Increased Sympathetic Nerve Activity in Renovascular Hypertension

Mats Johansson, MD; Mikael Elam, MD, PhD; Bengt Rundqvist, MD, PhD; Graeme Eisenhofer, PhD; Hans Herlitz, MD, PhD; Gavin Lambert, PhD; Peter Friberg, MD, PhD

Background—Increased sympathetic nerve activity may contribute to the progression of renovascular hypertension. Because previous results have been inconclusive, we investigated whether renovascular hypertensives show increased total and regional sympathetic nerve activity.

Methods and Results—Sixty-five patients underwent renal angiography and measurements of plasma renin activity and angiotensin II in conjunction with estimation of sympathetic nerve activity by means of radiotracer dilution and intraneural recordings of muscle sympathetic nerve activity (MSNA). Age-matched healthy subjects (n = 15) were examined for comparison. Total body norepinephrine (NE) spillover, an index of overall sympathetic nerve activity, was increased by 100% and MSNA by 60% in the hypertensive patients compared with healthy subjects (P < 0.01 for both). A subgroup of 24 patients with well-defined renovascular hypertension (cured or improved hypertension after renal angioplasty) showed similar increases in total body NE spillover compared with the group at large. Patients with arterial plasma renin activity and angiotensin II levels above median had higher values for total body NE spillover than patients below median (P < 0.01).

Conclusions—This study unequivocally demonstrates elevated sympathetic nerve activity in patients with renovascular hypertension. The adrenergic overactivity may contribute to the blood pressure elevation and perhaps also to the high cardiovascular mortality in renovascular hypertension.

Key Words: hypertension ■ nervous system, sympathetic ■ renin

Although there is general agreement on the importance of the renin-angiotensin system for increased blood pressure in the early phase of renovascular hypertension,1 other mechanisms such as sodium retention and activation of the sympathetic nervous system may contribute to progression of the disorder.2-3 Previous results regarding involvement of the sympathetic nervous system in human renovascular hypertension have been contradictory.4,5 Whereas Miyajima et al4 previously reported increased muscle sympathetic nerve activity (MSNA) in renovascular hypertension, Grassi et al5 recently reported similar values for MSNA in secondary hypertensives compared with healthy controls. The latter study comprised a mixture of patients with hypertension secondary to renovascular disease or pheochromocytoma. Apart from these studies, there is little information about sympathetic nerve function in renovascular hypertension, particularly norepinephrine (NE) spillover or the combination of the technique with microneurography.

To investigate whether sympathetic nervous activity is elevated in renovascular hypertension, we studied a large population of patients with renovascular hypertension and age-matched healthy control subjects. Importantly, the study included a subgroup of 24 well-defined patients with renovascular hypertension as established from cure or improvement of hypertension after renal angioplasty. Sympathetic nerve activity was assessed by isotope dilution to determine total body NE spillover. To also assess efferent sympathetic nerve traffic, we obtained simultaneous recordings of MSNA and measurements of NE spillover in a subset of patients.

Methods

Subjects
The local ethical and isotope committees at Sahlgrenska University Hospital approved all studies, and all subjects gave their consent to participate in the study.

Hypertensive Patients (n = 65)
The study comprised patients with hypertension who were undergoing a clinical investigation for renovascular hypertension involving renal vein blood sampling for assessment of plasma renin activity (PRA) (Table 1). All patients had hypertension and renal artery stenosis ≥50% according to angiography. Renal angioplasty was performed in 40 patients. A patient was considered cured of hypertension if revascularization was followed by normotension (mean diastolic blood pressure ≤ 90 mm Hg) without additional antihypertensive therapy. A patient was considered improved if...
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TABLE 1. The Study Population

<table>
<thead>
<tr>
<th>Hypertensive Patients (n=65)</th>
<th>Healthy Subjects (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57±2</td>
</tr>
<tr>
<td>Sex, females/males</td>
<td>33/32</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26±1</td>
</tr>
</tbody>
</table>

Values represent numbers in each group or mean±SEM.

