Phenotypic Evidence of Faulty Neuronal Norepinephrine Reuptake in Essential Hypertension

Magdalena S. Rumantir, David M. Kaye, Garry L. Jennings, Mario Vaz, Jacqueline A. Hastings, Murray D. Esler

Abstract—Previous reports suggest that neuronal norepinephrine (NE) reuptake may be impaired in essential hypertension, perhaps because of dysfunction of the NE transporter, although the evidence is inconclusive. To further test this proposition, we applied phenotypically relevant radiotracer methodology, infusion of tritiated NE and quantification of NE metabolites, to 34 healthy lean subjects (body mass index <27.0 kg/m²), 19 overweight (body mass index >28.0 kg/m²) but otherwise healthy normotensive subjects, 13 untreated lean patients with essential hypertension, and 14 obesity-related hypertensive subjects. Spillover of NE from the heart was increased in lean hypertensives only (mean±SD 33.4±20.6 versus 16.1±11.7 ng/min in lean normotensives, P<0.05), but this could have resulted from high cardiac sympathetic nerve firing rates, faulty NE reuptake, or both. The arterial plasma concentration of 3-methoxy-4-hydroxyphenyllglycol, an extraneuronal metabolite of NE, was elevated in lean hypertensives only (3942±1068 versus 3055±888 pg/mL in healthy subjects, P<0.05). The fractional extraction of plasma tritiated NE in passage through the heart, determined on the basis of neuronal NE uptake, was reduced in lean essential hypertensives (0.65±0.19 versus 0.81±0.11 in healthy subjects, P<0.05). Cardiac release of the tritiated NE metabolite [3H]dihydroxylphenyllglycol, produced intraneuronally by monoamine oxidase after uptake of [3H]NE by the transporter, was reduced in lean hypertensives only (992±1435 versus 4588±3189 dpm/min in healthy subjects, P<0.01). These findings suggest that neuronal reuptake of NE is impaired in essential hypertension. Through amplification of the neural signal, such a defect could constitute a neurogenic variant of essential hypertension. In obesity-related hypertension, there was no phenotypic evidence of NE transporter dysfunction. (Hypertension. 2000;36:824-829.)

Key Words: nervous system, sympathetic ▪ norepinephrine ▪ obesity ▪ heart ▪ hypertension, obesity

The exact pathophysiology of the sympathetic nervous system remains to be delineated. Available evidence indicates that in a substantial proportion of patients, essential hypertension is neurogenic, with documentation of high rates of spillover of norepinephrine (NE) from the heart and kidneys.1 The increased cardiac and renal spillover of NE is of great interest, although this cannot be measured directly; clinical microneurographic measurements in hypertensive patients have documented activation of sympathetic efferents in another sympathetic outflow, that to the skeletal muscle vasculature.2

There are additional potential neural mechanisms, however, that could contribute to high intrasynaptic concentrations of NE and increased NE spillover in essential hypertension and to the development and maintenance of neurogenic variants of hypertension, but these have not been definitively investigated. That there might be an increase in the density of sympathetic innervation in human hypertension, as is well documented in the Japanese spontaneously hypertensive rat,3 remains one such possibility. Facilitation of neuronal NE release by epinephrine released from sympathetic nerves as a cotransmitter4 and impairment of neuronal NE reuptake after its release from sympathetic nerves are others.

Previous reports from 3 laboratories5–7 suggest that neuronal NE reuptake may be impaired in some patients with essential hypertension, perhaps due to dysfunction of the NE transporter, although the evidence is inconclusive. In these earlier studies, the half-time of the rapid disappearance phase of tritiated NE removal from plasma on termination of an intravenous infusion of the tracer, which is primarily, but not exclusively, dependent on neuronal NE uptake was found to be prolonged in some patients with essential hypertension.5–7 In the present study, we applied more specific radiotracer methods and focused in particular on neuronal processing of tritiated NE by the heart.

