High-NaCl intake impairs dynamic autoregulation of renal blood flow in ANG II-infused rats

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Submitted 17 May 2010; accepted in final form 12 August 2010

Saeed A, DiBona GF, Marcussen N, Guron G. High-NaCl intake impairs dynamic autoregulation of renal blood flow in ANG II-infused rats. Am J Physiol Regul Integr Comp Physiol 299: R1142–R1149, 2010. First published August 18, 2010; doi:10.1152/ajpregu.00326.2010.—The aim of this study was to investigate dynamic autoregulation of renal blood flow (RBF) in ANG II-infused rats and the influence of high-NaCl intake. Sprague-Dawley rats received ANG II (250 ng·kg⁻¹·min⁻¹·sc) or saline vehicle (sham) for 14 days after which acute renal clearance experiments were performed during thiobutabarbital anesthesia. Rats (n = 8–10 per group) were either on a normal (NNa; 0.4% NaCl)- or high (HNa; 8% NaCl)-NaCl diet. Separate groups were treated with 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (tempol; 1 M in drinking water). Transfer function analysis from arterial pressure to RBF in the frequency domain was used to examine the myogenic response (MR; 0.06–0.09 Hz) and the tubuloglomerular feedback mechanism (TGF; 0.03–0.06 Hz). MAP was elevated in ANG II-infused rats compared with sham rats (P < 0.05). RBF in ANG II HNa was reduced vs. sham NNa and sham HNa (6.0 ± 0.3 vs. 7.9 ± 0.3 and 9.1 ± 0.3 ml·min⁻¹·g kidney wt⁻¹, P < 0.05). Transfer function gain in ANG II HNa was significantly elevated in the frequency range of the MR (1.26 ± 0.50 dB, P < 0.05 vs. all other groups). Gain values in the frequency range of the MR and TGF were significantly reduced by tempol, suggesting a pathogenetic role for superoxide in the impaired RBF autoregulatory response.

METHODS

HYPERTENSION IS A COMMON CAUSE of kidney injury and end-stage renal disease and accelerates loss of kidney function in patients with chronic kidney disease, regardless of the underlying etiology (19). However, the risk of renal injury is variable, and the pathophysiological mechanisms by which hypertension causes renal parenchymal injury are complex and incompletely understood (5, 13, 27, 28, 34). The renal blood flow (RBF) autoregulatory response, mediated mainly by the myogenenic response (MR) and the tubuloglomerular feedback mechanism (TGF), stabilizes RBF and glomerular filtration rate (GFR), despite wide variations in arterial blood pressure (AP) (9, 23). RBF autoregulation (RBFA) may also serve a protective function, particularly in hypertension, by preventing transmission of systemic AP to the glomerular capillaries (2, 28). A role for autoregulatory capacity as a determinant of vulnerability to renal injury has been suggested in the 5/6 renal ablation model in rats (4) and in genetic models characterized by impaired RBFA (37, 38). In addition, treatment with dihydropyridine calcium channel blockers, which interfere with RBFA by impairing the MR, has been shown to increase the susceptibility to hypertensive glomerular injury in rats subjected to 5/6 nephrectomy (17).

Increased dietary NaCl has been shown to accelerate renal injury in hypertension (1, 5, 32). However, the pathophysiological mechanisms by which increased NaCl intake enhances renal injury in hypertension are multiple and not fully elucidated. Takenaka et al. (35) showed that preglomerular arterioles in Dahl salt-sensitive rats, on a high-NaCl diet, exhibited reduced myogenic responsiveness to increased AP, suggesting a role for impaired RBFA as a cause of renal injury in this model (35). In addition, several studies indicate that reactive oxygen species contribute to renal injury in NaCl-sensitive forms of hypertension (18, 36). In the present study, we hypothesized that the combined influence of ANG II, and a high-NaCl intake, could lead to impaired autoregulation of RBF in this hypertensive model. In addition, we speculated that superoxide (O₂⁻), a reactive oxygen species whose production is stimulated by ANG II (15, 27), could be involved in the abnormal RBF autoregulatory response. To examine this hypothesis we used transfer function (TF) analysis that enabled us to analyze the separate contributions of the MR and the TGF mechanism to dynamic autoregulation of RBF in chronically ANG II-infused Sprague-Dawley rats on either a normal or a high-NaCl diet.

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II HNa (n = 8). In separate groups, the membrane-permeable superoxide dismutase mimetic 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (tempol) was administered in the drinking water (1 M) throughout the 14-day period: 5) sham NNa+tempol (n = 8); 6) sham HNa+tempol (n = 10); 7) ANG II NNa+tempol (n = 10); and 8) ANG II HNa+tempol (n = 10). Tempol administered by drinking water in this concentration has been demonstrated to significantly reduce AP and markers of oxidative stress in several hypertensive rat models (15, 18, 36) including in ANG II HNa (30).

