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# Effect of felodipine on myocardial and renal injury induced by aldosterone-high salt hypertension in uninephrectomized rats

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## Abstract

It has been recently shown that calcium channel blockers might have a protective effect on cardiac fibrogenesis induced by aldosterone. The objective of this study was to evaluate the protective effect of felodipine, a dihydropyridine calcium channel blocker, against heart and kidney damage caused by aldosterone-high sodium intake in uninephrectomized rats. Wistar rats were divided into three groups: CNEP (uninephrectomized + 1% NaCl in the drinking water, N = 9); ALDO (same as CNEP group plus continuous infusion of 0.75 µg/h aldosterone, N = 12); ALDOF (same as ALDO group plus 30 mg·kg<sup>-1</sup>·day<sup>-1</sup> felodipine in the drinking water, N = 10). All results were compared with those of age-matched, untreated rats (CTL group, N = 10). After 6 weeks, tail cuff blood pressure was recorded and the rats were killed for histological analysis. Blood pressure (mmHg) was significantly elevated ( $P < 0.05$ ) in ALDO ( $180 \pm 20$ ) and ALDOF ( $168 \pm 13$ ) compared to CTL ( $123 \pm 12$ ) and CNEP ( $134 \pm 13$ ). Heart damage (lesion scores - median and interquartile range) was 7.0 (5.5-8.0) in ALDO and was fully prevented in ALDOF (1.5; 1.0-2.0). Also, left ventricular collagen volume fraction (%) in ALDOF ( $2.9 \pm 0.5$ ) was similar to CTL ( $2.9 \pm 0.5$ ) and CNEP ( $3.4 \pm 0.4$ ) and decreased compared to ALDO ( $5.1 \pm 1.6$ ). Felodipine partially prevented kidney injury since the damage score for ALDOF (2.0; 2.0-3.0) was significantly decreased compared to ALDO (7.5; 4.0-10.5), although higher than CTL (null score). Felodipine has a protective effect on the myocardium and kidney as evidenced by decreased perivascular inflammation, myocardial necrosis and fibrosis.

Key words: Aldosterone; Dihydropyridine; Fibrinoid necrosis; Vascular remodeling; Hypertension; Myocardial remodeling

## Introduction

Aldosterone-high salt intake produces a variety of deleterious actions on the cardiovascular system of uninephrectomized rats. Previous studies have shown inflammation and oxidative stress within the arteriolar wall of the heart and kidney, and inflammatory tissue remodeling (1,2). The histological features of malignant or accelerated hypertension have also been described in this model (1,3).

The mechanisms by which aldosterone induces cardiac tissue damage are not fully understood and seem to involve genomic and non-genomic effects (4). Genomic action is the classical mechanism by which aldosterone binds to mineralocorticoid receptors (MR) and promotes protein synthesis. Non-genomic effects of aldosterone occur within minutes, inducing vasoconstriction (5). Also, vascular smooth muscle

cells from spontaneously hypertensive rats show an increased aldosterone-induced non-genomic signaling mediated by c-Src. This signaling appears to be important in the profibrotic and proinflammatory actions of aldosterone in this genetic model of hypertension (6).

Recently, it has been demonstrated that cardiomyocytes have putative aldosterone-regulated MR genes (7). Accordingly, the first event leading to cardiovascular inflammation and remodeling may be associated with inappropriate MR activation. In addition, Dietz et al. (8) have recently reported that dihydropyridine calcium channel blockers (CCBs) have MR antagonist activity and that a number of widely used dihydropyridine CCBs, including felodipine, compete for aldosterone binding to MR and have the effect of MR antagonists on cardiomyocytes. L-

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type calcium channel antagonists are effective in decreasing blood pressure and have been shown to be renoprotective. However, whether these protective effects are due to blood pressure reduction or to a specific pharmacologic blockade is still a matter of debate.

We, therefore, hypothesized that felodipine may protect the heart and kidney from damage by decreasing the perivascular inflammation induced by aldosterone-high salt intake in uninephrectomized rats. The objective of this study was to determine whether felodipine, a dihydropyridine calcium channel blocker, is effective in preventing heart and kidney damage in uninephrectomized rats subjected to aldosterone-high sodium intake.

## Material and Methods

### Animals

Twelve-week-old male Wistar rats ( $300 \pm 20$  g) were obtained from the Central Animal Facility of the Botucatu Medical School, São Paulo State University, SP, Brazil. The animals were housed under standard environmental conditions and maintained on a commercial rat laboratory diet (Purina Labina, Brazil) and tap water *ad libitum*. The experimental protocol was approved by the Animal Care and Use Committee of our Institution and conformed to the NIH Guidelines on the Care and Use of Laboratory Animals (Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, 1996).

