Vasodilatory Effects of C-Type Natriuretic Peptide on Forearm Resistance Vessels Are Distinct From Those of Atrial Natriuretic Peptide in Chronic Heart Failure

Motoyuki Nakamura, MD; Naoshi Arakawa, MD; Hiroaki Yoshida, MD; Shinji Makita, MD; Katsuhiko Hiramori, MD

Background C-type natriuretic peptide (CNP) is a newly identified peptide that is structurally related to atrial natriuretic peptide (ANP). Although it has been suggested that CNP is released from the endothelium for the regulation of local vascular tone, no data are available concerning the vasodilatory response to CNP in humans.

Methods and Results Strain-gauge plethysmography was used to determine the vasodilatory effects of intra-arterially infused CNP compared with the effects of ANP infusion in 11 patients with chronic heart failure (CHF) and 11 age-matched healthy controls. Graded doses of CNP and ANP (8, 16, 32, and 48 pmol·min⁻¹·dl⁻¹ tissue volume) were administered randomly into the nondominant brachial artery, and forearm blood flow (FBF) was measured. No significant changes in systemic blood pressure and heart rate were found during the study. Both the absolute and percent FBF responses to ANP relative to the baseline value were significantly lower in CHF patients than in healthy controls (P<0.01), whereas the responses to CNP were similar. The calculated forearm spillover of cyclic GMP (cGMP) was significantly lower in CHF patients receiving the highest dose of ANP (P<0.02), whereas changes in cGMP spillover after the equimolar dose of CNP were significantly higher (P<0.02), despite the lesser potency of CNP.

Conclusions In patients with CHF the peripheral vasodilatory effect of ANP is attenuated, but CNP-induced peripheral vasorelaxation is preserved, with CNP being less potent for equimolar doses. (Circulation. 1994;90:1210-1214.)

Key Words • natriuretic peptides • endothelium • heart failure

Recently discovered C-type natriuretic peptide (CNP) is structurally homologous to atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), both of which originate from the heart.¹⁻³ Unlike these two previously identified cardiac peptides, CNP has been reported to be found in human cultured endothelial cells,⁴⁻⁵ vascular wall,⁶ and circulation.⁴⁻⁷ Endothelial gene expression and secretion of CNP may be stimulated by several vasoactive substances, including cytokines.⁵⁻⁶ Systemic administration of CNP in dogs has been reported to markedly decrease systemic blood pressure and cardiac output without accompanying natriuresis and diuresis.⁹⁻¹⁰ Furthermore, the stimulatory effect of CNP on cyclic GMP (cGMP) production in cultured vascular smooth muscle cells has been reported to be more potent than that of ANP.¹¹⁻¹² These observations suggest that CNP may be an important local regulator of vascular tone or growth. However, no data are available concerning its effects on peripheral vasodilation and local cGMP production in humans, especially in patients with chronic heart failure in which the vasodilatory effect of ANP has been reported to be attenuated.¹³

The aim of the present study was to investigate the effects of CNP on peripheral vasorelaxation and local cGMP production and to compare these effects with those of ANP in patients with chronic heart failure (CHF) and in healthy volunteers. This was achieved by venous occlusion plethysmography and local intra-arterial infusion of low-dose CNP and ANP.

Methods

Subjects

Eleven patients with CHF admitted to the Iwate Medical University Hospital were enrolled in this study (Table 1). The etiology of heart failure was valvular heart disease in 9 patients and congenital heart disease in 2 patients. In terms of the clinical severity of CHF, 9 patients were in New York Heart Association (NYHA) functional class II and 2 patients in NYHA class III. All patients had significant cardiomegaly with an increased cardiothoracic ratio (mean, 68%; range, 59% to 80%). The mean±SEM values of pulmonary capillary wedge pressure and cardiac index (n=9) obtained in this admission were 20±2 mm Hg and 2.5±0.2 L·min⁻¹·m⁻², respectively. At the time of the experiment, all patients were clinically stable with no signs or symptoms of heart failure at rest. All were treated by oral administration of digitals, diuretics, or both. In patients receiving nitrates or other vasodilators, administration of these drugs was discontinued at least 48 hours before the study commenced. For 2 patients who were treated with small doses of enalapril (2.5 to 5 mg/d), the drug was stopped 2 weeks before the study. None of the patients had gross peripheral edema or clinical evidence of peripheral vascular disorders, nor did any exhibit diabetes mellitus, hypertension, or hypercholesterolemia.
Table 1. Clinical Characteristics of Healthy Controls and Patients With Chronic Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>CHF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Female/male</td>
<td>3/8</td>
<td>7/4</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>52±5</td>
<td>57±4</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>128±4</td>
<td>117±5</td>
<td>NS</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>66±2</td>
<td>63±4</td>
<td>NS</td>
</tr>
<tr>
<td>FAV, mL</td>
<td>867±35</td>
<td>653±33</td>
<td>.01</td>
</tr>
</tbody>
</table>

