Differential Regulation of Natriuresis by 20-Hydroxyeicosatetraenoic Acid in Human Salt-Sensitive Versus Salt-Resistant Hypertension

Cheryl L. Laffer, MD, PhD; Michal Laniado-Schwartzman, PhD; Mong-Heng Wang, PhD; Alberto Nasjletti, MD; Fernando Elijovich, MD

Background—Twenty-hydroxyeicosatetraenoic acid (20-HETE) is a cytochrome P450 metabolite of arachidonic acid that produces vasoconstriction and inhibition of renal tubular sodium transport. In Dahl rats, a 20-HETE deficiency plays a role in salt-sensitive (SS) hypertension. In humans, there are no data on regulation of 20-HETE by salt intake or on a role for this compound in SS hypertension.

Methods and Results—Thirteen salt-resistant (SR) and 13 SS hypertensive subjects had urine 20-HETE excretion measured during salt-loading and depletion. In all patients, 20-HETE was 66.6% higher in the salt-replete (1.75 ± 0.25 mg/h) than in the salt-depleted state (1.05 ± 0.16 mg/h, P < 0.003). There was no difference in 20-HETE excretion between SR and SS patients in either state of salt balance. In SR patients, sodium excretion during salt-loading correlated with 20-HETE (r = 0.61, P < 0.03) but not with blood pressure. In contrast, in SS patients, sodium excretion did not correlate with 20-HETE but did correlate with blood pressure (r = 0.66, P < 0.02). Finally, in the SS group only, there was a negative correlation between body mass index and 20-HETE excretion (r = -0.79, P < 0.002) that was present during both salt-loading and depletion.

Conclusions—We demonstrate for the first time that 20-HETE excretion is regulated by salt intake in hypertension. We find a disrupted relationship between sodium excretion and 20-HETE in SS patients, which results in dependence of their salt excretion on blood pressure and may be related to the magnitude of their obesity. We conclude that salt-sensitivity of blood pressure in essential hypertension may result from impairment of a natriuretic mechanism dependent on 20-HETE. (Circulation. 2003;107:574-578.)

Key Words: blood pressure ■ obesity ■ sodium

Salt-sensitivity of blood pressure (BP) is a major cardiovascular risk factor independent of BP, and it predicts higher mortality in normotensive and hypertensive subjects. Kidney transplantation experiments between salt-sensitive (SS) and salt-resistant (SR) rats suggest that salt-sensitivity of BP is due to a local or humoral “renal factor” that impairs either natriuresis or vasodilatory adaptive responses to volume overload. Renal 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE), the major cytochrome P450 (CYP) metabolite of arachidonic acid in mammals, is a vasoconstrictor that inhibits tubular sodium transport. Abnormalities in the production or actions of this eicosanoid are obvious candidates for a causative role in salt-sensitivity of BP. Increased CYP expression or 20-HETE synthesis, with exaggerated 20-HETE vasoconstriction and impairment of renal hemodynamic adjustments to a salt load, participate in the hypertension of spontaneously hypertensive rats (SHR) and of mice with genetically altered ratios of CYP 4A isoforms. In contrast, a deficit in the inhibitory effects of 20-HETE on renal sodium transport is involved in salt-dependent hypertension of Dahl SS rats. These animals have diminished CYP 4A protein and 20-HETE contents in the outer renal medulla, with increased medullary thick ascending limb chloride transport, a shift in pressure natriuresis, and hypertension. Outer medullary perfusion of 20-HETE or induction of CYP 4A by clofibrate corrects these abnormalities, whereas intrarenal CYP inhibitors reproduce them in normotensive rats. Finally, the CYP 4A2 genotype cosegregates with SS hypertension in an F2 cross between Dahl SS rats and normotensive Lewis rats, and also with salt-induced hypertension but not spontaneous hypertension in an F2 cross between SHR and normotensive Brown Norway rats. These studies provide substantial evidence that CYP 4A genes and the product of their catalytic activity, 20-HETE, are important factors in the hypertension and salt-sensitivity of BP in rodents.
The human kidney synthesizes 20-HETE\textsuperscript{24,25} and excretes it in urine.\textsuperscript{26,27} We measured urinary 20-HETE in SS and SR essential hypertensive patients during salt-loading (saline infusion and high-salt diet) and after achieving a salt-depleted state with furosemide and low-salt diet. We documented regulation of urine 20-HETE excretion by changes in salt intake in humans. More importantly, we show that SS subjects have a disrupted relationship between sodium excretion and 20-HETE compared with SR patients. We propose that SS essential hypertension may result from an impaired natriuretic mechanism dependent on 20-HETE.

