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EFFECT OF EPLERENONE ON HYPERTENSION-ASSOCIATED RENAL DAMAGE IN RATS: POTENTIAL ROLE OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (PPAR-γ)

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Several factors, including mineralocorticoids, have been implicated in the renal damage associated with hypertension. Peroxisome proliferator activated receptor gamma (PPAR-γ) agonists improve renal damage associated with different pathologies. Therefore, our hypothesis was that mineralocorticoid receptor blockade ameliorates renal damage associated with hypertension and that this improvement may be mediated by PPAR-γ. Spontaneously hypertensive rats (SHR) were treated with either vehicle or eplerenone, a mineralocorticoid receptor antagonist, at two different doses: 30 and 100 mg/kg/day for 10 weeks. Age-matched Wistar Kyoto rats (WKY) were used as a normotensive reference group. SHR showed tubulointersticial fibrosis and mild tubular atrophy. These alterations were accompanied by increases in renal cortex gene expression of transforming growth factor beta (TGF-β) connective tissue growth factor (CTGF) and phosphorylated Smad2 protein levels, factors involved in the fibrotic response. Interleukin 1-beta (IL-1β) and tumor necrosis factor alpha (TNF-α) gene expression were also increased. By contrast, lysyl oxidase (LOX) expression and PPAR-γ protein levels were decreased in SHR as compared with normotensive animals. Only the high dose of eplerenone was able to reduce blood pressure and partially prevent LOX down-regulation in SHR. Both eplerenone doses significantly ameliorated interstitial fibrosis and tubular atrophy, reduced TGF-β, CTGF and cytokine gene expression, and decreased Smad2 activation, while normalizing PPAR-γ protein levels. Conclusions: Mineralocorticoid receptor activation participates in hypertension-associated renal damage. This effect seems to involve stimulation of both fibrotic and inflammatory processes mediated (at least in part) by a down-regulation of PPAR-γ that can favour an up-regulation of the TGF-β/Smad signalling pathway.

Key words: *aldosterone, eplerenone, fibrosis, hypertension, inflammation, kidney, PPAR-γ*

INTRODUCTION

Much evidence has shown a close relationship between high blood pressure and renal function. While renal disease accelerates hypertension, the kidney is a target organ of hypertension (1, 2). Increases in pressure load to the renal vasculature induce glomerular haemodynamic changes resulting in mechanical stretch of glomerular capillaries and mesangial cells, which can lead to vascular lesions of hyaline atherosclerosis and subsequent fibrosis, narrowing of the vessel and ischemia (3, 4). High blood pressure levels are associated with glomerular hypertension and subsequent hyperfiltration leading to proteinuria (5). Reabsorption processes of filtered proteins can initiate renal interstitial injury by activating inflammatory factors including interleukin-1 beta (IL-1β, IL-6) or tumor necrosis factor alpha (TNF-α), which promote infiltration of inflammatory cells. In addition, hyperfiltration can also favour the development of tubulointerstitial fibrosis through collagen accumulation. This process involves the production of different growth factors such as transforming growth factor beta (TGF-β) or connective tissue growth factor (CTGF), which can be upregulated through TGF-β/Smad signalling (6-8), as well as enzymes like lysyl oxidase (LOX) responsible for collagen assembly (9). Inflammatory processes can further contribute to glomerulosclerosis and tubulointerstitial fibrosis (10), which represents the final manifestation of chronic renal disease (11).

Mineralocorticoid receptor is a member of the nuclear receptor superfamily. These receptors are located in the cytosol of most target cells, including epithelial cells, smooth muscle cells and endothelial cells. Upon binding of their ligands, among them aldosterone, deoxycorticosterone and glucocorticoids such as cortisol, they translocate to the nucleus in order to modulate gene expression (12, 13). Interestingly, however, it has recently been described that in cardiomyocytes it is a constitutively chromatin-bound factor mainly located in the nucleus (14). Mineralocorticoid receptor is essential for controlling sodium transport in epithelial tissues such as the kidney and colon. The renal effect of mineralocorticoids has long been thought to involve sodium and potassium excretion in the distal nephron (15). However, interest has emerged regarding its

pathophysiological role in the progression of renal disease through pro-inflammatory, pro-fibrotic and pro-oxidant properties, as has been demonstrated for aldosterone. Clinical and experimental studies have shown associations between high aldosterone levels and renal deterioration (16, 17). In addition, administration of mineralocorticoid receptor antagonists to different models of hypertension has been found to reduce renal alterations (18-20).