FBF change to ACh<sub>20</sub>, mL·min<sup>-1</sup>·dL<sup>-1</sup> tissue (n=7) 4.8±1.3 4.7±2.2 NS

Plasma cGMP level, pmol·mL<sup>-1</sup>

Before ANP 3.3±0.3 7.8±1.2 .01

After ANP 7.4±0.7 13.4±1.8 .01

Before CNP 3.2±0.3 9.0±1.5 .01

After CNP 3.9±0.6 13.4±1.8 .01

Basal plasma ANP level, fmol · mL<sup>-1</sup> 8±2 34±5 .01

CHF indicates chronic heart failure; SBP, systolic blood pressure; HR, heart rate; FAV, forearm volume; cGMP, cyclic GMP; ANP, atrial natriuretic peptide; CNP, C-type natriuretic peptide; FBF, forearm blood flow; and ACh<sub>20</sub>, acetylcholine (20 nmol · min<sup>-1</sup> · dL<sup>-1</sup> tissue).

To determine the normal value of changes in forearm blood flow induced by intra-arterial infusion of the experimental agents, 11 age-matched healthy subjects were recruited (Table 1). They were evaluated carefully by physical examination, routine blood tests, ECG, and chest radiograph, and no abnormalities were detected. Although two smokers were included in the control group and one smoker was included in the CHF group, smoking was prohibited during the study period.

Measurements of Forearm Blood Flow

Forearm blood flow (FBF) was measured by venous occlusion plethysmography with subjects in a supine position. In brief, after forearm volume was measured by water displacement, a 20-gauge cannula (PA-04020, Arrow International Inc) was inserted into the nondominant arm with subjects under local anesthesia (1% lidocaine) for regional infusion of experimental agents and for monitoring systemic blood pressure and heart rate. A gallium–indium–silastic strain gauge (Medasonic SG-24) was placed carefully around the forearm approximately 3 cm below the elbow, and a congesting cuff was attached to the upper arm. At least 20 minutes after this procedure, hand circulation was excluded by inflation of a wrist cuff to suprasystolic pressure. The upper arm–congesting cuff was inflated by a rapid cuff inflator (Hokanson E20) to 40 mm Hg for 7.5 seconds in each 15-second cycle using a time switch. The last four consecutive flow curves for each recording period were analyzed with a computer-assisted digital board, and the mean values were used for statistical analysis.

For examining the accuracy and reproducibility of the plethysmography, reactive hyperemic FBF response after two ischemic stimuli (1-minute and 5-minute upper arm arterial occlusions) has been measured on two occasions at least 1 hour apart in another set of healthy volunteers (n=6). The FBF (mean±SEM) at rest and after 1-minute and 5-minute upper arm occlusions on the two occasions were as follows: 2.5±0.2, 4.5±0.8, and 10.5±2.0 mL·min<sup>-1</sup>·dL<sup>-1</sup> tissue in the first measurement and 2.5±0.2, 4.4±0.4, and 10.0±1.3 mL·min<sup>-1</sup>·dL<sup>-1</sup> tissue in the second measurement, respectively.