Surgical Preparation and Measurements

Rats were anaesthetized with thiobutabarbital (Inactin; 120 mg/kg ip), placed on a heating table, and surgically prepared for renal clearance experiments as previously described (29). An AP catheter was inserted via the femoral artery and was connected to a pressure transducer (Smiths Medical, Kirkchasey, Germany) for monitoring of arterial pressure [AP; pulsatile and mean (MAP)] and heart rate using a data acquisition program (MP 150; Biopac Systems, Santa Barbara, CA). The left kidney was exposed by a flank incision and immobilized in a plastic cup. The left ureter was catheterized for urine collection. Rectal and kidney temperatures were kept at 37°C. A perivascular ultrasonic transit-time flow probe (0.7 VB) was placed around the left renal artery and connected to a flowmeter (model T206, filter 100 Hz) with an ultrasonic transit-time flow probe (0.7 VB) was placed around the left renal artery and connected to a flowmeter (model T206, filter 100 Hz) for measurement of RBF (Transonic Systems, Ithaca, NY). GFR was determined by measuring renal 51Cr-EDTA clearance (Amersham Laboratories, Buckinghamshire, UK) as described (29). Blood was sampled at the start and completion of two consecutive 20-min urinary clearance periods, and mean values of plasma radioactivity were used to calculate GFR. Arterial blood samples (0.3 ml) were replaced by equivalent volumes of 4% BSA in isotonc saline. Rats were infused with 10 ml·kg⁻¹·h⁻¹ of isotonc saline throughout. Rats were killed by an overdose of pentobarbital sodium, and the kidneys were excised and weighed. Kidneys were immersion-fixed in paraformaldehyde and prepared for histological analyses. Renal vascular resistance (RVR) was calculated as MAP (mmHg)/RBF (ml·min⁻¹·g kidney wt⁻¹), and filtration fraction (FF) was estimated as GFR/RBF.

TF Analysis

Data used to examine the dynamic relationship between AP and RBF, i.e., dynamic autoregulation of RBF, were sampled at 62.5 Hz yielding 75,000 data points for each 20-min period (150,000 data points for the two 20-min periods). Processing of AP and RBF data was performed off-line by using previously developed software routines written for Matlab 7.14 (The MathWorks, Natick, MA). After subtracting the mean value from the data files, they were digitally low-pass filtered (3.0 Hz cut-off frequency, finite-impulse response, order 50) and then resampled to a rate of 6.25 Hz. These 6.25-Hz data files were split into blocks of 2,048 data points, yielding a frequency discrimination of 0.003 Hz. Power spectral density (PSD) of AP and RBF was calculated, as described (10, 11). The TF spectra were calculated from AP (input) and RBF (output). The TF gain was taken as the quotient of the cross spectrum of input and output divided by the power spectrum of the input (10, 11). Coherence is a frequency domain estimate of a linear correlation (i.e., squared coherence, akin to coefficient of determination) between two signals indicating the degree to which the variance in one signal can be explained by a linear operation on the other signal (10, 11). The coherence spectra were calculated from AP (input) and RBF (output). The coherence function was taken as the quotient of the square of the cross spectrum of input and output divided by the product of the power spectral densities of AP and RBF (10, 11). These algorithms involved a Hanning window with 50% overlap of the blocks (12 blocks in the two 20-min recording periods). To permit comparison among rats, the TF gain (magnitude) values over the frequency range have been normalized to the mean value of the renal vascular conductance for the entire data set. After conversion of the normalized TF gain values into decibels (20 log (gain)), a mean spectrum was calculated from the consecutive spectra in each rat, and these were subsequently averaged for all rats. The TF gain corresponds to the ratio of the amplitude of normalized fluctuations in RBF divided by those of AP. In the presence of RBFA, fluctuations of RBF are attenuated vs. those of AP causing the TF gain to be negative. Thus, positive TF gain values indicate impaired RBFA (9, 23). Phase and coherence spectra were similarly calculated and averaged. Data over the range of frequencies for the MR (0.08–0.18) and the TGF mechanism (0.03–0.06) were analyzed (9, 23). The slope of gain reduction in the frequency range of the MR was determined by least squares fitting of the linear region of gain reduction, and the phase peak was estimated as the average phase value within the same frequency interval. In addition, to assess the contribution of the MR to RBFA, mean gain values in the frequency range of 0.06–0.09 Hz were used to minimize corruption by TGF (<0.06 Hz) and myogenic transients (>0.09 Hz) (38). To assess the effect of filtering at either 30 Hz or 100 Hz, we made two consecutive recording periods in a single rat with 30 Hz filter/62.5 Hz sampling followed by 100-Hz filter/62.5-Hz sampling. Each of these data sets was subjected to the same processing and analysis as described above. The results showed that over the frequency range of interest (0.01–1.0 Hz) there were no significant differences in PSD for AP and RBF or in TF gain, phase, or coherence in the TGF (0.03–0.06 Hz) or MR (0.08–0.18 Hz) frequency ranges. To determine the threshold for coherence above which it exceeds zero with a certain significance level, we used the method described by Koopmans (26), which depends on the total number of samples, the total number of blocks, and the nature of the tapering window. In this study with large sample numbers, coherence values > 0.1 are significantly different from zero at P < 0.001.

