Mineralocorticoid Receptor Antagonism Attenuates Cardiac Hypertrophy and Prevents Oxidative Stress in Uremic Rats

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Abstract—Chronic renal failure causes left ventricular hypertrophy, but the molecular mechanisms involved remain unknown. We, therefore, investigated whether the mineralocorticoid receptor is implicated in the cardiac hypertrophy observed in uremic rats and whether mineralocorticoid receptor blockade could be protective in chronic renal failure. Experimental groups were: control rats, uremic rats (NPX) with 5/6 nephrectomy (5 weeks), and NPX rats fed with spironolactone for 5 weeks. Systolic blood pressure was increased in both NPX rats and NPX rats fed with spironolactone for 5 weeks. Echocardiography revealed concentric left ventricular hypertrophy in uremia, which was attenuated by spironolactone. Enlarged cardiomyocyte size was observed in both left and right ventricles of NPX rats, an effect that was prevented by spironolactone. Mineralocorticoid receptor antagonism attenuated the increase of ventricular brain natriuretic peptide mRNA levels induced by nephrectomy. Left ventricular gene expressions of aldosterone synthase, mineralocorticoid receptor, and hydroxysteroid dehydrogenase type 2 were the same in the 3 groups, whereas gene expression of the glucocorticoid receptor was significantly diminished in chronic renal failure rats. No significant differences in cardiac aldosterone were observed between control rats and NPX rats, although NPX rats fed with spironolactone for 5 weeks showed increased plasma aldosterone levels. However, a significant increase in serum and glucocorticoid-inducible kinase-1 mRNA expression and protein was present in the NPX group; spironolactone treatment significantly reduced serum and glucocorticoid-inducible kinase-1 mRNA and protein in the left ventricle. Uremic rats exhibited a significant increase of superoxide production and reduced nicotinamide-adenine dinucleotide phosphate oxidase subunits expression (NOX-2, NOX-4, and $p47^{phox}$) in the left ventricle, which was prevented by the mineralocorticoid receptor antagonist. Our findings provide evidence of the beneficial effects of spironolactone in cardiac hypertrophy and cardiac oxidative stress in chronic renal failure. (***Hypertension*****. 2008;52:1-6.)**

Key Words: aldosterone ■ mineralocorticoid receptor ■ cardiac hypertrophy ■ SGK1 ■ oxidative stress ■ hydroxysteroid dehydrogenase type 2

Left ventricular hypertrophy (LVH) is one of the most common cardiac abnormalities in uremic patients and represents a leading cause of death. Although some studies have shown that correction of hypertension and/or anemia in dialysis patients significantly decreased LVH, other studies have shown that LVH did not change or that it can even progress, in spite of adequate blood pressure control in uremic patients.[1] Experimental studies evidenced similar cardiovascular abnormalities in models of chronic uremia,[2,3] and correction of anemia or treatments with several antihypertensive agents did not prevent hypertrophy in uremic rats.[3–5] These findings are in favor of a blood pressure–independent effect of uremia that could function as a prohypertrophic factor.

The pathophysiological role of aldosterone in heart hypertrophy has received important support from experimental and clinical studies.[6–8] The cardioprotective action of aldosterone antagonists proved to be independent of hemodynamic effects.[7] Previous reports have demonstrated the presence of mineralocorticoid receptor (MR) and the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD)-2 in heart[9] and blood vessels.[10] This prompts the notion that either changes in cardiac MR expression or 11β-HSD2 activity could be involved in cardiac hypertrophy.[11–13] Also, the possibility of local synthesis of aldosterone in the heart has been studied by different groups; however, the origin of aldosterone in the heart is controversial.[14–16]

Cardiomyocyte hypertrophy because of aldosterone led to enhanced MR signaling, as judged by the ability of aldosterone to induce serum and glucocorticoid-inducible kinase-1 (SGK1) gene transcription.[17] SGK1 has been implicated in cardiac hypertrophy[18] and is activated in response to growth

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factors or oxidative stress.[19,20] In addition, a number of recent studies have provided increasing evidence that support a key role for reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidases in the production of superoxide and reactive oxygen species in mineralocorticoid disease models.[21–23]

The purpose of the present study was to evaluate whether aldosterone contributes to the cardiac hypertrophy observed in chronic renal failure. We chose spironolactone, as the MR antagonist, in 5/6 nephrectomy rats. In addition, to gain insight in the pathophysiological MR-dependent mechanism, we measured the gene expression of MR, glucocorticoid receptor (GR), 11β-HSD2, and SGK1 in the left ventricle (LV) of experimental animals. We also measured cardiac aldosterone levels and enzymes activities of 11β-HSD1 and 11β-HSD2. Finally, markers of oxidative stress were analyzed in the LV from controls (sham) rats, 5/6 nephrectomy (NPX) rats, and 5/6 nephrectomy rats with spironolactone treatment during the entire period of 5 weeks (NPXspi).

