0013-7227/04/$15.00/0
Printed in U.S.A.

Endocrinology 145(11):4796–4802

doi: 10.1210/en.2004-0295

Origin of Aldosterone in the Rat Heart

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Aldosterone has been demonstrated in the perfusate of the *ex situ* rat heart and heart homogenates; however, the origin of aldosterone in the heart is controversial, with some reporting a primary role for extraadrenal synthesis within the heart, and others finding that all of the aldosterone in the heart is sequestered from the circulation. In an attempt to resolve this controversy, we measured the aldosterone and corticosterone contents of plasma and hearts of rats on a normal salt (NS), low salt (LS), or high salt (HS) diet, adrenalectomized (ADX+HS), and ADX with aldosterone replacement or deoxycorticosterone excess (ADX+HS+DOC) before tissue harvest. The sodium content of the diet had no significant effect on corticosterone levels in the plasma or heart. LS significantly increased, whereas HS decreased the aldosterone content of plasma and heart compared with NS. Corticosterone levels in both plasma and heart and aldosterone levels in plasma of ADX-HS rats were undetectable in most individuals and were extremely low in very few. Although plasma aldosterone was undetectable, aldosterone was measurable in 30% of the hearts of 84 ADX+HS rats, albeit at low levels. The aldosterone and corticosterone contents of the hearts of ADX+HS+DOC were similar to those of ADX+HS, indicating that aldosterone synthase and 11β-hydroxylase, not substrate, are the limiting factors for extraadrenal synthesis of corticosteroids in the heart. In conclusion, we found that the level of aldosterone content in the healthy rat heart *in vivo* is significantly lower than that reported elsewhere and reflects plasma levels in intact rats. (*Endocrinology* 145: 4796–4802, 2004)

CIRCULATING ALDOSTERONE LEVELS correlate positively with left ventricular mass in patients with hypertension as well as in population-based studies (1, 2), a phenomenon that has been modeled in experimental animals (3–5). The importance of excessive cardiac mineralocorticoid receptor (MR) activation was confirmed by the results of the RALES (Random Aldactone Evaluation Study) and EPHESUS (Eplerenone Post-MI Heart Failure Efficacy and Survival Study) trials, demonstrating that patients in congestive heart failure (CHF) clearly benefit from an MR antagonist at a dose that did not alter blood pressure (6, 7). MR antagonists mitigate the symptoms of patients receiving an angiotensin-converting enzyme inhibitor in whom circulating aldosterone levels are presumed not to be elevated, suggesting that MR activation is in excess of physiological need (6, 7).

At about the same time that an expanded, nonepithelial cell role was being established for aldosterone, interest in the *de novo* extraadrenal synthesis of adrenal corticoids was revived by the demonstration in the brain of cytochrome P450 side-chain cleavage ($P450_{scc}$) enzyme activity, which is required for the first step of steroid synthesis from cholesterol (8). Within a few years, mRNA for all enzymes of aldosterone synthesis had been described in the heart and vascular system (9–15). Species, strain, and pathophysiological differences have been partially responsible for confusion about the existence of aldosterone synthase message in the heart. Aldosterone synthase mRNA is expressed in some strains of rat, but not mouse heart, and although Wistar and spontaneously hypertensive (SHR) rat hearts express aldosterone synthase mRNA under basal and stimulated conditions, it was not found in Sprague Dawley rat hearts unless the rats were chronically treated with angiotensin II (16). mRNA for $P450_{scc}$, 3β-ol-dehydrogenase, 21-hydroxylase, and 11β-hydroxylase, but not aldosterone synthase, were detected in healthy adult human heart samples using RT-PCR and Southern blotting at 100- to 10,000-fold lower concentrations than those found in the adrenal (9, 17). Although the healthy adult human heart appears not to express aldosterone synthase mRNA, fetal and failing adult hearts do (17–19).

