Myocardial Production of Aldosterone and Corticosterone in the Rat

PHYSIOLOGICAL REGULATION

(Received for publication, July 23, 1997, and in revised form, November 17, 1997)

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Increasing evidence suggests that mineralo- and glucocorticoids modulate cardiovascular homeostasis via the effects of circulating components generated within the adrenals but also through local synthesis. The aim of this study was to assess the existence of such a steroidogenic system in heart.

Using the quantitative reverse transcriptase-polymerase chain reaction, the terminal enzymes of corticosterone and aldosterone synthesis (11β-hydroxylase and aldosterone synthase, respectively) were detected in the rat heart. This pathway was shown to be physiologically active, since production of aldosterone, corticosterone, and their precursor, deoxycorticosterone, was detected in both the homogenate and perfusate of isolated rat hearts using radioimmunoassay after Celite column chromatography. Perfusion of angiotensin II or adrenocorticotropic hormone for 3 h increased aldosterone and corticosterone production and decreased deoxycorticosterone, suggesting that aldosterone and corticosterone are formed within the isolated heart from a locally present substrate.

Chronic regulation of this intracardiac system was then examined. As in adrenals cardiac 11β-hydroxylase and aldosterone-synthase mRNAs were independently regulated by 1 week’s treatment with either low sodium and high potassium diet (which increased aldosterone synthase mRNA level only), angiotensin II (which raised level of both mRNAs), or adrenocorticotropic (which stimulated the 11β-hydroxylase gene exclusively). Changes in cardiac steroid levels during treatment were not directly related to their plasma levels suggesting independent regulating mechanisms. This study, therefore, provides the first evidence for the existence of an endocrine cardiac steroidogenic system in rat heart and emphasizes its potential physiological and pathological relevance.

Glucocorticoids (corticosterone in the rat and cortisol in humans) and mineralocorticoids (mainly aldosterone in both species) are synthesized from cholesterol, predominantly in the adrenal cortex. The two forms of the cytochrome P-450 enzyme which catalyze the final step of these synthetic pathways are encoded by two closely related genes CYP11B1 and CYP11B2, respectively (1) but display differences in their enzymatic activity, regulation, and tissue distribution (2). P-450 11β-hydroxylase (11β-OHase) synthesizes corticosterone from 11-deoxycorticosterone (DOC) in the zona fasciculata reticularis and is mainly regulated by adrenocorticotropic hormone (ACTH). P-450 aldosterone (Aldo)-synthase, which catalyzes synthesis of aldosterone from DOC, is present only in the zona glomerulosa. Its activity is principally controlled by angiotensin II (Ang II) and potassium and more weakly by ACTH and sodium (3, 4). While ACTH is a chronic inhibitor of aldosterone secretion, it is also a potent stimulator of its synthesis in some acute conditions (5, 6). Two other P-450c11 genes, CYP11B3 and CYP11B4 were recently cloned from a rat genomic library (7). CYP11B3 was 97% identical to CYP11B1 and encoded an enzyme with activities intermediate between those of 11β-OHase and Aldo-synthase (8), whereas CYP11B4 appeared to be a pseudogene (7).

Besides this classical adrenal biosynthetic pathway, extra-adrenal sites of steroid hormone production have been identified (9), for example in brain (10) and more recently in vessels. Indeed, aldosterone and corticosterone production and 11β-OHase and Aldo-synthase gene expression have been demonstrated in mesenteric rat artery and in endothelial and smooth muscle cells isolated from human pulmonary artery (11–13). Moreover, Hatakeyama et al. (13) showed that this vascular aldosterone potentiates Ang II-induced hypertrophy of cultured vascular smooth muscle cells, suggesting a physiological role for this locally generated steroid. To date, there is no information regarding aldosterone or corticosterone synthesis within the heart. However, Knox and Lockett (14) have previously demonstrated that isolated hearts produce a substance whose physicochemical properties are consistent with those of aldosterone. The detection of 3β-hydroxysteroid dehydrogenase activity (which produces progesterone from pregnenolone) in rat heart also indicates the potential for steroid metabolism in cardiac tissue (15).

Several lines of evidence indicate that glucocorticoids and aldosterone may influence cardiac function. (i) Glucocorticoid and mineralocorticoid receptors have been identified in human and rodent heart (16–18); (ii) 11β-hydroxysteroid dehydrogenase, which converts glucocorticoids to their inactive 11-keto metabolites and confers mineralocorticoid specificity to aldosterone target tissues (19), has also been detected in cardiac heart.
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cells (20); (iii) aldosterone triggers cardiac fibrosis (21, 22) and electrolyte imbalance (23, 24); and (iv) glucocorticoids regulate the cardiac expression of a subset of steroid-responsive genes (25, 26). We hypothesize that mineralocorticoids and glucocorticoids modulate cardiac homeostasis not only via the effects of circulating components generated within the adrenal glands, but also through local synthesis. The aim of this study was, therefore, to assess the existence of such a local system in the rat heart.

