Steroidogenic Enzyme Gene Expression in the Human Heart*

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ABSTRACT
Corticosteroids have specific effects on cardiac structure and function mediated by mineralocorticoid (MR) and glucocorticoid (GR) receptors. Aldosterone and corticosterone are synthesized in the rat heart. To see whether they might also be synthesized in the human cardiovascular system, we examined the expression of genes for steroidogenic enzymes as well as genes for GR, MR, and 11-hydroxysteroid dehydrogenase (11-HSD2; which maintains the specificity of MR). Human samples were from left and right atria, left and right ventricles, aorta, apex, intraventricular septum, and atrioventricular node as well as whole adult and fetal heart. Using RT-PCR, messenger ribonucleic acids encoding cholesterol side-chain cleavage enzyme (CYP11A), 3β-HSD2, 21-hydroxylase (CYP21), 11β-hydroxylase (CYP11B1), GR, MR, and 11-HSD2 were detected in all samples with the exception of the ventricles, which did not express CYP11B1. Aldosterone synthase (CYP11B2) messenger ribonucleic acid was detected in the aorta and fetal heart, but not in any region of the adult heart, and 17α-hydroxylase (CYP17) was not detected in any cardiac sample. Levels of steroidogenic enzyme gene expression were typically 0.1% those in the adrenal gland. These findings are consistent with autocrine or paracrine roles for corticosterone and deoxycorticosterone, but not cortisol or aldosterone, in the normal adult human heart. (J Clin Endocrinol Metab 85: 2519–2525, 2000)

GLUCOCORTICOIDS such as cortisol and mineralocorticoids such as aldosterone are secreted into the blood by the adrenal cortex; they are thereby able to affect distant target tissues by interacting with specific nuclear receptors. Both classes of hormones affect the cardiovascular system. High levels of glucocorticoid receptors (GR) are expressed throughout the heart, and glucocorticoids affect cardiac contractility (1) and cardiac weight (2) as well as vascular tone. Additionally, mineralocorticoid receptors (MR) and the kidney isozyme of 11β-hydroxysteroid dehydrogenase (11-HSD2; which maintains the specificity of MR). Human samples were from left and right atria, left and right ventricles, aorta, apex, intraventricular septum, and atrioventricular node as well as whole adult and fetal heart. Using RT-PCR, messenger ribonucleic acids encoding cholesterol side-chain cleavage enzyme (CYP11A), 3β-HSD2, 21-hydroxylase (CYP21), 11β-hydroxylase (CYP11B1), GR, MR, and 11-HSD2 were detected in all samples with the exception of the ventricles, which did not express CYP11B1. Aldosterone synthase (CYP11B2) messenger ribonucleic acid was detected in the aorta and fetal heart, but not in any region of the adult heart, and 17α-hydroxylase (CYP17) was not detected in any cardiac sample. Levels of steroidogenic enzyme gene expression were typically 0.1% those in the adrenal gland. These findings are consistent with autocrine or paracrine roles for corticosterone and deoxycorticosterone, but not cortisol or aldosterone, in the normal adult human heart. (J Clin Endocrinol Metab 85: 2519–2525, 2000)

Materials and Methods

Materials
Human glyceraldehyde-3-phosphate dehydrogenase PCR primers, AdvanTaq Plus PCR kits, PCR MIMIC construction kits, and 1β-Strand complementary DNA (cDNA) synthesis kits were obtained from CLONTECH Laboratories, Inc. (Palo Alto, CA). Hybond N nylon membrane, [γ-32P]ATP (3000 Ci/mmol), Rapid-Hyb buffer, and Hyperfilm-MP were obtained from Amersham Pharmacia Biotech (Arlington Heights, IL). Oligonucleotides were synthesized by Bio-Synthesis, Inc (Lewisville, TX).