Aldosterone production has been reported in cultured vascular endothelial cells (12), vascular smooth muscle cells, mesenteric artery, brain, and isolated perfused hearts (10–12, 14, 15, 20–24). Aldosterone release by the perfused heart has been reported to be increased by angiotensin II, ACTH, a low salt diet (LS), and heart failure and to be greater in stroke-prone spontaneously hypertensive rats (SHRsp), compared with Wistar-Kyoto (WKY) rats (10, 15, 22, 23). The release of aldosterone into the perfusate as well as the elevated content of aldosterone in rat heart homogenates have been interpreted as *de novo* synthesis. Two very similar studies in humans resulted in diametrically opposite results and conclusions. In both studies, plasma levels of aldosterone in the heart effluent (anterior interventricular vein and coronary sinus) were compared with those in the aortic root in patients with left ventricular dysfunction and in healthy controls. Mizuno *et al.* (25) reported that peripheral blood concentrations of aldosterone in CHF patients were not different from those in control subjects; however, aldosterone levels were significantly higher in the blood draining from the heart than

Abbreviations: ADX, Adrenalectomized; CHF, congestive heart failure; DOC, deoxycorticosterone; HS, high salt diet; LS, low salt diet; MR, mineralocorticoid receptor; NS, normal salt diet; $P450_{scc}$, cytochrome P450 side-chain cleavage; poly(A)$^+$, polyadenylated.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

that in that from the aortic root in patients with CHF, but not in control individuals. This study supports the idea that aldosterone synthesis is increased in the failing heart and that the therapeutic response to MR inhibition is due to blocking the effect of aldosterone synthesized within the heart. Tsutamoto *et al.* (26) also found that circulating levels of aldosterone in patients with cardiac dysfunction were not different from those in control subjects. However, the aldosterone concentrations in coronary sinus samples were lower than those in the aortic root in both patients with heart failure and normal controls, suggesting that the heart extracted aldosterone from the circulation. Administration of the MR antagonist spironolactone decreased the extraction of aldosterone from plasma in patients with CHF (27).

In addition to clinical inconsistencies, there are those of enzyme expression and kinetics that make the interpretation of studies in this area difficult. The concentration of mRNA for all steroidogenic enzymes in rat heart and vascular tissue is much lower than that in the adrenal. The combined mass of the heart, endothelial, and vascular smooth muscle cells is very large, certainly much greater than the cell mass of the zona glomerulosa. Therefore, a significant total amount of enzyme expression and aldosterone synthesis is conceivable. Reported conversion rates of tritiated deoxycorticosterone (DOC) to aldosterone and corticosterone in heart homogenates are as high as those in zona glomerulosa, even though expression of aldosterone synthase is 1000 times less (10, 15, 22, 23, 28, 29). With these conversion rates, it is difficult to explain why adrenalectomy results in undetectable plasma concentrations of corticosterone and aldosterone unless the heart does not release these steroids, or precursors are not available for synthesis.

The following studies were undertaken in the rat to revisit reported discrepancies about the content and level of production of aldosterone in the heart by comparing levels of aldosterone in plasma and hearts of healthy rats under physiological conditions that alter adrenal production of aldosterone and in adrenalectomized (ADX) rats. The establishment of a baseline production of aldosterone within the heart will make it possible to assess normal and aberrant regulation of heart aldosterone synthesis, the role heart aldosterone synthesis may play in disease, and potential therapeutic regimens to normalize production.

Materials and Methods

Female Wistar rats (Harlan Sprague Dawley, Indianapolis, IN) in a given experiment were of the same age and shipment. Husbandry and all procedures followed the NIH Guide for the Care and Use of Laboratory Animals and were performed in an Association for Assessment of Laboratory Animal Care-accredited facility, and the animal care and use protocols were approved by the Veterans Affairs institutional animal care and use committee. Adrenalectomies were performed through bilateral flank incisions under isoflurane anesthesia delivered by a standard anesthetic machine with buprenorphine for postoperative analgesia. ADX rats were provided 0.9% saline [high salt diet (HS)] to drink. Aldosterone (4.8 μg/d) was delivered sc by miniosmotic pump (ALZET, Cupertino, CA). Rats were fed *ad libitum* a standard normal salt diet (NS; 0.3% NaCl; Harlan Teklad, Madison, WI), LS (<0.03% NaCl), or HS (standard chow plus 0.9% saline to drink). DOC (5 mg) was administered sc as an emulsion of 1:1 saline and oil to provide a large excess of substrate for extraadrenally expressed 11β-hydroxylase and aldosterone synthase enzymes in ADX rats. One to 3 d before euthanasia and tissue collection, the rats were transported to a quiet room adjacent to the animal surgical suite to acclimatize them to the routine. The rats were mask-induced with isoflurane delivered in oxygen with a standard anesthesia machine, 4–5 ml blood were drawn from the left ventricle into an EDTA Vacutainer (Becton Dickinson, Franklin Lakes, NJ), and the beating heart was removed, trimmed, opened, and blotted, then frozen in liquid nitrogen within 1–3 min of the rat being taken from its cage. Rats weighing less than 10 g (the average weight of rats in the control group the day before tissue harvest) were eliminated from the experiment. An ELISA for human hemoglobin (Bethyl Laboratories, Inc., Montgomery, TX) using rat hemoglobin to construct the standard curves was used to measure hemoglobin in hearts and plasma harvested in the same way to determine blood contamination of the hearts. It was determined that blood contamination of the hearts was negligible and consistent between the five paired heart and plasma samples measured. Therefore, the aldosterone and corticosterone measured in the hearts were within the tissue, not due to contamination with blood.

