Inhibitory Effect of Natriuretic Peptides on Aldosterone Synthase Gene Expression in Cultured Neonatal Rat Cardiocytes

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Background—Although previously thought to be synthesized solely in adrenal cortex, we have recently showed that aldosterone is also produced in and the expression of CYP11B2 mRNA was induced in the failing or hypertensive human ventricle. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are cardiac hormones with wide biological effects, including inhibition of renin and aldosterone production. We hypothesized that natriuretic peptides reduce the expression of CYP11B2 mRNA in the heart.

Methods and Results—To test this hypothesis, we examined whether endogenous or exogenous natriuretic peptides reduce the expression of CYP11B2 mRNA using real-time reverse transcription-polymerase chain reaction. By using HS 142–1, a functional guanylyl cyclase-A type receptor antagonist, we showed that angiotensin II (AngII) pretreated with HS 142–1 increased CYP11B2 mRNA expression (1.62±0.12-fold, HS 142–1+AngII 10⁻⁷ mol/L versus AngII 10⁻⁷ mol/L alone, P<0.0001). The treatment with exogenous (10⁻⁶ mol/L) ANP and BNP reduced CYP11B2 mRNA expression (ANP, P=0.0042; BNP, P=0.0012).

Conclusions—We showed that endogenous and exogenous natriuretic peptides reduced CYP11B2 mRNA expression in cultured neonatal rat cardiocytes. This may inhibit the cardiac renin-angiotensin-aldosterone system by suppressing the gene expression of CYP11B2 and restraining cardiac hypertrophy and fibrosis. (Circulation. 2003;107:807-810.)

Key Words: angiotensin hormones natriuretic peptides polymerase chain reaction
Cell Cultures
The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health in Bethesda, Md. Co-cultures of myocytes and non-myocytes were prepared as previously described.\textsuperscript{17} HS 142–1 (10\textmu g/mL)\textsuperscript{16} was added 10 hours before the addition of AngII to block GC-A receptors.

RT-PCR for CYP11B2 and GAPDH: Design of Primers and Probes
Oligonucleotide primers and TaqMan probes for rat CYP11B2 were designed from the GenBank databases (D14092, D14093) using Primer Express version 1.0.2 (Applied Biosystems Inc). The forward primer was 5'-ACTCCGTGGCCTGAGACG -3' (exon2); the reverse primer was 5'-TGGTGACAGCACGTTTGGG-3' (exon3); and the TaqMan probe was 5'-TGGCGCTTCAACCGACTGAACTG-3' (exon3). In addition, primers and the TaqMan probe for rat GAPDH were purchased from Applied Biosystems.

Isolation of Total RNA
Total RNA was extracted as previously described.\textsuperscript{10}

RT-PCR
RT-PCR was carried out with the extracted RNA in an ABI PRISM 7700 Sequence Detector (Applied Biosystems) using 96 samples per assay (50 \mu L per tube). RT was carried out for 1 cycle at 50 °C for 2 minutes, 60 °C for 30 minutes, and 95 °C for 5 minutes; the PCR protocol consisted of 40 cycles at 94 °C for 15 sec and 60 °C for 1 minute

cGMP Assay
The cGMP content of each sample in the cultured media were measured by specific radioimmunoassay for cGMP using YAMASA Cyclic GMP Assay Kit (Yamasa Corporation).

Statistical Analysis
Data were expressed as mean±SD. Statistical analysis was performed using 1-way ANOVA followed by multiple comparisons using Fisher’s protected least-significant difference and unpaired Student’s \( t \) test, as appropriate. \( P<0.05 \) was considered significant.

Results
Effect of AngII and HS 142-1 on Expression of CYP11B2 mRNA in Co-Cultures
Figure 1A shows the level of CYP11B2 mRNA expression in co-cultures after 48 hours of treatment with AngII (10\textsuperscript{-7} mol/L). HS 142–1 tended to increase CYP11B2 mRNA expression (1.18±0.1-fold versus control, \( P=0.0913 \)) after 48 hours of treatment, and a significant increase was observed after 72 hours of treatment (1.37±0.09-fold versus control, \( P=0.0065 \)). Whereas the treatment with AngII alone had no significant effect on CYP11B2 mRNA expression (0.91±0.11-fold versus control, \( P=0.4779 \)), AngII pretreated with HS 142–1 increased CYP11B2 mRNA expression (1.62±0.12-fold, HS 142–1 + AngII 10\textsuperscript{-7} mol/L versus AngII 10\textsuperscript{-7} mol/L alone, \( P<0.0001 \)).

