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## EFFECTS OF RENAL PERFUSION PRESSURE ON RENAL MEDULLARY H<sub>2</sub>O<sub>2</sub> AND NO PRODUCTION

Chunhua Jin<sup>1</sup>, Chunyan Hu<sup>2</sup>, Aaron Polichnowski<sup>1</sup>, Takefumi Mori<sup>1,2</sup>, Meredith Skelton<sup>1</sup>, Sadayoshi Ito<sup>2</sup>, and Allen W. Cowley Jr.<sup>1</sup>

<sup>1</sup>Department of Physiology, Medical College of Wisconsin, Milwaukee, WI 53226

<sup>2</sup>Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

### Abstract

Studies were designed to determine the effects of increases of renal perfusion pressure (RPP) upon the production of H<sub>2</sub>O<sub>2</sub> and NO<sub>2</sub><sup>-</sup>+NO<sub>3</sub><sup>-</sup> (NO<sub>x</sub>) within the renal outer medulla. Sprague-Dawley rats were studied with either the renal capsule intact or removed to ascertain the contribution of changes of medullary blood flow (MBF) and renal interstitial hydrostatic pressure (RIHP) upon H<sub>2</sub>O<sub>2</sub> and NO<sub>x</sub> production. Responses to three 30 minute step changes of renal perfusion pressure (RPP; from ~85 to ~115 to ~145 mmHg) were studied using adjustable aortic occluders proximal and distal to the left renal artery. Medullary interstitial H<sub>2</sub>O<sub>2</sub> determined by microdialysis increased at each level of RPP from 640 to 874 to 1593 nmol/L as did H<sub>2</sub>O<sub>2</sub> urinary excretion rates and these responses were significantly attenuated by decapsulation. Medullary interstitial NO<sub>x</sub> increased from 9.2 to 13.8 to 16.1 μmole/L with parallel changes in urine NO<sub>x</sub>, but decapsulation did not significantly blunt these responses. Over the range of RPP, MBF (laser-Doppler flowmetry) rose ~30% and RIHP from 7.8 to 19.7 cmH<sub>2</sub>O. RIHP and the natriuretic and diuretic responses were significantly attenuated with decapsulation but MBF was not affected. The data indicate that pressure-induced increases of H<sub>2</sub>O<sub>2</sub> emanated largely from increased tubular flow rates to mTAL and NO largely from increased MBF to the vasa recta. The parallel pressure-induced increases of H<sub>2</sub>O<sub>2</sub> and NO indicate a participation in shaping the “normal” pressure-natriuresis relationship and explain why an imbalance in either would affect the blood pressure salt-sensitivity.

### Keywords

renal medullary oxidative stress; hydrogen peroxide; nitrate and nitrite; nitric oxide; pressure-natriuresis; renal medullary blood flow

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Renal oxidative stress is enhanced in many animal models of hypertension and renal disease and is associated with renal fibrosis, vasoconstriction, apoptosis, and a reduction of urinary excretion of sodium (UNaV)<sup>1</sup>. It has been demonstrated that either a reduction of NO production or an increase in renal oxidative stress within the renal medulla can produce hypertension and renal injury<sup>1,2,3,4,5,6,7</sup>. The mechanisms leading to excess production of reactive oxygen species within the kidney are beginning to be understood. It is evident, for example, that both elevations of hormones such as angiotensin II (Ang II) and increased tubular or extracellular sodium concentrations can stimulate the production of superoxide (O<sub>2</sub><sup>-</sup>) within

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**Corresponding author:** Allen W. Cowley, Jr, Ph.D. Department of Physiology Medical College of Wisconsin 8701 Watertown Plank Road Milwaukee, Wisconsin 53226 Phone: 414-456-8724 FAX: 414-456-6546 Email: cowley@mcw.edu.

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the medullary thick ascending limbs of Henle (mTAL) and contributes to hypertension and renal injury<sup>8,9,10</sup>.

The elevation of renal perfusion pressure (RPP) with hypertension can contribute importantly to the progressive renal injury generally observed in hypertension<sup>11</sup> as demonstrated in two rat models of hypertension; infusion of low pressor angiotensin II (AngII) + high salt diet<sup>8</sup>, and the Dahl salt-sensitive rat strain<sup>12</sup> in which RPP to one kidney was chronically protected from elevated pressures using a computerized servo-controlled balloon occluder implanted between the distal left and proximal right renal arteries<sup>8</sup>. In the kidneys exposed to high RPP, molecules related to pathways of oxidative stress such as TGF- $\beta$  and NF- $\kappa$ B, exhibited enhanced expression compared to the pressure-protected kidney. Kidneys exposed to the higher perfusion pressure also exhibited greater glomerular and medullary tubular sclerosis and interstitial fibrosis with an exaggerated expression of genes related to pathways of oxidative stress and apoptosis<sup>12</sup> when compared to the pressure-protected kidney. These results indicate that RPP in some manner stimulated oxidative stress and contributed to the progression of hypertension and renal injury.

