Regulation of Plasma Endothelin by Salt in Salt-Sensitive Hypertension

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Background—Salt dependency of blood pressure (BP) characterizes most models of experimental hypertension in which endothelins play a significant vasoconstrictor role. Despite this, there are no data on the regulation of plasma endothelin by salt balance in human hypertension.

Methods and Results—Plasma endothelin was measured in 47 patients with essential hypertension. Endothelin, catecholamine, and plasma renin activity (PRA) responses to 24-hour sodium deprivation (↓Na) were assessed in 29 of these patients. Endothelin was higher in hypertensive patients (4.6±0.2 fmol/mL) than in 20 control subjects (3.3±0.3 fmol/mL, P<0.002), was correlated with BP, and was negatively associated with PRA (P<0.04). Salt-sensitive, salt-resistant, and indeterminate groups were defined by the tertiles of the t statistic for the difference in BP before and after ↓Na. Systolic BP falls were −15±1, −2±2, and −9±1 mm Hg, respectively. PRA, its response to ↓Na, and its level after ↓Na were lowest (albeit nonsignificant) in salt-sensitive patients. Baseline catecholamine and endothelin levels did not differ among the groups. In response to ↓Na, catecholamines increased more significantly in salt-sensitive patients (+2.4±0.9 nmol/L) than in the other groups (0.4±0.2 and 0.7±0.2 nmol/L for indeterminate and salt-resistant groups, respectively; P<0.03), whereas endothelin increased in the salt-sensitive group (0.8±0.3 fmol/mL), decreased in the salt-resistant group (−0.4±0.3 fmol/mL), and sustained minimal change in the indeterminate group (0.2±0.3 fmol/mL) (P<0.04). Thus, endothelin levels in the salt-depleted state were highest in salt-sensitive patients (5.2±0.4 fmol/mL) versus the other groups (3.4±0.4 and 4.4±0.4 fmol/mL for salt-resistant and indeterminate groups, respectively) (P<0.02). Changes in endothelin during ↓Na and levels after ↓Na were correlated with changes in catecholamines (P<0.02).

Conclusions—Our data suggest that salt-depleted salt-sensitive hypertensives with blunted renin responses exhibit enhanced catecholamine-stimulated endothelin levels and may therefore respond better than unselected patients with essential hypertension to endothelin receptor blockers. (Circulation. 2001;103:263-268.)

Key Words: endothelin ■ sodium ■ catecholamines ■ renin ■ hypertension

Endothelins, powerful endothelium-derived vasoconstrictor peptides, were discovered in 1988. Their potency, prolonged pressor action, stimulatory effects on the sympathetic nervous system, and cell growth have raised the possibility that abnormal regulation or action of these peptides participates in the pathophysiology of experimental and human hypertension and hypertensive complications. Recent research has demonstrated that endotheins play a role in the maintenance of blood pressure (BP) and vascular hypertrophy of experimental salt-dependent hypertension, eg, the deoxycorticosterone acetate-salt and Dahl salt-sensitive rat models.

In human essential hypertension, circulating levels of endothelins have been found to be elevated by some investigators. Expression of endothelin-1 in the endothelium of small resistance arteries is enhanced in patients with moderate to severe hypertension. Because endothelins have a predominantly paracrine action, with secretion from the endothelium toward the medial layer of the vessel, their plasma levels primarily reflect spillover to the circulation, not necessarily the degree of activation of the endothelin system. However, unequivocal evidence for a role of endothelins in human hypertension was provided by the demonstration that bosentan, a specific antagonist of endothelin type A (ET₁α) and endothelin type B (ET₂) receptors, significantly lowers BP in these patients. Analogous to the data in rats, salt sensitivity of BP in humans may constitute a phenotype that predicts BP depen-
dence on the vasoconstrictor action of endothelins. For example, plasma endothelin is higher in (1) hypertensive African American subjects than in hypertensive white subjects,13 (2) low-renin essential hypertensive subjects than in their normal- or high-renin counterparts,14 (3) obese hypertensive subjects than in obese normotensive subjects,15 and (4) salt-sensitive (SS) hypertensive subjects than in salt-resistant (SR) hypertensive subjects, classified with a dietary protocol.16 Furthermore, endothelin is correlated with plasma insulin levels in essential hypertensive subjects.17

