Role of nitric oxide as a key mediator on cardiovascular actions of atrial natriuretic peptide in spontaneously hypertensive rats

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Costa MA, Elesgaray R, Caniffi C, Fellet A, Mac Laughlin M, Arranz C. Role of nitric oxide as a key mediator on cardiovascular actions of atrial natriuretic peptide (ANP) in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 298: H778–H786, 2010. First published September 25, 2009; doi:10.1152/ajpheart.00488.2009.—The objective was to study atrial natriuretic peptide (ANP) effects on mean arterial pressure (MAP) and cardiovascular nitric oxide (NO) system in spontaneously hypertensive rats (SHRs), investigating the receptors and signaling pathways involved. In vivo, SHRs and Wistar-Kyoto (WKY) rats were infused with saline (0.05 ml/min) or ANP (0.2 μg·kg⁻¹·min⁻¹) for 1 h. MAP and nitrates and nitrates excretion (NOx) were determined. NO synthase (NOS) activity and endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) NO expression were measured in the heart and aorta. In vitro, heart and aortic NOS activity induced by ANP was determined in the presence of iNOS and nNOS inhibitors, natriuretic peptide receptor (NPR)-A/B blocker, G protein, and calmodulin inhibitors. As a result, ANP diminished MAP and increased NOx in both groups. Cardiovascular NOS activity was higher in SHRs than in WKY rats. ANP increased NO activity but the activation was lower in SHRs than in WKY rats. ANP had no effect on NOS isoform expression. NOS activity induced by ANP was not modified by iNOS and nNOS inhibitors. NPR-A/B blockade blunted NOS stimulation via ANP in ventricle and aorta but not in atria. Cardiovascular NOS response to ANP was reduced by G protein and calmodulin inhibitors in both groups. In conclusion, in atria, ventricle, and aorta, ANP interacts with NPR-C receptors, activating Ca²⁺-calmodulin eNOS through G protein. In ventricle and aorta, NOS activation also involves NPR-A/B. The NOS response to ANP was impaired in heart and aorta of SHRs. The impaired NO-system response to ANP in hypertensive animals, involving alterations in the signaling pathway, could participate in the maintenance of high blood pressure in this model of hypertension.

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THE CARDIOVASCULAR ACTIONS of nitric oxide (NO) and atrial natriuretic peptide (ANP) are essential to the regulation of blood pressure, participating in the regulation of cardiac function, vascular tone, and salt and water balance (16, 21, 22, 25).

NO is synthesized by the NO synthase (NOS) enzyme, which exists in three isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS), expressed in cardiovascular tissues (1, 14).

ANP, mainly produced in the cardiac atria and ventricles in response to the stretching of the cardiac wall, exerts its biological actions through an interaction with two specific membrane-bound guanylyl cyclase receptors: natriuretic peptide receptor (NPR)-A and NPR-B (24, 27). There is a third natriuretic receptor subtype, NPR-C, which has been proposed primarily as a clearance receptor removing natriuretic peptides from the circulation, but now other biological functions have been reported (2, 8, 10).

In previous studies we have demonstrated that NO system activation would be one of the mechanisms involved in ANP vascular, cardiac, and renal actions in normotensive rats. ANP would exert its hypotensive, natriuretic, and diuretic effects, at least in part, through the activation of NOS (5, 6). Furthermore, we have reported that NPR-C would mediate the activation of NOS by ANP in cardiac atria (8, 13). In this regard, William et al. (32) demonstrated that natriuretic peptides stimulate the Na⁺-K⁺ pump via an NPR-C and NO-dependent pathway. Additionally, we have also shown that ANP could interact with NPR-A and/or -B in ventricle, kidney, and artery, resulting in NOS activation (8, 13).

Spontaneously hypertensive rats (SHRs) constitute an animal model of genetic origin that is used in the study of the mechanisms involved in human essential hypertension.

With respect to ANP studies, high-plasma levels of the peptide were observed in this model of hypertension (18, 20). In addition, Tremblay et al. (28) showed a genetic overexpression of the NPR-A, associated with cGMP overproduction, which could explain the augmented biological response to ANP in SHRs.