Since it was found that increased delivery of NaCl to the mTAL by microperfusion results in increased production of superoxide in this tubular segment of the outer medulla<sup>13</sup>, we hypothesized that elevations of RPP with a resulting increased delivery of NaCl would produce oxidative stress in the this region of the kidney. Given that elevations of RPP are known to increase medullary blood flow (MBF) and renal interstitial hydrostatic pressure (RIHP), these parameters were also determined in order to indirectly assess the mechanisms responsible for observed changes of H<sub>2</sub>O<sub>2</sub> in the outer medulla. The study therefore determined if kidneys of normal Sprague Dawley rats when subjected to acute increases of RPP (~85 to ~115 to ~145 mmHg) responded with increased levels of H<sub>2</sub>O<sub>2</sub> within the outer medulla and whether these responses were driven by increases of MBF and RIHP by comparing responses in kidneys with intact renal capsules and to responses in decapsulated kidneys. Also, changes in NO production in response to elevations of RPP were also studied to determine how these events may be interrelated.

## Methods

### Experimental Animals

Male Sprague Dawley (SD) rats were used in all protocols (Protocol 1 and 2 used Harlan SD, Madison, WI.; Protocol 3 used SLC SD rats, Shizuoka, Japan) aged 10-11 weeks. Rats were anesthetized with ketamine (20mg/kg, i.m.) and inactin (50mg/kg, i.p), and placed on a temperature-controlled surgical table to maintain body temperature at 37°C. All procedures were approved by the Institutional Animal Care and Use Committee.

### Surgical Preparation

In studies to determine the relationship between three levels of RPP (80-85, 110-115, and 140-145 mmHg) and H<sub>2</sub>O<sub>2</sub> or NOx production in a single kidney, the left kidney was isolated, the renal artery denervated, and an adjustable micro-Blalock clamp placed around the aorta proximal and distal to the renal arteries to raise or lower RPP. Superior mesenteric and celiac arteries were tied off to achieve the highest increase of RPP to 140-145 mmHg. The left ureter was catheterized for collection of urine and the femoral artery and vein catheterized for measurement of aortic renal perfusion pressure (RPP) and for infusion of solutions respectively. An intravenous infusion of 3% bovine serum albumin in saline was given at a rate that maintained blood volume constant as estimated by hematocrit. Following one hour of equilibration, RPP was measured for two 30-min. baseline periods to ensure the stability of the preparation before RPP was changed and adjusted for measurements at the three levels of

RPP described above. Urine was collected for 30-minute corresponding to the duration of each step change in RPP for determination of urine sodium and volume as well as either H<sub>2</sub>O<sub>2</sub> or NO<sub>x</sub>. Three distinct protocols were carried out:

**Protocol 1. Effects of step increases in RPP upon production of renal medullary H<sub>2</sub>O<sub>2</sub> or NO<sub>x</sub> in rats with intact kidney capsule and in rats with the capsule removed**

In these studies, a linear microdialysis fiber (320 μm OD, 5mm membrane window, LM-5, BAS Inc., West Lafayette, IN) was inserted longitudinally using a 30-gauge needle from the lower to the upper pole of the kidney to pass through the outer medulla and anchored in place on the kidney surface with cyanoacrylate adhesive. This dialysis fiber was perfused with 0.9% NaCl at a rate of 2μl/min throughout the study. Dialysate was collected continuously throughout each 30 min. period for determination of either H<sub>2</sub>O<sub>2</sub> or NO<sub>x</sub>. The placement of the fiber in the outer medulla was confirmed at the end of the experiment by careful visual examination, and rats with incorrectly placed fibers were discarded from the study. In one group of rats, the renal capsule remained intact while in a separate group of rats, the capsule was removed as previously described<sup>14</sup>. Two separate groups of rats were surgically prepared in the identical manner but RPP was maintained constant at control levels throughout the entire study. H<sub>2</sub>O<sub>2</sub> was determined in one time control group and NO<sub>x</sub> in the second time control group (“time control”; Table S1A and B; please see <http://hyper.ahajournals.org>).

**Protocol 2. Effect of step increases of RPP on medullary renal interstitial hydrostatic pressure (RIHP) or medullary blood flow (MBF) in rats with intact kidney capsule and in rats with the capsule removed**

Since simultaneous implantation into the same kidney of the microdialysis fiber, the implanted polyethylene catheter for RIHP measurement, and the optical fiber for measurement of MBF was too disruptive to normal function, separate groups of rats were studied. For the determination of RIHP, rats were prepared as described above except rather than implanting a microdialysis fiber, a polyethylene catheter (PE50) with a polyethylene matrix in the tip was implanted into the outer medulla of the kidney as previously described<sup>14</sup>. For rats in which MBF was determined, an optical fiber was inserted into the outer medulla as previously described<sup>15</sup>. RIHP or MBF was measured continuously during the step changes in RPP. These measurements were made in groups of rats with the kidney capsule either intact or removed.