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. Three members - PPAR-α, PPAR-β/δ and PPAR-γ - have so far been identified (21). PPAR activity has traditionally been limited to glucose homeostasis and lipid metabolism. However, several studies have shown that PPAR-γ can modulate renal fibrosis through the inhibition of TGF-β pathway, which is considered to be a key mediator in the progression of renal fibrosis (22, 23). In addition, PPAR-γ is capable of interacting with NF-κB and suppressing its activity (transrepression) (18, 24), thereby regulating inflammatory responses. These anti-fibrotic and anti-inflammatory effects could underlie the beneficial effects of PPAR-γ agonists observed in diabetic and non-diabetic nephropathies (22, 25, 26). It is unclear, however, whether hypertension-associated renal damage is accompanied by changes in PPAR-γ expression or whether PPAR-γ expression is affected by mineralocorticoid receptor activation, which can participate in hypertension-related renal damage. The aims of this study were thus to evaluate whether hypertension-associated renal damage is accompanied by changes in renal levels of PPAR-γ and to consider the effect of a selective mineralocorticoid receptor antagonist, eplerenone, in spontaneously hypertensive rats (SHR). In order to elucidate whether changes produced by eplerenone are due to blood-pressure reduction, we explored two different doses of this mineralocorticoid receptor antagonist: one producing no significant decrease in arterial pressure and the other with marked effects on blood pressure.

MATERIALS AND METHODS

Experimental design

Studies were performed in male SHR (22 weeks old, n=24) from Charles River Laboratories (Barcelona, Spain). Animals were divided into three groups in function of being treated for ten weeks with either vehicle or eplerenone, a mineralocorticoid receptor antagonist, mixed in chow at two different doses: 30 (E30) and 100 (E100) mg/kg/day, respectively. Doses were chosen from previous studies (18, 27); one produced no significant change in systolic arterial pressure (SAP) (E30) and the other reduced SAP to a large extent (E100). A group of same-aged Wistar Kyoto rats (WKY, n=8) were used as a normotensive reference group. SAP was also measured using a tail-cuff pletysmograph (Narco Bio-Systems, Houston, TX) as previously described (22). Animals were killed by decapitation upon conclusion of the treatment. Kidneys were isolated for histological analysis and expression levels of the following genes were measured: TGF-β, CTGF, LOX, IL-1β, TNF-α and PPAR-γ gene expression. Nuclear protein levels of PPAR-γ and phosphorylated Smad2 were also measured. Isolation and manipulation of tissues were always performed under sterile conditions.

The Animal Care and Use Committee of Universidad Complutense approved all experimental procedures according to guidelines for ethical care of experimental animals of the European Community.

Metabolic parameters

Plasma concentrations of cholesterol, high-density lipoprotein cholesterol and glucose were measured using specific quantitative sandwich enzyme immunoassays (R&D Systems, MN, USA).

Renal histology

Renal cortex segments from each rat were fixed in 10% sodium phosphate-buffered paraformaldehyde, embedded in paraffin and cut into sections (4 μm) (18). Kidney injury score was calculated using the following semiquantitative index: 0 - no changes; 1 - focal changes that involve 25% of the sample; 2 - changes affecting >25 to 50%; 3 - changes involving >50 to 75%; 4 - lesions affecting >75%. Injury score was calculated as the sum of this semi-quantitative assessment of glomerular damage (mesangial cell proliferation and matrix expansion), tubulointerstitial injury (tubular dilation and/or atrophy and interstitial fibrosis) and inflammatory cell infiltrate (28). Collagen content was quantified upon Masson's trichrome staining on serial microscopic fields with automated image analysis (KZ 300 imaging system 3.0 (Zeiss, Munchen-Hallbergmoos, Germany) and expressed as percent of stained area to total area. Glomerular area was measured in 20 completely round glomeruli of each animal and randomly chosen from all renal cortex. Glomerular area was calculated after manually tracing each glomeruli with the same previously indicated automated image software. Two independent pathologists scored kidney injury in a blind fashion.