To test this hypothesis, we investigated 11β-Ohase and Aldo-synthase gene expression in rat heart by a quantitative polymerase chain reaction after reverse transcription. We then measured basal aldosterone and corticosterone production in the isolated rat heart using Celite column chromatography coupled with radioimmunoassay. Finally, we examined the regulation of this cardiac endocrine system using 1 week of treatment with a low sodium/high potassium diet, Ang II, or ACTH. This study provides direct evidence that local pathways of aldosterone and corticosterone synthesis exist in rat heart. Furthermore, this cardiac steroid production is regulated by the classical stimuli of adrenal steroid biosynthesis.

EXPERIMENTAL PROCEDURES

Animals

The study, which was conducted in accordance with both institutional guidelines and those formulated by the European community for the use of experimental animals (L555-86/609/EEC), was performed using 2-month-old male Wistar rats (Ifa Credo, Lyon, France). For chronic experiments (protocol 2 below), animals were randomly divided into four groups, each group receiving one of the following treatments: (i) a low sodium and high potassium group (0.01% Na and 2% K in chow); (ii) Ang II (100 ng/kg/min) infused via a subcutaneous osmotic minipump; and (iv) a sham-operated group, with an osmotic minipump containing only physiological serum.

Systolic blood pressure was measured by the tail-cuff method. After 1 week of treatment, rats were anesthetized by intraperitoneal injection of potassium citrate (25, 26). We hypothesize that mineralocorticoids and glucocorticoids regulate electrolyte imbalance (23, 24); and (iv) glucocorticoids regulate the cross-reactivity of each specified antibodies with different biological steroids has been previously described (29). Radioimmunoassay was performed in a 0.25 M phosphate buffer (pH 7), containing sodium azide (2 g/liter) and gelatin (1 g/liter). The accuracy of each series of assays was determined by the addition of various known amounts of unlabelled steroid. To verify that the hormones released into perfusate were not degraded in Krebs buffer, exogenous steroids were added during perfusion.

Incubation of Cardiac Homogenate with 11\(^{-}\)H|Deoxycorticosterone

To confirm that immunooassays measured bona fide steroids and did not cross-react with other steroid compounds, cardiac homogenate was incubated with 1\(^{-}\)H|DDOC. Briefly, hearts from control rats were perfused with or without Ang II (10 \(^{-}8\) M) as described in experimental protocol 1. After 3 h, perfusion was stopped, and the heart was minced with scissors and washed twice in physiological serum. The heart was then homogenized using a chilled glass homogenizer in ice-cold buffer (pH 7.4) containing 0.25 M sucrose, 15 mM malate, 5 mM MgCl\(_2\), 10 mM EGTA, 10 mM Tris-HCl, 10 mM KH\(_2\)PO\(_4\), 0.075% bovine serum albumin (fraction V, Pentex, Miles), and protease inhibitors (1 mM phenylmethysulfonyl fluoride, 5 \(\mu\)M pepstatin, 1 \(\mu\)M leupeptin, and 0.1 \(\mu\)M aprotinin). Insoluble matter was removed by centrifugation. The supernatant was then incubated aerobiocally for 1 h at 37 °C in 2 ml of buffer (composition as above) containing 90 pmol of deoxy|1-2,3-\(^{-}\)H|corticosterone (specific activity, 47 Ci/mmol; Amersham Corp., Les Ulis, France), as described previously (30). Steroids were then extracted by chloroform and separated as described under “Hormone Assay.” Unlabeled steroids were added with chloroform to the mixture, and the radioactivity of the corresponding fraction was counted using a scintillation counter. Aliquot of each fraction were used for radioimmunoassay to assay unlabeled steroids and to calculate recovery. The protein concentration was determined according to the method of Bradford (28), with bovine serum albumin used as a standard.

Total RNA Extraction

Each heart was separated into its four constituent chambers. Total RNA was extracted from these cardiac tissues and the adrenal glands according to Trizol reagent protocol (Life Technologies, Inc., Cergy Pontoise, France). The yields of total RNA extracted were similar in all four cardiac chambers, in control and treated hearts, and in non-perfused and perfused hearts. The quality of RNA was confirmed by ethidium bromide staining in 1% agarose gel.

Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Primers—Oligonucleotide primers (Bioprobe Systems, Montreuil, France) were chosen in homologous parts of the coding region of 11β-Ohase and Aldo-synthase genes (Fig. 1, panel A). The sense primer was 5′-ACTCCGTGCGCTGAGACG-3′ (position 363–581 bp, exon II) and the antisense primer 5′-CTGTGGTGGACTTGAA-3′ (position 768–949 bp, exon IV) according to the sequence published by Nomura et al. (1). We obtained a PCR product of 346 bp for each transcript after RT-PCR amplification (Fig. 2A, left panel).

The CYP11B3-specific sense primer was 5′-CGTGGAGGTCCGTTTAC-3′ (position 322–340 bp, exon II), and the antisense primer was 5′-AGTGTCTTCCTACACCT-3′ (position 768–749 bp, exon IV) (Fig. 1, panel B) according to the sequence published by Mukai et al. (7),
CYP11B3-specific primers are compared with the 11β-OHase nucleotide sequence in Fig. 1, panel B. We obtained a PCR product of 445 bp after RT-PCR amplification (see Fig. 3), as described previously (8).

**Internal Standard Preparation**—To quantify transcripts of 11β-OHase and Aldo-synthase enzymes by RT-PCR, target mRNA was coamplified with a defined concentration of specific internal standard (cRNA). The PCR product was subcloned into a pSP(64) poly(A) vector (Promega, Charbonnières, France). Sequence analysis of the subcloned cRNA. The PCR product was then linearized with a specific oligonucleotide primer for RT-PCR amplification and comparison with 11β-hydroxylase nucleotide sequences. The dashes refer to the bases that are identical in the two sequences.

**Differentiation by Enzymatic Digestion of 11β-OHase and Aldo-Synthase mRNA**—PCR products were then size-differentiated using XhoI, which hydrolyzed the Aldo-synthase PCR product into two fragments of 177 and 169 bp (right panel). Panel B, 11β-hydroxylase and Aldo-synthase mRNA densities in the four cardiac chambers of adult rat heart. Values are mean ± S.E., n = 6 per group. *p < 0.05 atria versus ventricles. Abbreviations: MW, molecular weight standards IX (Boehringer Mannheim); Stand, internal standard; 11βH, 11β-hydroxylase; AS, Aldo-synthase; RA, right atrium; LA, left atrium; RV, right ventricle; and LV, left ventricle.

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Fig. 1. Panel A, schematic organization of the rat 11β-hydroxylase and Aldo-synthase genes. Top, schematic organization of the gene with the location of exons shown by boxes and introns by horizontal lines. The exons are numbered from the 5′ end of the gene, with exon 1 containing the first codon, ATG. Bottom, the mRNA with the limits of exons, the positions of the primers ( ) and the XhoI site ( ). Panel B, schematic organization of the rat CYP11B3 gene. Top, schematic organization of the gene; middle, the mRNA with the limits of exons; bottom, specific oligonucleotide primers used for RT-PCR amplification and comparison with 11β-hydroxylase nucleotide sequences. The dashes refer to the bases that are identical in the two sequences.

![Diagram of CYP11B3 gene](image-url)
Statistical Analysis

Statistical significance was estimated between two groups using one-way analysis of variance and group-to-group comparison using Student’s t test. Tests were considered significant when \( p < 0.05 \). All values presented are the mean ± S.E.

RESULTS

11β-OHase and Aldo-Synthase mRNA Levels and Steroid Production in Normal Hearts

Cardiac Gene Expression—11β-OHase and Aldo-synthase mRNA levels were higher in atria than in ventricles (1.5- and 1.3-fold for the 11β-OHase mRNA level and 1.3- and 1.4-fold for the Aldo-synthase mRNA level in right and left atria versus right and left ventricles, respectively). 11β-OHase mRNA levels were 7-fold higher than those of Aldo-synthase in each cardiac cavity (Fig. 2, A and B). CYP11B3 gene expression was undetectable in the heart of 2-month-old rats (Fig. 3). In contrast, a low level of CYP11B3 mRNA was observed in the heart of 21-day-old rats. We also observed a similar developmental regulation in the adrenal glands, consistent with previous reports (8).

Cardiac Steroid Production—Hearts of control rats were perfused for 15 min with Krebs buffer to wash out plasma steroids before measurement. They were then perfused for 3 h with Krebs buffer, in the presence or absence of Ang II or ACTH, according to protocol 1 as described under “Experimental Procedures.”

