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Potassium Supplementation Reduces Cardiac and Renal Hypertrophy Independent of Blood Pressure in DOCA/Salt Mice

Qing Wang, Andrea A. Domenighetti, Thierry Pedrazzini, Michel Burnier

Abstract—We have demonstrated previously that deoxycorticosterone acetate (DOCA)/salt induces cardiac hypertrophy and left ventricular dysfunction independent of blood pressure (BP) in 1–renin gene mice. Because these mice also develop hypokalemia and metabolic alkalosis caused by mineralocorticoid excess, we investigated whether correcting hypokalemia by dietary potassium supplementation would prevent the DOCA/salt-induced cardiac hypertrophy, cardiac dysfunction, and electrocardiographic changes in normotensive, 1–renin gene and hypertensive, 2–renin gene mice. All mice were studied after 5 weeks of DOCA and salt administration. Potassium was given by adding 0.4 or 0.6% KCl to the drinking water. Our results show that correction of hypokalemia and metabolic alkalosis prevents cardiac hypertrophy and normalizes cardiac function without affecting BP in normotensive, 1–renin gene mice. In hypertensive, 2–renin gene mice, potassium supplementation induces a significant decrease in BP. The decrease in BP and correction of kalemia are associated with a significant but partial correction of cardiac hypertrophy. In both group of mice, electrocardiographic alterations were measured after administration of DOCA/salt, which could be corrected by potassium supplementation. Thus, these results show that correction of hypokalemia and metabolic alkalosis does prevent the development of cardiac hypertrophy and normalizes cardiac function independent of BP in normotensive, 1–renin gene mice that receive excess mineralocorticoid and salt. In 2–renin gene, hypertensive mice, potassium supplementation also prevents the development of cardiac hypertrophy, but the effect cannot be separated from the decrease in BP. (*Hypertension*. 2005;46:547-554.)

Key Words: hypokalemia ■ metabolism ■ hypertrophy ■ heart failure ■ deoxycorticosterone ■ mouse

There is substantial clinical and experimental evidence that potassium depletion, or hypokalemia, has a negative impact on the cardiovascular system as well as on the kidney and contributes to the pathogenesis of hypertension, stroke, ventricular arrhythmias, and renal injury.[1–7] Thus, even mild potassium depletion has been shown to produce a substantial impairment of cardiac function in dogs and healthy human subjects.[8,9] Several studies have also demonstrated that hypokalemia or mineralocorticoids that induce hypokalemia can affect cardiac structure, leading to cardiac necrosis and fibrosis in experimental animals and humans.[8,10–13] Conversely, potassium supplementation appears to be rather cardioprotective and nephroprotective. Indeed, a high potassium intake and prevention of hypokalemia have been associated with a decrease in blood pressure (BP) in hypertensive animals and humans.[14–19] Potassium supplementation has also been shown to prevent stroke; to reduce mortality in stroke-prone spontaneously hypertensive and Dahl salt-sensitive rats; and to prevent renal glomerular, tubular, and vascular lesions in rats.[14,17,20,21]

Together with systemic hypertension and metabolic alkalosis, hypokalemia is the clinical hallmark of mineralocorticoid excess. Several experimental studies in rats have demonstrated that excess mineralocorticoids due to the administration of aldosterone or deoxycorticosterone acetate (DOCA) together with salt induce cardiac hypertrophy and, in some cases, cardiac interstitial and perivascular fibrosis.[12,13,22] In these models, the development of left ventricular hypertrophy and cardiac fibrosis has been attributed essentially to the increase in BP and the excess of mineralocorticoid and salt. Whether hypokalemia, which is always present in these situations, also contributes to the development of left ventricular hypertrophy independent of BP changes has so far been difficult to ascertain.[23] We have recently reported that mice treated with DOCA and 1% NaCl develop hypokalemia, metabolic alkalosis, renal and cardiac hypertrophy, and left ventricular dysfunction.[24,25] In these mice, left ventricular hypertrophy develops in the absence of systemic hypertension in 1–renin gene mice and in parallel with the increase in BP in hypertensive, 2–renin gene mice.[24] These 2 mouse models provide a good opportunity to investigate the respective roles of hypokalemia and hypertension in the development of cardiac hypertrophy in response to mineralocorticoid

excess. Therefore, we investigated whether correcting hypo-kalemia by dietary potassium supplementation affects DOCA/salt-induced cardiac hypertrophy and subsequent dysfunction in 1– and 2–renin gene mice. Our results show that indeed hypokalemia contributes to the development of cardiac hypertrophy independent of BP changes in 1–renin gene mice.

