Chronic hyperaldosteronism in a transgenic mouse model fails to induce cardiac remodeling and fibrosis under a normal-salt diet

Qing Wang, Sophie Clement, Giulio Gabbiani, Jean-Daniel Horisberger, Michel Burnier, Bernard C. Rossier, and Edith Hummler. Chronic hyperaldosteronism in a transgenic mouse model fails to induce cardiac remodeling and fibrosis under a normal-salt diet. Am J Physiol Renal Physiol 286: F1178–F1184, 2004. First published February 3, 2004; 10.1152/ajprenal.00386.2003.—Primary aldosteronism causes severe hypertension in humans (Conn’s syndrome) with cardiac hypertrophy, characterized by a fibrosis more severe than that observed in patients with essential hypertension. This suggests that aldosterone by itself may have specific and direct effects on cardiac remodeling through the activation of the cardiac mineralocorticoid receptor. Experimental evidence obtained in studying uninephrectomized rats treated with aldosterone or deoxycorticosterone (DOC) together with salt loading has led to similar conclusions. To examine the direct consequences of chronically elevated aldosterone levels on cardiac pathophysiology, we analyzed a mouse model (α-epithelial Na channel −/−/Tg) that is normotensive under normal-salt diet but exhibits chronic hyperaldosteronism. Sixteen-month-old transgenic rescue mice that were kept under a regular salt diet that contains a small amount of sodium (0.3% Na+) displayed a compensated PHA-1 phenotype with normal body weight, normal kidney index, normal blood pressure, but 6.3-fold elevated plasma aldosterone levels compared with the age-matched control group. Peripherial resistance of distal colon to aldosterone was shown by a significant decrease of the amiloride-sensitive rectal potential difference, and its diurnal cyclicity was blunted. Despite chronically high plasma aldosterone levels, these animals do not show any evidence of cardiac hypertrophy, remodeling, or fibrosis, using collagen staining and anti-α-skeletal and α-smooth actin immunohistochemical labeling of heart sections. Cardiac fibrosis as seen in DOC- or aldosterone/salt-treated animal models is therefore likely to be due to the synergistic effect of salt, aldosterone, and other confounding factors rather than to the elevated circulating aldosterone levels alone.

IN THE KIDNEY, ALDOSTERONE exerts its physiological effect on distal nephron through interaction with the mineralocorticoid receptor (MR) and participates in the control of sodium reabsorption and potassium secretion, thus playing an important role in the regulation of blood volume and blood pressure (BP) (19). Primary aldosteronism in humans (Conn’s syndrome) is characterized by high plasma aldosterone and low renin hypertension, with variable degree of hypokalemia and metabolic alkalosis. Those patients showed more myocardial fibrosis than essential hypertensive patients (21, 27). It has also been proposed that the mineralocorticoid hormone aldosterone is involved in the pathogenesis of congestive heart failure (CHF) and that an increase of plasma aldosterone may be a risk factor in cardiovascular pathologies (35, 39). For example, the CONSENSUS study showed a relationship between plasma aldosterone concentration and mortality in patients with CHF, although the underlying mechanism is not known (29). In contrast, hypertension, heart failure, and myocardial fibrosis do not seem to be frequent in populations with lifelong exposure to a high-potassium/low-sodium diet, which should induce chronically high aldosterone levels. At least, we were not able to find any reports of such an association in the literature.

In addition to the indirect effects of aldosterone on cardiac function during heart failure resulting from sodium retention, expansion of extravascular space, and hypervolemia and hypertension, experimental evidence suggests that aldosterone may exert direct effects on the heart. This direct effect on the heart requires the presence in cardiac cells of its specific MR. MRs with high affinity for both aldosterone and the physiological glucocorticoid cortisol and corticosterone are present in nonepithelial tissues, including the central nervous system and heart (9, 10). It has also been reported that aldosterone synthesis and secretion occur in the heart, suggesting that this local renin-angiotensin-aldosterone system (RAAS) could exert specific autocrine or paracrine effects on the cardiovascular system (24, 25). Thus the MR is expressed in cardiomyocytes, endothelial cells, and fibroblasts (9). Additionally, the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) that converts cortisol into cortisone (or corticosterone to 11-dehydrocorticosterone), thereby protecting the MR from the illicit glucocorticoid occupancy, is coexpressed with the MR in the human heart, suggesting that cardiac tissue possesses the cellular machinery required for aldosterone-specific action, although the enzyme level is 100-fold lower in the heart than in the typical aldosterone-sensitive cells (9).