### Experimental protocol

Laparotomy and unilateral nephrectomy were performed under sodium thiopental anesthesia (50 mg/kg, intraperitoneally). Immediately after surgery and recovery from anesthesia, the rats were randomly divided into three groups as follows: CNEP (N = 9) - rats maintained on high NaCl (1%) in the drinking water. ALDO (N = 12) - rats maintained on high NaCl as the CNEP group and continuously infused with aldosterone (Sigma, USA; 0.75 µg/h), via minipumps implanted subcutaneously (Alzet minipumps mod. 2002, Alza Corp., USA). ALDOF (N = 10) - same as the ALDO group plus felodipine (30 mg·kg<sup>-1</sup>·day<sup>-1</sup>) in the drinking water. All results were compared to an additional control group of animals that were not unilaterally nephrectomized and that did not receive high NaCl, aldosterone or felodipine (N = 10).

Tail cuff blood pressure and body weight were recorded before and 6 weeks after surgery. At the end of the 6-week period, all rats were anesthetized with sodium thiopental (50 mg/kg, *ip*) and killed by thoracotomy and blood samples were taken from the heart for electrolyte and creatinine measurements. The heart and the remaining kidney were excised and weighed. Left and right ventricles were weighed separately.

### Histopathological analysis

The morphometric analysis of the myocardium was performed as described previously (9). Transverse sections of the

left (LV) and right (RV) ventricles and a sample of the kidney were fixed in 10% buffered formalin and embedded in paraffin. Five-micron sections of myocardium and kidney were stained with hematoxylin-eosin. Also, ventricular sections were stained with the collagen-specific stain picrosirius red (sirius red F3BA in aqueous saturated picric acid).

*Interstitial myocardial collagen volume fraction (CVF)*. The CVF of the entire section was determined using a Leica microscope (objective lens 40X) attached to a video camera and connected to a personal computer equipped with image analyzer software (Image-Pro Plus 3.0, Media Cybernetics, USA). The components of cardiac tissue were identified according to their color (i.e., red for collagen fibers, yellow for myocytes, and white for interstitial space). The digitized profiles were transferred to the computer and CVF was calculated as the sum of all connective tissue areas divided by the sum of the connective tissue and myocyte areas. On the average, 35 microscopic fields were analyzed. Perivascular collagen was excluded from this analysis.

### Scores of myocardial damage

Two pathologists blind as to the source of the hematoxylin-eosin-stained tissue section, independently examined the slides and rated the damage using the criteria shown in Table 1. This approach emphasized cellular necrosis and inflammatory phenomena. Arteriole remodeling was analyzed by calculating the relative wall thickness (RWT) and relative perivascular fibrosis (RPF) as follows:  $RWT = (WA - LA) / LA$ , where WA is the area of vascular wall, and LA is the lumen area, and  $RPF = (FA - WA) / WA$ , where FA is the perivascular fibrosis area.

All analyses were performed using two separate sets of vessels as follows: vessels with luminal diameters less than 50 µm and vessels with lumen diameters between 50-100 µm. At least four vessels of each set were measured in all sections and only vessels with a circular cross-section were analyzed.

### Statistical analysis

Differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by pairwise multiple comparison (Bonferroni *t*-test). ANOVA on ranks followed by the Dunn test was used to compare scores among groups. Spearman's correlation was used to compare LV and RV damage scores and to analyze the association between LV or kidney score damage with systolic blood pressure. Differences were considered to be statistically significant when  $P < 0.05/k$ , where k is the number of statistical comparisons (Bonferroni correction).

## Results

Morphometric data and blood pressure results are presented in Table 2. Systolic blood pressure increased in both the ALDO and ALDOF groups indicating a non-antihypertensive effect of felodipine. Accordingly, both hypertensive groups presented increased LV and RV weight. The increase in