Methods

Mice and Experimental Protocol

Seven-week-old, male, wild-type backcross N_{5-6}(129Ola/C57BL/6J) mice (weight, 22 to 26 g) obtained from the Institute of Pharmacology, University of Lausanne, Lausanne, Switzerland, that were homozygous for either the *Ren-1c* gene locus (1–renin gene mice) or the *Ren-1d/Ren-2* gene loci (2–renin gene mice) were used throughout these experiments. The characteristics of these mice have been described earlier.[24] The DOCA/salt model was induced as published earlier.[24] Mice were given 1% NaCl to drink. Control mice also underwent uninephrectomy without DOCA administration but received tap water as the drinking fluid, as described previously. All mice received regular food, containing 3 mg Na^+ and 7.5 mg K^+/g pellet (U.A.R.). In the potassium-supplementation groups, DOCA/salt-treated mice received either 0.4% or 0.6% KCl in the drinking water. Considering that the amount of fluid intake in the DOCA/salt-treated mouse is ≈25 mL/d, the potassium intake in DOCA/salt- and supplementary potassium–treated mice ranged between 75 and 100 mg/d, including the amount of potassium derived from the food (≈3 g pellet per day, containing ≈22.5 mg K^+). In the potassium-supplemented groups, potassium intake was 3- to 4-fold higher than in control DOCA/salt-treated mice (receiving ≈22.5 mg K^+ per day). All mice were treated for 5 weeks. Each group of mice consisted of at least 8 individuals.

BP, HR, and Heart and Kidney Indices

BP and heart rate (HR) were recorded intra-arterially with a computerized data-acquisition system as described earlier.[24] Thirty minutes after implantation of the catheter into the carotid artery, the arterial line was connected to a pressure transducer, and BP and HR were monitored every 20 seconds for 20 minutes. Once BP was measured and blood sampled for laboratory values, mice were killed, and the heart and kidneys were rapidly excised, washed with cold PBS, dried with soft facial tissue, and weighed. To determine cardiac and renal hypertrophy, cardiac and kidney weight indices were calculated according to heart and kidney weight (mg) to body weight (g) ratios.

Determination of Cardiac Contractility

Determination of cardiac contractility was performed as described previously.[25] In brief, the mouse was anesthetized with 1% to 2% halothane mixed with O_2. The right carotid artery was dissected and exposed for ≈5 mm of its length. A custom-made Pebax 03 fluid-filled catheter was advanced into the left ventricle through the right carotid artery.[25] The correct position of the catheter tip in the left ventricle was then confirmed by the waveform of left ventricular pressure visualized on a Hewlett-Packard monitor. The arterial line was connected to the computerized data acquisition system to record left ventricular pressure, left ventricular end-diastolic pressure (LVEDP), LV$dP/dt_{max}$, LV$dP/dt_{min}$, and the time constant of isovolumic relaxation (Weiss τ) at a sampling rate of 1000 Hz.[25]

Biochemical Analyses

To measure blood pH and serum and urinary Na^+ and K^+ values, 75 μL and 400 to 500 μL, respectively, of blood was drawn from the carotid artery into hematocrit capillaries (75 mm/75 μL) and 0.6-mL Multivette tubes containing gel/clot activator (Sarstedt). Blood pH was measured with the 248pH/blood gas analyzer (Rapidlab, Bayer). Serum and urinary Na^+ and K^+ values were measured by flame photometry (model 943, Instrumentation Laboratory). Blood was

drawn from the arterial catheter after the BP measurements were completed. Studies performed in our laboratory have demonstrated that blood sampling via an intra-arterial catheter generates less stress in mice than does decapitation or retro-orbital blood sampling. Indeed, plasma norepinephrine levels were 6-fold higher with decapitation than with the use of a carotid artery catheter and were almost 2-fold higher with retro-orbital blood sampling.[26]

In Vivo ECG Recordings and Analysis

Six-lead, surface-limb ECG measurements (I, II, III, aVF, aVL, and aVR) were recorded in mice under halothane anesthesia. Anesthetized (≈1 minute halothane) mice were positioned prone on a custom-made ECG recording platform that included a gas mask for continuous halothane administration. Ag/AgCl gel-coated ECG electrodes (Unomedical) were attached to the 2 front and left rear paws of the mouse. The electrodes were connected to a standard 6-leads ECG amplifier module (EMKA Technologies), which included high- and low-pass filters (set to 0.05 and 500 Hz, respectively) and a gain selection device (set to 1000-fold). Signals were digitized continuously at 1 kHz and recorded with the IOX data acquisition system (EMKA Technologies).

ECG Analysis

ECGAuto software (EMKA Technologies) was used to analyze the data recorded. For each mouse, qualitative analysis of cardiac rhythm and measurements of HR were performed on 30 minutes of continuous experimental recording. Quantitative analysis of interval durations and wave surfaces was carried out on a 30-second interval taken ≈1 minute after the beginning of each 30-minute recording. Within the chosen 30-second period, electrical complexes were averaged in blocks of 5 to minimize background noise and to increase the wave recognition power of the software.

ECG recordings were analyzed as described by Royer et al.[27] The corrected QT interval (QTc) was calculated according to the formula by Mitchell et al[28]: QTc=QT/(RR/100)$^{1/2}$, where the RR interval was determined automatically by the software by averaging individual RR intervals for the 30-second period.

