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Different Protective Actions of Losartan and Tempol on the Renal Inflammatory Response to Acute Sodium Overload

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The aim of this work was to study the role of local intrarenal angiotensin II (Ang II) and the oxidative stress in the up-regulation of pro-inflammatory cytokines expression observed in rats submitted to an acute sodium overload. Sprague–Dawley rats were infused for 2 h with isotonic saline solution (Control group) and with hypertonic saline solution alone (Na group), plus the AT1 receptor antagonist losartan (10 mg kg^{-1} in bolus) (Na–Los group), or plus the superoxide dismutase mimetic tempol (0.5 mg min^{-1} kg^{-1}) (Na–Temp group). Mean arterial pressure, glomerular filtration rate, and fractional sodium excretion (FE_{Na}) were measured. Ang II, NF-κB, hypoxia inducible factor-1α (HIF-1α), transforming growth factor β1 (TGF-β1), smooth muscle actin (α-SMA), endothelial nitric oxide synthase (eNOS), and RANTES renal expression was evaluated by immunohistochemistry. Ang II, NF-κB, and TGF-β1 and RANTES early inflammatory markers were overexpressed in Na group, accompanied by enhanced HIF-1α immunostaining, lower eNOS expression, and unmodified α-SMA. Losartan and tempol increased FE_{Na} in sodium overload group. Although losartan reduced Ang II and NF-κB staining and increased eNOS expression, it did not restore HIF-1α expression and did not prevent inflammation. Conversely, tempol increased eNOS and natriuresis, restored HIF-1α expression, and prevented inflammation. Early inflammatory markers observed in rats with acute sodium overload is associated with the imbalance between HIF-1α and eNOS expression. While both losartan and tempol increased natriuresis and eNOS expression, only tempol was effective in restoring HIF-1α expression and down-regulating TGF-β1 and RANTES expression. The protective role of tempol, but not of losartan, in the inflammatory response may be associated with its greater antioxidant effects.
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In normal physiological conditions, the antioxidative function of nitric oxide (NO) and superoxide dismutase (SOD) activity in the tissues, contrabalances superoxide anion (O_2^-) oxidative effects, maintaining its production at a minimal level. However, this balance is altered when tissular NO production by the endothelial nitric oxide synthase (eNOS) or the adequate removal of NO by O_2^- is impaired, allowing O_2^- accumulation in the tissues (Majid and Kopkan, 2007). Consequently, O_2^- activates transcription nuclear factor NF-κB, which in turn modulates the induction of genes involved in renal inflammatory and fibrogenic responses through release of cytokines and accumulation of inflammatory cells in the kidney (Li and Zhuo, 2008). Several studies have demonstrated that a disbalance between NO and O_2^- in the kidney determines the initial condition of oxidative stress, which alters renal hemodynamia and the excretory function, leading to the development of sodium retention, hypertension, and renal injury (Majid and Kopkan, 2007). It is well known that a high salt intake leads to the development of hypertension in Dahl salt-sensitive rats, which is associated with an increase in oxidative stress and a decrease in the antioxidant capacity (Kitiyakara et al., 2003; Rodríguez-Iturbe et al., 2004). NO deficiency is also known to impair kidney function and contributing to the development of salt-sensitive hypertension (Tolins and Shultz, 1994; Tian et al., 2007). Moreover, an antioxidant treatment increases NO bioavailability, contributing to reduce renal damage in salt-sensitive rats (Zhou et al., 2008).

It is known that O_2^- might increase PKC-α activity in thick ascending limb and trigger the activation of the NHE-3 exchanger and Na–K–2Cl co-transporter, which in turn stimulate tubular sodium reabsorption (Silva et al., 2006). Furthermore, studies carried out in vitro in macula densa have shown that the transport of luminal NaCl to the inside of tubular cells induces the depolarization of the tubular cell membrane, which appears to be the signal for NADPH oxidase activation and O_2^- production (Liu et al., 2007; Sachse and Wolf, 2007). In addition, mechanic factors like the cellular stretch, the tubular flow (Hong and Garvin, 2007; Garvin and Hong, 2008), and/or fluid hypertonicity (Kültz, 2007; Lim et al., 2007) are able by themselves to increase O_2^- production. This cascade of events leads to the development of a vicious circle in which a small increase in sodium transport begets an increase in O_2^- production.

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Intrarenal angiotensin II (Ang II) is also linked to oxidative stress and the inflammation observed in salt-sensitive models (Bader and Ganten, 2008). Besides its actions on hydrosaline balance regulation, the attention has been recently focused on the possibility that intrarenally formed Ang II behaves like a true cytokine, modulating the induction of genes involved in renal inflammatory and fibrogenic responses (Liao et al., 2008). In this way, Ang II increases O_2^- production through the activation of NADPH oxidase, which is a major source of O_2^- in the kidney (Pendergrass et al., 2009). Ang II activates transforming growth factor β1 (TGF-β1) expression, stimulates collagen and fibronectin synthesis, and participates in renal failure development, controlling the deposition and remodeling of extracellular matrix (Wolf, 2006). Then, the increase in intrarenal Ang II production or sodium tubular transport as well as the mechanic effect mediated by the increase in the tubular flow or the stretch can generate oxidative stress and tissular inflammation in the kidney.

Studies carried out in vivo in our laboratory have shown that an acute sodium overload up-regulated intrarenal Ang II and the expression of diverse pro-inflammatory and pro-fibrogenic markers in renal tissues (Rosón et al., 2006a,b, 2008). However, up to date it remains unclear which are the pathophysiological mechanisms involved in the early expression of inflammatory markers after an acute sodium overload.

Based on these antecedents, the aim of our study was to evaluate the role of tubular Ang II signaling through the AT1 receptor (AT1-R) and the participation of the oxidative stress and NO in the development of renal pro-inflammatory response observed in rats submitted to an acute hypertonic sodium overload. The AT1-R antagonist losartan or the SOD mimetic tempol was administered to sodium overloaded rats in order to block renal Ang II effects and to inhibit oxidative stress production, respectively.