A panel of first strand cDNA samples from various parts of the human cardiovascular system (CLONTECH Laboratories, Inc. Human Cardiovascular Multiple Tissue cDNA Panel) included left and right atria (LA and RA), left and right ventricles (LV and RV), apex, atrioventricular node (AV node), intraventricular septum (IV septum), whole hearts obtained from both adult and fetal donors, and aorta. The manufacturer prepared pooled samples from polyadenylated [poly(A)'] messenger ribonucleic acid (mRNA) from normal (nondiseased) tissues from at least three independent donors who had died suddenly or of traumatic causes. Samples were free of contaminating genomic DNA. The amount of cDNA in each sample was adjusted by the manufacturer to yield equivalent levels of several housekeeping genes, including β-actin, calcium-binding protein 45a, glyceraldehyde 3-phosphate dehydrogenase, hypoxanthine guanine phosphoribosyl transferase, major histocompatibility complex class I human leukocyte antigen-C allele, human leukocyte antigen-C4 (carried by virtually all individuals), phospholipase A2, ribosomal protein S9, and ubiquitin. We generated human adrenal first strand cDNA from poly(A)’ mRNA (CLONTECH Laboratories, Inc.) and adjusted its concentration to produce equivalent levels of glyceraldehyde-3-phosphate dehydrogenase compared to equivalent volumes of samples from the cardiovascular cDNA panel.

PCR analysis of mRNA expression
Oligonucleotide primers used for RT-PCR (Table 1) were located in two different exons of each gene (except for 3β-HSD2) to avoid amplification of any contaminating genomic DNA. Amplifications were performed in a GeneAmp 9600 thermal cycler equipped with a heated lid. PCR blanks and positive controls were included in each analysis. All reactions included 0.5 mg cDNA in a 40-μL reaction volume. Initial denaturation was performed at 94°C for 30 s; other reaction conditions are listed in Table 2.

Aliquots (7.5 μL) of the PCRs were taken at four cycle intervals, subjected to electrophoresis in agarose, and blotted to Hybond N nylon membranes. Membranes were prehybridized in Rapid-Hyb buffer for 15 min. Oligonucleotides internal to each amplified segment (Table 1) were labeled with [γ-32P]ATP using T4 polynucleotide kinase (Roche,
Indianapolis, IN) and hybridized with blots for a hotel 5 Ci in Rapid-Hyb buffer. Blots were washed twice at room temperature and twice (20 min each time) at 50°C with 15 mmol/L NaCl, 1.5 mmol/L sodium citrate, and 0.1% SDS, except for the CYP11A and 11-HSD2 blots, for which the high stringency washes were performed at 45°C. Autoradiography was performed at 280°C using Hyperfilm-MP in the presence of two intensifying screens for 3–18 h, with the exception of the GR blots, which were exposed to film for 20 min at room temperature (Fig. 1).

Competitive PCR

Levels of specific mRNA transcripts were determined using competitive PCR (Fig. 2). Internal standards (MIMICS) for each transcript were generated using a PCR MIMIC kit (CLONTECH Laboratories, Inc.) and the primers listed in Table 3. These primers each contained a target gene primer sequence 5′ of a sequence complementary to a heterologous DNA fragment (a BamHI/EcoRI fragment of the v-erbB gene). Consequently, the desired primer sequences were incorporated at the ends of the MIMIC fragment during an initial PCR amplification. During the subsequent competitive PCR, the resulting DNA fragments competed with genuine cDNA templates for the same primers.

Thus, serial dilutions of each MIMIC standard (10^−6 to 1 attomoles/μL) were added to PCRs containing 0.1 ng of each cDNA sample in 12.5 μL. Aliquots were subjected to electrophoresis in ethidium bromide-stained agarose and photographed. When the bands corresponding to the genuine template and the MIMIC were of approximately equal intensity, it could be concluded that the cDNA sample contained the same molar concentration of the genuine template and the MIMIC.

Results

Cortisol biosynthesis requires five enzymatic conversions (14): cholesterol is converted to pregnenolone by cholesterol side-chain cleavage enzyme (CYP11A), and pregnenolone is successively converted to 17-hydroxypregnenolone, 17-hydroxyprogesterone, 11-deoxycortisol, and cortisol by 17-hydroxylase (CYP17), 3α-hydroxysteroid dehydrogenase (3β-HSD2), 21-hydroxylase (CYP21), and 11β-hydroxylase (CYP11B1), respectively. To synthesize aldosterone, cholesterol is successively converted to pregnenolone, progesterone, and deoxycorticosterone by the actions of CYP11A, 3β-HSD2, and CYP21, respectively. The final conversion of
deoxycorticosterone to aldosterone involves three successive oxidations mediated by the same enzyme, aldosterone synthase (CYP11B2). With the exception of 3β-HSD2, which is a short-chain dehydrogenase, all of these enzymes are cytochromes P450.