Methods

Twenty-six essential hypertensive subjects (on therapy or with systolic BP >140 mm Hg or diastolic BP >90 mm Hg) were recruited at the University of Texas Medical Branch (UTMB). The research was approved by the Institutional Review Board and subjects gave informed consent. They maintained usual salt intake for 2 weeks, whereas those receiving antihypertensive therapy discontinued it for the same period. Body mass index (BMI, weight in kg/height in meters squared) was recorded. Blood counts, chemistries, serum creatinine, and electrolytes were measured in the laboratories of UTMB. Electrocardiographic left ventricular hypertrophy (LVH) was diagnosed if the Cornell index ([RaVL+SV3]mm × QRS msec) was greater than 2440 mm · ms⁻¹.

Patients were admitted to the Clinical Research Center to study the effect of changes in sodium intake on BP\textsuperscript{28} and on urine 20-HETE. Salt-loading (day 1) was achieved with a diet containing 160 mEq NaCl (metabolic kitchen) and with 2 L normal saline infused from 8 AM to 10 PM. Salt-depletion (day 2) was produced by a 10 mEq NaCl diet and three 40-mg doses of oral furosemide.

Urine collection for the salt-loading period included the 24 hours of day 1, whereas that for the salt-depleted period began 4 hours after the last dose of furosemide. This starting point was chosen to guarantee that the patients were in a negative sodium balance. The latter was calculated by subtracting sodium excretion from sodium intakes for the respective days.

Urine specimens were kept refrigerated and aliquots were frozen at −80°C at the end of each period for measurement of 20-HETE. Urine creatinine and sodium were measured in fresh samples. To compare sodium excretion rates between periods, results were expressed in mmol/h.

BPs were recorded with a mercury sphygmomanometer and the appropriate size cuff before admission and with an ambulatory monitor (Spacelabs 90207, readings every 15 minutes throughout the study) during admission. Average BPs from noon to 10 PM on days 1 and 2 were used for classification of the patients into the SS or SR groups. On day 1, these BP recordings started after the saline infusion, and on day 2, they started after the second dose of furosemide. The number of valid BP readings in the 26 patients was 36±2 on day 1 and 34±2 on day 2. A fall of 10 mm Hg in systolic BPs from day 1 to day 2 was the cutoff used to define a patient as SS.

Blood samples for routine tests, plasma renin activity (PRA), and plasma catecholamines (epinephrine plus norepinephrine) were obtained at baseline (before saline infusion at 8 AM of the first day) and at the end of the salt-loading and salt-depleted periods. PRA was measured by radioimmunoassay and plasma catecholamines by radioenzymatic assay (BioTrak TRK 995, Amersham).

Deuterated 20-HETE (4 ng, internal standard) was added to 10 mL of freshly thawed urine to measure 20-HETE. After incubation with 1 ng of \textit{Escherichia coli} β-glucuronidase (Sigma Chemical Co, 2 hours, 37°C) and acidification with formic acid (0.2 mol/L, pH 4), samples were extracted with ethyl acetate, evaporated under nitrogen, resuspended in 1 mL of methanol, re-evaporated under nitrogen, and resuspended in 30 μL of methanol. Suspensions underwent thin layer chromatography (TLC, silica gel G and upper phase of ethyl acetate:water:iso-octane:acetic acid 110:100:50:20, v/v/v/v) and the area corresponding to 20-HETE standard was scraped, resuspended in 1 mL of water, acidified (10% formic acid), and extracted with ethyl acetate. Extracts were evaporated, converted to pentfluoroheptanoyl bromide derivative (20-HETE–PFB), and subjected to a second TLC (ethyl acetate:hexane:acetic acid 150:350:0.5, v/v/v). The area of 20-HETE–PFB was scraped, extracted with ethyl acetate, evaporated, and incubated with bis(trimethylsilyl)-trifluoroacetamidomethane to obtain the PFBB-trimethylsilyl (TMS) derivative. The dry concentrate was dissolved in 50 μL of octane, and this solution was used for negative ion-chemical ionization (NCI) gas chromatography (GC)/mass spectrometry (MS) analysis.\textsuperscript{29} Total 20-HETE content in the purified sample was estimated by comparison of ion intensities at m/z 391:393 versus a standard curve of the molar ratio 20-HETE–PFB–TMS/20-20-deuterated-HETE–PFB–TMS constructed by NCI-GC/MS analysis. The concentration of 20-HETE in the samples (ng/mL range) was multiplied by the total urine volume (ie, excretion rate) and divided by the number of hours of the period. Results are reported as 20-HETE in μg/h.

