Attenuation of inhibitory effect of CNP on the secretion of ANP from hypertrophied atria

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Kim, Suhn HEE, Jeong HEE Han, Sung HEE Lim, Sook Jeong Lee, Sung Zoo Kim, and Kyung Woo Cho. Attenuation of inhibitory effect of CNP on the secretion of ANP from hypertrophied atria. Am J Physiol Regulatory Integrative Comp Physiol 281: R1456–R1463, 2001.—It has been shown that atrial natriuretic peptide (ANP) influences proliferation of cardiac cells. To define the possible role of C-type natriuretic peptide (CNP) in cardiac hypertrophy, the influence of CNP on the secretion of ANP was studied with the use of perfused nonbeating atria from monocrotaline-treated rats. Increases in atrial volume caused proportional increases in ANP secretion that were markedly suppressed by CNP (10^{-6} M) in nonhypertrophied left atria and control right atria but not in hypertrophied right atria. However, increases in atrial volume and mechanically stimulated extracellular fluid (ECF) translocation by CNP were similar to those in the control group. Therefore, the secretion of ANP in terms of ECF translocation was decreased by CNP in nonhypertrophied left and control right atria but not in hypertrophied atria. However, the inhibitory effect of 8-bromo-cGMP on the secretion of ANP was observed in both atria. The cGMP productions from perfused hypertrophied atria and their membranes exposed to CNP were significantly lower than those from nonhypertrophied atria. No significant difference in natriuretic peptide receptor-B transcript was found. Therefore, attenuation of the inhibitory effect of CNP on the ANP secretion in hypertrophied atria may be due to lack of cGMP production. The results showing the relief of CNP-induced negative inhibition of ANP secretion by atrial hypertrophy suggest that CNP may be a contributing factor to delay the development of cardiac hypertrophy.

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stretch and endothelin-1 in isolated perfused nonbeating rat atria (20). However, it is not clear whether the intracardiac cross talk of natriuretic peptides is modified by atrial hypertrophy. Therefore, to determine the physiological role of CNP in atrial hypertrophy, the modification of the inhibitory effect of CNP on ANP secretion was investigated in hypertrophied atria obtained from monocrotaline (MCT)-treated rats.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats weighing 230–250 g were used. Rats were given a single subcutaneous injection of 60 mg/kg MCT or saline (18) and were killed at 4–5 wk.

**Blood collection and tissue preparation.** On the day of the experiments, rats were killed by decapitation, and blood was collected into a prechilled tube containing aprotonin (200 kalilkrein inhibitory units/ml), soybean trypsin inhibitor (SBTI; 50 No-benzoyl-l-arginine ethyl ester units/ml), phenylmethylsulfonyl fluoride (PMSF; 600 μM/ml), and EDTA (2.7 mM/ml). Blood was centrifuged at 10,000 g for 15 min at 4°C, and plasma ANP was extracted using Sep-Pak C18 cartridge (Waters, Milford, MA). Both atria were separated, weighed, and kept in 2 ml of 0.1 N acetic acid at 4°C. Tissues were boiled for 10 min, homogenized with Polytron homogenizer, and centrifuged at 10,000 g for 15 min at 4°C. The concentrations of ANP in plasma extracts and tissue homogenates were measured by radioimmunoassay (RIA) as described below.

**Isolated perfused atrial preparation.** Rats were killed by decapitation, and an isolated perfused atrial preparation was made by the method described previously (7, 19). Briefly, both atria were separately dissected from the heart, and a Tygon cannula containing three small catheters was inserted into the atrium. The cannulated atrium were transferred, fitted into the organ chamber containing buffer solution (36.5°C), and fixed with a water-tight silicone rubber cap. The atrium was immediately perfused with oxygenated HEPES buffer solution at a rate of 0.4 ml/min with a peristaltic pump. The composition of the buffer solution was as follows (in mM): 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 25 NaHCO3, 10 HEPES, and 10 glucose plus 1% bovine serum albumin (BSA). The pericardial buffer solution containing [3H]inulin to measure the translocation of extracellular fluid (ECF) was also oxygenated by silicone tubing coils located inside the organ chamber. The pericardial space of the organ chamber was sealed and connected with a calibrated microcapillary tube, by which changes in atrial volume were monitored. The perfusate was collected at 2-min intervals at 4°C. After two collection periods, atrial distension was induced for 2 min by elevation of the position of the outflow catheter tip to 1 cmH2O, and atrial contraction was induced by lowering the position of the catheter tip to the basal level. Atrial pressure was subsequently increased from 0 to 2, 4, 6, and 10 cmH2O for 2 min every 8 min.