**Table 1.** Criteria for histological damage score.

	Score
Myocardial scores	
1. Myocyte lesions	
1.1. Necrosis of few cells	+1
1.2. Microinfarction	+3
1.3. Infarction (area >0.05 cm <sup>2</sup> )	+5
Note: Frequent lesions (>3 foci)	+1
2. Benign vascular damage	
2.1. Media hypertrophy	+1
2.2. Wall hyalinization (subendothelial)	+1
2.3. Arteriolar fibroelastosis	+1
Note: Frequent damage (>3 vessels)	+1
3. Severe vascular damage	
3.1. Transmural fibrinoid necrosis	+4
3.2. Myointimal proliferation	+4
Note: Frequent damage (>3 vessels)	+1
4. Perivascular lesion	
4.1. Limited inflammatory infiltrate	+1
4.2. Inflammatory infiltrate with perivascular myocyte damage	+3
Note: Frequent damage (>3 vessels)	+1
Kidney scores	
1. Benign vascular damage	
1.1. Media layer hypertrophy	+1
1.2. Wall hyalinization (subendothelial)	+1
1.3. Arteriolar fibroelastosis	+1
Note: Frequent damage (>3 vessels)	+1
2. Severe arterial damage	
2.1. Glomerulonecrosis	+4
2.2. Transmural fibrinoid necrosis	+4
2.3. Myointimal proliferation	+4
Note: Frequent damage (>3 vessels)	+1
3. Other lesions	
3.1. Acute tubular necrosis	+1

**Table 2.** Morphometric and blood pressure data.

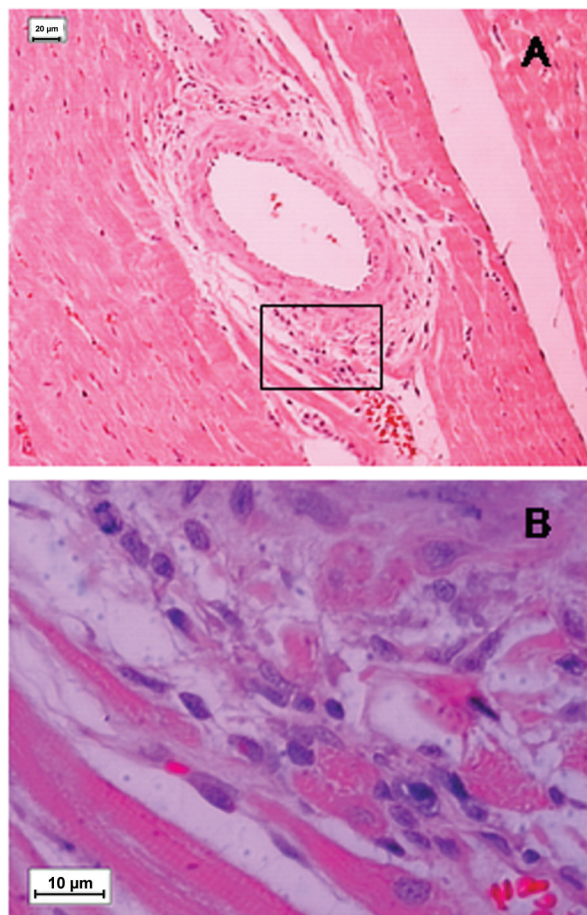
Group	BW (g)	SBP (mmHg)	LVW/BW (mg/g)	RVW/BW (mg/g)	CVF <sub>LV</sub> (%)	CVF <sub>RV</sub> (%)
CTL	389 ± 21 <sup>a</sup>	123 ± 12 <sup>a</sup>	1.82 ± 0.12 <sup>a</sup>	0.61 ± 0.05 <sup>a</sup>	2.9 ± 0.67 <sup>a</sup>	4.0 ± 0.34 <sup>a</sup>
CNEP	414 ± 34 <sup>a</sup>	134 ± 13 <sup>a</sup>	1.85 ± 0.08 <sup>a</sup>	0.60 ± 0.09 <sup>a</sup>	3.4 ± 0.44 <sup>a</sup>	3.6 ± 0.94 <sup>a</sup>
ALDO	399 ± 32 <sup>a</sup>	180 ± 20 <sup>b</sup>	2.51 ± 0.29 <sup>b</sup>	0.69 ± 0.08 <sup>b</sup>	5.1 ± 1.61 <sup>b</sup>	5.6 ± 1.42 <sup>b</sup>
ALDOF	416 ± 29 <sup>a</sup>	168 ± 13 <sup>b</sup>	2.22 ± 0.13 <sup>c</sup>	0.69 ± 0.06 <sup>b</sup>	2.9 ± 0.48 <sup>a</sup>	3.6 ± 0.44 <sup>a</sup>

Data are reported as means ± SD. CTL = control; CNEP = uninephrectomy plus 1% salt in the drinking water; ALDO = same as CNEP plus 6-week subcutaneously infused aldosterone; ALDOF = same as ALDO plus 6-week oral felodipine; BW = body weight; SBP = systolic blood pressure; LVW = left ventricle weight; RVW = right ventricle weight; CVF<sub>LV</sub> = collagen volume fraction of left ventricle; CVF<sub>RV</sub> = collagen volume fraction of right ventricle. Means with different superscript letters are significantly different (P < 0.05) in all pairwise multiple comparison procedures (ANOVA and Bonferroni *t*-test).