Infusions
Para-aminomhippurate (PAH, Merck Sharp & Dohme; dosing dependent on estimated glomerular filtration rate [GFR]) and tracer doses of 1,-2,5,6-[3 H]NE (40 to 60 Ci/mmol; New England Nuclear) were infused into a peripheral vein. An infusion rate of 1.0 to 1.5 μCi of [3 H]NE per minute was used.

TABLE 2. Clinical Data on the Hypertensive Group (n=65)

| Duration of hypertension, y (mean±SEM) | 11±1 |
| Smoker, %                               | 49   |
| Diabetes mellitus, %                    | 11   |
| Congestive heart failure, %             | 5    |
| Ischemic heart disease, %               | 31   |
| Bilateral renal artery stenosis, %      | 22   |
| Renal artery stenosis >50%, %           | 100  |
| Renal artery stenosis >70%, %           | 35   |
| Occlusion of renal artery, %            | 9    |
| Cr-EDTA clearance, mL · min⁻¹ · 1.73 m⁻², mean±SEM | 64±5 |

Infusions were begun. Samples were collected into ice-chilled tubes containing heparin or EDTA and glutathione. Plasma was separated by centrifugation and stored at −80°C until assayed for catecholamines, PRA, and angiotensin (Ang) II. Renal plasma flow was derived from total infusion clearance of PAH corrected for renal fractional extraction.

In 10 hypertensive patients, MSNA recordings and measurements of total body NE spillover were performed simultaneously, whereas MSNA examinations in healthy subjects were performed on another occasion. The intraindividual reproducibility of repeated recordings of resting MSNA is well established.

Assays
Catecholamines were extracted from plasma (1 mL) and samples of infusate (10 μL) by alumina adsorption and were separated by high-performance liquid chromatography.10 Timed collection of [3 H] eluate leaving the electrochemical cell permitted separation of [3 H]-labeled NE for subsequent counting by liquid scintillation spectroscopy. Interassay coefficients of variation were 4.6% for endogenous NE and 3.2% for [3 H]NE

PRA was measured according to the method of Giese et al11 with radioimmunoassay used for Ang I. Reference values are 0.2 to 2.0 ng Ang I · mL⁻¹ · h⁻¹. Ang II was assayed according to the methods of Kappelgaard et al12 and Morton and Webb.13

Calculations
Total body NE spillover (S TB) was measured by the radiotracer method14 and calculated according to the formula

\[ S_{TB} = I/S_{A} \]

where I is the infusion rate of tritium-labeled NE (disintegrations per minute [dpm/min]) and S A is the specific activity of NE in arterial plasma (dpm/pmol), calculated as

**Sympathetic Nerve Recording**
Multunit postganglionic sympathetic nerve activity was recorded with a tungsten microelectrode with a tip diameter of a few microns inserted into a muscle-innervating fascicle of the peroneal nerve at the fibular head. A reference electrode was inserted subcutaneously 1 to 2 cm from the recording electrode. Details regarding the recording technique and the criteria for MSNA have been provided previously.6–8 The number of MSNA bursts, which occur in bursts strictly coupled to the cardiac rhythm, was counted by inspection of the mean voltage neurogram.9 Two independent laboratory colleagues who were not part of the study and had no knowledge of the study protocol performed the analysis. Nerve activity was expressed as the average burst frequency (bursts/min).

**Experimental Protocol**
Baseline blood samples were taken simultaneously from a radial artery and 1 or both renal veins at steady state ≥30 minutes after the infusions were begun. Samples were collected into ice-chilled tubes containing heparin or EDTA and glutathione. Plasma was separated by centrifugation and stored at −80°C until assayed for catecholamines, PRA, and angiotensin (Ang) II. Renal plasma flow was derived from total infusion clearance of PAH corrected for renal fractional extraction.

