Atrial and Brain Natriuretic Peptides Stimulate the Production and Secretion of C-Type Natriuretic Peptide From Bovine Aortic Endothelial Cells

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Abstract
C-type natriuretic peptide (CNP) is a member of the natriuretic peptide family which is produced in vascular endothelial cells and may play an important paracrine role in the vasculature. We sought to determine the regulation of CNP production by other vasoactive peptides from cultured aortic endothelial cells. The vasoconstrictors endothelin-1 and angiotensin II had little effect on the basal secretion of CNP. In contrast, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) strongly stimulated the secretion of CNP. BNP caused as much as a 400-fold enhancement above the basal accumulated secretion of CNP over 24 h at a concentration of 1 μM; this was 20 times greater than the stimulatory effect of ANP. BNP and ANP also significantly enhanced the production of new CNP (translation) and mRNA expressed in the BAEC. In contrast, C-ANP-423, a truncated form of ANP which selectively binds to the natriuretic peptide clearance receptor, did not stimulate CNP secretion. The enhanced production and secretion of CNP, caused by either ANP or BNP, was significantly prevented by LY 83583, an inhibitor of cGMP generation, and was also attenuated by KT 5823, an inhibitor of cGMP-dependent protein kinase. Our results indicate that ANP and BNP can stimulate CNP production through a guanylate cyclase receptor on endothelial cells. BNP is a much more potent stimulator of CNP secretion, compared to ANP. Our findings suggest that the vasodilatory, and anti-mitogenic effects of ANP and BNP in the vasculature could occur in part through CNP production and subsequent action if these interactions occur in vivo. (J. Clin. Invest. 1995. 95:1151–1157.) Key words: translation • guanylate cyclase • protein kinase

Introduction
The natriuretic peptide family (1) is comprised of three proteins, each derived from a separate gene. Atrial natriuretic peptide (ANP) (2) and brain natriuretic peptide (BNP) (3) circu-
late in plasma after secretion from the heart in adult humans. The third family member, C-type natriuretic peptide (CNP), was originally described in the central nervous system, where it is found in much greater concentrations than either ANP or BNP (4). CNP is derived after processing from a prepro hormone of 126 amino acids, and a pro-hormone of 53 amino acids (5). Recently, CNP has been found to be produced in endothelial cells, where its secretion can be regulated by cytokines and growth factors (6, 7).

CNP has insignificant natriuretic and diuretic properties compared to ANP (4). CNP acts as a venodilator and a vasodepressor in the dog (4, 8, 9) and inhibits vascular smooth muscle cell (VSMC) proliferation in vitro (10, 11). After binding to both the natriuretic peptide guanylate cyclase B (GC-B) and clearance receptors on VSMC (12), CNP could potentially modulate local vasoconstrictor tone or influence the in vivo proliferation of vascular smooth muscle, though there is no direct evidence for this at present. Identifying the vascular factors that regulate CNP production or action might lead to a better understanding of the dynamic events that occur in the vasculature.

The interaction of CNP with important vasoactive peptides is essentially unknown. In these studies, we examined the effects of the other members of the natriuretic peptide family on CNP production and secretion, and compared these to the actions of the vasoconstrictor peptides endothelin-1 (ET-1) and angiotensin II (Ang). We found that ANP and BNP strongly stimulated the production and secretion of CNP, with BNP being much more potent. In contrast, ET-1 and ANG had little effect on CNP secretion. We also determined the type of natriuretic peptide receptor which mediated these actions of ANP and BNP, and established the signaling mechanism involved.

Methods
Endothelial cell cultures and experiments. Bovine aortic endothelial cell cultures (BAEC) were prepared as previously described (13, 14). The BAEC were seeded at a density of 77,000 cells/cm² on 100 mm or 6-well culture plates, passaged once, and cultured in DME with 10% FBS, then used for experiments ~1 wk after cell preparation. The cells displayed the typical morphologic characteristics of endothelial cells and virtually all cells showed positive fluorescence with an antibody to factor VIII. The cultures were devoid of any cells having the appearance of smooth muscle cells.