Kidney Histology

Kidneys were processed using routine techniques, and 3-μm thick transverse sections through the hilar area were prepared and stained with hematoxylin and eosin, periodic acid-Schiff, and elastin-vanGieson’s. Histopathological changes were scored semiquantitatively (0–3) by an investigator (N. Marcussen) blinded to treatment group. The scores: 0, when no pathologic changes were present; 1, when few of the structures showed changes and the changes were mild; 2, when moderate changes were present; and 3, when severe changes were present in the structures under investigation. For glomerular parameters, the percentage of pathologically altered glomeruli was estimated. Cortical arteries (i.e., interlobar, arcuate, and interlobular arteries) and arterioles (i.e., afferent and efferent arterioles) were scored separately.

Statistical Analysis

All values are means ± SE. Analyses were performed using one-way ANOVA. Normality was tested with the Shapiro-Wilk test, and equality of variances was assessed with the Levene’s test. If data were not normally distributed or had unequal variances, Kruskal-Wallis one-way ANOVA on ranks was used. An unpaired t-test and Mann-Whitney U-test was used when appropriate. Bonferroni corrections were made for multiple comparisons. To reduce the number of comparisons, no statistical analyses were made between ANG II NNa and sham HNa. Group ANG II NNa+tempol was compared with ANG II NNa and sham NNa, and group ANG II HNa+tempol was compared with ANG II HNa and sham HNa. In all cases, a P value < 0.05 was considered statistically significant. The statistical software SPSS 17.0 (SPSS, Chicago, IL) was used.

RESULTS

Kidney Function and Renal Hemodynamics

Effects of NaCl intake and ANG II. Group sham HNa showed elevated RBF and reduced FF compared with sham rats on a normal NaCl diet, sham NNa (Table 1). In addition,
left ventricular weight (LVW) was elevated in sham HNa compared with sham NNa, although there was no significant difference between groups in MAP (Table 1).

In ANG II-infused rats on a normal NaCl diet (ANG II NNa) MAP, LVW, and RVR were increased compared with sham NNa (Table 1). Similarly, MAP, LVW, and RVR were significantly elevated and RBF was reduced, in group ANG II HNa compared with both sham NNa and sham HNa (Table 1). In addition, GFR and FF were significantly elevated in ANG II HNa compared with sham NNa (Table 2, Figs. 1 and 2). In sham HNa, gain values in the TGF frequency range (0.03–0.06 Hz) were comparable to those in sham NNa (Table 2, Figs. 1 and 2). In ANG II NNa, gain values reflecting the regulatory action of the MR (0.06–0.09 Hz) were not significantly different from those in sham NNa, and there was a clear transition in gain from positive to negative values did not occur in ANG II HNa, and the corresponding local maximum increment in phase, indicating active MR (Figs. 1 and 2). In sham HNa, gain values in the TGF frequency range (0.03–0.06 Hz) were comparable to those in sham NNa (Table 2, Figs. 1 and 2).

Effects of ANG II. In ANG II NNa, gain values reflecting the regulatory action of the MR (0.06–0.09 Hz) were not significantly different from those in sham NNa, and there was a clear transition in gain from positive to negative values (Table 2, Figs. 1 and 2). In the frequency range of the MR (0.08–0.18 Hz), the normal positive-to-negative transition in gain (with decreasing frequency) and the slope of gain reduction, were comparable in groups sham NNa and sham HNa (Table 2, Figs. 1 and 2). The positive-to-negative transition in gain in both sham groups was associated with the expected local maximum increment in phase, indicating active MR (Figs. 1 and 2). In sham HNa, gain values in the TGF frequency range (0.03–0.06 Hz) were comparable to those in sham NNa (Table 2, Figs. 1 and 2).