Gene expression

Total RNA was isolated from renal cortex using RNeasy™ (Qiagen). Five μg of total RNA was reverse transcribed using 250 U of moloney murine leukemia virus reverse transcriptase (MMLV-RT, Sigma), 0.7 U of RNase inhibitor and 2 μM random hexamers in 25 mM Tris-HCl (pH 8.3), 37 mM KCl, 1.5 mM $MgCl_2$, 10 mM DTT, dNTP's 0.4 mM each in a final volume of 50 μl (29). Gene expression was quantified by real-time PCR using TaqMan™ primers and probes for TGF-β, CTGF, IL-1β, TNF-α, PPAR-γ, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Roche) and LOX (Applied Biosystems) (*Table 1*). Real-time PCR was performed using a fluorescence temperature cycler (Smart Clycler, Cepheid, Sunnyvale, California, USA). The $2^{-\Delta\Delta CT}$ method analyzes relative changes in gene expression from real-time quantitative PCR experiments (30). Data were normalised by GAPDH levels and expressed as % relative to controls (WKY).

Western blot

Renal cortex samples were homogenized in lysis buffer and then separated by 12% sodium dodecyl sulphate-polyacrilamide gel electrophoresis under reducing conditions. After electrophoresis, samples were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, Massachusetts, USA). The membranes were blocked in PBS containing 0.15 Tween-20 and 5% dry skimmed milk for 1 hour at room temperature, and were then incubated in the same buffer with specific PPAR-γ antibody (Santa Cruz Biotechnology, Inc., USA) for 18 hours at 4°C and phosporylated Smad2 (Millipore, USA). After washing, detection was made through incubation with peroxidase-conjugated secondary antibody, and developed using an ECL chemiluminescence kit (Millipore, Bedford, Massachusetts, USA). As a loading control we used β-actin protein (1:2500) (Sigma). In addition, Red Ponceau staining was used to show the quality of proteins and efficacy of protein transfer to the membrane (not shown). The autoradiographs were scanned using

Table 1. Primers and probes used in quantitative real-time PCR analysis.

Gene	Primers
IL-1β	Sense 5′ GCTACCTATGTCTTGCCCGT 3′ Antisense 5′ CTCCAGTGCAGCTGTCTAAT 3′ Probe 5′ FAM-TGACTCGTGGGATGATGACGACCTG-BHQ1 3′
TNF-α	Sense 5′ GGTGATCGGTCCCAACAAGGA 3′ Antisense 5′ CACGCTGGCTCAGCCACTC 3′ Probe 5′ FAM-TGGCCCAGACCCTCACACTCAGATCA-TAMRA 3′
TGF-β	Sense 5′ GGGCTTTCGCTTCAGTGCT 3′ Antisense 5′ TCGGTTCATGTCATGGATGGT 3′ Probe 5′ Cy5-TCAGTCCCAAACGTCGAGGTGACCTG-BHQ 3′
CTGF	Sense 5′ TGGCCCTGACCCAACTATGAT3′ Antisense 5′ GCACTTTTTGCCCTTCTTAATGTT 3′ Probe 5′ FAM-AAGCCAACTGCCTGGTCCAGACCA-DB 3′
PPAR- γ	Sense 5' TGTCATTATTCTCAGTGGAGACCG 3' Antisense 5' CAGCAGGTTGTCTTGGATGT 3' Probe 5' Cy3-TCGATGGGCTTCACGTTCAGCAA-DB 3'
GADPH	Sense 5' TTGTCAGCAATGCATCCTGC 3' Antisense 5' CGGCATGTCAGATCCACAAC 3' Probe 5' TxRed-TGGCCCCTCTGGAAAGCTGTGGCG-BBQ2 3'
LOX*	Rn00566984_m1

*TaqMan™ gene expression Assay (Applied Biosystems).

Table 2. Lipid and glucose levels of spontaneously hypertensive rats (SHR) treated or not with two doses of eplerenone (E30: 30 mg/kg/day and E100: 100 mg/kg/day) for two weeks. Wistar Kyoto rats (WKY) were used us normotensive reference group. Values are mean +S.E.M. of 8 rats. *p<0.05 *vs.* WKY; #p<0.05 *vs.* SHR.

