20-HETE and Furosemide-Induced Natriuresis in Salt-Sensitive Essential Hypertension

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

Abstract—Cyclooxygenase metabolites of arachidonic acid modulate the natriuretic effect of furosemide. It is not known whether 20-HETE, a monoxygenase metabolite of arachidonic acid that also inhibits sodium transport, participates in the action of furosemide. We measured urine sodium (UNaV) and 20-HETE during furosemide diuresis (40 mg three times over 12 hours) in 12 salt-sensitive (SS) and 11 salt-resistant (SR), salt-replete hypertensive subjects (126±24 mmol/24 hours positive sodium balance produced by 160-mmol-sodium diet and 2 L saline infusion). Individual systolic blood pressure decreases from the salt-replete to the salt-depleted state were the index of salt-sensitivity. SS had low plasma renin with blunted responses to changes in salt balance, inappropriate plasma aldosterone, and an increased aldosterone/renin ratio. UNaV by furosemide was less in SS (263±25 mmol/12 hours) than in SR (351±25 mmol/12 hours, P<0.02) patients. 20-HETE was not different between SS and SR patients before (1.92±0.38 versus 1.37±0.34 μg/h) or after furosemide (1.52±0.27 versus 2.01±0.40 μg/h), but furosemide changed 20-HETE excretion in opposite direction in SR (0.63±0.26) versus SS (−0.40±0.17, P<0.005) patients. In all patients together, %Δ20-HETE by furosemide correlated with %ΔUNaV (r=0.56, P<0.01) and negatively with salt-sensitivity of blood pressure (r=−0.55, P<0.01). In SS, Δ20-HETE by furosemide correlated with Δaldosterone/renin ratio (r=0.60, P<0.05), whereas 20-HETE during furosemide had a negative correlation with body mass index (r=−0.73, P<0.01). Our data suggest that 20-HETE modulates the natriuretic response to furosemide, and impaired natriuresis of SS involves a mechanism that alters the 20-HETE response to furosemide and is linked to salt-sensitivity of blood pressure. (Hypertension. 2003;41[part 2]:703-708.)

Key Words: hypertension, sodium dependent ■ natriuresis ■ furosemide ■ arachidonic acid ■ sodium ■ human ■ risk factors

Furosemide produces direct inhibition of the sodium-potassium-chloride cotransporter (NKCC) in isolated cells.1-3 The mechanism for this action is not fully elucidated but involves a 9-kDa upward shift in the apparent molecular mass of NKCC in rat renal tubules.4 In intact kidney (whole animals and humans), furosemide also stimulates COX-2 mRNA expression and the production of the sodium transport inhibitor prostaglandin E2 (PGE2).5-9 Activation of this COX-dependent pathway exerts major modulation on the natriuretic action of furosemide, as demonstrated by the markedly blunted effect of this drug in experimental animals or patients given nonspecific or COX-2-specific inhibitors.9-11

Eicosanoids that inhibit tubular sodium transport are also produced by cytochrome P-450 (CYP 450) monoxygenase metabolism of arachidonic acid (AA); 20-HETE is the major metabolite of this pathway in mammalian kidney. This compound inhibits sodium transport at the proximal tubule Na-K-ATPase12 and, most importantly, at the thick ascending limb NKCC.13 The inhibitory action on the NKCC cotransporter is possibly mediated by impaired apical K'-channel recycling of K'.14

Dahl salt-sensitive (SS) rats exhibit a deficit in outer renal medullary synthesis of 20-HETE, which has been implicated in their increased thick ascending limb chloride transport, shifted pressure-natriuresis, and salt-dependent hypertension.15 In humans, we have shown that the relationship between urine excretion of 20-HETE and sodium differs between SS and salt-resistant (SR) hypertensive patients, suggesting a role for this eicosanoid in determining salt-sensitivity of blood pressure (BP).16 Despite increasing evidence that the inhibitory actions of 20-HETE on sodium transport may be of physiological and clinical importance, there are no data on whether this eicosanoid modulates or participates in the pharmacological action of furosemide.

