Impaired pressure natriuresis resulting in salt-sensitive hypertension is caused by tubulointerstitial immune cell infiltration in the kidney

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Franco M, Tapia E, Bautista R, Pacheco U, Santamaria J, Quiroz Y, Johnson RJ, Rodriguez-Iturbe B. Impaired pressure natriuresis resulting in salt-sensitive hypertension is caused by tubulointerstitial immune cell infiltration in the kidney. Am J Physiol Renal Physiol 304: F982–F990, 2013. First published January 30, 2013; doi:10.1152/ajprenal.00463.2012.— Immune cell infiltration of the tubulointerstitium in experimental models of hypertension is a key role in the impairment of pressure natriuresis that results in salt-dependent hypertension. We evaluated the relationship between the renal inflammation and pressure natriuresis in the model of SSHTN that results from transient oral administration of NaNO2-nitro-l-arginine methyl ester (l-NAME). Pressure natriuresis was determined in Wistar rats that received 4 wk of a high-salt (4% NaCl) diet, starting 1 wk after stopping l-NAME, which was administered alone (SSHTN group, n = 17) or in association with mycophenolate mofetil (MMF; MMF group, n = 15). The administration of MMF in association with l-NAME is known to prevent the subsequent development of SSHTN. Control groups received a high (n = 12)- and normal (0.4%)-salt diet (n = 20). Rats with SSHTN had increased expression of inflammatory cytokines and oxidative stress. The severity of hypertension correlated directly (P < 0.0001) with the number of tubulointerstitial immune cells and angiotensin II-expressing cells. Pressure natriuresis was studied at renal arterial pressures (RAPs) of 90, 110, 130, and 150 mmHg. Glomerular filtration rate was similar and stable in all groups, and renal blood flow was decreased in the SSHTN group. Significantly decreased natriuresis (P < 0.05) was found in the SSHTN group at RAPs of 130 and 150 mmHg, and there was an inverse correlation (P < 0.01) between the urinary sodium excretion and the number of tubulointerstitial inflammatory cells (lymphocytes and macrophages) and cells expressing angiotensin II. We conclude that tubulointerstitial inflammation plays a key role in the impairment of pressure natriuresis that results in salt-dependent hypertension in this experimental model.

angiotensin II-positive cells; lymphocytes; macrophages; mycophenolate mofetil; urinary sodium excretion

It is assumed that the role played by renal inflammation is related to the impairment in the renal capacity to excrete sodium, since the central event in the pathogenesis of salt-induced hypertension is impairment in the pressure-natriuresis relationship. Pressure natriuresis refers to the increment in urinary sodium excretion (UNaV) that occurs when blood pressure rises and is the consequence of complex modifications in renal interstitial hydrostatic pressure, medullary blood flow, nitric oxide, reactive oxygen species, prostaglandins, and angiotensin II activity (8, 30, 36) that result in coordinated decreases in the surface distribution of apical sodium-hydrogen exchangers and basolateral Na-K ATPase activity (27). In SSHTN, the pressure-natriuresis relationship is less steep and shifted to the right so that higher blood-pressure levels are necessary to drive the natriuretic response required to maintain sodium balance (41). Tubulointerstitial inflammation may impair UNaV via several mechanisms. Inflammation may lead to a loss of peritubular capillaries, tubular injury, and fibrotic interstitial changes. If these changes are severe and extensive, they may cause salt wasting, but milder changes that do not render inoperative the sodium reabsorption mechanisms are associated with SSHTN. This may be due to reduced oxygen delivery to regions of the kidney that function normally under relative hypoxia, causing a further reduction in oxygen partial pressure with the generation of oxidative stress and impaired pressure natriuresis (16). In addition, the infiltration of immune-competent cells in the renal interstitial space is associated with inflammation-induced generation of reactive oxygen species and increased local angiotensin II activity (7, 41, 42, 51) that suppress natriuresis.

