Postnatal changes in inhibitory effect of C-type natriuretic peptide on secretion of ANP

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Abstract

To define developmental changes in atrial natriuretic peptide (ANP) secretion and in the cross talk between C-type natriuretic peptide (CNP) and ANP, we performed experiments in isolated perfused nonbeating cardiac atria isolated from rabbits between 1 and 8 wk of age. Changes in atrial pressure resulted in increases in atrial volume that rose with age and reached the peak value at 4 wk. A rise in volume change increased ANP secretion with concomitant translocation of extracellular fluid (ECF) into the atrial lumen, which increased with age and reached the peak value at 4 wk. The positive relationship between stretch-induced ANP secretion and ECF translocation shifted upward and leftward with age. CNP suppressed stretch-induced ANP secretion in the 8-wk-old group but not in the 2- and 4-wk-old groups without differences in ECF translocation and atrial volume. Therefore, the ANP secretion in terms of ECF translocation was markedly suppressed by CNP in the 8-wk-old group but not in the 2- and 4-wk-old groups. The production of cGMP by CNP in atrial tissue membranes was markedly attenuated in young rabbits. However, 8-bromo-cGMP suppressed stretch-induced ANP secretion in 2- and 8-wk-old groups. Natriuretic peptide receptor-B mRNA was similar in both groups. Therefore, we conclude that the inhibitory effect of CNP on atrial ANP secretion is developmentally regulated, being absent during normal cardiac development in young animals and intact in adult animals.

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MATERIALS AND METHODS

Animals. New Zealand White rabbits, aged 1, 2, 3, 4, or 8 wk, were used. All animal experimentation described in this study was conducted in accordance with the guidelines of the American Association for Accreditation of Laboratory Animal Care.

Isolated perfused atrial preparation. An isolated perfused atrial preparation was made as previously described (7, 20). After anesthesia with thiopental sodium (20 mg/kg), a Tygon catheter was inserted into the left atrium and secured with ligatures. The cannulated atrium was transferred, fitted into an organ chamber containing buffer solution (36.5°C), and fixed with a watertight silicone rubber cap. The atrium was immediately perfused with oxygenated HEPES buffer solution at a rate of 0.1–1.0 ml/min with peristaltic pump. The buffer solution composition was (in mM) 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 25 NaHCO₃, 10 HEPES, and 10 glucose and 0.1% BSA. The pericardial buffer solution, which contained [³H]inulin to measure the translocation of extracellular fluid (ECF), was also oxygenated by silicone tubing coils located inside the organ chamber (6). Gas pressure and pH in perfusate were monitored via periodic sampling and measured with a Corning 175 automatic pH-blood gas system (PO₂ = 486.4 ± 20.7 mmHg, pH = 7.40 ± 0.08; Corning Medical and Scientific, Medfield, MA). The pericardial space of the organ chamber was sealed and connected with a calibrated microcapillary tube, through which changes in atrial volume were monitored. After stabilization for 30 min, the perfusate was collected in 2-min intervals (4-min interval in the case of 1-wk-old rabbits because of low flow rate) at 4°C. Atrial distension was induced for 2 min (4 min in the case of 1-wk-old rabbits) by elevating the position of the out-flow catheter tip to 2 cmH₂O, and atrial contraction was induced by lowering the position of the catheter tip to basal level. Changes in atrial volume in terms of atrial distension followed by reduction (distension and reduction volume, DRV) were measured by changes in water column through a calibrated microcapillary tube. Atrial pressure was subsequently increased from 0 to 1, 2, 4, or 6 cmH₂O for 2 min (4 min in the case of 1-wk-old rabbits) every 8 min.

