Renovascular hypertension is 1 of the most prevalent causes of secondary hypertension in humans, and the 2-kidney 1-clip model (2K1C) of renovascular hypertension was experimentally developed by Goldblatt in 1934. The reduction of renal artery blood flow by approximately 50% by placing a silver clip in 1 of the renal arteries results in increased renin release, which leads to activation of the renin angiotensin aldosterone system (RAAS) and a gradual increase in systemic mean arterial pressure (MAP).

The development and/or maintenance of renovascular hypertension are associated with arterial baroreceptor dysfunction, increased oxidative stress, and sympathoexcitation. Recently published data indicate that this model, in the intermediate phase (5–8 weeks after clipping), has a strong neurogenic component. In fact, chronic treatment with rilmenidine, a centrally acting sympatholytic drug, promotes the reduction of MAP, renal sympathetic nerve activity (rSNA), and renin secretion in rabbits, indicating the pivotal role of sympathoexcitation in renovascular hypertension.

However, the underlying mechanisms leading to sympathoexcitation remain undetermined. The autonomic dysfunction in 2K1C, including sympathoexcitation and baroreceptor impairment, is in part related to the increased actions of Angiotensin II (AngII), which...
leads to oxidative stress.\textsuperscript{7} In fact, AT1 blocker and treatment with a nonenzymatic antioxidant, vitamin C, significantly improves the cardiovascular and autonomic function in the 2K1C model.\textsuperscript{7,8}

We recently showed that in the intermediate phase of 2K1C, the increase in MAP is associated with a significant increase in rSNA to the clipped\textsuperscript{9} and unclipped\textsuperscript{10} kidneys, which characterizes this phase of renovascular hypertension as neurogenic. Additionally, it has been proposed that circulating AngII and dietary salt are converging signals that trigger neurogenic hypertension. Indeed, in the model of hypertension induced by AngII infusion, hypertension is more severe in animals that received a 2\% NaCl compared to the animals that received 0.4\% NaCl in drinking water. In addition, there was an increase in superoxide formation in the rostral ventrolateral medulla suggesting that the severity of hypertension caused by increased sodium intake was also dependent on oxidative stress.\textsuperscript{11}

Moreover, it has been shown that a mild increase in sodium intake did not alter MAP in normotensive rabbits.\textsuperscript{12} However, the association of the infusion of a low dose of AngII and 0.9\% NaCl in drinking water for 21 days increased rSNA and triggered hypertension, indicating the synergistic actions of AngII and NaCl on autonomic dysfunction.\textsuperscript{13} Notwithstanding, in the 2K1C model of hypertension, in the intermediate phase, which is characterized by sympathoexcitatory, there is no evidence that a discrete increase in sodium diet may affect autonomic dysfunction and sympathoexcitation. Thus, the major aim of the present study was to evaluate the effects of a mild increase in sodium intake on rSNA and baroreceptor sensitivity. Furthermore, the effects of increased sodium intake on systemic oxidative stress and RAAS markers in clipped and unclipped kidneys in the 2K1C model of hypertension were also addressed.

**METHODS**

Male Wistar rats (150–180 g) were purchased from the Central Animal House of the Federal University of São Paulo (UNIFESP/EPM). The rats were housed in collective cages in a temperature-controlled room set to a 12:12 hour light–dark cycle with free access to standard rat chow (Nuvilab) or a high-sodium diet and water. All protocols were approved by the ethical committee of the UNIFESP/EPM (CEP 0609-10).

**Experimental protocol**

To obtain 2K1C animals, the rats were anesthetized (IP) with ketamine (40 mg/kg) and xylasine (20 mg/kg) and the left renal artery was partially obstructed by a silver clip (0.2 mm).\textsuperscript{14} Four weeks after surgery, the animals were fed for 2 weeks with a normal sodium diet (0.4\% NaCl) or a high-sodium diet (2\% NaCl).