The present investigations were directed to test the assumption that a consequence of tubulointerstitial inflammation is the blunting of the pressure-natriuresis response. In these studies, we selected an experimental model of SSHTN that follows recovery from nitric oxide synthase inhibition with NaNO2-nitro-l-arginine methyl ester (l-NAME) (34). In this model, 3 wk of l-NAME administration cause hypertension and significant renal injury and inflammation during the period l-NAME is given. Blood pressure returns to normal 1 wk after l-NAME is omitted, but if a high-salt diet is administered subsequently, hypertension develops in the following weeks. Treatment with mycophenolate mofetil (MMF) during l-NAME administration does not modify the blood-pressure elevation, due to inhibition of nitric oxide synthase, but ameliorates renal histological damage and inflammation caused by l-NAME and prevents the subsequent development of SSHTN (34). Important for the selection of this model in the present experiments are the findings that the histological damage that could, by itself, cause impairment in sodium excretion improves after l-NAME is discontinued, and several weeks later, the l-NAME-
treated groups (with or without MMF treatment) present mild and comparable renal injury. At this time, the salient feature is that salt-induced hypertension is associated with tubulointerstitial infiltration of immune cells, a characteristic that is common to most, if not all, models of SSHTN (6, 34, 37–40, 42, 43).

**MATERIALS AND METHODS**

Male Wistar rats (250–320 g) were housed in institutional animal facilities with free access to food and water. All studies were performed in accordance with the Mexican Federal Regulation for Experimentation and Care (NOM-062-ZOO-2001), and the investigation was approved by Bioethics and Investigation Committees of Instituto Nacional de Cardiologia Ignacio Chavez. The following experimental groups were studied: 1) SSHTN group (n = 17), which received l-NAME (Sigma, St. Louis, MO) in the drinking water at a concentration of 70 mg/100 ml for 3 wk, followed (after a washout period of 1 wk and return to normal blood pressure) by the administration of a high-salt diet (4% NaCl Teklad diet; Harlan Laboratories, Madison, WI) for 4 wk; and 2) the MMF group (n = 15), which received l-NAME, as in the SSHTN group, but in addition, received MMF (CellCept; Roche Pharmaceutical, Nutley, NJ) by gastric gavage in daily doses of 20 mg/kg during the initial 3 wk of the study (nitric oxide synthesis inhibition) and then stopped; thus this drug was not given during the subsequent 5 wk that preceded the pressure-natriuresis studies (1 wk of washout and 4 wk of a high-salt diet). MMF is relatively insoluble in water and therefore, was suspended by vigorous agitation before administration, as in previous studies (38, 44). Control groups received a high (4% NaCl)-salt diet (C-HSD; n = 12) and normal (0.4% NaCl)-salt diet (C-NSD; n = 20).

Additional groups of rats were studied to assess the renal injury resulting from l-NAME administration. In these studies, rats that received l-NAME (n = 6) and l-NAME + MMF (n = 7) were killed at the end of 3 wk of l-NAME administration, and renal histology and immunohistology were examined. Control rats (n = 5) were studied at the same time. All of these rats were receiving a normal-salt diet.

Finally, whereas MMF was given only during the l-NAME administration and therefore, had been stopped 5 wk after the pressure-natriuresis studies (1 wk of washout and 4 wk of a high-salt diet), the possible lingering effects of MMF administration on the pressure natriuresis were evaluated in separate experiments done in normal rats studied 4 wk after they received MMF for 3 wk. These studies showed a normal pressure natriuresis response (data not shown).

**Pressure-natriuresis experiments.** Pressure-natriuresis experiments were performed after 4 wk of a high-salt diet, as described by Wang et al. (52). Rats were anesthetized with pentobarbital sodium (30 mg/kg intraperitoneally) and placed on a surgical table with temperature control to maintain body temperature at 37°C. A patent airway was maintained with a PE-240 tube inserted in the trachea. A catheter in the left jugular vein was used for fluid administration, and a catheter was inserted in the left femoral artery to measure arterial pressure that was monitored continuously with a pressure transducer (model P23XL; Gould, Hato Rey, Puerto Rico) and recorded on a polygraph (Grass Instruments, Quincy, MA), as well as to take blood samples. The left kidney was exposed and placed in a luteal holder, covering the kidney surface with Ringer solution. A 2-mm flow probe was placed around the left renal artery for measuring renal blood flow (RBF) by an electromagnetic flow meter (model T10; Transonic Systems, Ithaca, NY) in seven to 10 rats of each experimental and control groups. An adjustable aortic clamp was placed above the left renal artery and used to control distal blood pressure. To obtain data at renal arterial pressure (RAP) of 150 mmHg in normotensive rats (seven rats from the C-NSD group, five rats from the C-HSD group, and six rats from the MMF group), the blood pressure was elevated by transient occlusion of carotid arteries (1), 20 min before beginning the first pressure-natriuresis evaluation, and was maintained for the rest of the experiment. Progressive reduction in RAP in steps of ~20 mmHg was maintained at a stable level by means of the aortic clamp; urine and blood collections were obtained at 150, 130, 110, and 90 mmHg. The left ureter was catheterized for urinary collections. Rats were maintained euvoletic by infusion of 10 ml/kg body wt of isotonic rat plasma during surgery, followed by an infusion of 10% polyfructosan (Inutest; Laevesan-Gesellschaft, Austria) in 0.9% sodium saline solution at a 2.2-ml/h rate, and replacement of blood samples was obtained for analysis. The measurements were started after 1 h of stabilization. After 10 min equilibration at each blood-pressure level, 30-min urinary collections were obtained for analyses. Plasma samples were obtained at midpoint in urinary collections. At the end of the experiment, the kidneys were removed, weighted, and harvested; the animals were killed by a pentobarbital overdose; and the kidneys were weighed and harvested for histology and immunohistology studies.