Methods

Male Sprague–Dawley rats (10–12 weeks old; 270–350 g body weight) were used. Animals were housed at controlled temperature (23 ± 2°C) and exposed to a daily 12-h light–dark cycle (lights on from 07:00 a.m. to 07:00 p.m.) with free access to tap water and standard rat chow (Isidro Casanova, Buenos Aires, Argentina). Experiments were conducted in accordance with the Institutional University of Buenos Aires guidelines for the care and use of research animals.

The rats were intraperitoneally anesthetized with urethane. After then, a tracheotomy was performed and a PE-90 tube (3-cm long) was inserted into the trachea to maintain an open airway. The left femoral vein was catheterized with a Silastic cannula (0.12 mm i.d.) for continuous infusion. The right carotid artery was also catheterized with a T4 tube for blood sampling and continuous mean arterial pressure (MAP) measurement, by means of a Statham GOULD P23ID transducer coupled to a Grass Polygraph 79D. The bladder was cannulated for urine collection using a PE-75 cannula. A 45-min infusion with isotonic saline solution, 0.15 M NaCl (ISS) allowed reaching a steady diuresis and permitted urine collection in all groups. Then, all the animals were infused for 120 min, at the same rate of 0.04 ml min^{-1} (Syringe Infusion Pump, Sage™, Orion, Mass, USA). The following groups were studied: group *C* (control): infused with SSI (NaCl 0.15 M), and three experimental groups subjected to an acute sodium overload, respectively, named: group *Na*: infused with hypertonic saline solution (NaCl 1.0 M); group *Los*: infused with NaCl 0.15 M + losartan (Merck, Buenos Aires, Argentina) (10 mg kg^{-1} in bolus); group *Temp*: infused with NaCl 0.15 M + tempol (Sigma-Aldrich Inc., Saint Louis, Missouri, USA) (0.5 mg min^{-1} kg^{-1}); group *Na–Los*: infused with NaCl 1.0 M + losartan (10 mg kg^{-1} in bolus) and group *Na–Temp*: infused with NaCl 1.0 M + tempol (0.5 mg min^{-1} kg^{-1}). Two blood samples were collected at 60 and 120 min and urine was collected along 30 min periods (from 0 to 120 min) for sodium, potassium, and creatinine measurements. MAP was continuously monitored during all the procedures.

Urine and blood measurements

Urinary and plasmatic sodium, potassium, and creatinine were measured by standard methods using an autoanalyzer. Creatinine clearance was assessed in order to evaluate the glomerular filtration rate (GFR). GFR and sodium fractional excretion (FE_{Na}) were calculated according to a standard formula. Urinary flow (UV) is expressed as μl min^{-1}; plasmatic sodium and potassium as mEq L^{-1}, sodium as μmol min^{-1}, GFR as ml min^{-1}, and FE_{Na} as percentage.

Kidney processing for histological examination

At the end of the infusion period, the left kidney was perfused with ISS through the abdominal aorta until the blood was washed out and the parenchyma showed a pale appearance. The kidney was rapidly excised, decapsulated, longitudinally cut, and harvested for immunohistochemical studies.

Tissues were fixed in phosphate-buffered 10% formaldehyde (pH 7.2) and embedded in paraffin. For immunohistochemistry, sections were deparaffined and rehydrated, and endogenous peroxidase activity was blocked by treatment with 0.5% H_2O_2 in methanol for 20 min. Samples were sectioned to 3 μm thickness, stained with Masson's trichromic, and were examined by light microscopy for tubular injury and interstitial fibrosis. Immunostaining of Ang II, hypoxia inducible factor-1α (HIF-1α), NF-κB, TGF-β1, endothelial nitric oxide synthase (eNOS), smooth muscle actin (α-SMA), and RANTES were detected using the following specific monoclonal antibodies: human anti-Ang II (Peninsula, CA; dilution of 1:500), rabbit anti-HIF-1α (Novus Biologicals, Inc., Littleton, CO; dilution: 1:1,000), rabbit anti-NF-κB p65 (Santa Cruz Biotechnology, Inc, California, USA), rabbit anti-TGF-β1 (Santa Cruz Biotechnology, Inc, California, USA), rabbit anti-eNOS (Santa Cruz Biotechnology, Inc, California, USA), mouse anti-α-SMA (Santa Cruz Biotechnology, Inc, California, USA), and goat anti-RANTES (Santa Cruz Biotechnology, Inc, California, USA; dilution: 1:200), respectively. Immunostaining was carried out by means of a commercial modified avidin–biotin–peroxidase complex technique (Vectastain ABC kit, Universal Elite, Vector Laboratories, Burlingame, California, USA) and counterstained with hematoxylin.

Histological sections were observed in a Nikon E400 light microscope (Nikon Instrument Group, Melville, NY). All measurements were carried out using image analysis software (Image-Pro Plus ver. 4.5 for Windows, Media Cybernetics, LP, Silver Spring, MD). The identification of different nephron segments was based on main cell characteristics, which were observed in the histological Pictures (Venkatachalam and Kriz, 1992).

Immunoreactivities for Ang II, HIF-1α, NF-κB, TGF-β1, RANTES, α-SMA, and eNOS are expressed as percentage of positive stained area ± SEM.

Statistical analysis

Results from urine and blood measurements and MAP levels are expressed as mean ± SEM. Gaussian distribution was evaluated by the Kolmogorov and Smirnov method, and comparisons among groups were carried out using ANOVA followed by the Newman–Keuls test. *P*-values <0.05 were considered significant.