Materials and Methods

A detailed Materials and Methods section is provided in the online data supplement (available at http://hyper.ahajournals.org).

Animals

Male Sprague-Dawley rats (150 to 180 g) were separated into 3 groups: NPX, NPXspi (15 mg/kg of body weight per day), and sham rats. Spironolactone was added to the diet immediately after nephrectomy. The ethics committee of the University Los Andes approved the protocols for animal experimentation according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

At the end of 5 weeks, blood and urine samples were collected. Cardiac aldosterone was determined as described.[24] Blood pressure (BP) was measured by the indirect tail cuff method. Also, direct BP measurements were carried out for accurate evaluation of this parameter.

Echocardiographic Studies

2D M-mode echocardiography was performed at the end of the experimental period by a single investigator who was unaware of the nature of the experimental groups.[25]

Real-Time Semiquantitative RT-PCR

Gene expression was quantitatively analyzed by real-time PCR.[26] Results were expressed as target gene/18S ribosomal RNA (rRNA).

Western Blot Analysis

Western blotting was performed as described previously.[27]

Histology

Equatorial ventricular sections were stained with hematoxylin-eosin to determine cardiomyocyte cross-sectional area. Images of sections were analyzed in their entirety (≥300 cells per image, 20 images per ventricle).

11β-HSD Activity Assay

LV protein homogenates were assayed for 11β-HSD1 and 11β-HSD2 activity, as described previously.[10]

Superoxide Anion Assay

Superoxide anion accumulation was measured by lucigenin-enhanced chemiluminescence in LV segments.[28]

Table 1. Characteristics of NPX Rats

Parameters	Sham	NPX	NPXspi
Body weight, g	330.2±9.9	300.1±12.1*	293.1±11.3*
Plasma sodium, mEq/L	139.6±0.7	138.8±1.2	143.1±3.5
Plasma potassium, mEq/L	4.2±0.1	5.1±0.2*	5.5±0.2*
Plasma chloride, mEq/L	103.2±0.7	105.3±1.0	108.5±2.7
Plasma creatinine, mg/dL	0.45±0.04	0.83±0.09*	0.84±0.05*
Plasma aldosterone, ng/dL	27.2±3.0	27.4±4.3	46.7±4.7*
Plasma corticosterone, ng/dL	446±41	312±77	321±53
Hematocrit, %	46.6±0.6	33.8±1.6	36.0±1.3

Values are means±SEs (n=12 for each group).
*$P<0.05$ vs sham.

Statistical Analysis

All of the data are expressed as means±SEMs. Statistical analysis was performed using unpaired 1-way ANOVA or Kruskal-Wallis test when appropriate (Stata 9.0). Significance was accepted at the 5% level.

Results

Biochemical and Cardiovascular Parameters

Plasma aldosterone was similar in NPX and sham groups but was elevated in NPXspiro rats (Table 1). The plasma corticosterone concentration did not differ significantly between groups. Plasma potassium was elevated in both NPX groups. No significant differences in plasma electrolytes values, creatinine, or hematocrit were observed when NPX and NPXspiro rats were compared, although there was a tendency toward increased plasma sodium, plasma chloride, and hematocrit in the NPXspiro group. Creatinine clearance was as follows: sham, 13.8±2.6 mL/min per kilogram of body weight; NPX, 3.6±0.4 mL/min per kilogram of body weight; and NPXspiro, 3.9±0.5 mL/min per kilogram of body weight (n=4 for each group; $P<0.05$, sham versus NPX and sham versus NPXspiro).