**Chemical analyses.** Blood and urine samples were collected for determination of glomerular filtration rate (GFR), UNaV, and fractional excretion of sodium (FENa,E). Urine volume was determined gravimetrically. Polyfructosan concentrations were determined as described by Davidon and Sackner (5) and sodium concentrations by flame photometry.

**Histology and immunohistology.** All histological evaluations were done without prior knowledge of the experimental group being studied. Histological studies were done in kidney sections of nine rats from the C-NSD group, eight rats from the C-HSD group, 10 rats from the SSHTN group, and 10 rats from the MMF group. Light microscopy was performed using formalin-fixed sections stained with periodic acid-Schiff and hematoxylin and eosin. Glomerulosclerosis index score and tubulointerstitial damage were evaluated as described in previous studies (34, 39, 40). The sclerosis in the glomeruli was graded from 0 to +4: grade 0 = normal, grade +1 = <25% involvement of the glomerular tuft, grade +2 = 25–50% involvement of the glomerular tuft, grade +3 = 50–75%, and grade +4 = sclerosis occupying >75% of the glomerular tuft. The glomerulosclerosis score was obtained as follows: [(1 × n glomeruli with +1) + (2 × n glomeruli with +2) + (3 × n glomeruli with +3) + (4 × n glomeruli with +4)] × 100/total number of glomeruli examined. The tubulointerstitium was evaluated in successive fields examined at 20× magnification in the entire cortical and juxtamedullary areas of each specimen using computer-assisted image analysis (Olympus BX51 system microscope and DP70 digital microscope camera with Sigma Scan Pro 5 software, San Jose, CA). Tubulointerstitial damage (tubular dilatation, tubular atrophy, and interstitial fibrosis) was graded on a 0–5 scale: 0 = no changes, grade 1 = <10%, grade 2 = 10–25%, grade 3 = 25–50%, grade 4 = 50–75%, and grade 5 = 75–100.

**Identification and quantification of tubulointerstitial immune cell infiltration.** Immunoperoxidase methodology was used to indentify lymphocytes and macrophages in the glomeruli (positive cells/glomerular cross-section) and tubulointerstitial regions (positive cells/mm²). Macrophages were identified with mouse CD68 anti-MAb and lymphocytes with mouse anti-rat CD3 (Biosource International, Camarillo, CA). CD4- and CD8-positive cells were identified with the corresponding MAb (anti-rat CD4, clone W3/25, mouse IgG1; and anti-rat CD8, no azide, clone OX-8, mouse IgG1), obtained from Cedarlane (Hornby, Canada). Angiotensin II-positive cells were identified with rabbit anti-ANG II human IgG (Peninsula Laboratories, San Carlos, CA). Peroxidase-conjugated goat anti-mouse and IgG (Stressgen Bioreagents, Victoria, BC, Canada) and donkey anti-rabbit (Accurate Chemical & Scientific, Westbury, NY) were used as secondary antibodies. Infiltrating immune cells and angiotensin II-positive cells were rarely found in the glomeruli, and only the data in tubulointerstitial areas are given.