Aldosterone and corticosterone assays

Plasma (0.5 ml) was extracted with 5 ml dichloromethane, reconstituted in 200–250 μl ELISA buffer (20 mM sodium phosphate, 100 mM sodium chloride, 0.01% thimerosal, and 0.05% Tween 20), and 50 μl were used to measure aldosterone by ELISA using a specific monoclonal antibody and protocol produced and characterized by us, as previously described (30, 31). The reconstituted extract was diluted 1:100, and corticosterone was measured by ELISA using a sheep polyclonal antibody, as described by us (32). All assays were performed in triplicate, included blanks, and were run more than once for most experiments to assure reproducibility. Plasma DOC was measured by RIA (33) in 0.5 ml plasma extracted with 7% dichloromethane in hexane.

Measurement of aldosterone and corticosterone in the heart

The heart was weighed, and 5 ml water were added containing 4000 dpm tritiated aldosterone for estimation of recoveries. It was homogenized using a Polytron (Brinkmann Instruments, Westbury, NY), the homogenate was extracted with 25 ml dichloromethane, and the aqueous phase was removed. To clean the sample, the organic phase was passed through a silica gel column (Silica Gel Grade 62, Sigma-Aldrich Corp., St. Louis, MO) that had been prewashed with 5 ml dichloromethane, where the steroids were adsorbed. The steroids were then eluted with 5 ml dichloromethane containing 7% methanol. The organic extract was evaporated under vacuum and reconstituted in 250 μl ELISA buffer. A 50-μl aliquot was used for estimation of recoveries, and 50 μl were used in triplicate for the aldosterone assay. The sensitivity of the ELISA for aldosterone was 1 pg/well, and that for corticosterone was 10 pg/well. A dilution of 1:100 (vol/vol) was used for measurement of corticosterone in intact rats, and a 1:10 (vol/vol) dilution was used for ADX rats. Results were expressed as picograms per gram of tissue for aldosterone and nanograms per gram of tissue for corticosterone, and as picograms (aldosterone) and nanograms (corticosterone) of plasma per milliliter. Dichloromethane was screened and redistilled when necessary to obtain negligible background signals for the steroid ELISAs. Recoveries were measured, and values were corrected for each tissue sample. Heart tissue protein was also measured using the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL) to be able to compare the present studies with those of previously reported. The protein content of rat hearts was 65 $\pm$ 10 ($\pm$SD) mg/g tissue (n = 10).

mRNA expression for the genes coding for aldosterone synthase and 11β-hydroxylase (CY11B2 and CY11B1, respectively) was measured in the hearts and adrenal glands of female Wistar rats on NS (n = 3) and HS and LS (plus 0.15% KCl in the water) diets for 12 d (n = 10). Tissues were removed, frozen in liquid nitrogen, and stored at −70 C until RNA extraction. Polyadenylated [Poly(A)$^+$] mRNA was extracted from tissues with the Micro Poly(A) Pure or Poly(A) Pure Kit (Ambion, Houston, TX). RNA pellets were resuspended in 0.1 mM EDTA and treated with deoxyribonuclease using the DNA-free kit and were filtered through Spin columns (Ambion). PCR primers and probes (Table 1) were designed with Primer3 software (http://genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). Annealing temperature was set at 60 C for primers and 70 C for probes during primer/probe design. For RT, 500 ng poly(A)$^+$ RNA were heated at 65 C for 5 min with 250 ng random

