Atrial Natriuretic Peptide and C-Type Natriuretic Peptide in Spontaneously Hypertensive Rats and Their Vasorelaxing Actions In Vitro

Chi-Ming Wei, Cheol H. Kim, Ali A. Khraibi, Virginia M. Miller, John C. Burnett, Jr

Abstract The present study determined circulating concentrations of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) in Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) and also investigated the vasorelaxing action of ANP and CNP on isolated contracted aorta. We also defined the vasorelaxing action of a novel and newly synthesized 27-amino acid chimera of ANP and CNP termed vasonatrin peptide (VNP), which we compared with ANP and CNP in WKY rats and SHR. Plasma and urinary cyclic GMP and sodium excretion were also investigated. Plasma ANP was increased in SHR in contrast to no change in circulating CNP. Plasma and urinary cyclic GMP and sodium excretion were no different between WKY rats and SHR. In WKY rats maximal relaxations to VNP in aortic rings without endothelium were greater than those to ANP and CNP. In SHR aortic rings the potency of VNP relaxation was preserved, the actions of ANP were enhanced, and the actions of CNP were markedly impaired. In association with these vasorelaxing actions, these data suggest that (1) circulating CNP is not different in SHR and WKY rats, but the aortic relaxing action of CNP is markedly impaired in SHR; (2) endogenous plasma ANP is significantly increased in SHR without associated increases in plasma or urinary cyclic GMP; (3) there is an increase in aortic relaxation to exogenous ANP in SHR; and (4) VNP has a potent endothelium-independent aortic relaxing action in both WKY rats and SHR. These data suggest differential regulation of ANP and CNP and their vascular actions in SHR. These data also suggest that VNP could have an important therapeutic role in hypertension. (Hypertension. 1994;23[part 2]:903-907.)

Key Words • peptides • atrial natriuretic peptide • muscle, smooth, vascular • antihypertensive agents • rats, inbred WKY • rats, inbred SHR

Atrial natriuretic peptide (ANP) is a potent natriuretic and vasoactive peptide that is produced by atrial myocytes and is important in the control of cardiorenal homeostasis. ANP binds to a specific particulate guanylyl cyclase receptor termed natriuretic peptide receptor-A (NPR-A), which is expressed in endothelial and in renal epithelial cells and mediates its biological actions. Previous reports have demonstrated that the vasoactive and natriuretic actions of ANP require its COOH terminus.

C-type natriuretic peptide (CNP) is a newly identified 22-amino acid peptide that like ANP has a 17-amino acid ring formed by a disulfide bond. Unlike ANP, CNP lacks the COOH terminus amino acid. CNP is genetically distinct from ANP and functions biologically via a separate particulate guanylyl cyclase receptor, natriuretic peptide receptor-B (NPR-B), which is expressed in vascular smooth muscle cells and in the kidney. While ANP and CNP both decrease cardiac preload and arterial pressure, ANP-mediated decreases in arterial pressure are in part secondary to increases in sodium excretion, which is minimal with CNP. In contrast, CNP is a potent endothelium-independent venodilator and ANP is not. Both CNP and ANP possess arterial vasorelaxing actions. Furthermore, ANP is of cardiac origin and functions as a circulating hormone, whereas CNP is synthesized and released from endothelial cells, consistent with a paracrine role in the control of vascular tone.

In previous studies, repeated studies have also demonstrated that plasma ANP is also elevated in spontaneously hypertensive rats (SHR) in association with upregulation of the NPR-A receptor. However, the biological importance of elevated plasma ANP in SHR remains controversial. To date, plasma CNP and CNP-mediated vasorelaxation in hypertension remain undefined. The importance of defining the vascular relaxing actions of natriuretic peptides in SHR is emphasized by reports that endothelium-dependent relaxation to acetylcholine is reduced in hypertensive rats and in essential hypertensive patients. We recently synthesized a new 27-amino acid peptide termed vasonatrin peptide (VNP). VNP is a chimera of ANP and CNP and possesses the 22-amino acid ringed structure of CNP and the 5-amino acid COOH terminus of ANP. We demonstrated in vitro and in vivo that VNP possesses the venodilatory actions of CNP, the natriuretic actions of ANP, and unique peripheral arterial vasodilatory actions not associated with either. This recent report suggested that VNP may mediate its unique augmented vasorelaxing actions via activation of...
both biological natriuretic peptide receptors (NPR-A and NPR-B).

