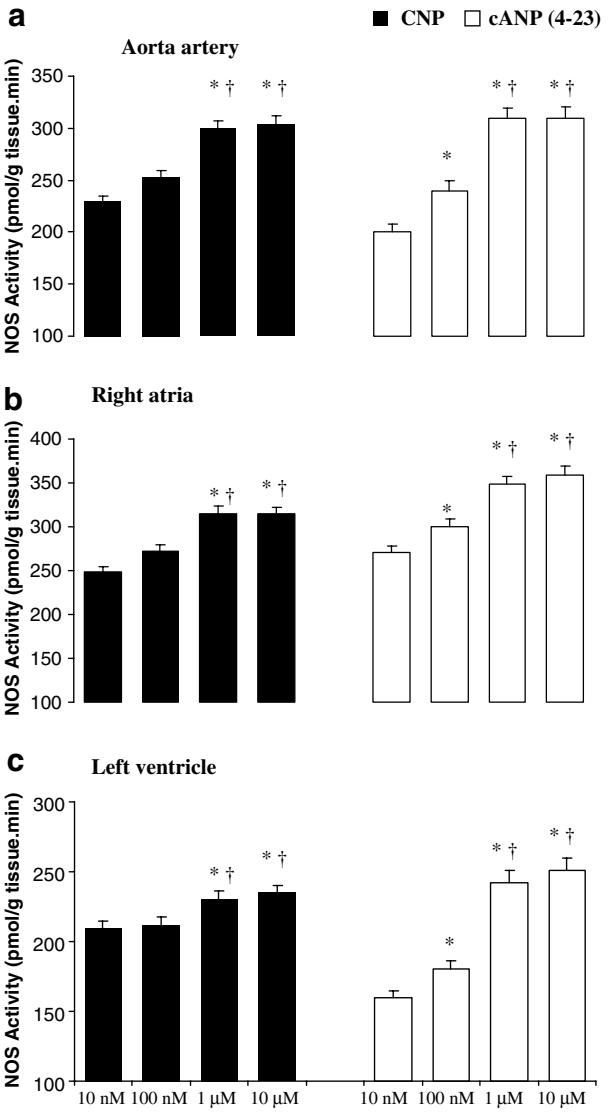


Fig. 3. Concentration-dependent stimulation of NOS activity in aorta artery (a), right atria (b) and left ventricle (c) by CNP and cANP(4-23). Values are means ± SE ($n = 8$). $^{*}p < 0.01$ vs. 10 nM; $^{\dagger}p < 0.001$ vs. 100 nM.

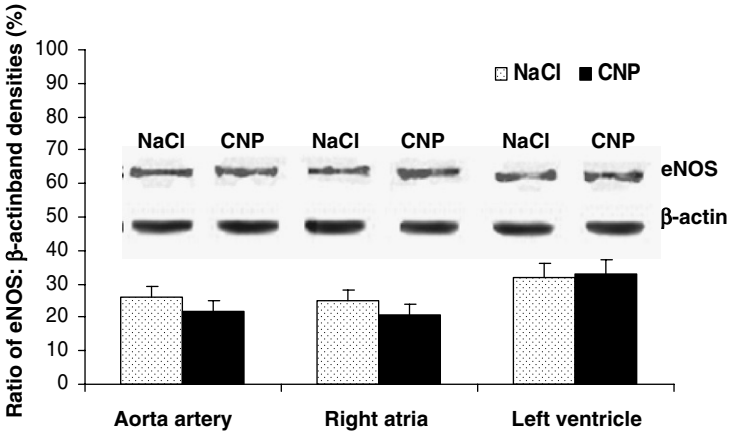


Fig. 2. Representative Western blot analysis of aorta artery, right atria and left ventricle of NaCl and CNP infusion groups to an anti-eNOS antibody. Data are means ± SEM, $n = 8$ rats/group. All experiments were performed in triplicate. Each blot was normalized with the expression of marker β-actin from the same gels.