BP was elevated in both NPX groups, and spironolactone had no significant effect on systolic BP measured by the tail-cuff method (NPX: 139.7±5.7 mm Hg; NPXspiro: 135.4±6.6 mm Hg; sham: 108.7±2.5 mm Hg; n=12 for each group) or by direct measurements of mean BP (115.4±9.0 mm Hg, 115.1±10.3 mm Hg, and 82.5±4.4 mm Hg, respectively; n=4 for each group). Echocardiography revealed a significant increase in intraventricu-

Table 2. Echocardiographic Parameters

Parameter	Sham	NPX	NPXspi
Heart rate, bpm	300.6±13.6	303.9±13.9	298.3±13.3
IVSTd, mm	1.58±0.04	1.99±0.11*	1.70±0.04†
LVPWd, mm	1.63±0.04	2.00±0.08*	1.80±0.06†
LVIDd, mm	7.11±0.31	6.36±0.21*	6.05±0.19*
LVIDs, mm	3.91±0.23	3.08±0.21*	3.05±0.16*

IVSTd indicates interventricular septal wall thickness; LVPWd, left ventricular posterior wall dimension; LVIDd, left ventricular inner dimension in diastole; LVIDs, left ventricular internal dimension at the end of systole (n=8–12 each group). Values are means±SEs.
*$P<0.05$ vs sham.
†$P<0.05$ NPX vs NPXspi.

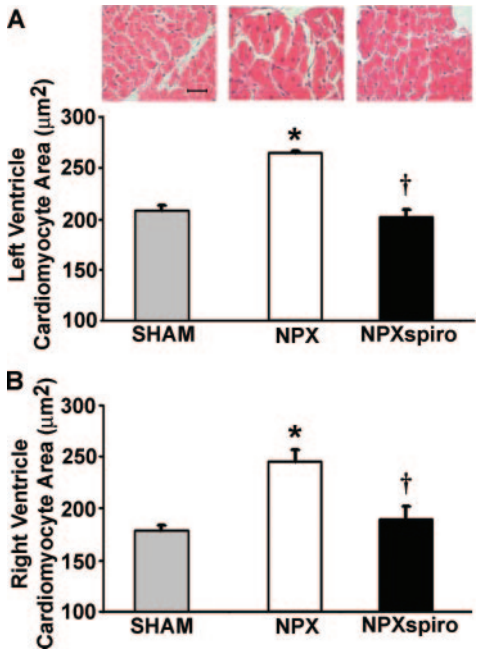

Figure 1. Cardiac myocyte cross-sectional area (CSA) in the 3 groups of rats, hematoxylin-eosin staining. A, Top representative micrographs of cross sections of the LV cardiomyocyte of sham (left), NPX (middle), and NPXspiro (right) rats; magnification: ×400; bar: 40 μm. Bars represent mean values of CSA obtained after examining LV cardiomyocyte morphometry of 6 rats for each group. B, Same as A for right ventricle. *$P<0.05$ vs sham; †$P<0.05$ vs NPX.

lar septal wall thickness and left ventricular posterior wall dimension in NPX rats that was partially but significantly prevented by spironolactone (Table 2).

Cardiac hypertrophy in NPX rats was confirmed by organ weight determination (please see the data supplement). Treatment with spironolactone resulted in a significant reduction of LVH as compared with the NPX group. As illustrated in Figure 1, an enlarged cross-sectional cell area was observed in cardiomyocytes of NPX rats as compared with sham rats. Left and right ventricle cellular hypertrophy was completely prevented by spironolactone.

Significant increases in atrial natriuretic peptide and brain natriuretic peptide (BNP) mRNAs were observed in LV samples of NPX rats. Spironolactone treatment prevented the increment in atrial natriuretic peptide mRNA abundance (Figure 2A) and ameliorated BNP mRNA abundance (Figure 2B).

SGK1 Expression in LV of Uremic Rats

We subsequently explored how heart MR signaling was affected by chronic renal failure. As shown in Figure 3, both protein and mRNA SGK1 expression were significantly increased in uremic rats. A significant reduction in both parameters was observed in NPXspiro rats as compared with the NPX group.

Activity and Expression of Steroids Metabolic Genes

In most nonepithelial tissues, 11β-HSD2 is expressed at minimal levels, but it is not known whether the enzyme is