	WKY	SHR	EPLE 30	EPLE 100
Cholesterol (mg/dl)	69.4±2.42	45±1.41*	45±3.88*	47.5±4.89*
HDL (mg/dl)	39.60±1.12	26.25±0.63*	26.00±1.48*	28.50±2.28*
HDL/Cholesterol	0.57±0.01	0.58±0.02	0.59±0.02	0.61±0.03
Glucose (mg/dl)	116.00±6.15	106.25±2.56	104.83±3.52	89.83±5.24*#

the GS-800 calibrated densitometer (Quantity One; Bio-Rad, Madrid, Spain), obtaining densitometric arbitrary units (18).

Statistical analysis

Results are expressed as mean ±S.E.M. from 8 rats by group unless otherwise specified. Data was analyzed using a one-way analysis of variance, followed by a Bonferroni test if differences were noted (GraphPad Software Inc 4.0, San Diego, CA, USA). A p-value of 0.05 or less was considered to be significant.

RESULTS

Systolic arterial pressure, body and relative kidney weight

SHR presented higher systolic arterial pressure levels than WKY (190±4 *vs.*124±3 mmHg, p<0.05). Only the high dose of eplerenone (E100) reduced blood pressure levels (155±3 mmHg, p<0.05), while no significant changes in blood pressure were observed with the low dose (E30, 187±4 mmHg). Both body weight (WKY: 440±3 ; SHR: 460±10 ; E30: 442±7 ; E100: 450±11 g) nd relative kidney weight (WKY: 0.317±0.002 g; SHR: 0.321±0.007 g; E30: 0.332±0.008 g; E100: 0.330±0.005 g) were similar among groups.

Metabolic parameters

Plasma levels of cholesterol and HDL were lower in SHR (p<0.05) as compared to WKY (*Table 2*). Any dose of eplerenone modified these parameters, and the ratio HDL/total cholesterol was similar in all groups studied. Neither hypertension nor eplerenone treatment modified plasma levels of glucose (*Table 2*).

Renal morphology

No damage was observed in kidneys from WKY. By contrast, kidneys from SHR showed mild tubular atrophy (0.96±0.06) that was reduced with both doses of eplerenone (E30: 0.5±0.02 and E100: 0.4±0.02; p<0.05). Kidneys from SHR also presented areas of interstitial fibrosis as observed in *Fig. 1A*, and an important increase in collagen content (*Fig. 1B*). Both doses of eplerenone reduced tubular atrophy and the interstitial fibrosis (*Fig. 1A* and *1B*). No glomerular damage was observed in hypertensive rats as compared with normotensive ones. SHR did not present interstitial infiltrate. Hypertensive animals overall showed higher glomerular volume than WKY (2108.7±42 *vs*. 1711.8±50 $\mu m^3 x10^3$, p<0.05) and any dose of eplerenone was able to modify it (E30: 2116.9±113 $\mu m^3 x10^3$; E: 100: 2120.9±50 $\mu m^3 x10^3$).

Renal gene and protein expression

Hypertension seemed to be associated with a reduction in LOX gene expression, since SHR showed lower levels than controls (*Fig. 1C*). Only the high dose of eplerenone was able to partially normalise LOX expression in SHR (*Fig. 1C*).

TGF-β and CTGF mRNA levels were higher (p<0.05) in kidneys from SHR compared to those from WKY (*Fig. 2A* and