RIA of ANP. The concentration of immunoreactive ANP in atrial perfusates and tissue homogenates was measured using specific RIA as described previously (6, 7). For RIA, 50 or 100 µl of atrial perfusates were directly used. In terms of different concentrations of atrial ANP, tissue homogenates were diluted by 10 to 500-fold and 50 µl was used for the RIA. RIA was performed in Tris-acetate buffer (0.1 M, pH 7.4) containing neomycin (0.2%), EDTA (1 mM), soybean trypsin inhibitor (50 µg, bovine trypsin inhibitor/ml), aprotonin (200 kallikrein inhibitory units/ml), phenylmethylsulfonyl fluoride (0.4 mg%), sodium azide (0.02%), and BSA (1%). Standard and samples were incubated with anti-ANP antibody and ¹²⁵I-labeled ANP for 24 h at 4°C. The bound form was separated from the free form using charcoal suspension. RIA for ANP was done on the day of the experiments, and all samples in an experiment were analyzed in a single assay. The secreted amount of ANP was expressed as nanograms of ANP per minute per gram of tissue wet weight.

The molar concentration of ANP release was calculated as follows (5, 6)

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\text{ANP released (µM) = } \frac{\text{ANP in perfuse (pg/min/g⁻¹)}}{\text{ECF translocation (µmol/min/g⁻¹) × 3,063}}
\]

The denominator 3,063 refers to the molecular mass for ANP (1–28) (in Da), since the ANP secreted was found to be mainly the processed ANP (6).

Measurement of ECF translocation. The ECF translocated from the atria was measured as described previously (5, 6). Radioactivity in perfuse and pericardial buffer solution was measured with a liquid scintillation counter, and the amount of ECF translocated through atrial wall was calculated as follows

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\text{ECF translocation (µL/min/g⁻¹) = } \frac{\text{total radioactivity perfuse (cpm/min) × 1,000}}{\text{radioactivity in pericardial reservoir (cpm/µl) × atrial wet wt (mg)}}
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Activation of particulate guanylyl cyclase in atrial membranes. Particulate guanylyl cyclase (GC) activity was measured by determination of cGMP generated in protein aliquots of atrial tissue membranes, as described previously (23). Briefly, left atrial tissues obtained from rabbits of different ages were 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 27,000 rpm. The homogenates were centrifuged at 1,500 g for 10 min at 4°C, and the supernatants were recentrifuged at 40,000 g for 60 min at 4°C. The membrane pellets were washed three times with 50 mM Tris-HCl (pH 7.4) and resuspended in this solution. Protein contents were determined using a biocinchonic acid assay kit. Particulate GC activity was measured in protein aliquots of tissue membranes as described previously (23). 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 isobutyl-1-methylxanthine, 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 (NPs). Incubations were stopped by the addition of 375 µl of cold 50 mM sodium acetate (pH 5.8) and 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 equilibrated RIA (23, 28). To prepare iodinated cGMP, we used 2'-O-nonosucinyl-guanosine 3',5'-cyclic monophosphate tyrosyl methyl ester (cGMP-TME; Sigma Chemical, St. Louis, MO). Iodinated cGMP-TME was made using the chloramine-T method and purified by a QAE Sephadex A-25 column (Sigma Chemical) (28). The specific activity of the iodinated tracer determined by the RIA technique was 215 Ci/mmol (18). Antiserum for cGMP was purchased commercially (Calbiochem-Novabiochem, San Diego, CA). 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 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. Nonspecific binding was <2.4%. The 50% intercept was at 0.74 ± 0.03 pmol/tube (n = 10). The intra- and interassay coefficients of variation was 4.2% (n = 15) and 7.1% (n = 8), respectively.