We thought that because the disposition of NE after its release is more dependent on neuronal reuptake in the heart than in all other organs,8 incomplete grades of impairment of...
NE transporter function would be most likely to be phenotypically evident there. We were encouraged in this line of thinking by the recent description of a missense mutation of the NE transporter gene in a family kindred with the postural tachycardia syndrome\(^9\) in whom the exaggerated reflex increase in heart rate with standing, which is a cardinal feature of the disorder, was due to the NE transporter fault. We investigated 4 categories of experimental subjects: lean and obese patients with essential hypertension and comparable groups with normal blood pressure. The inclusion of obese people both with and without hypertension was in recognition of the fact that although not all overweight people develop hypertension, the predisposing influences to blood pressure elevation in the obese are unknown. Dysfunction of the NE transporter might be such a predisposing factor, given that obesity-related hypertension does have an important neurogenic element.\(^2,10\)

**Methods**

Neurochemical indices of the phenotype of impaired neuronal reuptake of NE were studied. The indices chosen for investigation were those previously shown to be sensitive markers of reduced neuronal NE reuptake in situations of reuptake impairment, specifically after pharmacological uptake blockade with the tricyclic antidepressant desipramine,\(^5,7,8\) and in patients with transporter absence accompanying sympathetic nerve degeneration (pure autonomic failure).\(^11\) We measured the spillover rate of NE to plasma for the whole body and from the heart (Figure 1); the arterial plasma concentration of the extraneuronal metabolite of NE, 3-methoxy-4-hydroxyphenylglycol (MHPG) (Figure 1); the removal rate of NE from plasma (for the whole body, plasma NE clearance, 0.6 to 0.8\(^\text{mL/min}\\text{m}\)) for the investigation, which was approved by the Ethics Review Committee of the Alfred Hospital.

**General Procedure**

Subjects were studied in the morning while supine with abstinence from smoking, food, tea, and coffee for 12 hours before the experiment. All received a tracer infusion of \(^3\)H-labeled NE (specific activity 11 to 25 Ci/mmol, New England Nuclear) intravenously at 0.6 to 0.8 \(\mu\)Ci/min for the measurement of NE kinetics by isotope dilution.\(^8,11,12\) Whole body spillover of NE to plasma was calculated in all subjects (n=80) from arterial samples obtained via a 21-gauge cannula placed percutaneously under local anesthesia in the brachial or radial artery. Regional plasma NE kinetics was also measured for the heart (n=57), with coronary sinus venous sampling performed as previously.\(^8,11,12\)

**Biochemical Analysis**

The plasma concentration of neurochemicals was determined with HPLC with electrochemical detection.\(^11,13\) Timed collection of the
eluate leaving the detection cell with a fraction collector permitted the separation of $^{3}$H-labeled NE and $[^3]$H)DHPG for counting with liquid-scintillation spectroscopy.

### Calculation of Whole Body and Cardiac Plasma NE Kinetics and Cardiac $[^3]$H)DHPG Spillover

Whole body rates of NE plasma spillover and clearance were calculated as follows:

$$\text{NE plasma clearance} = \frac{[^3]H}\text{NE infusion rate (dpm/min)}}{[^3]H}\text{NE concentration (dpm/mL)}$$

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

NE spillover from the heart was calculated with the following equation:

$$\text{Cardiac NE spillover} = \frac{(\text{NE}_a - \text{NE}_c) + (\text{NE}_a - \text{NE}_c) - \text{CSPF}}{\text{NE}_a}$$

where $\text{NE}_a$ and $\text{NE}_c$ are the arterial and coronary sinus venous plasma concentrations of NE, respectively; $\text{NE}_a$ is the fractional extraction of tracer NE across the heart; and CSDF is the coronary sinus plasma flow.

The transcardiac fractional extraction of plasma tritiated NE was calculated as follows:

$$\text{Fractional transcardiac }[^3]H\text{NE extraction} = \frac{[^3]H}\text{NE available} - \frac{[^3]H}\text{NE in heart}}{[^3]H}\text{NE available}}$$

The cardiac spillover of $[^3]H)DHPG$ was calculated with the Fick principle from the venoarterial plasma $[^3]H)DHPG$ concentration difference across the heart and the CSDF, as follows:


### Reetest Reliability of the Measures

Intra-assay coefficients of variation were 4.6% for plasma NE at a concentration of 200 pg/mL, 5.0% for MHPG at a concentration of 1000 pg/mL, 3.9% for DHPG at a concentration of 1000 pg/mL, 5.3% for $^3$H-labeled NE at a concentration of 600 dpm/mL, and 8.2% for $[^3]$H)DHPG at a concentration of 40 to 100 dpm/mL. With replicate determinations of whole body and regional NE spillover, there is a high level of reproducibility of the measurements. Within-day coefficients of variation for total and cardiac NE spillover under resting conditions are 5.9% and 6.2%.