**Inflammatory cytokines and oxidative stress.** Renal cortex from five animals of experimental (SSHTN and MMF) and control (C-NSD) groups were used for Western blots (IL-2, IL-6, and nitrotyrosine abundance) and malondialdehyde (MDA) determinations. For the Western blot analyses, 40 µg of protein was treated with 12% SDS-PAGE and transferred onto a nitrocellulose membrane, which
Table 1. Data after 3 wk of l-NAME treatment

<table>
<thead>
<tr>
<th></th>
<th>Control NSD (n = 5)</th>
<th>l-NAME (n = 6)</th>
<th>l-NAME + MMF (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>136.2 ± 4.27</td>
<td>175.5 ± 3.05a</td>
<td>176 ± 3.6b</td>
</tr>
<tr>
<td>Plasma creatinine, mg/dl</td>
<td>0.30 ± 0.03</td>
<td>0.40 ± 0.03</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>Proteinuria, mg/24 h</td>
<td>2.8 ± 1.39</td>
<td>12.6 ± 4.80</td>
<td>11.5 ± 3.87</td>
</tr>
<tr>
<td>GS index, 0–400</td>
<td>0</td>
<td>65.3 ± 7.00b</td>
<td>45.7 ± 3.81</td>
</tr>
<tr>
<td>T1 damage score, 0–5</td>
<td>0.5 ± 0.21</td>
<td>1.96 ± 0.28a</td>
<td>1.25 ± 0.154</td>
</tr>
<tr>
<td>CD68 + cells/mm²</td>
<td>3.4 ± 1.12</td>
<td>88.6 ± 9.33a</td>
<td>381.1 ± 5.28b</td>
</tr>
<tr>
<td>CD3 + cells/mm²</td>
<td>0.6 ± 0.58</td>
<td>75.3 ± 7.96d</td>
<td>42.5 ± 5.17a</td>
</tr>
<tr>
<td>AII + cells/mm²</td>
<td>0.8 ± 0.58</td>
<td>28.5 ± 3.51a</td>
<td>12.5 ± 1.23a</td>
</tr>
</tbody>
</table>

Studies done in kidney sections harvested after 3 wk of No-nitro-l-arginine methyl ester (l-NAME) administration in the drinking water (l-NAME group) and l-NAME plus daily mycophenolate mofetil (MMF) treatment (MMF group). Control groups received no treatment. All rats were in a normal (0.4%)-salt diet (NSD). SBP, systolic blood pressure; GS, glomerulosclerosis; T1, tubulointerstitial; AII, angiotensin II-positive. Data are means ± SE. *Values higher than controls (P < 0.01 or lower). Differences between l-NAME and l-NAME + MMF groups are *P < 0.05, *P < 0.001 (multigroup variance analysis), and &P < 0.01.

was washed and probed with polyclonal antibody (1:1,000) against IL-2, IL-6, and nitrotyrosine (Santa Cruz Biotechnology, Santa Cruz, CA), and 1:1,000 goat horseradish peroxidase-labeled anti-rabbit IgG (Santa Cruz Biotechnology). Finally, enhanced chemiluminescence (ECL) detection solution was added, and hyperfilm ECL (GE Healthcare, Chalfont St. Giles, UK) was exposed to the membrane. Each membrane was stripped of bound antibody and reprobed with anti-β-actin on the same membrane for quantitative comparison. The bands were analyzed with a Kodak electrophoresis documentation and analysis system (EDAS 290).

MDA content was determined by the method of Ohkawa et al. (31). Briefly, kidney slices for MDA were placed in a cold mixture of 100 mM KCL and 0.003 EDTA, homogenized, and centrifuged at 600 g for 15 min. Supernatants of this preparation were used for MDA content determination, as described previously (39, 45).

Statistical analyses. GFR, RBF, UNaV, and the FNaE (percent of filtered) are expressed in relation to left-kidney weight. Multigroup ANOVA and Tukey Kramer post-tests were used to examine differences between groups. Serial changes were studied with paired t-tests. Correlations were analyzed with Pearson’s correlation coefficient. Data are shown as means ± SE. Two-tailed P values < 0.05 were considered significant. A commercially available statistical package (InStat; GraphPad Software, La Jolla, CA) was used for statistical calculations.