Experimental studies performed in rats that were uninephrectomized to stimulate renal hypertrophy and in addition exposed to a high-salt diet and chronic (1–2 mo) administration of aldosterone also suggested that aldosterone excess might lead to a detrimental effect on cardiovascular functions, characterized by severe hypertension, left ventricle hypertrophy, and development of cardiac fibrosis (4, 17, 39). These effects can be mimicked by the administration of the mineralocorti-
coid deoxycorticosterone acetate (DOCA), but not by the glucocorticoid corticosterone (39), and antagonized by the administration of MR antagonist, such as spironolactone (3) or the water-soluble potassium canrenoate (41). Importantly, in their initial studies, Brilla and Weber (4) showed that rats, uninephrectomized and similarly infused with aldosterone, but on a low-salt diet with water to drink, had no elevation of BP or cardiac collagen over control. This raises the question whether cardiac fibrosis in the high-salt/aldosterone-infused animal models is directly due to aldosterone on cardiac function, to sodium load, to consequences of the renal action of aldosterone, or to a combination of those factors. Previously, applied experimental protocols did not allow to distinguish between these possibilities.

To address this question, we used a mouse model in which the endogenous α-subunit of the epithelial sodium channel (α-ENaC; Scnn1a) is replaced by the rat α-ENaC subunit but under the control of the heterologous cytomegalovirus promoter (8). The resting ENaC activity regulates sodium excretion in the distal nephron (8). Within the first 2 wk after birth, these mice develop severe pseudohypoaldosteronism type 1, characterized by metabolic acidosis, urinary salt wasting and growth retardation, and about one-half of them die. Those that survive to an adult age develop a compensated pseudohypopaldosteronism with normal acid-base and electrolyte values under a regular-salt diet but with an over sixfold elevation of plasma aldosterone compared with wild-type littermate controls (8). To examine the direct consequences of a chronically, lifelong exposure to elevated plasma aldosterone levels in renal and cardiac pathophysiology, we analyzed old (≥14 mo old) experimental animals with respect to cardiac histology and function and found no signs of cardiac remodeling and fibrosis.

**MATERIALS AND METHODS**

**Animals.** Protocols involving animals were reviewed and approved by the state authorities (Service Vétérinaire Cantonal, Lausanne, Switzerland). Transgenic rescue mice (Scnn1a+/−/Scnn1amθ/+TgρEnαC, α-ENaC heterozygous mutant ±transgenic (Scnn1amθ/+TgρEnαC), and α-ENaC wild-type (Scnn1a−/−) mice were obtained by interbreeding mice heterozygous mutant for α-ENaC allele (Scnn1amθ/+) ± the transgene TgρEnαC on an outbred (NMRI) genetic background. Throughout the experiments, only males were considered. PCR-based genotyping for the gene-targeting status (+/+, +/−, and −/−) and the transgene (Tg+, Tg−) was performed by using specific primers, as described (8). Animals were kept under standard light (12:12-h light-dark cycle) and standard food conditions (0.3% Na+) with free access to tap water and were analyzed at the age of 15 to 16 mo.

**BP measurements.** BP and heart rate were recorded intra-arterially, with a computerized data-acquisition system (Notocord Systems SA, Croissy, France), as described (34, 36). Briefly, for placement of the intra-arterial catheter, mice were anesthetized (1 to 2% halothane with oxygen). The right carotid artery was exposed for a length of ~4 mm. A PE-10/PE-50 catheter filled with 0.9% NaCl solution containing heparin (300 IU/ml) was inserted into the vessel and tunneled to exit at the back of the neck. The mouse was set freely moving into the cage for 3–4 h. BP was recorded in conscious mice by connecting the catheter to a pressure transducer, using the computerized data-acquisition system at a sampling rate of 500 Hz. BP and heart rate were then monitored continuously for 15 to 30 min.