Data are presented as mean±SEM. Comparisons between SS and SR subjects were made with unpaired \( t \) tests. Differences between periods were analyzed with paired \( t \) tests. Correlation coefficients were calculated with Pearson’s method. These tests and single linear and bivariate regression analyses were performed with JMP software (version 3.0.2, SAS Institute). A probability less than 5% was used to reject the null hypothesis.

Results

Patient Characteristics

Subjects were 47±1 years old, with similar representation of blacks (n=12) and whites (n=14). Females constituted 73% of the total. Obesity (BMI >30 kg/m²) was present in 77% and LVH was present in 19%. There were no differences in demographic and clinical characteristics between the SR and SS groups (Table 1). Baseline PRA was lower (albeit not significantly) in SS patients than in SR patients, whereas plasma catecholamines were not different between groups (Table 1).

Although outpatient BP (154±4/93±2 mm Hg) placed the group within stage 1 of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure classification, there was wide interindividual variability, with 42% of subjects exceeding this stage. Outpatient BPs and those of the salt-loading day were slightly higher (albeit not significantly) in SS than in SR subjects (Table 2). In contrast, on day 2, while patients were being salt-depleted, BPs of both groups were similar, as expected from the definition of these groups (significantly greater BP fall in SS than in SR patients, Table 2).

| TABLE 1. Demographic, Clinical, and Biochemical Data in SR and SS Patients |
|-----------------------------|-----------------------------|
|                             | SR (n=13) | SS (n=13) |
| Age, y                      | 44±3       | 49±1       |
| Race, W/B                   | 6/7        | 6/7        |
| Sex, F/M                    | 9/4        | 10/3       |
| BMI, kg/m²                  | 35.5±2.1   | 33.5±1.7   |
| % LVH, Cornell              | 23         | 15         |
| PRA, ngA/L·sec              | 0.66±0.20  | 0.27±0.08  |
| Plasma catecholamines, nmol/L| 2.21±0.29  | 2.48±0.45  |

Values are presented as n or mean±SEM. No significant differences were found between SR and SS.

W indicates whites; and B, blacks.
Sodium Balance and Renal Function

Sodium loading produced a positive balance of $170 \pm 30$ mmol in SR and $91 \pm 30$ in SS patients (not significant [ns]). Before the urine collections for the salt-depleted period (4 hours after last dose of furosemide), the negative sodium balances were $-165 \pm 25$ mmol (SR) and $-174 \pm 24$ (SS, ns). Continuation of low salt diet led to negative balances of $-194 \pm 29$ mmol (SR) and $-191 \pm 24$ (SS, ns) at the end of the salt-depleted period.

There were no significant differences between SR and SS patients in creatinine clearance, filtered sodium load, or sodium excretion during either period (Table 3), or in the reductions of creatinine clearance and filtered sodium load produced by salt-depletion (Table 3). In contrast, reduction in sodium excretion by salt-depletion was significantly greater in SS than in SR patients, despite its high magnitude in both groups (97.5 $\pm$ 0.5% SR and 98.7 $\pm$ 0.2% SS; Table 3), suggesting enhanced sodium conservation by SS subjects during salt-depletion.

Urine 20-HETE and Correlations With Clinical Variables

In all patients together, urine 20-HETE was 1.75 $\pm$ 0.25 $\mu$g/h during salt loading and 1.05 $\pm$ 0.16 during the salt-depleted period (Figure 1, left and middle gray bars). The 40% reduction from the former to the latter ($\Delta - 0.70 \pm 0.21$ $\mu$g/h) was statistically significant ($P<0.003$, Figure 1, right gray bar). White and black bars in Figure 1 depict the lack of significant differences between the SR and SS groups in urine 20-HETE during both periods and in the magnitude of the reduction of 20-HETE excretion by salt-depletion.

Despite similar urine sodium and 20-HETE excretion in the SR and SS patients, there was a marked difference in the relationship between these 2 parameters between groups. Hence, SR subjects exhibited a strong positive correlation between 20-HETE and sodium excretion during salt loading (Figure 2, left upper panel), whereas such relationship was absent in SS subjects (right upper panel). Although urine flow rate also correlated with 20-HETE in the SR group ($r=0.56$, $P<0.05$), a stepwise bivariate regression demonstrated that the independent correlate of 20-HETE excretion was sodium excretion, not urine flow rate. Correlations of sodium excretion with diastolic BP exhibited the opposite pattern. Hence, they were significant in SS patients during sodium loading (right lower panel) and absent in SR patients (left lower panel). During the salt-depleted period, there were no relationships between 20-HETE, sodium excretion, urine flow rates, and BPs in either group.

