Elevations in Renal Interstitial Hydrostatic Pressure and 20-Hydroxyeicosatetraenoic Acid Contribute to Pressure Natriuresis

Jan M. Williams, Albert Sarkis, Bernardo Lopez, Robert P. Ryan, Averia K. Flasch, Richard J. Roman

Abstract—This study examined the role of changes in renal interstitial pressure on the renal levels of cytochrome P450 metabolites of arachidonic acid and compared the effects of inhibition of the formation of 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids with 1-aminobenzotriazole on the pressure-natriuretic response versus that seen after administration of HET0016, a more selective inhibitor of the formation of 20-HETE. Renal interstitial pressure rose by 3.4±0.3 mm Hg, and the levels of 20-HETE in renal cortical tissue doubled when renal perfusion pressure was increased from 100 to 160 mm Hg. Removal of the renal capsule prevented the increase in renal interstitial pressure and 20-HETE levels after an elevation in renal perfusion pressure. Urine flow and sodium excretion increased 5-fold when renal perfusion pressure was increased from 100 to 160 mm Hg. The administration of 1-aminobenzotriazole (50 mg/kg, IP) or HET0016 (10 mg/kg IV bolus plus 1 mg/kg per hour of infusion) decreased the pressure-natriuretic response by 50% and inhibited the renal formation of 20-HETE and epoxyeicosatrienoic acids by 90% and 50%, respectively. Administration of a lower dose of HET0016 (1 mg/kg per hour, IV) selectively reduced the formation of 20-HETE by 80% without inhibiting renal epoxygenase activity and blunted the pressure-natriuretic response by 42%. These results indicate that elevations in renal perfusion pressure increase 20-HETE levels in the kidney secondary to a rise in renal interstitial pressure. They also suggest that 20-HETE, rather than epoxyeicosatrienoic acids, modulates the pressure-natriuretic response, because selective blockade of the formation of 20-HETE with HET0016 blunts the response to the same extent as that seen after inhibition of the formation of 20-HETE and epoxyeicosatrienoic acids with 1-aminobenzotriazole. (Hypertension. 2007;49[part 2]:687-694.)

Key Words: kidney • HET0016 • ABT • RIHP • 20-HETE • pressure natriuresis • proximal tubule

The concept that the kidney plays an essential role in the long-term control of arterial pressure is based on the pressure-natriuretic response; however, the mechanism by which this occurs is unknown. Previous studies have indicated that pressure natriuresis is associated with elevations in medullary blood flow and renal interstitial hydrostatic pressure (RIHP) and a fall in sodium transport in the proximal tubule. The fall in sodium transport is associated with inhibition of Na+/K+-ATPase activity and the redistribution of the sodium/hydrogen exchanger from the brush border to the subapical space in the proximal tubule after elevations in RIHP. The rise in RIHP seems to be important in triggering pressure natriuresis, because removal of the renal capsule blocks the fall in proximal tubular reabsorption and attenuates the natriuretic response after an elevation in renal perfusion pressure (RPP). However, the mechanism by which elevations in RPP and/or RIHP inhibits sodium transport in the proximal tubule remains to be determined.

Recent studies have indicated that cytochrome P450 (CYP) metabolites of arachidonic acid (AA) may be involved. In this regard, chronic blockade of the formation of epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE) with 1-aminobenzotriazole (ABT) or cobalt chloride attenuates pressure natriuresis and prevents the fall in Na+/K+-ATPase activity and the redistribution of the sodium/hydrogen exchanger from the brush border to the subapical space in the proximal tubule after elevations in RPP. These studies suggest that a CYP metabolite of AA may mediate the fall in sodium transport in the proximal tubule. However, direct evidence that elevations in RPP stimulate the formation or release of CYP metabolites of AA in the kidney is lacking. It also remains to be determined whether the effects of CYP inhibitors to blunt the pressure-natriuretic response are mediated by inhibition of the formation of 20-HETE and/or EETs. Thus, the present study examined the effects of an elevation in RPP on the concentration of CYP metabolites of AA in the renal cortex of rats and explored the role that changes in RIHP play in this response. We also compared the effects of
blockade of the formation of 20-HETE and EETs with ABT on the pressure-natriuretic response versus those seen using a more selective inhibitor of the formation of 20-HETE, N-hydroxy-N-(4-butyl-2-methylphenyl) formamide (HET0016).