Despite the apparent relationship between salt sensitivity of BP and a role for endothelin in the development or maintenance of these forms of hypertension, there are no studies to date exploring the regulation of plasma endothelin by changes in salt balance in human essential hypertension. We investigated the effect of salt deprivation (↓ Na) on plasma endothelin levels in salt-replete essential hypertensive patients. The BPs of participating subjects were classified as SS or SR according to their response to an established protocol of acute ↓ Na in an inpatient setting.18

Methods

Forty-seven patients with essential hypertension (either on antihypertensive therapy or with systolic BP >140 mm Hg or diastolic BP >90 mm Hg) were recruited from the clinics of the University of Texas Medical Branch and the Clinical Research Institute of Monteval. The research was approved by the Institutional Review Boards of both institutions, and all subjects gave informed consent. Patients maintained their usual dietary salt intake; those who were receiving antihypertensive therapy or with systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg were excluded. Patients underwent a protocol of acute ↓ Na after salt loading, similar to that described by Weinberger’s group (Grum et al)19. In brief, on awakening the morning after admission, they were placed on a diet containing 160 mEq NaCl and were given a 2-L infusion of normal saline from 8:00AM to 12:00 noon (HiNa day). On the following day, awakening the morning after admission, they were placed on a diet containing 10 mEq NaCl per 24 hours, and furosemide (40 mg) was given orally at 8:00 AM, 12:00 noon, and 4:00 PM (LoNa day).

Blood samples for routine tests, plasma renin activity (PRA), plasma catecholamines (epinephrine and norepinephrine), and plasma endothelin were obtained each morning at 8:00 AM, immediately before sodium loading, immediately before furosemide, and after the ↓ Na day. PRA was measured by radioimmunoassay,19 and plasma catecholamines were measured by radioenzymatic assay (BioTrak TRK 995, Amersham).

Plasma immunoreactive endothelin was measured by radioimmunoassay as previously described.8,10 In brief, blood was collected in potassium EDTA tubes, and endothelin was extracted from plasma by passage through C18 Sep-Pak cartridges (Waters Associates). The antibody against endothelin-1 was from Peninsula. The minimum detectable concentration of endothelin was 0.4 fmol/mL. Recovery of 5 fmol/mL endothelin-1 added to plasma was 75%. Cross-reactivity of the antibody was 17% with big endothelin-1, 7% with endothelin-2 and -3, and none with unrelated peptides. The interassay and intra-assay coefficients of variation were <15% and <10%, respectively.

| TABLE 1. Characteristics of Hypertensive Patients |
|-----------------|-----------------|
| All (N=47)       | Subset (N=29) SS Studies |
| Age, y          | 48±1            | 46±1           |
| Sex (F/M), n/n  | 32/15           | 22/7           |
| Race (AA/W), n/n| 15/32           | 15/14          |
| BMI, kg/m²      | 32±1            | 35±1           |
| BP, mm Hg       | 154±3.96±2      | 153±4.94±2     |
| Serum creatinine, μmol/L | 80.6±2.7          | 72.4±2.8       |
| Serum cholesterol, mmol/L | 5.23±0.14          | 5.02±0.17      |
| Triglycerides, mmol/L | 1.75±0.25          | 2.00±0.37      |
| HDL cholesterol, mmol/L | 1.32±0.05          | 1.27±0.06      |
| LDL cholesterol, mmol/L | 3.10±0.14         | 2.91±0.15      |
| PRA, ng A · L⁻¹ · s⁻¹ | 0.35±0.07         | 0.46±0.10      |
| Plasma catecholamines (E+NE), mmol/L | ... | 2.55±0.22      |
| Cornell index (EKG), mm · ms | ... | 1928±140       |