Results

Mean arterial pressure and plasma measurements

As we have previously reported (Hong and Garvin, 2007), the acute 2-h salt overload (Na group) did not increase MAP

(mmHg) compared with basal levels (basal 95 ± 2 vs. after salt overload 91 ± 2). When losartan was infused alone (Los group), it decreased MAP values compared with its own basal levels (basal: 96 ± 4 vs. after Los: 63 ± 4, $P < 0.01$). In addition, when losartan was simultaneously administered with sodium overload (Na–Los group), no change was observed compared with its own basal period levels (basal: 94 ± 4 vs. after Na–Los: 84 ± 6), but it decreased MAP with respect to C group after 120 min of ISS infusion (C: 98 ± 4 vs. after Na–Los: 84 ± 6, $P < 0.05$). On the other hand, tempol administration did not reduce MAP levels, neither when it was administered alone (basal: 92 ± 2 vs. after tempol 87 ± 2), nor when it was co-administered with sodium overload (Na–Temp, basal: 96 ± 5 vs. after tempol 96 ± 3). As we have previously reported (Rosón et al., 2006a), plasmatic Na concentration ($mEq\,L^{-1}$) increased after sodium overload, compared with the control group (C: 137.6 ± 0.4, Na: 154.0 ± 1.4, $P < 0.01$). Neither losartan nor tempol administration elicited significant changes in plasmatic Na concentrations in Na overloaded rats (Na–Los: 153.2 ± 1.6; Na–Temp: 154.1 ± 1.0). Plasmatic potassium levels ($mEq\,L^{-1}$) were altered neither by sodium overload nor by losartan or tempol, when compared to control animals (C: 2.8 ± 0.1, Na: 3.0 ± 0.1, Na–Los: 3.0 ± 0.1, and Na–Temp: 2.8 ± 0.1).

Urinary measurements

Figure 1 shows urine flow, urinary sodium excretion, and urinary sodium and potassium concentrations. We have previously reported that an acute sodium overload increased urine flow and sodium excretion (Rosón et al., 2006a). Losartan administration caused an increase in urine flow in acute sodium overloaded rats in a greater proportion than that of urinary sodium excretion, when compared with Na group; thus, urine sodium concentration diminished. In contrast, tempol administration increased both urinary sodium excretion and urinary flow in a similar proportion. Therefore, tempol did not decrease urinary sodium concentration as losartan did. Neither losartan nor tempol administration modified urinary potassium excretion, which was increased by the acute sodium overload.

Glomerular filtration rate and fractional sodium excretion

We have shown (Rosón et al., 2006a) that an acute sodium overload increased the GFR, inducing glomerular hyperfiltration. Losartan prevented that increase in Na group after the first 60 min infusion period. On the other hand, tempol did not modify the GFR in the Na group at any time (Fig. 2, top).

As shown in Figure 2 (bottom), the acute sodium overload increased FE_{Na}. When losartan or tempol were co-administered, a further increase in FE_{Na} was observed.

Immunohistochemical expression in renal sections

The analysis of renal sections obtained from rats subjected to sodium overload revealed increased Ang II, HIF-1α, NF-κB (Fig. 3), and TGF-β1 (Fig. 4) staining, which was mainly localized in proximal convolute tubules (PCT) and cortical collecting ducts (CCDs) (Fig. 5) and medullar collecting ducts (MCDs) (Fig. 6), with respect to control group. RANTES expression only increased in CCD (Fig. 5) and MCD (Fig. 6). Acute sodium overload reduced eNOS expression, compared to control

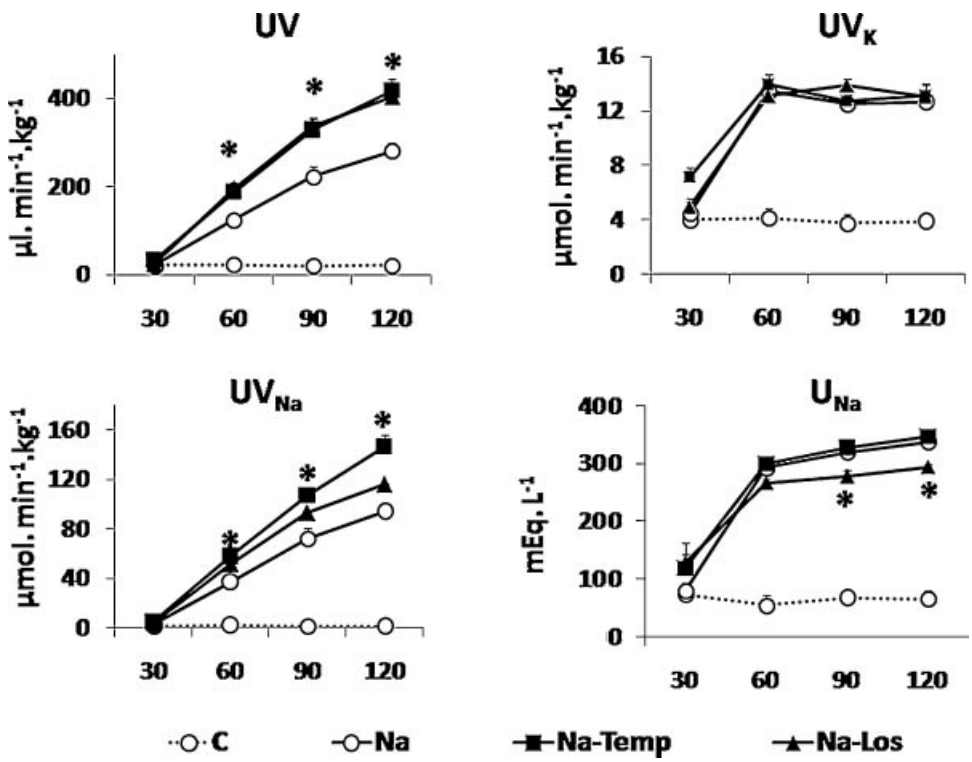

Fig. 1. UV, urinary flow; UV_K, potassium urinary excretion; UV_{Na}, sodium urinary excretion; U_{Na}, urinary sodium concentration along 120 min in control groups infused with isotonic saline (C) and experimental groups infused with sodium overload (Na); sodium overload plus losartan (Na–Los) and sodium overload plus tempol (Na–temp). C: Open circle, dash line; Na: open circle; Na–Los group: closed triangle; Na–temp (closed square). Values are expressed as mean ± SEM; n = 5–8. *$P < 0.05$ versus Na group.