**Protocol 3. Urinary H<sub>2</sub>O<sub>2</sub> responses of the left and right kidney within the same rat**—In another group of rats, the capsule of the left kidney remained intact while that of the right kidney was removed. RPP was then increased from 119 to 145 mmHg to both kidneys by tying off the celiac and superior mesenteric arteries and adjusting the final elevation of RPP with a ligature placed around the aorta distal to both renal arteries. Urine was collected bilaterally from catheters inserted into both ureters for measurement of changes of urinary H<sub>2</sub>O<sub>2</sub> excretion rates associated with the increase of RPP.

**Protocol 4. Effect of step increases of RPP on glomerular filtration rate (GFR)**

GFR was determined by inulin clearance of FITC-inulin<sup>16</sup>. FITC-inulin (5 mg/ml; Sigma) dissolved in BSA and saline was infused at 3 ml/hr for 60 min. prior to the collection of the first urine sample. Blood samples were taken at the midpoint of each urine collection period. Collected samples were diluted with phosphate buffered saline (pH 7.4) and the fluorescence measured with a microplate reader.

## Biochemical Measurements

H<sub>2</sub>O<sub>2</sub> concentration was determined in interstitial fluid collected by microdialysis and urine using a fluorescence spectrometric assay (Amplex Red Hydrogen Peroxide Assay kit, Molecular Probes, Eugene, OR) as previously described<sup>3,5,17</sup>. NO<sub>x</sub> was determined in

interstitial fluid collected by microdialysis and urine with absorbance spectrophotometer using the Greiss reaction (Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemical Company, MI) as previously described<sup>18</sup>. Since all of the nitrite in the sample is converted by the reaction to nitrate, the final determination is the sum of the converted nitrite plus the nitrate already in the sample. This sum is designated as NO<sub>x</sub> in this manuscript. Urine volume for calculation of urine flow rate (UV) was determined gravimetrically and urine sodium for calculation of urinary excretion of sodium (UNaV) measured by flame photometry.

### Statistical analysis

Data are presented as mean ± SE. For statistical comparisons, 2-way ANOVA with repeated measures was used followed by a Duncan's post hoc test. All statistical analyses were performed on the raw data.  $P < 0.05$  was considered to be statistically significant.

## Results

### Relationship of RPP to sodium and water excretion, renal medullary interstitial concentrations and urinary excretion of H<sub>2</sub>O<sub>2</sub> and NO<sub>x</sub> in rats with intact renal capsule and in rats with the renal capsule removed

Figure 1 (Panel A) summarizes changes of urine sodium excretion (UNaV) and medullary interstitial and urine H<sub>2</sub>O<sub>2</sub> in response to RPP adjusted to three levels in one group rats ( $n=7$ ) with an intact renal capsule compared to a group with the renal capsule removed ( $n=8$ ). In rats with the renal capsule intact, RPP was adjusted downward from resting control levels of  $112 \pm 2.0$  to  $84 \pm 0.8$  mmHg then to  $111 \pm 0.3$  mmHg (not different from pressure setting of time control group) and finally to  $143 \pm 0.3$  mmHg. Associated with these step increases of RPP, UNaV rose significantly from  $0.36 \pm 0.1$  to  $2.88 \pm 0.5$  to  $9.86 \pm 0.6$   $\mu\text{mol}/\text{min}$  per gram kidney weight (g kwt) and urine volume from  $4.3 \pm 0.5$  to  $18.3 \pm 3.1$  to  $54.4 \pm 4.9$   $\mu\text{L}/\text{min}$  per g kwt (data not shown). Elevation of RPP significantly increased medullary interstitial H<sub>2</sub>O<sub>2</sub> at each pressure step ( $640 \pm 47$ ,  $874 \pm 53$  and  $1593 \pm 120$  nmol/L;  $p < 0.05$ ). Parallel increases of urinary H<sub>2</sub>O<sub>2</sub> excretion were observed in these rats increasing significantly from  $0.18 \pm 0.01$ ,  $0.44 \pm 0.02$  to  $0.92 \pm 0.11$  nmol/min per g kwt with the increases of RPP. In a separate group of rats ( $n=8$ ), GRF was determined and found to autoregulate in a manner similar to that reported by others<sup>19</sup>. With RPP fixed at  $85 \pm 0.5$  mmHg, GFR averaged  $0.5 \pm 0.05$  ml/min/g kwt in the left kidney. When RPP was increased to  $109 \pm 1.1$  mmHg, GFR rose significantly to  $0.69 \pm 0.05$  ml/min/g kwt. GFR was not further increased ( $0.78$  ml/min/g) at the highest RPP fixed at  $139 \pm 2.1$  mmHg. RPP in the time control group ( $n=7$ ) was adjusted to remain constant at  $110 \pm 4.9$ ,  $111 \pm 5$  and  $110 \pm 4.8$  mmHg, respectively for the three collection periods. No significant changes in any measured variable were observed in the time control group (Table S1A; please see <http://hyper.ahajournals.org>).