The available data on the NO system in SHRs are limited and apparently contradictory. Nava et al. (23) have shown that NO synthesis could be enhanced in SHRs, probably as a counterregulatory mechanism activated to compensate for the increase in blood pressure (29). Several studies have found evidence suggesting that the activity and/or expression of the different NOS isoforms would be altered in this model and that a differential expression of NOS isoforms depends on age (3, 26, 30).

In view of the fact that there exists a close link between both NO and ANP and arterial blood pressure regulation, we can speculate that the alterations in the interaction between both systems would be involved in the development and/or maintenance of high levels of blood pressure in this experimental model of spontaneous hypertension.

To demonstrate our hypothesis, experiments were designed to investigate the effects of ANP on the NO system in SHRs, studying the changes in cardiovascular NOS activity and expression in response to peptide infusion. In addition, the signaling pathways implicated in the interaction between ANP and NOS were also investigated, identifying the natriuretic receptors and NOS isoforms involved in this experimental model of hypertension.
Materials and Methods

Animals. Sixteen-week-old male SHRs and Wistar-Kyoto (WKY) rats were purchased from the Instituto de Investigaciones Médicas A. Lanari, Facultad de Medicina (Universidad de Buenos Aires, Argentina). Rats were housed in a humidity- and temperature-controlled environment with an automatic 12-h:12-h light-dark cycle. They were fed standard rat chow from Nutrimentos Purina (Buenos Aires, Argentina) and tap water ad libitum up to the day of the experiments.

Experiment design. All experimental protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Publication No. 86-23, Revised 1996) and with regulation 6344/96 of Argentina’s National Institutes of Health (publication No. 86-23 regulations 6344/96 of Argentina’s National Institutes of Health, Publication No. 86-23, Re-vised 1996) and with regulation 6344/96 of Argentina's National Drug, Food, and Medical Technology Administration. Experimental procedures were approved by the Ethics Committee of the School of Biochemistry and Pharmacy at Buenos Aires University.

Protocol 1: effects of ANP infusion on mean arterial pressure and the NO system. Rats were anesthetized with urethane (1 g/kg body wt ip; Sigma-Aldrich, St. Louis, MO). The femoral vein and artery and the urinary bladder were cannulated with a polyethylene catheter for drug administration (NaCl or ANP infusion), mean arterial pressure (MAP) recording, and urine collection, respectively.

After surgery, an infusion of isotonic NaCl was started at a rate of 0.05 ml/min and maintained for 40 min to allow for the stabilization of hemodynamic and renal parameters. The first 30 min following stabilization were considered the control period, during which the rate of saline infusion was maintained at 0.05 ml/min. At the end of this period, one group of SHRs and WKY rats received first a bolus of ANP (5 μg/kg) and then an infusion with ANP (0.2 μg·kg⁻¹·min⁻¹) over 1 h, and another group continued with the isotonic saline infusion. NO₂-nitro-L-arginine methyl ester (L-NAME, an NOS inhibitor) was injected subcutaneously (3 mg/kg body wt) in another group of SHRs and WKY animals 1 h before bolus administration and ANP infusion (5 μg/kg and 0.2 μg·kg⁻¹·min⁻¹, respectively). The L-NAME dose used in this protocol induced no changes in MAP (34).

MAP was recorded and urine samples were collected at the end of the control and experimental periods in all groups of animals.

Arterial blood pressure was measured with a Statham P23 ID pressure transducer (Gould Instruments, Cleveland, OH) and recorded with a polygraph (Physiograph E & M, Houston, TX). Data were obtained using data acquisition software (Labtech Notebook, Laboratory Technologies, Wilmington, MA).

The concentration of nitrates and nitrates (NOx), end products derived from NO metabolism, was determined in urine samples according to the procedure described by Verdon et al. (31).

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

Determination of NOS activity. Tissue NOS activity was measured using [¹⁴C]L-arginine as substrate, as described previously (7, 9). Tissue slices (2 to 3 mm thick) were incubated 30 min at 37°C in Krebs solution with 0.5 μCi/ml [¹⁴C]L-arginine. The reaction was stopped by adding 500 μl stop buffer containing (in mmol/l) 0.5 EGTA, 0.5 EDTA, and 20 HEPEES (pH 5.5). Tissue samples were homogenized in the stop solution. The homogenates were centrifuged at 12,000 g for 20 min. The supernatants were then applied to a 1-ml Dowex AG 50W-X8 column (Na⁺ form, Bio-Rad), hydrated with the stop buffer, and eluted with 2 ml distilled water. The amount of [¹⁴C]L-citrulline was determined with a liquid scintillation counter (Wallac 1414 WinSpectral). Specific NOS activity was assessed in the presence of 10⁻⁴ M L-NAME (Sigma). NO production (measured as pmol of [¹⁴C]L-citrulline) in each tube was normalized to the weight of the tissue slices incubated with the substrate during equal periods of time and expressed as picomoles per gram wet weight per minute.