Homogenate—Aldosterone, corticosterone, and DOC were detected in the homogenate of isolated rat heart, until completion of perfusion, under baseline conditions (Fig. 4). Basal cardiac levels of these steroids were modified by Ang II and ACTH. Indeed, Ang II and ACTH enhanced aldosterone production by 3.5- and 3.4-fold, respectively and corticosterone production by 2- and 3-fold, respectively (Fig. 4, A and B). In contrast, DOC levels (Fig. 4C) fell 1.7- and 1.6-fold in response to 3 h of Ang II and ACTH perfusion, respectively.

Perfusate—Aldosterone, corticosterone, and DOC were all detected in the perfusate of isolated rat heart, until completion of perfusion, under baseline conditions (Fig. 5). Again, Ang II infusion produced a rise in levels of both aldosterone (8-fold) and corticosterone (1.4-fold). ACTH induced a rapid rise in aldosterone levels (4.9-fold, after 1 h of perfusion) (Fig. 5A) and a slower rise in corticosterone levels (1.5-fold, after 2 h of perfusion) (Fig. 5B). In contrast, DOC levels fell rapidly, 0.7-fold at 1 h, and were undetectable after 2 h in response to either Ang II or ACTH perfusion (Fig. 5C).

Dose-response Curves—The effects of increasing concentrations of Ang II and ACTH on cardiac steroid production are shown in Fig. 6. Levels of both aldosterone and corticosterone in cardiac homogenate rose from 1.8- to 4.6-fold and 1.6- to 3.9-fold, respectively, in response to increasing Ang II concen-
trations from 10^{-9} to 10^{-7} \text{ M} during 3 \text{ h} of perfusion. In contrast, DOC levels fell from 1.4-fold (10^{-9} \text{ M}) to 5-fold (10^{-7} \text{ M}) in a dose-dependent fashion. ACTH also raised aldosterone and corticosterone levels at a concentration of 10^{-9} \text{ M} (2.3- and 1.6-fold, respectively) and 10^{-8} \text{ M} (3.7- and 3-fold, respectively). In contrast, ACTH led to a dose-dependent 1.4-fold (10^{-10} \text{ M}) to 2.7-fold (10^{-8} \text{ M}) decrease in DOC levels. ACTH effects on cardiac steroid production seemed maximal at 10^{-8} \text{ M}. An ACTH and Ang II dose-related effect was also obtained in cardiac perfusate (data not shown).

**Conversion of \[^{3}H\]DOC to \[^{3}H\]Corticosterone and \[^{3}H\]Aldosterone in Cardiac Homogenate**—In control heart, 18 and 5\% of \[^{3}H\]DOC was converted to \[^{3}H\]corticosterone and \[^{3}H\]aldosterone, respectively (58.93 \pm 4.54 and 15.36 \pm 1.14 pmol/mg of protein/\text{h} for corticosterone and aldosterone, respectively). Ang II increased the conversion of \[^{3}H\]DOC to \[^{3}H\]corticosterone and \[^{3}H\]aldosterone by 1.9-fold (111.88 \pm 27.82 and 29.55 \pm 3.10 pmol/mg of protein/\text{h} for corticosterone and aldosterone, respectively; both \(p < 0.01\) versus control values).

**Effects of Chronic Treatment with Low Na\(^+\)/High K\(^+\) Diet, Ang II, or ACTH**

Rats were treated for 1 week with a low Na\(^+\)/high K\(^+\) diet, Ang II, or ACTH. At the time of sacrifice, hearts were excised and perfused according to protocol 2.

**Anatomical and Physiological Data**—Systolic blood pressure increased after 1 week of Ang II (58\%) and ACTH (17\%) treatment (Table I). Ang II-treated rats developed moderate left ventricular hypertrophy (23\% increase in left ventricular weight/right ventricular weight ratio).

**Plasma Hormones in Control and Treated Rats**—Plasma aldosterone levels (Table II) were increased by a low Na\(^+\)/high K\(^+\) diet (3.5-fold) and treatment with Ang II (4.2-fold), but reduced by ACTH (0.8-fold). Plasma corticosterone concentrations were increased by all three treatments (1.9-, 3.6-, and 2.3-fold for low Na\(^+\)/high K\(^+\), Ang II, and ACTH, respectively). Treatments with Ang II and ACTH raised plasma levels of DOC (4.2- and 2.3-fold, respectively). Plasma renin activity was affected only by a low Na\(^+\)/high K\(^+\) diet, which caused a significant increase (37\%).