BP was continuously monitored over the entire hospitalization with the Spacelabs monitor, as described above. The number of readings in the 29 patients for the period of noon to 10 PM was as follows: 37±1 for HiNa and 33±1 for LoNa. The effect of ↓ Na on BP was assessed by 2 different methods: (1) the absolute BP change in mm Hg from the salt-replete to the salt-depleted state (mean of all readings of the LoNa minus that of the HiNa days), and (2) the value of the t statistic (unpaired Student t test) for the comparison. In each patient, all of systolic BP readings of the HiNa and LoNa days (noon to 10 PM). This parameter,

\[
\bar{X}_{\text{HiNa}} - \bar{X}_{\text{LoNa}} \pm \sqrt{\left(\text{SD}_{\text{HiNa}}/n_{\text{HiNa}} + \text{SD}_{\text{LoNa}}/n_{\text{LoNa}}\right)^2},
\]

which is analogous to what has been described for the z-score, is a better metric of the salt sensitivity of BP than the absolute change expressed in millimeters of mercury (John Flack, personal communication, 2000). The t statistic and/or the z-score standardize the estimate of salt sensitivity for all subjects because they are independent of the absolute levels of BP and of the BP variances of both days of study. Positive and negative values of t represent reduction and increase of BP by ↓ Na, respectively. Hence, the more salt sensitive the patient is, the larger is the t value. Patients were classified into SS (n=10), indeterminate (IN, n=10), and SR (n=9) groups, according to whether their t values fell within the upper, middle, or lower tertiles of the distribution of the t statistic in the total population, respectively.

ECG left ventricular hypertrophy (LVL) was assessed by the Cornell ([(RAVL+SV₃)+QRS ms] · RV₄) and Sokolow-Lyon (SV₃+RV₄) mm indices, with maximum normal cutoffs being 2440 mm · ms and 35 mm, respectively.

Data are presented as mean±SE. Comparisons of means between 2 groups were carried out by unpaired Student t test, and those between 3 groups were carried out by 1-way ANOVA followed by contrasting of means with the Tukey-Kramer test. Correlation coefficients were calculated with the Pearson method. All statistical tests and fitting of regression lines were performed with the JMP software (version 3.0.2) of the SAS Institute. A value of P<0.05 was used to reject the null hypothesis.

Results

Clinical characteristics of all patients and those of the subset that underwent salt sensitivity studies are depicted in Table 1. Patients were middle-aged, with a predominance of females.
Although only one third of the total recruits were of African American origin, they constituted slightly more than half the patients who underwent salt-sensitivity studies. The average BP of the entire group were within stage I of the Joint National Committee on Prevention, Detection, and Treatment of High Blood Pressure classification. Mean serum creatinine and lipids were normal in the total group and in the subset. The latter had a normal mean index for ECG LVH. These data indicate that the subjects overall were uncomplicated essential hypertensive patients. However, there was variability in BP and clinical characteristics among individual subjects. Hence, 26 subjects exceeded the systolic or diastolic cutoffs (159 and 99 mm Hg, respectively) for stage I hypertension, 37% had hyperlipidemia (LDL cholesterol >3.36 mmol/L), and 24% had LVH by ECG (Cornell index >2440 mm · ms). The prevalence of obesity (body mass index >30 kg/m²) was 59% in the total group and 79% in the subset. PRA was below the minimum normal of 0.14 ng A₁·L⁻¹·s⁻¹ (A₁, angiotension I) in 44% of the total group and in 38% of those who underwent salt-sensitivity studies. Plasma catecholamines were measured only in the latter subgroup and were above the maximum normal value of 2.96 nmol/L in 24% of these subjects.