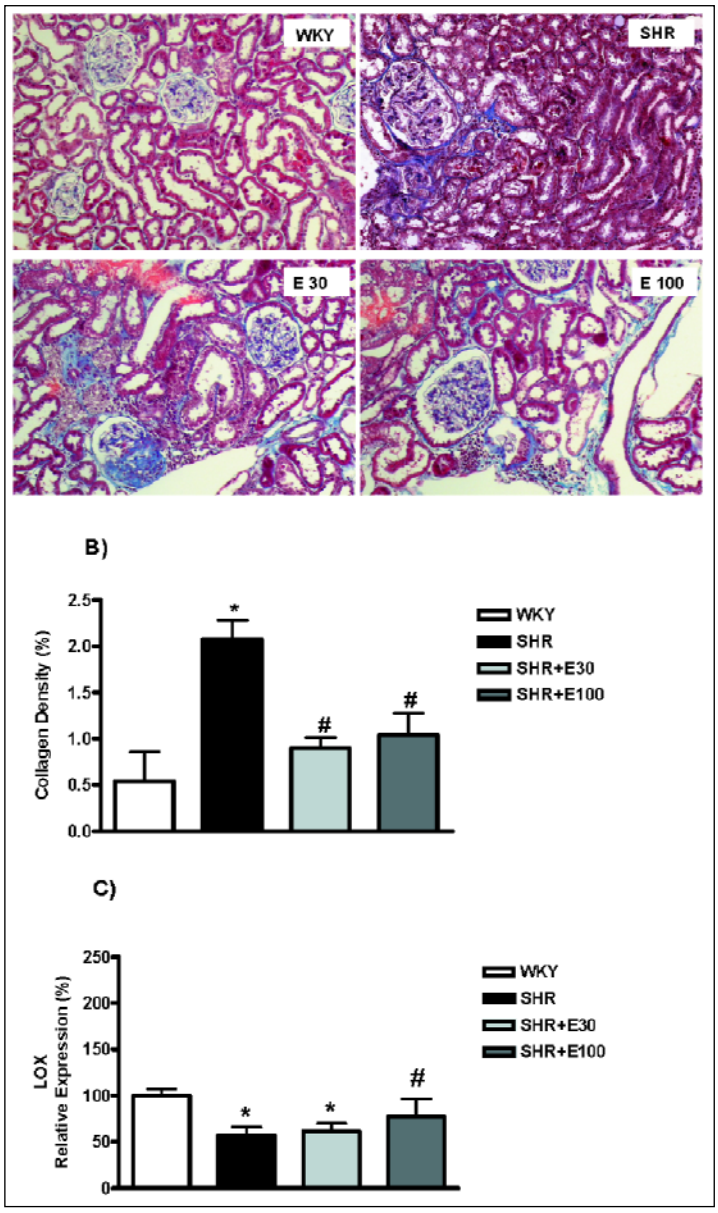

Fig. 1. (*A*) Masson's trichrome staining of renal cortex cross-sections from Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) treated or not with two doses of eplerenone (E30; 30 mg/kg/day and E100: 100 mg/Kg/day) for 10 weeks. WKY rats were used as normotensive reference group, and with a normal renal histology (magnification x400) (*B*) Collagen content (%) in the renal cortex from SHR treated as indicated in A. (*C)* Lysyl oxidase (LOX) mRNA levels in renal cortex homogenates from SHR treated as indicated in *A*. Results were normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression levels. Values are mean ±S.E.M. of 8 animals per group. *p<0.05 *vs*. WKY; #p<0.05 *vs*. SHR.

B, respectively). Accordingly, hypertensive animals exhibited enhanced nuclear levels of phosphorylated Smad2 (*Fig. 2C*). Both doses of eplerenone reduced TGF-β and CTGF mRNA expression in a similar manner (*Fig. 2A* and *2B*, respectively), while only the high dose was able to normalise levels of phosphorylated Smad2 (*Fig. 2C*).

IL-1β and TNF-α mRNA levels were higher ($p<0.05$) in kidneys from SHR compared to those from WKY (*Fig. 3A* and *Fig. 3B*, respectively). Both doses of eplerenone reduced IL-1β and TNF-α mRNA levels in a similar manner (*Fig. 3A* and *Fig. 3B*, respectively).

PPAR-γ nuclear protein expression was lower ($p<0.05$) in renal cortex from SHR compared to those from WKY (*Fig. 4*). Either dose of eplerenone normalised PPAR-γ expression (*Fig. 4*).

DISCUSSION

It has been shown that mineralocorticoid antagonists reduce blood pressure and improve vascular and renal function, but the underlying mechanisms are not entirely understood. Our group has recently reported that a mineralocorticoid antagonist (eplerenone) ameliorates aortic remodelling and improves endothelial function in SHR (18). Here we report that hypertension is associated with renal damage characterized by tubulointerstitial fibrosis, tubular atrophy and inflammation in this animal model. These changes were accompanied by a striking decrease in renal PPARγ expression and a concomitant activation of the TGF-β/Smad axis. Interestingly, treatment with eplerenone ameliorated interstitial fibrosis, tubular atrophy and inflammation, and reversed changes in PPAR-γ expression and TGF-β/Smad signalling. This supports that mineralocorticoid receptor activation might participate in the progression of renal damage associated with hypertension through a down-regulation of PPAR-γ which can favour the activation of the TGF-β pathway.

