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Adrenomedullary Secretion of Epinephrine Is Increased in Mild Essential Hypertension

Marie-Cécile Jacobs, Jacques W.M. Lenders, Jacques J. Willemsen, Theo Thien

Abstract  To assess whether patients with mild essential hypertension have excessive activities of the sympathoneuronal and adrenomedullary systems, we examined total body and forearm epinephrine spillovers and norepinephrine and epinephrine clearances in 47 subjects with mild essential hypertension (25 men, 22 women, aged 38.1±6.7 years) and 43 normotensive subjects (19 men, 24 women, aged 36.5±5.9 years). The isotope dilution method with infusions of tritiated norepinephrine and epinephrine was used at rest and during sympathetic stimulation by lower body negative pressure at -15 and -40 mm Hg. Hypertensive subjects had a higher arterial plasma epinephrine concentration (0.20±0.01 nmol·L⁻¹; mean±SE) than normotensive subjects (0.15±0.01) (P<.01). The increased arterial plasma epinephrine levels appeared to be due to a higher total body epinephrine spillover rate in the hypertensive subjects (0.23±0.02 nmol·min⁻¹·m⁻²) than the normotensive subjects (0.18±0.01) (P<.05) and not to a decreased plasma clearance of epinephrine. The arterial plasma noradrenaline level, total body forearm norepinephrine spillover rates, and plasma norepinephrine clearance were not altered in the hypertensive subjects. The responses of the catecholamine kinetic variables to lower body negative pressure were not consistently different between normotensive and hypertensive individuals. These data indicate that individuals with mild essential hypertension (1) have elevated arterial plasma epinephrine concentrations that are due to an increased total body epinephrine spillover rate, indicating an increased adrenomedullary secretion of epinephrine; (2) have no increased sympathoneuronal activity and no increased forearm norepinephrine spillover; and (3) have similar responses of both the sympathoneuronal and adrenomedullary systems to sympathetic stimulation by lower body negative pressure. (Hypertension. 1997; 29:1303-1308)

Key Words  epinephrine • norepinephrine • hypertension, essential • kinetics • adrenomedullary system

The sympathetic nervous system not only plays an important role in the regulation of blood pressure but is probably also involved in the pathogenesis of human essential hypertension. Several lines of evidence indicate that hypertensive individuals have an elevated sympathetic neural outflow. Biochemical measurements such as plasma catecholamine concentrations have been used extensively to document this. Several studies have reported on increased plasma NE or plasma EPI levels in hypertensive individuals, particularly in those younger than 40 years. However, plasma catecholamine concentrations are not a reliable index of sympathoneuronal and adrenomedullary activities for several reasons. First, plasma NE and EPI levels are determined not only by the rate at which these catecholamines enter plasma but also by their rates of removal. Second, sympathetic outflow to different organs is not uniform, and in particular, during sympathetic stimulation, sympathoneuronal responses show a differentiated pattern across different organs, depending on the kind of stimulus. Thus, venous and even arterial plasma NE and EPI concentrations have a limited value as measures of sympathetic and adrenomedullary activities. The isotope dilution method can provide more detailed information because there is a proportional relationship between the sympathetic nerve firing rate to an organ and the spillover rate of norepinephrine into the circulation. This method enables calculation of spillover and clearance rates of NE and EPI, both for the whole body and for specific vascular beds. Several groups have investigated NE kinetics in hypertensive patients, showing normal total body NE spillover with decreased neuronal uptake of NE or increased total body NE spillover in young hypertensive individuals. Other investigators, however, have found no significant difference in total body spillover of NE between normotensive and hypertensive individuals.

In contrast to NE kinetics, much less attention has been paid to EPI kinetics. Several studies have reported on increased plasma EPI levels in hypertensive individuals, but it is unclear whether these increased plasma EPI concentrations are due to an increased adrenomedullary secretion of EPI or to a diminished clearance of EPI from plasma. So far, no direct comparison between normotensive and hypertensive individuals has been carried out with regard to EPI kinetics.

The purpose of the present study was to assess simultaneously sympathoneuronal and adrenomedullary activities as measured by the isotope dilution technique, using simultaneous infusions of tritiated NE and tritiated EPI in untreated subjects with mild essential hypertension. Since excessive activity of the sympathoneuronal and adrenomedullary systems may be disclosed only during sympathetic stimulation, the kinetics of NE and EPI were also assessed during low and high intensities of LBNP.