All experimental procedures were performed 6 weeks after clipping. One day before cardiovascular and autonomic evaluations, the rats were anesthetized with ketamine (40 mg/kg) and xylasine (20 mg/kg) and the femoral vein (for drug injection) and artery (for arterial recording) were catheterized (PE-50 and PE-10).

**Metabolic parameters**

In the last week of treatment with a normal or high-sodium diet, the rats were housed in individual metabolic cages (Naogene). All metabolic parameters presented were measured on the second day.

**Systemic oxidative stress—determination of blood TBARs and total glutathione contents**

The determination of plasma lipid peroxidation was evaluated by TBARs production. The concentration of TBARs was determined at 535 nm.\textsuperscript{15}

For the determination of erythrocyte total glutathione (tGSH) levels, the red blood cells were washed 3 times with cold phosphate-buffered solution (10 mM sodium phosphate, 140 mM NaCl, pH 7.4) followed by protein precipitation using 2 M HClO\textsubscript{4}, 4 mM EDTA (1:1 with diluted samples). The supernatant of the acid extract was neutralized to pH 7.0, and reaction of GSH with DTNB was conducted at 412 nm, 25 °C.\textsuperscript{16} The erythrocyte levels of tGSH were corrected by Hb content, and they were assessed by reaction with Drabkin solution.

**Plasma renin activity measurements**

Plasma was collected and used to measure the renin activity by reverse phase high-performance liquid chromatography as previously reported.\textsuperscript{17}

**Plasma and urinary sodium measurements**

Plasma and urine sodium concentrations were measured using a Corning 410C Flame Photometer.

**Urinary angiotensinogen measurement**

Urinary angiotensinogen was measured using a commercial solid phase sandwich enzyme-linked immunosorbent assay (ELISA), as indicated by manufacturer’s instruction (Immuno-Biological Laboratories, Japan). The urinary levels of angiotensinogen were normalized by urinary creatinine content.

**Arterial baroreflex control of heart rate**

The baroreceptor control of heart rate (HR) was evaluated in conscious rats by bolus injection of sodium nitroprusside (20 µg/kg) or phenylephrine (10 µg/kg) to induce a decrease or elevation of arterial blood pressure, respectively.

The cardiac baroreflex sensitivity was evaluated by the mean index relating changes in HR to changes in MAP, and it was expressed as beats per mm Hg.

**Renal sympathetic nerve activity**

Animals were anesthetized with urethane intravenous (1.2 Sigma-Aldrich Co, EUA) and the trachea was cannulated for artificial ventilation. Body temperature was maintained at
37 °C with the use of a heating table. The renal nerve was retroperitoneally exposed, dissected, and placed in a silver bipolar electrode and immersed in mineral oil during the experiment. Renal nerve activity was amplified and filtered (20 K, 100–1000 Hz, Neurolog-UK) and collected for display and analysis using a PowerLab data acquisition system. At the end of the experiments, the background noise level was determined after hexamethonium bromide (30 mg/kg, IV) administration. The neural activity was analyzed “offline” using appropriate software (Spike Histogram, ADInstruments).

**Arterial baroreflex control of rSNA**

Intravenous infusion of sodium nitroprusside (200 µg/ml) or phenylephrine (100 µg/ml) during 1 minute was made to evaluate alterations in rSNA during changes in arterial pressure caused by the vasoactive drugs. The slope analyses represents the baroreflex gain (ΔrSNA/ΔMAP) for each animal from 5, 10, 15, 20, 25, 30, 35, and 40 mm Hg of MAP changes with the corresponding rSNA changes.