For experiments, subconfluent BAEC were incubated in DME media without serum for various times (time course) up to 24 h, or in the presence of different concentrations (1–1,000 nM) of ANP or BNP, ET-1, or Ang II. In other experiments, either LY 83583 (10⁻⁷ M) (inhibitor of guanylate cyclase activation) or KT 5823 (5 x 10⁻⁴ M) (inhibitor of cGMP-dependent protein kinase) (Calbiochem Corp., San Diego, CA) was added to the BAEC for 30 min before the natriuretic peptide, or by itself. The media was aspirated and frozen for subsequent radio immunoassay of CNP, and the RNA was extracted from both control (no peptides) and the various experimental plates (4 per condition) as previously described (14). The degradation of ANP and BNP was also compared after 0, 4, 8, and 24 h of exposure to the BAEC.

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1. Abbreviations used in this paper: Ang, angiotensin II; ANP, atrial natriuretic peptide; BAEC, bovine aortic endothelial cell cultures; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; ET-1, endothelin-1; GC, guanylate cyclase; VSMC, vascular smooth muscle cell.

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This was accomplished by calculating the area under the curves of the ANP or BNP specific peaks, seen after injecting the natriuretic peptide-containing cell culture medium onto a C18 reverse phase, high pressure liquid chromatography column. The samples were eluted over 30 minutes using a 10–40% acetonitrile: 0.1% trifluoroacetic acid gradient.

CNP radio immunoassay. CNP immunoreactivity was measured by a sensitive double antibody, non-equilibrium assay, using antibody obtained from Peninsula Laboratories (Belmont, CA). The sensitivity of the assay was 1.5–2 pg/tube and the intra and interassay coefficients of variation were always less than 10%. The antibody recognizes CNP-22 (rat, porcine, and human CNP-22 are identical) and CNP-53 (porcine) but was not reported to cross react with ANP or BNP. We found a cross-reactivity for these latter two peptides (1 μM) with CNP of less than 0.0001% in the RIA.

In vivo translation studies. BAEC were cultured for 7 d, then incubated in methionine-free DME medium with dialyzed 10% FBS for 1 h before experimentation (13, 14). The cells were then incubated with 250 μCi of [35S]methionine in the presence or absence of ANP or BNP, 100 nM, or LY 83583, 100 nM, or peptide plus LY 83583, for 8 h. The media was aspirated and saved, and the cells were then washed and lysed in lysis buffer for 1 h at 4°C (13, 14). The lysate and secretion media were then preclaried and specific labeled CNP protein was immunoprecipitated using polyclonal antibody to CNP. Antibody which was preadsorbed with CNP, 10−7 M, for 12 h before the immunoprecipitation procedure served as a specificity control. The immunoprecipitated protein was then solubilized in SDS-sample buffer (containing fresh mercaptoethanol as a reducing agent), and heated to 100°C for 5 min. The labeled protein was then electrophoretically resolved on a 4% stacking/10% spacing/16.5% separating polyacrylamide gel using a tricine buffer system (cell secretion media) or 12% separating gel using a glycine buffer system (intracellular lysate). For comparison, molecular weight markers were resolved under the same circumstances. The gel was then stained and destained, and subjected to fluorography, then autoradiography for 4–7 d. Each translation experiment was performed at least 3 times. To determine if the intracellular CNP precursor protein was extensively glycosylated, immunoprecipitated and labeled protein from control cells was denatured, then incubated with 0.4 U of N-Glycosidase F, 2 μM Neuraminidase and 2.5 μM α-Glycosidase (Boehringer Mannheim, Indianapolis, IN) for 20 h. The proteins were then separated in parallel and compared to non-deglycosylated proteins on SDS-PAGE as described.

S1 nuclease protection. The extracted RNA was hybridized with a P32-labeled cRNA probe, made from a template of a human cDNA for CNP (5) kindly provided by Dr Gordon Porter, Scios-Nova, Mountain View, CA). The cDNA was in EcorI–EcorI orientation in pSK and anti-sense and sense cRNA probes were promoted using T7 and T3 RNA polymerase, respectively. The cRNA probe spans exon 2 of the human CNP gene, and a transcript of approximately 237 bases was protected. Hybridization, S1 nuclease digestion and electrophoretic separation were carried out as previously described (13, 14). A HindIII-digested and P32-labeled cRNA for H-ras served as an RNA loading standardization probe. The gel was opposed to film with intensifying screens for 24 h, and the autoradiographic bands were compared by laser densitometry (LK8). Sense probes produced no hybridization.