Statistical Analysis

All results are presented as mean±SEM. Statistical comparisons between groups were performed by a 1-way ANOVA, followed by a Newman-Keuls test. For the ECG interval durations, a 2-way ANOVA was used to statistically evaluate differences between genotypes and treatments. $P<0.05$ was considered the minimal level of significance. Statistical analyses were performed with the PRISM statistical software package.

Results

Characteristics of DOCA/Salt Mice

Untreated, 1– and 2–renin-gene mice were not different from control, uninephrectomized mice that received tap water, in terms of body weight, BP, serum Na^+ level, HR, and cardiac weight. Baseline plasma renin activity was 3.9±0.4 ng · mL^{-1} · h^{-1}, BP was 121±2 mm Hg, cardiac weight was 111±1 mg, and serum K^+ was 4.9±0.1 mmol/L in untreated 1–renin gene mice (n=13). In untreated, 2–renin gene mice, plasma renin activity was 25.6±1.2 ng · mL^{-1} · h^{-1}, (n=11, $P<0.01$ vs 1–renin gene), BP was 141±3 mm Hg ($P<0.01$ vs 1–renin gene), cardiac weight was 125±4 mg ($P<0.01$ vs 1–renin gene), and serum K^+ was 4.9±0.1 mmol/L. These values were comparable to those measured in control, uninephrectomized, tap water–drinking mice (data not shown, see Wang et al[24]). The only difference was kidney size because of the uninephrectomy and the compensatory growth of the remaining kidney.

One-renin gene mice   Two-renin gene mice
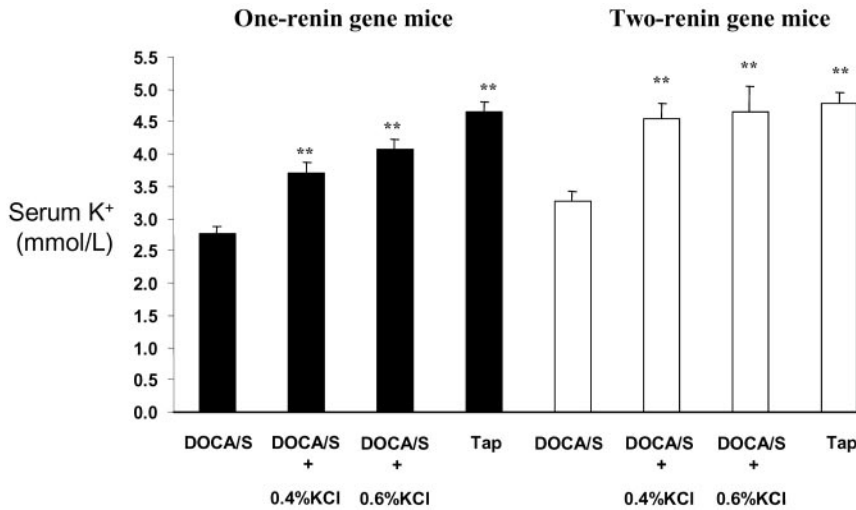
Figure 1. Potassium supplementation corrects hypokalemia in DOCA/salt 1– and 2–renin gene mice. DOCA/S indicates DOCA/salt; tap indicates control mice receiving tap water. Other abbreviations are as defined in text. Mean±SEM, n=8 to 12 mice in each group. **$P<0.01$ compared with DOCA/S group.

After 5 weeks of DOCA/salt administration, both 1– and 2–renin gene mice developed hypokalemia, with a serum K^+ value decreasing from 4.7 ± 0.1 (control mice) to 2.8 ± 0.1 mmol/L with DOCA/salt ($P<0.01$) in 1–renin gene mice and from 4.8 ± 0.2 (control mice) to 3.3 ± 0.1 mmol/L ($P<0.01$) in 2–renin gene, DOCA/salt mice (Figure 1). In parallel, metabolic alkalosis developed because blood pH rose from 7.27 ± 0.02 to 7.44 ± 0.02 and from 7.23 ± 0.02 to 7.39 ± 0.03 in 1– ($P<0.01$) and 2– ($P<0.01$) renin gene mice, respectively (Table 1). In 1–renin gene, DOCA/salt-treated mice, BP did not increase when compared with the control group (111 ± 2 vs 112 ± 3 mm Hg, $P=NS$; Figure 2), whereas in 2–renin gene mice, BP was higher at baseline and increased significantly from 142 ± 3 to 167 ± 3 mm Hg, ($P<0.01$) with the administration of DOCA/salt (Figure 2). As shown in Figure 3 as well as in Table 1, administration of DOCA/salt induced cardiac and renal hypertrophy, as judged by the marked increases in cardiac and kidney weights (Table 1) and cardiac and kidney weight indices.
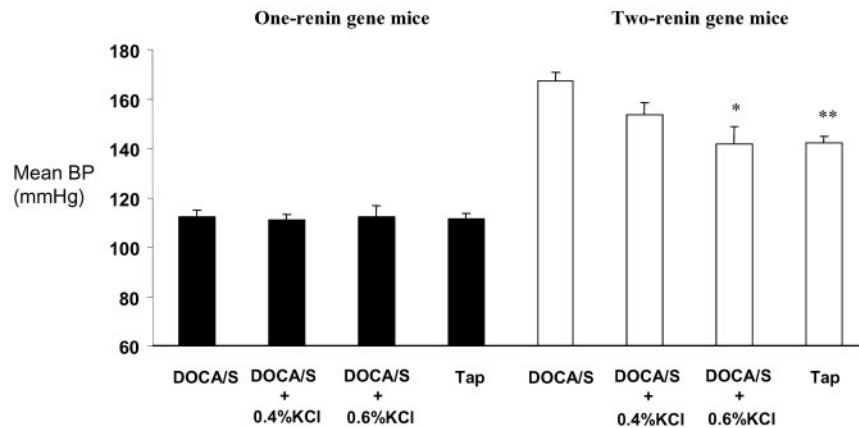
Effect of Potassium Supplementation