Using commercially available pooled samples from various anatomic regions of normal human hearts, we determined levels of transcripts encoding all of these enzymes. These were compared to levels in the human adrenal gland. Northern hybridization may not be sensitive enough to detect transcript concentrations that may only have physiological relevance at a local or paracrine level. Consequently, we used RT-PCR followed by Southern blot hybridization to detect transcripts of interest.

Consistent with previous reports (8, 15), MR and 11-HSD2 were present in human cardiovascular tissues. Levels were similar between anatomical regions, with the exception of LA and RA, which appeared to express higher levels of 11-HSD2 mRNA than the other regions. GR was expressed at much higher levels than MR. Hybridization bands corresponding to GR mRNA were readily detectable after 26 PCR cycles in all samples after 20-min exposure at room temperature, whereas bands corresponding to MR mRNA could be seen after 38 PCR cycles following overnight exposure at ~80°C. CYP11A was detectable after 30 PCR cycles in all anatomical regions. CYP21 mRNA was detected in the LA, RA, aorta, and adult and fetal heart samples after 30 PCR cycles, whereas expression in the remaining regions (LV, RV, apex, IV septum, and AV node) could best be seen after 34 cycles.

Transcripts for 3β-HSD2 were detected after either 30 (LA, RA, LV, aorta, adult heart, and fetal heart) or 34 (RV, IV septum, and AV node) cycles. Expression levels varied somewhat between anatomical regions, with the highest expression in the LA and the lowest in the RV.

CYP11B1 transcripts were readily detectable in all heart samples after 26 or 30 PCR cycles, with the exception of the
ventricles, in which no transcripts could be detected even after 42 PCR cycles and prolonged exposure of the blot. Expression of CYP11B2 was limited to the fetal heart and aorta, and the hybridization bands could be seen after 34 PCR cycles.

In another attempt to detect CYP11B2 transcripts (16), we used primers that were not gene specific to simultaneously amplify a segment of both CYP11B1 and CYP11B2 (nucleotides 163–380) in which CYP11B2, but not CYP11B1, contains a \textit{Pst}\textsubscript{I} restriction site. The use of a radioactively end-labeled PCR primer and digestion with this enzyme allows CYP11B1 and CYP11B2 transcripts to be distinguished after PAGE and autoradiography. Again, no CYP11B2 transcripts could be detected in adult heart (not shown). We also did not detect CYP17 transcripts other than in the adrenal gland (not shown).

To compare the amount of each steroidogenic enzyme mRNA in the human heart with that in the human adrenal gland, we quantitated steroidogenic enzyme mRNAs (as cDNA) in the aorta, fetal heart, and LA. The aorta and fetal heart were chosen due to the expression of CYP11B2 in these two samples, whereas the LA was chosen because of the relatively high expression levels of the remaining steroidogenic mRNAs. The results (mRNA molecules expressed in attomoles per \(\mu\)g cDNA) are presented in Table 4. The concentrations of CYP11A and CYP21 in all three anatomical regions were approximately 100–1,000 fold lower, and levels of 3\(\beta\)-HSD2 were 1,000–10,000-fold lower than those in the adrenal. CYP11B1 mRNA levels in the LA and aorta were estimated to be 1,000-fold lower than the levels in the adrenal (1 attomole/\(\mu\)g cDNA compared to \(10^{3}–10^{4}\) attomoles/\(\mu\)g cDNA). CYP11B1 levels could not be quantitated in the fetal heart due to amplification of a spurious band during PCR. The levels of CYP11B2 in the fetal heart and aorta were less than \(10^{-2}\) attomoles/\(\mu\)g cDNA; the cDNA was completely competed by the PCR MIMIC at the greatest dilution of the MIMIC used in the study. The level of CYP11B2 expression in the adrenal was \(10^{-2}\) attomoles/\(\mu\)g cDNA.
TABLE 4. Levels of steroidogenic enzyme mRNA molecules in human LA, aorta, fetal heart, and adrenal expressed in attomoles per μg cDNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>LA</th>
<th>Aorta</th>
<th>Fetal heart</th>
<th>Adrenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP11A</td>
<td>$10^{-1}$</td>
<td>$10^{-1}$</td>
<td>$10^{-1}$</td>
<td>$10^3$</td>
</tr>
<tr>
<td>3β-HSD2</td>
<td>$10^{-1}$</td>
<td>$10^{-1}$</td>
<td>1</td>
<td>$10^2$</td>
</tr>
<tr>
<td>CYP21</td>
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<td>1</td>
<td>NA</td>
<td>$10^3$</td>
</tr>
<tr>
<td>CYP11B1</td>
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<td>1</td>
<td>NA</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>CYP11B2</td>
<td>NA</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>$10^{-2}$</td>
</tr>
</tbody>
</table>