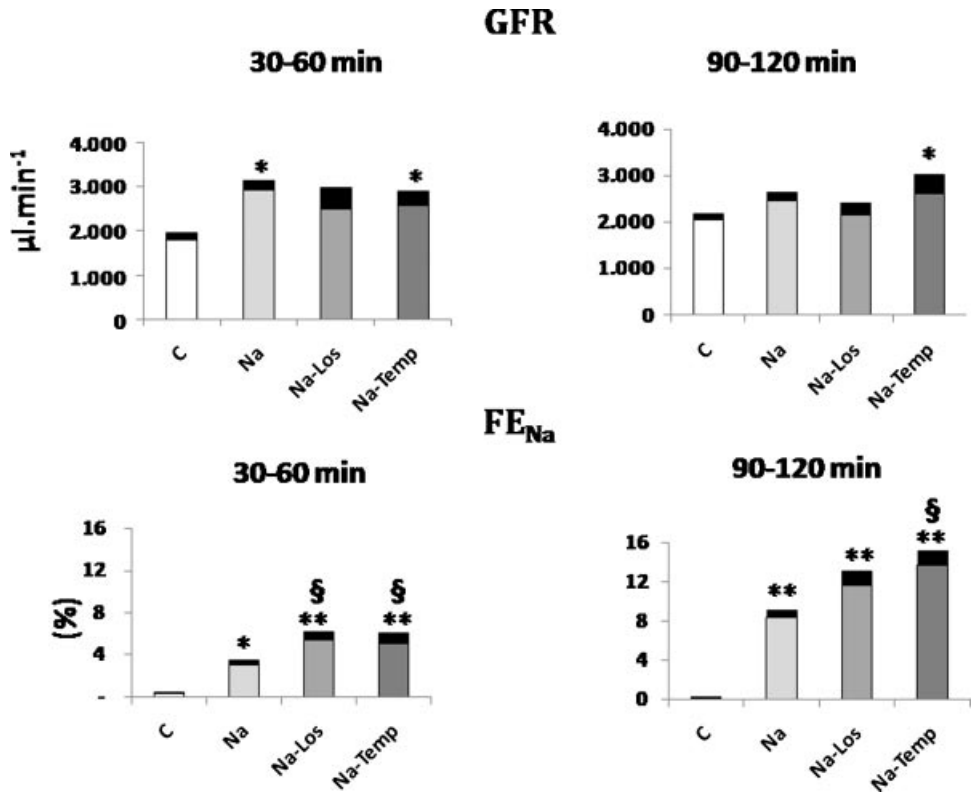

Fig. 2. Glomerular filtration rate (GFR, top) and fractional excretion sodium (FE_{Na}, bottom) along 60 and 120 min periods in control groups infused with isotonic saline (C) and experimental groups infused with sodium overload (Na); sodium overload plus losartan (Na–Los) and sodium overload plus tempol (Na–temp). Values are expressed as mean ± SEM; n = 5–8. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus C group; $^{\S}P < 0.05$ versus Na group.

group, in renal cortex (C: 5.49 ± 0.24%, Na: 4.30 ± 0.18%; $P < 0.05$) and medulla (C: 4.08 ± 0.21%, Na: 2.87 ± 0.13%; $P < 0.05$). Neither the staining with Trichromic-Maisson, nor the α-SMA immunoexpression revealed histopathological signs of fibrosis in Na group (data not shown).

Effects of losartan and tempol on renal proximal tubules

The renal staining of none of the inflammatory markers studied in rats infused with ISS was modified by losartan or tempol administration, in any of the areas examined (data not shown). Increased Ang II, HIF-1α, and NF-κB staining in Na overloaded rats was reduced in proximal tubules when losartan was administered (Fig. 3) but losartan failed to restore HIF-1α and to decrease TGF-β1 staining (Fig. 4). On the other hand, tempol caused a further inhibition of Ang II, HIF-1α, and NF-κB expression (Fig. 3) and restored TGF-β1 staining in Na overloaded rats, reaching similar levels to those observed in C group (Fig. 4). RANTES staining was neither modified by sodium overload nor by losartan or tempol co-administration (Fig. 4). In addition, losartan increased eNOS expression in renal cortex, compared with Na group (7.09 ± 0.35% vs. 4.30 ± 0.18%; $P < 0.001$) and medulla (6.18 ± 0.20% vs. 2.87 ± 0.13%; $P < 0.001$). Moreover, the increase in eNOS expression elicited by tempol was higher than that stimulated by losartan in renal cortex (10.44 ± 0.59% vs. 7.09 ± 0.35%; $P < 0.001$) and medulla (7.49 ± 0.49% vs. 6.18 ± 0.20%; $P < 0.05$).

Effects of losartan and tempol on cortical collecting ducts

Losartan reduced Ang II, HIF-1α, and NF-κB staining in CCDs of sodium overloaded rats, but it did not attenuate TGF-β1 and RANTES staining. Whereas tempol reduced Ang II, HIF-1α, NF-κB, and TGF-β1 staining in CCDs of sodium overloaded rats, it lacked any effect on RANTES staining, like losartan (Fig. 5).

Effects of losartan and tempol on medullar collecting ducts

Losartan and tempol markedly reduced Ang II, HIF-1α, and NF-κB expression in MCDs in acute sodium overloaded rats. While losartan did not attenuate TGF-β1 and RANTES staining, already overstained in these rats, tempol diminished the overexpression of both markers (Fig. 6).