Study Protocols

The study commenced 1 hour after subjects had consumed a light meal and was performed in a quiet room. After subjects had remained supine for a minimum of 20 minutes, baseline blood flow was recorded. Four step-up doses of ANP and CNP (both: 8, 16, 32, and 48 pmol·min<sup>-1</sup>·dL<sup>-1</sup> tissue) were given every 2 minutes. All subjects had had a routine blood pressure, heart rate, forearm blood flow (FBF), and physical examination before forearm infusion. The order of the agents was randomized, and a minimal recovery period of 20 minutes was interposed between experimental administrations to allow blood flow to return to baseline values. Venous blood samples were obtained from the experimental forearm before and after in both ANP and CNP experiments. Maximal infusion volume was 0.4 mL·min<sup>-1</sup> in this protocol. The experimental protocol used in this study was approved by our hospital ethics committee, and informed consent was obtained from all participants.

Plasma ANP Concentrations and cGMP Spillover

Baseline plasma ANP levels were determined by commercial radioimmunoassay kits (Shionogi), with an intra-assay variation of 7.9%. Plasma CNP concentrations obtained before and after ANP and CNP infusion were measured in duplicate by commercial radioimmunoassay kits (Yamasa Shoyu). Intra-assay variation and interassay variability were 3.1% and 2.9%, respectively. The difference between plasma CNP levels at the baseline and after the maximum dose of ANP or CNP was multiplied by the increase in FBF achieved by the corresponding maximum dose, and these values were taken as representing forearm cGMP spillover.

Statistical Analysis

Differences in baseline values between the experimental groups were tested using an unpaired t test or a χ<sup>2</sup> test. The difference in FBF changes between the two groups were tested using two-way ANOVA with repeated measurements and were followed by Fisher's least significant test. The FBF change after ANP and CNP infusion within each group were analyzed by one-way ANOVA with repeated measure. Data are presented as mean±SEM. All calculated P values are two-tailed, and a value of <.05 was considered significant.

Results

No significant difference in age, sex, systolic blood pressure, or heart rate was found between patients with CHF and healthy controls (Table 1). However, forearm volume in CHF patients was lower than that in healthy volunteers (653±33 versus 867±35 mL; P<.01). The mean baseline plasma concentration of ANP in patients with CHF was more than four times higher than in healthy controls (Table 1). No significant change in the plasma ANP level was found during the CNP infusion within either group (normal, from 10±2 to 8±2 fmol · mL<sup>-1</sup>; CHF, from 45±3 to 37±7 fmol · mL<sup>-1</sup>).

Forearm Blood Flow

No significant systemic effects on blood pressure or heart rate were observed during either experimental infusion (Table 2). Actual FBF values during the study are shown in Table 2. Both the absolute changes in FBF (Fig 1) and the percent changes in FBF relative to baseline values during ANP infusion were significantly lower in patients with CHF (absolute maximum change, +6.1±1.0 versus +1.8±0.8 mL · min<sup>-1</sup> · dL<sup>-1</sup> tissue; percent maximum change, +307±15% versus +214±25%;
TABLE 2. Mean±SEM Responses of Forearm Blood Flow, Systolic Blood Pressure, and Heart Rate During Forearm Infusion of ANP and CNP in Healthy Controls and Patients With CHF

<table>
<thead>
<tr>
<th></th>
<th>ANP, pmol · min⁻¹ · dL⁻¹ tissue</th>
<th>CNP, pmol · min⁻¹ · dL⁻¹ tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBF, mL min⁻¹ · dL⁻¹ tissue</td>
<td>2.9±0.4</td>
<td>3.2±0.5</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>128±5</td>
<td>123±5</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>64±3</td>
<td>64±3</td>
</tr>
<tr>
<td>Patients with CHF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBF, mL min⁻¹ · dL⁻¹ tissue</td>
<td>2.1±0.3</td>
<td>2.2±0.4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>120±5</td>
<td>111±6</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>63±4</td>
<td>61±3</td>
</tr>
</tbody>
</table>

ANP indicates atrial natriuretic peptide; CNP, C-type natriuretic peptide; FBF, forearm blood flow; SBP, systolic blood pressure; HR, heart rate; and CHF, chronic heart failure.

*P<.05, †P<.01 vs baseline values (0 pmol · min⁻¹ · dL⁻¹ tissue).

both P<.01). However, no significant difference in FBF changes induced by CNP infusion was found between the two groups, despite the changes' being less potent than those induced by ANP (absolute maximum change, +1.5±0.4 versus +0.9±0.2 mL · min⁻¹ · dL⁻¹ tissue; NS) (Fig 1). Percent change relative to baseline values was also similar (percent maximum change, +150±13% versus +143±8%; NS). Changes in FBF due to acetylcholine in CHF patients did not differ significantly from those in healthy controls (+4.8±1.3 versus +4.7±2.3 mL · min⁻¹ · dL⁻¹ tissue; NS) (Table 1).