We investigated this issue by measuring natriuretic and 20-HETE excretory responses to furosemide administration in salt-replete hypertensive patients classified into SS and SR subgroups. Our data suggest that the natriuretic response to furosemide (1) is modulated by 20-HETE in essential hypertensive subjects and (2) is impaired in SS subjects by a mechanism related to their altered 20-HETE response to furosemide.
Methods

Twenty-three hypertensive subjects (either 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), after protocol approval by the Institutional Review Board and with subjects' informed consent. Patients on antihypertensive therapy discontinued it for at least 2 weeks, and all subjects maintained their usual salt intake. Demographic and clinical characteristics were recorded. Body mass index (BMI) was calculated as weight (kg)/height (m²). BP (mercury sphygmomanometer, seated, after 5 minutes resting, in triplicate) was measured periodically for safety. The recording immediately before admission was deemed to be the outpatient baseline BP. Routine laboratory tests were performed at UTMB. An ECG was scored for left ventricular hypertrophy by using the Cornell index ([RaVL+SV3] mm×QRS msec, cutoff = 2440).

Patients were admitted overnight to the Clinical Research Center, and initial BPs were obtained by ambulatory monitors (SpaceLabs 90207) every 20 minutes from 6:00 to 8:00 AM (3.6±0.3 readings). Blood samples for measurement of plasma renin activity (PRA) and plasma aldosterone were obtained at 8:00 AM before any intervention. These BP and hormonal values constitute the baseline inpatient data, reflecting the ad libitum salt intake of the patients.

Over the ensuing 24 hours, a positive sodium balance (126±24 mmol for the 23 subjects) was produced by administering a high-salt diet (160 mmol NaCl, metabolic kitchen) and by infusing 2 L normal saline from 8:00 AM to 12:00 PM. Monitor BPs were recorded every 15 minutes from 12:00 PM until 10:00 PM (37±1 readings). A 24-hour urine sample was collected on ice; one fresh aliquot was used for measurement of sodium, and another one was stored at −80°C for measurement of 20-HETE. Blood sampling was repeated the following morning, at 8:00 AM. These BP, urine, and hormonal data reflect the salt-replete state.

The effect of furosemide was studied the next day. Three 40-mg oral doses were given at 8:00 AM, 12:00 PM, and 4:00 PM, and the individual salt-sensitivity index for correlation analyses. The value of the hormonal data reflect the actions of furosemide.

A third set of blood specimens was drawn. These BP, urine, and hormonal data reflect the actions of furosemide. These BP and hormonal values constitute the baseline inpatient data, reflecting the ad libitum salt intake of the patients.

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Patients were classified as SS if the fall in average systolic BP from the salt-replete to the salt-depleted state was at least 10 mm Hg. The value of the t statistic from the unpaired comparison between all readings of the salt-replete and salt-depleted days was used as the individual salt-sensitivity index for correlation analyses.

PRA and plasma aldosterone were measured by radioimmunoassay. 20-HETE was measured (after incubation of urine with 1 mg of Escherichia coli β-glucuronidase [Sigma Chemical Co], 2 hours, 37°C, extraction and thin layer chromatography steps) by negative ion-chemical ionization gas chromatography/mass spectroscopy analysis of a pentfluorobenzyl bromide-trimethylsilyl derivative. Concentration in the samples, in the nanograms per milliliter range, was multiplied by total urine volume and divided by the number of hours of the collection period, to express results as an hourly excretion rate (μg/h).

When comparing the 2 collection periods, and to account for their different duration, urine sodium excretion rates are given as hourly data. Aldosterone/renin ratios (ARRs) are expressed in pmol·L⁻¹·h⁻¹·nmol⁻¹. Data are presented as mean±SEM. Comparisons between SS and SR were made with unpaired t tests. Changes within groups were analyzed with paired t tests and correlation coefficients with Pearson’s method. These tests and single linear regression analyses were performed with JMP software (version 3.0.2, SAS Institute). A value of P<0.05 was used to reject the null hypothesis.

Results

Subjects were 47±2 years old, predominantly female (n=18), with similar numbers of blacks (n=12) and whites (n=11). Prevalence of obesity was 74%; that of left ventricular hypertrophy, 17%. Mean creatinine clearance was 117±6 mL·min⁻¹·1.73 m², and none of the patients had renal dysfunction. Table 1 shows that SS patients were somewhat older than were SR patients, but there were no significant differences in any of the other characteristics between the 2 groups.