**Analytic procedures.** Blood samples were drawn from the arterial catheter after the BP measurements were completed. Plasma aldosterone samples were taken in the afternoon, and the assay was performed coded in duplicate on plasma samples using 125I RIA (detection limit 6 pg/mg).

**Electrophysiological studies.** Sodium transport across the rectal epithelium was measured in vivo, under anesthesia, as the amiloride-sensitive rectal potential difference (PD), in the morning (10–12 AM) and in the afternoon (4–6 PM) in the old experimental and aged-matched control group, as described (33).

**Cardiac index and immunohistochemistry.** Hearts and kidneys were rapidly excised and rinsed in cold PBS. Heart weight was measured without the atria. Cardiac weight index (CWI) was determined as the heart weight to body weight ratio. For light microscopy, tissue samples were fixed in 10% neutral buffered formol and embedded in paraffin. Four-micrometer serial sections were stained by the following different histological methods: hematoxylin-eosin, blue alnine Masson’s trichrome, and the Miller technique (11) or used for immunohistochemistry with anti-α-skeletal actin (α-SKA1 (5); 1:40 dilution in Tris-buffered saline) or anti-α-smooth muscle actin (α-sm1l (26); 1:200 dilution in Tris-buffered saline). Immunoperoxidase staining was performed essentially as previously described (5). After being stained, sections were observed using a Zeiss Axiopt photomicroscope (Carl Zeiss, Oberkochen, Germany). Images were acquired with a high sensitivity Axioscan color camera (Zeiss), stored on optical disks (Sony, Tokyo, Japan), and printed with a digital Fujifilm Pictography 4000 printer (Fujifilm, Tokyo, Japan). For further quantification, another series of ~10 representative images showing the whole heart surface per animal was acquired with high sensibility Photonic Coolview color camera system (Zeiss), stored and printed, as described above. The percentage of α-SKA areas of positive cardiomyocytes was calculated, using the software KS400 (Kontron System, Zeiss Vision, Oberkochen, Germany), as previously described (5, 28).

The degree of fibrosis was evaluated by calculating the percentage of blue areas on Masson’s trichrome-stained sections, using the same software as described above (KS400: Kontron System, Zeiss). For each specimen, coded slides were carefully examined by two independent observers.

**Statistical analyses.** Results are shown as means ± SE from (n) experiments. Statistical significance was assessed using ANOVA followed by unpaired t-tests, when appropriate. A P value <0.05 was considered significant.

**RESULTS**

**Characteristics of the chronic compensated PHA-1 mouse.** To ensure that the phenotype observed in young animals was maintained throughout the lifespan of the animal, we tested the epithelial sodium transport in distal colon by measuring amiloride-sensitive rectal PD. The experimental (α-ENaC−/−/Tg) had a significantly lower amiloride-sensitive PD compared with that of the age-matched controls (α-ENaC+/−) both in the morning and in the afternoon (P < 0.001; Fig. 1). In the experimental group, we no longer found larger amiloride-sensitive PD in the afternoon than in the morning, indicating that the circadian rhythm was abolished. This decrease in ENaC-mediated sodium transport occurred in the context of an over sixfold elevation of plasma aldosterone levels in the old experimental group (P < 0.001; Fig. 2), demonstrating the peripheral resistance to the hormone and suggesting a chronic hypovolemia, compatible with a compensated PHA phenotype. These elevated plasma aldosterone levels are not different than the increase in 3-mo-old transgenic rescue animals (5.8-fold increase) (8), strongly suggesting that the experimental animals are lifelong exposed to significantly higher plasma aldosterone.
levels. In old experimental animals, we furthermore observed a less than 60% increased plasma corticosterone level ($P < 0.01$; Fig. 2). Kidney weight index ($P = 0.75$; Table 1) and mean BP measurements revealed no difference between both age-matched groups and were in the normal range ($P = 0.45$; Table 1).