Table 3
Changes in NOS activity induced by the addition of CNP, cANP(4-23) and L-NAME in heart and aorta artery

	NOS activity (pmol/g tissue min)				
	Basal activity	L-NAME	CNP	cANP(4-23)	CNP+cANP
Aorta artery	217.3 ± 6.3	109.8 ± 5.2*	300 ± 5.3*	308.5 ± 5.9*	316.1 ± 6.1*
Heart					
Right atria	233.6 ± 4.7	126.8 ± 3.4*	335.1 ± 4.4*	345.2 ± 6.2*	356.3 ± 5.8*
Left ventricle	175.3 ± 8.7	104.6 ± 9.7*	230.0 ± 5.7*	242.2 ± 8.9*	247.2 ± 4.7*

Values represent means ± SEM.
* $p < 0.01$ vs. basal activity. Data were analyzed by ANOVA followed by Bonferroni test.

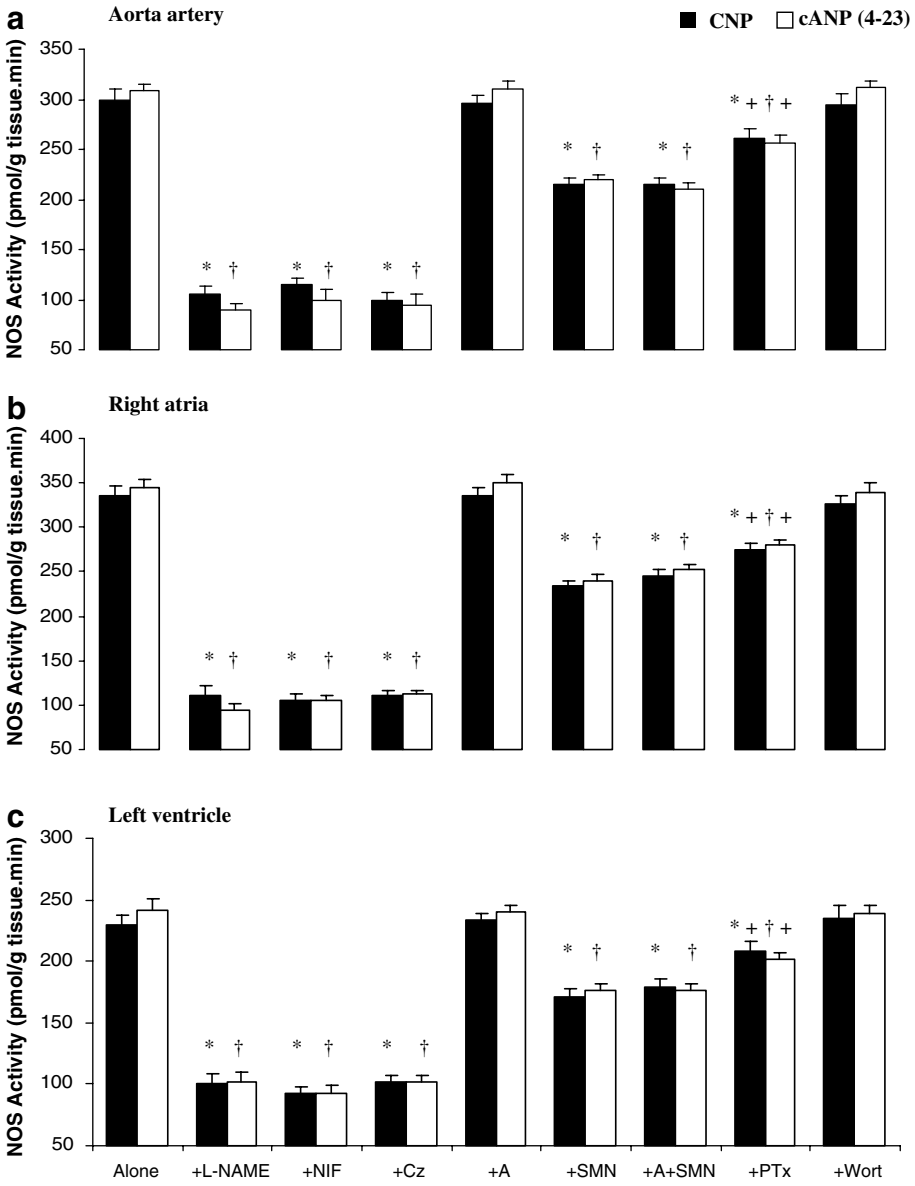


Fig. 4. Inhibition of cardiac and vascular NOS activity induced by CNP (1 μM) or cANP(4-23) 1 μM by the blockade of various steps in the signalling pathway. L-Nitro arginine metil ester (L-NAME; 1 μM), nifedipine (NIF; 1 μM), calmidazolium (Cz; 1 μM), anantin (A; 100 nM), suramin (SMN; 500 nM), pertussis toxin (PTx; 800 ng/mL) or wortmannin (Wort; 10 μM). (a) Aorta artery, (b) Right atria, (c) left ventricle. Values are means ± SE ($n = 8$). *$p < 0.01$ vs. CNP alone; †$p < 0.01$ vs. cANP(4-23) alone; +$p < 0.01$ vs. SMN.

that CNP actions on cardiac and vascular system involved, almost in part, NO-system activation.

However, we cannot through away that, the high plasma levels of CNP achieved by infusion can result in alterations in ANP and/or BNP levels, by competing for binding to NPR-C and reducing clearance, an thus may stimulate guanylyl cyclase-A. Although, the fact that NOS activation via CNP was not affected by NPR-A antagonist and it is abol-

ished by G protein inhibition, would indicate that the interaction between CNP and NO-system only involve NPR-C natriuretic receptor. In addition, cANP(4-23), specific NPR-C receptor agonist, induced a similar increase in NOS activity than the observed with CNP in all studied tissues, suggesting that this receptor would be involved in NOS activation induced by this peptide.