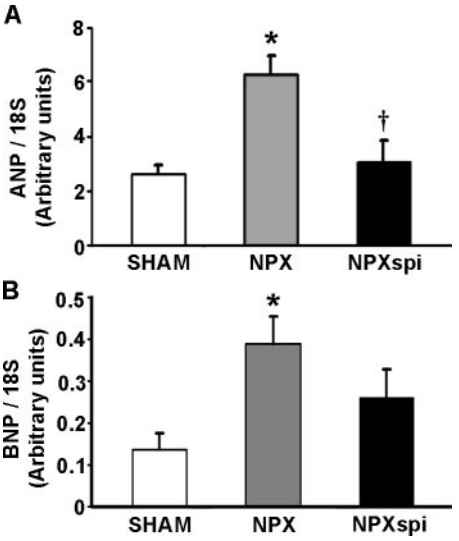

Figure 2. LV gene expression of natriuretic peptides analyzed by semiquantitative real-time RT-PCR. A, Atrial natriuretic peptide (ANP) and (B) BNP mRNA abundances normalized to 18S. Results are expressed as means±SEs; n=6 for each group of rats. *$P<0.05$ vs sham; †$P<0.05$ vs NPX.

upregulated in the heart of chronic renal failure rats. Therefore, we measured LV gene expression and activity of 11β-HSD2 of sham, NPX, and NPXspiro rats. The activity of 11β-HSD1 was also measured (please see the data supplement). 11β-HSD1 enzyme activity was 200 times higher than

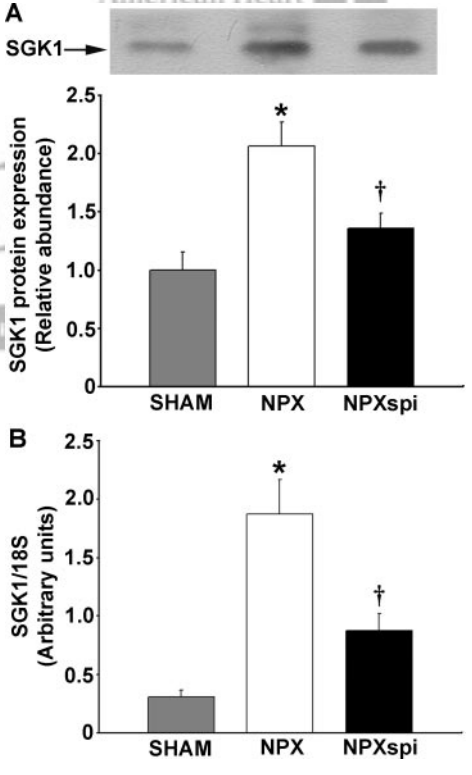

Figure 3. LV gene and protein expression of SGK1. A, Top representative Western blot of SGK1. Bars represent mean values of protein expression. B, SGK1 mRNA abundance normalized to the abundance of 18S rRNA (18S). Results are expressed as means±SEs; n=5 for each group of rats. *$P<0.05$ vs sham; †$P<0.05$ vs NPX.

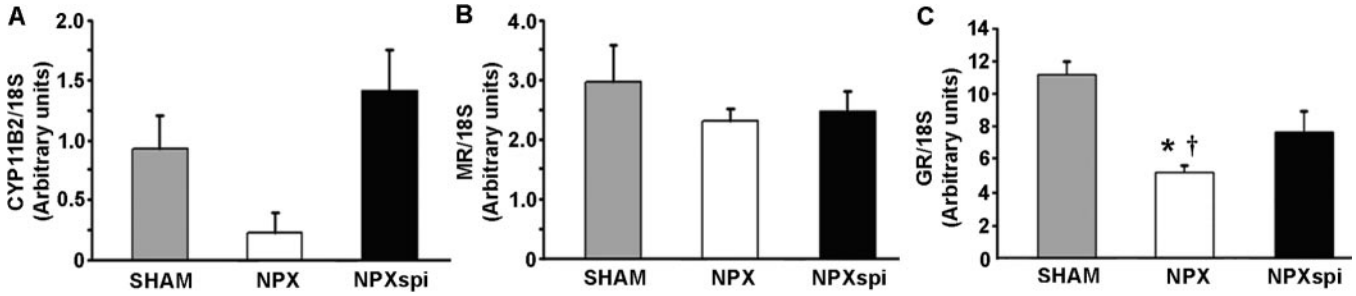

Figure 4. LV gene expression analyzed by semiquantitative real-time RT-PCR. A, Aldosterone synthase mRNA (CYP11B2). B, MR mRNA. C, GR mRNA. Results normalized to 18S and expressed as means±SEs; n=7 for each group of rats. *$P<0.05$ vs sham; †$P<0.05$ vs NPXspiro.

that of activity of 11β-HSD2. Both enzymatic activities were similar in the 3 groups of rats. All of the groups exhibited low gene expression of 11β-HSD2.