Competitive RT-PCR. Left atria from 2- and 8-wk-old groups were immediately removed, put into liquid nitrogen, and kept at −70°C until assayed. RT-PCR was performed as described previously (21, 23). Total RNA was extracted from atria using TRI reagent (MRC, Cincinnati, OH), according to the manufacturer’s suggested protocol. Total RNA concentrations were quantitated by ultraviolet spectrophotometry. One microgram of mRNA was suspended in 20 µl RT buffer containing 10 mM Tris (pH 8.3), 50 mM KCl, 5 mM MgCl₂, 1 mM each of dATP, dCTP, dGTP, and dTTP, 20 U RNase inhibitor, 2.5 µM random hexamers, and 150 U Moloney
leukemia virus RT (Perkin Elmer, Branchburg, NJ). mRNA was reverse transcribed at room temperature for 10 min and 42°C for 30 min. The reaction was stopped by heat inactivation for 5 min at 99°C and then chilled on ice. cDNA products were amplified by PCR with the following sense and antisense primers: NPR-B sense, 5'-AACGGGCGCATTTGTTATATCTGGCGGC-3' (730–756); NPR-B antisense, 5'-TTGATAGGATGCTCAGCACAAGTGTC-3' (1395–1421); NPR-B competitor sense, 5'-ATTGAGTTCACTATAGAGAATACACCGGGCGATTGTTATATCTGGCGGCATTTGTCGAC-3'; and NPR-B competitor antisense, 5'-TTTTTTTTACAGGATGCTCAGCACAAGTGTCGAGTTACAGGAGTGT-3'.

The NPR-B mRNA competitive protocol was made according to the manufacturer's suggested protocol (competitive DNA construction kit and competitive RNA transcription kit, Takara). Fifty microliters of PCR buffer contained 10 mM Tris (pH 8.3), 50 mM KCl, 2 mM MgCl₂, 200 μM each of dATP, dCTP, dGTP, and dTTP, 2.5 U Taq polymerase, and 1.5 pM each of NPR-B and different concentrations of the NPR-B competitor (10⁶, 5 × 10⁶, 10⁷, and 10⁸ copies). The temperature profile of amplification consisted of 30-s denaturation at 95°C, 1-min annealing at 58°C, and 2-min extension at 72°C for 40 cycles. PCR products (692 bp for NPR-B, 454 bp for the competitor) were separated in 3% agarose gels, and bands were visualized by ethidium bromide staining. Photographs of gels were taken, and the density was analyzed using a densitometer. The concentration of NPR-B mRNA was estimated by the amount of competitor at which the ratio of NPR-B and its competitor density was 1.

Statistical analysis. The results are given as means ± SE. The significance of differences was determined with Student's paired and unpaired t-test. ANOVA followed by the Duncan multiple range test (see Fig. 2) was also applied. The correlation coefficients were determined using least-squares linear regression analysis, and the comparison of slopes was performed by a parallelism test. Statistical significance was defined as P < 0.05.

RESULTS

Characteristics of stretch-induced ANP secretion in 1-wk-old rabbits. The basal rate of ANP secretion and ECF translocation was 0.79 ± 0.31 pg·min⁻¹·g⁻¹ and 4.59 ± 1.13 μl·min⁻¹·g⁻¹ (n = 11), respectively (Fig. 1, C and D). When atrial pressure was increased from basal level to 1, 2, 4, or 6 cmH₂O for 4 min by the elevation of the outflow tip and then decreased to basal level, atrial volume (DRV) was increased by 159.9 ± 22.4, 227.9 ± 36.6, 302.2 ± 50.7, or 434.3 ± 78.4 μl/g, respectively (Fig. 1B). The secretion of ANP was increased after reduction of atrial volume from stretch with peak values of 1.22 ± 0.42, 1.27 ± 0.45, 1.69 ± 0.46, and 5 ± 1.60 ng·min⁻¹·g⁻¹ (Fig. 1C). The translocation of ECF was also increased by stretch at the same period as ANP secretion with peak values of 8.46 ± 2.22, 10.25 ± 2.11, 13.90 ± 3.48, and 17.44 ± 4.54 μl·min⁻¹·g⁻¹ (Fig. 1D).