Methods

General
Experiments were performed on 105 male Sprague-Dawley rats weighing between 225 and 350 g purchased from Taconic Farms. The rats were housed in the Animal Care Facility at the Medical College of Wisconsin, which is approved by the American Association for the Accreditation of Laboratory Animal Care. The rats had free access to food and water throughout the study except when they were fasted the night before an experiment. All of the protocols were approved by the Medical College of Wisconsin Animal Care Committee.

Protocol 1: Effects of Elevations in RPP on the levels of CYP Metabolites of AA in the Renal Cortex
Rats were anesthetized with ketamine (30 mg/kg, IM, Phoenix Pharmaceutical Co) and inactin (50 mg/kg, IP, Sigma) and were placed on a heating table to maintain the body temperature at 37°C. Catheters were placed in the carotid and femoral arteries for the recording of arterial pressure above and below the renal arteries and in the femoral vein for intravenous infusions. The rats received an intravenous infusion of a 0.9% NaCl solution containing 2% albumin at a rate of 100 μL/min throughout the experiment. Vasopressin (52 pg/min), aldosterone (20 ng/min), and norepinephrine (100 ng/min) were included in the infusion solution to fix the circulating levels of hormones as described previously. An adjustable clamp was placed on the aorta between the renal arteries so that RPP to the left kidney could be controlled. After surgery and a 45-minute equilibration period, RPP was increased to ~160 mm Hg by tying off the celiac and mesenteric arteries. The right kidney was exposed to the elevated pressure, whereas RPP to the left kidney was controlled at 100 mm Hg using the aortic clamp. After 15 minutes, both kidneys were removed, and the renal cortex was rapidly frozen in liquid nitrogen for measurement of endogenous concentrations of CYP metabolites of AA. These experiments were performed in 3 groups of rats: group 1 (vehicle renal capsule intact; n=6) served as the control group and received an intravenous infusion of vehicle (11% sulfobutyl ether β-cyclodextrin, CyDex, Inc). In group 2 (vehicle, renal capsule removed; n=6), the renal capsules of both the left and right kidneys were partially removed to prevent the increase in RHP after elevations in RPP. In group 3 (HET0016; n=6), rats were treated with a low dose of HET0016 (1 mg/kg, IV) to block the formation of 20-HETE.

Additional experiments were performed in 7 rats to confirm that removal of the renal capsule lowered RHP. These rats were prepared as described above, and a polyethylene matrix capsule was implanted in the renal cortex for measurement of RHP as described previously. RHP was recorded as RPP to the left kidney was reduced from 160 to 100 mm Hg in steps of 20 mm Hg by tightening the clamp on the aorta between the renal arteries. The kidney was perfused at each level of RPP for 2 to 3 minutes until urination stopped. The clamp was then loosened, and the renal arteries were equilibrated for 15 minutes. After 15 minutes, RPP was increased to ~160 mm Hg by tying off the celiac and mesenteric arteries and releasing the aortic clamp, and urine and plasma samples were collected during a 15-minute control period. RPP was then increased to ~160 mm Hg by tying off the celiac and mesenteric arteries and releasing the aortic clamp, and urine and plasma samples were collected. At the end of each experiment, the left kidney was removed and weighed, and the renal cortex was frozen in liquid nitrogen for measurement of the renal metabolism of AA. Experiments were performed in 3 groups of rats: group 1 (n=11) served as the control group and received an intravenous infusion of vehicle; group 2 (n=9) received ABT (50 mg/kg, IP); and group 3 (n=7) received a 10 mg/kg IV bolus dose of HET0016 followed by a 1 mg/kg per hour continuous intravenous infusion.