**Table 3.** Plasma sodium, potassium and creatinine levels of the groups studied.

Group	Na <sup>+</sup> (mEq/dL)	K <sup>+</sup> (mEq/dL)	Creatinine (mg/dL)
CTL	143 ± 2.04 <sup>a</sup>	4.80 ± 0.58 <sup>a</sup>	0.65 ± 0.16 <sup>a</sup>
CNEP	154 ± 14.53 <sup>a/b</sup>	5.30 ± 0.42 <sup>a</sup>	1.02 ± 0.08 <sup>b</sup>
ALDO	164 ± 13.20 <sup>b</sup>	3.67 ± 0.11 <sup>b</sup>	1.14 ± 0.33 <sup>b</sup>
ALDOF	153 ± 10.84 <sup>a/b</sup>	3.85 ± 0.53 <sup>b</sup>	0.71 ± 0.04 <sup>a</sup>

Data are reported as means ± SD. Group abbreviations are defined in the legend to Table 2. Means with different superscript letters are significantly different ( $P < 0.05$ ) in all pairwise multiple comparison procedures (ANOVA and Bonferroni *t*-test).



**Figure 1.** Photomicrograph of rat myocardium stained with hematoxylin-eosin showing periarteritis in the ALDO group (uninephrectomized + 1% NaCl in the drinking water plus continuous infusion of 0.75 µg/h aldosterone; Panel A). The square in Panel A is presented at higher magnification in Panel B to illustrate the lymphomononuclear infiltrate and perivascular myocyte necrosis. Bar scale = 20 (Panel A) and 10 µm (Panel B).

CVF that occurred in both ventricles of the ALDO group was prevented in the felodipine-treated group.

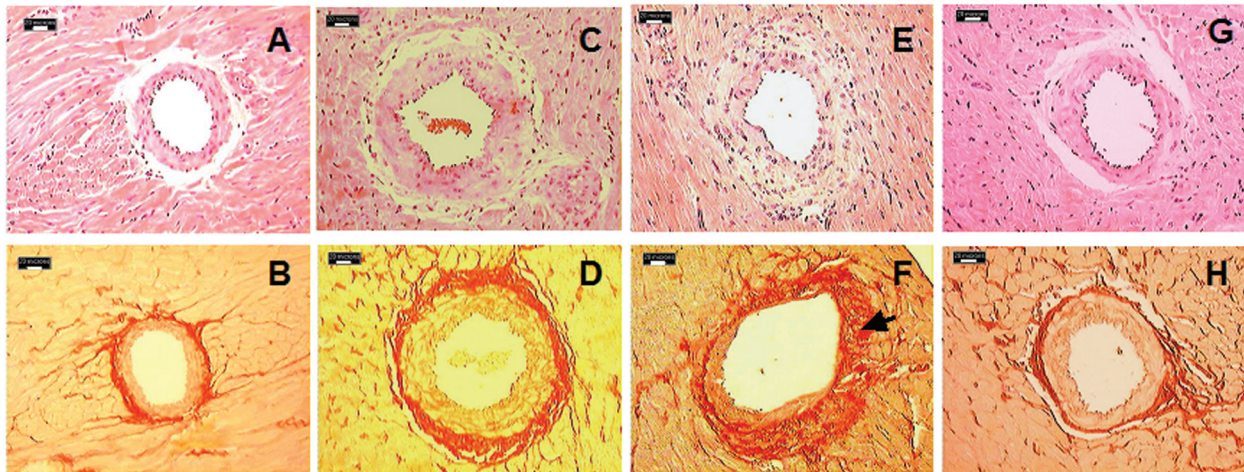
Serum electrolytes and creatinine data are shown in Table 3. Aldosterone infusion increased serum sodium level and decreased potassium level, effects that were not prevented by felodipine treatment. Conversely, felodipine prevented the increase in serum creatinine observed in the CNEP and ALDO groups.