Similar step increases of RPP were evaluated in the decapsulated group ( $83 \pm 0.6$  to  $109 \pm 1.0$  and then  $141 \pm 0.8$  mmHg;  $n=8$ ; Figure 1A). Elevation of RPP from 83 to 109 mmHg increased renal interstitial H<sub>2</sub>O<sub>2</sub> in the decapsulated kidney significantly and to nearly the same amount seen in the group with the intact capsule. However, when RPP was elevated from 109 to 141 mmHg, medullary interstitial H<sub>2</sub>O<sub>2</sub> was significantly attenuated in this group increasing only 15% compared to the 82% increase observed in the group with the intact capsule. Similarly urinary excretion of H<sub>2</sub>O<sub>2</sub> increased with each pressure step with the excretion in the intact kidney being significantly greater than the increase measured in the decapsulated kidney at the highest pressure step.

Figure 1B summarizes changes of medullary and urine NO<sub>x</sub> in response to similar changes of RPP in renal intact ( $n=7$ ) and decapsulated ( $n=6$ ) rats. With the renal capsule intact, RPP was adjusted downward from resting control levels of  $116 \pm 3.2$  to  $83 \pm 0.5$  mmHg, then to  $111 \pm 0.6$

mmHg and finally to  $140 \pm 2.3$  mmHg ( $n=7$ ). Associated with the step changes of RPP, rising from 83 to 111 to 140 mmHg, UNaV increased significantly from  $0.22 \pm 0.1$  to  $2.42 \pm 0.7$  to  $10.28 \pm 1.6$   $\mu\text{mol}/\text{min}$  per g kwt and UV from  $3.4 \pm 0.5$  to  $20.4 \pm 3.5$  to  $53.2 \pm 5.3$   $\mu\text{L}/\text{min}$  per g kwt (data not shown). Medullary interstitial NOx increased significantly when RPP was increased from 83 to 111 mmHg ( $9.2 \pm 2.8$  to  $13.8 \pm 3.9$ ;  $P < 0.05$ ). As RPP was increased further to 140 mmHg, medullary interstitial NOx rose to  $16.1 \pm 3.9$   $\mu\text{mol}/\text{L}$ . Similarly, urinary NOx excretion increased from  $1.6 \pm 0.9$  to  $7.0 \pm 2.9$  to  $11.1 \pm 4.8$   $\text{nmol}/\text{min}$  per g kwt at the three respective pressure steps. In decapsulated kidneys, the pressure-natriuresis response was significantly reduced compared to the intact group (UNaV  $0.25 \pm 0.1$  to  $3.15 \pm 0.6$  to  $6.24 \pm 0.9$   $\mu\text{mol}/\text{min}$  per g kwt; urine volume  $3.1 \pm 0.7$  to  $18.1 \pm 3.7$  to  $31.4 \pm 5.2$   $\mu\text{L}/\text{min}$  per g kwt-data not graphed). In contrast, to the H<sub>2</sub>O<sub>2</sub> responses, renal decapsulation did not significantly attenuate the relationship between RPP and medullary interstitial NOx ( $8.9 \pm 2.4$ ;  $15.9 \pm 3.2$ ; and  $16.9 \pm 3.3$   $\mu\text{mol}/\text{L}$ ), or urinary excretion of NOx ( $1.1 \pm 0.3$  to  $6.6 \pm 0.9$  to  $7.8 \pm 1.3$   $\text{nmol}/\text{min}$  per g kwt). No significant changes in any measured variable were observed in the time control group (Table S1B; please see <http://hyper.ahajournals.org>).

### Effect of step increases of RPP on medullary renal interstitial hydrostatic pressure (RIHP) and medullary blood flow (MBF) responses to decapsulation

In rats with intact renal capsules and implanted catheter for determination of RIHP ( $n=7$ ), as RPP was adjusted from  $82 \pm 0.6$  to  $111 \pm 0.8$  to  $142 \pm 0.6$  mmHg, RIHP increased significantly from  $7.8 \pm 0.8$ ,  $12 \pm 0.7$  to  $19.7 \pm 0.5$   $\text{cmH}_2\text{O}$  (Figure 2A). UNaV increased from  $0.1 \pm 0.04$  to  $2.2 \pm 0.5$  to  $7.2 \pm 0.9$   $\mu\text{mol}/\text{min}$  per g kwt while UV increased from  $2.5 \pm 0.4$ ,  $15.5 \pm 2.7$  to  $54.8 \pm 3.4$   $\mu\text{L}/\text{min}$  per g kwt with the three pressure steps. With decapsulation ( $n=7$ ), increase of RIHP was significantly attenuated rising from  $4.4 \pm 0.5$  to  $6.5 \pm 0.4$  to  $11.1 \pm 0.4$   $\text{cmH}_2\text{O}$  for the same pressure steps. The increase in UNaV was significantly blunted in the kidneys that were decapsulated compared to the kidneys with intact renal capsule ( $4.5 \pm 0.6$  vs  $7.2 \pm 0.9$   $\mu\text{mol}/\text{min}$  per g kwt;  $p < 0.05$ ). Similarly, UV was also attenuated by decapsulation averaging  $2.7 \pm 0.4$  to  $12.3 \pm 1.9$  to  $30.4 \pm 5.1$   $\mu\text{L}/\text{min}$  per g kwt.