**Effect of chronic hyperaldosteronism on cardiac function and morphology.** We next tested whether 6.3-fold chronically elevated plasma aldosterone levels affected cardiac function and morphology. First, morphologically, we observed no cardiac hypertrophy in the old experimental group (Table 1). The CWI (heart weight/body wt) and the heart beats per minute (HR) did not differ between both groups (Table 1). Histological aspect of cardiac tissue, following staining with hematoxylin and eosin, revealed no difference between the experimental (a) and control (b) mice regarding the size of cardiomyocytes (Fig. 3A, a and b). We next investigated the $\alpha$-SKA expression, a known marker of development of myocardial muscle hypertrophy (22, 23, 38). Figure 3A, c and d, shows representative sections of experimental and control hearts stained with anti-$\alpha$-SKA. The percentage of $\alpha$-SKA-positive areas was determined (Fig. 3B). No significant difference ($P = 0.174$) was observed between both groups, further confirming the absence of hypertrophy in mice exposed chronically to higher plasma aldosterone levels.

Masson’s trichrome, which allows to detect positive blue collagen fibers in the conjunctive tissue, revealed a generally low fibrosis in both groups (Fig. 4A, a and b, top insets) and the percentage of collagen-positive areas in heart sections was not significantly different (control group: 1.03 ± 0.62% vs. experimental group: 1.22 ± 0.22%; Fig. 4B). Miller staining showed similar results (data not shown). The myocardial sections were likewise immunostained for $\alpha$-sm1, the typical actin isoform of myofibroblasts known to be present in fibrotic cardiac tissue (31, 37). This staining appeared to be exclusively located in vessel walls (Fig. 4A, c and d, top insets), and no $\alpha$-sm1-positive myofibroblasts were noted. This gave us proof about the absence of fibrosis in the myocardium of both groups (Fig. 4A, c vs. d).

**DISCUSSION**

Heart failure is one of the most frequent causes of morbidity and mortality in Western countries. Clinical studies proposed a critical role of aldosterone in heart remodeling, based on the findings in the RALES (randomized aldactone evaluation study) and EPHESUS (eplerenone post acute myocardial infarction heart failure efficacy and survival study) trials that evidenced a beneficial effect of mineralocorticoid antagonists on both mortality and morbidity in heart failure (13). MR inhibition with spironolactone was accompanied by a 30% improvement in mortality and 35% less hospitalization in patients with severe CHF yielding evidence of a benefit from this selective aldosterone blockade (12, 13). On the other hand, these clinical studies do not allow differentiating whether the beneficial effect of spironolactone or eplerenone was due to a direct action of the antagonist on heart MR or to an indirect effect on MR in other renal or nonrenal tissues.

In experimental animal studies, aldosterone excess, combined with a high-salt diet, caused cardiac fibrosis, although the contribution of high-salt intake in causing this phenotype is not well understood. In our present study, we analyzed mice that present a model for renal salt wasting with respect to the renal and cardiac phenotypes. These mice develop long-life chronically increased plasma aldosterone levels when kept on a regular (0.3% Na$^+$) diet (Fig. 2). In this mouse model, ENaC-mediated sodium transport presents the limiting factor because adult mice kept under a very-low-salt diet (0.01% Na$^+$) show acute pseudohypoaldosteronism with continuous weight loss, whereas mice kept under a moderate low-salt diet (0.1% Na$^+$)
can still compensate for the urinary or colonic salt losses (Hummler E, unpublished data). Because circadian changes in sodium handling in the distal nephron can be monitored by variations in amiloride-sensitive rectal PD (PDamil) (33), we measured in vivo the rectal PDamil in these transgenic rescue mice. We found that the lowered ENaC-mediated sodium transport reflects the diminished ENaC activity in the kidney (Fig. 1). This coincides well with the findings in humans or in other ENaC-modified mouse models that develop pseudohypoaldosteronism type 1 like the β-ENaC mutant mice presenting a partial knockout of the Scnn1b gene locus (14). Human patients with pseudohypoaldosteronism type 1 similar to the mouse PHA-1 models carrying ENaC mutations activate the RAAS system, resulting in increased plasma aldosterone levels (2). Contrary to the described DOCA/salt models, our mice do not develop renal hypertrophy or hypertension (Table 1), suggesting that primarily, the high-salt intake is the causative factor in those models. Our daytime measurements of BP show no significant difference in BP between wild-type and transgenic rescue animals. If measurements at other times of the cycle would show either a relative increase or no difference, our conclusions would only be strengthened. If these measurements would disclose a period of relative hypotension in the mutant animals, our conclusions that high aldosterone levels, when appropriate to physiological conditions such as low-sodium balance, are not by themselves detrimental to the heart or the kidney, are still valid and are supported by the results.