Histological alterations in the heart were as follows: 1) perivascular inflammatory infiltrate with lymphomononuclear cells, including mastocytes, which was associated with edema and perivascular myocyte necrosis in ALDO rats (Figure 1, panels A and B). This abnormality was rarely seen in the ALDOF group; 2) media layer hypertrophy in coronary arteries was present in all groups except the control group (Figure 2); 3) interstitial lymphomononuclear inflammatory infiltrate, including mastocytes, in areas of discrete myocyte necrosis. These abnormalities were more frequently present in the ALDO group than in the ALDOF group; 4) transmural fibrinoid necrosis was observed only in the ALDO group. In this case, media layer hypertrophy was replaced by arteriolar wall fibrosis (Figure 2, panels E and F); 5) areas of myocardial infarction with extensive myocyte necrosis, interstitial hemorrhage and polymorphonuclear cells. These lesions were either isolated or confluent and were found only in the ALDO group.

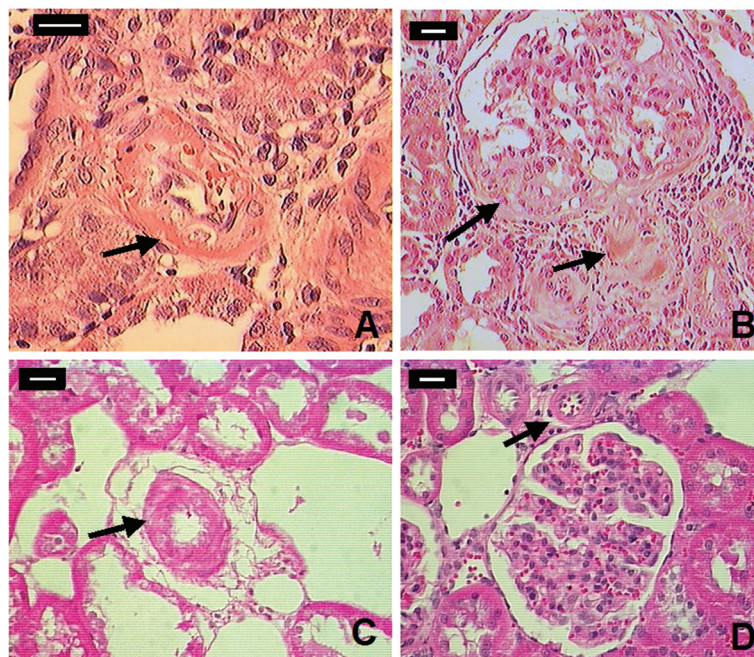
Histological alterations in the kidney were as follows: 1) acute tubular necrosis plus intensive apoptosis and tubular regeneration phenomena. These were present to a greater extent in the ALDO group as compared to the ALDOF group; 2) evidence of malignant hypertension such as arteriolar fibrinoid necrosis (Figure 3, panel A), fibrinoid necrosis of afferent arterioles and glomerulonecrosis (Figure 3, panel B). All animals from the ALDO group presented at least one of these lesions. Only two animals from the ALDOF group presented some of these lesions; 3) changes suggesting benign hypertension such as muscular media layer hypertrophy and arteriosclerosis were observed in both the ALDO and ALDOF groups (Figure 3, panel C). Eight of 10 ALDOF animals presented no glomerular damage (Figure 3, panel D).

LV vascular remodeling results are presented in Table 4. For vessels smaller than 50 µm there was an increased perivascular fibrosis in the CNEP group. Arterioles with a 50-100-µm internal diameter showed increased RWT in the ALDO and ALDOF groups compared to controls, with no statistical difference from CNEP. There was increased perivascular fibrosis in the ALDOF group.

Tissue damage scores are presented for both ventricles and the kidney in Figure 4. Both ventricles in the ALDO group had the highest score (median and interquartile range: LV = 7.0 and 5.5-8.0, RV = 6.5 and 5.0-9.5), compared to CNEP (LV = 0.0 and 0.0-0.75; RV = 0.0 and 0.0-1.0) and controls (LV = 0.0 and 0.0-1.0, RV = 0.0 and 0.0-0.25). Felodipine significantly prevented heart injury as indicated



**Figure 2.** Photomicrograph of rat myocardium showing no damage in the control group (Panels A and B), arteriolar hypertrophy and perivascular fibrosis without inflammation in the CNEP group (uninephrectomized + 1% NaCl in the drinking water; Panels C and D) and arteriolar transmurial necrosis (arrow) in the ALDO group (uninephrectomized + 1% NaCl in the drinking water plus continuous infusion of 0.75  $\mu\text{g}/\text{h}$  aldosterone; Panels E and F). Felodipine (ALDOF group) prevented myocardium perivascular damage (Panels G and H). Upper and lower panels show hematoxylin-eosin and picrosirius red staining, respectively. Bar scale = 20  $\mu\text{m}$ .