In rats with intact renal capsules and optical fibers implanted for the measurement of MBF ( $n=5$ ), as RPP was adjusted from  $84 \pm 0.5$  to  $111 \pm 0.9$  to  $141 \pm 2.1$  mmHg, MBF increased  $13 \pm 6\%$  above the lowest pressure at the intermediate step and then  $25 \pm 5\%$  at the highest pressure step (Figure 2B). UNaV increased from  $0.3 \pm 0.1$  to  $3.4 \pm 0.4$  to  $10.0 \pm 0.8$   $\mu\text{mol}/\text{min}$  per g kwt while UV increased from  $4.2 \pm 0.6$  to  $22.2 \pm 2.5$  to  $72.2 \pm 11.2$   $\mu\text{L}/\text{min}$  per g kwt with the three pressure steps. In contrast to the RIHP, MBF was not significantly attenuated by decapsulation ( $n=6$ ). However, since the rise of RIHP was clearly blunted by decapsulation, increases in UNaV and urine volume were also blunted in this group of rats.

### Changes in urinary oxidative stress with differing RPP between two kidneys

The relationship between RPP, urinary H<sub>2</sub>O<sub>2</sub> excretion, and the influence of renal interstitial hydrostatic pressure (RIHP) were further confirmed in another group of rats ( $N=7$ ) by comparing responses within the same rat. The renal capsule was removed from the left kidney while the capsule of the right kidney remained intact. In these rats, the aortic occluder was placed below both of the renal arteries so RPP increased from  $119 \pm 3.6$  to  $145 \pm 5.3$  mmHg to both kidneys as shown in Figure 3. Urinary H<sub>2</sub>O<sub>2</sub> excretion of the intact right kidney increased from  $0.12 \pm 0.02$  to  $0.48 \pm 0.09$   $\text{nmol}/\text{min}$  per g kwt ( $p < 0.05$ ), an increase significantly greater than the response of the decapsulated left kidney in which H<sub>2</sub>O<sub>2</sub> excretion was increased slightly with the pressure step and did not reach significance. These data support the results shown above and emphasize the importance of changes of RIHP upon the production of renal H<sub>2</sub>O<sub>2</sub>.



## Discussion

### Renal medullary oxidative stress as driven by acute increases of renal perfusion pressure

The concept of pressure-natriuresis and the relationships between RPP, medullary blood flow (MBF), renal interstitial hydrostatic pressure (RIHP), and UNaV has been well established<sup>20, 21</sup>. It was the goal in the present study to establish how these factors were involved in the observed increases of H<sub>2</sub>O<sub>2</sub> and NO in response to elevations of RPP. The present study provides the first evidence that acute increases of RPP elevate the production of H<sub>2</sub>O<sub>2</sub> in the outer medulla and that this response is triggered by an increase of RIHP.

Since blood flow to the renal medulla of the rat is poorly autoregulated, increases of RPP are transmitted to the vasa recta circulation<sup>21,22</sup> and as RPP and vasa recta flow increase, vasa recta capillary hydrostatic pressure increases producing parallel increases in RIHP due to a net filtration of fluid into the renal interstitial space<sup>14,23</sup>. Pressure-natriuresis results from reduction of Na<sup>+</sup> reabsorption both in the proximal tubules<sup>24,25</sup> and in the deep medullary nephrons. An increase of RPP with an increase of RIHP signals the release of 20-HETE within the proximal tubules<sup>24</sup> which inhibits Na<sup>+</sup>-K<sup>+</sup>-ATPase activity and results in the internalization of the sodium/hydrogen exchanger from the brush border and a reduction in Na<sup>+</sup> reabsorption. This results in a greater delivery of NaCl to the distal tubules<sup>24,25</sup> and natriuresis which is further enhanced by washout of the medullary solute gradient that affects the passive reabsorption of Na<sup>+</sup> in deep medullary loops of Henle<sup>1,21</sup>. It is the increased delivery of NaCl to the mTAL that we hypothesized would stimulate the enhanced production of O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> in this tubular segment when RPP is increased. Since removal of the renal capsule significantly blunted the rise of RIHP, pressure-natriuresis, and the rise of H<sub>2</sub>O<sub>2</sub> within the outer medulla, (all by approximately 40%), we conclude that NaCl delivery to the mTAL was critical for this response. It is possible that changes of RIHP could in some way have directly stimulated H<sub>2</sub>O<sub>2</sub> production in various tubular segments, however, such responses have not yet been explored. Although the precise origin of H<sub>2</sub>O<sub>2</sub> production was not determined in the present study, it is recognized that the mTAL can be a major source of oxygen free radical (O<sub>2</sub><sup>-</sup>) production<sup>4,9,10,13</sup> and increased luminal flow and NaCl delivery to the mTAL can stimulate O<sub>2</sub><sup>-</sup> production<sup>13,26</sup>. It is of interest that changes of urinary H<sub>2</sub>O<sub>2</sub> excretion in the present study were remarkably parallel to those seen in the microdialysis samples of the outer medullary interstitial fluid. This suggests that urinary H<sub>2</sub>O<sub>2</sub> excretion could serve as a good marker of renal medullary oxidative stress since H<sub>2</sub>O<sub>2</sub> is relatively stable in aqueous solutions and a more specific assay than other currently used markers of renal oxidative stress such as 8-isoprostanes determined by enzyme immunoassay, and products of lipid peroxidation as determined by a colorimetric assay for thiobarbituric acid reactive substances.