Discussion

In the present study, we demonstrate that the early expression of inflammatory markers observed in the kidney of rats submitted to sodium overload was associated with the simultaneous decrease in eNOS expression and increase in HIF-1α expression, which suggests greater oxidative stress. Both losartan and tempol had a natriuretic effect and inhibited Ang II up-regulation. However, only tempol showed an antiinflammatory effect accompanied by an antioxidant action, since it increased eNOS expression and lowered HIF-1α expression to control level. On the contrary, AT1 receptor inhibitor losartan, though it increased eNOS expression, did not restore HIF-1α expression and did not prevent the overexpression of inflammatory markers in renal tubules. These results demonstrate an antiinflammatory effect of tempol associated with an antioxidant action, independently of renal Ang II expression.

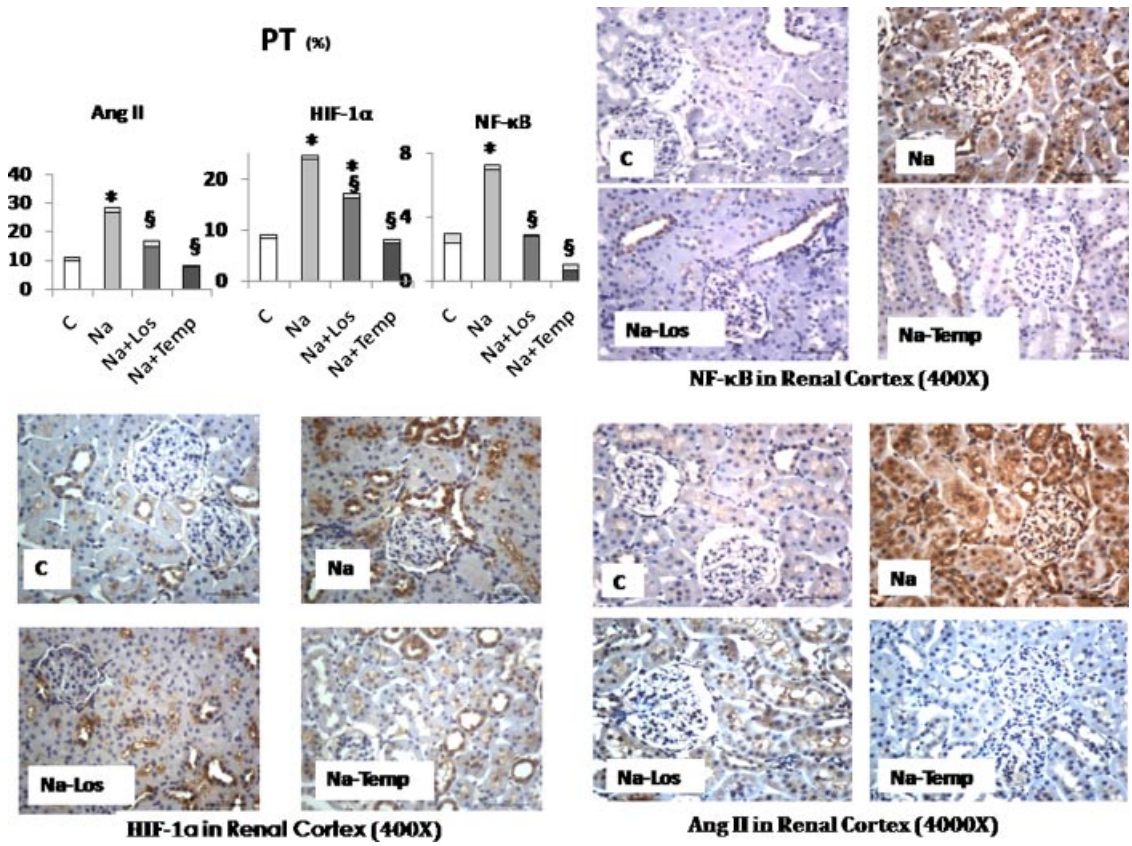

Fig. 3. Immunohistochemical expression of Ang II, HIF-1α, and NF-κB in PCT in rats submitted to 120 min infusions: control groups infused with isotonic saline (C) and experimental groups infused with sodium overload (Na); sodium overload plus losartan (Na–Los) and sodium overload plus tempol (Na–temp). Values are expressed as mean ± SEM; n = 5–8. *$P < 0.05$ and **$P < 0.01$ versus C group; §$P < 0.05$ versus Na group. Representative images of positive staining (original magnification 400×) of Ang II (top right), HIF-1α (bottom, left), and NF-κB (bottom, right) in renal cortex. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In earlier studies from our laboratory we have observed that rats submitted to sodium overload showed hyperfiltration, an increased Na tubular reabsorption, accompanied by enhanced renal Ang II and HIF-1α expression and inflammatory markers in renal tubules (Rosón et al., 2006a). In the present study, besides we observed lower eNOS expression. These considerations suggest that the oxidative stress produced by an imbalance between the production of NO and O_2^- in the kidney could play a role in the pathogenesis of the inflammation. It is well known that several factors, like the hyperfiltration as well as the peripheral sympathetic nervous system or the oxidative stress, regulate angiotensinogen expression and consequently, Ang II production in proximal tubules (Quan and Baum, 2002; Gociman et al., 2004). Furthermore, Ang II induces, through AT1 receptor stimulation, angiotensinogen gene expression, and in consequence, Ang II expression is enhanced by a positive feed back mechanism (Eggena et al., 1993; Merjan et al., 2001). It is also known that renal tubular Ang II regulates NADPH oxidase-dependent O_2^- production, and consequently the HIF-1α stabilization (Kaewpila et al., 2008). On the other hand, Ang II regulates tubular sodium transport, stimulates Na+–K+– ATPase activity and ATP consumption, decreases ATP concentration, leading to a diminished eNOS expression (Silva and Garvin, 2009). In order to evaluate the participation of intrarenal Ang II in the inflammatory response, we studied losartan effects, as a specific antagonist of AT1 receptors. Losartan normalized Ang II and NF-κB expression in Na group reaching similar levels to controls animals, but it failed to restore GFR and HIF-1α immunoexpression, which was partially decreased. Moreover, losartan did not modify RANTES and TGF-β1 expression, although it simultaneously enhanced eNOS expression. In addition, losartan exerted a natriuretic effect without changing the MAP. While losartan inhibited partially the hyperfiltration, it normalized the Ang II expression to control levels. We assume that another cause independent from the hyperfiltration could be involved in Ang II expression, that is, sympathic nervous activity or oxidative stress produced by a mechanical effect (Quan and Baum, 2002; Garvin and Hong, 2008). However, Ang II, besides its known binding to AT1 or AT2 plasma membrane receptors, it may be internalized and translocated to the nucleus, where it would directly interact with the receptor located near the nuclear membrane (Merjan et al., 2001). In this way, specific AT1 receptors have been shown at the nuclear membrane, where they induce the transcription of renin and angiotensinogen mRNA modulating their synthesis (Eggena et al., 1993). Then, we cannot exclude that losartan modulates the signaling pathway of Ang II independently of hyperfiltration. These facts could explain why, while GRF inhibition was a partial effect, on the other hand, losartan normalized Ang II expression to control levels. Independent of the cause that produces up-regulation of Ang II expression, losartan enhanced eNOS expression but could neither restore HIF-1α expression nor prevent the inflammatory response. Our results suggest that