**11\beta-OHase and Aldo-Synthase mRNA Levels in Treated**

![Fig. 5](image-url)  **Time course of aldosterone (panel A), corticosterone (panel B), and deoxycorticosterone (panel C) production in the coronary sinus effluent (perfusate) of isolated perfused rat hearts under baseline conditions (\(\odot\)) and during 3 h of Ang II (10^{-8} \text{ M}, \text{\odot}) or ACTH (10^{-8} \text{ M}, \text{[]}) perfusion.** Steroids are extracted from cardiac perfusate with chloroform and assayed using radioimmunoassay after Celite column chromatography. Values are mean \(\pm\) S.E., \(n = 6\) per group. \(*p < 0.05, **p < 0.01, ***p < 0.001\) versus baseline.

![Fig. 6](image-url)  **Dose-response curves for aldosterone (panel A), corticosterone (panel B), and deoxycorticosterone (panel C) production in the homogenate of isolated perfused rat heart after 3 h of Ang II (0 to 10^{-7} \text{ M}, \text{\odot}) or ACTH (0 to 10^{-7} \text{ M}, \text{[]}) perfusion. Ang II indicates angiotensin II and ACTH adrenocorticotropic hormone. Values are mean \(\pm\) S.E., \(n = 5\) per group. \(*p < 0.05, **p < 0.01, ***p < 0.001\) versus baseline conditions (i.e. perfusion without Ang II or ACTH).**
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TABLE I
Effects of chronic treatment with low Na+/high K+ diet, Ang II, or ACTH on anatomical and physiological parameters

| Treatment | Body weight | Left ventricle weight | Right ventricle weight | Left ventricle weight/right ventricle weight | Systolic blood pressure |
|-----------|-------------|-----------------------|------------------------|---------------------------------------------|------------------------|
| Sham-operated | 301 ± 20 | 447 ± 29 | 152 ± 11 | 3.00 ± 0.61 | 134 ± 10 |
| Low Na+/high K+ | 295 ± 22 | 409 ± 28 | 162 ± 14 | 2.69 ± 0.42 | 140 ± 10 |
| Ang II | 318 ± 20 | 529 ± 18 | 144 ± 7 | 3.70 ± 0.44 | 212 ± 18*** |
| ACTH | 275 ± 19 | 523 ± 19 | 136 ± 8 | 3.57 ± 0.46 | 157 ± 15* |

* n = 6 for each group. Values are mean ± S.E. * p < .05, *** p < .001, versus sham-operated rat.

TABLE II
Effects of chronic treatment with low Na+/high K+ diet, Ang II, or ACTH on plasma concentrations of aldosterone, corticosterone and deoxycorticosterone

| Treatment | Aldosterone | Corticosterone | Deoxycorticosterone | Plasma renin activity |
|-----------|-------------|----------------|---------------------|-----------------------|
| Sham-operated | 335 ± 47 | 71 ± 12 | 966 ± 203 | 16 ± 5 |
| Low Na+/high K+ | 1,180 ± 200*** | 136 ± 21*** | 730 ± 135 | 22 ± 2* |
| Ang II | 1,395 ± 177*** | 253 ± 36*** | 4,097 ± 1,090*** | 10 ± 5 |
| ACTH | 266 ± 24*** | 164 ± 27*** | 2,105 ± 378*** | 17 ± 4 |

* n = 6 for each group. Values are mean ± S.E. * p < .05, *** p < .001, versus sham-operated rat.

Hearts—Figs. 7 and 8 (A and B) illustrate the changes in left ventricle 11β-OHase and Aldo-synthase mRNA levels in response to a low Na+/high K+ diet, Ang II, or ACTH. A low Na+/high K+ diet increased Aldo-synthase mRNA levels by 2-fold, whereas 11β-OHase mRNA levels remained unchanged. Ang II increased the concentrations of 11β-OHase and Aldo-synthase mRNA 4- and 3.4-fold, respectively. ACTH raised the 11β-OHase mRNA level 3.2-fold but had no effect on Aldo-synthase mRNA. Similar regulations were found in the right ventricle (Fig. 8, C and D).

Cardiac Steroid Production in Treated Hearts—Hearts from rats treated for 1 week with a low Na+/high K+ diet, Ang II, or ACTH were perfused for 15 min to wash out plasma steroids. Concentrations of aldosterone, corticosterone, and DOC were then measured in the homogenate and perfusate (Table III). A low Na+/high K+ diet raised levels of aldosterone by 3.8- and 11.3-fold and corticosterone by 3.7- and 1.7-fold in both homogenate and perfusate, respectively. Ang II treatment increased concentrations of aldosterone (4.4- and 16.7-fold), corticosterone (2.9- and 2.5-fold), and DOC (3.6- and 4.9-fold) in both homogenate and perfusate, respectively. Interestingly, ACTH enhanced aldosterone production 4.5-fold in the homogenate and 15-fold in the perfusate, but decreased plasma aldosterone levels (0.8-fold) (see Table II).