To define the inhibitory effect of CNP on the ANP secretion by atrial hypertrophy, both atria from MCT rats and control right atria were exposed to CNP (10–26) (in Da), since the ANP secreted was found to be mainly the processed ANP (7).

**Measurement of ECF translocation.** We have previously reported a two-step sequential mechanism of ANP secretion from atria: 1) atrial release of ANP into interstitial space by atrial stretch and 2) the secretion of released ANP into atrial lumen concomitantly with ECF translocation by contraction (5). The translocation of ECF is dependent on atrial volume change. The ECF translocated from the atria was measured as described previously (5). Radioactivity in atrial perfusate and pericardial buffer solution was measured with a liquid scintillation counter, and the amount of ECF translocated through the atrial wall was calculated as follows

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\text{ECF translocation (μl \cdot min}^{-1} \cdot \text{g}^{-1}) = \frac{\text{total radioactivity in perfusate (cpm/min)} \cdot 1,000}{\text{radioactivity in pericardial reservoir (cpm/μl)} \cdot \text{atrial wet wt (mg)}}
\]

where cpm is counts/min.

Preparation of perfused atrium for measurement of cGMP production. At the end of the experiments, atria were separated from atrial cannula, lightly blotted, quickly frozen in liquid nitrogen, and stored at −70°C until assay, as described elsewhere (26). Atrial tissues were minced in 2 ml of ice-cold trichloroacetic acid (TCA; 6%) solution and homogenized at 4°C by three 30-s bursts of maximal speed using Tissue Tearer (Biospec Products, Racine, WI). After centrifugation at 1,000 g for 10 min at 4°C, supernatant was transferred to a polypropylene tube, subjected to ether extraction three times, and then dried using a Speed-Vac concentrator (Savant, Hicksville, NY). Dried samples were resuspended with 200 μl of sodium acetate buffer.

**Measurement of particulate guanylyl cyclase activity in atrial membranes.** Atrial tissue was homogenized at 4°C in 30 mM phosphate buffer (pH 7.2) containing 120 mM NaCl and 1 mM phenanthroline by three 30-s bursts of maximal speed using Tissue Tearer (Biospec Products). The homogenate was centrifuged at 1,500 g for 10 min at 4°C, and supernatant was recentrifuged at 40,000 g for 60 min at 4°C. The membrane pellet was washed three times with 50 mM Tris-HCl (pH 7.4) and resuspended in this solution. Protein contents were determined by bichinonic acid assay kit (Sigma Chemical, St. Louis, MO).

Particulate guanylyl cyclase (GC) activity was measured by the determination of cGMP generated in atrial tissue membranes according to a method described previously (22, 26). Five-microgram protein aliquots of the suspension were incubated at 37°C for 15 min in 50 mM Tris-HCl (pH 7.6; containing 1 mM isobutylmethylxanthine, 1 mM GTP, 0.5 mM ATP, 15 mM creatine phosphate, 80 μg/ml creatine phosphokinase, and 4 mM MgCl2) and 1 μM natriuretic peptides. Incubations were stopped by addition of 375 μl of cold 50 mM sodium acetate (pH 5.8) and by boiling for 5 min. Samples were then centrifuged at 10,000 g for 5 min at 4°C.

**RIA of cGMP.** The amount of cGMP generated in the supernatant was measured by RIA (22, 26). Briefly, 2’-O-monosuccinylguanosine 3’,5’-cyclic monophosphate tyrosyl methyl ester (cGMP-TME; Sigma Chemical) was iodinated by the chloramine-T method. Iodinated cGMP-TME was purified by a QAE Sephadex A-25 column (Sigma Chemical),

\[\text{ANP released (μM)} = \frac{\text{immunoreactive ANP (pg \cdot min}^{-1} \cdot \text{g}^{-1})}{\text{ECF translocation (μl \cdot min}^{-1} \cdot \text{g}^{-1}) \cdot 3,060}\]

The denominator 3,060 refers to the molecular mass for ANP-(1–28) (in Da), since the ANP secreted was found to be mainly the processed ANP (7).
and the specific activity of the iodinated tracer determined by RIA technique was 215 Ci/mmol (17).