A search for further univariate relationships between 20-HETE excretion and other clinical or biochemical variables (in all patients combined or in the SR and SS subgroups) failed to document relationships with age, race, sex, PRA, or plasma catecholamines in either the salt-loading or the salt-depleted periods. In contrast, 20-HETE correlated with diastolic BP during salt loading in SS subjects but not in SR subjects (Figure 3, upper panels), and it exhibited a very strong negative correlation with BMI in SS patients that was not present in SR patients (Figure 3, lower panels). The relationship between 20-HETE and BMI was also present in SS after salt-depletion.

### TABLE 2. Blood Pressures in the Different Stages of the Study

<table>
<thead>
<tr>
<th>Period</th>
<th>SR (n=13)</th>
<th>SS (n=13)</th>
<th>Δ Between Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Office</td>
<td>$152 \pm 6/90 \pm 4$ (111 $\pm$ 4)</td>
<td>$157 \pm 6/96 \pm 3$ (116 $\pm$ 4)</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>$145 \pm 5/87 \pm 3$ (107 $\pm$ 4)</td>
<td>$156 \pm 5/92 \pm 3$ (115 $\pm$ 3)</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>$141 \pm 6/86 \pm 4$ (105 $\pm$ 4)</td>
<td>$143 \pm 4/89 \pm 3$ (107 $\pm$ 3)</td>
<td></td>
</tr>
<tr>
<td>Δ Day 2 – Day 1</td>
<td>$-5 \pm 1/7$ – $1/2$ – $2 \pm 1$</td>
<td>$-13 \pm 1/11$ – $4 \pm 1$ – $(8 \pm 1)$†</td>
<td></td>
</tr>
</tbody>
</table>

Blood pressures given as systolic $\pm$ SEM/diastolic $\pm$ SEM. Office indicates sphygmomanometric recordings during ambulatory monitors obtained during the admission. *Significant falls in blood pressures from day 1 to day 2. †Significantly greater blood pressure falls in SS than in SR patients.

### TABLE 3. Creatinine Clearance and Sodium Excretion in SR and SS Patients

<table>
<thead>
<tr>
<th></th>
<th>Salt-Loading Period</th>
<th>Salt-Depleted Period</th>
<th>Δ Between Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance, mL/sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>2.47 $\pm$ 0.10</td>
<td>2.07 $\pm$ 0.23</td>
<td>$-0.40 \pm 0.17$</td>
</tr>
<tr>
<td>SS</td>
<td>2.27 $\pm$ 0.20</td>
<td>1.93 $\pm$ 0.23</td>
<td>$-0.33 \pm 0.22$</td>
</tr>
<tr>
<td>$P$ for SS vs SR</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Filtered Na load, mmol/hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>1242 $\pm$ 81</td>
<td>1031 $\pm$ 113</td>
<td>$-211 \pm 82$</td>
</tr>
<tr>
<td>SS</td>
<td>1151 $\pm$ 95</td>
<td>975 $\pm$ 121</td>
<td>$-176 \pm 113$</td>
</tr>
<tr>
<td>$P$ for SS vs SR</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Urine sodium excretion, mmol/hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>12.1 $\pm$ 1.3</td>
<td>2.4 $\pm$ 0.6</td>
<td>$-9.6 \pm 1.4$</td>
</tr>
<tr>
<td>SS</td>
<td>15.4 $\pm$ 1.2</td>
<td>1.4 $\pm$ 0.2</td>
<td>$-14.0 \pm 1.3$</td>
</tr>
<tr>
<td>$P$ for SS vs SR</td>
<td>NS</td>
<td>NS</td>
<td>$&lt;0.04$</td>
</tr>
</tbody>
</table>

NS indicates not significant. $P$ for SS vs SR is from the unpaired t tests between the 2 groups.

**Figure 1.** Urinary excretion of 20-HETE during the salt-loading (left) and salt-depleted (middle) periods, and the difference between these periods (right). Data in all 26 patients analyzed together are shown by the gray bars. Data in the 13 patients in each subgroup, salt-resistant and salt-sensitive, are depicted by the white and black bars, respectively. ns indicates no difference between salt-resistant and salt-sensitive groups in either period or in the effect of salt-depletion. †Significantly reduction in 20-HETE excretion by salt-depletion.
eicosanoid in essential hypertension. First, assuming that renal outer medullary 20-HETE regulates renal tubular sodium transport in humans as it does in rats, we hypothesized that changes in salt balance would alter its renal content and hence its urine excretion. Second, we hypothesized that SS patients, analogous to Dahl SS rats, would have a deficit in renal 20-HETE compared with SR patients that could be detected by its diminished urine excretion during salt loading.

