Renal Endothelin ET\textsubscript{A}/ET\textsubscript{B} Receptor Imbalance Differentiates Salt-Sensitive From Salt-Resistant Spontaneous Hypertension

Lars Rothermund, Susanne Luckert, Peter Koßmehl, Martin Paul, Reinhold Kreutz

Abstract—It is unclear why a subgroup of patients with essential hypertension develop salt-sensitive hypertension with progression of target organ damage over time. We evaluated the role of the renal endothelin (ET) system in the stroke-prone spontaneously hypertensive rat (SHRSP) model of salt-sensitive spontaneous hypertension (SS-SH) compared with the spontaneously hypertensive rat (SHR) model of salt-resistant spontaneous hypertension (SR-SH).

Both strains were studied after either sham-operation on a normal diet (Sham) or after unilateral nephrectomy and high NaCl loading (NX-NaCl) with 4% NaCl in diet for 6 weeks (n=10, respectively). Systolic blood pressure (SBP) increased only in SHRSP-NX-NaCl compared with SHRSP-Sham (250±6 versus 172±5 mm Hg, \(P<0.0001\)). SBP remained unchanged in SHR-NX-NaCl compared with SHR-Sham. In SHRSP-NX-NaCl animals, urinary albumin and ET-1 excretion, renal ET-1 mRNA expression, glomerulosclerosis index, and tubulointerstitial damage index were elevated compared with SHRSP-Sham (\(P<0.05\), respectively), whereas no significant changes were found in SHR after NX-NaCl. Urinary sodium excretion (\(U_{\text{Na}}\)) was significantly reduced by 38% in SHRSP-NX-NaCl compared with SHR-NX-NaCl (\(P<0.005\), respectively). SHR animals showed a similar increase in both renal ET\textsubscript{A} and ET\textsubscript{B} receptor densities after NX-NaCl (2.2-fold, \(P<0.05\)). In contrast, SHRSP-NX-NaCl developed a significantly more pronounced increase in ET\textsubscript{A} compared with ET\textsubscript{B} binding (4.7-fold versus 2.4-fold, \(P<0.05\), compared with SHRSP-Sham, respectively), resulting in a significant 2.1-fold increase in ET\textsubscript{A}/ET\textsubscript{B} receptor ratio only in the SHRSP-NX-NaCl (\(P<0.05\)). Thus, activation of the renal ET system together with an increased ET\textsubscript{A}/ET\textsubscript{B} receptor ratio may contribute to the development and progression of SS-SH. (Hypertension. 2001;37:275-280.)

Key Words: sodium ■ hypertension, sodium-dependent ■ endothelin ■ receptors, endothelin ■ rats, spontaneously hypertensive ■ rats, stroke-prone SHR

There are abundant experimental data indicating that the endothelin (ET) system plays an important role in the pathogenesis of salt-sensitive hypertension and the hypertensive target organ damage in this disease.\(^1,2\) This evidence has been in large part derived from experimental rat models in either deoxycorticosterone acetate (DOCA)-salt hypertension\(^3\) or the genetic Dahl\(^4\) and Sabra salt-sensitive\(^5\) hypertensive rat models. It is unclear, however, whether the activation of the renal ET system is also of significance during the progression from spontaneous hypertension to salt-sensitive spontaneous hypertension (SS-SH) with more severe target organ disease. In particular, the relative contributions of the renal ET receptor subtypes to the development and progression of spontaneous hypertension toward SS-SH are largely unknown. Most studies did not investigate the regulation of ET receptor subtypes in the kidney through the direct determination of receptor density and affinity but rather drew indirect conclusions from the application of selective or unselective ET receptor antagonists. Recently, Orth et al\(^6\) reported on a striking salutary effect of chronic ET\textsubscript{A} receptor blockade with complete normalization of kidney damage in uninephrectomized stroke-prone spontaneously hypertensive rats (SHRSP) after high-salt diet. Interestingly, this finding was independent of an antihypertensive effect of the compound. However, this study lacks any further analysis of the renal ET system, including ET\textsubscript{A} and ET\textsubscript{B} receptor regulation in the SHRSP model studied.