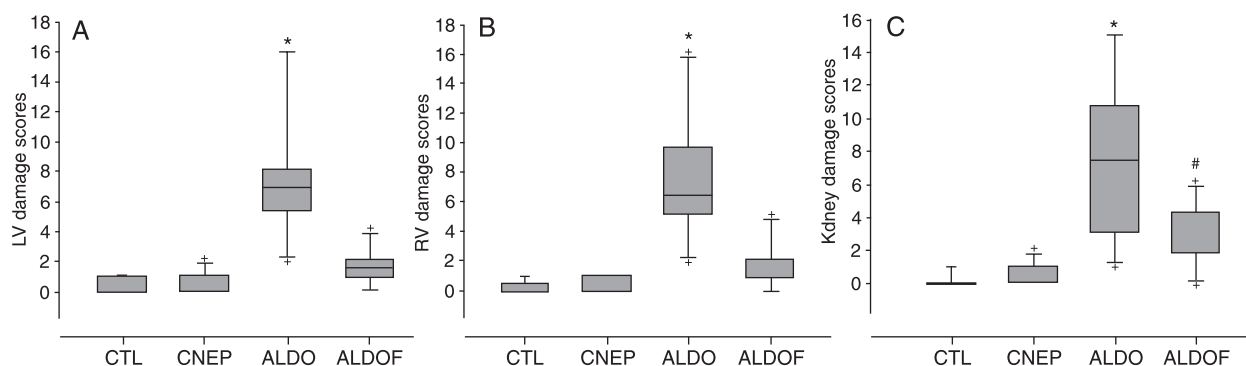


**Figure 3.** Photomicrograph of a rat kidney from the ALDO group (uninephrectomized + 1% NaCl in the drinking water plus continuous infusion of 0.75  $\mu\text{g}/\text{h}$  aldosterone) showing arteriolar fibrinoid necrosis (arrow, Panel A), fibrinoid necrosis of afferent arterioles and glomerulonecrosis (arrows, Panel B). Felodipine prevented most of the kidney damage in the ALDOF group (Panels C and D). Panel C shows muscle layer hypertrophy (arrow) and Panel D shows a typically preserved glomerulus (arrow). Bar scale = 20 microns.

**Table 4.** Left ventricular coronary artery remodeling data.

Group	Arterioles (<50 μm)		Arterioles (50-100 μm)	
	RWT	RPF	RWT	RPF
CTL	0.68 ± 0.110 <sup>a</sup>	0.32 ± 0.103 <sup>a</sup>	0.46 ± 0.13 <sup>a</sup>	0.21 ± 0.067 <sup>a</sup>
CNEP	0.66 ± 0.110 <sup>a</sup>	0.39 ± 0.094 <sup>b</sup>	0.53 ± 0.089 <sup>a/b</sup>	0.26 ± 0.073 <sup>a/b</sup>
ALDO	0.69 ± 0.085 <sup>a</sup>	0.39 ± 0.144 <sup>a/b</sup>	0.58 ± 0.091 <sup>b</sup>	0.21 ± 0.097 <sup>a</sup>
ALDOF	0.69 ± 0.110 <sup>a</sup>	0.36 ± 0.121 <sup>a/b</sup>	0.59 ± 0.095 <sup>b</sup>	0.29 ± 0.064 <sup>b</sup>

Data are reported as means ± SD. Group abbreviations are defined in the legend to Table 2. RWT = relative wall thickness; RPF = relative perivascular fibrosis. Means with different superscript letters are significantly different (P < 0.05) in all pairwise multiple comparison procedures (ANOVA and Bonferroni t-test).