RESULTS

Blood pressure and histological findings induced by 3 wk of l-NAME administration are shown in Table 1. As in previous studies (34), MMF administration did not modify the hypertension induced by inhibition of nitric oxide synthase or the return to normal blood pressure, 1 wk after the discontinuation of l-NAME (l-NAME; basal = 120 ± 1.7 mmHg, 1 wk = 154 ± 3.1, 2 wk = 163 ± 3.2, 3 wk = 175.5 ± 3.05, 1 wk after discontinuation of l-NAME = 136 ± 30; l-NAME + MMF: basal = 117 ± 1.4, 1 wk = 151 ± 3.6, 2 wk = 151 ± 3.6, 3 wk = 176 ± 3.6, 1 wk after discontinuation of l-NAME = 139 ± 2.4). As shown in Table 1, l-NAME administration resulted in renal injury (glomerulosclerosis and tubulointerstitial damage) and renal infiltration of lymphocytes (CD3 + cells), macrophages (CD68 + cells), and angiotensin II + cells in tubulointerstitial areas. Treatment with MMF during the l-NAME resulted in a significant reduction (albeit not normalization) of renal injury and tubulointerstitial inflammation.

General data obtained prior to the pressure-natriuresis experiments (5 wk after discontinuation of l-NAME) are shown in Table 2. As expected, systolic blood pressure was higher in the rats from the SSHTN group. As in previous studies (34), MMF treatment prevented the subsequent development of SSHTN, and the systolic blood pressure in the MMF group was not significantly different from any of the study groups and essentially comparable in the SSHTN group and the MMF group. Representative microphotographs are shown in Fig. 1.

Renal immunohistologic findings present in kidneys harvested immediately after the pressure-natriuresis experiments are shown in Table 3. During the 5 wk that elapsed after discontinuation of l-NAME (with and without MMF) and the pressure-natriuresis studies, the renal injury and tubulointerstitial inflammation were reduced with respect to the findings at the end of l-NAME treatment (Table 1). At the time when the pressure-natriuresis studies were performed (Table 3), the glomerulosclerosis and tubular damage, although higher in the SSHTN group, were not significantly different in any of the study groups and essentially comparable in the SSHTN group and the MMF group. In contrast, interstitial macrophages (CD68-positive cells), lymphocytes (CD3-positive cells, as well as CD4 and CD8 T cells), and angiotensin II-positive cells were several-fold higher in the group with SSHTN than in the control groups and the MMF group. Representative microphotographs are shown in Fig. 1.

Renal inflammatory cytokines and oxidative stress measurements are shown in Fig. 2. IL-2 (Fig. 2A), IL-6 (Fig. 2B), nitrotyrosine (Fig. 2C), and MDA (Fig. 2D) were increased in the SSHTN group and were suppressed in the group that had been administered MMF previously.

Table 2. General results

<table>
<thead>
<tr>
<th></th>
<th>C-NSD (n = 20)</th>
<th>C-HSD (n = 12)</th>
<th>SSHTN (n = 17)</th>
<th>MMF (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>301.4 ± 4.92</td>
<td>308.0 ± 3.09</td>
<td>302.0 ± 1.35</td>
<td>307.5 ± 3.85</td>
</tr>
<tr>
<td>Prior to pressure natriuresis</td>
<td>438.9 ± 10.72</td>
<td>448.4 ± 16.44</td>
<td>450.0 ± 20.02</td>
<td>452.5 ± 8.69</td>
</tr>
<tr>
<td>Left-kidney weight, g</td>
<td>1.39 ± 0.23</td>
<td>1.45 ± 0.036</td>
<td>1.56 ± 0.040</td>
<td>1.56 ± 0.058</td>
</tr>
<tr>
<td>Food ingestion, g/day</td>
<td>32.0 ± 0.88</td>
<td>30.0 ± 2.29</td>
<td>26.5 ± 1.41</td>
<td>26.5 ± 1.41</td>
</tr>
<tr>
<td>Urine volume, ml/24 h</td>
<td>20.4 ± 4.51</td>
<td>20.0 ± 2.70</td>
<td>26.0 ± 5.38</td>
<td>22.0 ± 3.45</td>
</tr>
<tr>
<td>Proteinuria, mg/24 h</td>
<td>8.25 ± 1.03</td>
<td>8.62 ± 0.85</td>
<td>9.11 ± 1.20</td>
<td>8.77 ± 0.92</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>116.0 ± 1.08</td>
<td>118.8 ± 1.54</td>
<td>157.9 ± 1.91a</td>
<td>132.7 ± 1.64a</td>
</tr>
</tbody>
</table>