Renal Metabolism of AA
Microsomes were prepared from the kidneys of vehicle-, ABT-, and HET0016-treated rats as described previously. The metabolism of AA was determined by incubating the microsomes (0.5 mg of protein) with a saturating concentration of [1-14C]AA (0.1 μCi, 42 μmol/L, Amersham Biosciences) for 30 minutes at 37°C. The products were extracted with ethyl acetate and separated by high-performance liquid chromatography on a C18-reverse-phase column (Thermo Hypersil-Keystone), and the metabolites were monitored with a radioactive flow detector, as described previously. Values are expressed as picomoles of product formed per minute per milligram of protein.

Protocol 2: Effects of CYP450 Inhibitors on RIHP and the Pressure-Natriuretic Response
Rats were prepared for the study of pressure natriuresis and measurement of RIHP as described above. A catheter was inserted into the left femoral artery for the collection of urine. [1H-3H]-inulin (2 μCi/mL) and LiCl (20 mmol/L) were added to the infusion solution for the measurement of the glomerular filtration rate (GFR) and the fractional excretion of lithium. RPP was lowered to 100 mm Hg by tightening the aortic clamp. After a 45-minute equilibration period, urine and plasma samples were collected during a 15-minute control period. RPP was then increased to ~160 mm Hg by tying off the celiac and mesenteric arteries and releasing the aortic clamp, and urine and plasma samples were recollected. At the end of each experiment, the left kidney was removed and weighed, and the renal cortex was frozen in liquid nitrogen for measurement of the renal metabolism of AA. Experiments were performed in 3 groups of rats: group 1 (n=11) served as the control group and received an intravenous infusion of vehicle; group 2 (n=9) received ABT (50 mg/kg, IP); and group 3 (n=7) received a 10 mg/kg IV bolus dose of HET0016 followed by a 1 mg/kg per hour continuous intravenous infusion.

Renal Metabolism of AA
Microsomes were prepared from the kidneys of vehicle-, ABT-, and HET0016-treated rats as described previously. The metabolism of AA was determined by incubating the microsomes (0.5 mg of protein) with a saturating concentration of [1-14C]AA (0.1 μCi, 42 μmol/L, Amersham Biosciences) for 30 minutes at 37°C. The products were extracted with ethyl acetate and separated by high-performance liquid chromatography on a C18-reverse-phase column (Thermo Hypersil-Keystone), and the metabolites were monitored with a radioactive flow detector, as described previously. Values are expressed as picomoles of product formed per minute per milligram of protein.

The results of the studies performed above suggested that the 10 mg/kg dose of HET0016 inhibited the renal synthesis of 20-HETE but also reduced the formation of EETs. Therefore, additional studies were performed to find a more selective dose of HET0016. In these experiments, rats were anesthetized with inactin (n=10) or isoflurane (n=6) because we suspected that inactin may reduce renal epoxygenase activity. The rats were prepared for the study of pressure natriuresis, and the left kidney was collected to determine the baseline production of CYP metabolites of AA. The rats then received an intravenous infusion of vehicle or the low dose of HET0016 (1 mg/kg per hour) for 1 hour, and the right kidney was collected for the measurement of CYP activity. Subsequent studies were performed using the low dose of HET0016 on the pressure-natriuretic response in rats anesthetized...
with isoflurane. Experiments were performed on 4 groups of rats: group 1 (n=10) received an intravenous infusion of vehicle; group 2 (n=6) was treated with vehicle, and the renal capsule of the left kidney was removed; group 3 (n=9) received an intravenous infusion of HET0016 at a dose of 1 mg/kg per hour for 1 hour; and group 4 (n=6) was also infused with HET0016 at a dose of 1 mg/kg per hour, and the renal capsule of the left kidney was removed.

Statistical Methods
Data are presented as mean±SE. Significance of differences in mean values was determined using a 1- or 2-way repeated-measures ANOVA and the Holm–Sidak test for preplanned comparisons. A P<0.05 was considered to be statistically significant.