Postnatal changes in ANP secretion and ECF translocation. Figure 2 shows changes in tissue weight and ANP content of left atria, DRV, ECF translocation, and ANP secretion from isolated perfused atria in response to increased intra-atrial pressure by 6 cmH₂O in rabbits of different ages. Mechanically stimulated ECF translocation and ANP secretion were calculated by subtracting the mean value of the previous two observations from the peak value. Atrial weight gradually increased with age (Fig. 2A). The tissue ANP content in the left atria was markedly increased from 0.65 ± 0.16 μg at 1 wk to 1.46 ± 0.25 μg at 2 wk and reached the peak value at 3 wk (Fig. 2B). DRV gradually increased and reached the peak value at 4 wk (Fig. 2C). Mechanically stimulated ECF translocation and ANP secretion markedly increased with age and reached the peak value at 4 wk (Fig. 2, D and E). There were positive correlations between DRV, mechanically stimulated ECF translocation, and ANP secretion in all groups (Fig. 3). The leftward shift of these relationships with age happened suddenly between weeks 3 and 4, coincident with a marked increase in both ECF translocation and ANP secretion (Fig. 3B).

Postnatal changes in inhibitory effect of CNP on ANP secretion. To evaluate postnatal changes in inhibitory effect of CNP on stretch-induced ANP secretion, we tested isolated perfused nonbeating atria from rabbits of three different ages (2, 4, and 8 wk old). Figure 4, A and B, shows positive correlations between DRV and mechanically stimulated ECF translocation (y = 0.04x + 5.03; r² = 0.49; P < 0.001; Fig. 4A) and mechanically stimulated ECF translocation and ANP secretion (y = 0.11x – 0.34; r² = 0.44; P < 0.005; Fig. 4B) in control atria from the 2-wk-old group. CNP caused a slight increase in ANP secretion. Therefore, the relationships between stretch-induced ANP secretion and ECF translocation (y = 0.14x – 0.04; r² = 0.54; P < 0.001; Fig. 4B) shifted leftward with CNP. In the 4-wk-old group, CNP did not cause any significant changes in relationships between DRV, ECF translo-
significant difference in the slopes (Fig. 4, C and D). In the 8-wk-old group, however, CNP caused suppression of stretch-induced ANP secretion. The relationship between ANP secretion and ECF translocation (Fig. 3) showed a remarkable increase with age. Therefore, we evaluated the responsiveness of cGMP production using different doses of CNP in atrial tissue membranes from 2- and 8-wk-old groups (Fig. 7B). Basal cGMP production in the 2-wk-old group was 15.53 ± 2.78 pmol·min⁻¹·mg protein⁻¹ (n = 7), which was higher than in the 8-wk-old group (8.87 ± 1.15 pmol·min⁻¹·mg protein⁻¹, n = 7, P < 0.005). In the 2-wk-old group, the addition of CNP at doses of 10⁻⁷, 10⁻⁹, or 10⁻¹¹ M did not significantly increase cGMP production. CNP at higher doses of 10⁻⁵ or 10⁻⁶ M increased cGMP production from 16.98 ± 3.12 to 19.12 ± 3.33 (P < 0.05) or 20.26 ± 3.67 pmol·min⁻¹·mg protein⁻¹ (P < 0.01), respectively. However, in the 8-wk-old group, cGMP production was significantly increased by the lowest dose of CNP, and CNP-stimulated cGMP production was dose dependent. Therefore, the ratio of cGMP production by CNP to the control value was higher in the 8-wk-old group than in the 2-wk-old group (Fig. 7B).