In the present study, we set out to investigate the renal regulation of the ET system and the specific contribution of renal ET receptor subtype density and affinity in our SHRSP model of SS-SH compared with a model of salt-resistant spontaneous hypertension (SR-SH) represented by the spontaneously hypertensive rat (SHR). We studied the SHR and SHRSP strains after either sham operation on a normal diet or
Methods

Animals and Experimental Design
Male SHR/Fub and SHRSP/Fub rats were obtained from our colony at the Freie Universität Berlin (Fub), Benjamin Franklin Hospital. Animals were housed with constant room temperature (21°C) and humidity (75%) under a controlled light-dark cycle. At the age of 6 weeks, animals were randomly assigned to 1 of 4 groups (n = 10 each): SHR and SHRSP rats were fed a normal diet (0.2% NaCl) and underwent a sham-operation (Sham) or were fed a high-salt diet (4% NaCl),

Ureteral Stenosis (NX) and underwent uninephrectomy (NX-NaCl). Normal tap water was available ad libitum. For sham operation or NX, each animal was anesthetized with ether inhalation, and a left nephrectomy was performed via a retroperitoneal incision. Blood samples as well as 24-hour urine samples were taken in week 6 after surgery. Thereafter, animals were killed during ether anesthesia, and the kidney was rapidly excised, rinsed in a 0.9% NaCl solution, blotted dry, and weighed. A midcoronal section of the right kidney was immersed in Dubosq-Brasil solution and embedded in paraffin for histological studies. The remaining renal tissue was immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

Blood Pressure Measurement
Systolic blood pressure (SBP) was determined in awake rats at the age of 12 weeks using a tail cuff and pressure transducer in conjunction with a computerized pressure delivery and chart recording system (TSE Biosystems GmbH).

Urine and Biochemical Analysis
After completion of the blood pressure measurements, animals were placed in metabolic cages for 1 day for adaptation. The next day, 24-hour urine samples were collected for determination of urinary protein excretion (U P), albumin excretion (U A), and sodium excretion (U Na). U P was measured according to Bradford’s method, and U A and U Na were determined by ELISA with a rat specific antibody (ICN Biomedicals). Creatinine and sodium concentrations were measured with standard techniques.

Determination of Urine ET-1 Concentration
A commercially available enzyme immunoassay for ET-1, suitable for direct measurement of ET-1 in urine samples, was carried out according to the instructions of the manufacturer (Immundiagnostik GmbH).

Binding Assays for ET A and ET B Receptors
To analyze the renal expression of both known ET receptor subtypes (ET A and ET B), binding assays were performed in the presence or absence of the subtype-specific ET receptor ligands as described in detail previously. A cDNA fragment for ECE-1 (ECE-1) was determined by Northern blot analysis as described in detail elsewhere. A commercially available enzyme immunoassay for ET-1, suitable for direct measurement of ET-1 in urine samples, was carried out according to the instructions of the manufacturer (Immundiagnostik GmbH).

Northern Blotting
The mRNA expression level of ET-1 and ET-converting enzyme-1 (ECE-1) was determined by Northern blot analysis as described in detail elsewhere. A cDNA fragment for ECE-1 was amplified by RT-PCR from a 3’ portion of the rat cDNA between nucleotides 949 and 1678 (GenBank accession No. D2963); all known isoforms of ECE-1 were detected by Northern blot analysis.

Morphological Investigation of the Kidney
After the samples were embedded in paraffin, they were cut into 3-μm sections and stained with periodic acid–Schiff’s, followed by hematoxylin counterstaining. Glomerulosclerosis index (GSI) was assessed with a semiquantitative scoring method as described previously. GSI was defined as the mean value of all scores obtained. All parameters were assessed independently by 2 investigators in a blinded manner.

Statistical Analysis
All data are expressed as mean ± SEM. Statistical analysis was performed using 2-way ANOVA followed by Bonferroni’s adjustment and by Mann-Whitney U test. Differences were considered significant at the level of P < 0.05.