Hypertension was associated with marked renal inflammation in SHR. Indeed, higher IL-1β and TNF-α mRNA expression levels were observed in renal cortex from SHR as compared with that from WKY. Similarly, higher levels of other inflammatory markers have been reported in kidneys of different models of hypertension, including SHR (31-33). Elevated plasma, vascular, cardiac and brain cytokine levels in SHR have

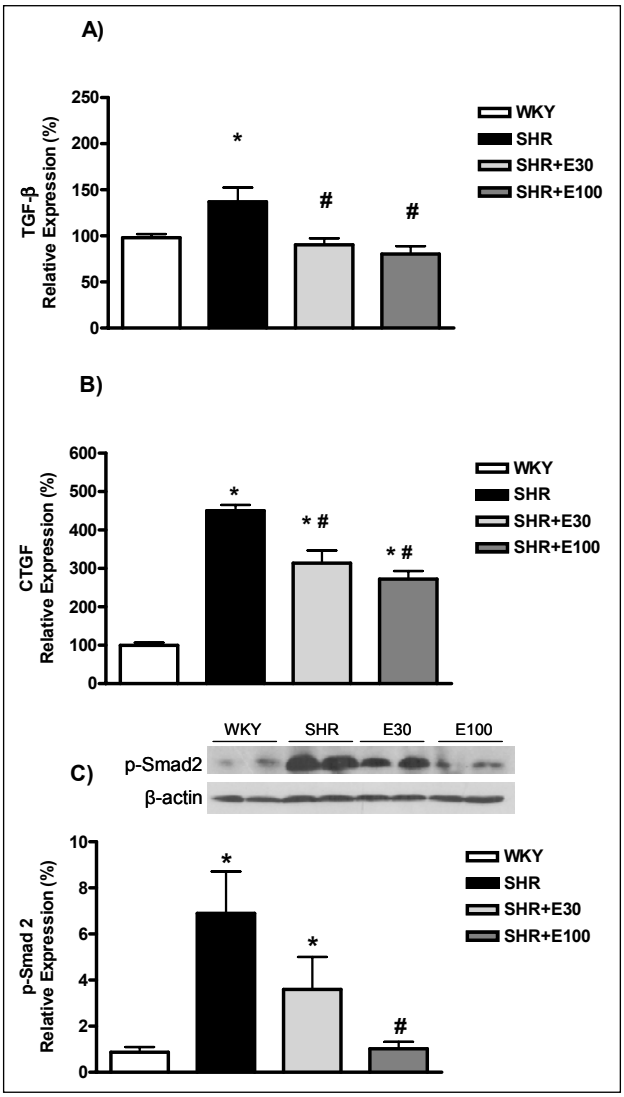

Fig. 2. mRNA levels of transforming growth factor beta (TGF-β) (*A*) and connective tissue growth factor (CTGF) (*B*) were evaluated by real-time PCR in renal cortex from spontaneously hypertensive rats (SHR) treated or not with two doses of eplerenone (E30: 30 mg/kg/day and E100: 100 mg/kg/day) for 10 weeks. Wistar Kyoto rats (WKY) rats were used as normotensive reference group. Results were normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression levels. (*C*) Nuclear levels of phosphorylated Smad2 (p-Smad2) were assessed by Western blot in the renal cortex from SHR treated as indicated in *A*. β-actin protein levels were used as a loading control. Values are mean ±S.E.M. of 8 rats. *p<0.05 *vs*. WKY; #p<0.05 *vs*. SHR.