### Statistical Analysis

Results are presented as mean±SD. Statistical comparisons of groups were assessed with ANOVA or the Mann-Whitney U test for data that were not normally distributed. The null hypothesis was rejected at $P<0.05$.

### Results

The general characteristics of the experimental groups are shown in Table 1. The majority of participants were men. All lean hypertensive patients had a BMI of <27 kg/m², but overall BMI was higher than that in lean subjects with normal blood pressure, with mean values of 25.1 and 23.1 kg/m², respectively ($P<0.05$). Heart rates and intra-arterial blood pressures directly recorded at rest in the cardiac catheterization laboratory on the test day are included in Table 1. Heart rates in both lean and obese hypertensive groups were elevated compared with those of normotensive subjects (Table 1).

The mean plasma concentration of NE was 45% higher in lean hypertensive subjects than in lean healthy volunteers ($P<0.05$) (Table 2), and the total NE spillover rate was 20% higher (difference not statistically significant) (Figure 3). Obese normotensive and hypertensive subjects did not differ from healthy subjects in these measurements of whole body NE kinetics. The concentration in arterial plasma of the extraneuronal NE metabolite MHPG was elevated in lean hypertensives only (3942 ± 1068 pg/mL) compared with that in healthy subjects (3056 ± 888 pg/mL) (Table 2). The ratio of the plasma concentration of NE to that of its extraneuronal metabolite DHPG, which provides a semiquantitative index of whole body NE neuronal reuptake, was higher in lean hypertensives than in healthy controls (0.30 versus 0.20, $P<0.01$, Figure 3).

NE spillover from the heart was increased in lean hypertensive patients (33.4 ± 20.6 ng/min) compared with that in healthy lean subjects (16.1 ± 11.7 ng/min, $P<0.05$, Figure 3). Cardiac NE spillover was significantly higher in lean than obese hypertensives ($P<0.05$, Figure 3). Cardiac NE spillover in the normotensive obese subjects was suppressed compared with that in the lean normotensives ($P<0.05$, Figure 3).

Extraction of tritiated NE from plasma during transcardiac passage was reduced in lean patients with hypertension (65% versus 81%; $P<0.05$), as was release from the heart of its metabolite, $[^3]$H)DHPG, formed intraneuronally via monoamine oxidase (Figure 4). Total NE plasma clearance for the whole body was 18% lower in lean hypertensives than in

### TABLE 1. Characteristics of Subject Groups

<table>
<thead>
<tr>
<th>Participant</th>
<th>Age, y</th>
<th>Gender, M/F</th>
<th>BMI, kg/m²</th>
<th>Systolic BP (mm Hg)</th>
<th>Diastolic BP (mm Hg)</th>
<th>Heart Rate, bpm</th>
<th>Infusion Time, min</th>
<th>Infusion Rate, dpm/min</th>
<th>Tritiated NE (dpm/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal BP</td>
<td>38.1±17.3</td>
<td>32/2</td>
<td>23.1±2.2</td>
<td>139±16</td>
<td>71±7</td>
<td>63±12</td>
<td>85±23</td>
<td>1.44±0.44</td>
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</tr>
<tr>
<td>High BP</td>
<td>42.9±12.2</td>
<td>10/3</td>
<td>25.1±2.7*</td>
<td>165±18*</td>
<td>86±14*</td>
<td>79±15*</td>
<td>71±19</td>
<td>1.32±0.64</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normal BP</td>
<td>43.6±11.0</td>
<td>19/0</td>
<td>30.2±1.8*</td>
<td>136±14</td>
<td>73±7</td>
<td>61±9</td>
<td>78±31</td>
<td>1.19±0.42*</td>
<td></td>
</tr>
<tr>
<td>High BP</td>
<td>47.6±11.3</td>
<td>12/2</td>
<td>30.5±1.8*</td>
<td>167±10*</td>
<td>91±7*</td>
<td>72±8*</td>
<td>77±27</td>
<td>1.11±0.37*</td>
<td></td>
</tr>
</tbody>
</table>

BP indicates blood pressure. Values are expressed as mean±SD. *$P<0.05$ vs lean normotensive individuals.
healthy subjects \((P=0.059)\) (Figure 4). The arterial plasma concentration of \([3\text{H}]\text{DHPG}\) was 28% lower in lean hypertensives than in healthy lean volunteers \((P=0.09, \text{Figure 4})\) and lower still in both obese experimental groups; in the latter cases, this may be attributable to the somewhat lower tritiated NE infusion rates (Table 1). The ratio of the plasma concentrations of tritiated DHPG to tritiated NE was substantially lower in lean hypertensives than in lean healthy subjects \((\text{mean} \ 0.041 \text{ versus } 0.064, P<0.05)\).