Additionally, we also showed that CNP increased NOS activity in cardiac ventricle and atria and aorta artery in *in vitro* experiments, suggesting that NOS stimulation induced by the peptide observed in *in vivo* infusions would be independent of the hemodynamic changes induced by CNP.

On the other hand, Murthy et al. demonstrated, in gastrointestinal smooth muscle, that eNOS is activated by two G proteins (G_{i1} and G_{i2}) that couple to NPR-C [11,23]. The signaling cascade, postulated by Murthy et al., involves G-protein dependent stimulation of Ca^{2+} influx and activation of eNOS bound to calmodulin; in turn, NO activates soluble guanylyl cyclase resulting in formation of cGMP [11].

Our results showed that cardiac and vascular NOS activity stimulated by CNP was blunted by suramin and was partially abolished by PTx. These results suggest that another G protein different from Gi would be also involved in this mechanism. Thus, the fact that NOS stimulation via CNP was blunted when G protein was inhibited and that NPR-A/B receptors are not associated to G proteins, would suggest that the activation of NOS induced by CNP only involves NPR-C receptor.

Moreover, the increased NOS activity induced by CNP and cANP(4-23) was blunted by the antagonist of calmodulin and the block of Ca^{2+} influx, suggesting that signaling cascade is triggered by agonist-induced Ca^{2+} influx and it is mediated by Ca^{2+}/calmodulin-dependent NOS.

Our findings demonstrate that NO-system activation would be one of the mechanisms involved in CNP vascular and cardiac actions. NPR-C natriuretic receptor would mediate the activation of NOS by CNP in heart and aorta artery. CNP would interact with NPR-C receptor coupled via Gi and/or other G protein, which activates Ca^{2+}-calmodulin dependent NOS. CNP would increase the activity of eNOS inducing no changes in its expression.