Results
Effects of Elevations in RPP on the Levels of CYP Metabolites of AA
We detected large peaks with an m/z of 319 and retention times corresponding with 14,15-DiHETE and 11,12-DiHETE in ethyl acetate extracts of the renal cortex of rats (Figure 1A). The largest peak that eluted at 18 minutes produced an MS2 spectrum with secondary ions at m/z of 301, 275, 273, 257, and 245 that is identical to that seen using a authentic 20-HETE standard. The concentration of 20-HETE doubled in the renal cortex after an elevation in RPP in rats with an intact renal capsule (Figure 1B). This was associated with a rise in RIHP of 3 to 4 mm Hg (Figure 2). Removal of the renal capsule prevented the increase in RIHP and renal cortical 20-HETE levels after an elevation in RPP. Infusion of the low dose of HET0016 (1 mg/kg per hour) reduced baseline RIHP and 20-HETE levels and abolished the increase in renal cortical 20-HETE levels in response to an elevation in RPP.

The effects of elevations in RPP on the levels of other metabolites of AA in the kidney are presented in the Table. The levels of 11, 12-DiHETE and 5-HETE in the renal cortex...
increased in the rats treated with vehicle when RPP was elevated, whereas the levels of other HETEs and EETs were not significantly altered. Removal of the renal capsule and treatment with HET0016, both of which reduced baseline RIHP, lowered the levels of 5-HETE, 11,12-DiHETE, and 14,15-DiHETE in renal tissue at elevated RPP.

**Effects of CYP450 Inhibitors on the Pressure-Natriuretic Response**

A comparison of the effects of ABT and HET0016 on the pressure-diuretic and -natriuretic responses is summarized in Figure 3. Urine flow and sodium excretion increased 5-fold when RPP was increased in vehicle-treated rats. Fractional excretion of lithium, a marker of proximal tubular reabsorption, rose significantly from 35±1% to 55±3% of the filtered load. Administration of ABT (50 mg/kg) or HET0016 (10 mg/kg bolus dose followed by 1 mg/kg per hour of infusion) decreased the pressure-natriuretic response by 50% and inhibited the renal formation of 20-HETE and EETs by 90% and 50%, respectively. Both ABT and HET0016 significantly reduced the fractional excretion of lithium after RPP was elevated to 42±2% and 43±3%, respectively. Baseline GFR was not significantly different and averaged 1.1±0.1 mL/min per gram in all of the groups at an RPP of 100 mm Hg. GFR remained unaltered in all of the treatment groups after RPP was elevated.

**Figure 2.** Effects of the removal of the renal capsule and the acute treatment with HET0016 (1 mg/kg for 1 hour) on RIHP. Values are mean±SE. †P<0.05 from the corresponding value measured in the vehicle-treated rats with an intact renal capsule.

**Figure 3.** Effects of acute treatment with vehicle, ABT (50 mg/kg, IP), or HET0016 (10 mg/kg and 1 mg/kg per hour, IV) on the diuretic (A) and natriuretic (B) responses to an elevation in RPP. Values are mean±SE. *P<0.05 from the corresponding value in the kidneys perfused at a low RPP (100 mm Hg) within a treatment group. †P<0.05 from the corresponding value measured in the vehicle-treated group.

**Effects of Selective Blockade of the Renal Synthesis of 20-HETE and Removal of the Renal Capsule on the Pressure-Natriuretic Response**

The effects of the low dose of HET0016 on the synthesis of 20-HETE, EETs, DiHETEs, and HETEs by renal cortical microsomes are summarized in Figure 4. Basal formation of 20-HETE was similar in rats anesthetized with inactin or isoflurane, but the formation of EETs was markedly reduced in rats anesthetized with inactin compared with the levels seen in the rats anesthetized with isoflurane. HET0016 reduced the synthesis of 20-HETE by >80% in rats anesthetized with inactin or isoflurane. Production of EETs tended to decrease after HET0016 in the rats anesthetized with inactin, but the baseline production of EETs was already too low to determine whether HET0016 inhibited epoxygenase activity in this group. In contrast, HET0016 increased renal epoxy-