**Western blotting analyses**

Kidneys were removed and placed in a solution buffer with protease inhibitors (40 µg/ml PMSF, 0.5 µg/ml leupeptin, and 0.7 µg/ml pepstatin) for homogenization. The homogenate was centrifuged (2,400 g for 10 minutes at 4 °C) to remove any particulate material, nuclei, and mitochondria from the supernatant. The protein concentration was determined by the method of Lowry. Protein samples were solubilized in sample buffer and (2% SDS, 20% glycerol, 100 mM dithiothreitol, 50 mM Tris pH 6.8, 0.05% bromophenol blue) and separated by SDS-PAGE using 7.5% polyacrylamide gels. Proteins were then transferred to PVDF microporous membrane (Millipore Immobilon-P, Millipore, Bedford, MA). After transfer, PVDF membrane was incubated with a blotto solution (5% nonfat dry milk and 0.1% Tween 20 in PBS, pH 7.4) to block nonspecific binding for 1 hour. The membranes were incubated with primary antibody, diluted in blotto (1:2,000), overnight at 4 °C with the primary antibody angiotensinogen (Santa Cruz), ACE (Santa Cruz), and ACE2 (Abcam). Afterward, the membranes were incubated for 1 hour with secondary antibody IgG from Zymed Laboratories (1:2,000). The detection was made by chemiluminescence (ECL) (GE Healthcare, Piscataway, NJ).

**Statistical analysis**

Data are presented as means ± SE. The data were analyzed by analysis of variance (1-way ANOVA) followed by Newman–Keuls post hoc test. Values were considered statistically significant when P < 0.05.

**RESULTS**

**Effects of a 2% NaCl diet on urinary parameters and cardiovascular function**

Water intake and urinary volume were significantly increased in the 2K1C rats compared to the control group (CTRL). Additionally, treatment with a high-sodium diet further increased water intake and urinary volume in the 2K1C rats. The water balance did not change between the groups (Table 1).

There was no difference in the urinary sodium excretion/day between the groups (CTRL vs. 2K1C) after the consumption of a normal sodium diet. The consumption of a high-sodium diet significantly increased the sodium excretion/day in both groups; however, no difference was observed between the CTRL and 2K1C rats. No significant differences in plasma sodium levels were observed between the groups (Table 1).