### Table 1. Physiological parameters of old control and experimental mice

<table>
<thead>
<tr>
<th>Physiological Parameters</th>
<th>Control Group</th>
<th>Experimental Group</th>
<th>P Value</th>
</tr>
</thead>
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<tr>
<td>Age, year</td>
<td>15±0.5 (12)</td>
<td>16±0.6 (12)</td>
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<td>Body weight, g</td>
<td>45.9±0.9 (12)</td>
<td>44.7±0.6 (12)</td>
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<td>Kidney weight, mg</td>
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<td>729±27 (12)</td>
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<tr>
<td>Kidney weight index, mg/g</td>
<td>16.5±0.4 (12)</td>
<td>16.3±0.5 (12)</td>
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<tr>
<td>Mean BP, mmHg</td>
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<td>113±4 (12)</td>
<td>0.45</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>522±20 (11)</td>
<td>515±20 (12)</td>
<td>0.81</td>
</tr>
<tr>
<td>Heart weight, mg</td>
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<td>177±5 (12)</td>
<td>0.76</td>
</tr>
<tr>
<td>Cardiac weight index, mg/g</td>
<td>3.8±0.06 (12)</td>
<td>4.0±0.1 (12)</td>
<td>0.11</td>
</tr>
</tbody>
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Values are means ± SE. n, Nos. in parenthesis. BP, blood pressure.

Fig. 3. Evaluation of heart hypertrophy. A: serial sections from the experimental (α-ENaC−/−Tg; a) and control (α-ENaC+/+; b) mice were stained with hematoxylin eosin. No difference in the size of cardiomyocytes between both groups was detected. Scale bar: 10 μm. Evaluation of myocardiary hypertrophy using the anti-α-skeletal actin (α-SKA) antibody revealed no change in the number of positively stained cardiomyocytes in the experimental (c) vs. the control (d) group. Representative pictures are shown and insets in c and d represent a higher magnification. Scale bar: 400 μm in c and d, 50 μm in insets. B: α-SKA-positive areas were quantified, using the software KS400 and the percentage was plotted. Bar represents SE. No difference was found between both groups (P = 0.17).
Although we cannot exclude that in the mutant mice a hypotensive period during the circadian cycle neutralized a potential "fibrotic effect" of high aldosterone levels, we do not know about any experimental evidence supporting it.

Further evidence for the critical role of salt intake for renal and cardiac phenotypes comes from the recent study showing that DOCA-treated C57BL/6J wild-type mice do not develop renal and cardiac hypertrophy when kept under low-sodium diet (0.05% Na\(^+\)) (32), whereas DOCA/salt-treated wild-type mice developed cardiac and renal hypertrophy in the absence of hypertension (34). We also found that the rectal aldosterone- and amiloride-sensitive ENaC-mediated sodium transport does not follow the diurnal cyclicity and is blunted in transgenic rescue mice (33) (Fig. 1).

In our study, the old transgenic rescue mice do not develop cardiac hypertrophy or cardiac fibrosis, despite elevated plasma aldosterone levels pointing to the role of high-salt intake in experimental models additionally to aldosterone excess in generating cardiac remodeling (Figs. 3 and 4). At least in two other mouse models, the implication of aldosterone alone in the development of heart fibrosis seems questionable.