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, transferred to a nitrocellulose membrane (Bio-Rad, Munich, Germany), and then incubated with rabbit polyclonal anti-NOS antibodies (1/500 dilution) and a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody. The bands were visualized with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody at room temperature for 1 h.

Table 1. Effects of ANP infusion on MAP in SHRs and WKY rats

<table>
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<tr>
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<th>NaCl</th>
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<th>ANP</th>
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<th>L-NAME-ANP</th>
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<tr>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
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<td>WKY</td>
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<tr>
<td>100 ± 6</td>
<td>105 ± 5</td>
<td></td>
<td>104 ± 4</td>
<td>85 ± 3</td>
<td>105 ± 4</td>
<td>97 ± 4^b,d</td>
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<tr>
<td>∆</td>
<td>5 ± 3</td>
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<td>19 ± 4^f</td>
<td>142 ± 5</td>
<td>165 ± 5</td>
<td>151 ± 3^e</td>
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<tr>
<td>SHR</td>
<td>167 ± 8^a</td>
<td>6 ± 4</td>
<td>163 ± 4</td>
<td>5^c,e</td>
<td>165 ± 5</td>
<td>151 ± 3^e</td>
</tr>
<tr>
<td>∆</td>
<td>173 ± 6</td>
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<td>21 ± 4^d</td>
<td>14 ± 2^h,i</td>
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Values are means ± SE; n = 8 rats/group. C, control period, NaCl infusion at 0.05 ml/min for 30 min; E, experimental period, NaCl infusion or bolus atrial natriuretic peptide (ANP, 5 μg/kg) + infusion ANP (0.2 μg·kg⁻¹·min⁻¹) or NO₂-nitro-L-arginine methyl ester (l-NAME, 3 mg/kg body wt) 30 min before ANP treatment over 1 h. MAP, mean arterial pressure (in mmHg); ∆, change induced by each treatment on MAP. *P < 0.001 vs. C NaCl Wistar-Kyoto (WKY) rat; **P < 0.001 vs. E NaCl WKY; ***P < 0.01 vs. E NaCl WKY; ****P < 0.001 vs. E ANP WKY; ^P < 0.01 vs. E ANP spontaneously hypertensive rat (SHR); _P < 0.01 vs. E NaCl WKY; A_ P < 0.01 vs. A_ ANP WKY; A_ P < 0.01 vs. A_ ANP SHR; a_ P < 0.01 vs. A_ ANP SHR; a_ P < 0.01 vs. A_ l-NAME + ANP WKY.
Table 2. Effects of ANP infusion on NOx in SHRs and WKY rats

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<th></th>
<th>NaCl</th>
<th>ANP</th>
<th>L-NAME-ANP</th>
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<tr>
<td></td>
<td>C</td>
<td>E</td>
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<tr>
<td>WKY</td>
<td>0.97 ± 0.10</td>
<td>1.02 ± 0.12</td>
<td>0.92 ± 0.12</td>
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<tr>
<td>Δ</td>
<td>0.11 ± 0.07</td>
<td>0.08 ± 0.05</td>
<td>0.68 ± 0.05</td>
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<tr>
<td>SHR</td>
<td>1.50 ± 0.10</td>
<td>1.42 ± 0.10</td>
<td>1.48 ± 0.05</td>
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Values are means ± SE, n = 8 rats/group. NOx, nitrates and nitrites excretion (in nmol/min; 100 g body wt); Δ, change induced by each treatment on NOx. *P < 0.001 vs. C NaCl WKY; †P < 0.001 vs. E NaCl WKY; ‡P < 0.01 vs. E NaCl WKY; ‡‡P < 0.01 vs. E ANP WKY; ‡§P < 0.01 vs. E ANP SHR; ‡¶P < 0.01 vs. Δ NaCl WKY; ††P < 0.01 vs. Δ ANP WKY; †‡P < 0.01 vs. Δ NaCl SHR; †§P < 0.01 vs. Δ ANP SHR; †¶P < 0.01 vs. Δ L-NAME + ANP WKY.