**Table 1: Effects of Elevations in RPP on CYP Metabolites**

<table>
<thead>
<tr>
<th>CYP Metabolite, ng/g of Tissue</th>
<th>Vehicle (100 mm Hg)</th>
<th>&gt;150 mm Hg</th>
<th>Renal Capsule Removed (100 mm Hg)</th>
<th>&gt;150 mm Hg</th>
<th>HET0016 (100 mm Hg)</th>
<th>&gt;150 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HETE</td>
<td>0.50±0.12</td>
<td>1.21±0.29*</td>
<td>0.33±0.15</td>
<td>0.49±0.23†</td>
<td>0.12±0.04</td>
<td>0.18±0.06†</td>
</tr>
<tr>
<td>12-HETE</td>
<td>1.71±0.50</td>
<td>2.49±0.66</td>
<td>2.67±0.93</td>
<td>3.69±1.24</td>
<td>1.08±0.34</td>
<td>1.20±0.20</td>
</tr>
<tr>
<td>15-HETE</td>
<td>2.41±0.60</td>
<td>3.52±0.89</td>
<td>2.18±0.62</td>
<td>2.61±0.74</td>
<td>0.79±0.17</td>
<td>1.15±0.23†</td>
</tr>
<tr>
<td>11,12-DiHETE</td>
<td>0.50±0.16</td>
<td>0.84±0.22*</td>
<td>0.31±0.09</td>
<td>0.35±0.08†</td>
<td>0.21±0.04</td>
<td>0.32±0.06†</td>
</tr>
<tr>
<td>14,15-DiHETE</td>
<td>0.47±0.13</td>
<td>0.67±0.16</td>
<td>0.26±0.08</td>
<td>0.30±0.08†</td>
<td>0.19±0.04</td>
<td>0.35±0.08†</td>
</tr>
</tbody>
</table>

Values are mean±SE.

*P<0.05 vs the corresponding low RPP value within a group and †P<0.05 vs vehicle.
genase activity significantly in rats anesthetized with isoflu-rane, but it had no effect on the renal formation of DiHETEs or other HETEs (lipoxygenase, cyclooxygenase, and CYP derived).

The effects of removal of the renal capsule and low dose of HET0016 on the pressure-natriuretic response in rats anesthetized with isoflurane are presented in Figure 5. In vehicle-treated rats, urine flow and sodium excretion increased 3-fold when RPP was increased from 100 to 150 mm Hg. Removal of the renal capsule reduced the pressure-diuretic and -natriuretic response by 40%. Blockade of the synthesis of 20-HETE with the low dose of HET0016 had a similar effect. HET0016 had no effect on the pressure-natriuretic response in rats in which the renal capsule was removed. GFR was not significantly different and averaged $1.2\pm0.1$ mL/min per gram in all 4 of the groups. GFR was not significantly altered in any group when RPP was elevated. Levels of 20-HETE in the renal cortex fell by $>90\%$ in rats treated with HET0016 versus that seen in vehicle-treated rats (Figure 1B) and averaged $0.018\pm0.004$ and $0.027\pm0.005$ ng/g at RPP of 103 and 154 mm Hg, respectively.

**Discussion**

The results of previous studies indicating that blockade of CYP enzymes with cobalt chloride or ABT blunts the pressure-natriuretic response suggested that 20-HETE or EETs may serve as a mediator of this response. However, direct evidence that elevations in RPP increase the intrarenal levels of CYP metabolites of AA was lacking, and the mechanism by which elevations in RPP increase synthesis or release eicosanoids in the kidney was unknown. The results of the present study indicate that an acute elevation in RPP to 150 mm Hg increases RIHP by 4 mm Hg and the concentrations of 20-HETE, 11, 12-DiHETE, and 5-HETE in the renal cortex of rats. Removal of the renal capsule prevents the rise in RIHP and the levels of these eicosanoids after elevations in RPP. It also blunts the pressure-natriuretic response by 40%. These results indicate that elevations in RPP stimulate the synthesis and/or release of CYP metabolites of AA in the renal cortex secondary to elevations in RIHP. These findings are completely consistent with the results of previous studies suggesting that a CYP eicosanoid may mediate the inhibition of sodium transport in the proximal tubule after an elevation in RPP. Of these eicosanoids that we found were significant elevated, 20-HETE seems to be the most likely candidate to be the mediator, because it has been shown to inhibit sodium transport in the proximal tubule by inhibiting Na⁺, K⁺-ATPase activity secondary to protein kinase C-induced phosphorylation of the serine 23 residue in this enzyme. Although a contribution of 11, 12-DiHETE and 5-HETE to the pressure-natriuretic response cannot be excluded, the effects of these compounds on sodium transport in the proximal tubule have not been studied previously, and there is no evidence that they can mimic the effect of
elevations in pressure and inhibit Na+, K+-ATPase or alter the distribution of sodium transporters.