### Table 1. Effects of high sodium intake on body weight, water intake, food intake, electrolyte balance, cardiovascular and autonomic parameters and urinary angiotensinogen in control and 2K1C rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTRL</th>
<th>2K1C</th>
<th>CTRL 2% NaCl</th>
<th>2K1C 2% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>323 ± 15 (5)</td>
<td>285 ± 36 (5)</td>
<td>319 ± 7 (5)</td>
<td>322 ± 21 (5)</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>25 ± 1 (8)</td>
<td>24 ± 1 (8)</td>
<td>23 ± 2 (8)</td>
<td>25 ± 1 (8)</td>
</tr>
<tr>
<td>Water intake (ml/day)</td>
<td>30 ± 1 (8)</td>
<td>47 ± 3 (8)*</td>
<td>40 ± 3 (8)</td>
<td>62 ± 7 (8)*</td>
</tr>
<tr>
<td>Urinary flow (ml/day)</td>
<td>13 ± 1 (8)</td>
<td>25 ± 2 (8)*</td>
<td>20 ± 2 (8)</td>
<td>50 ± 5 (8)*</td>
</tr>
<tr>
<td>Water balance (ml/day)</td>
<td>18 ± 1 (8)</td>
<td>15 ± 2 (8)</td>
<td>14 ± 1 (8)</td>
<td>16 ± 4 (8)</td>
</tr>
<tr>
<td>Na⁺ excretion (mmol/day)</td>
<td>3.8 ± 0.2 (8)</td>
<td>3.9 ± 0.3 (8)</td>
<td>9.3 ± 0.5 (8)*</td>
<td>8.2 ± 1 (8)*</td>
</tr>
<tr>
<td>Na plasma (mmol/l)</td>
<td>140 ± 2.6 (8)</td>
<td>140 ± 2.8 (8)</td>
<td>139 ± 0.8 (8)</td>
<td>141 ± 2 (8)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>100 ± 3 (10)</td>
<td>174 ± 5 (12)*</td>
<td>104 ± 6 (5)</td>
<td>158 ± 9 (9)</td>
</tr>
<tr>
<td>rSNA (pps)</td>
<td>88.5 ± 9 (5)</td>
<td>136 ± 12 (7)*</td>
<td>85 ± 5 (5)</td>
<td>136 ± 10 (5)</td>
</tr>
<tr>
<td>Plasma renin activity (nmol/ml/h)</td>
<td>6 ± 0.7 (6)</td>
<td>4.1 ± 0.8 (6)</td>
<td>4.6 ± 0.9 (6)</td>
<td>4.5 ± 0.9 (6)</td>
</tr>
<tr>
<td>Urinary angiotensinogen (AGT/creatinine ratio)</td>
<td>3.0 ± 0.7 (6)</td>
<td>52.9 ± 15.6 (6)*</td>
<td>—</td>
<td>29.6 ± 12 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Body weight in g, food intake in g, water intake in ml/day, urinary flow in ml/day, water balance in ml/day, daily Na⁺ excretion in mmol/day, Na⁺ plasma in mmol/l, MAP in mm Hg, rSNA in pps, plasma renin activity, and urinary angiotensinogen of CTRL and hypertensive animals (2K1C) treated with a normal 0.4% NaCl diet or 2% NaCl diet for 2 weeks. *P < 0.05 vs. CTRL group, **P < 0.05 vs. 2K1C group (1-way ANOVA with post hoc Newman–Keuls test). Abbreviations: AGT, angiotensinogen; ANOVA, analysis of variance; CTRL, control; MAP, mean arterial pressure; rSNA, renal sympathetic nerve activity; 2K1C, 2-kidney 1-clip model.
A significant increase in MAP was observed in the 2K1C rats 6 weeks after clipping compared to the CTRL group. However, the 2% NaCl diet did not cause any further change in MAP either in the 2K1C or CTRL groups (Table 1). The basal rSNA was significantly increased in the 2K1C rats compared to the CTRL group, and again, a 2% NaCl diet did not cause any additional change in the basal rSNA in either group (Table 1).

Urinary angiotensinogen was significantly increased (>15-fold) in the 2K1C rats. After 2% NaCl diet, a nonsignificant reduction in the urinary angiotensinogen was found (Table 1).

Effects of a 2% NaCl diet on arterial baroreflex control of HR

The bradycardic response induced by phenylephrine was attenuated in the 2K1C rats compared to the CTRL group (CTRL: $-2.1\pm0.3$, $n=4$; 2K1C: $-0.9\pm0.14$ bpm/mm Hg, $n=5$), as shown in Figure 1A and B. Treatment with 2% NaCl significantly increased baroreflex sensitivity in the 2K1C rats (CTRL 2% NaCl: $-1.9\pm0.2$, $n=5$; 2K1C 2% NaCl: $-1.5\pm0.14$ bpm/mm Hg, $n=9$). There was also an increase in the baroreceptor sensitivity in the CTRL group treated with 2% NaCl in response to sodium nitroprusside (Figure 1C and D) compared to the CTRL group (CTRL: $-3.4\pm1$, $n=5$; 2K1C: $-1.6\pm0.2$, $n=7$; CTRL 2% NaCl: $-5.5\pm0.5$, $n=5$; 2K1C 2% NaCl: $-1.5\pm0.2$ bpm/mm Hg, $n=9$).

Effects of a 2% NaCl diet on arterial baroreceptor control of rSNA

The arterial baroreceptor reflex sensitivity to rSNA control was attenuated in the 2K1C rats compared to the CTRL group as evaluated by the slope of each group ($-2.1\pm0.4$, $n=5$ vs. $0.7\pm0.2$ potentials per second (pps)/mm Hg, $n=7$) (Figure 2B). Individual values are presented in Tables 2 and 3 and Figure 2A and C. Figure 2B and D show the group data. Treatment with a 2% NaCl diet significantly increased the baroreceptor reflex sensitivity to rSNA control only in the 2K1C group, as shown in Figure 2B (CTRL 2% NaCl: $-1.7\pm0.3$, $n=6$; 2K1C 2% NaCl: $-1.5\pm0.1$ pps/mm Hg, $n=7$). Individual values are presented in Table 2, and Figure 2B shows the group data.