We unequivocally demonstrated that urine 20-HETE excretion is affected by salt balance, with values 66% higher during salt loading than during salt depletion. To our knowledge, this is the first demonstration of regulation of eicosanoid metabolism by salt balance in humans. Upregulation of a putatively natriuretic system during salt loading is the expected physiological response, as observed for the CYP isoform that produces the major natriuretic epoxygenase metabolite of arachidonic acid, 11,12-EET.29

Changes of CYP 4A and 20-HETE by salt in animal models have been of opposite direction to those in our patients, ie, reductions by high salt and increases by low salt,18,23,30 but these experiments were carried out in isolated organs or subcellular fractions and bear no relation to our studies. In addition, major differences in the catalytic activity of CYP 4A isoforms in different segments of the nephron have been demonstrated with antisense oligonucleotides, suggesting that regulation by a stimulus such as salt may differ depending on the isoform and nephron segment involved.31 Finally, most studies on effects of salt on CYP 4A gene or protein measured 4A1, 4A2, and 4A3, the predominant isoforms in rats. In humans, 20-HETE is synthesized mostly by CYP 4A11 and 4F2,24 and nothing is known about effects of salt on these isoforms.

We do not know whether our findings reflect salt-induced stimulation of transcription of CYP isoforms, translation of CYP mRNA, salt-induced release of preformed 20-HETE from glycerolipid stores, or other mechanisms. Regardless of this, increased urinary 20-HETE (ie, increased availability of a sodium transport inhibitor) during salt loading compared with salt-depletion supports a physiologically significant role for 20-HETE in the regulation of renal sodium handling in humans.

Urine excretion of 20-HETE was not different between SS and SR patients during either salt loading or salt depletion, thereby failing to confirm our second hypothesis. However, a possible role for 20-HETE in SS hypertension emerged from analysis of its differential correlates in both groups of patients. In SR subjects, urine sodium excretion correlated with 20-HETE but not with BP, and BP did not change between the salt-loaded and the salt-depleted states. In contrast, in SS subjects, in whom there was no relationship between sodium excretion and 20-HETE, the salt load increased BP in a manner related to the magnitude of sodium excretion. These data suggest that a defect in the ability of 20-HETE to inhibit tubular sodium transport in SS patients during salt-loading leads to a shift in the pressure natriuresis curve and to an increase in BP. It is conceivable that ineffective 20-HETE–mediated natriuresis results in unopposed 20-HETE–mediated vasoconstriction, which would account for the positive correlation between 20-HETE and BP observed in SS patients during salt loading.

The correlation of 20-HETE with the urine flow rate of SR subjects might be thought to be responsible for the relation...
between 20-HETE and sodium excretion if the diuresis produced by salt loading diminishes tubular reabsorption of 20-HETE. Several observations argue against this possibility. First, the correlation of 20-HETE with urine flow was weaker than that with sodium excretion in univariate analyses. Second, it was not present in SR patients during salt depletion or in SS patients during either period. Finally, multivariate analysis showed that sodium excretion, not urine flow rate, was the independent correlate of 20-HETE excretion.

Finally, we do not have an explanation for the strong negative correlation (in both states of salt balance) between 20-HETE and BMI in SS subjects only (despite similar 20-HETE excretion and obesity in SR and SS patients). It is noteworthy, however, that agonists for the peroxisome proliferator activated receptors-α (PPARα), the fibrin acids, stimulate transcription and activation of CYP 4A.32 Crosstalk between PPARα and CYP monoxygenases participates in regulation of fatty acid oxidation and energy metabolism.33 Because PPARαs are expressed in the kidney34 and their physiology is altered in obesity,35 it is conceivable that an abnormality in these transcriptional receptors affects CYP 4A11- or CYP 4F2-dependent synthesis of renal 20-HETE in humans. This could explain the high prevalence of obesity in the SS hypertensive phenotype.

In conclusion, we demonstrate that renal 20-HETE is regulated by salt intake in essential hypertension, the first demonstration of such regulation in humans. Furthermore, we show that the relationship between 20-HETE and sodium excretion is different between the SS and SR groups, suggesting that abnormalities in the actions of this eicosanoid on sodium excretion may be a major factor responsible for the salt-sensitivity of BP.

Acknowledgments

This work was supported by grant MO1 RR00073 NCRR (Drs Laffer and Eliovich), National Institutes of Health grant PO1 34300 (Drs Nasjletti and Laniado-Schwartzman), and American Heart Association grant 99-302777 (Dr Wang).

References

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_Circulation_. 2003;107:574-578; originally published online January 6, 2003;
doi: 10.1161/01.CIR.0000046269.52392.14
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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