Norepinephrine Overflow and Renin Pattern of the Individual Kidney in Patients With Unilateral Renal Artery Stenosis

Peter Friberg, Reinhard Volkmann, Gert Jensen, and Mattias Aurell

This study was performed to determine divided renal efferent sympathetic nerve activity from kidneys in seven patients with renin-positive, unilateral renal artery stenosis before and 30 minutes after an acute intravenous dose of 1.25 mg enalaprila. Renal norepinephrine release was calculated from split renal plasma flow, venoarterial plasma concentration gradients across the kidney, and the fractional extraction of tritiated norepinephrine. All patients had unilateral renin secretion, the affected kidney increasing its plasma renin activity gradient 1.7-fold, whereas no statistically significant change was noted on the contralateral side in response to enalaprilat. Total norepinephrine release to plasma and norepinephrine plasma clearance (assessed by isotope dilution) were similar before and after administration of enalaprilat (approximately 400 ng/min and 1.0 l/min), despite a 26% fall in mean arterial pressure (from 125 mm Hg, p<0.01). Heart rate remained unchanged. After enalaprilat, norepinephrine venoarterial difference increased in the renin-secreting kidney (from 264 to 396, SED=57 pg/ml, p<0.05), whereas it increased only slightly in the contralateral kidney (from 149 to 256, SED=72 pg/ml, NS). Tritiated norepinephrine extraction fell approximately 25% (p<0.01) in both kidneys. Thus, renal norepinephrine spillover increased from 49 to 62, SED=9 ng/min (NS) and from 81 to 129, SED=17 ng/min (p<0.05) from the affected and the contralateral kidney, respectively. Hence, in this relatively small study in patients with renovascular hypertension, no evidence for increased renal nerve activity could be observed in the affected kidney, despite its marked renin production. Furthermore, it appears that angiotensin converting enzyme inhibition may influence renal neuronal uptake of norepinephrine, as enalaprilat reduced fractional extraction of norepinephrine. (Hypertension 1991;17:1003-1009)

It generally is believed that the renin-angiotensin system plays an important role in renal hypertension. The involvement of the sympathetic nervous system, however, is less clear. Direct recording of muscle sympathetic nerve activity has been reported to be elevated in patients with renal artery stenosis compared with controls. This increase was normalized after percutaneous transluminal angioplasty on the affected renal artery. Although increased muscle sympathetic nerve activity may prevail in renovascular hypertension, the possible involvement of the renal nerves is less clear.

Studies of the sympathetic nervous system by measurements of norepinephrine (NE) have yielded increased levels of NE in the renal vein both during resting conditions and in response to acute blood pressure reduction by hydralazine in patients with unilateral renal artery stenosis. Moreover, Gordon et al showed increased levels of NE in left adrenal vein in humans with renal hypertension on the basis of renal artery stenosis. These findings have been taken as evidence for an increased renal sympathetic nerve activity. The experimental justification for this is that NE overflow into the renal venous drainage is largely proportional to the rate of renal nerve stimulation.

Besides the characteristic of efferent sympathetic nerve activity being markedly regionalized, it is important to note, however, that the plasma NE concentration is influenced not only by NE spillover from the organ into the blood but also by its clearance. This means that not only regional plasma flow is important but also the fractional extraction of NE across the organ, because all organs show a bidirectional NE flux. NE extraction probably is best determined by using a constant rate infusion of tritiated NE. Hence, interpretation for sympathetic nerve activity of data based on analyzing only plasma NE in arterial and venous blood draining a certain organ should be undertaken with great care.
Several studies have been aimed to analyze the possible interaction between the sympathetic nervous system and the renin-angiotensin system, particularly in experimental models in vivo and in vitro. A potential increase in NE overflow in renal veins in renin-dependent hypertensive patients could be explained by an increase in plasma renin activity (PRA) and hence increased production of angiotensin II, which in turn could facilitate NE release and potentiate the effects of NE postsynaptically.

The aim of this study was to elucidate efferent, split renal sympathetic nerve activity, using the [3H]NE tracer technique, and its interaction with the renin-angiotensin system in hypertensive patients with an angiographically verified unilateral stenosis. Thus, these patients were diagnostically investigated for renin dependence by means of bilateral renal vein catheterization. Stenotic, renin-positive, and contralateral kidneys were compared before and after an acute dose of the angiotensin converting enzyme (ACE) inhibitor enalaprilat.