Results

Body Weight, Kidney Weight, and Creatinine Clearance
The data for body weight (BW), normalized kidney weight (K/BW), and creatinine clearance (C crea) are presented in Table 1. BW was significantly lower in the SHRSP-NX-NaCl compared with SHRSP-Sham and SHR-NX-NaCl. K/BW was higher in SHRSP-Sham than SHR-Sham, increased in both strains after NX-NaCl, and was higher in SHRSP-NX-NaCl than in SHRSP-Sham (P < 0.05, respectively). C crea was significantly decreased in SHRSP-NX-NaCl compared with SHRSP-Sham and SHRSP-NX-NaCl (P < 0.05, respectively).

Blood Pressure
SBP is shown in Figure 1. We observed no significant difference in SBP between SHR-Sham and SHRSP-Sham. SBP was not elevated in SHR-NX-NaCl compared with SHR-Sham. In contrast, SBP was significantly increased in SHRSP-NX-NaCl compared with SHRSP-Sham or SHR-NX-NaCl (P < 0.001, respectively).

Sodium and Volume Excretion
The data for U Na, urinary volume excretion (U v), and urinary Na concentration are presented in Figure 1. U Na increased 9.1-fold in SHR-NX-NaCl compared with SHR-Sham and 6.8-fold in SHRSP-NX-NaCl compared with SHRSP-Sham (P < 0.0001, respectively). However, sodium excretion was significantly lower in SHRSP-NX-NaCl than in SHR-NX-NaCl (P < 0.005). U v increased 4.0-fold in SHR-NX-NaCl compared with SHR-Sham (P < 0.01) and 7.8-fold in SHRSP-NX-NaCl compared with SHRSP-Sham (P < 0.0001). Thus, urinary Na concentration was significantly elevated in SHRSP-NX-NaCl compared with SHR-Sham and SHRSP-NX-NaCl (P < 0.001, respectively) but remained unchanged in SHRSP-NX-NaCl compared with SHRSP-Sham.

Table 1. BW, K/BW, and C crea

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>BW, g</th>
<th>K/BW, mg/g</th>
<th>C crea, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-Sham</td>
<td>259±6.3</td>
<td>3.1±0.06*</td>
<td>0.66±0.10</td>
</tr>
<tr>
<td>SHR-NX-NaCl</td>
<td>259±6.3</td>
<td>5.5±0.10</td>
<td>0.77±0.11</td>
</tr>
<tr>
<td>SHRSP-Sham</td>
<td>231±10.4</td>
<td>4.2±0.16</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td>SHRSP-NX-NaCl</td>
<td>176±9.6†</td>
<td>8.1±0.39†</td>
<td>0.38±0.09†</td>
</tr>
</tbody>
</table>

*P < 0.05 vs SHRSP-Sham. †P < 0.05 vs SHR-NX-NaCl.
Proteinuria, Albuminuria, and Urinary ET-1 Excretion

The data for $U_P$, $U_{alb}$, and urinary ET-1 excretion ($U_{ET-1}$) are presented in Figure 2. $U_P$ and $U_{alb}$ did not differ between SHR-Sham and SHRSP-Sham and did not change in SHR-NX-NaCl compared with SHR-Sham. However, a significant increase in both parameters was observed in SHRSP-NX-NaCl compared with SHRSP-Sham and compared with SHR-NX-NaCl ($P<0.0001$, respectively).

$U_{ET-1}$ was unchanged in SHR-Sham compared with SHRSP-Sham and did not differ in SHR-NX-NaCl compared with SHR-Sham. A significant increase in $U_{ET-1}$ was observed in SHRSP-NX-NaCl compared with SHRSP-Sham and compared with SHR-NX-NaCl ($P<0.0001$, respectively).