Plasma levels of immunoreactive endothelin were higher in the whole group of hypertensive patients (4.6±0.2 fmol/mL) and also in the subset of 29 who participated in salt-sensitivity studies (4.4±0.3 fmol/mL) than in 20 normotensive control subjects from our laboratory (3.3±0.3 fmol/mL, P<0.002). However, Figure 1 shows that there was overlap of endothelin levels between hypertensive and normotensive subjects.

Baseline plasma endothelin of hypertensive patients exhibited a positive correlation with systolic and diastolic BPs obtained in the office and with the average systolic and diastolic BPs obtained with ambulatory monitors. The variability of plasma endothelin was explained better by ambulatory than by office BPs (Figure 2).

Plasma endothelin levels were higher in male (5.2±0.4 fmol/mL) than in female (4.3±0.2 fmol/mL) hypertensive patients (P<0.04), but not different between white (4.8±0.2 fmol/mL) and African American (4.0±0.5 fmol/mL) patients (P=NS, not shown). The sex difference in plasma endothelin was probably due to higher BPs in males (159±5/102±3 mm Hg) than in females (152±3/93±2 mm Hg) (P=NS/ P<0.01). Baseline plasma endothelin (1) was not higher in patients with hyperlipidemia (LDL cholesterol >3.36 mmol/L) or LVH (Cornell index >2440 mm · ms) than in their counterparts and (2) was not correlated with age, serum creatinine, serum lipids, or indices for LVH in the ECG. In contrast, there was a significant negative correlation between baseline plasma endothelin and PRA (r=−0.32, P<0.04), best fit by the logarithmic function shown in Figure 3.

Baseline systolic and diastolic BPs of patients classified as SS (155±7/96±4 mm Hg), IN (152±7/93±4 mm Hg), and SR (153±7/91±4 mm Hg) were not significantly different. The systolic BP changes of these 3 groups in response to ↓ Na were −15±1, −9±1, and −2±2 mm Hg, respectively.

Table 2 shows PRA, catecholamine, and endothelin levels in the SS, IN, and SR groups before and after ↓ Na (ie, in the
Table 2. PRA, Catecholamines, and Endothelin

<table>
<thead>
<tr>
<th></th>
<th>SR</th>
<th>IN</th>
<th>SS</th>
<th>P (ANOVA)</th>
</tr>
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<tbody>
<tr>
<td>PRA, ng/A \cdot L^{-1} \cdot s^{-1}</td>
<td></td>
<td></td>
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<tr>
<td>Before ↓ Na</td>
<td>0.29±0.08</td>
<td>0.25±0.07</td>
<td>0.12±0.03</td>
<td>NS</td>
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<td>After ↓ Na</td>
<td>0.97±0.27</td>
<td>0.96±0.39</td>
<td>0.61±0.37</td>
<td>NS</td>
</tr>
<tr>
<td>Δ Due to ↓ Na</td>
<td>0.68±0.21</td>
<td>0.71±0.35</td>
<td>0.49±0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma catecholamines, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before ↓ Na</td>
<td>2.24±0.34</td>
<td>2.47±0.31</td>
<td>2.70±0.47</td>
<td>NS</td>
</tr>
<tr>
<td>After ↓ Na</td>
<td>2.96±0.41</td>
<td>2.91±0.28</td>
<td>5.09±1.35</td>
<td>NS</td>
</tr>
<tr>
<td>Δ Due to ↓ Na</td>
<td>0.72±0.18</td>
<td>0.44±0.18</td>
<td>2.39±0.91</td>
<td>&lt;0.03</td>
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<tr>
<td>Plasma endothelin, fmol/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Before ↓ Na</td>
<td>3.73±0.49</td>
<td>4.19±0.43</td>
<td>4.43±0.33</td>
<td>NS</td>
</tr>
<tr>
<td>After ↓ Na</td>
<td>3.37±0.40</td>
<td>4.38±0.38</td>
<td>5.23±0.38</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Δ Due to ↓ Na</td>
<td>-0.36±0.27</td>
<td>0.19±0.27</td>
<td>0.80±0.32</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