Effects of a 2% NaCl diet on systemic oxidative stress markers

TBARs levels were significantly increased in the 2K1C group compared to the CTRL group. A high-sodium diet increased oxidative stress in the CTRL group but not in the 2K1C group (CTRL: $0.9\pm0.08$, $n=5$; 2K1C: $1.9\pm0.14$, $n=8$; CTRL 2% NaCl: $2.13\pm0.16$, $n=8$; 2K1C 2% NaCl: $2.27\pm0.17$ nmol/ml, $n=6$). There was no difference in the erythrocyte GSH levels among the groups (CTRL: $7.4\pm0.4$, $n=7$; 2K1C 2% NaCl: $7.2\pm0.7\mu$mol/gHb, $n=6$) (Figure 3A).
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Significant increase in AGT levels was observed in the clipped (CTRL: 100 ± 12, n = 5; 2K1C: 279 ± 89%, n = 5) but not in the unclipped kidneys of the 2K1C rats compared to the CTRL group (CTRL: 83 ± 15, n = 6; 2K1C: 85 ± 12%, n = 4). Increased sodium intake significantly decreased AGT levels in the clipped and unclipped kidneys (clipped: 64 ± 26, n = 6; unclipped: 33 ± 11%, n = 4) (Figure 4C and F).

There was an increase in angiotensin-converting enzyme (ACE) protein expression in the clipped (CTRL: 100 ± 8, n = 5; 2K1C: 148 ± 16%, n = 6) but not in the unclipped kidneys in the 2K1C rats compared to the CTRL group (CTRL: 101 ± 7, n = 6; 2K1C: 98 ± 17%, n = 6). High-sodium intake decreased ACE protein expression in both kidneys.

Table 2. Values of reduced rSNA in response to 5, 10, 20, 25, 30, 35, and 40 mm Hg MAP increases by intravenous infusion of phenylephrine (100 µg/kg)

<table>
<thead>
<tr>
<th>ΔMAP (mm Hg)</th>
<th>CTRL</th>
<th>2K1C</th>
<th>CTRL 2% NaCl</th>
<th>2K1C 2% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>25.5 ± 8 (4)</td>
<td>-9.1 ± 4 (6)</td>
<td>-13.5 ± 2 (5)</td>
<td>-16.5 ± 4 (7)</td>
</tr>
<tr>
<td>10</td>
<td>28.8 ± 7 (6)</td>
<td>17.8 ± 4 (7)</td>
<td>-25.3 ± 2 (6)</td>
<td>-34 ± 7 (7)</td>
</tr>
<tr>
<td>15</td>
<td>48.3 ± 7 (6)</td>
<td>19.7 ± 4 (8)*</td>
<td>-32.5 ± 4 (6)</td>
<td>-37.4 ± 7 (7)*</td>
</tr>
<tr>
<td>20</td>
<td>53.3 ± 7 (6)</td>
<td>24 ± 4 (8)*</td>
<td>-47.6 ± 7 (6)</td>
<td>-53.8 ± 8 (7)*</td>
</tr>
<tr>
<td>25</td>
<td>57.8 ± 9 (6)</td>
<td>25.7 ± 3 (8)*</td>
<td>-49.3 ± 9 (6)</td>
<td>-59.5 ± 7 (7)*</td>
</tr>
<tr>
<td>30</td>
<td>72.8 ± 10 (6)</td>
<td>40.1 ± 6 (8)*</td>
<td>-61.6 ± 7 (6)</td>
<td>-63.1 ± 8 (7)*</td>
</tr>
<tr>
<td>35</td>
<td>80 ± 13 (6)</td>
<td>40.6 ± 4 (8)*</td>
<td>-70.5 ± 8 (6)</td>
<td>-67.5 ± 7 (7)*</td>
</tr>
<tr>
<td>40</td>
<td>81.6 ± 12 (6)</td>
<td>58 ± 6 (8)</td>
<td>-73.6 ± 9 (6)</td>
<td>-70.4 ± 5 (7)</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. CTRL group, *p < 0.05 vs. 2K1C group (1-way ANOVA with post hoc Newman–Keuls test).