The present study was designed to determine circulating ANP and CNP in SHR. In addition, we sought to define the vascular effects of ANP and CNP and the chimera VNP in both SHR and Wistar-Kyoto (WKY) rats.

**Methods**

**In Vivo Studies**

Experiments were conducted in accordance with the Animal Welfare Act. All rats used in these studies were male WKY rats or SHR purchased from Harlan Sprague-Dawley (Indianapolis, Ind) and fed a normal Purina rat chow containing 0.1 mg sodium per gram, with free access to water. The WKY rats or SHR were anesthetized with thiobutabarbital (Inactin; BYK Gulden) (100 mg/kg IP). Body temperature was maintained between 36°C and 38°C by a heating pad. Tracheostomy was performed, but the animals were not artificially ventilated. Polyethylene catheters (PE-50; Becton Dickinson Co) were placed in the carotid artery for the collection of blood samples and to monitor mean arterial pressure and heart rate and in the right atrium through the right jugular vein to monitor right atrial pressure. A PE-90 catheter was placed in the bladder for urine collection. Urine was collected for 15 minutes, and urine volume was determined. Plasma and urine samples were stored for electrolytes and cyclic GMP (cGMP) analysis.

Blood for plasma cGMP, ANP, and CNP analysis were collected in EDTA tubes, immediately placed on ice, and centrifuged (2500 rpm) at 4°C. Plasma was separated and stored at −20°C until assay. Urine for cGMP determination was heated to >90°C before storage. Plasma and urinary cGMP, ANP, and CNP were determined by a specific radioimmunoassay as previously described.\(^{16,24}\)

**In Vitro Studies**

Rings cut from aorta obtained from WKY rats and SHR were suspended for the measurement of isometric force in organ chambers filled with aerated (95% O\(_2\)/5% CO\(_2\)) modified Krebs-Ringer bicarbonate solution (composition in mmol/L: NaCl 118.3, KCl 4.7, CaCl\(_2\) 2.5, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 25.0, Ca-NAEDTA 0.026, and dextrose 11.1; control solution) at 37°C. In half of the rings, the endothelium was removed by gently rubbing the initial surface with a cotton swab wetted with control solution. Each ring was stretched to the optimal point on its length-tension curve as determined by the tension developed to norepinephrine (3×10\(^{-7}\) mol/L) at each level of stretch. The presence of endothelium was determined at the beginning of the experiment by relaxation to acetylcholine (log molar) during a contraction to norepinephrine at optimal length. The maximal tension of each ring was determined by norepinephrine (10\(^{-4}\) mol/L). To study responses to ANP, CNP, and VNP, the rings were contracted with phenylephrine (10\(^{-4}\) mol/L). These contractions averaged 30% to 40% of the maximal contraction to norepinephrine. The peptides were added cumulatively once the contraction had stabilized.

The following drugs were used: acetylcholine chloride (Sigma Chemical Co), human ANP and human CNP (Peninsula Laboratories), VNP (Mayo Clinic), L-norepinephrine bitartrate (Sigma), and phenylephrine bitartrate (Sigma). All drugs were dissolved in distilled water immediately before study, and the concentrations are reported as the final molar concentration (mol/L) in the organ chamber.

**Statistical Analysis**

Results are expressed as mean±SEM. In organ chamber studies, n equals the number of rats from which rings were taken. Rings with and without endothelium were studied in parallel, and Student's t test for unpaired observations was used to determine statistical significance among the responses of rings with and without endothelium. In in vivo studies the data were analyzed using ANOVA for repeated measures followed by Fisher's least significant difference test when appropriate within the group. Data between groups were analyzed by Student's unpaired t test. Statistical significance was determined at P<.05.

**Results**

Mean arterial pressure was greater in SHR compared with WKY rats without changes in heart rate and right atrial pressure (Table 1). Urine volume was significantly less in SHR, and urinary sodium excretion was not statistically different between the two groups. Plasma ANP was greater in SHR compared with WKY rats (526±44 versus 70±7 pg/mL, P<.05), while plasma CNP was not different between SHR and WKY rats (6.9±1.1 versus 5.6±0.3 pg/mL, respectively) (Fig 1). Plasma cGMP and urinary cGMP were not different between WKY rats and SHR (Table 1).

ANP, CNP, and VNP caused concentration-dependent relaxation in rings with and without endothelium in WKY rats (Fig 2). In WKY rats, maximal relaxation to VNP in aortic rings without endothelium was 99±2%, while plasma CNP was not different between SHR and WKY rats (90±4%) and CNP (82±8%)(Table 2).