NA, Not applicable.

Discussion

Possible autocrine or paracrine cardiac effects of glucocorticoids

We detected human cardiac expression of the mRNAs for many of the enzymes involved in the formation of adrenal corticosteroids, supporting the possibility of local production of corticosteroids and a physiological role for these hormones in cardiac function. CYP11A, 3β-HSD2, CYP21, and CYP11B1 were expressed within all cardiac regions studied, with the exception of the ventricles, which did not express CYP11B1. These findings suggest that specific regions within the human heart could synthesize active glucocorticoids such as corticosterone de novo; corticosterone is the major glucocorticoid in many mammals and has at least one third the molar potency of cortisol in most bioassays (17). Cortisol cannot be synthesized de novo in the human heart due to lack of expression of CYP17. In principle, cortisol could be produced locally from circulating 17-hydroxyprogrenolone or 17-hydroxyprogesterone, but it is not known whether the concentrations of these steroids within the heart are sufficient for appreciable conversion to take place.

Most of the mRNAs encoding steroidogenic enzymes were present at levels approximately 1000-fold lower than those in the adrenals. However, it should be remembered that because the heart is much larger than the adrenals, the total masses of mRNA for many of the steroidogenic enzymes in the entire heart are probably at least 1% of those in the adrenals. Corticosteroid biosynthesis of 1% of the quantity synthesized by the adrenal gland seems potentially adequate to produce local (i.e. autocrine or paracrine) effects in cardiac tissue, because locally produced steroids are not diluted into the entire circulation. Corresponding levels of enzymatic activity and local steroid production have not been measured in the human heart, and therefore, additional data are necessary to determine whether cardiac steroidogenesis is physiologically significant in humans. Although such studies have been carried out in rats (13), they may be difficult to conduct in humans due to limited sample availability.

The effects of glucocorticoids are presumably mediated by changes in gene expression within the cardiovascular system. For example, glucocorticoids stimulate the expression of the genes for atrial natriuretic peptide (ANP) and Na⁺/K⁺-adenosine triphosphatase (18–22). ANP acts on a number of organs (e.g. kidney, adrenal, vasculature, and brain) to promote natriuresis, diuresis, and vasodilatation, thereby affecting cardiac preload and afterload (23). Because ANP is secreted mainly by the atria, the relatively high level of CYP11B1 expression in the atria suggests a role for glucocorticoids produced within the atria in autocrine or paracrine regulation of ANP production.

Possible autocrine or paracrine cardiac effects of mineralocorticoids

We were able to confirm previous studies demonstrating MR and 11-HSD2 expression in the human heart (8, 9) and vasculature (15). Coexpression of MR and 11-HSD2 in the same cells could allow aldosterone to have direct, specific effects on the heart and blood vessels regardless of whether aldosterone is synthesized only in the adrenal cortex or also within the cardiovascular system itself. Such effects include induction of cardiac fibrosis and hypertrophy at doses that do not affect blood pressure; these effects are blocked by spironolactone, an aldosterone antagonist (10–12). These effects may be physiologically significant in humans, considering that spironolactone significantly reduces morbidity and mortality from congestive heart failure (24). Aldosterone may also be involved in the control of ionic homeostasis and intracellular pH within the heart (25–28).