Baseline systolic and diastolic BPs (outpatient-sphygmomanometer and inpatient-monitor) were not significantly different between SS patients and SR patients (Table 2). In contrast, systolic BP of the salt-replete stage was significantly higher in SS patients than in SR patients. The fall in systolic BP owing to salt-depletion was significantly greater in SS patients than in SR patients (as expected from the criterion used to define the 2 groups). This led to similar BPs in SR and SS patients after salt deprivation (Table 2).

Baseline PRA was lower in SS (0.26±0.07 ngA I /L/sec) than in SR (0.75±0.23 ngA I /L/sec, P<0.05) patients (Figure 1, top left). Salt loading reduced PRA significantly in both groups (P<0.02), but the magnitude of this reduction was blunted in SS patients (−0.11±0.05 ngA I ·L⁻¹·s⁻¹) compared with SR patients (−0.41±0.17 ngA I ·L⁻¹·s⁻¹), and the absolute PRA of the salt-replete state was still significantly lower in the SS group. Furosemide significantly stimulated PRA of SS patients (0.79±0.29 ngA I ·L⁻¹·s⁻¹, P<0.02) but not that of SS patients (0.53±0.30 ngA I ·L⁻¹·s⁻¹, P=NS) (Figure 1, top right). In summary, SS patients had lower PRA and blunted PRA responses to both salt loading and salt deprivation by furosemide.

Plasma aldosterone was not significantly different between SR (534±85 pmol/L) and SS (654±95 pmol/L) patients at baseline (Figure 1, middle left). The figure shows that there is no significant difference between SR and SS patients (n=11) at baseline or after salt loading, and the absolute PRA of the salt-replete state was still significantly lower in the SS group. Furosemide significantly stimulated PRA of SS patients (0.79±0.29 ngA I ·L⁻¹·s⁻¹, P<0.02) but not that of SS patients (0.53±0.30 ngA I ·L⁻¹·s⁻¹, P=NS) (Figure 1, top right). In summary, SS patients had lower PRA and blunted PRA responses to both salt loading and salt deprivation by furosemide.

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<th>TABLE 1. Clinical Characteristics of the Patients</th>
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<td>Gender, F/M</td>
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<td>LHW, N/Y</td>
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SR indicates salt-resistant patients; SS, salt-sensitive patients; NS, not statistically significant; WA, white; BA, African-American; BMI, body mass index; LHW, left ventricular hypertrophy by electrocardiogram; and CrCl, creatinine clearance.

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<th>TABLE 2. Blood Pressures During the Phases of the Protocol</th>
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BP indicates blood pressures: systolic (mm Hg) ± SEM/diastolic (mm Hg) ± SEM; outpatient baseline, sphygmomanometric readings in the clinic; inpatient baseline, monitor readings before interventions; salt-replete, monitor readings during the salt-load; salt-depletion, monitor readings during the furosemide and low-salt diet period; and ∆SBP, fall in systolic BP (mm Hg, salt-depletion minus salt-loading).
were no appreciable effects produced by salt-loading or furosemide on the aldosterone levels of SS patients. In contrast, in SR patients these maneuvers produced the expected significant suppression and stimulation of plasma aldosterone, respectively (middle left and middle right panels).

The ARR was significantly higher in SS patients than SR patients at baseline (Figure 1, bottom left), owing to lower PRA in SS patients. The difference in ARR between groups was exaggerated after salt-repletion, owing to lack of aldosterone suppression by salt loading in SS patients. Finally, although furosemide did not change ARR of SR patients, it did reduce it significantly in SS patients (Figure 1, bottom left and right), a consequence of unchanged aldosterone levels despite some increase in PRA in this group. In summary, changes of ARR in SS patients reflect blunted renin-angiotensin-aldosterone responses to salt loading and salt depletion in these subjects.

Furosemide significantly increased the natriuresis of both SS and SR patients, above that of the salt-replete period. However, this increase in natriuresis was significantly less in SS patients (6.3 ± 3 mmol/h) than in SR patients (18 ± 3 mmol/h, P < 0.02) (Figure 2, left). The impaired natriuretic effect of furosemide in SS resulted in decreased sodium excretion during administration of this agent in this group (263 ± 25 mmol/12 hours) compared with SR patients (351 ± 25 mmol/12 hours, P < 0.02; data not shown).