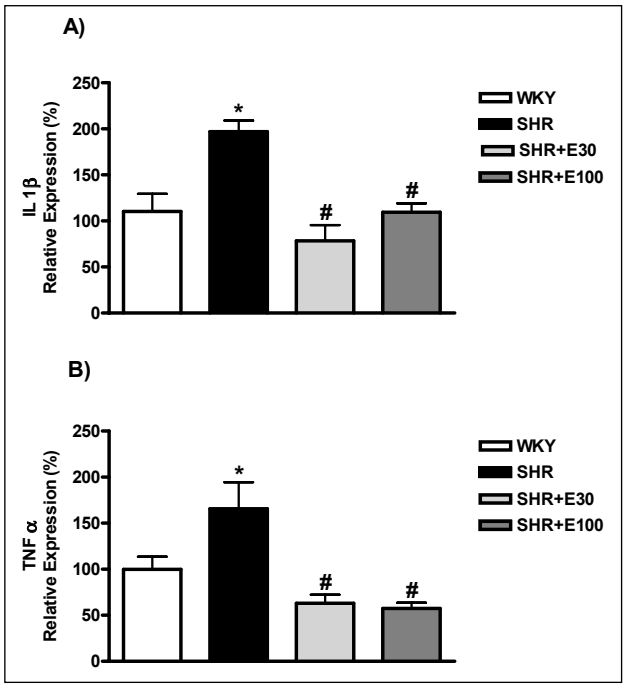

Fig. 3. Interleukin 1-beta (IL-1β) (*A*) and tumor necrosis factor alpha (TNF-α) (*B*) mRNA levels were analyzed by real-time PCR in renal cortex from spontaneously hypertensive rats (SHR) treated or not with two doses of eplerenone (E30: 30 mg/kg/day and E100: 100 mg/kg/day) for 10 weeks. Wistar Kyoto rats (WKY) were used as normotensive reference group. Values are mean ±S.E.M. of 8 rats shown as percent relative expression *vs*. WKY rats. Results were normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression levels. *p<0.05 *vs*. WKY; #p<0.05 *vs*. SHR.

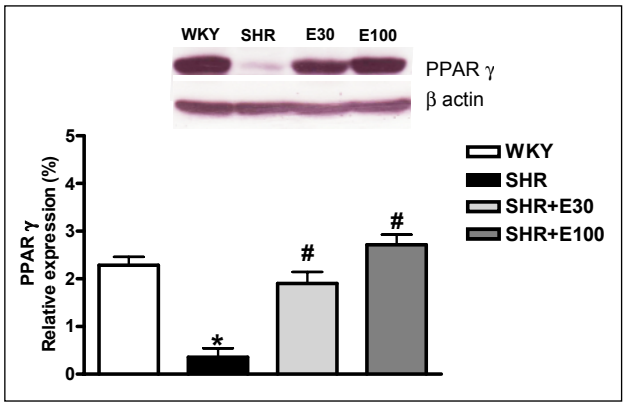

Fig. 4. Peroxisome proliferator activated receptor gamma (PPAR-γ) protein levels in nuclear extracts of renal cortex homogenates from spontaneously hypertensive rats (SHR) treated or not with two doses of eplerenone (E30: 30 mg/kg/day and E100: 100 mg/kg/day) for 10 weeks. Wistar Kyoto rats (WKY) were used as normotensive reference group. β-actin protein levels were used as a loading control. Values are mean ±S.E.M. of 8 rats. *p<0.05 *vs*. WKY; #p<0.05 *vs*. SHR.

been previously reported (29, 33), suggesting that high blood pressure is associated with a generalised inflammatory process. This might not only further contribute to the progression of renal damage by glomeruloesclerosis and tubulointerstitial fibrosis stimulation, but also to the maintenance of hypertension through different mechanisms, including sodium retention (10). Additional mechanisms can affect sodium balance in hypertension since the function and regulation of renal Na,K-ATPase is altered in SHR (34, 35). Na,K-ATPase is an essential component of body Na homeostasis, and taking into account that the ion transport mediated by this enzyme consumed between 20-30% of renal metabolic energy (36), it is possible to speculate that any alteration in this transporter can have relevant consequences on renal function.

We have previously reported that a reduction in blood pressure is one of the mechanisms accounting for the

