Aldosterone Induces Angiotensin-Converting-Enzyme Gene Expression in Cultured Neonatal Rat Cardiocytes

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Background—The cardiac renin-angiotensin-aldosterone system is activated in failing hearts in proportion to the severity of the disease. We hypothesized that a positive feedback mechanism might exist within this system and contribute to the progression of the heart failure.

Methods and Results—To test this hypothesis, we examined whether angiotensin II or aldosterone induces the expression of angiotensin-converting-enzyme (ACE) mRNA in cultured neonatal rat ventricular cardiocytes. Expression of ACE mRNA was detected and quantified using real-time reverse transcription–polymerase chain reaction. Exposure to angiotensin II (10⁻⁵ mol/L) for 24 hours had no significant effect on the expression of ACE mRNA (0.760.5-fold versus control, P=NS), but similar treatment with aldosterone (10⁻⁵ mol/L) induced a 23.3±7.9-fold increase (P<0.01) in ACE mRNA expression. The effect of aldosterone was both time- (maximal effect, 24 hours) and dose-dependent (EC₅₀, 4×10⁻⁷ mol/L), and it was significantly (P<0.01) inhibited by spironolactone, a specific mineralocorticoid receptor antagonist.

Conclusions—Aldosterone upregulates ACE mRNA expression, which is blocked by spironolactone in neonatal rat cardiocytes. Thus, spironolactone may suppress the progression of heart failure by blocking the effects of aldosterone and angiotensin II. (Circulation. 2001;104:137-139.)

Key Words: angiotensin ■ aldosterone ■ polymerase chain reaction ■ cells
Health. We prepared neonatal rat cardiocyte cultures, consisting of myocytes and nonmyocytes in their native proportion, as previously described. Prepared cardiocytes were plated at a density of 1.0×10^5 cells/cm^2 in gelatin-coated 6-well plates (Becton Dickinson).

The cardiocytes were cultured for 30 hours in 10% FCS (Hazleton Biologies)–containing medium and then transferred to serum-free medium containing 0.1% BSA (Sigma) for an additional 10 hours. After this preconditioning period, which was used to examine the effects of aldosterone or angiotensin II, the cultures were provided with fresh medium containing 0.1% BSA, to which angiotensin II (Peptide Institute) or aldosterone (Sigma) was added for selected periods of time. To block aldosterone receptors, spironolactone (10^-6 to 10^-4 mol/L; Sigma) was added 1 hour before the addition of the agonists.

Real-Time RT-PCR for ACE and GAPDH

Design of Primers and Probes
Oligonucleotide primers and TaqMan probes for rat ACE were designed from the GenBank databases (U03734) using Primer Express version 1.0 (Perkin-Elmer Applied Biosystems, Inc), as previously described.15,17 The forward primer was 5'-GAAGACGACTTACAGTGTAGCC-3', the reverse primer was 5'-GGAGACGACTTACAGTGTAGCC-3', and the TaqMan probe was 5'-AATGGCCACGTCCCGGAAAT-3'. In addition, primers and the TaqMan probe for rat GAPDH were purchased from Perkin-Elmer Applied Biosystems.

Isolation of Total RNA
Total RNA was extracted from cardiocytes cultured in 6-well plates using a Qiagen RNeasy kit, as previously described. The RNA was then treated by DNase I (Qiagen) to minimize genomic DNA contamination.

RT-PCR
RT-PCR was performed with the extracted RNA in an ABI PRISM 7700 Sequence Detector (Perkin-Elmer Applied Biosystems) using 96 samples per assay (50 µL 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 of 94°C for 15 seconds and 60°C for 30 seconds. To verify that the amplified products were the target genes, the products from one sample tube were sequenced.

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

Results

Quantification by Real-Time RT-PCR
The linear range of the standard curve for target gene mRNAs (ACE and GAPDH) was determined using the total RNA isolated from the lung tissue of neonatal rats (Figure 1). The threshold cycle was plotted as a function of initial template concentration to generate the standard curve. With this system, the intra-assay and interassay coefficients of variation were 19.5% and 16.9% (n=8 to 10), respectively.

Effect of Angiotensin II and Aldosterone on Expression of ACE mRNA in Cardiocyte Cultures
Figure 2 shows the level of ACE mRNA expression by cardiocytes after 24 hours of treatment with either angiotensin II (10^-8 mol/L) or aldosterone (10^-5 mol/L). Although angiotensin II had no significant effect on ACE expression (0.7±0.5-fold versus control, P=NS), aldosterone treatment increased ACE mRNA expression 23.3±7.9-fold (P<0.01).

Characteristics of Aldosterone Effects on Expression of ACE mRNA
Examination of the time-dependency of effects revealed that aldosterone (10^-5 mol/L) induced significant increases in ACE mRNA expression within 6 hours (versus 0 hours); expression levels then peaked after 24 hours (Figure 3a).

The concentration-dependency of the upregulation of ACE mRNA expression by aldosterone was assessed by examining the effects of exposing cardiocytes to 10^-8 to 5×10^-5 mol/L aldosterone for 24 hours. As shown in Figure 3b, the effect of aldosterone was concentration-dependent. The EC₅₀ was 4×10^-7 mol/L.

As shown in Figure 3c, spironolactone significantly and dose-dependently inhibited the upregulation of ACE mRNA expression induced by treating cardiocytes with aldosterone for 24 hours.

Discussion
In the present study using real-time RT-PCR, we found that aldosterone induces the expression of ACE mRNA in cultured neonatal rat cardiocytes. Although earlier studies have described the upregulation and progression of cardiac RAAS activity in heart failure, the mechanism responsible remains unclear.1,2 This study indicates that a positive feedback pathway exists from...
In summary, our results directly demonstrate that aldosterone increases the expression of ACE mRNA and that this effect is blocked by spironolactone, an inhibitor of the mineralocorticoid receptor, in neonatal rat cardiomyocytes. Thus, a positive feedback pathway from aldosterone to ACE exists within the local cardiac RAAS.

References
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