Expression of PreproET-1 and ECE-1 mRNA

We observed no significant difference in kidney mRNA expression of preproET-1 between SHR-Sham and SHRSP-Sham. PreproET-1 expression did not change in SHR-NX-NaCl compared with SHR-Sham but was significantly elevated in SHRSP-NX-NaCl compared with SHRSP-Sham and with SHR-NX-NaCl (2.2±0.47 versus 0.09±0.10 and 1.03±0.13, respectively; $P<0.05$). Overall, no significant differences were detected for renal ECE-1 mRNA levels (data not shown).

ET$_A$ and ET$_B$ Receptor Binding

The data for kidney ET receptor binding are presented in Figure 3. Kidney ET$_A$ receptor density increased in SHR-NX-
NaCl and SHRSP-NX-NaCl compared with SHR-Sham and SHRSP-Sham ($P<0.0001$, respectively). The increase in ET $A$ receptor density was significantly more pronounced in SHRSP-NX-NaCl than in SHR-NX-NaCl ($P<0.003$). No significant difference in ET $A$ receptor affinity was observed between the 2 strains or in response to the treatment protocol (Table 2).

Kidney ET $B$ receptor density increased similarly in both SHR-NX-NaCl and SHRSP-NX-NaCl compared with SHR-Sham and SHRSP-Sham ($P<0.05$, respectively). In contrast to ET $A$ receptor density, no interstrain difference in ET $B$ receptor density was detected. However, ET $B$ receptor affinity was significantly reduced in SHRSP-NX-NaCl compared with SHRSP-Sham and SHR-NX-NaCl (Table 2, $P<0.05$). We observed no significant difference in kidney ET $A$/ET $B$ receptor ratio between SHR-Sham and SHRSP-Sham. ET $A$/ET $B$ receptor ratio was not elevated in SHR-NX-NaCl compared with SHR-Sham. In contrast, ET $A$/ET $B$ receptor ratio was significantly increased in SHRSP-NX-NaCl compared with SHRSP-Sham as well as with SHR-NX-NaCl ($P<0.01$, respectively).

**Glomerulosclerosis and Tubulointerstitial Damage Indices**

The GSI and TDI data are shown in Figure 4. GSI and TDI did not differ between SHR-Sham and SHRSP-Sham and were unchanged in SHR-NX-NaCl compared with SHR-Sham. GSI as well as TDI were significantly increased in SHRSP-NX-NaCl compared with SHR-NX-NaCl ($P<0.01$ and $P<0.0001$, respectively).

**Discussion**

The main question of the present study was whether an activated renal ET system contributes to the development and progression of SS-SH compared with SR-SH.

We demonstrated that the renal ET system is indeed activated in our SHRSP model of SS-SH after unilateral
nephrectomy and high-NaCl dietary exposure. Unlike the SHR model of SR-SH, SHRSP-NX-NaCl animals develop severe hypertension and kidney damage. We show that an important maladaptive functional change in the progression of hypertensive kidney damage in SS-SH is the manifestation of reduced $U_{\text{Na}^-}$ in SHRSP-NX-NaCl. SS-SH and renal failure in the SHRSP-NX-NaCl model is characterized by an increased renal ET$_A$/ET$_B$ receptor ratio.

**Development of SS-SH and Kidney Damage**

The observed development of decreased $C_{\text{crea}}$ together with marked elevation of glomerulosclerosis and tubulointerstitial damage in SHRSP-NX-NaCl is in agreement with a recent study that reports similar findings in another SHRSP strain. In addition, we observed a significant elevation in $U_{\text{P}}$ and $U_{\text{alb}}$, indicating manifestation of renal damage in SHRSP-NX-NaCl. In contrast, no increase in SBP or any significant changes in $C_{\text{crea}}$, GSI, TDI, $U_{\text{P}}$, or $U_{\text{alb}}$ were observed in SHR-NX-NaCl. These findings clearly demonstrate that the SHRSP strain represents a model of SS-SH that is highly susceptible to progression of its spontaneous hypertension toward severe hypertension in response to dietary salt excess, unlike the SHR strain, which maintains the SR-SH phenotype even after unilateral nephrectomy.