Standards or samples were introduced in a final volume of 100 μl of 50 mM sodium acetate buffer (pH 4.8), and 100 μl each of diluted cGMP antiserum (Calbiochem-Novabiochem, San Diego, CA) and iodinated cGMP were added. After incubation at 4°C for 24 h, the bound form was separated from the free form by charcoal suspension. The measurement of cGMP generated was done on the day of experiments, and all samples in an experiment were analyzed in a single assay. Nonspecific binding of iodinated tracer was 2.4%. The 50% intercept was at 0.74 ± 0.03 pmol/tube (n = 10). The intra- and interassay coefficients of variation were 4.2% (n = 15) and 7.1% (n = 8), respectively. Results of the determinants were expressed as picomoles of cGMP generated per milligram of protein per minute.

RT-PCR. RT-PCR was performed as described previously (21, 22). Total RNA was extracted from atria by use of TRI reagent (RNA/DNA/protein isolation reagent; MRC, Cincinnati, OH). One microgram of mRNA was suspended in 20 μl of RT buffer and reverse transcribed at room temperature for 10 min and at 42°C for 30 min. The reaction was stopped by heat inactivation for 5 min at 99°C and then chilled on ice. Complementary DNA products were amplified by PCR using primers. The sequences (5'-3') of the oligonucleotides and sizes of PCR products for NPR-B were as follows: sense, AACGGGCGCAT-TGTGATATCTGCGGC; and antisense, TTATCACAGGAT-GGTCG-TCCAAGTCA (692 bp). The temperature profile of amplification consisted of 30-s denaturation at 95°C, 1-min annealing at 60°C, and 2-min extension at 72°C for 30 (for glyceraldehyde-3-phosphate dehydrogenase; GAPDH) or 40 cycles (NPR-B). PCR products were separated in 2% agarose gels, and bands were visualized by ethidium bromide staining. The specificity of the amplified sequences was confirmed by DNA sequencing.

Statistical analysis. The results were given as means ± SE. Statistical significance of differences was performed by Student's t-test. The correlation coefficients were determined using least-squares linear regression analysis, and the com-
parison of slopes between ANP secretion and ECF translocation was performed by parallelism test. The critical level of significance was set at $P$ value $< 0.05$.

RESULTS

The tissue weight of the right heart increased after injection of MCT. The ratio of right to left atrial weight increased from $1.04 \pm 0.06$ to $2.67 \pm 0.15$ ($P < 0.001, n = 18$) at 4 wk, and plasma concentration of ANP markedly increased ($152.2 \pm 23.5$ vs. $460 \pm 45.4$ pg/ml, $P < 0.01$). The atrial concentration of ANP markedly decreased ($124.4 \pm 12.2$ vs. $268.7 \pm 26.2$ ng/mg, $P < 0.01$) in hypertrophied right atria but not in nonhypertrophied left atria of MCT rats.

Stretch-induced ANP secretion by CNP from hypertrophied right atria compared with nonhypertrophied left atria. To evaluate changes in stretch-induced ANP secretion by CNP from nonhypertrophied left atria in MCT rats, isolated perfused nonbeating atria were used. The basal rate of ANP secretion was $5.83 \pm 1.69$ ng-min$^{-1}$g$^{-1}$ ($n = 10$), which was suppressed by CNP ($1.32 \pm 0.34$ ng-min$^{-1}$g$^{-1}$; $n = 11, P < 0.01$; Fig. 1C). When atrial pressure was increased from basal level to 2, 4, 6, or 10 cmH$_2$O for 2 min by the elevation of outflow tip, atrial volume (distension and reduction volume; DRV) was increased in proportion to atrial pressure. Increases in DRV caused proportional increases in ANP secretion that were suppressed by CNP. The basal rate of ECF translocation was not changed, but the mechanically stimulated ECF translocation was slightly increased by CNP (Fig. 1D). Therefore, the secretion of ANP in relation to ECF translocation (ANP concentration) was markedly decreased by CNP (Fig. 1E).