With potassium supplementation (0.4% and 0.6% KCl in the drinking water), urinary K^+ excretion increased and the urinary Na^+ to K^+ ratio decreased dose-dependently ($P<0.01$, Table 1). Serum K^+ increased significantly and dose-dependently and reached normal values both in 1– and 2–renin gene mice when compared with control mice and mice that received DOCA/salt treatment only ($P<0.01$; Figure 1). Similarly, blood pH was normalized with administration of the potassium supplement ($P<0.01$, Table 1). In 2–renin gene mice, potassium supplementation induced a significant decrease in mean BP, from 167 ± 3 mm Hg (DOCA/salt) to 157 ± 4 mm Hg on a 0.4% KCl diet and to 142 ± 7 mm Hg on a 0.6% KCl diet ($P<0.05$, Figure 2). In 1–renin gene mice, potassium supplementation completely reversed DOCA/salt-induced cardiac hypertrophy with no change in BP (Figures 2 and 3). Furthermore, the decrease in BP and the correction of kalemia in 2–renin gene mice was also associated with a significant decrease in cardiac hyper-

TABLE 1.   Physiological Parameters in Control and DOCA/Salt Mice Without and With Potassium Supplementation

	1-Renin Gene Mice				2-Renin Gene Mice			
Parameters	Tap Water	DOCA-Salt	DOCA-Salt+0.4% KCl	DOCA-Salt+0.6% KCl	Tap Water	DOCA-Salt	DOCA-Salt+0.4% KCl	DOCA-Salt+0.6% KCl
Number of mice	11	12	13	10	11	13	11	9
Body wt (g)	27±0.3	27±0.4	28±0.4	27±0.3	29±0.5	29±0.6	29±0.4	25±0.8
Heart rate (beats/min)	587±19	572±12	559±25	570±17	572±17	597±16	583±13	586±21
Serum Na^+ (mmol/L)	148±0.7	151±1.0	152±1.3	151±0.8	148±0.9	152±0.9	148±0.9	151±1.8
Blood pH	7.27±0.02†	7.44±0.02	7.33±0.02†	7.33±0.02†	7.23±0.02†	7.39±0.03	7.29±0.02†	7.23±0.03†
Cardiac weight (mg)	107±2†	132±4	119±3†	109±2†	126±3†‡	158±4‡	146±3†‡	122±5†‡
Kidney weight (mg)	203±5†	339±9	300±8†	276±6†	210±6†	336±10	323±11†	262±15†
Urinary Na concentration (mmol/L)	86±6*	139±3	147±6	157±6	51±7*	136±5	140±6	128±10
Urinary K concentration (mmol/L)	75±5*	37±3	77±5†	92±2†	75±5†	30±3	58±3*	74±7†
Urinary Na/K	1.18±0.09†	4.17±0.33	1.96±0.09†	1.73±0.08†	0.68±0.08†‡	4.87±0.39	2.44±0.11†	1.76±0.10†

Value are mean±SEM.

*$P<0.05$; †$P<0.01$, vs. DOCA-salt group; ‡$P<0.01$, vs. one-renin gene group.

Figure 2. Potassium supplementation lowers BP in 2–renin gene, DOCA/salt mice but not in 1–renin gene, DOCA/salt normotensive mice. DOCA-S indicates DOCA-salt. Other abbreviations are as defined in text. Mean±SEM, n=10 to 18 mice in each group. *$P<0.05$ and **$P<0.01$ compared with DOCA/S group.

trophy (Figure 3). Nevertheless, even with the 0.6% KCl supplement, cardiac weight index remained greater than that in control mice. In contrast to cardiac weight, kidney weight decreased but was not normalized by potassium supplementation, and this finding was independent of the renin gene background (Figure 3). As shown in Table 2, significant correlations were found between serum K^+ and plasma pH and cardiac and renal indices. Of note, BP was correlated with cardiac and renal indices in 2–renin-gene mice only.