Healthy Subjects (n=15)
The subjects in the control group were of similar age and body mass index as the hypertensive patients (Table 1). None had a history of neurological or cardiovascular disease. A comprehensive clinical evaluation in conjunction with hematology, routine serum biochemistry, and ECG testing were all within normal ranges.

Catheterization
Patients were studied in the morning in a catheterization laboratory. All subjects refrained from smoking and from drinking coffee for the 12 hours preceding the study. Patients undergoing a clinical investigation for renal artery stenosis were hospitalized 4 days before the investigation, and while they were inpatients, they were kept on a low-salt diet (40 mmol/24 hours). Diuretics and calcium channel blockers were withdrawn 2 days before the investigation.

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TABLE 4. Hemodynamics, NE, PRA and Ang II Plasma Concentrations, NE Kinetics, and MSNA in Hypertensive Patients and Age-Matched Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive Patients (n=65)</th>
<th>Healthy Subjects (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>123±2*</td>
<td>98±3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>73±2*</td>
<td>62±2</td>
</tr>
<tr>
<td>Renal plasma flow, mL/min</td>
<td>399±26*</td>
<td>697±47</td>
</tr>
<tr>
<td>Arterial NE, pmol/mL</td>
<td>4.35±0.29*</td>
<td>1.71±0.16</td>
</tr>
<tr>
<td>Total body NE clearance, mL/min</td>
<td>1841±115</td>
<td>2047±124</td>
</tr>
<tr>
<td>Arterial PRA ng Ang I · mL⁻¹ · h⁻¹</td>
<td>14.0±3.0*</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Arterial Ang II, pg/mL</td>
<td>36±5*</td>
<td>11±1</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>54±3*</td>
<td>34±4</td>
</tr>
</tbody>
</table>

Data are mean±SEM. *P<0.01 vs healthy subjects.

\[ S_{A_{NE}} = \frac{[\text{H}]NE_A}{NE_A} \]

where \([\text{H}]NE_A\) is the arterial plasma concentration of tritium-labeled NE (pmol/mL) and \(NE_A\) is the arterial plasma concentration of endogenous NE (pmol/mL).

Total body NE clearance (CL\(_{TB}\)) was calculated as

\[ CL_{TB} = I/[\text{H}]NE_A \]

Statistical Methods

Results are expressed as mean±SEM.

Student’s \(t\) tests for unpaired observations were used. Parameters not normally distributed were logarithmically transformed before the parametric test. If a nonnormal distribution was retained, the Mann-Whitney \(U\) test for unpaired comparisons was used. The relation between 2 variables was assessed by calculating the rank correlation coefficient according to Spearman. Statistical significance was defined as \(P<0.05\).

Results

Mean arterial pressure was elevated by 26% in hypertensive patients, whereas renal plasma flow was reduced by 43% compared with healthy subjects (\(P<0.01\) for all; Table 4). Arterial plasma concentrations of NE were elevated by 154% in renovascular hypertensives, whereas arterial PRA and Ang II were 23- and 3-fold increased, respectively, compared with controls (\(P<0.01\) for all; Table 4). Total body NE spillover and MSNA were elevated by 100% and 60%, respectively, in renovascular hypertensives compared with healthy subjects (\(P<0.01\); Figure 1), whereas total body NE clearance did not differ between the groups (Table 4).

Twenty-four patients whose hypertension was cured or improved after renal angioplasty also showed elevated total body NE spillover compared with healthy controls (6854±957 vs 3456±350 pmol/min, respectively; \(P<0.05\)). Values for this subgroup of 24 patients did not differ from the group of renovascular hypertensives as a whole.

Patients taking ACE inhibitor or \(\beta\)-blocker treatment for hypertension whose therapy was withdrawn 3 to 4 days before the investigation showed similar values for total body NE spillover compared with other hypertensives. Patients taking calcium channel blockers did not differ in total body NE spillover compared with the other hypertensives.