Postnatal changes in NPR-B mRNA. To determine whether the different response of ANP secretion to CNP in young rabbits may be due to the lack of NPR-B mRNA, NPR-B mRNA in the left atrium was measured in 2- and 8-wk-old rabbits using competitive RT-PCR. Basal NPR-B mRNA levels were lower in the 2-wk-old group than in the 8-wk-old group (8.87 ± 1.15 pmol·min⁻¹·mg protein⁻¹, n = 7, P < 0.005). In the 2-wk-old group, the addition of CNP at doses of 10⁻⁷, 10⁻⁹, or 10⁻¹¹ M did not significantly increase cGMP production. CNP at higher doses of 10⁻⁵ or 10⁻⁶ M increased cGMP production from 16.98 ± 3.12 to 19.12 ± 3.33 (P < 0.05) or 20.26 ± 3.67 pmol·min⁻¹·mg protein⁻¹ (P < 0.01), respectively. However, in the 8-wk-old group, cGMP production was significantly increased by the lowest dose of CNP, and CNP-stimulated cGMP production was dose dependent. Therefore, the ratio of cGMP production by CNP to the control value was higher in the 8-wk-old group than in the 2-wk-old group (Fig. 7B).

Postnatal changes in particulate GC activity. To evaluate postnatal changes in GC activity in cardiac atria, we measured the amount of cGMP production stimulated by NPs in tissue membrane fractions of left atria. As shown in Fig. 7A, ANP, BNP, and CNP (10⁻⁶ M, n = 3) caused increases in cGMP production, which gradually increased with age. In particular, CNP-stimulated cGMP production showed a remarkable increase with age. Therefore, we evaluated the responsiveness of cGMP production using different doses of CNP in atrial tissue membranes from 2- and 8-wk-old groups (Fig. 7B). Basal cGMP production in the 2-wk-old group was 15.53 ± 2.78 pmol·min⁻¹·mg protein⁻¹ (n = 7), which was higher than in the 8-wk-old group (8.87 ± 1.15 pmol·min⁻¹·mg protein⁻¹, n = 7, P < 0.005). CNP at higher doses increased cGMP production significantly, whereas the lowest dose did not increase cGMP production. In the 8-wk-old group, cGMP production was significantly increased by the lowest dose of CNP, and CNP-stimulated cGMP production was dose dependent. Therefore, the ratio of cGMP production by CNP to the control value was higher in the 8-wk-old group than in the 2-wk-old group (Fig. 7B).

Postnatal change of CNP effect. The developmental changes in ANP secretion and ECF translocation (Fig. 2) were compared between ANP secretion and ECF translocation in atria from rabbits of different ages. The positive correlations between ANP secretion and ECF translocation (Fig. 3A) and the ratio of cGMP production by CNP to the control value were reproducible. 8-Bromo-cGMP (10⁻⁴ M) caused a marked suppression of stretch-induced ANP secretion in 2- (Fig. 6A) and 8-wk-old groups (Fig. 6B). Therefore, ANP concentration was markedly suppressed by CNP in the 8-wk-old group but not in the 2- and 4-wk-old groups.

To determine whether the different age response of ANP secretion to CNP may be due to the amount of cGMP production, we evaluated the response of ANP secretion to 8-bromo-cGMP, a cell-permeable cGMP. As shown in Fig. 6, an increase in ANP secretion in response to given pressure was reproducible. 8-Bromo-cGMP (10⁻⁴ M) caused a marked suppression of stretch-induced ANP secretion in 2- (Fig. 6A) and 8-wk-old groups (Fig. 6B). Therefore, ANP concentration was markedly suppressed in both groups (Fig. 6C).