Plasma cGMP Levels and Forearm cGMP Spillover

As shown in Table 1, forearm intra-arterial infusion of ANP apparently increased venous plasma cGMP levels in both groups (normal, from 3.3±0.3 to 7.4±0.7 pmol · mL⁻¹; P<.01; CHF, from 7.8±1.2 to 13.4±1.8 pmol · mL⁻¹; P<.01). Although the increase during CNP infusion tended to be of lesser magnitude than during ANP infusion, venous plasma cGMP levels rose from 3.2±0.3 to 3.9±0.6 pmol · mL⁻¹ in healthy controls and from 9.0±1.5 to 13.4±1.8 pmol · mL⁻¹ in the CHF group (both P<.05; Table 1).

As shown in Fig 2, although the calculated cGMP spillover was increased significantly in the ANP study in both groups, the increase in CHF patients was significantly less than that in the control group (12.0±2.4 versus 31.0±6.4 pmol · min⁻¹ · dL⁻¹ tissue; P<.02). In contrast, although CNP was less potent in its induction of cGMP spillover, the level in the CHF group was higher than that in the control group (+4.4±1.3 versus +0.7±0.2 pmol · min⁻¹ · dL⁻¹ tissue; P<.02) (Fig 2).

Discussion

This study demonstrated that in patients with CHF the forearm vasodilatory effect of ANP is attenuated compared with that in healthy controls. However, the response of FBF to intra-arterial infusion of CNP was similar despite CNP being less potent than ANP for equimolar doses. Furthermore, the forearm cGMP spillover induced by ANP in patients with CHF was significantly lower than that in healthy controls, whereas CNP-stimulated cGMP production was significantly
higher in patients with CHF despite the overall response being less potent than that stimulated by ANP. Although studies examining the physiological effects of CNP in humans are few at present, systemic administration of synthetic CNP in dogs has been shown to profoundly reduce mean arterial pressure and cardiac output without natriuresis or diuresis.\textsuperscript{5,10} Stingo et al\textsuperscript{4} have demonstrated that a high molecular form of CNP (CNP-53) was present in cultured human endothelial cells and a low molecular form (CNP-22) in human plasma obtained from healthy volunteers. Suga et al\textsuperscript{11,6} found that production of CNP and expression of CNP mRNA were markedly stimulated by cytokines (ie, transforming growth factor–β, basic fibroblastic growth factor, and tumor necrosis factor–α) in cultured human endothelial cells. Moreover, in cultured vascular smooth muscle cells, CNP has been reported to stimulate cGMP production and to lead to a potent antiproliferative effect.\textsuperscript{11,12} These observations suggest that CNP is released from endothelial cells and may have an important role in local regulation of vascular tone or structure.

The CNP-induced cGMP spillover from the forearm in patients with CHF was apparently higher than in healthy controls, whereas CNP-induced vasorelaxation of forearm resistance vessels was similar in the two groups. Any interpretation of these results must be cautious, because the origin of forearm cGMP spillover could not be determined by the present study. Vascular smooth muscle cells as well as vascular endothelium have been reported to express natriuretic peptide family receptors, which coupled to guanylate cyclase system.\textsuperscript{15–16} However, because CNP receptors have been reported to be distinct from ANP receptors\textsuperscript{17} and to localize principally in vascular smooth muscle cells,\textsuperscript{18} the main source of forearm cGMP spillover during CNP infusion may be vascular smooth muscle cells.