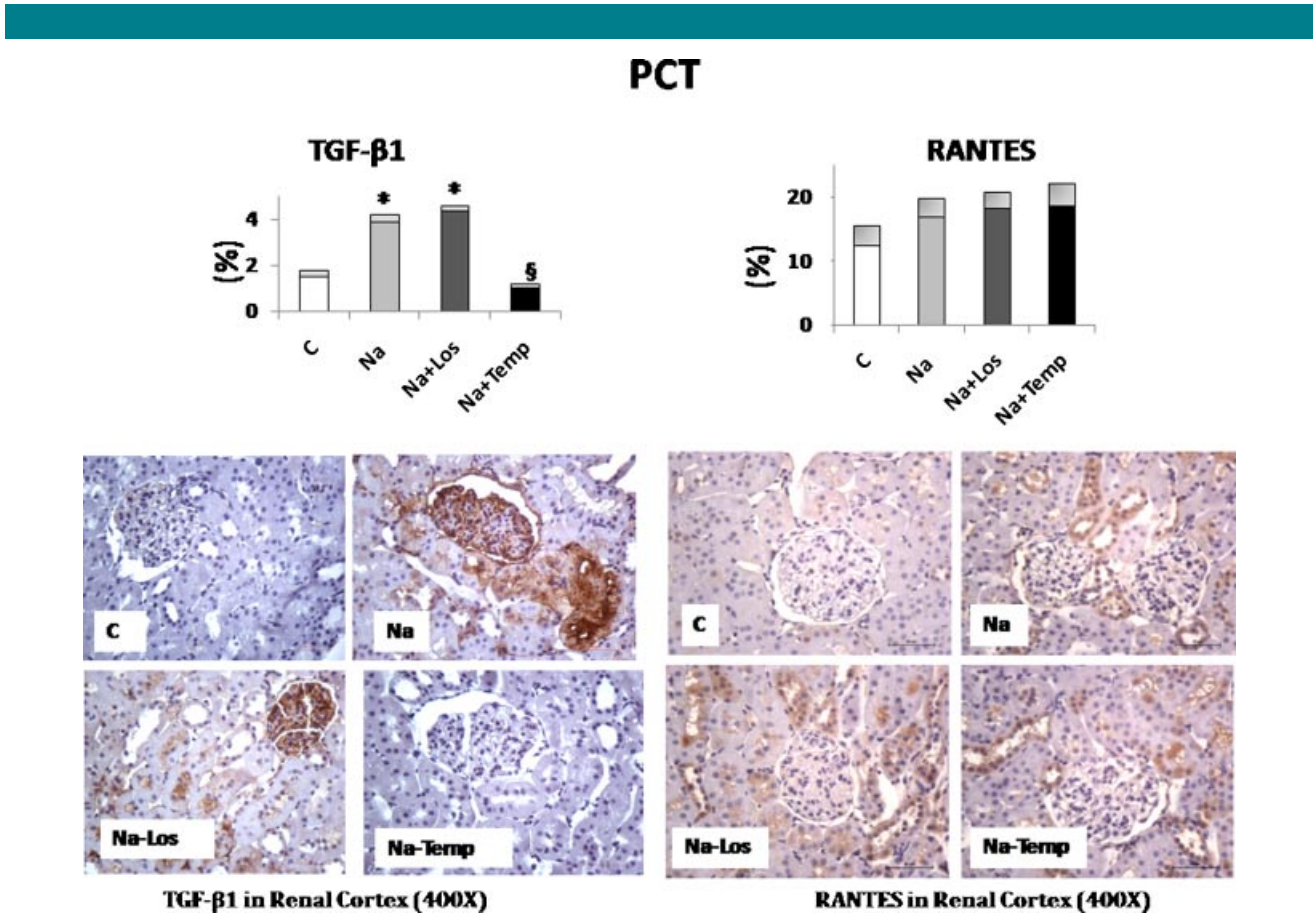

Fig. 4. Immunohistochemical expression of TGF-β1 and RANTES in PCT in rats submitted to 120 min infusions: control groups infused with isotonic saline (C) and experimental groups infused with sodium overload (Na); sodium overload plus losartan (Na–Los) and sodium overload plus tempol (Na–temp). Values are expressed as mean ± SEM; n = 5–8. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus C group; $^{\S}P < 0.05$ versus Na group. Representative images of positive staining (original magnification 400×) of TGF-β1 (bottom, left) and RANTES (bottom, right) in renal cortex. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

another pathway, independent of the up-regulation of renal Ang II, may be responsible of HIF-1α increase in sodium overloaded rats. Besides, since losartan increased natriuresis and $FE_{Na,}$ we suggest that increased renal Ang II is more involved in Na tubular transport.