Postnatal changes in particulate GC activity. To evaluate postnatal changes in GC activity in cardiac atria, we measured the amount of cGMP production stimulated by NPs in tissue membrane fractions of left atria. As shown in Fig. 7A, ANP, BNP, and CNP (10⁻⁶ M, n = 3) caused increases in cGMP production, which gradually increased with age. In particular, CNP-stimulated cGMP production showed a remarkable increase with age. Therefore, we evaluated the responsiveness of cGMP production using different doses of CNP in atrial tissue membranes from 2- and 8-wk-old groups (Fig. 7B). Basal cGMP production in the 2-wk-old group was 15.53 ± 2.78 pmol·min⁻¹·mg protein⁻¹ (n = 7), which was higher than in the 8-wk-old group (8.87 ± 1.15 pmol·min⁻¹·mg protein⁻¹, n = 7, P < 0.005). In the 2-wk-old group, the addition of CNP at doses of 10⁻⁷, 10⁻⁹, or 10⁻¹¹ M did not significantly increase cGMP production. CNP at higher doses increased cGMP production significantly, whereas the lowest dose did not increase cGMP production. In the 8-wk-old group, cGMP production was significantly increased by the lowest dose of CNP, and CNP-stimulated cGMP production was dose dependent. Therefore, the ratio of cGMP production by CNP to the control value was higher in the 8-wk-old group than in the 2-wk-old group (Fig. 7B).
10^6, 10^7, or 10^8 copies, the PCR product of NPR-B was gradually decreased (Fig. 8). The amount of NPR-B mRNA in the 2-wk-old group was not significantly different from the 8-wk-old group (n = 3).

DISCUSSION

The present study clearly shows postnatal changes in atrial compliance, stretch-activated ANP secretion, and GC-B activity and in the intracardiac role of CNP on the regulation of ANP secretion in rabbits.

It has been reported (30, 39) that atrial ANP mRNA and its content gradually increase after birth but ventricular ANP and its content abruptly decrease, although the mechanisms involved for the tissue-specific regulation of ANP expression are not well defined. To evaluate the responsiveness of ANP secretion to atrial stretch during development, we measured atrial compliance and stretch-activated ANP secretion in 1-, 2-, 3-, 4-, and 8-wk-old rabbits, using isolated perfused atria.

Fig. 4. Relationships between mechanically stimulated ECF translocation and DRV (A, C, and E) and stretch-induced ANP secretion and ECF translocation (B, D, and F) in control (Cont) and C-type natriuretic peptide (CNP)-treated atria from 2- (A and B), 4- (C and D), and 8-wk-old (E and F) groups.

Fig. 5. Changes in interstitial ANP concentration at 6 cmH_2O caused by CNP (10^{-6} M) in 2-, 4-, and 8-wk-old groups. CNP caused a suppression of ANP concentration in the 8-wk-old group but not in the 2- and 4-wk-old groups. **P < 0.01, significantly different from corresponding control group.

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nonbeating atria. In the 1-wk-old group, increases in atrial volume induced by increased atrial pressure caused proportional increases in ECF translocation and ANP secretion, which have a close relationship, as previously shown in adult rabbits (5, 6). This means that ANP released from atria in response to stretch is secreted sequentially into atrial lumen along with the translocation of ECF in 1-wk-old rabbits. Atrial compliance increased progressively with age and reached the peak value at 4 wk (3.7-fold increase compared with 1-wk-old rabbits). Increases in atrial volume caused increases in ECF translocation in all age groups.

Fig. 6. Effect of 8-bromo-cGMP (8-BrcGMP; $10^{-4}$ M) on ANP secretion in 2- (A) and 8-wk-old groups (B). The same atrial pressure (6 cmH$_2$O) was applied every 10 min, and 8-BrcGMP was infused after fraction 15. 8-BrcGMP caused a suppression of stretch-induced ANP secretion and changes in ANP concentration (C). *$P < 0.01$, significantly different from corresponding control group.

Fig. 7. cGMP production by natriuretic peptides (all $10^{-6}$ M) in atrial tissue membranes (A) from rabbits of different ages and dose response of cGMP production with various concentrations of CNP in 2- and 8-wk-old groups (B). BNP, brain natriuretic peptide; F, full-term fetus; 1 day, 1-day-old rabbits. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, significantly different from 2-wk-old group; # $P < 0.05$, ## $P < 0.01$, significantly different from the lowest dose of CNP.