Figure 1. Mean±SEM for total body NE spillover (top) and MSNA (bottom; MSA) in hypertensives and healthy subjects. **Statistically significant difference vs healthy subjects (\(P<0.01\)).

(7032±579 pmol/min for patients taking calcium channel blockers versus 7324±1241 pmol/min for other hypertensives). Positive relationships were found between total body NE spillover and both PRA and Ang II (\(r=0.36\) and \(r=0.30\), respectively; \(P<0.05\)). Patients with arterial plasma concentrations of PRA and Ang II above median had higher total body NE spillover than patients below median (\(P<0.01\); Figure 2). In patients with arterial PRA above median, MSNA was higher than in patients below median (60±5 versus 49±2 bursts/min; \(P=0.05\)). In hypertensive patients in whom simultaneous measurements of total body NE spillover and MSNA were performed, a close relationship was found between these variables (Figure 3). Patients with GFR below median had similar values for total body NE spillover as patients above median (6806±661 versus 7185 pmol/min for patients above median), and there was no correlation between GFR and total body NE spillover.

Discussion

The present study firmly established increased sympathetic nerve activity in renovascular hypertension by using 2 independent techniques for examination of sympathetic outflow. Overall sympathetic outflow, as assessed from measurements of total body NE spillover, was increased 2-fold compared with healthy controls and correlated closely with MSNA, supporting the contention of an elevated state of efferent sympathetic traffic in renovascular hypertension. Increased total body NE spillover in renovascular hypertension therefore primarily reflects increased sympathetic nerve firing and...
transmitter release rather than simply a presynaptic modulatory action on NE release (eg, by Ang II) or altered disposition of NE after its release.

Experimental renovascular hypertension is divided into 3 stages.2 Removal of the affected kidney will cure or improve hypertension only in the first and second phases, whereas hypertension will persist in the third phase, which indicates that changes in the contralateral kidney are of importance for maintenance of blood pressure elevation. In the first phase, vasoconstrictor effects of Ang II are the main cause of hypertension, whereas other mechanisms such as sodium retention or increased sympathetic activity may be involved together with Ang II in the second and third phases of experimental renovascular hypertension. Although human renovascular hypertension will not have the clear phase distinction of experimental renovascular hypertension, there are data supporting other mechanisms operating in conjunction with Ang II vasoconstriction to maintain the blood pressure increase.2,4

The present study adds important new information to existing knowledge about sympathetic nerve activity in human renovascular hypertension because previous data in these patients have been inconclusive.4,5 Grassi et al5 reported normal MSNA in secondary hypertension, whereas Miyajima et al4 found increased MSNA in renovascular hypertensives compared with healthy controls and primary hypertensives. The small size of the study population, its heterogenous nature, and the fact that patients with an adrenal pheochromocytoma and renovascular hypertension were lumped together may have confounded the results of the former study.

Patients in the present study had longstanding hypertension, whereas increased MSNA and total body NE spillover have been observed primarily in borderline and younger primary hypertensives.15,16 Although one has to be cautious when comparing the present results with previously reported data in primary hypertensives, the level of sympathetic activation appears higher in renovascular hypertension. Esler et al15 reported a 38% increase in total body NE spillover compared with healthy control subjects (largely due to higher NE release in hypertensive patients aged <40 years), whereas a 100% increase in total body NE spillover compared with healthy subjects was found in the present study. Elevated MSNA has been shown in patients with accelerated primary hypertension with an activated renin-angiotensin system and retinopathy.17 The latter results corroborate with the findings of the present study. Data indicating interactions between the renin-angiotensin and the sympathetic nervous systems18,19 suggest that activation of the former system may be a factor contributing to elevated sympathetic nerve activity. After angioplasty of unilateral renal artery stenosis in humans, MSNA decreased concomitantly with a fall in plasma concentrations of Ang II.4 The present group of renovascular hypertensives had high circulating levels of PRA and Ang II. Although only weak relationships were found between total body NE spillover and indexes of the renin-angiotensin system, patients with PRA and Ang II above the median had increased adrenergic drive compared with patients with values below the median. Although a causal relationship was not established, our data lend some support for a facilitatory role of the renin-angiotensin system in adrenergic activity in human renovascular hypertension. Ang II may exert a facilitatory effect on NE release by stimulation of prejunctional Ang II receptors on peripheral sympathetic nerves20 or by an action on the central nervous system.19 Although the close relationship between total body NE spillover and MSNA suggests a central mechanism, a facilitatory effect on NE release in the periphery by Ang II cannot be ruled out. Clearly, the contribution of increased activity of the renin-angiotensin system cannot explain a major part of the variability in total body NE spillover. Other mechanisms behind this adrenergic overactivity in hypertensive patients must be considered.

