The T8590C Polymorphism of CYP4A11 and 20-Hydroxyeicosatetraenoic Acid in Essential Hypertension

Cheryl L. Laffer, James V. Gainer, Michael R. Waterman, Jorge H. Capdevila, Michal Laniado-Schwartzman, Alberto Nasjletti, Nancy J. Brown, Fernando Elijovich

Abstract—A role for a deficit in transport actions of 20-hydroxyeicosatetraenoic acid (20-HETE) in hypertension is supported by the following: (1) diminished renal 20-HETE in Dahl-S rats; (2) altered salt- and furosemide-induced 20-HETE responses in salt-sensitive hypertensive subjects; and (3) increased population risk for hypertension in C allele carriers of the T8590C polymorphism of CYP4A11, which encodes an enzyme with reduced catalytic activity. We determined T8590C genotypes in 32 hypertensive subjects, 25 of whom were phenotyped for salt sensitivity of blood pressure and insulin sensitivity. Urine 20-HETE was lowest in insulin-resistant, salt-sensitive subjects (F=5.56; P<0.02). Genotypes were 13 TT, 2 CC, and 17 CT. C allele frequency was 32.8% (blacks: 38.9%; whites: 25.0%). C carriers (CC+CT) and TT subjects were similarly distributed among salt- and insulin-sensitivity phenotypes. C carriers had higher diastolic blood pressures and aldosterone:renin and waist:hip ratios but lower furosemide-induced fractional excretions of Na and K than TT. The T8590C genotype did not relate to sodium balance or pressure natriuresis. However, C carriers, compared with TT, had diminished 20-HETE responses to salt loading after adjustment for serum insulin concentration and resetting of the negative relationship between serum insulin and urine 20-HETE to a 1-µg/h lower level of 20-HETE. The effect of C was insulin independent and equipotent to 18 µU/mL of insulin (Δ20-HETE=2.84–0.054×insulin–0.98×C; r²=0.53; F=11.1; P<0.001). Hence, genetic (T8590C) and environmental (insulin) factors impair 20-HETE responses to salt in human hypertension. We propose that genotype analyses with sufficient homozygous CC will establish definitive relationships among 20-HETE, salt sensitivity of blood pressure, and insulin resistance. (Hypertension. 2008;51:767-772.)

Key Words: hypertension ■ obesity ■ arachidonic acid ■ cytochrome P450 ■ insulin ■ insulin resistance

The monoxygenase derivative of arachidonic acid synthesized by CYP450 enzymes, 20-hydroxyeicosatetraenoic acid (20-HETE), is a candidate for participation in blood pressure regulation and hypertension. This could be via excess of its potent vasoconstrictor actions or deficiency of its inhibition of tubular transport, normally exerted at the ubiquitous renal tubular Na-K-ATPase.

Studies in Dahl-S rats9–11 and in angiotensin II–induced hypertension in mice12 support the view that a diminished effect of 20-HETE on renal transport is an important mechanism in experimental hypertension. Human population studies on polymorphisms of CYP4A11 that diminish its catalytic activity to synthesize 20-HETE13,14 are consistent with this interpretation.

Our studies in essential hypertension documented abnormal 20-HETE responses to physiological15 and pharmacological16 natriuresis and negative relationships with serum insulin but not diminished urinary excretion of 20-HETE, as expected from observations in rodents. Because Dahl-S rats are not only salt sensitive but also insulin resistant,18 we decided to investigate whether excretion of 20-HETE is diminished in hypertensive subjects who exhibit both phenotypes concomitantly. Also, population studies on CYP4A11 did not describe physiological phenotypes in participating subjects. Therefore, we genotyped the CYP4A11 T8590C locus in a series of hypertensive subjects characterized for salt sensitivity of blood pressure and insulin sensitivity, in an attempt to understand the mechanism for the possible association of the C allele with hypertension.