Abbreviations: ANOVA, analysis of variance; CTRL, control; MAP, mean arterial pressure; rSNA, renal sympathetic nerve activity; 2K1C, 2-kidney 1-clip model.

**Effects of a 2% NaCl diet on intrarenal renin angiotensin system**

A significant increase in AGT levels was observed in the clipped (CTRL: 100 ± 12, n = 5; 2K1C: 279 ± 89%, n = 5) but not in the unclipped kidneys of the 2K1C rats compared to the CTRL group (CTRL: 83 ± 15, n = 6; 2K1C: 85 ± 12%, n = 4). Increased sodium intake significantly decreased AGT levels in the clipped and unclipped kidneys (clipped: 64 ± 26, n = 6; unclipped: 33 ± 11%, n = 4) (Figure 4C and F).

There was an increase in angiotensin-converting enzyme (ACE) protein expression in the clipped (CTRL: 100 ± 8, n = 5; 2K1C: 148 ± 16%, n = 6) but not in the unclipped kidneys in the 2K1C rats compared to the CTRL group (CTRL: 101 ± 7, n = 6; 2K1C: 98 ± 17%, n = 6). High-sodium intake decreased ACE protein expression in both kidneys.
when compared to 2K1C as illustrated in Figure 4A and D (clipped: 105 ± 10, n = 6; unclipped: 47 ± 10%, n = 4).

As shown in Figure 4B and E, ACE2 protein expression was reduced in the unclipped kidney (CTRL: 110 ± 12, n = 6; 2K1C: 55 ± 16%, n = 6), and there was no alteration in the clipped kidney (CTRL: 100 ± 9, n = 5; 2K1C: 102 ± 8%, n = 6). The consumption of a high-sodium diet decreased ACE2 expression in the clipped but not the unclipped kidney (clipped: 63 ± 11, n = 5; unclipped: 58 ± 8, n = 5).

Effects of a 2% NaCl diet on plasma renin activity

There were no differences in plasma renin activity (PRA) between the CTRL and 2K1C group, and the treatment with a high-sodium diet did not change PRA as shown in Table 1.

### DISCUSSION

The major new findings of the present study are as follows: (i) increased sodium intake significantly enhanced arterial baroreceptor sensitivity to the control of rSNA and HR in 2K1C rats; (ii) these changes were not related to changes in the basal values of MAP, HR, rSNA, systemic oxidative stress, or PRA; and (iii) high-sodium intake significantly reduced intrarenal and urinary AGT and ACE expression in the kidneys of 2K1C rats.

It is well known that in 2K1C rats, there is an impairment of the arterial baroreceptor control of efferent sympathetic activity that could trigger sympathoexcitation and RAAS activation. In the present study, we observed an increase in ACE and AGT protein expression in clipped kidney and decreased baroreflex control sensitivity to HR and rSNA in 2K1C rats. After the consumption of a high-sodium diet, there were decreased levels of ACE and AGT in both kidneys (clipped and nonclipped), lower urinary excretion of AGT as well as an improvement in baroreflex sensitivity without further increases in MAP and rSNA. However, it is not possible to state whether the improvement of baroreceptor function is a cause or a consequence of reduction of intrarenal RAAS.