Kidney weight was obtained at the end of pressure natriuresis experiments. Mean arterial pressure (MAP) was obtained by direct intra-arterial determinations prior to the pressure natriuresis experiments. Experimental (salt-sensitive hypertension (SSHTN) and MMF) and the control (C) high-salt diet (C-HSD) groups had been on a high-salt diet, whereas the C-NSD group was on a normal-sodium diet for 4 wk. Food ingestion means were done during the preceding 30 days; proteinuria and urine volume were measured the day before pressure natriuresis studies. *P < 0.01 vs. the rest.
RBF in the experimental groups at RAPs of 90, 110, 130, and 150 mmHg are shown in Fig. 3. The rats with SSHTN had lower values of RBF, and this was corrected by MMF treatment (Fig. 3A). Differences in GFR in the groups under study were not significantly different (Fig. 3B), and consequently, filtration fraction was increased in rats of the SSHTN group (Fig. 3C). The control groups (C-NSD and C-HSD) and the MMF group did not show significant modifications in the GFR.

Table 3. Histology and immunohistology at the time of pressure natriuresis studies

<table>
<thead>
<tr>
<th></th>
<th>C-NSD (n = 7)</th>
<th>C-HSD (n = 7)</th>
<th>SSHTN (n = 10)</th>
<th>MMF (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS index, 0–400</td>
<td>0.6 ± 0.24</td>
<td>0.6 ± 0.40</td>
<td>4.8 ± 2.63</td>
<td>3.4 ± 1.69</td>
</tr>
<tr>
<td>TI damage score, 0-5</td>
<td>0.6 ± 0.24</td>
<td>0.8 ± 0.37</td>
<td>1.8 ± 0.58</td>
<td>1.2 ± 0.20</td>
</tr>
<tr>
<td>CD68 + cells/mm²</td>
<td>7.82 ± 0.57</td>
<td>10.14 ± 0.66</td>
<td>35.65 ± 2.29a</td>
<td>22.99 ± 1.49b</td>
</tr>
<tr>
<td>CD4 + cells/mm²</td>
<td>7.76 ± 0.63</td>
<td>11.66 ± 0.84</td>
<td>43.17 ± 2.76a</td>
<td>26.77 ± 2.15b</td>
</tr>
<tr>
<td>CD8 + cells/mm²</td>
<td>6.78 ± 0.78</td>
<td>2.67 ± 1.09</td>
<td>29.3 ± 3.80c</td>
<td>17.9 ± 2.29b</td>
</tr>
<tr>
<td>CD3 + cells/mm²</td>
<td>3.16 ± 1.08</td>
<td>6.28 ± 0.60</td>
<td>18.9 ± 2.12d</td>
<td>9.81 ± 0.49c</td>
</tr>
<tr>
<td>AII + cells/mm²</td>
<td>8.09 ± 0.60</td>
<td>10.63 ± 0.64</td>
<td>32.06 ± 2.39e</td>
<td>19.25 ± 0.42a</td>
</tr>
</tbody>
</table>

Histology and immunohistology in the C-NSD and C-HSD groups and the experimental groups (SSHTN and MMF). Values are means ± SE. *P < 0.01 vs. the rest; **P < 0.01 vs. C-NSD and C-HSD; ***P < 0.001 vs. C-NSD and C-HSD, and P < 0.05 vs. MMF; #P < 0.001 vs. the rest; ^P < 0.05 vs. C-NSD.

RBF in the experimental groups at RAPs of 90, 110, 130, and 150 mmHg are shown in Fig. 3. The rats with SSHTN had lower values of RBF, and this was corrected by MMF treatment (Fig. 3A). Differences in GFR in the groups under study were not significantly different (Fig. 3B), and consequently, filtration fraction was increased in rats of the SSHTN group (Fig. 3C). The control groups (C-NSD and C-HSD) and the MMF group did not show significant modifications in the GFR.
In contrast, rats with SSHTN increased the GFR from 110 to 150 mmHg RAP (P < 0.05; Fig. 3B). UNaV and FNaE at the corresponding RAPs are shown in Fig. 4A and B, respectively. The essentially unchanged sodium-excretion rate in the SSHTN group contrasts with the values in the C-NSD and C-HSD that increase from 90 to 130 mmHg of RAP. Rats from the MMF group had values of UNaV and FNaE that were not significantly different from those in rats from the control groups.