**Methods**

Thirteen hypertensive patients were diagnostically investigated for suspect renin-dependent hypertension. Two had bilateral renal artery stenosis, and the remaining 11 had unilateral stenosis. Seven patients were found to be renin positive (see below) and therefore were included. The patients were hospitalized for 5 days and were put on a sodium-restricted diet of less than 25 mmol per 24 hours to further stimulate renin secretion. Antihypertensive therapy was withdrawn 3 days before examination.

**Catheterization**

On the study day, a cannula was introduced percutaneously into the left radial artery (left brachial artery in one patient) for blood pressure monitoring and blood sampling. In addition, left and right renal veins were catheterized via both femoral veins using the Seldinger technique. In two patients the renal veins were reached via the same femoral vein. The catheters were positioned under fluoroscopic control, and the positions then were checked regularly by means of oxygen saturation.

In the right arm, para-aminohippurate (PAH, Merck Sharp & Dohme, Philadelphia) and levo-7 [3H]NE (New England Nuclear, Boston) (approved by the Ethical and Isotope Committees, respectively, at Sahlgren's Hospital, Göteborg) were infused into peripheral veins.

**Renal Plasma Flow**

PAH was infused, after a priming bolus dose, at a rate individualized to each patient's body weight and serum creatinine level. The concentrations of PAH in duplicate arterial and renal vein samples were determined, and the arterial concentration at steady state was used to calculate total body clearance. Renal plasma flow then was derived from total body PAH clearance corrected for renal fractional extraction of PAH. Split renal function was assessed by 99mTc-diethylenetriamine pentaacetic acid (DTPA) gamma camera renography before and after 25 mg captopril given orally. In our laboratory this dose has been shown to give moderate blood pressure reduction. The precaptopril and postcaptopril scan was not used as a diagnostic tool but rather for split estimation of glomerular filtration rate. The procedure was, for practical reasons, performed 3 days before the invasive measurements and was used to give an approximate estimation of percent divided glomerular filtration rate. These values then were used subsequently in association with the PAH infusion clearance (extraction) data to calculate renal plasma flow. This experimental procedure has two disadvantages. First, two different ACE inhibitors were used, which may alter systemic (renal) hemodynamics. However, both drugs have been shown to increase renal blood flow. Second, the blood pressure reduction was less pronounced during the gamma camera renography (captopril) investigation compared with the invasive procedure (enalaprilat), which might be a drug effect per se but also may be due to the newly introduced and thereby less hemodynamic active sodium restriction and antihypertensive therapy withdrawal.

**Total and Divided Renal Norepinephrine Spillover to Plasma**

Measurements of NE spillover to plasma from both kidneys and the body as a whole were used to estimate split renal and overall sympathetic activity (integrated nerve firing rate). At steady state during peripheral intravenous infusion at a tracer dose (0.80 μCi/min) of levo-7 [3H]NE (specific activity, 14–20 Ci/mmol), the total NE spillover to plasma and total plasma NE clearance can be calculated using the following equations:

\[
\text{Total NE spillover} = \frac{[\text{3H}]\text{NE infusion rate (dpm/min)}}{\text{arterial plasma NE specific activity (dpm/pg)}}
\]

**Total NE clearance**

\[
\text{Total NE clearance} = \frac{[\text{3H}]\text{NE infusion rate (dpm/min)}}{\text{arterial plasma [3H]NE concentration (dpm/ml)}}
\]

where dpm is disintegrations per minute of tritiated NE.

The rate of NE spillover from left and right kidney was calculated according to the Fick principle with adjustment for NE uptake across the organ using the fractional extraction of [3H]NE:

\[
\text{Left or right renal NE spillover} = \left[ (\text{NE}_V - \text{NE}_A) + \text{NE}_A \times \text{NE}_E \right] \times \text{RPF}
\]