### Discussion

Typical neurochemical consequences of neuronal NE reuptake impairment are facilitation of NE spillover from sympathetic nerves to plasma, reduced clearance of NE from plasma for the body as a whole and by individual organs, and alteration in the pattern of NE metabolites due to reduction in neuronal metabolism and augmentation of extraneuronal metabolism.\(^5\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^11\)\(^,\)\(^12\)\(^,\)\(^15\) Neurochemical indices of this type were applied to test for the phenotype of impaired neuronal reuptake of NE in patients with essential hypertension. By amplifying the neural signal, leading to greater concentrations of NE in the synaptic cleft, such a defect in neuronal NE reuptake could constitute a neurogenic variant of essential hypertension.

Previous reports that involved measurement of the whole body plasma kinetics of tritiated NE suggest that neuronal NE reuptake may be impaired in essential hypertension.\(^5\)\(^,\)\(^7\) although this evidence was inconclusive. In the present study, we applied recently developed, more specific radiotracer methods, including a measure of neuronal processing of tritiated NE by the heart. Because the disposition of NE after its release is more dependent on neuronal reuptake in the heart than in all other organs,\(^8\)\(^,\)\(^11\) less-than-complete grades of NE transporter dysfunction would be most likely to be phenotypically evident there. In lean patients with essential hypertension, the spillover of NE from the heart was increased, cardiac extraction of radiolabeled NE from plasma was reduced, and the intraneuronal conversion of the tracer to tritiated DHPG within the heart was low, such as to strongly suggest that the intracardiac neuronal uptake of NE was subnormal. These changes noted in the heart were, however, quantitatively less than expected on the basis of total absence of neuronal NE reuptake.\(^8\)\(^,\)\(^11\)

For the whole body, the changes seen were proportionally less than those in the heart. The ratio of the arterial plasma concentration of the intraneuronal metabolite of NE, DHPG, to that of NE and the ratio of the plasma concentrations of tritiated DHPG and tritiated NE, both of which are inversely

![Figure 3. NE spillover rates for the heart and the whole body in lean and obese subjects with either normal blood pressure (NBP) or high blood pressure (HBP) (top). Spillover of NE from the heart was increased in lean essential hypertensives. The concentration of the extraneuronal NE metabolite MHPG in arterial plasma and the ratio of plasma NE to DHPG concentrations, an index of neuronal NE reuptake,\(^8\)\(^,\)\(^15\) is shown in the same groups (bottom). *\(P<0.05\), **\(P<0.01\) vs NBP.](http://hyper.ahajournals.org/)

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### TABLE 2. NE Plasma Kinetics and NE Metabolites

<table>
<thead>
<tr>
<th>Participant</th>
<th>NE Plasma Kinetics</th>
<th>NE Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NA Plasma Concentration, pg/mL</td>
<td>NA Total Spillover, ng/min</td>
</tr>
<tr>
<td>Lean Normal BP</td>
<td>202±97</td>
<td>547±241</td>
</tr>
<tr>
<td>Lean High BP</td>
<td>293±102*</td>
<td>657±305</td>
</tr>
<tr>
<td>Obese Normal BP</td>
<td>200±89</td>
<td>493±213</td>
</tr>
<tr>
<td>Obese High BP</td>
<td>225±88</td>
<td>587±276</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

*\(P<0.05\) vs lean normotensive individuals.
related to whole body NE neuronal reuptake, were lower in lean hypertensives than in healthy subjects. In contrast, the values for whole body NE spillover (20% increased) and total plasma NE clearance (18% reduced), although qualitatively altered in the directions expected on the basis of impairment of neuronal NE uptake, did not differ significantly from those found in healthy subjects. This could have resulted from a partial impairment of neuronal NE reuptake, suggested by a comparison with total NE plasma clearance values reported when reuptake is totally absent (plasma clearance 25% to 40% reduced), but it probably also reflects the lesser importance of NE uptake to overall NE disposition in organs such as the kidneys and skeletal muscle (compared with the heart), which make an important contribution to whole body plasma NE kinetics.