A high-sodium diet potentiates arterial baroreceptor discharge, and baroreceptor denervation is known to trigger increased blood pressure after the consumption of a high-salt diet. Considering that electrical stimulation of the carotid sinus inhibits renin secretion and that RAAS activation has an important role in blood pressure and sodium excretion, the decrease in renin release that is caused by baroreflex activation is likely an important mechanism to prevent an increase in MAP. Baroreceptor dysfunction could be a principal mechanism that contributes to neurohumoral activation and subsequent alteration in vascular resistance, sodium...
and water balance and renin secretion. Indeed, it is has been suggested that blood pressure variability may constitute an important mechanism to target organ damage independently of mean blood pressure.23 Our hypothesis is that the improvement in baroreflex sensitivity, after the consumption of a high-sodium diet prevents a further increase in ABP in 2K1C rats. Interestingly, we observed an improvement in baroreceptor function in CTRL rats after the consumption of a high-salt diet, suggesting that this is an adaptative physiological mechanism that is related to cardiovascular homeostasis during elevated salt ingestion. An interesting previous study from Jarcki et al. described that in conditions of constant renal perfusion in anesthetized dogs, alterations of carotid sinus pressure caused variations of renin secretion, indicating the important influence that arterial baroreceptors have on renin release independent of changes in intrarenal perfusion.24

In the present study, 2K1C rats maintained an adequate sodium balance before and after high-sodium intake considering that the water balance and plasma sodium was maintained. Previous evidence showed that an 8% NaCl diet that was fed to 2K1C animals decreased the renin levels and did not cause any further increase in blood pressure, suggesting that the 2K1C model is not a salt sensitive model.25 The increase in baroreflex sensitivity that is associated with an appropriate sodium balance after the consumption of a high-sodium diet contributes to the prevention of a further increase in MAP. Thus, our data show that this higher sensitivity in baroreceptor control of HR

Figure 4. (a) Protein expression of ACE in the clipped kidney. (b) Protein expression of ACE in the unclipped kidney. (c) Protein expression of ACE2 in the clipped kidney. (d) Protein expression of ACE2 in the unclipped kidney. (e) Angiotensinogen level in the clipped kidney. (f) Angiotensinogen level in the unclipped kidney. CTRL and hypertensive animals (2K1C) treated with a 0.4% NaCl or 2% NaCl diet for 2 weeks. *P < 0.05 vs. CTRL group, †P < 0.05 vs. 2K1C group (1-way ANOVA with post hoc Newman–Keuls test). Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; AGT, angiotensinogen; ANOVA, analysis of variance; CTRL, control; 2K1C, 2-kidney 1-clip model.
and rSNA is a compensatory mechanism that contribute in part to a salt-resistant effect on baseline MAP and rSNA in the neurogenic phase of renovascular hypertensive rats treated with 2% NaCl.

It has been shown that a diet with an increased amount of salt leads to a reduction in intrarenal RAAS. In 2K1C rats that were treated with a moderate high-sodium diet, we observed a significant reduction of intrarenal RAAS that could foster renal protection considering that intrarenal AngII modulates vascular resistance, increases sodium reabsorption and affects the tubuloglomerular feedback. Thus, suppression of the intrarenal RAAS in the 2K1C rats on high salt associated with increased in baroreceptor function provide a salt-resistant effect on 2K1C hypertension.

Interestingly, a high-salt diet (3%) lead to activated intrarenal RAS and worsened hypertension while a low-salt diet (0.03%) lead to the opposite effect in spontaneously hypertensive rats. The discrepancy in the responses to high-sodium diet between the renovascular model and spontaneously hypertensive rats is not surprising, considering that the mechanisms underlying the increase in blood pressure are distinct in these models. One key factor is the neurogenic influence in these models of hypertension. In spontaneously hypertensive rats, lesions of an AngII and osmosensitive region of the anterior hypothalamus, the anteroventral third ventricle, does not prevent the development of hypertension. However, hypertension in the intermediate phase of 2K1C model depends upon the integrity of the anteroventral third ventricle region, which indicates that the central nervous system plays an important role in the onset and progression of increased blood pressure in this model.