where NE_v is plasma NE concentration in the right or left renal vein (picograms per millilitre); NE_a is arterial plasma NE concentration (picograms per
TABLE 1. Total Body and Split Renal Norepinephrine Kinetics Before and After Enalaprilat (1.25 mg i.v.) in Seven Subjects With Renin-Positive, Unilateral Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before enalaprilat</th>
<th>30 minutes after enalaprilat</th>
<th>SED</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial NE (pg/ml)</td>
<td>427±73</td>
<td>470±53</td>
<td>38</td>
<td>NS</td>
</tr>
<tr>
<td>Total NE clearance (ml/min)</td>
<td>1,040±150</td>
<td>930±150</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>Total NE spillover rate (ng/min)</td>
<td>387±56</td>
<td>412±65</td>
<td>58</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Involved kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal venoarterial NE difference (pg/ml)</td>
<td>264±75</td>
<td>396±104</td>
<td>57</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>[^{3}H]NE extraction (%)</td>
<td>56±6</td>
<td>41±5</td>
<td>3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Renal plasma flow (ml/min)</td>
<td>127±47*</td>
<td>123±48†</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>NE clearance (ml/min)</td>
<td>64±18*</td>
<td>49±16†</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>NE spillover rate (ng/min)</td>
<td>49±16*</td>
<td>62±22†</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Contralateral kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal venoarterial difference (pg/ml)</td>
<td>149±38</td>
<td>256±97</td>
<td>72</td>
<td>NS</td>
</tr>
<tr>
<td>[^{3}H]NE extraction (%)</td>
<td>50±3</td>
<td>38±3</td>
<td>5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Renal plasma flow (ml/min)</td>
<td>250±36</td>
<td>318±57</td>
<td>22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NE clearance (ml/min)</td>
<td>110±13</td>
<td>106±18</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>NE spillover rate (ng/min)</td>
<td>81±13</td>
<td>129±23</td>
<td>17</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are mean±SEM; SED, standard error of the difference; NE, norepinephrine; \[^{3}H\]NE, fractional extraction of tritiated NE.
* p<0.01, † p<0.001, comparisons between involved and contralateral kidney, respectively.

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milliliter); NE<sub>E</sub> is fractional extraction of tritiated NE in a single passage through the respective kidney: (A−V)/A; and RPF is right or left renal plasma flow (milliliters per minute).

In addition, renal clearance of NE from plasma was calculated using the following relation:

Left or right renal NE clearance = NE<sub>E</sub> × RPF  (4)

**Plasma Renin Activity**

Patients were considered renin positive when a secretion index<sup>18</sup> ([vene−artery]/artery) was greater than 0.5 only in the affected kidney during resting or stimulated conditions, if at the same time resting arterial PRA was greater than 2.0 ng angiotensin I/ml/hr or rose more than 1.5 times after the acute dose of enalaprilat.

**Study Protocol**

During resting conditions, simultaneous blood samples for subsequent analyses of NE, PRA, angiotensin II, and PAH were obtained from right and left renal veins and left radial artery approximately 1 hour after the \[^{3}H\]NE and PAH infusions were started. Enalaprilat (1.25 mg) then was administered over 4 minutes, and 5 minutes later renal vein and arterial blood were withdrawn for analyses of PRA and angiotensin II. After an additional 25 minutes, another blood sampling was performed (similar to that during resting conditions). Arterial pressure and heart rate were monitored continuously.

**Biochemical Measurements**

All blood samples for catecholamine measurements were collected on ice into chilled test tubes containing reduced glutathione and EDTA and were centrifuged at 4°C for 15 minutes; plasma was separated for storage at −80°C for subsequent assay. Plasma NE concentration was measured by means of cation-exchange high-performance liquid chromatography with electrochemical detection.<sup>19</sup> Sensitivity of the assay was approximately 15 pg/ml, and the coefficient of variation between assays was 10−12%. Some of the kidney NE samples injected onto the chromatographic column were fractionally collected and subsequently counted by scintillation counter. The counts of the NE peak did not, however, differ from counts obtained in an unseparated sample; hence, not all samples were separated with regard to \[^{3}H\] activity. A reference sample of the infused \[^{3}H\]NE was always taken and analyzed with respect to separated \[^{3}H\]NE activity to determine recovery of tritiated NE. PRA and levels of angiotensin II concentrations were measured by means of radioimmunoassay.<sup>20−22</sup> The reference values for PRA are 0.2−2.0 ng angiotensin I/ml/hr.

**Statistical Analysis**

Results are expressed as mean±standard error of the difference (SED). Statistical significance was assessed by Student's t test for paired observations. A value of p<0.05 was considered statistically significant.