Effect of Potassium Supplementation on Cardiac Contractility

Cardiac function was measured in the conscious state in all groups (Table 3). LVEDP was elevated in both DOCA/salt-treated 1– and 2–renin gene mice but was significantly higher in 2–renin gene mice ($P<0.01$). Accordingly, LV$dP/dt_{min}$ (mm Hg/s) was significantly reduced and Weiss ô, the relaxation time constant, was prolonged in DOCA/salt-treated mice compared with controls ($P<0.01$, Table 3). In addition, LV$dP/dt_{max}$ was significantly decreased in 1–renin gene, DOCA/salt mice compared with control mice, whereas no difference was found in 2–renin gene mice (Table 3). HR was comparable in all groups.

In 1–renin gene mice, all functional cardiac parameters were restored by potassium supplementation. Importantly, BP, left ventricular systolic pressure, nor HR was modified by the potassium diet (Table 3). In contrast, in 2–renin gene, DOCA/salt mice, potassium supplementation significantly lowered BP and left ventricular systolic pressure, but LV$dP/dt_{max}$ was unaffected ($P>0.05$, Table 3). However, LV$dP/dt_{min}$, LVEDP, and τ
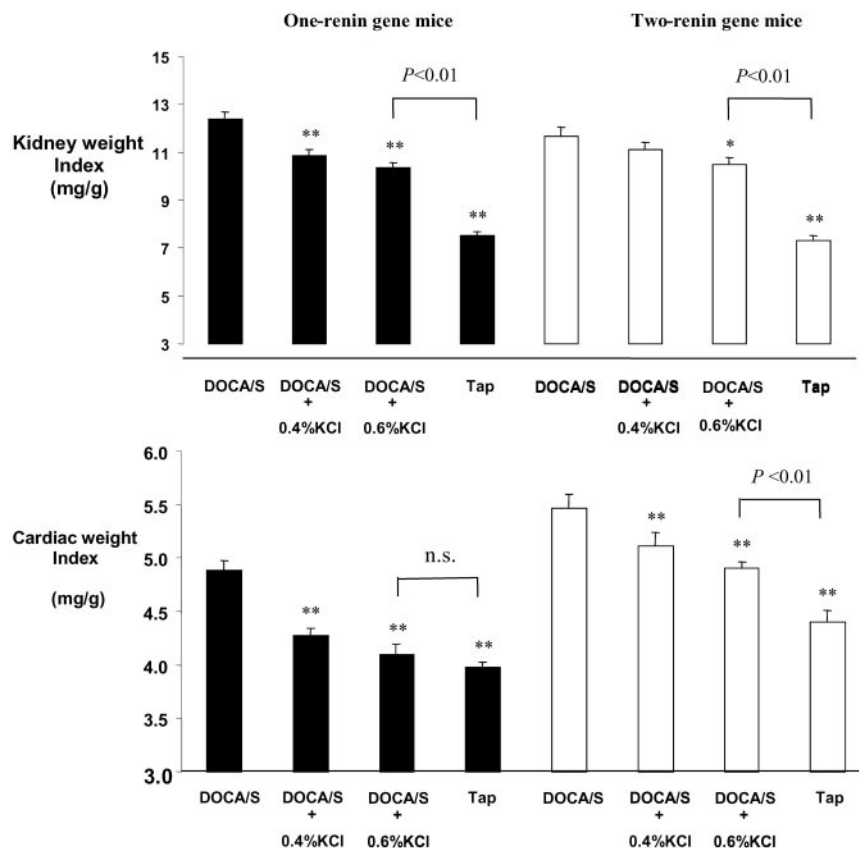
Figure 3. Upper panel, Potassium supplementation reduces kidney weight index in 1–renin gene, DOCA/salt mice but not in 2–renin gene mice. DOCA/S indicates DOCA/salt. Other abbreviations are as defined in text. Lower panel, Potassium supplementation dose-dependently reduces cardiac weight index in 1– and 2–renin gene DOCA/salt-treated mice. DOCA/S indicates DOCA/salt. Other abbreviations are as defined in text. Mean±SEM, n=10 to 18 mice in each group. **$P<0.01$ compared with DOCA/S group.

TABLE 2. Correlations Between Parameters of Mineralocorticoid Excess and the Development of Cardiac and Renal Hypertrophy

Parameters	1-Renin Gene Mice	2-Renin Gene Mice
Serum K$^+$ and CWI	y=−0.38x+5.75, r=0.71, n=44, $P<0.001$	y=−0.35x+6.48, r=0.58, n=41, $P<0.001$
Serum K$^+$ and KWI	y=−1.9x+17.6, r=0.76, n=44, $P<0.001$	y=−1.1x+14.8, r=0.49, n=41, $P<0.001$
Blood pH and CWI	y=6.1x−40, r=0.77, n=23, $P<0.0001$	y=3.1x−17.8, r=0.66, n=24, $P=0.0002$
Blood pH and KWI	y=15.8x−106, r=0.56, n=23, $P=0.005$	y=13.6x−89, r=0.54, n=24, $P=0.0037$
Mean BP and CWI	y=0.015x+2.72, r=0.26, n=47, $P=0.12$	y=0.025x+1.17, r=0.69, n=34, $P<0.001$
Mean BP and KWI	y=0.004x+11.05, r=0.02, n=47, $P=0.89$	y=0.082x−2.49, r=0.56, n=34, $P=0.0006$

were also normalized with potassium supplementation in DOCA/salt, 2–renin gene mice.