Protocol 2: effects of ANP on NOS activity in isolated heart and aortic artery. Signaling cascade involved in interaction between ANP and NOS in SHRs. SHRs and WKY rats were euthanized by decapitation, and NOS activity was measured in the aortic artery, right atria, and left ventricle using [14C]-arginine as substrate. Tissue slices (2 to 3 mm thick) were incubated for 30 min at 37°C as described in protocol 1, but agonists (15 min after incubation was started) and/or antagonists (at the beginning of the 30-min incubation period) were added during the incubation period according to the protocol described in Fig. 1.

Concentration-dependent stimulation of NOS activity by ANP in all studied tissues had been performed in previous studies (data not shown). The lowest concentration of ANP that induced the maximum effect on NOS was used in the experimental protocol.

Statistical analysis. All values are expressed as means ± SE. The Prism program (Graph Pad Software, San Diego CA) was used for statistical analysis. The mean and standard deviation or median values of each variable for each group were calculated. The results of each variable for each experimental group were analyzed with a two-way analysis of variance where one factor was the different treatments and the other was the genotypes (WKY or SHRs). The effects of each parameter on the NO system in hypertensive animals (Table 2). As expected, L-NAME treatment reduced NOx excretion in all experimental conditions.

We then verified whether the increase in NOx excretion was associated with the stimulation of cardiovascular NOS. NOS activity in response to saline or ANP infusion is shown in Fig. 2. In all studied tissues, NOS activity was higher in SHRs than in WKY rats. ANP infusion enhanced this activity in both groups of animals. NOS stimulation induced by ANP was lower in WKY than in SHRs, indicating a lower effect of ANP on the NO system in hypertensive animals (Table 2). As expected, L-NAME treatment reduced NOx excretion in all experimental conditions.

Protocol 1: effects of ANP infusion on MAP and the NO system. When we determined MAP and the systemic production of NO in this model of hypertension, we found that both parameters, MAP and NOx excretion, were significantly higher in SHRs than in WKY rats (Table 1). ANP infusion reduced MAP in both groups of animals. NOS blockade with L-NAME did not modify MAP in either SHRs or WKY animals that received NaCl infusion (M. A. Costa, unpublished observation).

When rats were pretreated with L-NAME before ANP infusion, the effect of the peptide on MAP diminished in both groups, SHR and WKY. However, L-NAME had a greater effect on the MAP response to ANP in WKY than in SHRs.

The reduction in MAP induced by ANP was accompanied by a rise in NOx excretion, but the increase in NOx was more marked in WKY than in SHRs, indicating a lower effect of ANP on the NO system in hypertensive animals (Table 2). As expected, L-NAME treatment reduced NOx excretion in all experimental conditions.

Results

Fig. 2. Effect of NaCl and ANP infusion on cardiac and vascular NOS activity in spontaneously hypertensive rats (SHRs) and Wistar-Kyoto (WKY) rats. Data corresponding to the difference (Δ) between saline and ANP treatment in each group are shown between brackets. Data are means ± SE; n = 8 rats/group. *P < 0.001 vs. WKY + NaCl; †P < 0.01 vs. WKY + ANP; ‡P < 0.01 vs. WKY + NaCl; ††P < 0.01 vs. WKY + ANP; †‡P < 0.01 vs. ΔWKY.
both groups of animals. Heart nNOS expression was more marked in SHRs than in WKY rats. ANP induced no changes in protein level of eNOS, nNOS, and iNOS isoforms, neither in the heart nor artery in both groups (Fig. 3).

Protocol 2: effects of ANP on NOS activity in isolated heart and aortic artery. Signaling cascade involved in interaction between ANP and NOS. In vitro experiments showed that ANP increased cardiac and vascular NOS activity in both groups.
NOS activity induced by ANP was blunted when L-NAME was added previously, verifying that activity measured was specific from NOS (M. A. Costa, unpublished observation).

Similar to in vivo experiments, cardiac and vascular NOS activation induced by ANP was lower in SHRs than in WKY rats.