Methods

Thirty-two subjects with essential hypertension (blood pressure >140/90 mm Hg or taking antihypertensive medications) consented to participate in a protocol approved by the institutional review board. Genotyping for T8590C and salt sensitivity of blood pressure were determined in all of the subjects, whereas phenotyping for insulin sensitivity and measurement of 20-HETE excretion were completed in 25. Studies were conducted in the General Clinical Research Center ≥2 weeks after discontinuation of therapy and maintenance on usual salt intake. Salt sensitivity of blood pressure...
Table 1. Clinical and Biochemical Characteristics of C Carriers (CC+CT) and TT Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>All Subjects (n=32)</th>
<th>Subjects With IS ≡ SS Data (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC+CT (19)</td>
<td>TT (13)</td>
</tr>
<tr>
<td>Clinical variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>45±2</td>
<td>48±2</td>
</tr>
<tr>
<td>Race, black/white</td>
<td>13/6</td>
<td>5/8</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>155±5/98±2</td>
<td>ns/&lt;0.05</td>
</tr>
<tr>
<td>Na balance&lt;sup&gt;salts-loaded&lt;/sup&gt;, mmol</td>
<td>135±26</td>
<td>140±30</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>140±29</td>
<td>125±10</td>
</tr>
<tr>
<td>RAAS and electrolyte excretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARR, (pmol/L/ngAI/L/s)</td>
<td>3625±561</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PRA&lt;sub&gt;salts-depleted&lt;/sub&gt;, ngAI/L/s</td>
<td>0.61±0.21</td>
<td>1.10±0.29</td>
</tr>
<tr>
<td>ΔAldo&lt;sub&gt;salts-loaded&lt;/sub&gt;, pmol/L</td>
<td>−33±56</td>
<td>−170±53</td>
</tr>
<tr>
<td>F&lt;sub&gt;EK&lt;/sub&gt;, %</td>
<td>1.9±0.2</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>F&lt;sub&gt;Eu&lt;/sub&gt;, %</td>
<td>11.3±0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Obesity/insulin sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>34±2</td>
<td>35±2</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.91±0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Serum insulin, µU/mL</td>
<td>31.8±2.8</td>
<td>30.9±3.6</td>
</tr>
<tr>
<td>SI&lt;sub&gt;HOMA&lt;/sub&gt;, mL/LµU&lt;sub&gt;µM&lt;/sub&gt;</td>
<td>0.154±0.020</td>
<td>0.146±0.025</td>
</tr>
</tbody>
</table>

IS indicates insulin sensitivity; SS, salt sensitivity; RAAS, renin-angiotensin-aldosterone system; ARR, aldosterone:renin ratio; PRA, plasma renin activity; Aldo, aldosterone; F<sub>EK</sub>, fractional excretion of Na during furosemide; F<sub>Eu</sub>, fractional excretion of K during furosemide; SI<sub>HOMA</sub>, insulin-sensitivity index. A blank space on the P value column or ns indicates a not statistically significant difference between groups; P values for trends (0.05<P<0.1) are given.

Results

Table 1 shows clinical and biochemical data in the entire population and in the 25 subjects characterized for salt sensitivity of blood pressure, insulin sensitivity, and 20-HETE excretion. The whole population was middle aged (47±2 years), obese (body mass index: 34±1 kg/m²), and predominantly female (24 women and 8 men): 18 subjects were black, and 14 were white. Average blood pressure was 154±4/94±2 mm Hg, but there was wide variability in the severity of blood pressure elevation, with 44% patients having stage 2 hypertension, as defined by Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure criteria.

Genotypes for the T8590C polymorphism of CYP4A11 were 13 homozygous TT (40.6%), 2 homozygous CC (6.3%), and 17 heterozygous CT (53.1%). The frequency of the C allele was 32.8%, somewhat higher in blacks (38.9%) than in whites (25.0%), analogous to what has been reported in the Tennessee population. Because of the small number of CC homozygous subjects, all of the analyses were carried out as comparisons between carriers of the C allele (CC+CT, hereafter designated as C) versus homozygous TT.

There were no major differences between the fully phenotyped patients and the entire group. Table 1 shows that age, creatinine clearance, and sodium balance after salt loading did not differ between C and TT subjects. Racial distribution was assessed by means of a salt load (24 hours of a 160-mmol Na diet+2 L of saline infusion in 4 hours) followed by salt depletion by diet (10 mmol/d) and furosemide (120 mg over 8 hours) on the second day. A patient was deemed salt sensitive if daytime average systolic blood pressure (Spacelabs 90207 monitors) decreased ≥10 mm Hg from the salt-loading to the salt-depletion day.

Insulin sensitivity was determined with the homeostatic model assessment method, in which the index SI<sub>HOMA</sub> is calculated as 22.5<sup>glucose<sub>insulin</sub></sup>.<sup>−10</sup>. To reduce variability, each subject’s SI<sub>HOMA</sub> was obtained with fasting insulin and glucose data on 3 consecutive days. A patient was deemed insulin resistant if the average SI<sub>HOMA</sub> was <0.161 mL<sub>L</sub>µU<sub>µM</sub> and a cutoff derived from maximum normal fasting glucose (5.56 mmol/L) and maximum normal fasting insulin (25 µU/mL) in our laboratory.