Urine excretion of 20-HETE was not different between SR and SS patients, either in the salt-replete state (SR, 1.38 ± 0.34 μg/h; SS, 1.92 ± 0.38 μg/h) or after furosemide administration (SR, 2.01 ± 0.40 μg/h; SS, 1.52 ± 0.27 μg/h). However, the changes produced by furosemide in 20-HETE excretion were significant in both groups, were of opposite direction (79% ± 30% increase in SR and 15% ± 9% decrease in SS) and were significantly different between them (Figure 2, right).

20-HETE excretion and natriuresis data, taken together, suggest that furosemide-induced natriuresis will be greater in patients who sustain an increase in 20-HETE excretion in response to this drug. This was confirmed by analyzing the furosemide-induced changes in urine 20-HETE and sodium excretion as continuous variables in all patients together. Figure 3 (left) depicts a significant positive correlation between these variables, indicating that the greater the 20-HETE stimulation by furosemide, the greater was the natriuretic response to this agent, particularly in SR subjects. More importantly, the right panel of Figure 3 shows a significant negative relationship between the 20-HETE response to furosemide and the salt-sensitivity index of individual patients. This indicates that the less the stimulation of 20-HETE by furosemide (or the more its inhibition), the more SS the patient was.

Finally, we investigated, within the SS group, whether any of the characteristics associated with this phenotype was
Figure 3. Scattergrams in all patients combined. Left, Relationship between changes in urine excretion of 20-HETE produced by furosemide (%Δ urine 20-HETE) and the increase in natriuresis produced by this drug (%ΔUNaV). Right, Relationship between %Δ urine 20-HETE by furosemide and the individual salt-sensitivity index for each patient (see Methods). Data in SS patients are plotted with ○, whereas those in SR subjects are shown in □. Correlation coefficients for the data in both groups combined, and their statistical significance, are indicated on the graphs.

Figure 4. Scattergrams in SS patients only. Left, Relationship between ∆ARR and changes in urine excretion of 20-HETE produced by furosemide. Right, the negative relationship between BMI and absolute excretion of 20-HETE during furosemide administration. Correlation coefficients and their statistical significance are indicated on the graphs.

Discussion

Elucidation of the mechanisms leading to salt-sensitivity of BP has become crucial because of the recent demonstration that this pathophysiological feature is an independent risk factor for cardiovascular morbidity in both normotensive and hypertensive subjects.20 By using a protocol almost identical to that used in the preceding publication,20 we classified essential hypertensive subjects into SS and SR subgroups. SS subjects exhibited typical characteristics of this phenotype, including significantly higher BPs during a salt load but not during salt depletion, low plasma renin activity and blunting of its responses to salt loading and salt depletion,21 inappropriate lack of suppression of plasma aldosterone during ad libitum salt intake and salt loading,22 and a high ARR (a consequence of the 2 previous abnormalities23), which was exaggerated by salt loading and diminished by salt-depletion.

From experimental models, it is known that salt-sensitivity of BP is linked to increased sodium reabsorption by renal tubules, eg, increased chloride currents in the thick ascending limb of the Dahl SS rat.24 However, it is uncommon to detect impaired natriuresis in the intact SS animal or human, because the hypertensive response to salt restores natriuresis to its appropriate level (ie, shift in the pressure natriuresis curve). We have now shown that exaggerated salt transport of SS subjects can be unmasked and made apparent as blunted natriuresis when salt excretion is pharmacologically produced by the loop diuretic furosemide. This suggests that the defect in sodium transport of SS, whatever its mechanism, affects the response to this drug.