Effects of high-NaCl diet. In the frequency range of the MR (0.08–0.18 Hz), the normal positive-to-negative transition in gain (with decreasing frequency) and the slope of gain reduction, were comparable in groups sham NNa and sham HNa (Table 2, Figs. 1 and 2). The positive-to-negative transition in gain in both sham groups was associated with the expected local maximum increment in phase, indicating active MR (Figs. 1 and 2). In sham HNa, gain values in the TGF frequency range (0.03–0.06 Hz) were comparable to those in sham NNa (Table 2, Figs. 1 and 2).

Effects of ANG II+ high-NaCl diet. In the frequency range of the MR the normal transition in gain from positive to negative values did not occur in ANG II HNa, and the corresponding local maximum in phase was missing, indicating an impaired

### Table 1. Kidney function and renal hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Sham NNa</th>
<th>Sham HNa</th>
<th>Ang II NNa</th>
<th>Ang II NNa + Tempol</th>
<th>Ang II HNa</th>
<th>Ang II HNa + Tempol</th>
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<tr>
<td>Body weight, g</td>
<td>300 ± 15</td>
<td>318 ± 9</td>
<td>283 ± 10</td>
<td>297 ± 11</td>
<td>281 ± 16</td>
<td>267 ± 9f</td>
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<td>MAP, mmHg</td>
<td>118 ± 3</td>
<td>120 ± 3</td>
<td>146 ± 3a</td>
<td>149 ± 5</td>
<td>156 ± 4b</td>
<td>159 ± 5c</td>
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<td>LVW, g/kg body wt</td>
<td>2.18 ± 0.05</td>
<td>2.48 ± 0.044</td>
<td>2.81 ± 0.09a</td>
<td>2.72 ± 0.07a</td>
<td>2.87 ± 0.09ab</td>
<td>2.81 ± 0.09f</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>379 ± 6</td>
<td>359 ± 6</td>
<td>387 ± 6</td>
<td>376 ± 13</td>
<td>392 ± 10c</td>
<td>339 ± 10d</td>
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<tr>
<td>GFR, ml·min⁻¹·g kidney wt⁻¹</td>
<td>1.15 ± 0.06</td>
<td>1.00 ± 0.08</td>
<td>1.28 ± 0.08</td>
<td>0.96 ± 0.07b</td>
<td>1.40 ± 0.04p</td>
<td>0.85 ± 0.04d</td>
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<td>RBF, ml·min⁻¹·g kidney wt⁻¹</td>
<td>7.9 ± 0.3</td>
<td>9.1 ± 0.3c</td>
<td>7.1 ± 0.4</td>
<td>6.5 ± 0.5c</td>
<td>6.0 ± 0.3b</td>
<td>5.0 ± 0.3d</td>
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<tr>
<td>RVR, mmHg/ml·min⁻¹·g kidney wt⁻¹</td>
<td>15.1 ± 0.6</td>
<td>13.2 ± 0.5</td>
<td>20.9 ± 1.2c</td>
<td>24.9 ± 2.5a</td>
<td>26.4 ± 1.6</td>
<td>29.5 ± 3.0f</td>
</tr>
<tr>
<td>FF, GFR/RBF, %</td>
<td>14.7 ± 1.0</td>
<td>10.7 ± 0.8b</td>
<td>18.2 ± 1.2</td>
<td>16.0 ± 1.7</td>
<td>23.5 ± 0.7b</td>
<td>17.6 ± 1.24f</td>
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<tr>
<td>UF, µl·min⁻¹·g KW</td>
<td>4.24 ± 0.76</td>
<td>5.69 ± 0.01</td>
<td>8.30 ± 2.31</td>
<td>16.25 ± 5.29c</td>
<td>32.80 ± 5.85c</td>
<td>32.20 ± 5.92d</td>
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<tr>
<td>USN,V, µmol/min</td>
<td>0.62 ± 0.15</td>
<td>2.13 ± 0.42c</td>
<td>0.46 ± 0.09</td>
<td>1.30 ± 0.49</td>
<td>6.08 ± 0.88c</td>
<td>4.23 ± 1.19</td>
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<tr>
<td>FFNa, %</td>
<td>0.36 ± 0.09</td>
<td>1.42 ± 0.19c</td>
<td>0.26 ± 0.05</td>
<td>0.94 ± 0.30</td>
<td>3.07 ± 0.47c</td>
<td>3.45 ± 0.90</td>
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</table>

Data are means ± SE of two 20-min clearance periods in thiobutabarbital anesthetized rats (see METHODS). NNa, normal NaCl; HNa, high NaCl; MAP, mean arterial pressure; LVW, left ventricular weight; GFR, glomerular filtration rate; RBF, renal blood flow; RVR, renal vascular resistance; FF, filtration fraction; UF, urine output, and absolute and fractional urinary sodium excretion. aP < 0.05 vs. sham NNa, bP < 0.05 vs. sham HNa, cP < 0.05 vs. all other groups excluding groups with tempol, dP < 0.05 vs. corresponding Ang II group without tempol, eP < 0.05 vs. sham NNa, and fP < 0.05 vs. sham HNa.