TABLE 1. Real-time PCR primers/probes

Gene	Genebank accession no.	Primers/probes	Sequence
11β-Hydroxylase (CYP11B1)	NM_012537	Sense	AAG AAC ACT TTG ATT CCT GGG ATA
		Antisense	GAT GGC ATC CAT TGA CAG AGT A
		Probe	HEX-AGG GTC GCC AAC AGT CCT GGA GTG TCA-BHQ1
Aldosterone synthase (CYP11B2)	NM_012538	Sense	TAT AGA AGC CAG CAA CTT TGC AC
		Antisense	AGT CAA GCT TCT GGG TAA GAA CAG
		Probe	FAM-TGG TAG CCT GAA GTT CAT CCA TGC CCT-BHQ1
GAPDH	X02231	Sense	GTC TCA TAG ACA AGA TGG TGA AG
		Antisense	GAA CAT GTA GAC CAT GTA GTT GAG
		Probe	TR-CGT ATC GGA CGC CTG GTT ACC AG-BHQ2

GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; HEX, hexchloro-fluorescein; FAM, Fluorescein; TR, Texas Red; BHQ, Black Hole Quencher.

hexamers primers and 1 μl 10 mM deoxy-NTPs (U.S. Biochemical Corp., Cleveland, OH) in a final volume of 12 μl. Superscript II ribonuclease H^- reverse transcriptase (Invitrogen Life Technologies, Inc., Carlsbad, CA) was used for RT reaction following the manufacturer's protocol. SUPERase In RNase inhibitor (Ambion) was added at a concentration of 1 U/μl. The Multiplex Quantitative PCR was carried out in a final volume of 50 μl/well containing 1 μl RT product, 1× buffer provided with the polymerase, 6 mM $MgCl_2$, 0.2 mM deoxy-NTPs, 0.2 mM of each primer and probe (see Table 1), and 2.5 U SureStart *Taq* DNA polymerase (Brilliant Quantitative PCR Core Reagent Kit, Stratagene, La Jolla, CA). The cycling conditions consist of one cycle of 10 min at 95 C to activate the hot start polymerase and 50 cycles of 15 sec at 95 C, 15 sec at 60 C, and 1 min at 72 C. The PCR was carried out in an i-Cycler thermal cycler (Bio-Rad Laboratories, Hercules, CA), and real-time data were collected during the extension phase of the PCR cycle. For the standard curve, PCR products were cloned in the pCR2.1 TOPO vector (Invitrogen Life Technologies, Inc.) and were diluted to add $10–10^8$ molecules/PCR. Primers were synthesized by MWG Biotech International (High Point, NC) and Black hole quenched probes (Biosearch Technologies, Inc., Novato, CA).

Differences between groups were evaluated by ANOVA, followed by a Tukey contrast where appropriate (STATISTICA 6.0; StatSoft, Tulsa, OK). Where values in some animals were too low to measure, such as in ADX rats, a nonparametric evaluation using Kruskal-Wallis and the Dunn test was performed. Results are expressed as the mean ± SEM in the text and figures, except where indicated. Where homogeneity of variances between groups did not exist, logarithmic transformations were performed before the ANOVA.

Results

The specificity of primers and probes pairs for the CYP11B1 or CYP11B2 mRNAs was tested with increasing concentrations of plasmids carrying the cloned CYP11B1 or CYP11B2 cDNAs. PCR products were detected only with the specific primers/probes pairs, indicating no cross-reactivity (or at least $<10^{-8}$). The specificity was also checked by performing the PCR in the presence of 100 or 10,000 molecules/well of either CYP11B1 or CYP11B2 standard plasmids and increasing concentrations of the other one, always in the presence of 10^7 molecules/well of the glyceraldehyde-3-phosphate dehydrogenase standard plasmid. The value corresponding to the number of molecules per well for all genes was unaffected by the presence of the other templates. CYP11B2 mRNA in the ventricles of NS rats was 1.05×10^{-6}-fold that in the adrenal. Expression in the atria was 1.7×10^{-6}-fold that in the adrenal. CYP11B1 mRNA in the ventricles of NS rats was expressed at 2.95×10^{-6}-fold that in the adrenal. Expression in the atria was 1.2×10^{-6}-fold that in the adrenal. There was no difference in CYP11B1 mRNA in the ventricles of LS and HS rats; however, the difference in expression of CYP11B2 was significantly greater in LS than HS ventricles. The ratio of CYP11B2/glyceraldehyde-3-phosphate dehydrogenase message was 2967 ± 968.8 in LS and 157.92 ± 41.7 in HS ($P < 0.01$).