Methods

Subjects

Forty-seven subjects with mild essential hypertension and 43 normotensive control subjects participated in the study. Before...
Selected Abbreviations and Acronyms

EPI = epinephrine
FBF = forearm blood flow
LBNP = lower body negative pressure
NE = norepinephrine

entry into the study, all participants (aged 20 to 45 years) had a negative medical history and a normal physical examination. Thirty-one of the 47 hypertensive subjects had discontinued their antihypertensive medication for at least 4 weeks before the study, and the remaining 16 had not yet received any antihypertensive treatment. Blood pressure was measured at two occasions (in triplicate at each occasion after 10 minutes of supine rest) at 2-week intervals with a mercury sphygmomanometer. Mild hypertension was defined as a diastolic pressure between 90 and 105 mm Hg. All participants (aged 20 to 45 years) had a normal medical history and a normal physical examination. Secondary hypertension was excluded according to standard clinical criteria. Normotension was defined as a blood pressure of less than 140/90 mm Hg. Subjects with a body weight higher than the ideal body weight plus 10% were excluded from the study.11 The normotensive control subjects were recruited by means of a newspaper announcement. The study protocol was approved by the Hospital Ethics Committee, and all subjects gave their written informed consent.

Study Protocol and Procedures

All subjects were required to abstain from alcohol, nicotine, and caffeine-containing foods and beverages for at least 24 hours before each test session. The subjects were allowed to eat a light breakfast 2 hours before the study. All experiments were carried out in the morning in a temperature-controlled room (21°C to 22°C). During the study, the subjects remained supine. The lower body of the subjects was sealed at the iliac crests in an airtight Plexiglas LBNP box. The applied subatmospheric pressure was recorded by a manometer connected to the inside of the box.

After local anesthesia, a brachial artery was cannulated (Angiocath, 20 gauge, Deseret Medical, Becton Dickinson) for monitoring blood pressure and heart rate (Hewlett-Packard GmbH) and for drawing arterial blood samples. An intravenous cannula was inserted into a deep brachial vein in the ipsilateral arm for drawing venous blood samples. A venous cannula in the contralateral arm was used for simultaneous infusion of the radiotracers. FBF was recorded in the same arm that was also used for collection of arterial and venous blood samples, with the use of venous-occlusion strain-gauge plethysmography (Hokanson EC4, DE Hokanson) with air-filled cuffs.12 During FBF measurement and blood sampling, the hand circulation was excluded by inflation of a wrist cuff to 100 mm Hg above systolic pressure.13 After instrumentation, the subjects rested for 30 minutes. Thereafter, both radiotracers were infused, each as a bolus of 15 μCi • m⁻², followed by a constant infusion at a continuous rate of 0.35 μCi • min⁻¹ • m⁻² for a total duration of 90 minutes. During the last 3 minutes of the 30-minute rest period, baseline recordings of intra-arterial blood pressure, heart rate, and FBF were obtained, and arterial and venous blood samples were drawn for measurement of labeled and unlabeled plasma catecholamines. Blood pressure was recorded simultaneously with the FBF measurement. FBF was measured three times per minute. Thereafter, LBNP was applied at an intensity of −15 mm Hg for 15 minutes. Blood pressure, heart rate, and FBF recordings were made and blood samples collected in this sequence during the last 3 minutes of this LBNP period. After 30 minutes of rest, another LBNP period at −40 mm Hg for 15 minutes followed, with similar collection of blood pressure, heart rate, and FBF recordings and arterial and venous blood samples. The syringes containing the radiotracers were weighed before and after the infusion to verify the infusion rate. Samples of the infusates were taken at the end of each infusion.

[^3]H[NE] (levo-[ring-2,5,6-3H]-norepinephrine) (specific activity, 30 to 60 Ci/mmol) and [^3]HEPI (levo-[N-methyl-3H]-epinephrine) (specific activity, 50 to 85 Ci/mmol) (DuPont–New England Nuclear) were sterilized with the use of a microsyringe filter (0.22 μm) and diluted in 0.9% NaCl containing acetic (0.2 mol/L) and ascorbic (5.7 mmol/L) acids. Sterilization, dilution, and storage took place under nitrogen. The vials were stored until use at −80°C for a maximum of 3 months. Just before a study, an aliquot of each radiotracer was diluted in normal saline for intravenous infusion.