To analyze the isoform involved in NOS activation via ANP in this model, the experiments were performed in the presence of an inhibitor of iNOS (aminoguanidine) or an inhibitor of nNOS (7-nitroindazole). In all tissues in both groups, nNOS inhibition did not modify either basal or ANP-induced NOS activity (Fig. 3). Meanwhile, iNOS blockade provoked a decrease in basal NOS activity in both groups. This reduction was higher in SHRs than in WKY rats in all tissues (Table 3). In addition, the decrease in basal NOS activity induced by Ca$^{2+}$-calmodulin inhibition was more marked in WKY than in SHRs, which confirms that iNOS is the main isoform involved.
ANP-induced NOS activity in in vitro studies in heart and aortic artery in SHR and WKY rats

<table>
<thead>
<tr>
<th></th>
<th>Δ[Basal-AG]</th>
<th>Δ[ANP-(ANP+AG)]</th>
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<tbody>
<tr>
<td>Right atria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>−21.8 ± 3.9</td>
<td>−19.4 ± 4.8</td>
</tr>
<tr>
<td>SHR</td>
<td>−35.7 ± 3.1†</td>
<td>−34.6 ± 2.3†</td>
</tr>
<tr>
<td>Left ventricle</td>
<td></td>
<td></td>
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<tr>
<td>WKY</td>
<td>−30.0 ± 4.8</td>
<td>−33.3 ± 6.3</td>
</tr>
<tr>
<td>SHR</td>
<td>−62.6 ± 7.7*</td>
<td>−66.9 ± 9.1†</td>
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<tr>
<td>Aortic artery</td>
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<tr>
<td>WKY</td>
<td>−26.3 ± 3.4</td>
<td>−27.3 ± 2.6</td>
</tr>
<tr>
<td>SHR</td>
<td>−60.2 ± 10.7*</td>
<td>−66.3 ± 11.6†</td>
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Values are means ± SE; n = 8 rats/group. AG, amino guanidine; Δ NOS activity (in pmol·g tissue⁻¹·min⁻¹). *P < 0.01 vs. Δ [basal-AG] WKY; †P < 0.01 vs. Δ [ANP-(ANP+AG)] WKY.

in basol NOS activity observed in SHR (Fig. 4). The effect of iNOS blockade on NOS activity was similar in basol and in ANP-stimulated conditions in all studied tissues, indicating that the inducible isofrom is not stimulated by ANP.

The increase in NOS activity induced by ANP was abolished by calmodiloxin in the heart and artery of both SHR and WKY rats, implying a Ca²⁺-calmodulin-dependent NOS pathway (Fig. 4).

Although the selective agonist of the NPR-C receptor, cANP₄₋₂₃, increased NOS activity in all studied tissues, NOS stimulation in the ventricle and aorta was lower than the ANP induced increase. Only in atria did both ANP and cANP₄₋₂₃ provoke similar NOS induction.

On the other hand, the NPR-A/B receptor antagonist, anatin, did not modify NOS basal activity in the studied tissues in both groups (M. A. Costa, unpublished observation). NOS activity induced by ANP in the atrial artery and left ventricle was partially abolished by this natriuretic receptor antagonist in both groups; however, anatin did not modify NOS activity induced by ANP in right atria. This latter finding suggests that the NPR-A/B receptor would not be involved in this mechanism in atria but would participate in NOS activation via ANP in ventricle and aorta in WKY and SHR (Fig. 5).

The action of 8-bromo-cAMP (8-Br-cGMP) on NOS activity was determined to verify the involvement of guanylyl cyclase-coupled natriuretic receptors in the effect of ANP on NOS activity in the aortic artery and left ventricle. In all tissues, the addition of 8-Br-cGMP partially mimicked the effect of ANP on NOS activity (Fig. 5).

The experiments were performed in the presence of KT-5823 to evaluate whether cGMP signaling effects occur through PKG. Basal NOS activity in the heart and atria was not affected by the presence of the PKG inhibitor in both groups (M. A. Costa, unpublished observation). The inhibition of PKG induced no changes in NOS stimulation by ANP in atria, but it partially reduced the effect of the peptide in the atrial artery and left ventricle in both groups (Fig. 5).