### Table 2. Characteristics of the transfer function between arterial pressure and renal blood flow

<table>
<thead>
<tr>
<th></th>
<th>Sham NNa</th>
<th>Sham HNa</th>
<th>Ang II NNa</th>
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<tr>
<td>Gain, 0.03-0.06 Hz, dB</td>
<td>−3.45 ± 0.63</td>
<td>−5.84 ± 0.65</td>
<td>−2.68 ± 0.98</td>
<td>−2.42 ± 0.66</td>
<td>−0.02 ± 0.50b</td>
<td>−0.28 ± 1.04df</td>
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<tr>
<td>Gain, 0.06-0.09 Hz, dB</td>
<td>−3.17 ± 0.50</td>
<td>−5.55 ± 0.92</td>
<td>−2.56 ± 1.24</td>
<td>−1.80 ± 0.68</td>
<td>1.26 ± 0.50e</td>
<td>−2.22 ± 1.043f</td>
</tr>
<tr>
<td>Gain, slope 0.08-0.18 Hz, dB/decade</td>
<td>18.60 ± 1.68</td>
<td>18.62 ± 2.05</td>
<td>9.22 ± 3.05a</td>
<td>8.45 ± 1.88c</td>
<td>2.33 ± 1.11b</td>
<td>8.30 ± 2.92d</td>
</tr>
<tr>
<td>Phase, 0.08-0.18 Hz, rad</td>
<td>0.58 ± 0.37</td>
<td>0.61 ± 0.06</td>
<td>0.41 ± 0.07a</td>
<td>0.40 ± 0.06e</td>
<td>0.18 ± 0.03c</td>
<td>0.39 ± 0.10f</td>
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</table>

Data are means ± SE. Gain values in the frequency range 0.03–0.06 Hz correspond to the TGF mechanism and values in the range 0.06–0.09 Hz reflect the regulatory action of the MR (see METHODS). aP < 0.05 vs. Sham NNa, bP < 0.05 vs. Sham HNa, cP < 0.05 vs. all other groups excluding groups with tempol, dP < 0.05 vs. corresponding Ang II group without tempol, eP < 0.05 vs. Sham NNa, and fP < 0.05 vs. Sham HNa.
Gain values in the frequency range of 0.06 – 0.09 Hz were significantly elevated in ANG II HNa compared with sham NNa, sham HNa, and ANG II NNa (Table 2). In the TGF frequency range, gain values in ANG II HNa were significantly elevated compared with sham NNa and sham HNa (Table 2, Figs. 1 and 2).

Effects of tempol. Tempol had no statistically significant effects on dynamic RBFA assessed by TF analysis in groups sham NNa and sham HNa (data not shown). Similarly, in the ANG II NNa+/tempol group, tempol did not significantly affect TF variables, including the slope of gain reduction and phase values, in the frequency range of the MR (Table 2, Figs. 1 and 2). However, in ANG II HNa+/tempol, there was a clear transition in gain from positive to negative values in the frequency range of the MR, and this was associated with a local maximum in phase indicating an active MR (Fig. 4). Consequently, gain values were significantly reduced in ANG II HNa+/tempol compared with ANG II HNa in the frequency range of both the MR and the TGF (Table 2).

Kidney Histology

There were no statistically significant differences between groups in tubulointerstitial changes (i.e., inflammation, fibrosis, and tubular dilatation) or glomerular abnormalities (i.e., glomerular size variation, ischemia, focal segmental glomerulosclerosis, and global glomerulosclerosis) (data not shown). There was significant thickening of intimal and medial layers and hyaline deposition/necrosis of cortical arteries in both ANG II NNa and ANG II HNa compared with sham NNa (Fig. 5, Table 3). In addition, cortical arterioles (i.e., afferent and efferent arterioles) showed significant hyperplasia and hyalnosis compared with sham (Fig. 5, Table 3). However, the magnitude of arterial and arteriolar changes in ANG II NNa and ANG II HNa were not significantly different, and tempol had no significant effects on vascular abnormalities (Fig. 5, Table 3).