In hypertrophied right atria, the basal rates of ANP secretion and ECF translocation were not changed by the addition of CNP (Fig. 2, C and D). Increases in DRV

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**Fig. 3.** Relationships between changes in DRV, ECF translocation, and ANP secretion by CNP in nonhypertrophied left atria (A and B) and hypertrophied right atria (C and D) from MCT-treated rats and in right atria from control rats (E and F; $n = 8$). In nonhypertrophied left atria and control right atria, CNP shifted the relationships between ECF translocation and DRV (A and E) and ANP secretion and ECF translocation (B and F) rightward and downward. In hypertrophied right atria, however, CNP did not change the relationship between those parameters (C and D). ∆ECF translocation, change in ECF translocation.
caused proportional increases in ANP secretion and ECF translocation. CNP caused no significant change in ANP secretion but a slight decrease in ECF translocation (Fig. 2, C and D). Therefore, the secretion of ANP in relation to ECF translocation (ANP concentration) was not changed by CNP (Fig. 2E).

Figure 3 shows the relationships between DRV, changes in mechanically stimulated ECF translocation, and ANP secretion by CNP in nonhypertrophied left (Fig. 3, A and B) and hypertrophied right (Fig. 3, C and D) atria from MCT rats and control right atria (Fig. 3, E and F). There were positive correlations between DRV and ECF translocation in nonhypertrophied left atria (y = 0.021x + 8.86, r = 0.75, P < 0.001; Fig. 3A), hypertrophied right atria (y = 0.027x + 26.74, r = 0.61, P < 0.001; Fig. 3C), and control right atria (y = 0.012x + 35.8, r = 0.53, P < 0.001; Fig. 3E). There were also positive correlations between DRV and ECF translocation by CNP (y = 0.023x + 20.57, r = 0.75, P < 0.001 in Fig. 3A; y = 0.033x + 16.42, r = 0.79, P < 0.001 in Fig. 3C; and y = 0.019x + 31.1, r = 0.69, P < 0.001 in Fig. 3E), but the slopes were not significantly different from the corresponding control group. The linear correlations between ANP secretion and ECF translocation were shifted rightward and downward by CNP in nonhypertrophied left atria (y = 0.16x + 1.27 vs. y = 0.31x + 5.15, P < 0.05; Fig. 3B) and in control right atria (y = 0.58x + 2.57 vs. y = 0.90x + 4.33, P < 0.01; Fig. 3F). This means that CNP suppresses ANP release from atrial myocytes. In contrast, in hypertrophied right atria, CNP did not shift the relationships between ANP secretion and ECF translocation (y = 0.45x + 1.85 vs. y = 0.57x - 2.34, P = 0.42; Fig. 3D).

Therefore, the concentration of ANP was significantly suppressed by CNP in nonhypertrophied left atria and control right atria but not in hypertrophied right atria, as shown in Fig. 4A.

Stretch-induced ANP secretion by 8-BrcGMP from hypertrophied right atria. To determine whether the inhibitory effect of ANP secretion by 8-BrcGMP, a cell membrane-permeable cGMP, is attenuated in hypertrophied atria, 8-BrcGMP (10^-4 M) was perfused into both left and right atria from MCT rats. Figure 5 shows the suppression of stretch-induced ANP secretion by 8-BrcGMP in nonhypertrophied left (n = 6, Fig. 5A) and hypertrophied right atria (n = 6, Fig. 5B). Positive relationships between changes in stretch-induced ANP secretion and ECF translocation were shifted rightward and downward by 8-BrcGMP in both atria. The slopes between those parameters made by 8-BrcGMP were significantly different from corresponding control groups (0.35 ± 0.10 vs. 0.99 ± 0.14, P < 0.001 in nonhypertrophied left atria; 0.39 ± 0.07 vs. 1.10 ± 0.21, P < 0.05 in hypertrophied right atria).

Activation of particulate GC by CNP in hypertrophied perfused atria and tissue membranes. To determine whether an attenuation of the inhibitory effect of CNP by atrial hypertrophy may be due to the low amount of cGMP generation through NPR-B, an isolated perfused nonbeating atrium exposed to CNP was frozen in liquid nitrogen at the end of experiment, and cGMP was extracted. CNP (10^-6 M) elicited an increase in cGMP production in nonhypertrophied left atria [3.40 ± 0.54 (n = 7) vs. 2.21 ± 0.18 pmol/mg protein (n = 7), P < 0.025; Fig. 4B] and in control right atria [3.35 ± 0.42 (n = 7) vs. 2.42 ± 0.25 pmol/mg protein (n = 7), P < 0.05; Fig. 4B]. In hypertrophied right atria, however, no significant change in cGMP production by CNP was observed [1.90 ± 0.33 (n = 7) vs. 1.84 ± 0.29 pmol/mg protein (n = 7), P = 0.45; Fig. 4B].