Since NPR-C receptors are Gₛ, protein-coupled receptors, the effect of ANP on NOS activity in the presence of pertussis toxin, a Gₛ₁₂ protein inhibitor, was investigated. The toxin did not modify basal NOS activity in the heart and artery in both groups (M. A. Costa, unpublished observation). The results showed that the uncoupled NPR-C receptor/G protein complexes abolished NOS activity elicited by ANP in atria. NOS activity induced by ANP in the aortic artery and left ventricle was blunted by pertussis toxin (Fig. 5).

### DISCUSSION

In accordance with previous reports, our results confirmed that SHR exhibit a marked rise in arterial blood pressure associated with a significant increase in urinary excretion of NO metabolites, indicating that the L-arginine/NO pathway is upregulated in adult SHR.

Moreover, in the present study we demonstrated that basal NOS activity in the heart and atrial artery of hypertensive animals was higher compared with the activity observed in tissues from normotensive ones. In addition, basal activity of NOS was diminished when iNOS was blocked. The fact that the decrease in basal NOS activity induced by Ca²⁺-calmodulin inhibition was more marked in WKY that in SHR confirms that iNOS, a Ca²⁺-calmodulin-independent enzyme, was the main isofrom involved in basol NOS activity in SHR.

This model of hypertension shows controversial results concerning the expression of the different NOS isoforms. Vaziri et al. (30) demonstrated that 8-wk-old SHR present an elevation of thoracic aorta NOS activity coupled with significant increases in vascular iNOS and eNOS expression. According to these findings, Vapaatalo et al. (29) reported an augmented expression of eNOS in coronary endothelium in 12-wk-old SHR. Conversely, other authors (11, 12) showed a decrease in eNOS and iNOS expression in cardiac myocyte and vascular smooth muscle, respectively. With regard to these findings, our results have shown that the three isoforms of the enzyme were expressed in the right atria and left ventricle and that eNOS and iNOS were expressed in the aortic artery in both groups of animals. Accordingly, tissues from hypertensive rats showed greater protein levels of the three isoforms than normotensive ones. These results would suggest that the upregulation of NOS isoforms in vascular and cardiac tissues may play an important role in the compensatory mechanism in response to the elevation of systolic blood pressure during the development of hypertension in SHR.

However, we cannot discard the fact that the increment in oxidative stress induced by the elevation in peroxinitrites production may also be involved in maintenance of the high levels of blood pressure in this model of hypertension (15, 17).

Our in vivo studies showed that ANP treatment reduced MAP in both normotensive and hypertensive animals. NOS blockade blunted the ANP hypotensive effect in hypertensive animals and totally abolished this response in normotensive ones. These results would indicate that the NO system is involved in the hypotensive effect induced by ANP and that this response is impaired in hypertensive animals.

In the present study we have also shown that the acute infusion with ANP increased NOS activity in the heart and atrial artery in both groups. However, in agreement with our previous results in normotensive animals, the increase in NOS activity would not be associated with an increase in the protein expression of NOS isoforms in this model of hypertension. This fact indicates that ANP would exert a positive effect on NOS activity, without modifying the expression of the enzyme.

Additionally, we have also shown that ANP increased NOS activity in the cardiac ventricle and atria in vivo and in vitro experiments in both groups, suggesting that NOS stimulation...
induced by ANP infusion would be independent of the hemodynamic changes induced by this peptide.

With regard to the NOS isoform involved in ANP effects, we suggest that neither nNOS nor iNOS would be participating in the interaction between both systems in this model of hypertension. Moreover, increased NOS activity induced by ANP was blunted by the antagonist of calmodulin, suggesting that the signaling cascade is mediated by Ca\(^{2+}\)/calmodulin-dependent NOS. The present findings indicate that eNOS would be the isoform involved in this mechanism in this model of hypertension.

With respect to the natriuretic receptor involved in this mechanism, our results showed that the specific NPR-C receptor-selective agonist, cANP4-23, induced an increase in NOS activity, indicating that this receptor would be involved in NOS activation induced by ANP in the heart and aortic artery. However, this stimulation was lower than the one observed with ANP in the ventricle and aorta in both groups of animals. Additionally, the blockade of NPR-A/NPR-B did not modify the effect of ANP on NOS in atria, confirming that NPR-C would be the sole natriuretic receptor involved in the interaction between ANP and NOS in this tissue in hypertensive animals. Nevertheless, the studies in the aortic artery and left ventricle indicate that NPR-A/NPR-B would also be involved in the interaction between ANP and the NOS pathway in these tissues in SHR and WKY rats.