DISCUSSION

The main finding of the present study was that a high-NaCl intake in chronically ANG II-infused rats resulted in a marked impairment of the MR of RBFA. This abnormality was not seen in sham HNa and was significantly more pronounced than in ANG II NNa, indicating a synergistic effect of high-NaCl intake and elevated ANG II. In addition, this abnormality in RBFA in ANG II HNa was corrected by tempol, suggesting a role for O₂⁻⁻ in the impaired autoregulatory response.

In the frequency range of the MR, TF analysis showed a reduced slope of gain reduction and a diminished phase peak in ANG II NNa compared with sham NNa, indicating an impaired MR. This result differs from a previous study in which dynamic RBFA was assessed in conscious dogs infused with

![Fig. 1. Power spectral density (PSD) for arterial pressure (AP) and renal blood flow (RBF) and transfer function gain, phase, and coherence in anesthetized Sprague-Dawley rats after 14 days of ANG II (250 ng·kg⁻¹·min⁻¹ sc) or saline vehicle (sham) infusion. Rats were on either a normal (NNa)- or a high (HNa)-NaCl diet (see METHODS). Values are means ± SE.](http://ajpregu.physiology.org/)

![Fig. 2. Enlarged transfer function gain (top) and phase (bottom) in anesthetized Sprague-Dawley rats after 14 days of ANG II (250 ng·kg⁻¹·min⁻¹ sc) or saline vehicle (sham) infusion. Rats were on either an NNa or HNa diet (see METHODS).](http://ajpregu.physiology.org/)
ANG II to produce plasma ANG II levels within the physiological range (24). These investigators found that ANG II did not affect the efficiency of RBFA or the relative contribution of the MR and TGF components (24). The discrepancy between studies could be explained by marked differences in the experimental protocols, as the potential difference between conscious and anesthetized animals. In addition, in our study animals were hypertensive and infused with much higher ANG II concentrations and for a longer duration. However, several studies have demonstrated in experimental models closely resembling ours that RBFA is impaired in chronically ANG II-infused hypertensive animals (8, 21, 40) as well as in the nonstenotic kidney of Goldblatt hypertensive rats (31). Most of the studies on chronically ANG II-infused rats have been performed using the in vitro blood-perfused juxtamedullary nephron model in which responses of afferent arterioles to step-wise changes in perfusion pressure were analyzed (8, 21, 40). In contrast, our study presents data on dynamic autoregulation of whole kidney RBF during spontaneous fluctuations in AP in intact ANG II hypertensive animals. In addition, we extend previous findings by suggesting that chronic ANG II infusion selectively affects the MR component of RBFA in animals on a normal NaCl intake.

The major finding in the present study was the much more pronounced impairment in dynamic RBFA in ANG II HNa compared with ANG II NNa. This abnormality in ANG II HNa was characterized by an almost complete absence of a MR. This synergistic effect of ANG II and high-NaCl intake on dynamic RBFA represents a novel finding. One could hypothesize that the impairment in RBFA, mainly in group ANG II HNa, would make kidneys vulnerable primarily to reductions in AP and not to glomerular hypertension, as RVR was markedly elevated and RBF was reduced in these rats. However, despite the reduction in RBF, GFR tended to be elevated in ANG II HNa, and FF was significantly increased vs. the other groups. These results indicate that the increase in resistance predominantly occurred at the efferent glomerular arterioles and that glomerular capillary pressure was maintained or elevated despite reductions in RBF. In this situation it is reasonable to speculate that the aforementioned abnormalities in RBFA in ANG II HNa could eventually cause pressure-induced glomerular injury. In addition, it may seem contradictory that RVR was clearly increased in group ANG II HNa in view of the impaired MR demonstrated by TF analysis. However, it is important to recognize that impaired dynamic RBFA does not indicate a certain level of RBF at a certain level of AP, but more likely predicts whether the response of RBF following a dynamic change in arterial pressure will be normal. In line with

Fig. 3. PSD for AP and RBF, and transfer function gain, phase, and coherence in anesthetized Sprague-Dawley rats after 14 days of ANG II-infusion (250 ng·kg⁻¹·min⁻¹·sc) with or without tempol in drinking water (1 M). Rats were on an NNa diet (see METHODS). Tempol did not significantly affect transfer function variables. Values are means ± SE.