Fig. 8. Natriuretic peptide receptor-B (NPR-B) mRNA expression in left atrial tissue of 2- (A) and 8-wk-old (B) groups using competitive RT-PCR. Competitor, NPR-B competitor; lanes 1–4, $10^6$, $5 \times 10^6$, $10^7$, and $10^8$ copies of competitor, respectively; M, DNA molecular size marker (174 RF DNA, HaeIII cut).
groups, which reached the peak value at 3 wk (3-fold increase, compared with 1-wk-old group). Postnatal changes in stretch-induced ANP secretion were more prominent than those in atrial volume and ECF translocation (30-fold increase compared with 1-wk-old group). Additionally, the leftward shift of ANP secretion in terms of ECF translocation with age happened suddenly between weeks 3 and 4 even though atrial ANP content had already reached the peak at 3 wk. The accentuated response to stretch of ANP secretion may be partly due to developmental changes in body fluid metabolism or endogenous stimuli of ANP secretion. Therefore, it is very likely that the age-related increase in ANP secretion was due to increases in atrial compliance and atrial ANP content.

The natriuretic peptide family and their receptors have been found in atrial myocytes and fibroblasts (15, 28, 29, 31). NPR-B (GC-B) is expressed in atrial tissue (28). In the present study, we found that cGMP production by CNP was attenuated in younger rabbits and increased markedly with age. These results suggest that CNP-NPR-B signaling in cardiac atria is developmentally regulated. Recently, we (28) reported intracardiac cross talk between ANP and CNP, showing the negative regulation of ANP secretion by CNP in beating rabbit atria, and an absence of the inhibitory effect of CNP related to the low activity of the GC-B enzyme in hypertrophied atria (22). The above results suggest that the physiological effect of CNP may change with age. Therefore, to define postnatal changes in the inhibitory effect of CNP on ANP secretion, we evaluated the effect of CNP on the ANP secretion from isolated perfused nonbeating atria from 2-, 4-, and 8-wk-old groups. Interestingly, an inhibitory effect of CNP was observed in the 8-wk-old group but not in the 2- and 4-wk-old groups. In the 2-wk-old group, CNP caused an increase in ANP secretion. At present, we do not know why the effect of CNP on ANP secretion changes with age. The amount of cGMP production from atrial tissue membranes was significantly higher in the 8-wk-old group than in the 2-wk-old group. However, NPR-B mRNA was not significantly different in both groups. Therefore, we conclude that the inhibitory effect of CNP on atrial ANP secretion is developmentally regulated, being absent during normal cardiac development in young animals and intact in adult animals. These data also suggest that the absence of the inhibitory effect of CNP on ANP secretion in younger rabbits may be partly due to low responsiveness of GC-B to CNP.

What is the physiological significance of postnatal changes in CNP-stimulated cGMP production and the intracardiac effect of CNP? The presence of an atrial CNP system suggests the importance of the intracardiac role of the CNP system as well as the ANP and BNP systems. CNP has a similar structure to ANP and BNP, but its functions are quantitatively different. Currently, endogenous CNP is known to influence the proliferation of cardiac fibroblasts (7), cardiac contractility (1, 28), and the secretion of atrial ANP (28). Cardiac hypertrophy is observed in transgenic mice lacking NPR-A (32). However, there has not yet been a study on transgenic mice lacking NPR-B. Recently, we (22) found low activation of GC-B by CNP without a difference in mRNA level in hypertrophied atria. Taking into account all the above data, there appears to be a relationship between NPR-B and cardiac hypertrophy and development. We suggest that GC-B activity may be kept low during the rapid growth period of the heart and then increase with the inhibition of cardiac growth. If ANP secretion due to the absence of the inhibitory effect of CNP may contribute to the regulation of vascular smooth muscle tone to reduce cardiac overload during cardiac development. In contrast, ANP may also contribute to the regulation of cardiac hypertrophy to inhibit the proliferation of cardiac myocytes and fibroblasts. The latter effect of ANP is in opposition to the developmental changes caused by CNP. This may be due to quantitative differences or developmental changes in the functions of ANP and CNP. More study is required to fully understand the physiological role of cross talk between ANP and CNP systems in the cardiac development.

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