Statistics. Data from secretion studies were combined (n = 8–12 wells/condition) and then analyzed by calculating a mean and standard error for each treatment or group. Data from the different conditions were compared by analysis of variance; a multiple range test (Scheffe’s) was used for significant F values (P < 0.05). Secretion studies were carried out at least three times. RNA comparisons were quantified by laser densitometry of autoradiographs and data was normalized for RNA loading by creating a ratio of the density of the experimental RNA hybridized with the CNP probe, divided by the same amount of RNA hybridized with H-ras. A ratio was then established by comparing normalized experimental RNA to normalized control RNA which was extracted from non-treated endothelial cells. A value of 1 was arbitrarily assigned to the control. This resulted in values expressing the relative densities of the experimental conditions compared to the control. Protein bands from the translation studies were also compared by laser densitometry.

Results

CNP secretion. Over time, ANP, 100 nM, caused a maximal, 6.3-fold stimulation of CNP secretion from the cultured BAEC (Fig. 1A). The stimulation was significant at 2 h of incubation and increased to the highest concentration after 24 h (mean 24 h basal CNP secretion, 64.8 ± 5.8 (SEM) pg/ml, mean ANP-stimulated CNP secretion 410 ± 24 pg/ml, n = 10 wells/condition, P < 0.05). The accumulated secretion of CNP stimulated by 100 nM BNP was significantly greater than ANP at the same concentration and time points. BNP caused a maximal 13-fold increase above basal secretion at this concentration after 24 h of incubation (mean BNP-stimulated CNP secretion 849 ± 65 pg/ml) (Fig. 1A). In contrast, neither ET-1 nor Ang II, at concentrations as high as 1 μM, affected the basal secretion of CNP over 24 h (data not shown). Both ANP and BNP caused a dose-related stimulation of CNP secretion, and again BNP significantly stimulated more CNP secretion, including at a concentration of 10 nM (Fig. 1B). Over several concentrations, BNP was at least one log order of magnitude more potent than ANP in stimulating CNP. We then examined the possibility that the more potent stimulation by BNP, compared with ANP, resulted from there being less degradation of BNP over the 24 h period of exposure to the cells. We found that the exogenously added peptides were comparably degraded at 4, 8, and 24 h; for instance, ANP and BNP were degraded by ~67% at 24 h.

We also determined that the stimulation of CNP secretion by either ANP or BNP occurred through a guanylate cyclase receptor. This conclusion was reached based upon the fact that C-ANP 4–23, a specific agonist for the natriuretic peptide clearance receptor at the concentrations used in this study (13, 15), had no effect on CNP secretion (Fig. 1B). Furthermore, LY 83583, an inhibitor of natriuretic peptide-induced guanylate cyclase activation (13, 16), prevented either ANP or BNP from stimulating CNP secretion over 24 h by approximately 100 and 71%, respectively (Fig. 2). To provide further understanding of the signaling mechanism involved in the action of the natriuretic peptides, we also studied the effects of KT 5823, an inhibitor of cGMP-dependent protein kinase (17) at the concentration used in this study. We found that KT significantly reversed ANP and BNP-stimulated CNP secretion by 63 and 69%, respectively, (Table 1) (P < 0.05).

Translation studies. We next wanted to provide evidence that ANP and BNP regulate CNP production as well as secretion. We therefore metabolically labeled the BAEC, and assessed new CNP protein synthesis (translation) in response to the natriuretic peptides. We also hoped to gain some insight as to the intracellular, precursor forms of CNP found within the cell, or the processed forms secreted into the media, since this type of data has not been described previously. First, we separated proteins (which were secreted into the incubation medium) by SDS-PAGE. We observed two prominent protein bands in the secretion media which corresponded in size to CNP-22 and CNP-53 (Fig. 3A). Thus, both forms are secreted from the cultured cells. This indicates that processing from the high molecular weight, prepro CNP takes place entirely within the cell, giving rise to pro CNP-53 the proCNP is then partially processed within the cell or at the cell membrane, leading to the secretion of CNP-22, since our secretion media contains no serum-processing proteases. Within the cell lysate, we detected
Figure 1. (A) Effect of ANP or BNP, 100 nM, on the secretion of CNP from cultured bovine aortic endothelial cells over time. Data are the mean±SEM from three experiments combined (n = 10 wells/condition). (●——●) is control (absence of peptide) secretion, (▲——▲) is ANP, (●——●) is BNP. Error bars are not shown when the SEM is less than 5 pg/ml. Comparison of control to ANP or BNP-induced CNP secretion is different at all time points (P < 0.05) by ANOVA plus Scheffe’s test. Additionally BNP is significantly different than ANP at 6, 8, and 24 h. (B) Dose-related stimulation of ANP or BNP on CNP secretion at 24 h. Each bar represents the mean±SEM from three experiments combined (n = 12 wells/condition). * P < 0.05 by ANOVA plus Scheffe’s test compared to control. BNP-stimulated CNP secretion is significantly greater than ANP-stimulated CNP (P < 0.05) at 10-4 through 10-8 M.