Figure 1 is a composite graph showing the effects of different sodium chloride intakes and adrenalectomy in 9-wk-old rats (n = 10) assigned to the following four groups: intact NS, intact LS for 16 d, intact HS for 16 d, and ADX-HS for 16 d, and ADX for 2 d before tissue harvest. Plasma aldosterone was significantly increased by 16 d of LS [5127.3 ± 502.9 (±SEM), pg/ml] compared with NS (336 ± 62.4 pg/ml; $P < 0.000127$), whereas HS lowered it to 56.6 ± 11.2 pg/ml ($P < 0.01$). Plasma aldosterone in ADX-HS rats was measurable (barely) in only one of 11 rats. Plasma corticosterone in this rat was below the level of detection, indicating that she was indeed ADX. Levels of aldosterone in the hearts of the adrenal-intact rats were: NS, 220.8 ± 32.6 pg/g; LS, 4906.4 ± 508.7 pg/g; and HS, 87.2: ±12.3 pg/g. The aldosterone concentration in the hearts of the ADX rats was consistently 14.5 ± 5.6 pg/g in six of 11 ADX rats. The levels of aldosterone in the NS, LS, and HS hearts were significantly different from those in ADX rats ($P < 0.01$), as were those in LS compared with HS hearts ($P < 0.01$). Plasma corticosterone was measurable in one ADX rat (not the animal in which aldosterone was measured) at just above the limit of detection. Dietary salt did not have a predictable or significant statistical effect on plasma corticosterone levels in the intact animals. Heart corticosterone levels in the ADX rats were not measurable. Those in intact-NS rats were significantly different from those in intact-HS ($P < 0.01$).

Seven separate experiments, comprising 84 ADX rats receiving no other treatment, were performed. Heart aldosterone was detected in more than half the hearts from ADX rats in four experiments (duration of ADX, 2, 2, 7, and 8 d), was detectable in only a few hearts from ADX animals in two experiments (duration of ADX, 7 d), and was undetectable in all hearts from ADX rats in two experiments (duration of ADX, 7 d). Of these 84 rat hearts, only 25 contained aldosterone, providing a mean of 11.8 ± 2.6 pg/g aldosterone. When the data from experiments in which there was an HS-intact group were combined, aldosterone in the ADX hearts was 19.6 ± 5.3 pg/g compared with 127 ± 18 pg/g in the corresponding intact-HS hearts. The results of the two