On the other hand, recent studies have revealed that PI3 kinase/Akt pathway are an important signalling cascade mediating eNOS activation in vascular endothelial cells [24]. However the inhibition of PI3-kinasa/Akt pathway did not affect NOS activity exhibited in response to CNP in heart and aorta artery.

With regard to the actions of CNP in cardiac atria, Kim et al. attributed to CNP a role modulating negatively ANP secretion in rat atria [25]. In addition, Lee et al. postulated that ANP release would be controlled by CNP via NPR-B-cGMP mediated signaling which may in turn act via regulation of intracellular Ca^{2+}, in rabbit atria [26]. The present study brings evidences to postulate that NO-system activation via CNP and the consequent NO release, could be the mechanism responsible of cGMP increase, reducing ANP secretion.

With respect to cardiac ventricle, several authors have reported negative, positive or no inotropic effect for CNP in different myocardial preparations and several species [27,28]. By other side, Pierkes et al. reported that CNP has a biphasic effect, positive inotropic effect in the first stage and then a negative inotropic effect in isolated NPR-A deficient-mouse heart [29]. In addition, Brady et al. showed that the magnitude of the effect of CNP on contraction is similar to it reported for NO [30]. Interestingly, the reduction in contraction amplitude was associated with an increase of intracellular cGMP levels [31]. These results suggest that a cross-talk between both systems, NO-cGMP and CNP-cGMP, could regulate cardiac contractility. Taken in account these findings and our results, we suggest that the increase of NO production induced by CNP could be the mechanism that would mediate the negative inotropic effect of this peptide.

Respect to CNP antiproliferative effects, Tokudome et al. have shown that CNP inhibits the hypertrophy of cardiomyocytes, these effect would be associated to an increase of cyclic GMP concentration and hypertrophic responses induced by growth factor were blunted by NO [32]. In this regard, our results suggest that the increase in cGMP through the activation of NOS, could explain the inhibition of proliferation induced by CNP.

On the other hand, it was demonstrated that CNP has hyperpolarizing actions in mesenteric and coronary vascular beds, through NPR-C activation and G-protein-gated inwardly rectifying K^+ opening [33]. In addition, Brunner and Wolkart showed that relaxation of coronary microvasculature induced by CNP is partially mediated by NO-cGMP pathway [34]. In accordance with these findings, we demostrated that CNP would interact NPR-C to activate NOS with the subsequent augment of NO levels, and through this pathway, CNP might induce vasorelaxation in aorta artery and probably in other vascular beds.

In summary, our results demonstrate that the activation of NO-system would be one of the mechanisms involved in CNP vascular and cardiac actions. This peptide would interact with NPR-C receptor coupled via G proteins leading to the activation Ca^{2+}-calmodulin dependent NOS. The increase of NO production would induce the accumulation of cGMP, reducing cardiac myocyte contractility and ANP synthesis and secretion in right atria and inducing vasorelaxation.

Perspectives

Our findings may be construed to be not only of pharmacological meaning, but rather a step in the direction of a better understanding of the possibly complex role that CNP plays in the regulation of blood pressure, due to the activation of NO-system, in physiological and perhaps pathophysiological conditions.