In the present study, despite a lack of any change in PRA after the consumption of a high-sodium diet, there was a decrease in AGT and ACE protein expression in both kidneys—clipped and unclipped—suggesting that a preferential change in the tissue RAAS occurred in the studied phase of hypertension in the 2K1C rats (6 weeks after clipping). The activation of RAAS is closely related to autonomic dysfunction and cardiovascular alterations in the 2K1C model of hypertension. The blockade of AT1 receptors in 2K1C rats increased baroreflex sensitivity and decreased tonic rSNA and MAP in 2K1C. We suggest that the increase in baroreflex sensitivity that is triggered by salt is involved in the decreased levels of the RAAS components in both kidneys.

Furthermore, ACE2, which promotes Angiotensin 1–7 generation and has a role in cardiovascular protection, was unchanged in the clipped kidney and decreased in the unclipped kidney. In the present study, ACE2 levels decreased in clipped kidney after the consumption of a high-sodium diet. Despite the fact that we found a decrease in intrarenal and urinary AGT in response to sodium intake, the decrease in ACE2 could contribute to hypertension by reducing the protective effects of Angiotensin 1–7. In normotensive animals, the consumption of a high-salt diet decreased ACE, AGT, and ACE2 levels and increased renal oxidative stress. We did not observe any changes in systemic oxidative stress after the consumption of a high-salt diet, but we cannot exclude the possibility that the treatment altered intrarenal oxidative stress.

Increased oxidative stress is also correlated with baroreceptor dysfunction in renovascular hypertension. In the present study, the consumption of a high-sodium diet increased baroreflex sensitivity without any changes in systemic oxidative stress, which remained elevated in 2K1C animals. Thus, improved baroreceptor function was likely unrelated to changes in oxidative stress, at least on a systemic level. It is apparent that oxidative stress is not the only factor that promotes baroreflex dysfunction in renovascular hypertension. In the control group, there was a positive correlation between increased sodium intake, increased oxidative stress and the increased baroreflex control of rSNA.

A significant increase in water ingestion was observed in the 2K1C group compared to the control animals, and treatment with a high-sodium diet further increased the water intake, but only in the 2K1C group. There are several evidences showing increase in RAAS in the central nervous system in 2K1C rats. Such studies shows an increase in expression of AT1 receptor in areas involved in cardiovascular function such as the rostral ventrolateral medulla and paraventricular nucleus (PVN) and in areas involved in drinking behavior as subfornical organ (SFO). Thus, we suggest that in 2K1C an increased central action of AngII is responsible for the augment in drinking water. The sodium excretion and urinary volume was increased in the 2K1C rats after the consumption of a high-sodium diet, and there was no alteration in the plasma sodium and water balance. In normotensive animals, there was an increase in sodium excretion although the urinary volume was unchanged. It has previously been demonstrated that the increase in 2K1C sodium and water reabsorption occur via pressure natriuresis and diuresis. Therefore, the 2K1C animals possibly had a greater renal perfusion pressure to eliminate the same amount of sodium as the control animals. We suggest that there was no impairment in the sodium balance in the 2K1C rats, and there was no salt retention even after these animals consumed a high-salt diet.

Taken together, the results show that increased sodium intake in the neurogenic phase of renovascular hypertension in rats leads to alterations in renal and cardiac autonomic control and intrarenal RAAS components with no further increases in baseline MAP or rSNA. Therefore, increased arterial baroreceptor control associated with a suppression of the intrarenal RAAS in the 2K1C rats on high-salt diet provide a salt-resistant effect on hypertension and sympathoexcitation in renovascular hypertensive rats.

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DISCLOSURE

The authors declared no conflict of interest.
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