11β-OHase and Aldo-Synthase mRNA Levels in Control and Treated Adrenal Glands—As in the heart, basal adrenal concentration of 11β-OHase mRNA was 7.4-fold higher than that of Aldo-synthase mRNA (Fig. 8, E and F). Similar regulatory changes were observed in the adrenals as in left and right ventricles. A low Na+/high K+ diet increased Aldo-synthase mRNA levels only (4.2-fold). Treatment with Ang II raised the concentrations of both 11β-OHase and Aldo-synthase mRNA 2- and 8.5-fold, respectively, whereas treatment with ACTH stimulated 11β-OHase gene expression exclusively (3.9-fold), as described previously (4, 31, 32).

DISCUSSION

The main results of this study are: (i) 11β-OHase and Aldo-synthase, but not CYP11B3, genes are expressed in the heart of the 2-month-old rat; (ii) cardiac tissue produces the steroid hormones, aldosterone, corticosterone, and DOC; (iii) this myocardial system is regulated by the same stimuli as the adrenals (sodium, potassium, Ang II, and ACTH); and (iii) cardiac and plasma steroid concentrations seem independently regulated.

Evidence for a Cardiac System of Steroid Synthesis—In this study, we demonstrate cardiac expression of genes encoding key enzymes involved in the biosynthesis of adrenal gluco- and mineralocorticoids. Cardiac concentration of 11β-OHase and Aldo-synthase mRNAs was approximately 1000-fold lower in the heart than in the adrenals. However, the total amount of
both mRNA molecules in the whole heart was only 100-fold lower than in the adrenal glands. Such a ratio is comparable with that of angiotensin-converting enzyme mRNA, whose total quantity is about 150-fold lower in heart than in the lungs, one of the main sources for this enzyme (33). We could not detect CYP11B3 mRNA (the third P-450c11 gene) in the hearts of 2-month-old rats. However, CYP11B3 was expressed in cardiac tissue 21 days after birth. Thus, cardiac CYP11B3 gene expression is developmentally regulated, as previously described in the adrenal glands (8).

Cardiac levels of 11β-OHase mRNA were 7-fold higher than those of Aldo-synthase mRNA. Interestingly, we also found a similar ratio in adrenals consistent with previous reports (34). Extra-adrenal expression of these genes has already been demonstrated in brain (10) and blood vessels (11, 13). In contrast to cardiac muscle and adrenals, Aldo-synthase gene expression in vascular cells is higher than that of 11β-OHase (35), suggesting that the cardiac steroid biosynthetic pathway is similar to that of the adrenal glands but different from that of blood vessels.

The present study demonstrates for the first time that major adrenocortical hormones, i.e. aldosterone, corticosterone, and DOC, are synthesized within the heart. To quantify the cardiac steroid concentration and to avoid contamination by plasma steroids, measurements were performed in an isolated rat heart preparation. Steroid concentrations were determined using immunoassays in both cardiac homogenate (representing the quantity of intracellular hormones) and in cardiac perfusate (representing the quantity of steroids released into the coronary circulation). The cardiac homogenate is able to convert [3H]DOC to [3H]aldosterone and [3H]corticosterone, thus

### Table III

| Treatment       | Aldosterone Homogenate pg/mg of protein | Corticosterone Homogenate pg/mg of protein | DOC Homogenate pg/mg of protein |
|-----------------|----------------------------------------|-------------------------------------------|-------------------------------|
| Sham-operated   | 57 ± 14                                | 3 ± 2                                     | 493 ± 24                      |
| Low Na+/high K+ | 218 ± 47 *                             | 34 ± 7 ***                               | 493 ± 24                      |
| Ang II          | 249 ± 52 *                             | 50 ± 10 ***                              | 1,418 ± 266 *                 |
| ACTH            | 254 ± 54 **                            | 45 ± 8 ***                               | 1,029 ± 210 *                 |

* n = 6 for each group. Values are mean ± S.E. * p < .05, ** p < .01, *** p < .001, versus sham-operated rat.
increasing our confidence that the immunoassays measured the correct steroids and did not cross-react with other steroidal compounds.