During the salt-sensitivity protocol, urine from each period was collected for measurement of sodium excretion and 20-HETE, and blood samples were obtained for measurement of plasma renin activity and aldosterone. Serum insulin (Nichols Institute), plasma renin activity,<sup>20</sup> and aldosterone<sup>21</sup> were measured by radioimmunoassay. The method used for measurement of urine 20-HETE (negative chemical ionization, gas chromatography/mass spectroscopy of a 20-HETE derivative) has been reported previously in detail.<sup>19</sup> Genotyping for the T8590C polymorphism of CYP4A11 was carried out by PCR amplification performed with intron-specific primers on DNA extracted from buffy coats. Primer sequences, reaction conditions, DNA extraction, and genotyping methods can be found at www.bioventures.com.<sup>13</sup>

Data are presented as means±SEMs. Comparisons between 2 groups were made with unpaired <i>t</i> tests for continuous variables and χ<sup>2</sup> analysis for categorical variables. Comparisons among 4 groups were carried out by ANOVA and contrasting of means (Dunnett’s). Adjustment of 20-HETE by serum insulin was performed by ANCOVA, with the coefficient for insulin (β weight) obtained from multivariate regression with insulin as the covariate. Stepwise forward and backward multivariate regression analyses and all of the other tests were performed with JMP software (version 3.0.2, SAS Institute). A <i>P</i> value <0.05 was used to reject the null hypothesis. A posthoc power analysis confirmed that β error was <0.2 for n=25 and the results of the 2 tests and ANOVAs presented in the 3 figures.
showed a trend, consistent with allele frequencies, toward a larger number of blacks in the C group. However, this difference did not reach statistical significance. Diastolic blood pressure, but not systolic, was higher in C than in TT.

C subjects had significantly higher aldosterone:renin ratios during usual salt intake compared with TT subjects (a trend also observed in the fully phenotyped subgroup). This observation was independent of race in multivariate analysis (P for race=0.60). They also had diminished fractional excretion of K during furosemide administration and trends (P value not statistically significant) toward lower plasma renin activity17 and the relationship between the C allele and salt sensitivity of blood pressure. Urine 20-HETE was significantly lower in insulin-resistant, salt-sensitive subjects (0.88±0.17 µg/h) than in the other 3 groups, as depicted in Figure 1 and by the statistics in the legend.

The unadjusted urine 20-HETE response to salt loading tended to be lower in C than in TT subjects, but the difference was not significant (Figure 2, left). In contrast, after adjustment for individual serum insulin by covariate analysis, the 20-HETE response to salt was significantly diminished in C subjects compared with TT subjects (Figure 2, right). These results indicate that both the previously demonstrated inhibitory effect of serum insulin on 20-HETE excretion17 and the CYP4A11 polymorphism determine 20-HETE excretion.

The regression analysis in Table 2 quantifies these effects. In this model, the dependent variable was the increase in 20-HETE excretion produced by salt loading, and the regressors were serum insulin and a dummy variable representing

### Table 2. Multivariate Regression Model for the 20-HETE Response to Salt Loading (Micrograms per Hour)

| Parameter Estimates | Estimate | SE   | t Ratio | P>|t|  | Summary of fit and ANOVA |
|---------------------|----------|------|---------|------|--------------------------|
| Intercept           | 2.8449   | 0.5218 | 5.45 | <0.0001 |
| Insulin, µU/mL      | -0.0539  | 0.0137 | -3.92 | 0.0008 |
| Allele (C=0, T=1)   | 0.9808   | 0.3092 | 3.17 | 0.0048 |
| r²                  | 0.5263   |
| F ratio             | 11.130   |
| P>|F|                | 0.0006   |

C=0 and T=1 indicate dummy variables for carriers of the C allele vs homozygous TT subjects. Introduction of either the variable race or the interaction term insulin*allele as regressors in the model had no statistically significant effect (see text).
the C and TT groups. The model was significant. The effects of insulin and the C allele in reducing 20-HETE excretion were independent (the interaction term was not significant). Because there were more carriers of the C allele among black than among white subjects, we excluded that the effect of the C allele was because of race by determining the following: (1) the coefficient for race was not statistically significant ($P=0.92$); (2) including race as a regressor did not increase $r^2$ (0.53); and (3) race did not change the significance of the effect of the C allele in stepwise forward or backward procedures.