Data are mean±SE.

salt-replete and salt-depleted states) and also their changes in response to salt depletion. Baseline PRA (not shown) and PRA in the salt-replete and salt-depleted states were somewhat lower in the SS group than in the other 2 groups. Also, the PRA response to salt depletion was blunted in the SS group. However, none of these differences reached statistical significance. Baseline plasma catecholamine levels (not shown) and the levels after salt loading were not different among the groups. However, their responses to salt depletion were significantly larger in the SS group, leading to higher (albeit nonsignificant) plasma levels in salt-depleted SS patients. Finally, baseline endothelin (not shown) and endothelin after salt loading were not different among the SS, IN, and SR groups. However, the responses of plasma endothelin to salt depletion were in opposite directions for the SS (increase) and SR (decrease) groups. The change in the IN group was not statistically different from zero. The ANOVA for the mean changes in the 3 groups was significant, and the Tukey-Kramer test confirmed that this was due to the difference between the SS and SR groups. As a result of this, plasma endothelin in salt-depleted SS patients was significantly higher than that in the other 2 groups.

To confirm that different endothelin responses to ↓Na in the SS, IN, and SR groups were not due to the arbitrary cutoffs (tertiles of the t statistic) chosen to define these groups, we analyzed the relationship between individual salt sensitivity of BP and endothelin response to salt depletion as continuous variables. The scatterplots and regression analyses in Figure 4 show that there were continuous significant relationships between the change in plasma endothelin and either the fall in systolic BP or the t statistic for this fall during ↓Na. The variability of the plasma endothelin responses to ↓Na was explained better by the t statistic than by the absolute BP fall in mm Hg (21% versus 14%), supporting the view that the former is probably a better metric for salt sensitivity of BP (see Methods).

Finally, the changes in plasma endothelin due to ↓Na, as well as the resulting plasma endothelin levels in the salt-depleted state, were correlated with the changes in plasma catecholamines due to ↓Na (Figure 5). No such relationships were observed between endothelin and renin or between catecholamines and renin.

Discussion

Our hypertensive subjects exhibited significantly increased plasma endothelin levels, agreeing with observations by some but not all investigators. However, there was considerable scatter of individual values and overlap with those of the control subjects. Experimental studies predict that patients with either salt sensitivity or severe elevation of BP may have the highest levels of endothelin. The significant correlations between plasma endothelin and office or ambulatory BPs in the present study confirm the latter prediction. In contrast, plasma endothelin levels in SS subjects were not different from those in their SR counterparts when measured during habitual salt intake.

We found no other correlates of plasma endothelin in our patients. Slightly increased levels in males were probably due to higher BPs in subjects of that sex. The lack of racial differences was perhaps due to equal severity of hypertension or to equal prevalence of salt sensitivity of BP in our African American and white patients. Although significant correlations between ECG left ventricular mass and plasma endothelin have previously been reported in obese hypertensive patients, we could not confirm these by ECG indices of...

Figure 4. Relationships between changes in plasma ET (ΔET) and individual changes in systolic BP (ΔSBP, left) or t statistic for these changes (t for ΔSBP, right), produced by ↓Na in hypertensive patients. Correlation coefficients, their significance, and regression lines are shown on each panel.
LVH. This may be due to less accurate assessment of left ventricular size or less variability in left ventricular mass in our mildly hypertensive patients.

Acute changes in salt intake uncovered marked differences in hormonal responses between SS and SR patients. We cannot speculate whether these differences would have been elicited by a chronic (eg, dietary) protocol of salt loading and unloading.