The estimated molarity of aldosterone in myocardium is about 16 nM, a value 17-fold higher than the mean plasma value (0.93 nM). Several explanations for this cardiac concentration effect are possible. For instance, aldosterone degradation may be slower in cardiac tissue than in plasma, or may be segregated intracellularly once produced and/or locally delivered into the extracellular space instead of being released into plasma. In support of the latter hypothesis, aldosterone was almost undetectable in perfusate under base-line conditions. Furthermore, the concentration of aldosterone in the adrenal glands is about 130 mM, i.e. 140 times greater than the plasma concentration. Similar results have been reported for Ang II by Danser et al. (36), who found local Ang II concentrations 5–10 times higher in cardiac extracellular fluid than in plasma. Extremely high Ang I and Ang II levels (100-fold higher than in plasma) have also been measured in the interstitial fluid compartment of the dog heart (37). This in situ synthesis of a biologically active hormone may thus result in a far greater concentration within tissue that could be achieved via the bloodstream, supporting a putative physiological role. It is noteworthy that the situation was different for glucocorticoids; the concentration we found in plasma (205 nM) was close to that in the homogenate (143 nM).

The basal level of cardiac steroids (i.e. after wash out and before stimulation) presumably derives in part from the circulating pool and in part from endogenous synthesis. It is, however, important to note that variations from this value in the perfused heart preparation can be secondary to cardiac metabolism only. Addition of Ang II and ACTH to the perfusing buffer had two effects which provide conclusive evidence that steroids were produced in the heart: (i) aldosterone and corticosterone levels increased in both cardiac homogenate and perfusate, and (ii) the level of DOC (the precursor of both steroids) decreased simultaneously. Taken together, these experiments indicate that the biochemical components of a steroid biosynthetic pathway, i.e. 11β-OHase and Aldo-synthase, are present within rat heart and that this pathway is functional.

This study reinforces the concept that endogenous systems are present in cardiac tissue and that these systems exert autocrine and paracrine influences on local tissue function, as initially suggested by the demonstration of atrial natriuretic factor production by myocytes (38). The existence of an intrinsic cardiac renin angiotensin system and myocardial production of angiotensin I and II have also been described (25, 39). It has been postulated that such a system may be involved in chronic regulation of cardiovascular function (39). Recently, Huang et al. (40) have identified adrenergic cells in rodent and human heart, which constitutively release norepinephrine and epinephrine and thereby affect the spontaneous beating rate of cultured neonatal rat cardiomyocytes.

**Regulation of Cardiac Steroid Synthesis**—To assess the putative physiological role of this cardiac steroidogenic system, we investigated its regulation in response to chronic changes in sodium and potassium diet, Ang II, or ACTH, all of which control steroid biosynthesis in the adrenal glands (2). Seven days of low Na”/high K” diet resulted in increased concentrations of aldosterone and corticosterone in the heart associated with a rise in the level of Aldo-synthase mRNA but not 11β-OHase mRNA. These results suggest that a low Na”/high K” diet increases cardiac aldosterone synthesis in the adrenal glands (32, 41), acting at the transcriptional or post-transcriptional level. In contrast, the rise in corticosterone production induced by the diet suggests a regulation at pre-translational level. These functional differences in gene regulation may reflect structural differences in the 5’-flanking sequences of both P-450 enzymes gene promoters (7, 42).

Ang II is an important regulator of adrenal mineralocorticoid biosynthesis and secretion (43, 44), and it increases the levels of both 11β-OHase and Aldo-synthase mRNA in glomerulosa cells (31). The present study shows that the biosynthesis of aldosterone and corticosterone was also under the control of Ang II in cardiac muscle. Moreover, the levels of 11β-OHase and Aldo-synthase mRNA in the hearts of Ang II-treated rats were increased by the same order of magnitude as the respective hormones, suggesting transcriptional or post-transcriptional control of their biosynthesis, as in adrenals. Interestingly, DOC levels were increased in the cardiac homogenate and perfusate after 1 week’s treatment with Ang II, indicating that precursors of DOC (and thus the entire steroid biosynthetic pathway) may exist in the heart and may be stimulated by such treatment. In contrast, during short term treatment, only the late pathway (i.e. conversion of DOC to aldosterone) was activated, since cardiac DOC levels fell after 3 h of perfusion with Ang II.

ACTH is the most potent stimulator of adrenal steroid synthesis (44). However, stimulation of aldosterone secretion is transitory because chronic treatment with ACTH decreased aldosterone synthesis in adrenals (5, 6). In the present study, ACTH induced a rise in cardiac corticosterone, acting at a transcriptional or post-transcriptional level, but did not affect Aldo-synthase gene expression. Similar regulations have been previously described in cultured vascular or glomerulosa cells (31, 35). In contrast, ACTH induced different effects on aldosterone production in the heart and adrenals; cardiac aldosterone production increased 4.5-fold, whereas plasma levels were decreased slightly (0.8-fold). Several hypotheses could explain this discrepancy. (i) Regulation of steroidogenesis may be tissue-specific, and ACTH acts at a pretranslational level in the heart; (ii) the marked stimulation of the earlier steps of the cardiac biosynthetic pathway (indicated by the increase in corticosterone and DOC) was sufficient to overcome the lack of activation of Aldo-synthase gene transcription; and (iii) the kinetics of aldosterone regulation by ACTH may differ between the heart and adrenal glands. Indeed, ACTH acts on adrenal aldosterone synthesis in two phases, an initial rapid phase of activation and a second one of inhibition. Thus, after 1 week of treatment, aldosterone synthesis was still active in the heart but inhibited in the adrenals.