ECG Changes in DOCA/Salt Mice

Under 1% halothane anesthesia, 2–renin gene mice had a significantly higher HR and hence, shorter RR, P, PR, QT, and QRS interval durations than did 1–renin gene mice. Comparisons between DOCA/salt and control mice showed that PR, QT, and QTc intervals were significantly prolonged in treated mice relative to tap water–treated mice (Table 4). This effect was observed in both 1– and 2–renin gene mice. Potassium supplementation completely prevented PR, QT, and QTc prolongation in both mouse strains.

In DOCA/salt mice, a long QT-interval duration was associated with a higher incidence of arrhythmogenic events (Figure 4). In these mice, ECG measurements demonstrated the presence of 2 major forms of triggered activity: irregular occurrence of premature atrioventricular depolarizations (Figure 4B) and premature ventricular depolarizations dissociated from the supraventricular rhythm every third beat (Figure 4D). Triggered activity was observed in 6 of 8 mice in the 1–renin gene group (ie, 75% vs 0% for mice offered tap water, $P<0.05$) and in 5 of 8 animals in the 2–renin gene group (ie, 60% vs 0% for mice offered tap water, $P<0.05$). The 2 forms of triggered activity were not observed concomitantly. Potassium supplementation completely prevented the development of arrhythmias in both 1– and 2–renin gene mice (Figure 4C and 4F).

Discussion

Hypokalemia is known to have deleterious effects on the cardiovascular and renal systems.[1] The presence of hypokalemia in hypermineralocorticoid states has been shown repeatedly to affect both cardiac structure, leading to myocardial necrosis or fibrosis, and myocardial function, resulting in impaired cardiac contractility and ventricular arrhythmias.[12,13,23–25] However, whether hypokalemia contributes to the development of cardiac hypertrophy independent of BP has not clearly been demonstrated. The results of the present experiments show that potassium supplementation prevents the development of cardiac hypertrophy independent of BP in normotensive, 1–renin gene mice receiving excess mineralocorticoid and salt. In hypertensive, 2–renin gene mice, potassium supplementation also prevents the development of cardiac hypertrophy. In this case, however, because potassium supplementation results in significantly lower BP in DOCA/salt-treated animals, the beneficial effects of potassium cannot be separated from the decrease in BP. Our data also show that potassium supplementation has a greater effect on cardiac than on renal hypertrophy. Moreover, potassium supplementation corrects metabolic alkalosis in this model, which could also affect the development of cardiac hypertrophy. Finally, correction of hypokalemia improves cardiac function and decreases the incidence of arrhythmias.

The main observation of the present experiment comes from the results obtained in 1–renin gene mice. We have previously shown that these mice develop hypokalemia,

TABLE 3. Left Ventricular Hemodynamic Changes After Potassium Supplement

Parameters	1-Renin Gene Mice				2-Renin Gene Mice		
	Tap Water	DOCA-Salt	DOCA-Salt+0.4% KCl	DOCA-Salt+0.6% KCl	Tap Water	DOCA-Salt	DOCA-Salt+0.4% KCl
Number of mice	9	9	8	6	6	6	5
Body weight (g)	27±0.5	28±0.6	27±0.7	28±0.3	29±0.9	28±0.8	28±0.5
LVSP (mm Hg)	124±2	118±3	123±3	121±5	147±3†	184±3	165±7†
Heart rate (beats/min)	596±12	549±14	556±10	553±18	560±25	540±18	571±31
LVEDP (mm Hg)	1.2±0.8†	4.0±0.8	1.4±0.5†	1.4±0.7†	1.9±0.9†	13.3±1.6	2.0±0.6†
LVdP/dt$_{max}$ (mm Hg/s)	11137±319†	8169±472	10039±426†	10447±326†	15591±259	15417±576	15808±591
LVdP/dt$_{min}$ (mm Hg/s)	−8735±258†	−6015±309	−8109±26†	−8186±165†	−10372±376*	−8322±691	−11129±644*
Weiss tau, t (ms)	3.8±0.2†	7.7±0.7	4.3±0.4†	4.1±0.3†	5.6±0.4†	10.9±0.8	4.5±0.4†

Value are mean±SEM. LVSP: Left ventricular systolic pressure; LVEDP: Left ventricular end diastolic pressure; LVdP/dt$_{max}$ and LVdP/dt$_{min}$: Maximal rate of pressure rise and fall; Weiss tau: time consistent of isovolumic relaxation.

*$P<0.05$; †$P<0.01$, vs. DOCA-salt group.