Although the groups were relatively small and differences in renin did not reach statistical significance, our SS patients had somewhat lower baseline PRA and blunted renin responses to ↓Na. These 2 characteristics have been repeatedly observed in salt-sensitive hypertension (see review25). PRA responses to acute24 or to dietary23 salt depletion predict the BP fall in these patients. Because their PRA responses to other stimuli (eg, orthostasis24) are also blunted, the most likely explanation for these observations is an intrinsic defect in the responsiveness of the renin-angiotensin system and not an effect of preexisting volume expansion on the renin response to salt depletion.

The SS subjects in the present study had normal baseline catecholamines but an exaggerated increase in response to salt depletion. A role for hyperactivity of the sympathetic nervous system in salt-sensitive hypertension with a blunted renin-angiotensin system is controversial.21 However, these patients have exaggerated orthostatic rises in plasma catecholamines and impaired suppression of their plasma levels by a salt load.24 These observations, taken together with ours, suggest that overactivity of the sympathetic nervous system is present, either as a primary abnormality or as compensation for impaired responsiveness of the renin-angiotensin system in the salt-replete and salt-depleted states of SS hypertensive patients.

It has been suggested that the endothelin system is stimulated in a compensatory manner when a blunted renin-angiotensin system is incapable of maintaining BP.14 Although our SS patients did not have higher plasma endothelin compared with SR subjects, there was a significant negative correlation between endothelin and PRA in all patients. This could be explained by endothelin-induced inhibition of renin release25 but is more likely to represent higher endothelin levels secondary to a low renin state, as proposed by others.14,26

Finally, as demonstrated in the rat kidney, the normal response to salt depletion is inhibition of endothelin generation.25 This probably accounts for compensatory reduction of ET<sub>A</sub>-induced natriuresis. Therefore, the decrease of plasma endothelin by salt depletion in SR subjects is consistent with a physiological response, whereas the increase in SS patients suggests an abnormal response, perhaps triggered by blunting of the pressor role of the renin-angiotensin system in these subjects.

The mechanism by which plasma endothelin levels increase during ↓Na in SS patients is unknown. Correlations between catecholamine and endothelin responses to ↓Na suggest that synthesis, release, or spillover of endothelin may be stimulated by catecholamines in the vasculature or nervous system in salt-sensitive hypertension with a blunted renin-angiotensin system.26 The response of unselected hypertensive patients to bosentan, an ETA/ET<sub>B</sub> receptor blocker, was significant but not greater than the response of normotensive humans.12 This could imply a lack of a specific abnormality of the endothelin system in essential hypertension and simple removal of normal endothelin-dependent vasoconstrictor tone by bosentan. Alternatively, we suggest that the modest reduction of BP in the bosentan trial was due to lack of targeting of therapy to a specific hypertensive phenotype in which endothelin may play a specific pathogenic vasoconstrictor role. Our data suggest that salt-depleted (diet or diuretics) SS hypertensive patients should be the target population for endothelin receptor blockers.

Whether these patients will benefit more from pure ET<sub>A</sub> versus combined ET<sub>A</sub>/ET<sub>B</sub> blockers is unresolved. In heart failure, overexpression of vasoconstrictor ET<sub>A</sub> receptors in the smooth muscle predicts a more powerful action for ET<sub>A</sub>/ET<sub>B</sub> blockers.38 A recent demonstration of ET<sub>B</sub>-mediated vasoconstriction in the forearm vascular bed of essential hypertensive patients suggests that this may also be the case in hypertension.39

**Figure 5.** Scattergrams depicting relationships between changes in catecholamines produced by salt deprivation (ΔCAT due to ↓Na) and either changes in endothelin (ΔET) by ↓Na (left) or plasma ET after ↓Na (right). Correlation coefficients, their significance, and regression lines are shown in each panel.
In conclusion, SS hypertensive patients exhibit significantly increased plasma endothelin levels when in the salt-depleted state, probably via stimulation by catecholamines. This predicts an enhanced antihypertensive action for ETA or ETa/ETb blockers in this group of patients, a suggestion to be confirmed with the appropriate therapeutic trials.

Acknowledgments

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References

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