ANP, CNP, and VNP mediated endothelium-independent aortic relaxation in SHR (Fig 3). VNP caused potent aortic relaxation (90±3%), which was not significantly different between SHR and WKY (Table 2). There was enhancement of ANP-induced aortic relaxation with endothelium in SHR compared with WKY rats but no change in ANP-mediated relaxation without endothelium between SHR and WKY rats (Table 2). In contrast, maximal relaxation to CNP was significantly less in SHR compared with WKY rats (Table 2).

**Discussion**

The present study reports for the first time that plasma CNP concentrations are not different in SHR and WKY rats, but the aortic relaxing response to CNP is markedly impaired in SHR. In SHR endogenous plasma ANP is markedly increased without increases in plasma and urinary cGMP and sodium excretion. Finally, in isolated aorta of the SHR the relaxation response to exogenous ANP is enhanced. The present
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FIG 1. Bar graphs show plasma concentrations of atrial natriuretic peptide (ANP; top) and C-type natriuretic peptide (CNP; bottom). WKY indicates Wistar-Kyoto rats; SHR, Okamoto spontaneously hypertensive rats.

Study also demonstrates for the first time that a new synthetic peptide, VNP, a chimera of ANP and CNP, has potent endothelium-independent aortic relaxing actions in WKY rats, which are maintained in SHR. ANP is an important peptide that controls cardio renal homeostasis. In the present study plasma ANP was significantly increased in SHR. The increased plasma ANP may be due to several mechanisms, which include decreases in ANP clearance receptor activity, depressed neutral endopeptidase 24.11 activity (which degrades ANP), and enhanced cardiac production of ANP. At the level of the isolated aorta from the SHR, the aortic relaxing action of exogenous α-ANP was markedly increased in SHR in the presence of the endothelium compared with the WKY rat. This observation has important implications and suggests a unique interaction between ANP and the endothelium in the regulation of vascular tone in genetic hypertension. Although the present study cannot address the mechanism of this phenomenon, it should be noted that recent studies from Tremblay et al18 have reported upregulation of the NPR-A receptor in the SHR. Nonetheless, the increase in plasma ANP and its augmented vasorelaxing action in vitro suggest that the state of hypertension in the SHR may not be related to a hyporesponsiveness to endogenous ANP at the vascular level.

CNP is a newly identified vasoactive peptide of endothelial origin.13-15 The structure of CNP, like that of ANP, includes a 17-amino acid ring, but it lacks the COOH terminus. CNP binds to specific NPR-B receptors that are expressed in vascular smooth muscle cells. In congestive heart failure, plasma CNP concentration is not elevated, while cardiac tissue CNP concentration is significantly increased,16 suggesting a paracrine role for CNP in the failing heart. In the present study we report for the first time that plasma CNP concentrations are not different in SHR and WKY rats. In marked contrast, the aortic relaxing action of CNP is markedly attenuated in the SHR in aortic rings with or without endothelium. These data support the conclusion that the decreased CNP-mediated vasorelaxation in the SHR may have pathophysiological relevance to hypertension. Specifically, hypertension could result in down-regulation of NPR-B receptors or alterations in postreceptor signal transduction mechanisms. A limitation of the current study is the lack of cGMP data that could address whether the altered response to CNP is related to a reduced ability to stimulate cGMP production or if the defect is related to a postreceptor mechanism in which cGMP increases are intact but the vascular response is altered. Clearly, the mechanism is specific for CNP because the response to exogenous ANP in SHR was not altered.
Endothelium-dependent relaxation to acetylcholine is significantly decreased in hypertensive rats\(^{19,20}\) and essential hypertensive patients compared with normotensive control subjects.\(^{21,22}\) Furthermore, one study has reported no change,\(^ {25}\) whereas another has reported increased sensitivity of the smooth muscle cell to the endothelium-independent vasodilator sodium nitroprusside in hypertensive rats.\(^ {26}\) In the present study the response of aortic smooth muscle cell to ANP is preserved and actually enhanced in the presence of the endothelium, while the response to CNP is impaired in hypertension. Thus, these findings suggest differential regulation of vascular smooth muscle responsiveness to natriuretic peptide activity in SHR. The present study suggests not only that endothelial function is altered in hypertension but that vascular smooth muscle cell dysfunction may also be an important factor in hypertension.