Finally, from the coefficients in the model, it can be calculated that carrying the C allele results in a lesser effect of the C allele in stepwise forward or backward procedures.

Discussion

Our initial studies on a possible role for 20-HETE in human hypertension$^{15-17}$ were stimulated by the unequivocal evidence that a deficit in outer medullary CYP4A2 protein and 20-HETE is linked to salt sensitivity of blood pressure in Dahl-S rats. In addition to direct documentation of these deficits,$^9$ a series of publications characterized the functional consequences of decreased 20-HETE of renal medullary origin, including an increase in chloride transport, shift in pressure natriuresis, and salt-sensitive hypertension.$^{10}$ Moreover, these abnormalities can be ameliorated by infusion of 20-HETE in the renal medulla$^{18}$ or reproduced in control strains by inhibiting 20-HETE synthesis.$^{22}$ A role for a deficit of renal tubular 20-HETE in hypertension, via loss of its inhibitory actions on sodium transport,$^{5,7,8}$ has also been documented in other species. In C57BL/6J mice, which have very low renal production of 20-HETE, fenofibrate abolishes angiotensin II hypertension by stimulating CYP4a protein in renal tubules, not vessels.$^{12}$ In humans, polymorphisms in the major CYP isofoms that synthesize 20-HETE (M allele of V433M in CYP4F2$^{23}$ and C allele of T8590C in CYP4A11$^{13,14}$) produce enzymes with diminished catalytic activity. In the case of CYP4A11, there is proof in population studies for an association between the variant enzyme and hypertension.$^{13,14}$

The P450 4A1 and 4A2 gene loci cosegregate with blood pressure in F2 crosses of Dahl-S and control rats.$^9$ However, absolute proof that CYP genes are the cause of salt sensitivity of blood pressure has not yet been provided. There are 2 monogenic forms of hypertension produced by knocking out CYP4A4 genes in mice. One does not involve 20-HETE. This is the CYP4A10$^{[−/−]}$, in which salt-sensitive hypertension relates to diminished activity of the CYP2C44 epoxygenase with diminished synthesis of 11,12-epoxyeicosatetraenoic acid and unchanged CYP4A12 and renal 20-HETE.$^{24}$ The other depends on excessive 20-HETE vasoconstriction because of increased, rather than decreased, synthesis of the compound. This is the CYP4A14$^{[−/−]}$, which develops androgen-dependent hypertension associated with overexpression of CYP4A12.$^{25}$ Congenic Dahl-S strains harboring the entire region of chromosome 5 that contains the CYP4A genes from Lewis rats exhibit restored synthesis of outer medullary CYP4A protein and 20-HETE and have blunted blood pressure responses to salt and reduced proteinuria compared with Dahl-S strains.$^{11}$ However, experiments with different overlapping sections of the transferred chromosome raised the possibility that the CYP4A3 and A8 genes, not A1 and A2, may be responsible for the effect of retrogressing the entire region into the congenic strains.$^{26}$

In our early experiments, we measured 20-HETE in subjects with salt-sensitive hypertension in the expectation that urinary excretion might be reduced, analogous to decreased synthesis of the compound in Dahl-S kidney. Urine 20-HETE excretion most likely reflects its renal synthesis, because the urine is the most important efflux pathway for renal 20-HETE.$^{27}$ Although we detected abnormal regulation of physiological (salt loading)$^{15}$ and pharmacological (furosemide)$^{16}$ natriuresis by 20-HETE in these patients, we did not find reduced urinary excretion of the compound. Analogously, salt-sensitive young Sprague-Dawley rats have impaired 20-HETE–induced natriuresis despite high excretion levels of this compound, an abnormality that disappears as they age and become salt resistant.$^{28}$ Therefore, it is apparent that salt sensitivity of blood pressure may relate to either diminished

![Figure 3. Urine 20-HETE responses to salt loading in 4 groups of subjects grouped by both insulin-sensitivity phenotype (IS indicates insulin sensitive; IR, insulin resistant) and CYP4A11 genotype (C indicates carriers of the C allele of T8590C, either CC or CT genotype; TT, subjects homozygous for the T allele). The number of subjects in each group was as follows: IS-TT, 5; IS-C, 6; IR-TT, 7; and IR-C, 7. The F statistic and P value on the graph are for the ANOVA comparing the 4 groups. *Statistically significant difference in the 20-HETE response to salt between the IR-C and IS-TT groups (contrasting of means, Dunnnett’s test, $P<0.02$).](image-url)
synthesis or to defective natriuretic action of 20-HETE and perhaps to a combination of both.