A predominant high molecular weight form of CNP, at ~28 kD (Fig. 3 B). This was found despite treating the cell extract with freshly made reducing agent (2-mercaptoethanol) and SDS, agents which should prevent dimerization or aggregation, including with another intracellular protein. This isoform is substantially larger than the size of the prepro CNP (~13 kD), and we considered it likely to be glycosylated CNP precursor protein. No CNP-53 or CNP-22 was detected in the cell lysate. When we deglycosylated the control, immunoprecipitated protein, it did not change its migration, indicating that the unexpected mobility and size of the precursor protein on SDS-PAGE is not due to extensive glycosylation. We also separated the intracellular protein using both 12 and 16.5% acrylamide gels, and tricine and glycine buffer systems, but the protein ran very similarly under both conditions.

Importantly, we found that in the cell lysate, ANP and BNP each strongly stimulated the production of new CNP protein. Based on densitometry of the protein bands from four experiments combined, ANP maximally stimulated the production of the high molecular weight forms by 2.94±0.4-fold, compared with control. BNP was more potent, stimulating CNP production by 4.78±0.3-fold at 100 nM peptide. This was found to be dose-related, and was mediated through binding to a GC receptor, since LY 83583 very significantly blocked the stimulation of CNP production by either natriuretic peptide, while having no significant effect itself. The protein bands were identified as being specifically related to CNP, since antibody which was preabsorbed with CNP immunoprecipitated significantly less protein (Fig. 3 B, lane 9). ANP and BNP, 100 nM, also stimulated the ultimate production of CNP-53 and CNP-22 which was secreted into the culture medium, by approximately 3.14±0.3 and 4.19±0.4-fold, respectively (Fig. 3 A). This probably resulted from the comparable stimulation of precursor protein production. Again, LY 83583 substantially inhibited the stimulated translation of CNP, caused by either ANP or BNP.

mRNA studies. The regulation of the CNP gene by ANP and BNP was then determined by protection assay (Fig. 4 A). First, we found that over time, ANP or BNP, 10-7 M, each produced a significant increase in CNP steady state mRNA levels of at least 2–3-fold compared to control. This was first noted by 2 h, and extended over the 24-h experimental period. The stimulation was also concentration dependent (Fig. 4 B). Based upon combining results from two studies, and normalizing for RNA loading, ANP stimulated CNP gene expression 3.05, 1.87, and 1.3-fold at 10-6, 10-8, or 10-9 M, respectively. BNP induced a stronger 4.04, 2.90, and 1.91-fold increase in CNP mRNA at the same concentrations. Therefore, the stimulated increase in CNP mRNA expression in response to ANP or BNP is consistent with the enhanced production of CNP protein caused by these natriuretic peptides.

Discussion

CNP is a vasodilator in some species and an inhibitor of vascular smooth muscle proliferation in vitro (11) and probably in vivo (10), acting in paracrine and perhaps endocrine fashion after
synthesis and secretion from the endothelial cell (6). Hence, the regulation of CNP production and secretion by other vasoactive peptides is potentially important. Here, we report that ANP and BNP strongly stimulate the production and secretion of CNP from cultured aortic endothelial cells, albeit at relatively high concentrations of peptide. BNP is much more potent than ANP.

Table 1. Effects of KT 5823 on the ANP or BNP-stimulated Production of CNP Over 24 h from Cultured Endothelial Cells

<table>
<thead>
<tr>
<th>CNP</th>
<th>pp/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>ANP 100 nM</td>
<td>313 ± 8.2*</td>
</tr>
<tr>
<td>BNP 100 nM</td>
<td>651 ± 17*</td>
</tr>
<tr>
<td>KT 5823 50 nM</td>
<td>30 ± 2.7</td>
</tr>
<tr>
<td>ANP + KT 5823</td>
<td>105 ± 4.8</td>
</tr>
<tr>
<td>BNP + KT 5823</td>
<td>184 ± 36*</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM of data from two separate experiments combined (n = 9–12 wells/condition), results representative of a third study. * P < 0.05 by ANOVA plus Scheffe’s test for condition versus control. † P < 0.05 for ANP or BNP versus ANP or BNP plus KT.