Cardiac Expression of Aldosterone-Related Genes

The possible existence of local synthesis of aldosterone in the uremic heart was tested by measuring aldosterone content and aldosterone synthase (CYP11B2) mRNA. Aldosterone content was similar in the 3 experimental groups (sham: 26.8±4.5 pg/g of wet tissue; NPX: 31.2±8.2 pg/g of wet tissue; and NPXspiro: 29.8±5.7 pg/g of wet tissue; n=4 for each group). A relatively low amount of CYP11B2 mRNA, expressed as the ratio to 18S rRNA, was observed in the 3 groups of rats without significant differences among them (Figure 4A). In addition, RT-PCR analysis demonstrated no significant differences in MR gene expression among control and uremic animals (Figure 4B).

Recent studies have shown that alterations in the balance between GR and MR expression may determine a pathophysiological role of MR in the heart.[29,30] Hence, gene expression of GR in the LV of the 3 groups of rats was measured. As shown in Figure 4C, a 50% decrease in GR mRNA was found in NPX rats.

Oxidative Stress

The levels of 3 subunits of the NADPH oxidase system (NOX-2, NOX-4, and p47^{phox}) were measured in heart of sham, NPX, and NPXspiro animals. These components were measured by RT-PCR (NOX-2 and NOX-4) or by protein content (p47^{phox}). As shown in Figure 5, a commonality of response was observed for the 3 markers, with high values in the NPX group and complete protection with the MR antagonist. Consistent with these data, studies using the lucigenin-enhanced chemiluminescence method showed increased superoxide production in LV segments of NPX rats as compared with LV tissue from sham animals (Figure 5D). Finally, spironolactone prevented the increased superoxide production in the uremic LV.

Discussion

A number of studies indicate that MR activation exerts deleterious effects in the cardiovascular system.[6,11,29,30] However, whether MR is involved in heart hypertrophy of chronic renal failure has not been established.[2–4] In the present study, we detected concentric ventricular hypertrophy. Accordingly, morphometric studies confirmed increased cardiomyocyte size. Interestingly, we demonstrated that spironolactone ameliorated ventricular hypertrophy and cardiomyocyte hypertrophy. The beneficial effect of spironolactone suggested MR activation in uremia.

Several studies have suggested that LV hypertrophy in chronic renal failure is independent of BP levels.[2,4,31] We found equivalent increments of cardiomyocyte size in both right and LVs of NPX rats, despite the pressure differentials. Spironolactone ameliorated LVH without a significant reduction of BP. All of these data are consistent with a BP-independent mechanism. Nevertheless, we cannot discard the influence of renal protection exerted by spironolactone. It is known that uremia causes volume overload and increased ventricle wall stress. In this regard, NPXspiro animals showed a tendency toward lower body weight and increased plasma sodium, plasma chloride, and hematocrit as compared with NPX animals, suggesting a small reduction of intravas-

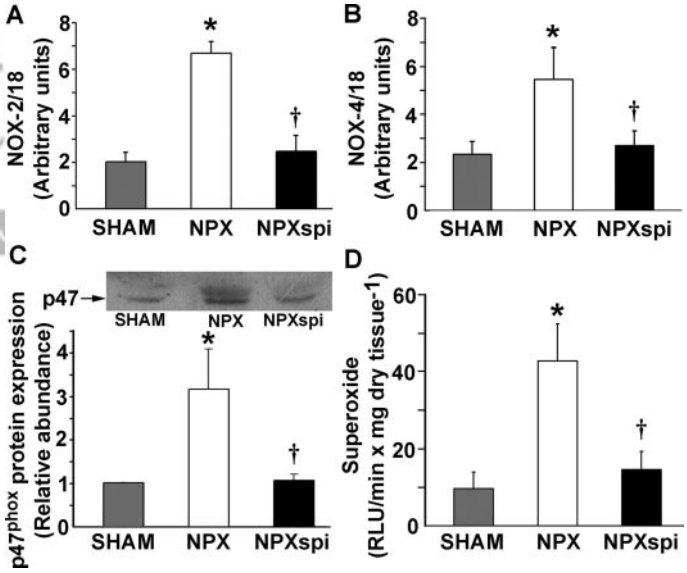

Figure 5. LV NADPH oxidase subunit expression and superoxide production. A, p47^{phox} protein expression. Top, Representative Western blot. Bars represent mean values of protein. B, NOX-2 mRNA abundance normalized to the abundance of 18S rRNA (18S). C, NOX-4 mRNA abundance normalized to the abundance of 18S rRNA (18S). D, Superoxide production, as determined by chemiluminescence of lucigenin in LV segments of sham, NPX, and NPXspiro rats. Data are expressed as means±SEs; n=4 to 5 in each group. *$P<0.05$ vs sham; †$P<0.05$ vs NPXspiro.