The mechanism underlying this differing nature of the CNP-induced cGMP spillover between the control group and the CHF group could not be explained by this study, but several possible explanations may be considered. First, Suga et al\textsuperscript{19} have reported that CNP-receptor expression in intact aortic media is weaker than in cell culture conditions and hypothesized a marked phenotype-related alteration in receptor expression for the natriuretic peptide family in vascular smooth muscle cells. If this alteration occurs in patients with CHF, the discrepancy between CNP-induced vasodilation and cGMP production in our study may therefore be explained by a postreceptor defect located at the level of the particulate guanylate cyclase system in vascular smooth muscle cells. Second, Wei et al\textsuperscript{20} demonstrated that the major action of CNP in canine peripheral arteries and veins in vitro was that of veno-relaxation and not arterial relaxation. Therefore, the increased release of cGMP after CNP infusion may represent the spillover from forearm venous systems. Third, Trachte and Drewett\textsuperscript{21} have recently reported that in rabbit isolated vessels CNP may not activate guanylyl cyclase. Thus, it may be possible that CNP infusion could have resulted in a displacement of ANP from the clearance receptor with increasing plasma ANP concentrations, which resulted in the observed changes in cGMP. However, in the present study, since there were no significant changes in the plasma ANP concentration during CNP infusion, such a possibility appears to be unlikely to account for our findings.

The present study also demonstrated impaired forearm blood flow during ANP infusion and decreased cGMP spillover in patients with CHF. Hirooka et al\textsuperscript{13} have shown that peripheral vasodilatory effects of ANP on forearm resistance vessels in patients with severe CHF were impaired compared with healthy controls despite a comparable increase in venous plasma cGMP levels. However, Tsutamoto et al\textsuperscript{22} recently measured cGMP production per ANP extraction in the peripheral vascular beds and showed a decreasing ratio in patients with severe CHF. This observation suggests a possible downregulation of ANP receptors coupled to guanylate cyclase in peripheral vascular smooth muscle cells. In fact, several in vitro studies have demonstrated that vascular smooth muscle during 8- to 24-hour exposure to ANP decreased receptor numbers as well as reduced responsiveness of intracellular cGMP accumulation to acute applied ANP.\textsuperscript{23–25} The present results are consistent with these previous observations and suggest that ANP receptors on peripheral vascular smooth muscle may be downregulated in patients with CHF.

Several recent studies have demonstrated that in patients with severe CHF, endothelium-dependent peripheral vasodilation induced by muscarinic receptor stimuli is attenuated and have suggested that there may be endothelial dysfunction in this pathological condition.\textsuperscript{26–28} This type of endothelial abnormality may cause the attenuated response of cGMP spillover from the endothelial cells after ANP infusion. However, in the present study endothelium-dependent vasodilation induced by acetylcholine in patients with CHF proved comparable to that in healthy controls. This observation suggests that endothelial dysfunction is not apparent in our patients. Thus, the cGMP spillover observed in our ANP study may be reflected by the ANP effect on vascular smooth muscle cells. The reasons why the acetylcholine-mediated forearm blood flow response in our CHF group appeared to be normal are not known in the context of this study. However, subjects of previous...
reports were mainly patients with severe heart failure (NYHA class III),26-28 whereas patients in the present study had relatively mild heart failure (NYHA class II). Furthermore, the etiology of heart failure is different for the previous subjects (ie, ischemic heart disease or cardiomyopathy) and the present study (ie, valvular heart disease). It is therefore likely that impairment of ANP-induced peripheral vasodilation can be attributed to the downregulation of ANP receptors in the forearm vascular smooth muscle cells in patients with CHF. This type of alteration, plus the abovementioned possible intracellular post-cGMP abnormality in our CNP study, may lead to the apparent attenuation of ANP-induced vasodilation in patients with CHF.

In summary, the vasorelaxing potency of CNP in vascular smooth muscle was found to be weaker than that of ANP as evaluated on an equimolar basis. Although CNP-induced cGMP spillover in patients with CHF was higher than in healthy controls, the vasodilatory effect of CNP was similar for the two groups. In contrast, ANP-induced peripheral vasorelaxation in patients with CHF was impaired with decreasing forearm cGMP spillover. It is not known whether the doses of CNP used in the present study were “physiological” or “pharmacological.” However, because the plasma level of CNP has been reported to be low, CNP may act as a paracrine rather than circulating hormone, and endothelial CNP production has been reported to be tremendously stimulated by cytokines,5,8 the plasma levels of which have been demonstrated to be elevated in CHF.29,30 It is therefore speculated that CNP may be an important local factor for regulating vascular tone in patients with CHF.

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References

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