**Figure 4.** Box-plot showing the damage score for the left (LV) and right (RV) ventricles (Panels A and B) and the kidney (Panel C). Myocardial injury but not kidney injury induced by aldosterone/salt was fully prevented by felodipine. \*P < 0.05 vs controls (CTL), CNEP and ALDOF; #P < 0.05 vs controls (ANOVA on ranks and the Dunn test). + = values exceeding the box-plot boundaries; CNEP = uninephrectomized plus 1% NaCl in the drinking water; ALDO = same as CNEP plus continuous infusion of 0.75 μg/h aldosterone; ALDOF = same as ALDO plus continuous infusion of 30 mg·kg<sup>-1</sup>·day<sup>-1</sup> felodipine.

by much lower damage scores compared to ALDO (LV = 1.5 and 1.0-2.0, RV = 1.0 and 1.0-2.0). Kidney damage scores were higher in ALDO (7.5 and 4.0-10.5) compared to controls (0.0 and 0.0-0.0) and CNEP (0.0 and 0.0-1.0). Felodipine partially prevented kidney injury. The damage scores for the ALDOF group (2.0 and 2.0-3.0) were intermediate between those for the CNEP and ALDO groups. However, while 9 of the 12 rats in the ALDO group presented at least one area of severe vascular damage, such damage was found only in 2 of 10 rats in the ALDOF group.

The LV and RV damage scores in the ALDO group were closely associated (R = 0.838, P < 0.001; Spearman's correlation). There was no significant association between kidney and myocardial damage scores or between systolic blood pressure and tissue injury.

## Discussion

We have demonstrated that felodipine given to aldos-

terone-high salt uninephrectomized rats prevents arterial inflammation, myocyte necrosis, myocardial fibrosis, and renal dysfunction.

Aldosterone-high salt-induced hypertension and myocardial and renal damage have been demonstrated by several groups of investigators (1,2,10,11). Also, the protective effects of angiotensin-converting enzyme inhibitors and AT1, mineralocorticoid and endothelin receptor blockers have been shown to be independent of blood pressure (12-14). These results suggest that aldosterone-salt-induced hypertension depends on multiple factors, including vasoconstriction and volume overload, and therefore would not be expected to be prevented by just one class of drugs.

Dihydropyridines, calcium channel blockers, are often used to treat arterial hypertension. A number of widely used dihydropyridine are able to inhibit the activation of MR. They have varied degrees of effect on MR and nifedipine and felodipine are the most potent MR inhibitors. Dihydropyridine may compete with aldosterone for binding

and block aldosterone-induced co-activator recruitment to MR (8). The aldosterone-induced MR is primarily inhibited by steroidal MR antagonists such as eplerenone, canrenate and spironolactone. Therefore, there is evidence that CCBs and aldosterone antagonists may have a common pathway concerning the beneficial effect of MR inhibition. Eventually, association of both drug classes might ameliorate arterial hypertension and prevent cardiac and kidney remodeling.

In this study in rats, felodipine (30 mg/kg) induced only a slight nonsignificant reduction of blood pressure and is therefore considered to be ineffective for antihypertensive treatment in this experimental model of hypertension. On the other hand, hypernatremia was partially prevented and hypokalemia following hyperaldosteronism was not modified by felodipine treatment. Dietz et al. (8) demonstrated that nimodipine, a dihydropyridine CCB, inhibited *in vivo* aldosterone-induced ENaC expression in the distal colon of nephrectomized rats. This suggests that this class of CCB would act *in vivo* as an MR antagonist. In humans, felodipine was reported to reverse the sodium retention induced by exogenous angiotensin II (15). Therefore, it is reasonable to suspect that our findings are related to the MR antagonism effect of felodipine and we may assume that felodipine increases sodium urinary loss and does not modify potassium excretion.

Interestingly, increased creatinine was found in the ALDO and CNEP groups, indicating that renal dysfunction was due to the uninephrectomy and salt overload state rather than to the hyperaldosteronism. The protective effect of felodipine agrees with data reported by Francischetti et al. (16) who demonstrated improved glomerular dynamics in hypertensive rats receiving felodipine. The authors considered this to be the result of nephrosclerotic prevention. Herein a similar positive finding was obtained in the absence of blood pressure normalization.

Recently, Fan et al. (17) showed kidney injury characterized by glomerular cell proliferation and renal interstitial fibrosis in this model of hypertension. Administration of the CCB azelnidipine attenuated the morphological changes of the kidneys. Our data are consistent with these results, indicating that dihydropyridine may elicit amelioration of aldosterone-induced kidney injury in uninephrectomized rats receiving 1% NaCl in the drinking water.

The myocardial hypertrophy and fibrosis, as well as perivascular and interstitial inflammation, observed in the ALDO group agreed with several other studies.

The mechanisms underlying myocardial fibrosis in aldosterone-salt hypertensive rats remain a matter of debate. Robert et al. (18) described increased myocardial collagen synthesis in aldosterone-high salt rats only 15 days after the beginning of aldosterone infusion, even though the plasma level of aldosterone was increased on the first day of infusion. The authors interpreted their results as evidence that the aldosterone-induced fibrosis is an indirect effect,

which would involve cellular necrosis. This would imply that aldosterone-salt-induced myocardial fibrosis is a reparative rather than a reactive process.