The relationship between the inflammatory infiltrate and UNaV and FNaE was examined at a RAP of 130 mmHg when a separation between experimental and control groups was present. The intensity of immune cell infiltration had a strong negative relationship with the UNaV rate (Fig. 5A) and with the FNaE (Fig. 5B). Similar negative relationships were found between the number of angiotensin II-positive cells and natriuresis (Fig. 6A) and FNaE (Fig. 6B).

Not unexpectedly, a strong (P < 0.0001), positive relationship exists between the severity of hypertension (determined prior to Fig. 2. Western blots of renal content of IL-2 (A), IL-6 (B), nitrotyrosine (C), and malondialdehyde (D) in experimental (SSHTN and MMF) and control [normal salt diet (C-NSD)] groups. Western blot data are expressed as optical density (O.D.) relative to β-actin. Gel pictures are samples for 1 run. Data correspond to n = 5 in each group. Molecular weight shown in nitrotyrosine corresponds to nitrosylated tyrosine-containing proteins. *P < 0.05; **P < 0.01; ***P < 0.001.

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The relationship between the inflammatory infiltrate and UNaV and FNaE was examined at a RAP of 130 mmHg when a separation between experimental and control groups was present. The intensity of immune cell infiltration had a strong negative relationship with the UNaV rate (Fig. 5A) and with the FNaE (Fig. 5B). Similar negative relationships were found between the number of angiotensin II-positive cells and natriuresis (Fig. 6A) and FNaE (Fig. 6B).

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DISCUSSION

There is a strong association between renal inflammation and SSHTN (37, 38). The novel findings of this study are the demonstration of a direct association between the degree of impairment in pressure natriuresis and the severity of tubulointerstitial inflammation. These studies, therefore, provide compelling evidence that interstitial inflammation, in the absence of significant renal damage (Table 3), plays in the impairment of pressure natriuresis that drives salt-induced hypertension.

The RBF was lower in the group with SSHTN, consistent with renal vasoconstriction, likely as the result of intrarenal oxidative stress and angiotensin II overactivity generated by inflammation (41, 51). GFR did not differ significantly in experimental and control groups in the range of RAP studied. No significant changes in GFR between 90 and 150 mmHg RAP were found in control and MMF groups; in contrast, the rats in the SSHTN group had a progressive increment in GFR, as RAP was varied from 110 to 150 mmHg (paired t-test, P < 0.01). In pressure-natriuresis studies done in various laboratories, GFR in control groups has been found to be at steady levels or showing only a mild increment (15, 20, 26, 44, 52). In hypertension induced by angiotensin II, Mattson et al. (26) and Wang et al. (52) found a progressive increment in GFR with increasing RAP pressure, similar to what we observed in the SSHTN group. In addition, inflammatory cytokines, such as transforming growth factor β, impair the autoregulation of glomerular hemodynamics via the generation of reactive oxygen species (13, 47) and may cause afferent arteriolar remodeling (7, 46). Taken together, these findings suggest that the loss of GFR autoregulation in the SSHTN group may be related to renal angiotensin II overactivity and renal inflammation. Since GFR was essentially the same in the experimental and control groups, and the RBF was lower in the SSHTN group, the filtration fraction in the SSHTN group was increased, as has been reported in oxidative stress associated with a high-salt...
diet (25) and in the chronic, slow pressor responses induced by angiotensin II infusion (19).

The role played by oxidative stress in the medullary regions of the kidney, restricting nitric oxide availability and causing dysfunction of the pressure-natriuresis response, has been reviewed recently (8, 30, 36, 48). Reduction of oxidative stress by hypoxia-inducible factor 1α (23) can improve pressure natriuresis. The generation of reactive oxygen species is an obligatory consequence of inflammation (51), and reduction in nitric oxide, resulting from oxidative stress, is expected to restrict the vasodilatation capacity and reduce interstitial cGMP, which is critical for the natriuresis response (24). The increased renal nitrotyrosine and MDA content in the SSHTN group (Fig. 2, C and D) support this notion.