Fig. 4. PSD for AP and RBF, and transfer function gain, phase, and coherence in anesthetized Sprague-Dawley rats after 14 days of ANG II-infusion (250 ng·kg⁻¹·min⁻¹·sc) with or without tempol in drinking water (1 M). Rats were on an HNa diet (see METHODS). Tempol did not significantly affect transfer function variables. Values are means ± SE.
our results, Elmarakby et al. (13) showed that autoregulatory responses of afferent arterioles to step-wise increases in perfusion pressure were blunted in ANG II-infused rats on high dietary NaCl (13). However, in that study no comparison was made to ANG II-infused animals on a normal NaCl intake. Interestingly, Zhao et al. (41) investigated the effects of ANG II and high-NaCl diet on afferent arteriolar responses to acetylcholine and sodium nitroprusside by using the in vitro blood-perfused juxtamedullary nephron preparation and found that vasodilator responses were significantly more attenuated in ANG II-infused rats on a high-NaCl diet compared with those on a normal NaCl diet (41). Although RBFA was not assessed,

Table 3. Arterial and arteriolar changes in kidney cortex

<table>
<thead>
<tr>
<th></th>
<th>Sham NNa</th>
<th>Ang II NNa</th>
<th>Ang II HNa</th>
<th>Ang II NNa + Tempol</th>
<th>Ang II HNa + Tempol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriolar hyperplasia</td>
<td>0</td>
<td>1.25 ± 0.16*</td>
<td>0.88 ± 0.30*</td>
<td>0.63 ± 0.18</td>
<td>0.50 ± 0.19</td>
</tr>
<tr>
<td>Arteriolar hyalinosis</td>
<td>0</td>
<td>0.38 ± 0.18</td>
<td>1.00 ± 0.13*</td>
<td>0.50 ± 0.27</td>
<td>0.62 ± 0.33</td>
</tr>
<tr>
<td>Thickening of the intimal layer of arteries</td>
<td>0</td>
<td>0.13 ± 0.13</td>
<td>0.13 ± 0.13</td>
<td>0.38 ± 0.18</td>
<td>0.13 ± 0.13</td>
</tr>
<tr>
<td>Thickening of the media layer of arteries</td>
<td>0.12 ± 0.13</td>
<td>1.50 ± 0.27*</td>
<td>1.63 ± 0.18*</td>
<td>1.38 ± 0.38</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>Hyaline deposition/necrosis of arteries</td>
<td>0</td>
<td>1.00 ± 0.27*</td>
<td>1.50 ± 0.27*</td>
<td>1.38 ± 0.42</td>
<td>0.88 ± 0.30</td>
</tr>
</tbody>
</table>

Data are means ± SE. Semiquantitative assessment of cortical arteries and arterioles (i.e., afferent and efferent arterioles) was performed by using a scale from 0 to 3 (see METHODS). Kruskal-Wallis test was used to compare the groups: Sham NNa, Ang II NNa, and Ang II HNa. Mann-Whitney U-test was used to compare group Ang II NNa vs. Ang II NNa+Tempol and group Ang II HNa vs. Ang II HNa+Tempol. Values are means ± SE. *P < 0.05 vs. Sham NNa.

Fig. 5. Renal cortical arteries and arterioles in Sprague-Dawley rats after 14 days of ANG II (250 ng·kg⁻¹·min⁻¹ sc) or saline vehicle (sham) infusion with or without tempol in drinking water (1 M). Rats were on either an NNa- or HNa diet (see METHODS). Top, left: sham NNa; top, right: ANG II NNa; bottom, left: ANG II HNa; and bottom, right: ANG II HNa+tempol. Black arrows indicate arterioles, and green arrows indicate arteries. Sections were stained with periodic acid-Schiff. Magnification, ×200. All ANG II-infused groups showed mild thickening of intimal and medial layers and hyaline deposition/necrosis of arteries. In addition, afferent and efferent arterioles showed mild hyperplasia and hyalinosis.
these results demonstrate that the combined influence of ANG II and high-NaCl intake produces more pronounced abnormalities in afferent arterioles, the main site of RBFA, than either stimulus alone.

In the present study, tempol attenuated abnormalities in TF gain values in the frequency ranges of the MR in ANG II HNa, indicating an important role for O₂⁻ in the impaired autoregulatory response specifically under the circumstance of both elevated ANG II and high-NaCl intake. However, tempol did not restore gain values in group ANG II HNa+tempol back to the normal levels seen in sham groups, but only to the levels observed in group ANG II NNa. In addition, tempol reduced AP only in ANG II HNa. In line with our results, previous studies have demonstrated that in ANG II-infused animals, high-NaCl intake further increased O₂⁻ generation and lipid peroxidation compared with animals on a normal NaCl intake (6, 22). The mechanisms by which tempol attenuated abnormalities in RBFA in ANG II HNa in the present study remain to be elucidated. One might speculate that the AP reduction, per se, could play a role as indicated by Inscho et al. (20) in ANG II-infused rats on a normal NaCl diet. However, in the present study, tempol clearly did not return AP to normal, suggesting that other, pressure-independent mechanisms might be involved. Interestingly, Sharma et al. (33) showed that transforming growth factor-β1 markedly attenuated RBFA in rats using the blood-perfused juxteduallary nephron technique and that this was associated with increased arteriolar O₂⁻ production (33). In addition, pretreatment with tempol prevented the impairment in RBFA induced by transforming growth factor-β1, indicating an important role for O₂⁻ in our study.