Acknowledgments

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References

[1] S. Moncada, R.M. Palmer, E.A. Higgs, Nitric oxide: physiology, pathophysiology and pharmacology, Pharmacol. Rev. 43 (1991) 109–142.
[2] M.R. Wilkins, J. Redondo, L.A. Brown, The natriuretic peptide family, Lancet 349 (1997) 1307–1310.
[3] S.P. D'Souza, M. Davis, G.F. Baxter, Autocrine and paracrine actions of natriuretic peptides in the heart, Pharmacol. Ther. 101 (2004) 113–129.
[4] H.H. Chen, J.C. Burnett Jr., C-type natriuretic peptide: the endothelial component of the natriuretic peptide system, J. Cardiovasc. Pharmacol. 32 (1998) S22–S28.
[5] A. Ahluwalia, R.J. MacAllister, A.J. Hobbs, Vascular actions of natriuretic peptides, Basic. Res. Cardiol. 99 (2004) 83–89.
[6] K.N. Pandey, Biology of natriuretic peptides and their receptors, Peptides 26 (2005) 901–932.
[7] L.R. Potter, S. Abbey-Hosch, D.M. Dickey, Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions, Endocr. Rev. 27 (2006) 47–72.
[8] M.B. Anand-Srivastava, Natriuretic peptide receptor-C signaling and regulation, Peptides 26 (2005) 1044–1059.
[9] M.B. Anand-Srivastava, P.D. Sehl, D.G. Lowe, Cytoplasmic domain of natriuretic peptide receptor-C inhibits adenylyl cyclase, J. Biol. Chem. 271 (1996) 19324–19329.
[10] M.A. Costa, R. Elesgaray, A.M. Balaszczuk, C. Arranz, Role of NPR-C natriuretic receptor in nitric oxide system activation induced by atrial natriuretic peptide, Regul. Pept. 135 (2006) 63–68.
[11] K.S. Murthy, B.Q. Teng, H. Zhou, J.G. Jin, J.R. Grider, G.M. Makhlouf, $G_{i\text{-}1}/G_{i\text{-}2}$-dependent signaling by single-transmembrane natriuretic peptide clearance receptor, Am. J. Physiol. Gastrointest. Liver. Physiol. 278 (2000) G974–G980.
[12] S. Suga, K. Nakao, K. Hosoda, M. Mukoyama, Y. Ogawa, G. Shirakami, H. Arai, S. Yoshihiko, Y. Kambayashi, K. Inouye, H. Imura, Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide, Endocrinology 130 (No 1) (1992) 229–239.
[13] W.K. Alderton, C.E. Cooper, R.G. Knowles, Nitric oxide synthases: structure, function and inhibition, Biochem. J. 357 (2001) 593–615.
[14] U. De Forstermann, H.W. Schmidt, J.S. Pollock, H. Sheng, J.A. Mitchell, T.D. Warner, M. Nakane, F. Murad, Isoforms of nitric oxide synthase: characterization and purification from different cell types, Biochem. Pharmacol. 42 (1991) 1849–1857.
[15] M.A. Costa, L.V. González Bosc, M.P. Majowicz, N.A. Vidal, A.M. Balaszczuk, C.T. Arranz, Atrial natriuretic peptide modifies arterial blood pressure through nitric oxide pathway in rats, Hypertension 35 (2000) 1119–1123.
[16] M.A. Costa, R. Elesgaray, A. Loria, A.M. Balaszczuk, C. Arranz, Atrial natriuretic peptide influence on nitric oxide system in kidney and heart, Regul. Pept. 118 (3) (2004) 151–157.
[17] J. Amin, O. Carretero, I. Sadayoshi, Mechanisms of action of atrial natriuretic factor and C-type natriuretic peptide, Hypertension 27 (1995) 684–687.
[18] T. Marumo, T. Nakaki, K. Hishikawa, R. Kato, T. Saruta, Natriuretic peptide—augmented induction of nitric oxide synthase through cGMP elevation in vascular smooth muscle cells, Endocrinology 136 (1995) 2135–2142.
[19] C.P. Verdon, B.A. Burton, R.L. Prior, Sample pretreatment with nitrate reductase and glucose-6-phosphate dehidrogenase quantitatively reduces nitrate while avoiding interference by NADP+ when the Griess reaction is used to assay for nitrite, Anal. Biochem. 224 (1995) 502–508.