Recent evidence suggests a role for the afferent renal nerves in the development of increased sympathetic activity

Figure 2. Mean±SEM for total body NE spillover in hypertensive patients with arterial PRA (top) or Ang II (bottom) above and below median. **Statistically significant difference vs patients below median (P<0.01).

Figure 3. Relation between simultaneously obtained MSNA (MSA) and total body NE spillover (n=10). Correlation coefficient was calculated according to Spearman.
in conditions associated with injured or ischemic kidneys. Patients with chronic renal failure and native kidneys showed increased MSNA compared with patients who underwent bilateral nephrectomy. Thus, increased central sympathetic outflow was apparently mediated by an afferent signal arising in the kidneys. In addition, Miyajima et al found that the increased MSNA in renovascular hypertensives was normalized 4 to 10 days after successful percutaneous angioplasty of a renal artery stenosis. Restoration of blood flow to the ischemic kidneys in these patients may have eliminated the afferent stimulus that provoked the increased adrenergic drive, although lowered Ang II concentrations may also have played a role.

In the present study, renal involvement was evident in terms of reduced GFR and reduced renal plasma flow in the hypertensive group. Hence, our patient group showed evidence of early renal insufficiency, which may be an initiator for the prevailing increase in sympathetic nerve activity. Even though no relationship was found between GFR and the indexes of sympathetic nerve activity, ischemic metabolites in affected kidneys may have conveyed an afferent stimulus to the central nervous system, thereby provoking increased adrenergic drive, although overall GFR was only slightly reduced.

Study Limitations
All patients were put on a low-salt diet to stimulate the renin-angiotensin system and increase the sensitivity for diagnosis of a functionally important renal artery stenosis. This procedure, however, was not followed in the healthy control group. It is important to stress that a rigorous low-salt diet has been shown to increase renal NE spillover without affecting total body NE spillover. That study was performed as a crossover design, so when the healthy subjects were given a normal sodium diet, renal NE spillover returned to normal. An important issue in the context of the present results is that in the former study, total body NE spillover did not change during sodium restriction, whereas in the present study, total body NE spillover was considerably elevated in renovascular hypertension. Furthermore, a recent study investigating the neurohormonal response to salt restriction in patients with primary hypertension showed no change in plasma concentration of NE during salt restriction. A second limitation to interpretation of the elevated adrenergic drive in hypertensives involves the possibility for a “drug or drug withdrawal” effect on sympathetic nerve activity. About half of the study population was undergoing long-term treatment with a calcium channel blocker, or ACE inhibitor. Hence, an important drug or drug withdrawal effect on overall sympathetic nerve activity seems unlikely.

In conclusion, sympathetic nerve activity is markedly elevated in patients with renovascular hypertension. This may contribute to the blood pressure elevation and perhaps also to the high cardiovascular mortality that have been reported in renovascular hypertension. Clonidine has been shown to markedly reduce blood pressure in renovascular hypertension, which suggests a role for antihypertensive therapy that diminishes central sympathetic outflow in patients who are not candidates for renal angioplasty.

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References


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