**Results**

Table 1 shows that total NE clearance and total NE spillover rate were unaffected by ACE inhibition. Renal NE venoarterial difference was largely unaltered between the kidneys during resting conditions, and the difference increased significantly in the in-
volved kidney after enalaprilat, leaving the difference in the contralateral kidney only slightly elevated (Table 1). Fractional extraction of tritiated NE did not differ between the kidneys before ACE inhibition (Table 1). The capacity of the involved kidney to clear NE from plasma was impaired compared with the contralateral side, being reduced by 42% from 110 ml/min (p<0.01) (Table 1, Figure 1). Moreover, resting renal NE spillover thus became significantly suppressed in the stenotic kidney in comparison with the contralateral kidney, being 49 and 81, SED=6 ng/min (p<0.01) for the former and latter kidney, respectively (Table 1, Figure 1). Besides the pronounced fall in mean arterial pressure (from 125 to 94, SED=8 mm Hg, p<0.01) and arterial angiotensin II levels (from 55 to 10, SED=12 pg/ml, p<0.01) after enalaprilat, arterial PRA increased, whereas PRA venoarterial difference on the involved side rose 169% from 16.1 ng angiotensin I/ml/hr (p<0.05), leaving the contralateral side unchanged (1.0 versus 4.0, SED=2 ng angiotensin I/ml/hr, NS) (Figure 2).

Fractional extraction of tritiated NE across both kidneys fell significantly after ACE inhibition. There was a tendency for a drop in renal NE clearance in the involved kidney, whereas no change was observed in the contralateral kidney in response to ACE inhibition (Table 1, Figure 1). However, the resultant renal NE spillover rate into renal vein plasma remained unchanged in the involved kidney, whereas it increased significantly in the contralateral kidney 30 minutes after ACE inhibition (Table 1, Figure 1).

Discussion

Using the [3H]NE kinetic method (inferring sympathetic nerve activity) in patients with renovascular hypertension, this study demonstrates a possible reduction of sympathetic nerve activity to the angiographically stenotic, renin-positive kidney compared with the contralateral one. In addition, no relation between low and high levels of endogenous angiotensin II and renal NE spillover rate was detected.

In animals and patients with unilateral renal artery stenosis, a number of studies have tried to evaluate "sympathetic activity" merely by analyzing changes in plasma NE, although one study has used direct peroneal sympathetic nerve activity recordings. For different reasons the interpretation of plasma NE results is difficult. The usefulness of plasma NE concentrations alone as an index of sympathetic activity is, however, limited, because plasma NE concentrations are determined by the rates of both entry into plasma and its clearance. During resting conditions, before enalaprilat was given, the involved kidney constituted only approximately 37% of the total renal NE spillover rate. The results further stress the importance of NE spillover rate measurements in individual organs, in this case with emphasis on the kidneys, because there was no change in total body NE spillover rate either before or after ACE inhibition.

The present study showed an increased NE concentration in the renal vein of the involved kidney after a "direct" renin stimulation by ACE inhibition, supporting previous results by Herlitz et al, who "indirectly" stimulated renin and hence angiotensin II production by hydralazine. Thus, it seems unimportant with regard to elevation of renal vein NE concentration which type of renin "stimulator" was used. The question then arises, whether this increased renal vein NE concentration in response to stimulation and even during basal conditions represents a true increase in sympathetic activity to the kidney or not.

It is important to note, however, that factors such as renal plasma flow influence NE spillover rate into plasma. Studies in dogs have shown that only substantial (more than 50%) reductions in renal plasma flow affect renal NE spillover rate, whereas fractional
extracted of radiolabeled NE was unchanged. On the other hand, NE kinetic studies have shown that renal sympathetic nerve stimulation in dogs increased substantially renal NE overflow in spite of a 50% reduction of blood flow. Hence, a discrepancy exists with respect to NE spillover rate depending on how renal blood flow is reduced.

Renal plasma flow in the stenotic kidney in the present patient group was reduced by approximately 50% compared with the contralateral kidney, which is in accordance with previous findings in renal hypertension. This reduction of flow in the affected kidney may contribute to the reduced renal NE spillover rate from this side. It is difficult, however, to compare renal NE kinetics in kidneys possibly adapted to chronic low renal plasma flow with an experimental, acute renal plasma flow reduction.