Further support for the view that the rise in 20-HETE levels likely contributes to the pressure-natriuretic response was derived from our studies comparing the effects of ABT, a nonspecific inhibitor of the formation of EETs and 20-HETE, with those of HET0016, which has been reported to be a highly selective inhibitor of the synthesis of 20-HETE, at least in vitro.29 We found that ABT and HET0016 given at a dose of 10 mg/kg had similar effects. Both drugs reduced the synthesis of 20-HETE in renal microsomes by >90% and attenuated the pressure-natriuretic response by ~50%. This was associated with a fall in the fractional excretion of lithium indicating that proximal tubular reabsorption was enhanced. Unfortunately, the 10-mg/kg dose of HET0016 was not selective and also reduced renal epoxygenase activity by 50%. Thus, we could not conclude whether 20-HETE or EETs mediate the pressure-natriuretic response on the basis of this series of experiments.

Additional experiments were, therefore, performed using a lower dose of HET0016 in animals anesthetized with isoflurane. We first confirmed (Figure 4) that this dose effectively blocks the synthesis of 20-HETE in renal microsomes after in vivo administration; that it had no effect on renal synthesis of 12-, 15-, or 5-HETEs (lipoxygenase activity); and that it increased, rather than inhibited, the renal formation of EETs. It also reduced the levels of 20-HETE in renal tissue by >80%, indicating that 20-HETE found in renal tissue is continually generated by CYP4A enzymes rather than released from the membrane by phospholipases. Selective blockade of formation of 20-HETE with the low dose of HET0016 blunted the pressure-natriuretic response to the same extent as that seen after inhibition of the formation of both 20-HETE and EETs with ABT or the high dose of HET00016. These findings indicate that whereas acute elevations in RPP increase the levels of several CYP metabolites of AA in the kidney, that 20-HETE, rather than EETs, is the likely mediator of the inhibitory effect on sodium transport in the proximal tubule.

The results of the present study are consistent with the hypothesis that pressure natriuresis is signaled by elevations in RPP that are transmitted to the medullary circulation and increase vasa recta capillary pressure and RIHP. The rise in RIHP then inhibits sodium transport in the proximal tubule by stimulating the synthesis of 20-HETE. The mechanism by which elevations in RIHP stimulate the formation of 20-HETE remains to be determined, but it may involve physical deformation of the tubules, elevations in intracellular calcium, activation of phospholipase A2, and the release of AA. The present finding that the concentration of several CYP- and lipoxygenase-derived metabolites of AA increase after an elevation in RPP supports the view that AA is probably released from membrane stores when RIHP is elevated. Our finding that removal of the renal capsule to prevent the rise in RIHP reduces the levels of 20-HETE and lipoxygenase- and epoxygenase-derived metabolites of AA by about the same extent after elevations in RPP further supports the view that the changes in the tissue levels of all of these metabolites are likely because of an RIHP-induced activation of phospholipase.