in these regards, causing as much as a 20-fold greater stimulation of CNP secretion. ANP and BNP also stimulate increased CNP gene expression, and significantly enhance the formation of new CNP protein (translation) in the cultured BAEC, both of the precursor and fully processed forms. For both these functions, BNP is more potent. The magnitude stimulation of the secretion of CNP over time in response to ANP or BNP does not precisely correlate with the magnitude stimulation of mRNA expression or protein production. However, the stimulated secretion of CNP in our studies represents accumulated secretion into the culture medium over as much as 24 h, on a background of increased production. Increased CNP secretion over time is
also seen at a lower level in the medium from control cells, 
where there is no significant change in mRNA expression. 
Furthermore, there is often a lack of a strict stoichiometric 
relation between secretion and production of peptide 
hormones.

These actions of ANP and BNP are mediated through gua-
nylate cyclase (GC) receptors expressed on the endothelial 
cells, since a specific ligand for the natriuretic peptide 
clearance receptor has no effect on CNP production/secretion. 
Furthermore, their effects on both CNP production and secretion 
occur through the generation of cGMP and the activation of cGMP-
dependent protein kinase. This is based upon the reversal of 
the stimulated CNP production/secretion by Ly 83583 and KT 
5823, inhibitors of cGMP production and related-kinase activation, 
respectively. Finally, the effects of ANP and BNP are 
specific, since ET-1 and Ang II have no significant effect on 
CNP secretion.

We found a predominant high molecular weight form 
of CNP in the labeled endothelial cells, but neither CNP-53 nor 
CNP-22 was found. The protein was not significantly glycosyl-
ated according to our findings, but we cannot rule out another 
form of post-translational modification. Large molecular weight 
forms of CNP have been detected by other investigators using 
gel permeation chromatography in immunoprecipitated lysates 
from the kidney (18). It has been proposed that the high mole-
cular weight forms may represent a non-specific interaction be-
tween CNP antibody and other large proteins (18). This is 
unlikely however, because when we use antibody which is satu-
rated with CNP before our immunoprecipitation of material 
from the labeled BAEC, virtually no bands are seen, indicating 
the specific nature of the interaction of the antibody with newly 
formed CNP precursor protein. To explain the aberrant behavior 
on SDS-PAGE, we suggest that the high molecular weight form 
is specifically linked to another protein which is resistant to 
mercaptoethanol or SDS, or that the prepro CNP is modified 
pseudo-translationally other than by the addition of sugar moieties 
(phosphorylated, farnesylated, etc.). Since CNP-53 and CNP-
22 are not found in the cell lysate, this suggests that the prohor-
mone is rapidly processed and secreted. Consistent with our 
findings, recent studies from Hagiwara et al. indicate that CNP 
is rapidly secreted and not stored after cell synthesis (19). In 
the cell medium, both CNP-53 and CNP-22 were found. To 
date, only CNP-22 has been reported in human plasma (20, 21).

Therefore, plasma enzymes may play a role in the processing of 
the pro CNP-53 to CNP-22, although the endothelial cell can 
also participate in posttranslational modifications/processing, 
as we determined.

Our results suggest that some of the previously observed 
effects of ANP (and BNP) in the vasculature could be explained 
by the stimulation of the secretion, and subsequent action of 
CNP. For instance, it is known that ANP can inhibit the prolifera-
tion of VSMC, and that this effect is probably mediated 
through the stimulation of cGMP (reviewed in reference 22). 
Therefore, we postulate that if in vivo, ANP acts as a growth 
inhibitor of VSMC through the generation of cGMP, this may be 
mediated in part through the stimulated release and subsequent 
action of CNP on smooth muscle cells. Porter et al. (11) recently 
showed that the anti-proliferative action of CNP greatly exceeds 
that of ANP for VSMC in culture. Furuya et al. (10) showed 
that in vivo, the intimal hyperplasia of rat carotid arteries 
induced by air-drying injury could be substantially suppressed 
by CNP infusion. Furthermore, TGF-β1 can inhibit the prolifera-
tion of VSMC and TGF-β has been observed to strongly stimu-
late CNP production from cultured endothelial cells (6). There-
fore, it is possible that in vivo, ANP or TGF-β might stimulate 
CNP secretion form the endothelial cell, which could contribute 
to the anti-mitogenic action on neighboring smooth muscle 
cells. However, in vitro results may not be readily extrapolatable 
to the in vivo situation, since it has been shown that the GC 
receptor subtype expressed on the in situ aorta is different from 
that expressed on cultured cells (23). CNP binds to the GC-B 
subtype of guanylate cyclase receptor, while ANP binds to the 
GC-A subtype (24). The subtype of GC receptor on a cell may 
therefore limit the response to CNP if the GC-B receptor is not 
expressed. Another function for ANP (and CNP) has been to 
inhibit the secretion of the vasoconstrictor and VSMC mitogen, 
ET-1 (13, 25). Inhibiting ET-1 provides a common mechanism
whereby the natriuretic peptides can cause vasodilation and the inhibition of VSMC proliferation.