Spillover rate of NE also might have been influenced by microcirculatory changes in the stenotic kidney, because neuronally released NE into plasma is dependent on factors like diffusional concentration gradients, capillary permeability to NE, and available surface area for exchange (see Reference 11). These factors may not be the same in the unaffected and affected kidney. The reduced NE spillover rate in the involved kidney also may be an adaptive, beneficial response, as has been suggested in the renal hypertensive rat model. Such a mechanism, by reducing efferent sympathetic nerve activity to the stenotic kidney, then would constitute a protection against severe hypertension by means of relatively increased sodium excretion and diminished renin secretion.

It is not entirely clear, however, if the resting NE overflow from the contralateral kidney really represents a true basal value, because it has been shown recently that sodium restriction (as in the present study) enhances renal NE overflow. It thus remains a possibility that the presently used low sodium diet (25 mmol sodium) preferentially could have stimulated sympathetic outflow to the unaffected kidney.

The present study shows, in accordance with Esler et al., that both kidneys remove approximately 16% of overall plasma clearance. Nevertheless, in assessing split renal NE clearance, a marked reduction of NE removal is seen in the stenotic kidney (Table 1), due to reduced renal plasma flow rather than to fractional extraction of [3H]NE, because the latter is largely equal in the involved and the contralateral kidney. Because total renal NE clearance is still within the normal range, the contralateral (normal) kidney probably has taken over some of the function of NE removal, constituting approximately 63% of total renal clearance. After ACE inhibition, the involved kidney further reduces its already diminished NE clearance, so that the contralateral kidney now constitutes approximately 68% of total renal NE removal. After enalaprilat administration, renal plasma flow increased only in the unaffected kidney and remained unchanged in the involved kidney. Fractional extraction of radiolabeled NE fell significantly—25% in both kidneys—hence leaving NE removal in the normal kidney the same as before ACE inhibition due to increased flow.

The reason for the fall in [3H]NE extraction after enalaprilat is puzzling. It mimics the response ob-
which might be due to either altered transmission of organ (postjunctional effect). The interaction between changes in the end organ, such as receptor number or affinity, that angiotensin II may interfere with sympathetic activity, although heart rate did not change (76 versus 73 beats/min, NS). The lack of response in the involved kidney may reflect an inability to respond to reflex sympathetic activation, which might be due to either altered transmission of NE from nerve endings, altered NE reuptake, or other factors.

High frequency electrical stimulation of renal nerves has been shown to decrease fractional extraction of labeled NE by the kidney. Hence, changes in renal nerve firing rate may influence NE extraction across the kidney. In the present study, it is likely that a modest increase in renal sympathetic nerve activity occurred, as evidenced by an elevation of NE overflow to the contralateral kidney after enalaprilat, which then might cause a fall in [3H]NE extraction, paralleling the situation in dogs. However, the stenotic kidney demonstrated a similar fall in [3H]NE extraction as the contralateral kidney but showed no increased renal NE overflow.

The enalaprilat-induced fall in systemic arterial pressure, which, at least to some extent, might be due to the influence of vasodilating renomedullary lipids, caused a statistically significant elevation of NE overflow to the contralateral kidney compared with the involved one, probably because of a small reflex activation of sympathetic activity, although heart rate did not change (76 versus 73 beats/min, NS). The lack of response in the involved kidney may reflect an inability to respond to reflex sympathetic activation, which might be due to either altered transmission of NE from nerve endings, altered NE reuptake, or changes in the end organ, such as receptor number or affinity.

It has been suggested, mainly in experimental animals, that angiotensin II may interfere with sympathetic regulation of cardiovascular function, either by exerting a facilitatory effect on NE release (prejunctional effect) or potentiating the NE effect on the end organ (postjunctional effect). The interaction between the renin-angiotensin system and the sympathetic nervous system also has been explored in human studies with somewhat controversial results. The present study could not identify any evidence for interaction between the two systems, because high angiotensin II concentration was associated with both low (stenotic kidney) and normal (contralateral kidney) NE overflow. On the other hand, low levels of angiotensin II, after enalaprilat, in fact were associated with an augmented NE spillover rate from the normal kidney. These differences probably are related to the fact that other investigators have tried to mimic a situation with either high angiotensin II or NE levels by infusing these substances, whereas the present study represents a situation in which the hormone or peptide is produced endogenously.

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**KEY WORDS**

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- renin
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