Our results also indicate that interstitial fibrosis in the ALDO group was a consequence of cellular damage, since prevention of inflammation and necrosis was associated with the absence of an abnormal accumulation of myocardial collagen. Also of relevance is the clear association between myocardial necrosis and vascular injury that accounts for the presence of ischemic myocardial necrosis in both ventricles. Others have suggested a similar mechanism (19). In the present study, the myocardial histological analysis identified at least three possible mechanisms of tissue damage, as follows: 1) a consequence of inflammation in the perivascular area; 2) secondary to ischemia if coronary arterioles were damaged enough to jeopardize the blood supply, and 3) a consequence of a lymphomononuclear infiltrate, including mastocytes, in interstitial vessels or of myocarditis foci. The cause of a lymphomononuclear infiltrate in the absence of cellular necrosis is not clear. However, several studies (1,19-22) have shown a blood pressure-independent association between inflammation and MR activation via the stimulation of immunocompetent cells. Monocyte-derived macrophages, rich in nicotinamide-adenine-dinucleotide phosphate (NADPH) oxidase, amplify the generation of reactive oxygen species (ROS) (23). ROS may also be generated by aldosterone-salt via MR activation. ROS production is associated with intracellular calcium elevation, protein kinase C activation and NADPH oxidase activation, creating a vicious cycle of inflammation and progressive necrosis and fibrosis (24).

Accordingly, the protective effect of felodipine would occur by anti-inflammatory and/or vasodilator actions resulting from an antagonizing action on either the MR or L-type calcium channels. Both mechanisms would lead to decreased levels of end products of lipid peroxidation, free radicals and hydroperoxides and to increased total antioxidant capacity (25). In the present study, significant vasodilation did not occur since arterial blood pressure remained elevated.

Our results show that felodipine prevented perivascular and interstitial inflammation and interstitial fibrosis in rats despite the presence of arterial hypertension and myocardial hypertrophy. Research with mibefradil, a T- and L-type CCB, has shown that the drug prevented myocardial fibrosis, although inflammation and necrosis were not assessed (26). Mibefradil, but not amlodipine, prevented glomerular damage in DOCA-salt hypertensive rats (27). Therefore, our findings, together with those of others, suggest that the effects of CCBs are drug class-dependent.

The heterogeneity of damage intensity from animal to animal is noteworthy. While several animals presented very extensive damage to the heart and/or kidney, others had only slight damage, which could be attributed to hypertension. Although this aspect is not highlighted in



the literature, it suggests that aldosterone-induced tissue damage clearly depends on the individual's response. We did not find a significant correlation between systolic blood pressure and either the kidney or the myocardial damage score in agreement with previous findings (28), suggesting a hypertension-independent mechanism for perivascular inflammation and myocardial necrosis.

In the present study, kidney and heart damage was analyzed in the same rat receiving an aldosterone-high salt diet. The results indicated that the presence of severe injury to the heart did not always indicate damage to the kidney or vice versa. This observation suggests that the physiological control and response to injury may differ depending on the target organ.

The distinct patterns of remodeling in large and small arterioles suggest a coronary vascular selectivity associated with this experimental model. Interestingly, coronary arteriole remodeling was not prevented by felodipine and occurred in the absence of hypertension and inflammation, indicating a reactive process induced by high salt intake in the perivascular area. This remodeling may not require a high level of circulating aldosterone and may involve a local

synthesis induced by high sodium (29). In normotensive WKY rats, a high salt diet alone has been shown to cause myocardial perivascular fibrosis (30). Normotensive rats fed a high salt diet underwent renal vascular and glomerular remodeling (31) and showed depressed reactivity to dilator agonists (32). Therefore, it is reasonable to assume that reactive perivascular fibrosis induced by high salt intake would cause a decreased coronary flow reserve and secondary myocardial remodeling.

The lack of increased perivascular fibrosis in the ALDO group could be explained by the marked exudative process still taking place at the end of the experiment. More than likely, a longer study period would have revealed a reparative process with increased fibrosis.

We have shown that felodipine has a protective effect on the myocardium and kidney as indicated by decreased perivascular inflammation and myocardial necrosis and fibrosis in aldosterone-high salt intake-hypertensive rats.

## Acknowledgments

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