Many biological effects induced by ANP are mediated by NPR-A and NPR-B through the activation of particulate guanylyl cyclase and enhancing cGMP formation, which in turn activates PKG (1). Our results showed that 8-Br-cGMP par-

![Fig. 5. Changes in ANP-induced cardiovascular NOS activity provoked by cANP4-23, NPR-C receptor-selective agonist; AN, NPR-A/B receptor antagonist; 8-Br-cGMP, stable analog of cGMP; KT-5823, cGMP-dependent PKG inhibitor; or PTx, G\(_i\) protein inhibitor, in SHRs and WKY rats. Data are means ± SE; \(n = 8\) rats/group. *\(P < 0.01\) vs. basal activity WKY; †\(P < 0.01\) vs. ANP-induced activity WKY; ‡\(P < 0.01\) vs. basal activity SHR; §\(P < 0.01\) vs. ANP-induced activity SHR.](http://ajpheart.physiology.org/)

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tially mimicked the effect of ANP on NOS activity in cardiac ventricle and aortic artery. Furthermore, NOS activation induced by ANP in these tissues was blunted when PKG was inhibited, indicating the participation of the cGMP/PKG pathway in the effects of ANP on NOS in ventricle and aorta, but not in atria, in this model of hypertension.

With respect to the guanylyl cyclase receptors in SHRs, studies performed in this model of hypertension have shown an increased affinity and expression of NPR-A in kidneys. On the other hand, Lee et al. (18) demonstrated impaired particulate guanylate cyclase activity in this model (28, 33). In this regard our results suggest that NOS stimulation via ANP through NPR-A/B, increasing cGMP in aorta and ventricle, might be altered in this model of hypertension.

With regard to NPR-C, in a previous work (4) we reported that renal NOS stimulation via ANP was partially blocked when G_i protein was inhibited in normotensive rats. In this regard, our present results show that NOS activation induced by ANP was inhibited by pertussis toxin in all studied tissues, indicating that G_i would also be involved in this mechanism in hypertensive animals.

Meanwhile, Marcil et al. (19) have demonstrated that G_i protein abundance and mRNA expression in the heart did not differ in SHRs and in WKY rats. Besides, Martin et al. (20) have shown greater expression of NPR-C in kidneys of SHRs compared with WKY rats. Our studies showed that the NOS response to ANP in hypertensive animals was impaired in all studied tissues when the interaction involves NPR-C and G_i protein pathway. In view of this fact, we could speculate that this receptor or any of its pathway steps might be altered in this model of hypertension.

Moreover, we cannot dismiss the possibility that NOS response to ANP in SHRs, a model in which basal activity was enhanced compared with normotensive animals, may be the upper limit of the enzyme response. This fact could also explain the impaired response to ANP observed in this model of hypertension.

**Conclusion.** Our results have shown that the cardiovascular effects of ANP would be mediated, at least in part, by an interaction with the NO system in this model of genetic hypertension, as shown previously in normotensive rats (6). In addition, we have demonstrated that ANP infusion augmented NOS activity in SHRs, without modifying its expression. According to our results, neither nNOS nor iNOS, the activity of which is exacerbated in this model of hypertension, would participate in the NO-system stimulation via ANP. In this regard, we postulate that ANP would induce eNOS stimulation, interacting with NPR-C in all studied tissues and activating a pathway that involves G_i,2 protein and C_g2-calmodulin. On the other hand, ANP would also interact with NPR-A/B in ventricle and aortic artery in these hypertensive animals, enhancing cGMP formation via particulate guanylyl cyclase, which in turn activates PKG.

The impaired NO-system response to ANP in hypertensive animals, involving alterations in both pathways through NPR-A/B or NPR-C, could participate in the maintenance of the high levels of arterial blood pressure in this model of genetic hypertension.

NOS activity is a hypotensive and natriuretic factor involved in the regulation of the cardiac function, vascular tone, and salt and water balance. This important physiological mechanism has not yet found a role in the pharmacological treatments of arterial hypertension and associated pathologies. Explaining the molecular mechanisms in models of arterial hypertension and their relationship with other regulating systems, like the NO system, would contribute to the development of new therapeutic strategies.

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**DISCLOSURES**

No conflicts of interest are declared by the author(s).

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