Effect of ANP and BNP on the Expression of CYP11B2 mRNA in Co-Cultures
Figure 1B shows the level of CYP11B2 mRNA expression in co-cultures after 48 hours of treatment with ANP (10\textsuperscript{-6} mol/L) and BNP (10\textsuperscript{-6} mol/L). ANP treatment reduced CYP11B2 mRNA expression (0.67±0.08-fold, \( P=0.0042 \)). BNP treatment also reduced CYP11B2 mRNA expression (0.61±0.09-fold, \( P=0.0012 \)).

cGMP and the Effect of ANP and BNP on CYP11B2 Gene Expression in Co-Cultures
Figure 2 shows the role of cGMP in the effect on CYP11B2 gene expression in co-cultures. The cGMP levels were significantly higher in the control study than in HS 142–1 treatment alone (78.1±3.93 fmol/tube, \( P=0.0003 \)). Also, the cGMP levels were significantly higher in ANP (10\textsuperscript{-6} mol/L) treatment than in the control study (88.2±1.88 versus 78.1±3.93 fmol/tube, \( P<0.0001 \)), and the cGMP levels were also significantly higher in BNP (10\textsuperscript{-6} mol/L) treatment than in the control study (92.78±5.89 versus 78.1±3.93 fmol/tube, \( P<0.0001 \)).

Discussion
Recent animal and human studies have shown that aldosterone is produced in the heart.\textsuperscript{5,7-9} In addition, using a cultured neonatal rat cardiocyte model, we revealed the presence of a vicious cycle, a positive feedback pathway
from aldosterone to angiotensin-converting enzymes within the local cardiac RAAS. This vicious cycle may activate the local cardiac RAAS in the failing heart and may contribute to cardiac hypertrophy and cardiac fibrosis in spite of the lowering the activity of the circulating aldosterone. 

It is well known that AngII stimulates the induction of aldosterone in the adrenal gland. As shown in Figure 1A, however, AngII (10^{-7} mol/L) alone did not affect CYP11B2 mRNA expression after 48 hours in the heart. One possible explanation for this difference is that the presence of natriuretic peptides in the cardiocytes counteracted the action of AngII, thereby reducing CYP11B2 mRNA expression. Thus, by blocking the function of natriuretic peptides with the use of HS 142–1, we showed that Ang II treatment increased CYP11B2 mRNA expression after 48 hours. Also, we showed that HS 142–1 treatment alone tended to raise CYP11B2 mRNA expression after 48 hours and significantly raised the expression after 72 hours. Therefore, this study clearly showed that endogenous natriuretic peptides reduced CYP11B2 mRNA expression in the heart.

In a rat experimental model, the concentrations of endogenous ANP and BNP in the culture media were reported to be from 10^{-8} mol/L to 10^{-10} mol/L. These levels of ANP and BNP are similar to those of ANP and BNP in patients with congestive heart failure. In congestive heart failure, we showed that endogenous natriuretic peptides suppressed CYP11B2 mRNA expression. On the other hand, both aldosterone and natriuretic peptides were increased in patients with heart failure. It seems that the results of these 2 studies are contradictory. This is partly because there may be some diminution of the effectiveness of natriuretic peptides in chronic heart failure to suppress cardiac aldosterone synthesis. Nonetheless, aldosterone secretion would be more prominent even in the heart if capability to secrete ANP and BNP were reduced.

The treatment with exogenous ANP (10^{-6} mol/L) and BNP (10^{-7} mol/L) suppressed CYP11B2 mRNA expression, as shown in Figure 1B. In treating patients with congestive heart failure, infusion of ANP and BNP ranging from 0.02 to 0.1 μg · kg^{-1} · min^{-1} makes plasma levels of ANP and BNP about 10^{-7} mol/L to 10^{-7} mol/L. Thus, these results raise a possibility that ANP and BNP infusion under the clinical condition would reduce aldosterone synthesis in the heart. Infusions of ANP (Carperitide) or BNP (Nomepitide) are now widely used in Japan and the United States. This is a new pathophysiological significance of its therapy; these drugs may be effective in the treatment of acute heart failure by suppressing the cardiac RAAS.

We also measured the cGMP content of each sample in the cultured media, as shown in Figure 2. This result reinforces our finding that endogenous and exogenous natriuretic peptides inhibit CYP11B2 mRNA expression in cultured neonatal rat cardiocytes.

In summary, we showed that natriuretic peptides reduced CYP11B2 mRNA expression in cultured neonatal rat cardiocytes. This may inhibit the cardiac RAAS by suppressing the gene expression of CYP11B2 and restraining cardiac hypertrophy and fibrosis.

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