The pressure natriuresis in rats of the control group and rats with mild inflammatory infiltration of immune cells (MMF group) showed a progressive increment of natriuresis, and at 130 mmHg RAP and 150 mmHg RAP, the UNaV rate was more than three times and 13 times, respectively, higher than the values at 90 mmHg RAP pressure. In contrast, the SSHTN group showed a suppression of the pressure natriuresis that was strikingly evident at RAP values of 130 and 150 mmHg.

In agreement with previous studies (6, 40, 43), we found that severity of SSHTN is correlated directly \((P < 0.001)\) with the intensity of the infiltration of immune-competent cells in the interstitial areas of the kidney. The association of immune cell infiltration and inflammatory activity is confirmed by the increment in renal expression of inflammatory cytokines (Fig. 2, A and B).

As reported by several investigators (2, 34, 35), L-NAME administration induces renal injury and inflammation, which are reduced after the discontinuation of L-NAME treatment. These findings were confirmed in the present studies, and at the time of the pressure-natriuresis experiments, the hypertensive rats of the SSHTN group and the normotensive rats of the other groups do not have significantly different histological damage. In contrast, the infiltration of immune cells and the number of angiotensin II-expressing cells in tubulointerstitial areas are significantly higher in the SSHTN group (Table 3). The causes responsible for the renal accumulation of lymphocytes and macrophages and its role in SSHTN are incompletely understood, but the role of immune reactivity in hypertension has been reviewed recently (10, 37, 42, 47), and recent work has identified the 70-kDa heat shock protein, overexpressed in the kidney, as a relevant autoantigen in SSHTN (33). In line with these emerging observations, recent investigations have shown that mice lacking lymphocyte responses develop increased natriuresis and blunted angiotensin II-induced hypertension (4).

In the present experiments, we have documented that the suppression of the natriuresis induced by tubulointerstitial inflammation is evident at RAPs of 130 and 150 mmHg when there is a strong, negative correlation between the UNaV and FNaE and the number of inflammatory and angiotensin II-expressing cells, as described before (6, 34, 39, 40, 43).

The renal inflammation was associated with accumulation of angiotensin II-positive cells. The activation of a functional renin-angiotensin system in the immune-competent cells (12, 17) and tubular cells (21, 28) likely plays a major role in the blunting of pressure natriuresis induced by renal inflammation. In previous studies, we have shown that in experimental models of SSHTN, the plasma angiotensin is reduced as a result of sodium retention; in contrast, the renal angiotensin activity is increased (6). These findings underline the independence of intrarenal angiotensin from physiologic modulation of plasma angiotensin (29) and indicate a primary role of intrarenal angiotensin in the pathogenesis of SSHTN (6). Whereas in the present studies, we only identified angiotensin II-expressing cells by immunohistology, previous work from our group has established that the number of angiotensin II-positive cells, the intensity of renal angiotensin II activity, and the severity of tubulointerstitial inflammation are correlated directly with one another (6, 50). Antinatriuretic effects of angiotensin II result from reduction in sodium-filtered load and increased sodium reabsorption (26, 29) and are mediated by angiotensin II type 1 receptors (3, 18, 20–22, 28), whereas natriuresis is mediated by angiotensin III and type 2 receptors (32).

Oxidative stress and angiotensin II activity are key components of renal inflammation. The increased renal MDA content and inflammatory cytokines in the SSHTN group (Fig. 3) confirm this association, and it is reasonable to assume the participation of these elements in the impairment of pressure natriuresis resulting from renal inflammation.

In conclusion, this investigation found that interstitial inflammation is associated with suppression of the natriuresis resulting from increments in RAP pressure. At RAP levels of 130 mmHg, the UNaV is inversely correlated with the number of inflammatory cells and angiotensin II-positive cells in the kidney. These findings add insight to the pathogenesis of SSHTN and its relation to renal inflammation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: M.F., R.J.J., and B.R-I. conception and design of research; M.F., R.J.J., B.R-I., and J.S. performed experiments; M.F., R.B., Y.Q., and B.R-I. analyzed data; M.F., R.B., Y.Q., and B.R-I. interpreted results of experiments; M.F., R.J.J., and B.R-I. prepared figures; M.F., R.J.J., and B.R-I. drafted manuscript; M.F. and B.R-I. edited and revised manuscript; M.F., R.J.J., and B.R-I. approved final version of manuscript.

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