In the present study, the low dose of HET0016 reduced the levels of lipoxygenase- and epoxygenase-derived metabolites of AA in the kidney at elevated RPP (Table 1), although it had no effect on the synthesis of these metabolites. This apparent discrepancy may be because of the fact that HET0016 reduced RIHP and that this may diminsh tissue-level eicosanoids by reducing the release of AA and the availability of substrate similar to what was seen in the rats in which the renal capsule was removed. The mechanism by which HET0016 alters RIHP remains to be determined, but previous studies have clearly indicated that other inhibitors of 20-HETE alter renal vascular tone and medullary blood flow.30,31

It is important to recognize that removal of the renal capsule to prevent elevations in RIHP or blockade of the synthesis of 20-HETE only blunted the pressure-natriuretic response by 50%. This means that at least half of this response occurs by a different mechanism. We and others have reported that elevations in RPP also inhibit sodium transport in the thin loop of Henle of deep nephrons secondary to washout of the medullary osmotic gradient and the loss of passive sodium reabsorption in this portion of the nephron.3,13,18,32 This mechanism is likely mediated by the changes in medullary blood flow and is not dependent on the formation of CYP metabolites of AA.

Previous studies have indicated that the development of hypertension in spontaneously hypertensive rats is associated with a reduction in medullary blood flow, RIHP, and the pressure-natriuretic response.5,10,12 Similarly, chronic reductions in medullary blood flow produced by a number of vasoconstrictors have also been reported to promote the development of salt-sensitive forms of hypertension.33 The present findings that elevations in RIHP increase 20-HETE levels in the renal cortex and that 20-HETE contributes to the pressure-natriuretic response may help explain the nagging question of how changes in medullary blood flow might influence the pressure-natriuretic relationship and the long-term control of arterial pressure. We propose that changes in medullary blood flow alter RIHP and that this sets the sensitivity of the pressure-natriuresis response by altering the basal production of 20-HETE and other metabolites of AA in the proximal tubules. The present results are also consistent with the large body of evidence suggesting that a deficiency in the renal formation of 20-HETE promotes the development of salt-sensitive hypertension.25,34–38 This conclusion is further supported by previous findings that the expression of CYP4A protein and the renal synthesis of 20-HETE is reduced in the kidney of Dahl salt-sensitive rats,25,34–37 along with DOCA-salt hypertension39 and angiotensin II hypertensive rats.40,41 It is also consistent with recent reports that knockout of the CYP4A1442 or CYP4A1038 genes produces hypertension in mice and our finding that chronic blockade of the formation of 20-HETE produces hypertension in salt-resistant strains of rats.43,44 Moreover, there are 3 recent genetic studies linking a T8590C polymorphism in the CYP4A11 gene that reduces the formation of 20-HETE to elevated blood pressure in the human population studies.45–47
Perspectives
The results of the present study indicate that elevations in RRP increase 20-HETE levels in the kidney secondary to a rise in RIHP. They also suggest that 20-HETE, rather than EETs, modulates the pressure-natriuretic response, because selective blockade of formation of 20-HETE with a low-dose HET0016 blunts the pressure-natriuretic response to the same extent as that seen after inhibition of the formation of both 20-HETE and EETs with ABT or a higher dose of HET0016. These results are consistent with the view that a deficiency in the formation of 20-HETE promotes the development of salt-sensitive forms of hypertension and provides a potential mechanism to explain how reductions in renal medullary blood flow may promote the development of hypertension by reducing RIHP and the renal production 20-HETE.

Acknowledgments
We thank Jennifer Goepfert of the Biochemical Core Laboratory for measuring the sodium concentration from the urine samples and Katherine Fredrich for determining the renal metabolism of AA from renal cortical microsomes.

Sources of Funding
This work was supported by National Institutes of Health grants HL29587 and HL36279 and a United Negro College Fund/Merck Postdoctoral Science Research Fellowship.

Disclosures
None.

References


Elevations in Renal Interstitial Hydrostatic Pressure and 20-Hydroxyeicosatetraenoic Acid Contribute to Pressure Natriuresis
Jan M. Williams, Albert Sarkis, Bernardo Lopez, Robert P. Ryan, Averia K. Flasch and Richard J. Roman

Hypertension. 2007;49:687-694; originally published online January 8, 2007; doi: 10.1161/01.HYP.0000255753.89363.47
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/49/3/687

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/