Particulate GC activity was measured by determination of cGMP generated in protein aliquots of atrial membranes. Basal cGMP production from hypertrophied right atrial membrane was not different from control right and nonhypertrophied left atrial membrane (n = 5; Fig. 6). CNP caused an increase in cGMP production in both groups. However, an increase in cGMP production by CNP in hypertrophied right atrial membrane was significantly lower than that in both groups (P < 0.05).

Gene expression of NPR-B in hypertrophied atria. Figure 7 shows RT-PCR products for NPR-B in both atria from control and MCT rats. Band of DNA was present in the lanes corresponding to the expected size of the products for NPR-B. The amount of PCR product for NPR-B corrected by GAPDH in hypertrophied right atria was not different from that in nonhypertrophied left atria and control right atria (Fig. 7, n = 4).
DISCUSSION

The present study clearly shows that atrial hyper-
trophy caused an attenuation of the inhibitory effect of
CNP on the ANP secretion, which may be partly due to
the low amount of CNP-stimulated cGMP production
in hypertrophied atria.

All of the natriuretic peptide family and their recep-
tors are found in atrial myocytes and fibroblasts (13,
28, 30). Many investigators have tried to find out the
intracardiac roles of natriuretic peptides and their
cross talk. However, there are several reports about
the possible intracardiac effect of ANP as an autocrine/
paracrine factor. ANP inhibits catecholamine- and
growth factor-induced DNA synthesis in cultured rat
cardiac myocytes and fibroblasts (3, 4). Recently, Horio
et al. (15) have also showed direct evidence for the
inhibitory regulation of hypertrophy by endogenous
ANP in cultured cardiac myocytes. Oliver et al. (32)
have found hypertension and cardiac hypertrophy with
interstitial fibrosis in NPR-A knockout mice. In con-
trast, transgenic mice overexpressing the ANP gene
have a low heart weight under normoxia and a blunted
right ventricular hypertrophy response to hypoxia-in-
duced pulmonary hypertension. Taken together, the
above reports suggest that ANP may play an important
role in the regulation of cardiac hypertrophy/growth as
an autocrine factor. Therefore, definition of the factors
for the regulation of ANP secretion from hypertrophied
heart is an important field for the understanding of
pathogenesis of cardiac hypertrophy.

Cardiac hypertrophy associated with congestive
heart failure induces reactivation of the ventricular
ANP gene, which is markedly declined after birth, as
well as atrial ANP. However, the significance of the
activated ANP system is still unknown. It is reported
that ANP may regulate its own release via NPR-A by
atrial myocytes in an autocrine/paracrine manner (27).
Recently, we found an accentuation of ANP secretion to
endothelin (ET)-1 in hypertrophied atria (20) and the
inhibitory regulation of ANP secretion by CNP via
NPR-B-cGMP pathway in isolated perfused beating
atria (26). Cardiac CNP level is extremely low, and
plasma CNP level did not change in congestive heart
failure (38). Therefore, the intracardiac effect of CNP
as a local hormone may play an important role in the
activating of the NPR-B gene by 8-bromo-cGMP (8-BrcGMP; 10−6
M) in nonhypertrophied left atria (A) and hypertrophied right atria
(B) from MCT-treated rats. 8-BrcGMP shifted the relationship between
changes in ECF translocation and ANP secretion rightward
and downward in both atria. MCT,LT-CONT, nonhypertrophied left
atria from MCT-treated rat as control (A, ●; n = 6); MCT,RT-CONT,
hypertrophied right atria from MCT-treated rat as control (B, ●; n = 6);
8-BrcGMP, 8-BrcGMP-perfused atria (○; n = 6).

Fig. 6. Comparison of cGMP production through activation of gua-
ylyl cyclase in atrial tissue membranes stimulated by CNP (10−6
M). CONT, control group (n = 6); CNP, CNP-perfused group (n = 6).
* P and #P < 0.05 vs. CNP-stimulated control right atria and non-
hypertrophied left atria, respectively.