[20] S. Vincent, H. Kimura, Histochemical mapping of nitric oxide synthase in the rat brain, Neuroscience 46 (1992) 755–784.
[21] A.L. Clavell, A.J. Stingo, C.M. Wei, D.M. Heublein, J.C. Burnett Jr., C-type natriuretic peptide: a selective cardiovascular peptide, Am. J. Physiol. (Regulatory Integrative Comp. Physiol. 33) 264 (1993) R290–R295.
[22] G. Barletta, C. Lazzeri, S. Vecchiarino, R. Del Bene, G. Messeri, A. Dello Sbarba, M. Mannelli, G. La Villa, Low-dose C-type natriuretic peptide does not affect cardiac and renal function in humans, Hypertension 33 (1998) 802–808.
[23] K.S. Murthy, B.Q. Teng, J.G. Jin, G.M. Makhlouf, G protein-dependent activation of smooth muscle eNOS via natriuretic peptide clearance receptor, Am. J. Physiol. Gastrointest. Liver Physiol. 275 (1998) C1409–C1416.
[24] X. Peng, S. Haldar, S. Deshpande, K. Irani, D.A. Kass, Wall stiffness suppresses Akt/eNOS and cytoprotection in pulse-perfused endothelium, Hypertension 41 (2003) 378–381.
[25] S.H. Kim, J.H. Han, S.H. Lim, S.J. Lee, S.Z. Kim, K.W. Cho, Attenuation of inhibitory effect of CNP on the secretion of ANP from hypertrophied atria, Am. J. Physiol. Regul. Integrative Comp. Physiol. 281 (2001) R1456–R1463.
[26] S.J. Lee, S.Z. Kim, X. Cui, S.H. Kim, K.S. Lee, Y.J. Chung, K.W. Cho, C-type natriuretic peptide inhibits ANP secretion and a trial dynamics in perfused atria: NPR-B-cGMP signaling, Am. J. Physiol. 278 (2000) H208–H221.
[27] A. Nir, D.F. Zhang, R. Fixler, J.C. Burnett Jr., Y. Eilam, Y. Hasin, C-type natriuretic peptide has a negative inotropic effect on cardiac myocites, European J. Pharmacol. 412 (2001) 195–201.
[28] M. Hirose, Y. Furukawa, F. Kurogouchi, K. Nakajima, Y. Miyashita, S. Chiba, C-type natriuretic peptide increases myocardial contractility and sinus rate mediated by guanylyl cyclase-linked natriuretic peptide receptors in isolated, blood-perfused dog heart preparations, Pharmacol. Exp. Ther. 286 (1998) 70–76.
[29] M. Pierkes, S. Gambaryan, P. Boknik, S.M. Lohmann, W. Schmitz, R. Potthast, R. Holtwick, M. Kuhn, Increased effects of C-type natriuretic peptide on cardiac ventricular contractility and relaxation in guanylyl cyclase A-deficient mice, Cardiovasc. Res. 53 (2002) 852–861.
[30] A.J.B. Brady, J.B. Warren, P.A. Poole-Wilson, T.J. Williams, S.E. Harding, Nitric oxide attenuates cardiac myocyte contraction, Am. J. Physiol. 265 (1993) H176–H182.
[31] T. Tokudome, T. Horio, T. Soeki, K. Mori, I. Kishimoto, S. Suga, F. Yoshihara, Y. Kawano, M. Kohno, K. Kangawa, Inhibitory effect of C-type natriuretic peptide (CNP) on cultured cardiac myocyte hypertrophy: interference between CNP and endothelin-1 signaling pathways, Endocrinology 145 (5) (2004) 2131–2140.
[32] A. Ahluwalia, A.J. Hobbs, Endothelium-derived C-type natriuretic peptide: more than just a hyperpolarizing factor, Trends Pharmacol. Sci. 26 (2005) 62–67.
[33] S.D. Chauhan, H. Nilsson, A. Ahluwalia, A.J. Hobbs, Release of C-type natriuretic peptide accounts for the biological activity of endothelium-derived hyperpolarizing factor, Proc. Natl. Acad. Sci. 100 (2003) 1426–1431.
[34] F. Brunner, G. Wolkart, Relaxant effect of C-type natriuretic peptide involves endothelium and nitric oxide-cGMP system in rat coronary microvasculature, Cardiovasc. Res. 51 (2001) 577–584.