TABLE 4.   Effect of Potassium Supplementation on ECG Recordings in 1- and 2-Renin Gene DOCA/Salt Mice

Parameter	1-Renin Gene Mice			2-Renin Gene Mice		
	Tap Water	DOCA-Salt	DOCA-Salt+0.6% KCl	Tap Water	DOCA-Salt	DOCA-Salt+0.6% KCl
H.R. (bpm)	403±10	397±12	400±5‡	462±11	477±15	466±12
RR (ms)	149±4	152±5	150±2‡	130±3	126±4	129±3
P_{dur} (ms)	14.2±0.3	13.7±0.7	14.3±0.4‡	12.6±0.4	13.2±0.9	12.4±0.2
PR (ms)	45.8±1.7	59.9±3.3*	48.2±0.9‡	43.5±0.8	55.1±1.4*	44.3±2.0
QRS (ms)	16.1±0.7	16.7±0.9	14.2±0.3†‡	15.0±0.7	15.6±0.4	13.5±0.5†
QT (ms)	60.9±1.0	71.4±1.7*	62.8±0.7‡	53.0±0.9	61.2±1.3*	54.1±0.9
QT_c (ms)	49.8±0.5	58.0±1.0*	51.3±0.8‡	46.5±0.6	54.5±1.0*	47.6±0.7

Analysis by 2-way ANOVA; values are mean±SEM.
*$P<0.05$ vs. Tap water and DOCA-salt 0.6% KCl (of same genetic background).
†$P<0.05$ vs. Tap water and DOCA-salt (of same genetic background).
‡$P<0.05$, Two-renin gene mice vs one-renin gene mice.

metabolic alkalosis, cardiac and renal hypertrophy, and cardiac dysfunction in the absence of hypertension when receiving DOCA and salt.[24,25] The mechanisms whereby these animals develop cardiac and renal hypertrophy have not been clearly established. Cardiac hypertrophy is associated with an upregulation of angiotensin II AT_1 receptors, and the hypertrophy is partially reversible during AT_1 receptor blockade with losartan.[24] Even though plasma K^+ levels were not changed by losartan, one cannot exclude the possibility that the potassium-retaining properties of AT_1 receptor antagonists could have played a role in the regression of hypertrophy. Small, transient changes in BP in response to DOCA/salt were excluded by measuring BP continuously for several days by telemetry (data not shown). The present data demonstrate that hypokalemia and metabolic alkalosis likely play a role in the pathophysiology of cardiac hypertrophy and dysfunction in this model. As mentioned earlier, correction of hypokalemia is more effective in the heart than the kidney,

suggesting that other mechanisms might be involved in the development of renal hypertrophy during mineralocorticoid exposure.

As described previously, 2–renin gene mice have a 10-fold higher plasma renin activity and a 100-fold higher plasma renin concentration than 1–renin gene animals.[24] Hence, these mice have high BP at baseline ($\approx$140 mm Hg vs 120 mm Hg in 1–renin gene mice), are salt-sensitive, and develop hypokalemia and metabolic alkalosis associated with cardiac and renal hypertrophy on administration of DOCA/salt. In contrast to 1–renin gene mice, the cardiac and renal hypertrophic response in 2–renin gene animals appears to depend on BP.[24] In the present study, these initial observations were reproduced, indicating that BP indeed plays an important role in the pathology of this model. Interestingly, a significantly lower BP was measured in potassium-supplemented, DOCA/salt, 2–renin gene mice. Along these lines, potassium supplementation was reported earlier to be beneficial in stroke-
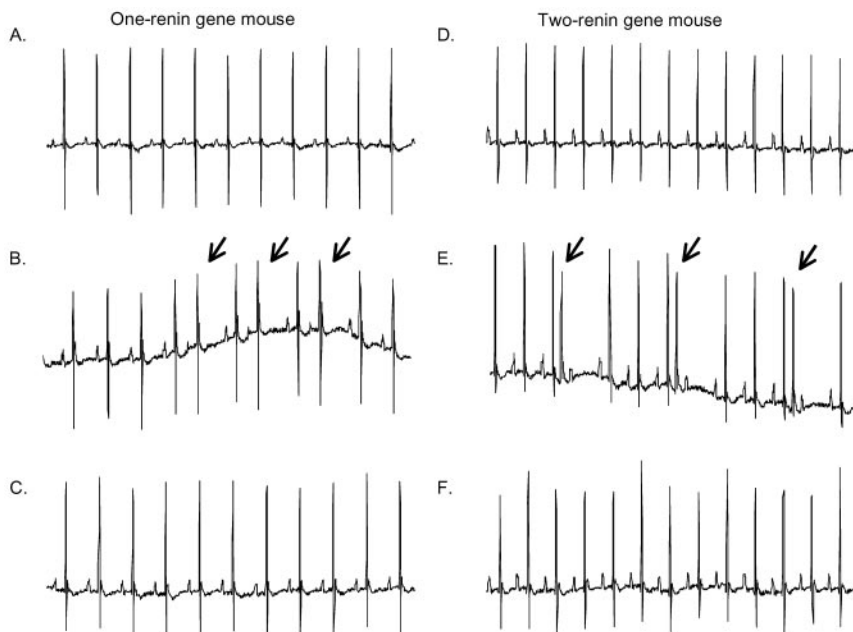
Figure 4. ECG recordings of 1– and 2–renin gene mice under control conditions (A and D), after administration of DOCA/salt (B and E), and after administration of DOCA/salt and a potassium supplement (C and F). Arrows indicate the arrhythmias observed when hypokalemia was present. Abbreviations are as defined in text.