Alternatively, one could speculate that tempol improved dynamic RBFA in ANG II HNa in the present study by reducing sympathetic nerve activity as suggested by a reduced AP and heart rate in group ANG II HNa+tempol. Previous studies have shown that chronic ANG II infusion increases O₂⁻ formation in the rostral ventrolateral medulla (6) and overall sympathetic activity (25) in rats on a high-NaCl diet compared with rats on a normal NaCl diet. In addition, tempol administered intracerebroventricularly prevents increases in AP and renal sympathetic nerve activity (RSNA) in response to ANG II injected intracerebroventricularly (7). Since it has previously been demonstrated that vasoconstrictor intensities of RSNA impair dynamic RBFA (12), it is possible that tempol improved RBFA in the present study by reducing RSNA. However, arguing against this hypothesis, Yoshimoto et al. (39) have shown that although whole-body sympathetic activity is elevated in ANG II-infused rats on a high-NaCl diet, RSNA is not.

In the present study, histological analyses showed modest but significant arterial and arteriolar changes in the renal cortex of both ANG II NNa and ANG II HNa. However, vascular changes were of similar extent in both groups and were unaffected by tempol. Although, dynamic RBFA was significantly more impaired in ANG II HNa compared with ANG II NNa, and attenuated by tempol, there was no correlation between structural vascular changes and autoregulatory capacity. Taken together, these findings indicate that abnormalities in dynamic RBFA could not be explained by structural alterations of the renal vasculature. Although impaired RBFA, assessed at steady-state using step changes in renal perfusion pressure has been shown to increase the susceptibility to hypertensive glomerular injury (2, 17, 37, 38), impaired dynamic RBFA in ANG II HNa did not result in increased glomerular injury as evidenced by semiquantitative analyses. Possibly, the short duration of the experiment could explain the limited glomerular changes. However, it is also possible that steady-state and dynamic analyses of RBFA provide important but different insights into the autoregulatory behavior of the renal vascular bed (3, 16).

Noteworthy, ANG II-infused rats on a high-NaCl diet showed significantly higher urinary water and Na⁺ excretion rates compared with group sham HNa. Food and water intake were not measured in the present study. However, as ANG II is a very potent dipsogenic factor and also increases Na⁺ appetite (14), it is reasonable to speculate that these effects of ANG II could contribute to the observed increases in urinary water and Na⁺ excretion in group ANG II HNa.

In conclusion, ANG II-infused rats on a high-NaCl diet developed marked impairments in dynamic RBFA, mainly affecting the MR, that were significantly more pronounced than in rats on a normal NaCl diet. In addition, these abnormalities were attenuated by tempol, suggesting a pathogenetic role for O₂⁻ in the impaired RBFA autoregulatory response.

**Perspectives and Significance**

Hypertension is an important cause of kidney injury and end-stage renal disease (19). However, the pathophysiologic mechanisms by which hypertension causes renal injury are multiple and incompletely understood (13, 27, 28, 34). The results of the present study suggest that a very-high-NaCl intake in ANG II-dependent forms of hypertension, in addition to elevating AP further, could increase the susceptibility to hypertensive glomerular injury by impairing RBFA. For instance, in patients with renovascular hypertension and unilateral renal artery stenosis, the avoidance of a high-NaCl intake might protect the nonstenotic kidney from progressive glomerular injury. Alternatively, pharmacological agents that target O₂⁻ may exert renoprotective effects by attenuating abnormalities in RBFA in the same situation. It remains to be investigated whether the effect of high-NaCl intake on RBFA in ANG II-infused rats is specific for this model or relevant also in other hypertensive states.

**ACKNOWLEDGMENTS**

The technical assistance of Elisabeth Grimberg is acknowledged.

**GRANTS**

This study was supported by grants from the Swedish Heart-Lung Foundation, the Göteborg Medical Society, the Swedish Medical Society, the Swedish Association for Kidney Patients, Swedish Society of Nephrology, and Brit Wennerström’s Research Foundation.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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