In summary, a low Na”/high K” diet and Ang II induced similar responses in the heart and adrenals. However, changes in cardiac tissue levels of aldosterone and corticosterone during treatment were not directly related to their plasma levels, suggesting independent regulating mechanisms. This hypothesis is supported by the differences between ACTH-induced regulation in the heart and adrenal glands.

Possible Role for Cardiac Aldosterone and Corticosterone Productions—The detection of glucocorticoid and mineralocorticoid receptors in the heart (16, 17), as well as 11β-hydroxysteroid dehydrogenase activity (20), strongly support the possibility of specific actions of aldosterone and corticosterone in the rat heart. The discovery of a local steroidogenic system that responds on both short and long term physiological stimuli suggests paracrine or autocrine actions for these cardiac-generated steroids. Furthermore, the higher aldosterone concentration in heart than in plasma supports also a putative physiological role.

The cardiac effects of mineralo- and corticosteroid hormones remain to be elucidated. Several results from the literature furnish, however, clues for possible cardiac functions. Steroid hormones may be involved in the control of ionic movements
since they increase intracellular Ca\textsuperscript{2+} in vascular smooth muscle cells with potential implications for the regulation of vascular tone (45, 46). Aldosterone has also been shown to increase gene expression of major isoforms of Na\textsuperscript{+},K\textsuperscript{+}-ATPase in isolated cardiac myocytes (24) and to modulate acid-base balance in cultured neonatal rat cardiac cells via effects on activity of Na\textsuperscript{+}/H\textsuperscript{+} and Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange (47).

Cardiac production of aldosterone and corticosterone was increased by Ang II. Interaction between the renin angiotensin and cardiac steroidogenic systems seems likely since dexa-methasone increased Ang II synthesis in the isolated heart (25) and cardiac AT-1 receptor mRNA in vivo (26). Furthermore, aldosterone increases AT-1 receptor density (48) and potentiates Ang II-stimulated hypertrophy (13) in vascular smooth muscle cells. These actions may thus sensitize cardiac responses to circulating or locally produced Ang II. The cardiac extracellular matrix is also a potential steroid target. Indeed, both corticosterone (49) and aldosterone (21, 22) induce cardiac fibrosis via an indirect unexplained mechanism (50).

In conclusion, we provide the first evidence for the existence of a cardiac endocrine system producing aldosterone and corticosterone in the rat. However, the synthesis of results using of a cardiac endocrine system producing aldosterone and corticosterone and extracellular matrix is also a potential steroid target. Indeed, muscle cells with potential implications for the regulation of vascular tone (45, 46). Aldosterone has also been shown to increase gene expression of major isoforms of Na\textsuperscript{+},K\textsuperscript{+}-ATPase in isolated cardiac myocytes (24) and to modulate acid-base balance in cultured neonatal rat cardiac cells via effects on activity of Na\textsuperscript{+}/H\textsuperscript{+} and Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange (47).

Cardiac production of aldosterone and corticosterone was increased by Ang II. Interaction between the renin angiotensin and cardiac steroidogenic systems seems likely since dexamethasone increased Ang II synthesis in the isolated heart (25) and cardiac AT-1 receptor mRNA in vivo (26). Furthermore, aldosterone increases AT-1 receptor density (48) and potentiates Ang II-stimulated hypertrophy (13) in vascular smooth muscle cells. These actions may thus sensitize cardiac responses to circulating or locally produced Ang II. The cardiac extracellular matrix is also a potential steroid target. Indeed, both corticosterone (49) and aldosterone (21, 22) induce cardiac fibrosis via an indirect unexplained mechanism (50).

In conclusion, we provide the first evidence for the existence of a cardiac endocrine system producing aldosterone and corticosterone in the rat. However, the synthesis of results using different complementary approaches will be necessary to define the functional effects and the overall physiological relevance of this system.

Acknowledgments—We thank Dr. L. Rappaport and Dr. A. Carayon for helpful discussions and Dr. B. Pendergast for kind help in preparing the manuscript. We also thank T. Dakhli for animal handling.

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