On the other hand, tempol, a SOD mimetic, which scavenges O_2^- production and enhances NO bioavailability (Wilcox and Pearlman, 2008), normalized Ang II, NF-κB, and TGF-β1 immunoexpression in PCT and CCD and RANTES in MCD to control levels, and augmented natriuresis without affecting MAP or hyperfiltration. These effects were simultaneously accompanied by HIF-1α normalization and eNOS expression, suggesting inhibition of oxidative stress. The oxidative stress produced by the increase of GFR or the tubular stretch may be the signaling for angiotensinogen–renin–Ang II cascade activation. Tempol inhibited Ang II staining restoring the levels observed in Na group and simultaneously maintained the hyperfiltration observed salt overloaded animals. These results suggest that hyperfiltration would not be the cause of Ang II up-regulation. However, we cannot exclude that the hyperfiltration is the cause of oxidative stress which may activate the angiotensinogen–Ang II cascade. Another possibility to justify tempol effects on renal Ang II would be based on its action over the renal sympathetic system. Renal sympathetic system stimulates Ang II production (Quan and Baum, 2002), and tempol by inhibiting this system, could be reducing Ang II synthesis (Shokoji et al., 2004).

The natriuretic action of tempol agrees with the literature. It has been shown that tempol produces natriuresis regulating Na–K–ATPase, Na+/H+ interchanger, Na+–K+–2Cl co-transporter and ENaC activities and increasing medullar renal blood flow (Chen et al., 2007; Wilcox and Pearlman, 2008). Furthermore, tempol prevents dopamine D1 receptors down-regulation in proximal tubules of rats submitted to oxidative stress, leading to a reduction in sodium reabsorption by proximal tubules (Fardoun et al., 2006). In addition, tempol administration to salt-sensitive rats fed a high salt diet prevented the enhancement of intrarenal angiotensinogen expression, decreasing this way antinatriuretic Ang II actions (Kobori and Nishiyama, 2004). However, considering that both tempol and losartan inhibited sodium transport and increased natriuresis, but only tempol decreased the overexpression of inflammatory markers, while losartan lacked these effects, we suggest that the beneficial effect of tempol was more related to its antioxidant role, than to its natriuretic effects.

Our results are in agreement with previous reports, which have shown that the acute response to tempol administration in anesthetized rats was an antioxidant effect, besides its diuretic and natriuretic actions (Chen et al., 2007).

Then, it is possible that another source of O_2^-, independent of Ang II–AT1 receptor signaling, may be responsible for the inflammatory marker overexpression in sodium overloaded rats, which were not modified by losartan. As known, some mechanical factors as hyperfiltration, tubular epithelial stretch

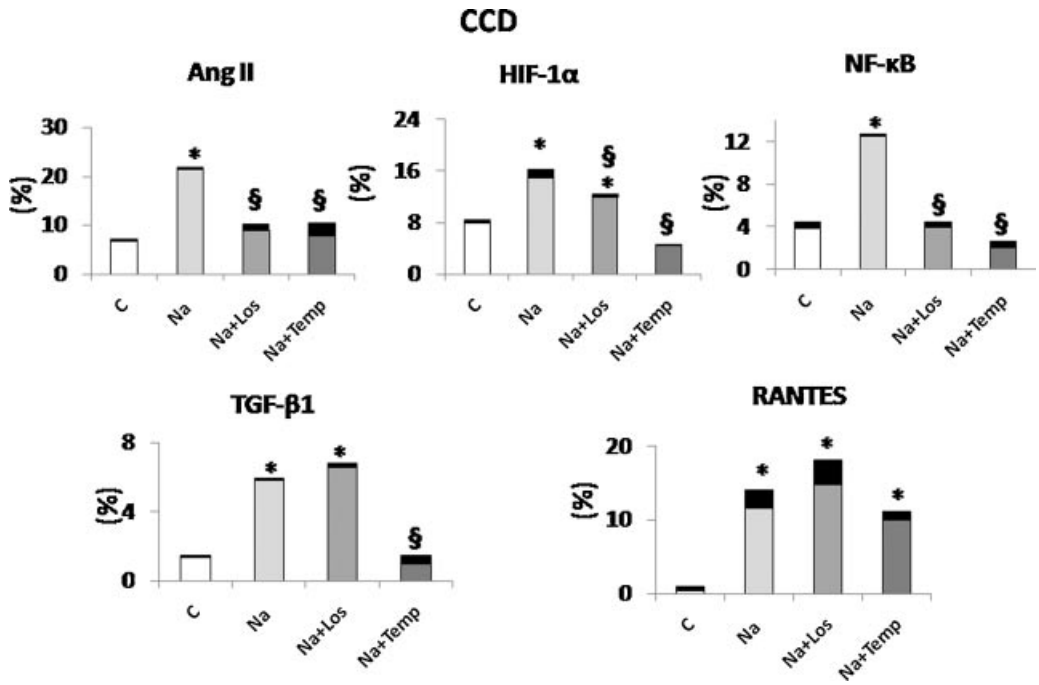

Fig. 5. Immunohistochemical expression of Ang II, HIF-1α, NF-κB, TGF-β1, and RANTES in CCD in rats submitted to 120 min infusions: control group infused with isotonic saline (C) and experimental groups infused with sodium overload (Na); sodium overload plus losartan (Na–Los) and sodium overload plus tempol (Na–temp). Values are expressed as mean ± SEM; n = 5–8. *$P < 0.05$ versus C group; §$P < 0.05$ versus Na group.