Stimulation of renal PGE2 by furosemide5–7,9 is a major mediator of its natriuretic action, as demonstrated by blunting of sodium excretion by inhibitors of COX.7,8,10,11 It is noteworthy that a decreased response of PGE2 to furosemide was described in essential hypertension a few years ago. This was particularly noticeable in low-renin hypertensive subjects, a subgroup that is commonly SS. It was suggested that the diminished capacity of the kidney of these patients to generate vasodilatory prostaglandins could underlie their renal hemodynamic abnormalities, their hypertension, and their low PRA levels.25 However, the suggestion that a defect in the COX pathway of AA metabolism participates in the pathogenesis of essential hypertension has been disputed by other investigators.26

Enhanced synthesis of PGE2 and other prostaglandins (eg, PGI2) by furosemide is owing to a dual mechanism, induction of expression of COX-2 mRNA,5–7 and stimulation of phospholipase A2 (PLA2) with release of AA from the cell membrane. The latter mechanism is important, as demonstrated by inhibition of vanadate-induced PGE2 synthesis and chloride transport by PLA2 antagonists in rabbit colon27 and by the abolition of the PGE2 response to furosemide in knockout mice that are −/− for a 85-kDa group IV cytosolic PLA2.28 Therefore, there is no need to invoke an action of furosemide on CYP 450 monooxygenases to account for furosemide-induced stimulation of excretion of 20-HETE, as observed in our SR patients. Increased substrate availability by PLA2 in response to furosemide could suffice to explain this finding. To our knowledge, there is no data on whether furosemide stimulates CYP 450 monooxygenases.

It seems reasonable to assume that an increase in an endogenous sodium transport inhibitor by an exogenous natriuretic agent is an appropriate response, as observed in
our SR patients who exhibited increased 20-HETE excretion associated with greater natriuresis when given furosemide. Furthermore, the continuous relationship between the effects of furosemide on 20-HETE and sodium excretion in all patients suggests that this eicosanoid contributes to the natriuresis produced by this drug. It would thus follow that the lack of such a response, ie, the observation that our SS subjects do not increase 20-HETE excretion in response to furosemide, may constitute an abnormality that not only accounts for the decreased natriuretic response to furosemide in this group but also may be linked to the pathogenesis of their hypertension. This is supported by the continuous relationship we observed in all patients between impaired 20-HETE responses to furosemide and salt-sensitivity of BP. Furthermore, our observations are consistent with the strong evidence supporting a role for reduced synthesis of renal outer medullary 20-HETE in the hypertension of Dahl SS rats, and with our previous demonstration of an abnormal relationship between 20-HETE and salt-induced natriuresis in SS hypertensive patients. The significance of the relationships observed in SS patients, between BMI and ARR and either the absolute levels of 20-HETE excretion during furosemide or the lack of stimulation of this eicosanoid by furosemide is not clear. Both characteristics, obesity and blunting of the renin-angiotensin-aldosterone system, are typical features of the salt-sensitive phenotype. Hence, relationships between them and impaired 20-HETE responses may merely reflect the magnitude or severity of salt-sensitivity of BP in SS patients. It is curious, however, that the relationship between BMI and 20-HETE excretion was present in SS patients, not SR patients, although both groups of patients were almost equally obese. In the present study, we cannot ascertain, however, whether there is a specific interaction between obesity and salt-sensitivity of BP that affects CYP 450 metabolism of AA.

Perspectives

Unraveling the mechanisms underlying salt-sensitivity of BP is no longer only a research issue, it has acquired clinical and therapeutic relevance because of its prognostic implications. We have built on the strong evidence for participation of 20-HETE in experimental salt-sensitive hypertension by investigating the issue in essential hypertensive patients. Our initial observation was a disruption of the relationship between salt and 20-HETE excretion during natriuresis produced by a salt load in SS patients. We now demonstrate a difference in the 20-HETE response to furosemide between SS and SR that is linked, in the former, to impaired natriuresis and to salt-sensitivity of BP. In addition to providing evidence for a possible participation of the monooxygenase pathway of AA metabolism in the action of loop diuretics, our present data strengthen the hypothesis that abnormalities in this pathway are involved in human SS hypertension. CYP 450 synthesis of 20-HETE can be manipulated pharmacologically (eg, stimulation by clofibrate or inhibition by 17 ODYA). Currently available agents are either too nonspecific or too toxic for use in humans. However, continuation of our research and confirmation of a role for 20-HETE in human SS hypertension will provide the necessary stimulus for development of pharmacologic agents suitable for human use. This would allow testing of the hypothesis that treatment of salt-sensitivity of BP may reduce cardiovascular morbidity independent of BP reduction.

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References


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