Finally, it should also be noted that although we have previously used microdialysis techniques to measure nitric oxide<sup>27</sup>, NOx<sup>18</sup>, superoxide<sup>18,28</sup> and H<sub>2</sub>O<sub>2</sub><sup>3,5</sup> in the renal medulla, these earlier studies utilized a needle type microdialysis probe (BR-2, BAS, Inc.) that provided a 2 mm length of membrane for dialysis. The linear microdialysis fiber (with nearly a 5 mm length of membrane) used in the current study provided a greater membrane surface area and enhanced the dialysis efficiency from 32% seen with the needle probe to 52%. The linear fiber maintained a more stable position in the face of increases of RPP. Since fluorescence units were not corrected for the efficiency of the membranes, the absolute values are greater than previously reported from our laboratory.

### Effects of increases of RPP upon renal medullary NO production

Medullary interstitial NOx concentrations and urinary NOx excretion levels also increased as RPP was increased, confirming observations of others<sup>29</sup>. This, however, was mostly associated with the increases of RPP from the lowest (85 mmHg) to the intermediate pressure step (110

mmHg). Changes in MBF following renal decapsulation have not been previously reported and a novel finding of the present study is that in contrast to the changes observed with RIHP, changes of MBF with RPP were unaffected by decapsulation. Microperfusion studies by Pallone et al.<sup>30</sup> found flowdependent increases of NO production in isolated perfused vasa recta of the outer medulla of the rat. A rise of RPP with an increase of MBF would therefore be expected to produce an increased endothelial release of NO and not be affected by renal decapsulation. Although we have reported that increased tubular flow and delivery of Na<sup>+</sup> to mTAL reduces NO production within isolated perfused mTAL<sup>13</sup>, since medullary interstitial NO<sub>x</sub> was increased in the present study, it appears that these levels were dominated by NO produced by the vasa recta vessels. These relationships are clearly complex and since medullary interstitial NO did not rise substantially between RPP of 110 to 140 mmHg even though MBF did rise significantly over this range RPP, it is evident that something else is going on at these pressure levels. It is possible that as RPP and MBF are increased to these higher levels, further increases of interstitial NO concentrations may be attenuated by medullary washout.

We conclude that elevations of RPP stimulate the release of both NO and H<sub>2</sub>O<sub>2</sub> within the renal outer medulla. The mechanisms responsible for the parallel release of these important vasoactive molecules differ whereby NO appears to be driven by increases of MBF while increases of H<sub>2</sub>O<sub>2</sub> results from increased mTAL delivery of NaCl. The balance of H<sub>2</sub>O<sub>2</sub> and NO production in the outer medulla are therefore important considerations in understanding the interrelationships between renal perfusion pressure, sodium excretion, and the long-term control of arterial blood pressure. The parallel increases of H<sub>2</sub>O<sub>2</sub> and NO produced by elevations of RPP appear to be important determinants of the “normal” pressure-natriuresis relationship.