cular volume. Atrial natriuretic peptide and BNP are volume-sensitive markers, and we observed that the increased NPs expression in hearts from NPX rats was ameliorated by spironolactone. Therefore, the effects discussed here could be partially mediated by indirect effects of spironolactone treatment, secondary to a reduction of intravascular volume and wall stress.[32] In addition to intravascular volume, natriuretic peptide expression depends on MR activation.[33] Yamamuro et al[34] found that in vitro aldosterone exposure induced cardiomyocyte hypertrophy and increased BNP transcription. Thus, reduced NPS expression in NPXspiro could also result from cardiomyocyte MR antagonism.

SGK1 mRNA and protein abundance were measured as functional readouts of MR activation, which could be implicated in heart hypertrophy.[15,17,35] We observed a dramatic increment in SGK1 expression in the uremic LV. Spironolactone attenuated LV SGK1 upregulation, supporting the hypothesis of heart MR activation in uremic rats.

Potential mechanisms leading to cardiac MR activation in the context of normoaldosteronemia were explored. There are reports that indicate aldosterone synthesis in the heart.[14,36] However, Gomez-Sanchez et al[15] showed that most aldosterone in the heart of healthy rats is derived from the circulation. Similar findings were obtained recently by Chai et al[24] We found no significant differences between the cardiac aldosterone content of sham and NPX rats and very low levels of CYP11B2 expression in the heart. Also, Ye et al[16] did not observe any increase in cardiac CYP11B2 in several rat models of cardiac pathology. Therefore, heart aldosterone synthesis appears not to be implicated in cardiac MR activation of experimental chronic renal failure.

We have also explored potential 11β-HSD2 upregulation as a mechanism implicated in heart hypertrophy, because transgenic mice selectively overexpressing 11β-HSD2 in cardiomyocytes developed severe heart hypertrophy.[29,30] In the present study, we found almost undetectable 11β-HSD2 expression and activity in the LV. These data suggest that cardiac MR could be primarily occupied by endogenous corticosterone.

Funder first proposed that glucocorticoid-MR complexes can be transcriptionally active as a result of the generation of reactive oxygen species.[37] We have now found increased NADPH oxidase components and increased production of superoxide in LV of NPX rats. In agreement with our results, Kennedy et al[38] demonstrated that uninephrectomized rats with cardiac hypertrophy had systemic and cardiac oxidant stress. Our experimental results suggest that oxidative stress induced by NADPH oxidase plays a major role in uremic heart hypertrophy through the cardiac MR activation. Interestingly, we observed that spironolactone prevented the upregulation of NADPH oxidase components and superoxide production. Thus, it is possible that the increased oxidative stress may also lead to further activation of MRs preoccupied by glucocorticoids.

Ouvrard-Pascaud and Jaisser[30] have shown that alterations in the balance between GR and MR expression may determine a pathophysiological role of MR in the heart. Our results demonstrate low GR levels and unaltered MR mRNA in the heart of uremic rats. Glucocorticoids have been thought to act as antagonists of the MR in cardiomyocytes and brain cells.[29,39] We speculate that the reduction on GR signaling could be a contributing factor to cardiac hypertrophy in NPX rats resulting in unbalanced MR-dependent signaling. Future studies should be directed to determine the function and interaction between MR and GR in uremic cardiac hypertrophy.

Perspectives

Altogether, our data indicate that spironolactone treatment could be preventive of the cardiac hypertrophy present in chronic renal failure. Although plasma aldosterone levels remained within the reference range in NPX rats, we found increased SGK1, natriuretic peptides, and NADPH oxidase subunits in uremia. All of these changes were ameliorated by MR antagonism. Clinical studies have indicated that 40% to 60% of chronic renal failure patients are hyperaldosteronemic.[40] Most reports prove that oxidative stress is present in end-stage renal disease patients, and oxidative stress would be a strong cofactor for the development of cardiovascular complications.[41] Considering the high prevalence of LVH in hemodialysis patients, our findings emphasize the need for clinical studies to determine the potential beneficial effects of MR blockade in patients with chronic renal failure.

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Disclosures

None.

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