**Activation of the Renal ET System**

In SHR-NX-NaCl, we observed no activation of the ET-system, whereas renal preproET-1 mRNA expression and $U_{\text{ET}-1}$ were significantly increased in SHRSP-NX-NaCl animals. Thus, the development of SS-SH and renal damage in SHRSP-NX-NaCl seems to be closely related to activation of the renal ET system. Similar findings were reported at the protein level in salt-sensitive hypertensive Dahl rats with enhanced blood pressure sensitivity to DOCA-salt treatment in ET$_B$ receptor–deficient rats. More recently, data obtained from ET$_B$ receptor–deficient mice and rats unambiguously demonstrated a hypertensive phenotype only after salt loading in ET$_B$ receptor–deficient animals. This form of hypertension was completely ameliorated by amiloride, a highly selective inhibitor of the epithelial sodium channel (ENaC) in the distal nephron. The most likely explanation for these findings is derived from previous elegant in vitro studies in distal nephron cells showing that ET-1 is capable of stimulating ENaC via the ET$_B$ receptor. In the present study, the SHR model of SR-SH showed a similar increase in both renal ET$_A$ and ET$_B$ receptor densities after NX-NaCl treatment and thereby maintains its ET$_A$/ET$_B$ receptor ratio constant. In contrast, SHRSP-NX-NaCl animals exhibited a significantly more pronounced increase in ET$_A$ compared with ET$_B$ binding, which resulted in a significant increase of ET$_A$/ET$_B$ receptor ratio in this model of SS-SH. The relevance of this finding may be further amplified by a reduced ET$_B$ receptor affinity observed only in SHRSP-NX-NaCl animals. Our data therefore suggest a role for the increased ET$_A$/ET$_B$ receptor ratio in combination with the reduced ET$_B$ receptor affinity in the pathogenesis of SS-SH and renal damage. Moreover, these data could, at least in part, explain the strong nephroprotective effect of selective ET$_A$ receptor blockade in another SHRSP-NX-NaCl model.
reported previously.6 Finally, increased renal ET-1 levels, as indicated by higher renal ET-1 mRNA expression and urinary ET-1 excretion in our SHRSP-NX-NaCl model, may therefore stimulate sodium reabsorption by ENaC via the increased renal ET<sub>A</sub>/ET<sub>B</sub> receptor ratio. Although it is well established that the nephron represents a major site for intrarenal ET-1 production and ET-1 binding,19 we cannot deduce from the current set of experiments where the induction of renal ET-1 and ET<sub>A</sub> receptor occurred in the SHRSP-NX-NaCl model. However, the observation that both renal Na<sup>+</sup> excretion and urinary Na<sup>+</sup> concentration were significantly reduced in SHRSP-NX-NaCl compared with SHR-NX-NaCl favors the potential relevance of the increased ET-1 expression and ET<sub>A</sub>/ET<sub>B</sub> receptor imbalance for the impairment of renal Na<sup>+</sup> handling in SHRSP-NX-NaCl.

We therefore conclude that activation of the renal ET system in conjunction with an increased ET<sub>A</sub>/ET<sub>B</sub> receptor ratio contributes to decreased U<sub>Na</sub><sup>+</sup>, and to higher susceptibility to the development of SS-SH and kidney damage in SHRSP-NX-NaCl compared with SR-SS in the SHR-NX-NaCl model. A possible mechanism is impairment of U<sub>Na</sub><sup>+</sup> via the stimulation of ENaC. Whether this unfavorable imbalance of renal ET<sub>A</sub>/ET<sub>B</sub> receptor ratio may also be involved in the progression of salt-sensitive hypertension in other experimental models and ultimately in patients with salt-sensitive hypertension remains to be investigated. The evaluation of this question appears of major clinical interest in face of the currently high prevalence of salt-sensitive hypertension and its impact on the development of end-stage renal disease. In this regard, ET<sub>A</sub> receptor antagonism may become a promising new approach in the pharmacological treatment of salt-sensitive hypertension.

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References

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