The differences in biological activity and receptor binding for ANP and CNP may be due in part to the presence or absence of a COOH terminus. We recently synthesized VNP, which is a chimera of CNP and ANP\(^ {12}\) and consists of the 22-amino acid ringed structure of CNP and the COOH terminus of ANP. In rats VNP demonstrated natriuretic actions similar to those of ANP.\(^ {12}\) In canine veins VNP has more potent femoral, saphenous, and renal vein relaxing actions than CNP; these actions were not observed with ANP.\(^ {12}\) VNP also produced more potent femoral, saphenous, and renal arterial relaxation than either ANP or CNP.\(^ {12}\) In the present study we demonstrated that VNP has potent relaxing actions in aorta of both WKY rats and SHR. Taken together, these data suggest that VNP is a unique synthetic chimera peptide that can relax both arteries and veins in the absence and presence of hypertension. Because VNP possesses characteristics of both ANP and CNP, VNP-mediated vasorelaxation may involve binding to both NPR-A and NPR-B receptors or may be through other mechanism(s) that are yet to be identified. A limitation of the present study is the absence of receptor binding studies to address these alternative mechanisms.

In conclusion, the present study also reports for the first time that plasma CNP concentrations are not different in SHR and WKY rats, but the aortic relaxing action of CNP is markedly attenuated in SHR. Second, endogenous plasma ANP is significantly increased in SHR without associated increase in plasma or urinary cGMP, although there is an increase in aortic relaxation to exogenous ANP in SHR. These data suggest differential regulation of ANP and CNP and their receptors in SHR. These data also suggest that VNP could have an important therapeutic role in hypertension. The alteration in arterial vascular responsiveness to CNP may have pathophysiological importance in the mechanism of genetic hypertension in SHR. Finally, the present study demonstrates for the first time that a new synthetic peptide, VNP, has a potent endothelium-independent aortic relaxing action in both WKY rats and SHR.

<table>
<thead>
<tr>
<th>Group</th>
<th>Endothelium</th>
<th>Maximal Relaxation, %</th>
<th>ED(_{50}), log mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=5)</td>
<td>With</td>
<td>87±6</td>
<td>8.0±0.3</td>
</tr>
<tr>
<td></td>
<td>Without</td>
<td>99±1</td>
<td>7.9±0.4</td>
</tr>
<tr>
<td>VNP</td>
<td>With</td>
<td>72±6</td>
<td>7.8±0.4</td>
</tr>
<tr>
<td></td>
<td>Without</td>
<td>89±4*</td>
<td>7.9±0.3</td>
</tr>
<tr>
<td>ANP</td>
<td>With</td>
<td>74±16</td>
<td>7.6±0.4</td>
</tr>
<tr>
<td></td>
<td>Without</td>
<td>82±8*</td>
<td>7.5±0.3</td>
</tr>
<tr>
<td>CNP</td>
<td>With</td>
<td>87±4</td>
<td>7.5±0.4</td>
</tr>
<tr>
<td></td>
<td>Without</td>
<td>90±3</td>
<td>7.4±0.4</td>
</tr>
<tr>
<td>SHR (n=5)</td>
<td>With</td>
<td>98±4†</td>
<td>8.0±0.4</td>
</tr>
<tr>
<td></td>
<td>Without</td>
<td>94±3</td>
<td>7.9±0.3</td>
</tr>
<tr>
<td>VNP</td>
<td>With</td>
<td>27±7††</td>
<td>7.5±0.3</td>
</tr>
<tr>
<td></td>
<td>Without</td>
<td>28±7††</td>
<td>7.5±0.4</td>
</tr>
</tbody>
</table>

ED\(_{50}\) indicates median effective dose; WKY, Wistar-Kyoto rats; VNP, vasonatrin peptide; ANP, atrial natriuretic peptide; CNP, C-type natriuretic peptide; and SHR, spontaneously hypertensive rats. Values are mean±SEM.

\(*P<.05\) vs VNP; "P<.05 vs WKY group; †P<.05 vs ANP.
Acknowledgments

This study was supported in part by grant MHA-103 from the American Heart Association, Minnesota Affiliate; a National Kidney Foundation of the Upper Midwest research grant; National Heart, Lung, and Blood Institute grants HL-36634, HL-07111, and HL-41533; and the Mayo Foundation.

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Atrial natriuretic peptide and C-type natriuretic peptide in spontaneously hypertensive rats and their vasorelaxing actions in vitro.
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Hypertension. 1994;23:903-907
doi: 10.1161/01.HYP.23.6.903

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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