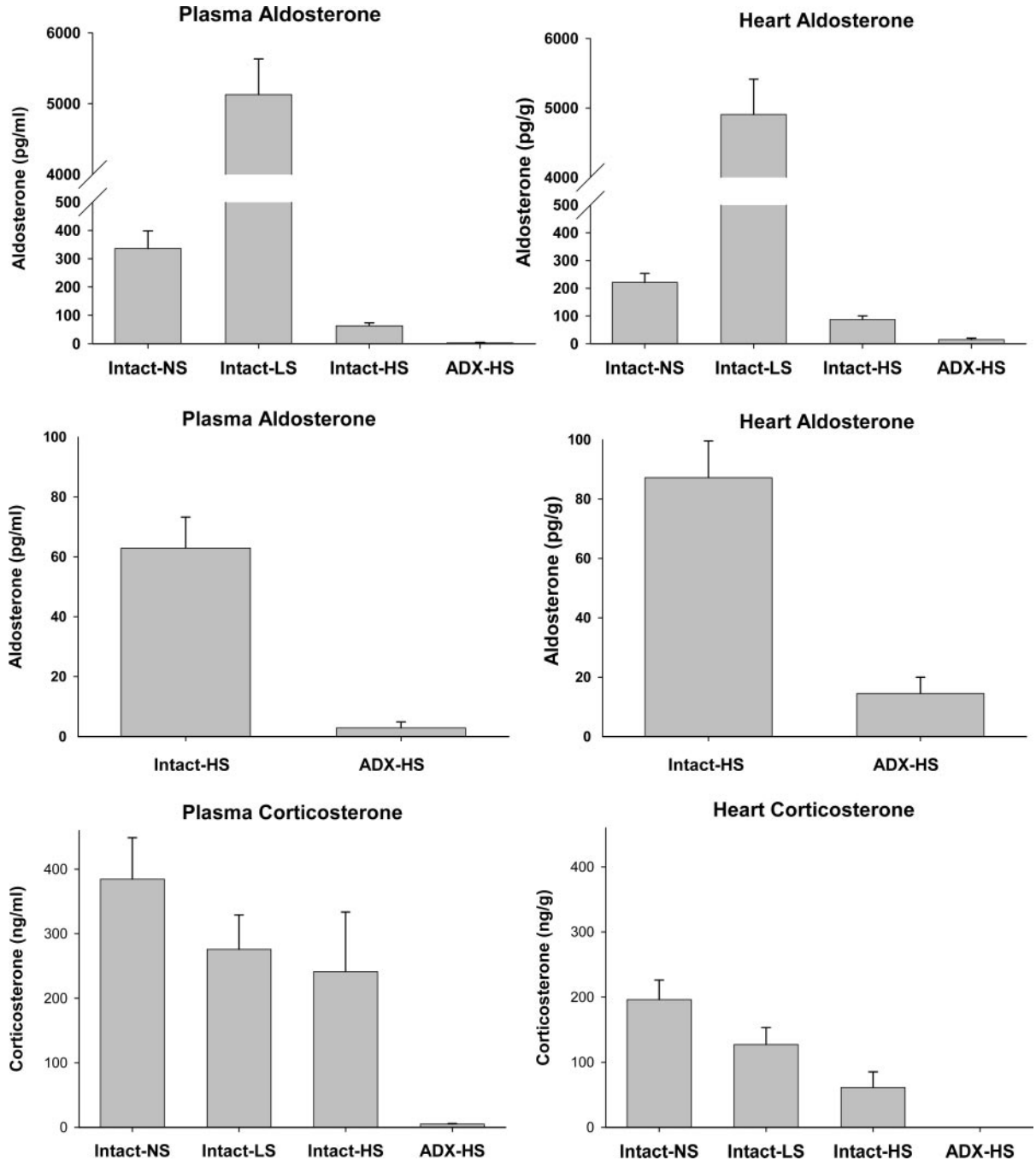

FIG. 1. Effects of NS, LS, and HS and adrenalectomy in 9-wk-old female Wistar rats on plasma and heart aldosterone and corticosterone contents. The *middle panel* is a redrawing of the HS and ADX using a smaller scale to demonstrate the differences. The dietary intervention was for 16 d; ADX was for 2 d. Values are the mean ± SE [n = 10 (NS) or 11].

experiments in which most hearts of ADX rats had clearly measurable aldosterone are shown in Figs. 1 and 2.

Figure 2 summarizes an experiment in which 12-wk-old rats (n = 9 or 10 depending on the group) were randomly assigned to three groups. Groups II and III were ADX, and group III received a miniosmotic pump (ALZET) sc that was primed to deliver 0.2 μg/h aldosterone upon insertion. All three groups in this study received HS thereafter, because ADX animals require supplemental sodium to maintain health. Tissues were harvested on d 8. Plasma and heart aldosterone levels of the aldosterone-replaced ADX rats were not statistically different from those of the intact rats; both were significantly greater than those of ADX animals (for the comparison of heart aldosterone: ADX-HS *vs.* intact-HS, $P < 0.0017$; and ADX-HS *vs.* ADX-HS plus aldosterone, $P < 0.01$). Plasma and heart corticosterone levels in the intact group were significantly greater than those in both ADX groups; levels were undetectable in most and at the limit of detection in a few individuals (intact-HS *vs.* ADX-HS and ADX-HS plus aldosterone, $P < 0.01$ for both plasma and heart).