Synthesis of aldosterone requires the expression of aldosterone synthase (CYP11B2). In contrast to the rat, in which CYP11B2 is expressed in the adult heart, we detected CYP11B2 mRNA within the human fetal heart, but not in the adult heart. We also failed to detect CYP11B2 in individual samples of cardiac infundibulum obtained from three children at surgery to correct tetralogy of Fallot and in an additional sample from a single adult heart (not shown). Thus, the data do not suggest a major physiological role for locally synthesized aldosterone in the normal human adult heart. However, the first strand cDNA samples used in the present study were prepared from poly(A)⁺ RNA pooled from multiple nondiseased tissue samples. Consequently, the data do not rule out the possibility of physiologically significant levels of CYP11B2 expression in the adult heart under conditions of sodium depletion and/or in pathological states such as heart failure or myocardial infarction. Increased local expression of CYP11B2 under such circumstances might promote fibrosis, wound repair, and remodeling. It is also possible that local expression of CYP11B2 might be confined to vascular sites (see below) rather than myocardium.

In any case, all regions of the heart express genes for all enzymes required to synthesize deoxycorticosterone (i.e. CYP11A, 3β-HSD2, and CYP21), which has significant mineralocorticoid activity. This steroid is thus potentially able to have autocrine or paracrine effects mediated by the MR.

The significance of CYP11B2 expression in the human fetal heart requires further study. Levels of CYP11B2 transcripts in the fetal heart were quite low (<10⁻² attomoles/μg cDNA), at least 1000-fold lower than those in the adrenal. Considering that aldosterone affects cardiovascular remodeling in pathological states, it may play a role in regulating fetal cardiac development, although individuals with genetic defects in aldosterone biosynthesis, such as 21-hydroxylase or aldosterone synthase deficiencies, do not have obvious defects in cardiovascular anatomy (14).
Possible autocrine or paracrine effects of corticosteroids in the aorta

Functional receptors for both mineralocorticoids and glucocorticoids are present in vascular tissue (15, 29–35). Glucocorticoids and mineralocorticoids both modulate vascular smooth muscle cell permeability to electrolyte ions, resulting in increased smooth muscle tone and responsiveness to various humoral and neurogenic vasoconstrictive agents (34, 36). Mineralocorticoids promote fibrosis and vascular remodeling when present in excess (12).

In the present study mRNAs encoding all steroidogenic enzymes necessary to synthesize corticosterone and aldosterone were detected in human aorta, supporting the notion of de novo production of both mineralocorticoids and glucocorticoids by this particular vascular tissue. Steroid 21-hydroxylase enzymatic activity has been previously demonstrated in human (37).

However, differences in steroidogenic enzyme gene expression may exist among other vascular sites and perhaps between species. For example, endothelial cells and smooth muscle cells derived from human pulmonary arteries apparently do not express either CYP11A or CYP11B1 mRNAs (15), whereas CYP11B1 as well as CYP11B2 are expressed in human umbilical vein endothelial cells, and aldosterone is apparently synthesized (38). The expression of CYP11B2 was greater than that of CYP11B1 in human umbilical vein endothelial cells, whereas in the present study, aortic CYP11B1 mRNA levels were at least 100-fold higher than those of CYP11B2 mRNA, similar to the ratio between levels of these transcripts in the adrenal. CYP11A and CYP11B1 transcripts have also been detected in rat mesenteric artery (39, 40).

Disparities between the studies may be due at least in part to differences in species or experimental preparations (cultured cells vs. fresh whole tissue), or, in fact, variations in steroidogenesis may exist within the vasculature. Supporting the idea that local steroidogenesis might be important only in certain vascular sites, MR was detected by immunohistochemical techniques in rabbit aorta and pulmonary artery, but not in smaller vessels (30).

Conclusions

Genes encoding all enzymes required for corticosterone biosynthesis are expressed in the aorta and in portions of the normal adult human heart, particularly the atria, whereas a gene encoding an enzyme specifically required for aldosterone biosynthesis is expressed only in the aorta. These findings are consistent with autocrine or paracrine roles for corticosterone and deoxycorticosterone, but not cortisol or aldosterone, in the normal adult heart, whereas aldosterone may have local effects in the vasculature.

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References