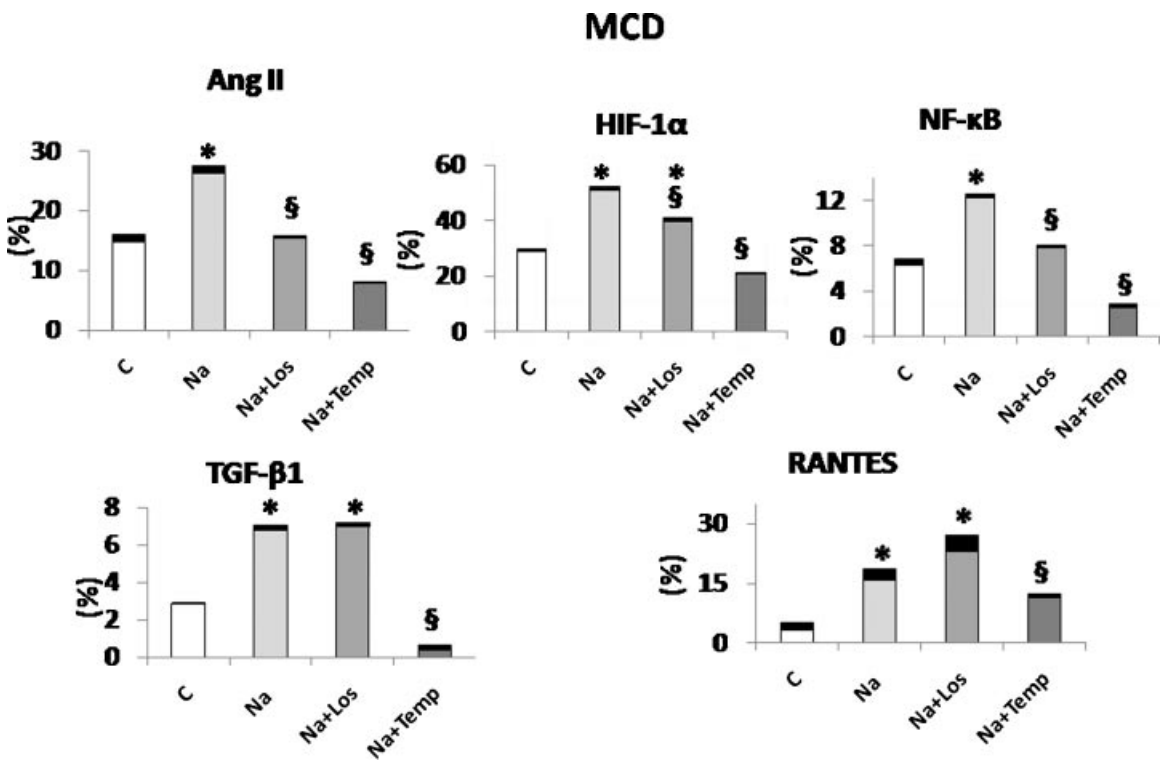

Fig. 6. Immunohistochemical expression of Ang II, HIF-1α, NF-κB, TGF-β1, and RANTES in MCD in rats submitted to 120 min infusions: control groups infused with isotonic saline (C) and groups infused with sodium overload (Na); sodium overload plus losartan (Na–Los) and sodium overload plus tempol (Na–temp). Values are expressed as mean ± SEM; n = 5–8. *$P < 0.05$ versus C group; §$P < 0.05$ versus Na group.

and/or the epithelial sodium transport can be able to generate oxidative stress, inducing in turn cytokines and chemokines overexpression (Hong and Garvin, 2007; Garvin and Hong, 2008). It has been demonstrated that TGF-β1 was up-regulated by the oxidative stress and inhibited by NO (Ying and Sanders, 2003; Ying et al., 2008). Therefore, O_2^- up-regulation as well as lower NO availability, may contribute, at least in part, to stimulate TGF-β1 production. We have observed that tempol, but not losartan, prevented the increase in TGF-β1 expression in rats submitted to sodium overload. Therefore, it is probable that TGF-β1 overexpression in sodium overloaded group was a consequence of the oxidative stress independently of Ang II.

RANTES overexpression was observed in CD but not in PCT. Renal interstitium hypertonicity is one of the main causes of oxidative stress by production of reactive oxygen species of mitochondrial origin (Yang et al., 2005; Zhou et al., 2006) and inflammation. Then, a higher interstitium hypertonicity in CD than in PCT may be related to different RANTES staining in those nephron segments. In addition, tempol favors renal blood flow and inhibits sodium transport, increasing this way natriuresis and diminishing medullar interstitial tonicity. Then, we suggest that the different response to tempol in PTC and MCD regarding RANTES staining may be related to tempol actions tending to diminish medullar tonicity and reduce the oxidative stress. Moreover, RANTES overexpression observed in CD of sodium overloaded rats was unchanged by losartan, but it was diminished by tempol only in renal medulla. High extracellular sodium concentration constitutes a challenge to the normal cell function and stimulates the inflammatory response in intestinal, bronchial, and renal epithelial cells (Tabary et al., 2000). A hyperosmotic stress results in activation of osmosensitive NF-κB, MAPK signaling pathways and expression of IL-8 and RANTES (Tabary et al., 2003). In addition to NF-κB, the genes expression of a variety of other transcription factors (AP-1, CREB) has been shown to be affected by salt-induced stress. Present results do not rule out the possibility that increased RANTES could be a consequence of Ang II stimulation of AT2 receptors (Wolf et al., 1997).

In conclusion, Ang II and the oxidative stress are involved in the activation of transcriptional factors in rats submitted to acute sodium overload. However, only tempol was effective in down-regulating early inflammatory markers TGF-β1 and RANTES expression. These effects can be associated with an enhanced eNOS and simultaneously decreased HIF-1a expression. Further studies are required to characterize and delineate these interactions at the level of the production of O_2^- and NO in the kidney.

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