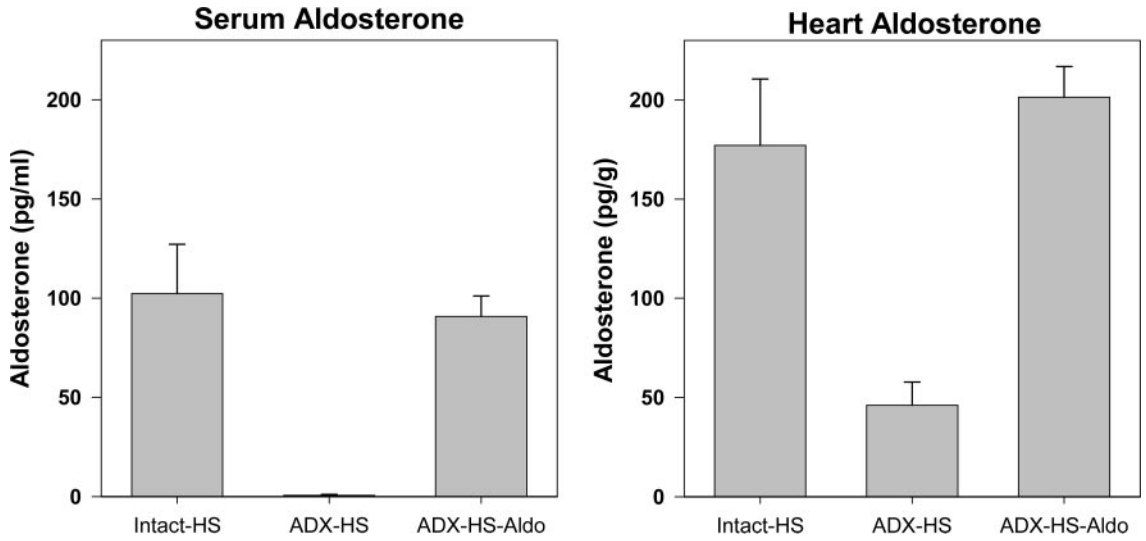

FIG. 2. Effects of adrenalectomy (ADX+HS) and adrenalectomy with aldosterone replacement by an indwelling continuous miniosmotic infusion pump (ADX+HS+aldo) in 12-wk-old female Wistar rats on plasma and heart aldosterone contents. The dietary intervention, ADX, and aldo replacement were performed for 8 d. Values are the mean ± SE [n = 9 (HS) or 10 (ADX+HS and ADX+HS+aldo)].

In two separate experiments a large excess of DOC (5 mg) was administered sc 3 h before tissue collection in ADX rats to determine whether substrate was a limiting factor for extraadrenal-expressed 11β-hydroxylase and aldosterone synthase enzymes. In one experiment there were eight rats per group ADX for 48 h before the study; in the other, nine rats per group were ADX for 7 d before receiving DOC. Plasma concentrations of DOC in untreated ADX rats were undetectable; the mean plasma DOC concentration in the rats receiving DOC injections was 41.2 ± 5 ng/ml. The aldosterone concentration in the hearts of ADX rats given no treatment was 26 ± 14 pg/g; that in ADX and DOC-treated rats was 39.67 ± 14.8 pg/g wet tissue weight. There was no statistical difference between the ADX and ADX plus DOC heart aldosterone concentrations.

Discussion

There are several reports that the heart is capable of synthesizing very significant amounts of aldosterone (10, 15, 22), yet the heart cannot replace the failing or absent adrenal gland. These studies were performed to clarify the controversy about the origin of aldosterone within the heart. We found that although plasma aldosterone concentrations in our NS rats (200–300 pg/ml) were comparable to those reported, aldosterone concentrations in their hearts reflected those in the circulation and were far lower than others have reported, even after correction of units of expression (10, 15, 22). Concentrations in our NS rat hearts averaged 200–250 pg aldosterone/g tissue, approximately 3.5 pg aldosterone/mg protein, 14–28 times lower that the values reported by Silvestre *et al.* (10, 23). Heart homogenates have been reported to have up to 17 times the concentration of aldosterone as plasma in non-ADX animals (10, 23); however, the only time that the aldosterone content in heart exceeded that in plasma was after adrenalectomy, and then only in the 30% of hearts from ADX rats in which aldosterone was detected. We used the same strain of rat, the Wistar, although our rats were obtained in the U.S., instead of France or Japan. The difference might be due to the high specificity of the monoclonal antibody developed by us, the specificity of which has been demonstrated by another laboratory unrelated to ours in animals treated with ACTH (34).