**Perspectives**—Pressure-natriuresis appears to come with the price of also producing oxidative stress within the outer medulla of the kidney unless offset by a parallel production of NO. This would appear to explain why excess levels of medullary H<sub>2</sub>O<sub>2</sub> can lead to a salt-sensitive form of hypertensive<sup>31</sup> while greater levels of medullary NO can reduce salt-sensitivity and lower arterial pressure<sup>32</sup>. One can also speculate that in the chronic state, if both the production of superoxide and NO occur with elevations of RPP, greater amounts of peroxynitrite would also be produced in renal outer medulla that may be associated with greater tissue fibrosis and injury. The present data suggest that the development of antioxidant agents that could effectively reduce medullary O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> production may be of greater clinical benefit than those that focus upon producing greater production of NO, such as L-arginine.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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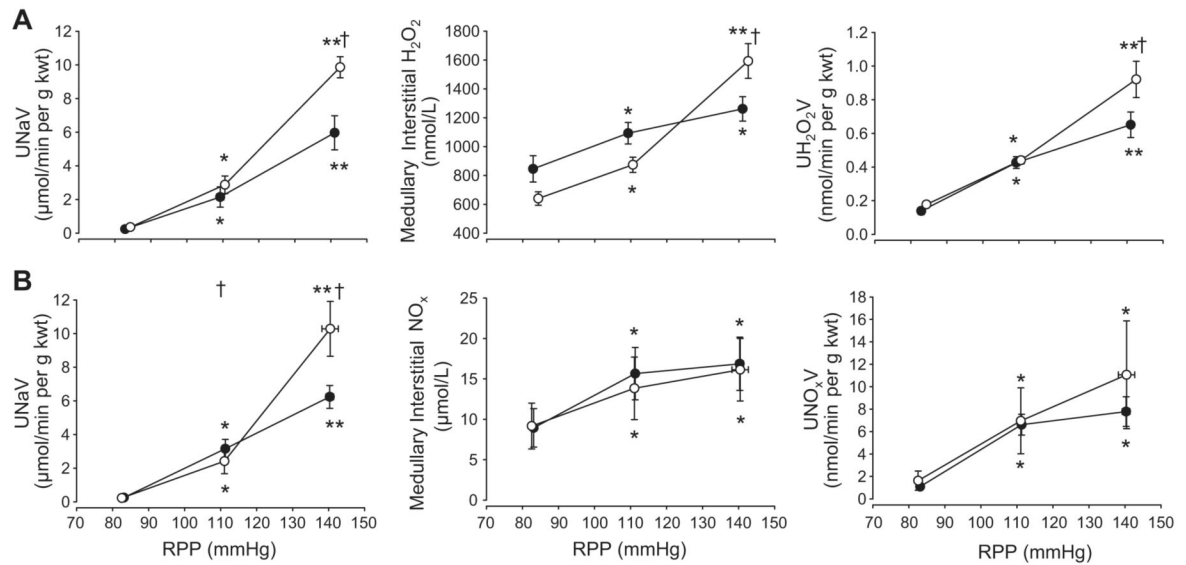
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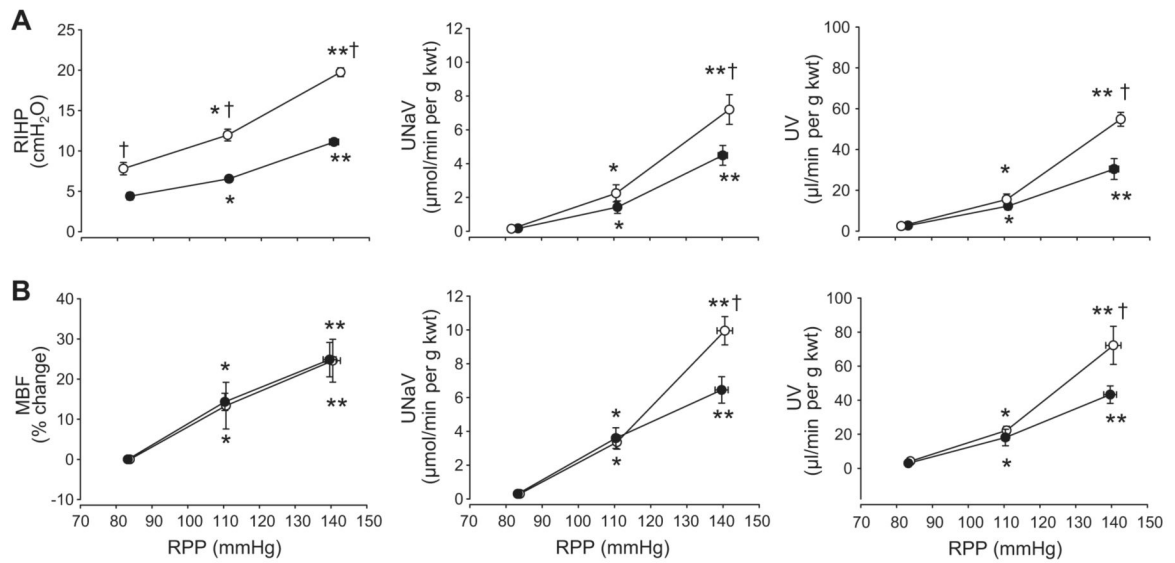