Fig. 7. Semiquantitative RT-PCR of mRNAs for natriuretic peptide
receptor (NPR)-B in both atria from control (CONT) and MCT-
treated rats. No significant differences in NPR-B mRNA corrected by
glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA be-
tween groups were observed. M, DNA molecular size marker (174 RF
DNA, Hae III cut); lanes 1–4, left atria; lanes 5–8, right atria.
pathogenesis of cardiac hypertrophy. However, there is no report on the modification of intracardiac cross talk of ANP and CNP by atrial hypertrophy. The answer for this question may give us some idea for the physiological role of the endogenous CNP system in the pathogenesis of cardiac hypertrophy. In the present study, we found that an inhibitory effect of CNP on the ANP secretion was markedly attenuated in hypertrophied right atria. The ANP release in terms of ECF translocation by CNP was not changed in hypertrophied atria but decreased in nonhypertrophied atria. CNP specifically activates NPR-B, followed by increased cGMP production (24). Therefore, to determine whether the activation of GC through NPR-B may be impaired in hypertrophied atria, we measured the amount of cGMP production in atrial tissue exposed to CNP. The amount of cGMP generation in both hypertrophied atrial tissues and its membranes exposed to CNP was significantly lower than that in nonhypertrophied atrial tissues and membranes. However, suppression of ANP secretion by 8-BrcGMP (26), a cell membrane-permeable cGMP, was not affected by atrial hypertrophy. Therefore, the attenuation of the inhibitory effect of ANP secretion by CNP in hypertrophied atria may be due to the low amount of cGMP generation in tissue membranes exposed to CNP. In the present study, mRNA for NPR-B in hypertrophied atria was not different from that in nonhypertrophied atria. These results are not consistent with the report showing increased mRNA levels for NPR-A and NPR-B and decreased mRNA levels for NPR-C in progressive hypertrophied rat heart (2). The discrepancy may be due to the differences in method for the induction of cardiac hypertrophy and degree of hypertrophy. The possible explanation for the defect of cGMP generation in hypertrophied atria may be the low activity of GC or the enhanced activity of phosphodiesterase.

What is the significance of relief of negative inhibitory limb of CNP on the ANP secretion in cardiac hypertrophy? Progressive cardiac hypertrophy may lead to the activation of ANP synthesis and release with subsequent high concentration of plasma ANP and low concentration of atrial ANP (8, 29–31, 34). ANP stimulated by stretch and ET-1 (20) in hypertrophied atria may cause vasodilation of pulmonary artery (25) and diuresis (14), followed by the reduction of pressure and volume overload, and also inhibit cardiac hypertrophy (15, 16) as one of the compensatory mechanisms. In addition, the present study shows evidence that CNP may be another factor participating in the maintenance of a high level of plasma ANP in cardiac hypertrophy. Under normal conditions, CNP endogenously synthesized from cardiac myocytes and fibroblasts (13, 28) may act as an autocrine/paracrine regulator by inhibiting ANP secretion (26), atrial dynamics (1, 26), and cardiac growth (4, 12, 33) through NPR-B. CNP also has systemic effects such as vasodilation and diuresis but these are relatively weak compared with ANP. In the course of cardiac hypertrophy, CNP may relieve the negative inhibition of ANP secretion, followed by reduction of overload to the heart, even though the mechanisms are not clear at present.

The results showing the relief of negative limb of ANP secretion by CNP in hypertrophied atria suggest that CNP may be a contributing factor to delay the development of cardiac hypertrophy secondary to MCT-induced pulmonary hypertension.

 Perspectives

Herein, we present an important finding that may provide a possible mechanism for the involvement of CNP in the development of cardiac hypertrophy. It has already been shown that ANP inhibits proliferation of cardiac fibroblasts, and cardiac hypertrophy with interstitial fibrosis is developed in NPR-A knockout mice. Although CNP also has the antigrowth effect of vascular smooth muscle, the involvement of CNP in the development of cardiac hypertrophy is not clear. Gene expression of cardiac CNP and its plasma level appear not to be influenced by cardiac hypertrophy, and a recent study using CNP knockout mice shows no evidence of cardiac hypertrophy. However, intracardiac action of CNP may be more important than that of ANP and BNP because of the high activity of cardiac GC-B compared with GC-A. CNP may act as a local hormone rather than a general hormone. Therefore, CNP may influence indirectly cardiac hypertrophy or development. Recently, we found the paracrine function of CNP in the heart to be a negative regulator of ANP secretion. The present study shows the attenuation of CNP-induced inhibition of ANP secretion by atrial hypertrophy due to the low activity of GC-B. Therefore, we suggest that CNP may be a contributing factor participating in the development of cardiac hypertrophy through the regulation of ANP secretion.

Additional study is required to elucidate the cellular and molecular basis of regulation of GC-B by cardiac hypertrophy.

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