LS increased and HS decreased aldosterone levels in both plasma and heart of intact rats, as would be expected if aldosterone in the heart were derived from the circulation, or the regulation of its synthesis were the same or similar in the heart as in the adrenal. The present findings are consistent with reports that LS increases aldosterone released by the isolated perfused heart (10), but not those that chronic sodium loading increases it (22). The differences between plasma aldosterone in NS and HS animals in our studies were larger that those reported previously. This probably reflects our use of rat chow containing 0.28–0.3% NaCl, the amount found in standard growth and maintenance chows in this country [Purina (Ralston Purina, St. Louis, MO) and Teklad], instead of the chow containing 0.4–0.6% NaCl used by others (10, 22).

Female rats were used in these studies because they are less likely to have ectopic adrenal tissue than males, as adrenal cortex primordia not uncommonly accompany the migrating testes and acquire significant synthetic ability upon chronic ACTH stimulation (35). In these studies both plasma and heart corticosterone concentrations in ADX rats were undetectable in almost all rats.

The disappearance of aldosterone in plasma in the human is biexponential, with an initial phase of 29 min and a second phase of about 120 min (36). By interpolation from the metabolic clearance rate of aldosterone in the rat, the disappearance of aldosterone from the circulation should be similar to that in humans (34). Because there is no known mechanism for long-term sequestration or protection from degradation of aldosterone, our data confirm reports that the rat heart may synthesize aldosterone *in vivo*, but in significantly smaller amounts than reported previously. This is consonant

with our finding of extremely low levels of expression of mRNA for aldosterone synthase and 11β-hydroxylase.

The aldosterone synthase enzyme is a partial processing enzyme that successively hydroxylates DOC to produce corticosterone, 18-hydroxycorticosterone, and aldosterone. The production of the intermediates, corticosterone and 18-hydroxycorticosterone, is significantly greater than that of the final product, aldosterone (37, 38). Thus, one would expect that corticosterone should be easily measured in the hearts of ADX rats if aldosterone were being synthesized. The finding that aldosterone in the heart was detectable in approximately 30% of hearts from ADX rats suggests that the heart is capable of synthesizing a very small amount of aldosterone. However, the finding that corticosterone, present at approximately 400 times the concentration of aldosterone in the intact heart, becomes undetectable in hearts of ADX rats suggests that no significant synthesis of corticosteroids occurs in the heart.

Although the total amount of mRNA in the whole heart was a fraction of that in the adrenal, the mRNA for CYP11B2 was significantly greater in the hearts of LS, compared with HS, rats in our studies. An increase in angiotensin II associated with LS is a primary stimulus of adrenal production of aldosterone, and pharmacological amounts of exogenous angiotensin II were reported to increase the aldosterone content in heart as well as that in serum (10).

The rate-limiting steps in the adrenal synthesis of aldosterone are the transport of cholesterol mediated by the steroidogenic acute regulatory protein to the inner mitochondrial membrane, where it is acted upon by $P450_{scc}$, and the conversion of DOC to aldosterone by the mitochondrial aldosterone synthase (39, 40). The rates of conversion of tritiated DOC to corticosterone and aldosterone by cardiac tissue have been reported to be as high as those by the adrenal zona glomerulosa, which expresses more than 1000 times more enzyme than the heart (10, 15, 22, 23). Unfortunately, documentation of the method for estimating this conversion rate was not described sufficiently to be reproduced (10, 23). We found no significant difference between the aldosterone content of hearts of ADX rats with no detectable plasma DOC or those with circulating values in excess of 40 ng/ml. This is consistent with our demonstration of very low levels of CYP11B2 expression in the heart, as well as reports that the mRNA for the other enzymes required for adrenal corticoid synthesis from cholesterol are expressed in rat hearts at concentrations several orders of magnitude less than those in the adrenal (9, 17).

Most aldosterone in the heart tissue of healthy rats is derived from the circulation. The use of MR antagonists in the treatment of heart failure has increased greatly and is expected to increase even more with the recent advent of new MR-selective antagonists with fewer side-effects (7). However, the MRs in some, if not most, nonepithelial tissues, notably the hippocampus and perhaps the healthy heart, are normally occupied by glucocorticoids. The effect of generalized long-term blockade of MR in patients is as yet unknown. Knowledge about the regulation of aldosterone content of heart tissue, regardless of the source, may offer a novel way of reducing it in the diseased organ without altering MR-mediated homeostasis elsewhere.

Acknowledgments

Received March 8, 2004. Accepted July 28, 2004.

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This work was supported by National Institutes of Health Grants HL-27737 and HL-27255 and medical research funds from the Department of Veterans Affairs.

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