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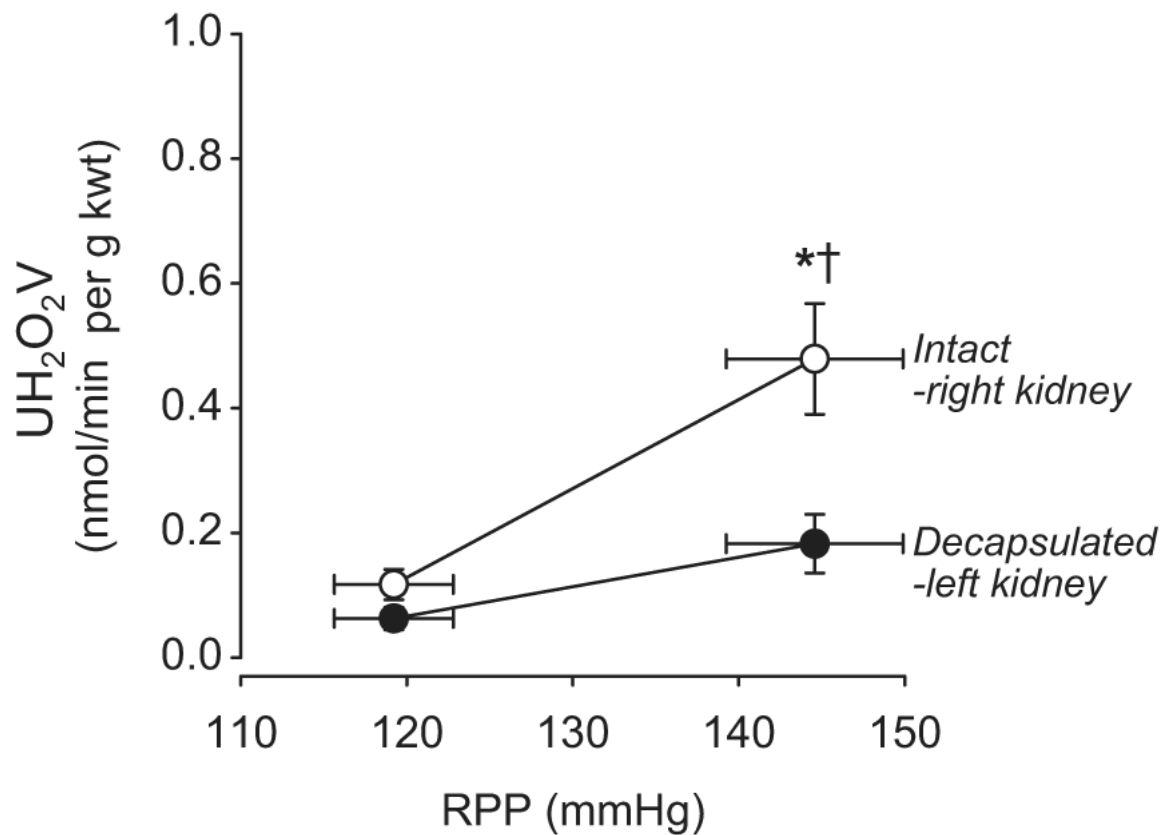


**Figure 1.**

Responses to changes in renal perfusion pressure (RPP) are shown in kidneys with intact capsules (open circles) and following decapsulation (closed circles). In Panel A, the changes in excretion of urinary sodium (UNaV; top figure), medullary interstitial H<sub>2</sub>O<sub>2</sub> (middle figure), and urinary excretion of H<sub>2</sub>O<sub>2</sub> (UH<sub>2</sub>O<sub>2</sub>V; bottom figure) following a change in RPP are shown. Panel B shows the change in excretion of urinary sodium (UNaV; top figure), medullary interstitial NO<sub>x</sub> (middle figure), and urinary excretion of NO<sub>x</sub> (UNO<sub>x</sub>V; bottom figure) following a change in RPP. Values are means ± SE. \* indicates significant change from the lowest pressure ( $p < 0.05$ ); \*\* indicates significant change from both the lowest and the intermediate pressure ( $p < 0.05$ ); † indicates significant difference from the decapsulated group at the same pressure step ( $p < 0.05$ ).



**Figure 2.** Changes in renal interstitial hydrostatic pressure (RIHP; Panel A) or medullary blood flow (MBF; Panel B) as well as urinary excretion of sodium (UNaV) and volume (UV) in response to changes in renal perfusion pressure (RPP) are shown in groups of rats with either intact (open circles) or decapsulated kidneys (closed circles). Values are means  $\pm$  SE. \* indicates significant change from the lowest pressure ( $p < 0.05$ ); \*\* indicates significant change from both the lowest and the intermediate pressure ( $p < 0.05$ ); † indicates significant difference from the decapsulated group at the same pressure step ( $p < 0.05$ ).



**Figure 3.**

Urinary oxidative stress determined as urinary excretion of H<sub>2</sub>O<sub>2</sub> (UH<sub>2</sub>O<sub>2</sub>V) increases with an increase in renal perfusion pressure (RPP) in the intact right kidney (open circles) but the decapsulated left kidney (closed circles) of the same rat showed significantly less excretion with the same change of pressure. Values are means  $\pm$  SE. \* significantly different from the baseline pressure in the same kidney ( $p < 0.05$ ). † significantly different from the decapsulated left kidney ( $p < 0.05$ ).