Later on we showed that our unexpected finding of a strong negative correlation between urine 20-HETE excretion and body mass index was explained by the observation that hyperinsulinemia is a major factor inhibiting urine 20-HETE excretion in humans. This is consistent with known insulin inhibition of CYP4A1, 2, and 3 isoforms in liver and kidney microsomes of the rat and suggests that insulin may also inhibit the isoforms that synthesize 20-HETE in humans. It is conceivable that inhibition of outer renal medullary CYP4A isoforms is a mechanism by which insulin increases sodium reabsorption at the thick ascending limb, because insulin also exerts this action at the proximal tubule by translational and posttranslational activation of the NHE3 isoform of the Na+/H+ exchanger, at the thiazide-sensitive Na+/Cl− transporter via diminished expression of WNK4 kinase; and at the epithelial sodium channel, via phosphorylation and translocation of the 3 channel subunits to the membrane.

This publication extends our previous observations in 2 regards. First, whereas Dahl-S rats are an inbred strain in which salt sensitivity of blood pressure coexists with insulin resistance, salt-sensitive hypertensive subjects are not always insulin resistant. We hypothesized that measuring 20-HETE excretion in patients characterized for both phenotypes would document reduced urine excretion of 20-HETE in hypertensive subjects with concomitant salt sensitivity of blood pressure and insulin resistance. We confirmed this hypothesis, which strengthens the view that, regardless of the genetics of CYP4A in hypertension, serum insulin is a major nongenetic determinant of the activity of the CYP4A system in the human kidney. Hence, insulin may regulate sodium and chloride transport and blood pressure via actions at the thick ascending limb.

Second, whereas in Dahl-S rats the hypertension relates to a deficit in CYP4A protein and renal medullary 20-HETE, in humans it relates to a variant enzyme (C allele of the T8590C polymorphism of CYP4A11), which probably determines diminished 20-HETE levels because of diminished catalytic activity, although this has never been shown in the population studies. We now demonstrate that the C allele of T8590C resets the inhibitory relationship between insulin and 20-HETE excretion toward lower levels of 20-HETE compared with that observed in homozygous TT subjects. Our data suggest that the C allele leads to reduced synthesis of 20-HETE at any given level of serum insulin and that the ultimate level of urine 20-HETE is the consequence of the genetic effect of the CYP4A11 polymorphism and the insulin-sensitivity phenotype of the subject. These suggestions are supported by the observation that stimulation of 20-HETE excretion by salt loading is absent in hypertensive carriers of the C allele who are insulin resistant. Aldosterone:renin ratios are increased, independent of race, in C-allele carriers. We have validated this observation in a separate cohort (n=45) from Vanderbilt University (N.J. Brown, unpublished data, 2007). Increased aldosterone:renin ratios perhaps explain more severe blood pressure elevation in C-allele carriers than in TT subjects, a possibility that remains to be explored further.

Decreased insulin-mediated glucose uptake and hyperinsulinemia are observed in normotensive offspring of hypertensive subjects, supporting the view that a genetic predisposition may contribute to both abnormalities. The sodium-retaining actions of insulin have been implicated in the pathogenesis of salt-sensitive hypertension, independent of obesity. Our data suggest that 1 of the plausible mechanisms underlying the relationships among insulin resistance, salt sensitivity of blood pressure, and essential hypertension is an abnormal synthesis of 20-HETE.

Perspectives
Regulation of renal sodium handling and salt sensitivity of blood pressure by CYP450 metabolites of arachidonic acid is complex and may involve genetically determined deficiencies of monoxygenases and epoxygenases, single nucleotide polymorphisms that affect catalytic activity of these enzymes, and interactions with phenotypes that affect expression of their genes. Our work has focused on the effects of insulin and the C allele of CYP4A11, both leading to reduced urine excretion of 20-HETE in human hypertension. Because of the potential effects of reduced 20-HETE actions on sodium reabsorption, shift in pressure natriuresis curves, and salt-sensitive hypertension, the observations are important in that they could be subject to therapeutic intervention with available agents. That is, stimulation of expression of the variant CYP4A11 enzyme by fibrates, reduction of serum insulin by thiazolidinediones, or the achievement of both effects by dual peroxisome proliferator-activated receptor-α/peroxisome proliferator-activated receptor-γ agonists may lead to increased 20-HETE synthesis, hence, increased 20-HETE–mediated natriuresis, diminution of salt sensitivity of blood pressure (an independent risk factor for cardiovascular mortality), and amelioration of hypertension.

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Disclosures
None.

References


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