In vivo, conditional downregulation of the MR within cardiomyocytes resulted in reversible dilated hypokinetic cardiomyopathy with increased heart index, cardiac remodeling with extensive interstitial fibrosis, whereas a conditional, cardiac-specific overexpression of the human MR exhibits a rather normal cardiac histology with no fibrosis (1, 20). In both models, the plasma aldosterone levels remained unchanged.

At variance with the previous data, Qin et al. (15) proposed that aldosterone drives cardiac hypertrophy and heart failure. Using a transgenic model overexpressing 11\(\beta\)-HSD2 mainly in cardiomyocytes, but also in tissues such as skeletal muscle, lung, spleen, and brain, they showed that mice were normotensive under 0.32% NaCl chow but spontaneously developed cardiac hypertrophy and fibrosis, a phenotype partially antagonized by the aldosterone antagonist eplerenone. The authors proposed that inappropriate activation of cardiac MR by aldosterone was causative of the cardiac phenotype. In addition, their data suggested a tonic inhibitory role of glucocorticoids, thereby preventing the proinflammatory effects of aldosterone under physiological conditions. This raises the question whether the hypercorticism observed in our model (Fig. 2B)
could have antagonized the deleterious effects of aldosterone, preventing the appearance of cardiac remodeling. We believe that it is unlikely, because it is only supported by indirect evidence. Consequences of enzyme overexpression that might cause changes in corticosterone, its inactive metabolite cortisone, or aldosterone concentrations in cardiomyocytes have not been reported by Qin et al. (15). In our study, the observed hypercorticism was modestly increased by 60%, whereas plasma aldosterone concentrations increased over 600%. This increase in plasma aldosterone might well change MR occupancy from <20% to saturation levels. On the other hand, assuming that more than 99% of corticosterone is bound to CBG, the free corticosterone in our study might be around 10 nM. A change from 10 to 16 nM would minimally change type 2 glucocorticoid receptor occupancy and would have indeed little effect in displacing aldosterone from MR and glucocorticoid receptor. It seems therefore unlikely that these modest changes in plasma corticosterone could have a tonic inhibitory role of aldosterone action on cardiomyocytes.

It has been proposed that aldosterone salt-induced cardiac fibrosis possibly involves ANG II action through upregulated AT1 receptor and with the cardiac AT1 receptor as the target for aldosterone, because the blockade of RAAS with either angiotensin I-converting enzyme inhibitors or ANG II receptor antagonists provided significant cardiovascular protection (for a review, see Ref. 18). High dietary salt intake can induce cardiac hypertrophy and collagen deposition (4, 6), and with a very-high-salt diet (8% NaCl), increased kidney weights have also been documented in rats (42). Takeda and colleagues (30) proposed that cardiac aldosterone and AT1 receptor synthesis in response to high-salt intake contributes to this cardiac hypertrophy and presumably to fibrosis, independently of the circulating RAAS. As reviewed by Young and Funder (40), the findings that AT1 receptors are elevated by high-salt intake (30) and are induced by aldosterone (16) may provide the key for the apparent synergy between salt load and mineralocorticoid administration in cardiac fibrosis. We have also found that ANG II AT1 receptor blockade with losartan reverses cardiac hypertrophy in normotensive one-rein gen mice receiving DOCA and salt (34). The present data go in the same line, suggesting that the cardiac hypertrophy and fibrosis as seen in DOCA- or aldosterone/salt-treated animal models may be due to the synergistic effect of salt and mineralocorticoid rather than to the elevated circulating aldosterone levels alone.

In conclusion, our results suggest that high aldosterone levels, when appropriate to physiological conditions such as low-sodium balance, are not by themselves detrimental to the heart or the kidney, but it is the co-occurrence of excessive salt loading (and in some cases, renal mass reduction) with inappropriate high aldosterone level that results in the described renal damage and heart remodeling. This observation further emphasizes the importance of a limited salt intake as a therapeutic approach to prevent the development of cardiovascular complications (7).

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