improvement of vascular inflammation elicited by eplerenone (27). In the present study, however, we show that in SHR eplerenone exhibits anti-inflammatory properties even at doses that did not modify blood pressure. These results suggest that besides a reduction in hemodynamic stress, additional mechanisms could be involved in the pro-inflammatory actions of mineralocorticoids. One of these potential mechanisms might be related to the strong down-regulation of PPAR-γ observed in SHR. Indeed, beyond its role regulating lipid and glucose metabolism, PPAR-γ exerts anti-inflammatory actions through different mechanisms including inhibition of NFκB activity (20, 21) and modulation of the renin-angiotensin system (24, 37). In this regard, the renal expression of this transcription factor was normalised in SHR treated with eplerenone. Similarly, spironolactone was able to reverse the reduction in PPAR-γ gene expression in renal cortex in another rat model of hypertension (L-NAME-hypertension) (38). We observed that at the highest dose eplerenone reduced glucose plasma levels. This would be consistent with an up-regulation of PPAR-γ not restricted to renal tissues. In agreement with this, eplerenone was able to increase PPAR-γ expression in adipose tissue from obese mice as compared with that from lean ones (39). However, these authors suggest that the effect of eplerenone on PPAR-γ expression is tissue-specific. Furthermore, in our study SHR and WKY exhibited similar glucose levels despite the fact that renal PPAR-γ expression was dramatically lower in SHR. Therefore, the observed up-regulation of PPAR-γ triggered by eplerenone could not be extrapolated to other tissues. Further studies would be necessary in order to explore this aspect, since eplerenone has demonstrated a neutral effect (or a slight decrease) on glucose levels (40). In agreement with previous studies, eplerenone did not modify plasma lipid levels (40, 41).

In agreement with previous studies (10, 42-44), in SHR high blood pressure levels were accompanied by hyperfiltration, as suggested by an increase in glomerular volume that was not normalized by eplerenone. This suggests that the decrease in blood pressure induced by eplerenone seems not to be large enough to modify the glomerular volume, supporting that mineralocorticoids do not affect intraglomerular pressure (45). SHR also showed tubulointerstitial fibrosis and tubular atrophy, although no glomerular fibrosis was observed. This indicates that glomeruli seem to be more resistant than tubuli to insult induced by high blood pressure levels. In fact, it has been shown in SHR that renal damage is less pronounced in outer cortex than in inner cortex, which shows less derangement than medulla (46). These alterations were observed without changes in kidney or body weight. By contrast, other authors have reported a reduction in both kidney and body weight in SHR as compared with normotensive animals (34). No clear explanation for this has been offered, although it could be the result of differences in experimental conditions. Under our experimental conditions the enzyme responsible for collagen assembly (LOX) (9) was down-regulated in renal cortex. This contrasts with the up-regulation of LOX observed in SHR heart (47). Our results do not preclude the active involvement of LOX in early stages of the tubulointerstitial fibrosis associated with hypertension, but they reveal a different modulation pattern of this enzyme in ongoing fibrosis in different organs. The inflammatory status in SHR kidneys could explain the decrease in LOX expression, which we and others have shown to be down-regulated by proinflammatory cytokines (48, 49). Additional mechanisms, however, should not be ruled out because only the higher dose of eplerenone, which reduced blood pressure levels, partially restored LOX expression, and despite the fact that both eplerenone doses were able to prevent the induction of IL-1β and TNF-α. Thus, it could be speculated that high pressure levels participate in the control of renal LOX.

Data from studies using mineralocorticoid receptor antagonists suggest that this receptor is involved in vascular and cardiac fibrosis associated with hypertension (6, 50). TGF-β and CTGF could be potential mediators in this process (7, 23); in fact, these growth factors promote extracellular matrix synthesis and mediate renal fibrosis associated with hypertension and other renal diseases (18, 51-53). Eplerenone reduced the expression levels of TGF-β and CTGF, which were significantly higher in the renal cortex of SHR than in WKY. In agreement with this, eplerenone was also able to reduce the high nuclear levels of phosphorylated Smad2 observed in SHR. Smad2 is a component of TGF-β signalling essential for the mediation of its biological effects (8, 54); therefore, it is likely that mineralocorticoid activation can trigger TGF-β/Smad signalling, which leads to CTGF up-regulation and subsequent renal fibrosis. This mechanism could also be related to the down-regulation of PPAR-γ observed in SHR. Indeed, PPAR-γ agonists inhibit TGF-β/Smad signalling and reduce renal interstitial fibrosis (22, 23), an effect that could account for the beneficial effects of these drugs reported in diabetic and non-diabetic nephropathies (22, 25, 26). Therefore, it is tempting to speculate that the down-regulation of renal PPAR-γ could be involved in the pro-fibrotic effects associated with mineralocorticoid receptor activation.

In summary, our data suggest that the down-regulation of PPAR-γ and the consequent activation of the TGF-β/Smad signalling pathways and inflammatory processes participate in the underlying mechanisms involving mineralocorticoid receptor activation in the progression of renal damage associated with hypertension.

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