Several potential confounders and limitations must be considered when evaluating these changes in NE spillover, disposition, and metabolism in lean hypertensive patients. The first is the possible relevance of sex, in that most participating subjects were male, and although it is not our experience, an influence of sex on sympathetic function and catecholamine metabolism has been described. Whether our findings apply equally to female hypertensive patients remains uncertain. A second consideration is that there are misgivings by some that sympathetic nervous activation in hypertension might simply represent an alerting response in the laboratory, perhaps contributed to by anxiety resulting from the recent diagnostic labeling of a patient as “hypertensive.” This possibility can be definitively excluded, however, because the pattern of sympathetic activation in lean hypertensive patients differs materially from that seen in mental stress responses. The sympathetic nervous activation present in essential hypertension involves the sympathetic outflow to skeletal muscle blood vessels, spares the sympathetic innervation of the skin and hepatomesenteric circulation, and is not accompanied by increased adrenal medullary secretion of epinephrine. None of these apply in mental stress responses, in which epinephrine secretion is increased and the sympathetic outflow to skeletal muscle vasculature typically is unchanged or reduced while skin and hepatomesenteric sympathetic activation is present.

A third potential confounding influence concerns the possibility that undetected dietary sodium restriction voluntarily adopted by hypertensive participants might have caused the observed reduction in NE reuptake and increase in cardiac NE spillover. This is unlikely, because available evidence indicates that in humans, sodium depletion increases renal, but not cardiac, NE spillover and actually increases neuronal NE uptake.

Near-total absence of activity of the neuronal NE transporter was recently reported in a family kindred with the postural tachycardia syndrome, in which a missense mutation of the NE transporter gene has been identified. The cardinal diagnostic features of this condition are exaggerated reflex increases in heart rate and plasma NE concentration with standing, attributable in the kindred reported although not in the majority of patients with the condition to the NE transporter fault. Given that orthostatic intolerance, sometimes associated with postural hypotension, rather than blood pressure elevation is typical of the disorder, it is pertinent to ask whether the idea that a defect in neuronal NE reuptake could cause hypertension remains credible. Probably so, given that there is evidence of an associated regional sympathetic neuropathy that involves multiple sites, including the legs and kidneys (but not the heart), in the postural tachycardia syndrome, which might be expected to modify the translation of a neuronal reuptake defect into blood pressure elevation. Unlike in the postural tachycardia syndrome, in essential hypertension, sympathetic nerve firing is commonly increased, and a combination of increased NE release coupled with faulty neuronal NE reuptake would be more likely to chronically elevate blood pressure.

It is possible that reduced neuronal NE reuptake also exists in the brain in patients with essential hypertension, such as to augment forebrain noradrenergic neurotransmission and elevate sympathetic nerve firing rates. Overflow from the brain...
of MHPG, the principal extraneuronal central nervous system NE metabolite, is increased 3-fold in patients with essential hypertension. Suprabulbar noradrenergic projections to the forebrain activate the sympathetic nervous system. There is good evidence that the stimulation of the sympathetic nervous system present is essential hypertension is, in fact, driven by increased forebrain NE turnover.

In obesity-related hypertension, there was no phenotypic evidence of NE transporter dysfunction. Obese subjects with and without hypertension differ in cardiac NE spillover, which is higher in the hypertensives. Although faulty neuronal reuptake of NE has been proposed as a basis for this differences in cardiac NE spillover, and as a predisposing factor in the development of the hypertension in the obese, a genetic fault that involves the NE transporter in obesity-related hypertension now appears to be excluded.

Screening of a mixed population of blood bank donors and psychiatric patients (with schizophrenia and bipolar affective disorder) recently lead to the identification of 13 DNA sequence variants of the NE transporter gene, of which 5 were interpreted as missense substitutions. Blood pressure status of the subjects with transporter gene mutations was not recorded in this report, and the degree of functional reduction in NE transporter activity associated with individual single nucleotide polymorphisms was not established. In a preliminary screening for these 5 missense substitutions in the NE transporter gene we conducted in 40 hypertensive patients (not the patients described in the present study), none of the substitutions were present. The transporter gene mutation recently described in the postural tachycardia syndrome kindred differs from the 5 identified earlier and is associated with almost total absence of transporter activity. A study in essential hypertension patients that incorporates neurochemical indices of the competency of NE neuronal reuptake such as those used in the present study, coupled with testing for coding region mutations in the transporter gene and functional assessment of any identified DNA sequence variants in an in vitro system, is now clearly needed.

Acknowledgments

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