prone spontaneously hypertensive and in Dahl salt-sensitive rats via reduction of BP.[16,17,29] Nonetheless, in the 2–renin gene model, correction of plasma K^+ values and metabolic alkalosis also appears to contribute to the prevention of left ventricular and renal hypertrophy, as indicated by the significant correlations obtained between serum K^+ levels or blood pH and cardiac and renal weight indices (Table 2). One limitation of the study is the rather short duration of potassium supplementation. Thus, we cannot exclude the possibility that prolonged potassium supplementation would have resulted in greater benefit. With time, the beneficial effect of potassium may also be overcome by the effect of high salt intake and mineralocorticoid excess.

Except for the decrease in BP observed in 2–renin gene mice, our experiments do not provide any clear explanation as to why potassium supplementation prevents the development of cardiac hypertrophy. However, several mechanisms could be involved. Thus, in vitro studies of neonatal cardiac myocyte cultures have shown that moderate to severe reductions in K^+ concentrations (from 5.3 to 1.0 mmol/L) in media produce molecular phenotypic alterations consistent with cardiac hypertrophy, such as upregulation of atrial natriuretic peptide and skeletal actin (SKA) upregulation.[30] This observation is concordant with our previous finding that cardiac α-SKA is upregulated in DOCA/salt-treated mice.[24] Potassium has also been shown in vitro to inhibit the proliferation of vascular smooth muscle cells and has been reported to improve endothelial function, 2 effects that may also contribute to the benefits of potassium supplementation.[31,32] Potassium supplementation may correct a DOCA/salt-induced imbalance of intracellular electrolytes, including K^+, Na^+, and Ca^{2+}, which are important in mediating cardiac hypertrophy and contractility.[33] Recent studies have suggested that the Na^+/H^+ exchange (NHE) system plays an important role in cardiac remodeling after myocardial infarction and may contribute to the development of cardiac hypertrophy induced by several hormonal pathways, including angiotensin II, endothelin-1, and α-adrenergic stimulation.[34–36] More recently, aldosterone has been reported to stimulate NHE-1 activity in vascular smooth muscle cells, thereby elevating intracellular pH by a nongenomic, protein kinase C–dependent mechanism.[37] Whether changes in serum K^+ or extracellular pH play a role in NHE activity and thereby influence the cardiac hypertrophic response observed in the DOCA/salt model has, to our knowledge, not been demonstrated so far but could be postulated. Finally, potassium may also have a direct effect on the cardiac renin-angiotensin system and hence, reduce cardiac hypertrophy. In this respect, we have reported previously that an angiotensin II receptor antagonist decreases cardiac hypertrophy in both 1– and 2–renin gene mice receiving DOCA/salt, even though the circulating activity of the renin-angiotensin system was low in 1–renin gene mice.

The present study also demonstrates that DOCA/salt treatment induces modifications in cardiac electrical activity in 1– and 2–renin gene mice (ie, increased arrhythmogenic activity and prolongation of PR and QTc intervals). Potassium supplementation completely prevents these changes, suggesting that hypokalemia is sufficient and necessary to induce these electrical modifications in DOCA/salt mice. At this stage, it could be speculated that hypokalemia would impair cardiomyocyte repolarization, leading to a prolongation of action potential duration and delayed afterdepolarizations.[38] A larger-amplitude afterdepolarization would increase the likelihood of reaching threshold for triggering a potentially arrhythmogenic action potential.[39] An increased Na^+/Ca^{2+} exchanger activity during the repolarization phase could also prolong action potential duration and provide more transient inward current for any given sarcoplasmic reticulum calcium release. These hypotheses require further investigation.

Perspectives
Taken together, the results of the present study provide evidence that hypokalemia and metabolic alkalosis contribute to the development of cardiac and renal hypertrophy and cardiac dysfunction under conditions of mineralocorticoid and salt excess. Correction of hypokalemia and metabolic alkalosis by dietary potassium supplementation prevents DOCA/salt-induced cardiac hypertrophy and left ventricular dysfunction. This observation may be very clinically relevant. Indeed, hypokalemia and metabolic alkalosis are the hallmarks of excess mineralocorticoids, but hypokalemia is also a common complication of diuretic use. Epidemiological studies have suggested that patients who develop hypokalemia do not benefit entirely from administration of a diuretic.[40] Moreover, K^+-sparing drugs, like blockers of the renin-angiotensin system and antialdosterone agents, have been shown to be very effective in reducing left ventricular hypertrophy.[41–43] Thus, greater clinical attention to K^+ balance and in particular to hypokalemia may be an effective approach to reduce cardiovascular events by preventing left ventricular hypertrophy, a known independent risk factor for cardiovascular complications.

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