It has been observed that upon systemic infusion of ANP, the blood pressure lowering effects are relatively modest when physiologic concentrations are reached in the blood (2). In some species, the vasodepression induced by CNP exceeds that of ANP and probably reflects a more pronounced decrease in cardiac output resulting from venodilation and reduced cardiac filling pressure (9, 10, 26). This differential effect has also been observed after infusion of ANP or CNP into the central nervous system (27). In contrast, CNP has been observed to have weak natriuretic properties (26), compared to ANP. These differential actions may again result from the subtype of GC receptor expressed in natriuretic peptide responsive tissues. In some studies, CNP has been shown to more potentely stimulate cGMP when compared to ANP, reflecting the presence of the GC-B receptor in the tissue studied (28), and perhaps this has a role in the different potency of action. Both peptides also bind the C receptor, but evidence to date indicates that most biological actions of CNP are mediated through the GC-B receptor (19).

Our results indicate that the regulation of CNP by ANP and BNP is mediated through the GC class of receptors. This is based upon our finding that (a) C-ANP 4-23, a specific ligand for the natriuretic peptide clearance receptor, has no effect on CNP secretion, and (b) inhibitors of cGMP generation or its related kinase completely reverse the stimulation of CNP production and secretion caused by either ANP or BNP. Other actions of ANP, for instance, the in vitro inhibition of endothelin production and secretion, are mediated through the clearance receptor (13). Both receptors are expressed on endothelial cells (21), in vitro and in vivo, and therefore, both classes of receptors could potentially mediate the overall biological effects of ANP and probably those of BNP. However, most presently defined in vivo actions of ANP appear to be mediated through the GC receptor.

ANP and BNP bind to the same receptors with roughly equal affinity (12), and to date, have been found to have essentially the same actions and similar potencies on a wide variety of vascular (29) and nonvascular functions (30). Thus, a fundamental question in the field is, what are the relative roles and particularly the unique functions for BNP, compared with ANP? To date, this question is unanswered. Our results indicate that BNP (compared to ANP) is much more potent in its ability to stimulate CNP production and secretion. Therefore, it would be important to know whether BNP acts similarly in vivo, because it may become therapeutically useful to preferably administer this peptide in order to stimulate CNP production in certain cardiovascular disorders. It is unclear as to what is the precise mechanism by which BNP more potently stimulates CNP, compared with ANP. In most studies (including our own unpublished observations) these two peptides stimulate cGMP generation equipotently from endothelial cells (12). However, we observed here that the inhibitor of cGMP generation, LY 83583, completely prevented ANP-induced CNP secretion, while it inhibited BNP-induced CNP ~ 30% less potently. KT 5823 reversed ANP or BNP-stimulated secretion roughly equally, but neither was complete. The latter finding is consistent with the observation that the relatively selective action of KT for cGMP-dependent protein kinase (PKG) at this concentration does not fully inhibit PKG activity. Our results lead us to hypothesize that BNP may act through the guanylate cyclase receptor, but may additionally activate a second, non-cGMP related mecha-
nism to stimulate CNP. Alternatively, there might be a distinct receptor for BNP on endothelial cells, which signals only in part through guanylate cyclase.

In summary, we report that members of the natriuretic peptide family (ANP and BNP) can regulate the production and secretion of another member (CNP). These effects are mediated through stimulating CNP gene expression and translation, and occur after ANP or BNP binds to a GC receptor. Our data allows the speculation that infusion of BNP, compared to ANP, is more likely to potently stimulate CNP secretion. If these effects are present in vivo, then several of the important actions of the natriuretic peptides in the vasculature could result in part from the stimulated secretion and subsequent action of C-type natriuretic peptide.

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