Analytic Methods

Blood samples were collected into prechilled tubes containing 0.25 mol/L EGTA and 0.2 mol/L glutathione and immediately placed on melting ice. Plasma was separated by refrigerated centrifugation and frozen until assayed within 2 weeks from collection. The samples were analyzed for concentrations of unlabeled and tritiated NE and EPI using high-performance liquid chromatography with fluorometric detection after selective precolumn derivatization of the catecholamines with the fluorescent agent 1,2-diphenylethylenediamine.14

The detection limits of unlabeled NE and EPI are 2.2 and 3.2 pmol/L, respectively. The intra-assay coefficients of variation of unlabeled NE and EPI at plasma levels of 1.31 and 0.11 nmol/L are 2.3% and 3.4%, respectively. At plasma levels of 1.02 and 0.15 nmol/L, interassay coefficients of variation are 8.5% and 7.2%, respectively. The detection limit of[^3]HNE and[^3]HEPI is 6 disintegrations per minute. The interassay coefficient of variation of[^3]HNE and[^3]HEPI is 7.0% in venous plasma samples.

Data Analysis

Forearm vascular resistance was calculated as the quotient of mean arterial blood pressure and FBF and was expressed in arbitrary units (AU). The average of the hemodynamic data during the 3-minute recording was taken.

The total body clearance rate of each catecholamine was calculated from the infusion rate of each tritiated catecholamine ([^3]HCA) and the steady-state arterial plasma concentration of each tritiated catecholamine ([^3]HCAarl) according to the formula Total Body CA Clearance (L •  min⁻¹) = ([[^3]H]CAarl (nmol • L⁻¹ • 100 mL⁻¹) • Total Body CA Clearance (L •  min⁻¹ • 100 mL⁻¹)). The regional kinetic variables of each catecholamine in the forearm are expressed per 100 mL forearm volume (FAV). Regional catecholamine spillover in the forearm was estimated from the following equation: Forearm Spillover (pmol • 100 mL⁻¹ • FAV • min⁻¹) = ([CAarl – CAm] + [CAarl • Fractional Extraction] • 100 mL • FAV). Where Fractional Extraction = ([[^3]H]CAarl -[^3]HCAarl) •[^3]HCAarl and FPP is forearm plasma flow in milliliters per 100 mL forearm volume per minute, calculated from the FBF and hematocrit. The forearm clearance of each catecholamine was calculated according to the formula Forearm Clearance (mL • 100 mL⁻¹ FAV • min⁻¹) = FFP • Fractional Extraction of Each Catecholamine.

Data are expressed as mean ± SE unless indicated otherwise. Differences between normotensive and hypertensive subjects were tested by the Mann-Whitney U test. For each variable, the responses to LBNP were tested by the Wilcoxon matched-pairs signed rank test. For calculation of correlations between hemodynamic and catecholamine kinetic variables, the Spearman correlation was used. A value of P < 0.05 was considered to be significant.
TABLE 1. Clinical Characteristics of Hypertensive and Normotensive Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>43</td>
<td>47</td>
</tr>
<tr>
<td>Men/women</td>
<td>19/24</td>
<td>25/22</td>
</tr>
<tr>
<td>Age, y</td>
<td>36.5±5.9</td>
<td>38.1±6.7</td>
</tr>
<tr>
<td>Quetelet index, kg - m⁻²</td>
<td>23.1±3.0</td>
<td>24.8±2.21</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>119±14</td>
<td>150±15</td>
</tr>
<tr>
<td>Diastolic pressure, mm Hg</td>
<td>73±9</td>
<td>98±8</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>67±10</td>
<td>72±11*</td>
</tr>
<tr>
<td>Urinary sodium, mmol/mmol creatinine</td>
<td>11.1±4.0</td>
<td>10.4±3.8</td>
</tr>
</tbody>
</table>

Values are mean±SD. *P<.05, †P<.01 vs normotensive subjects.

Results

Baseline Values

The descriptive characteristics of all participants are summarized in Table 1. The ages of the normotensive and hypertensive groups did not differ significantly, but the hypertensive subjects had a slightly but significantly higher quetelet index than the normotensive subjects. As expected, blood pressure was higher in the hypertensive than normotensive subjects, as was heart rate. FBF was similar in the groups (1.65±0.12 and 1.51±0.10 mL·100 mmL⁻¹·min⁻¹, respectively), and forearm vascular resistance was slightly higher in the hypertensive (81±4 AU) than the normotensive (70±4 AU) subjects (P<.05).

Plasma NE concentrations did not differ significantly between hypertensive and normotensive subjects (Table 2). No differences were found in total body NE spillover and clearance between hypertensive and normotensive subjects. Regional forearm NE spillover and clearance were also similar in the two groups (Table 2, Fig 1). There was no correlation between systolic, diastolic, or mean arterial pressures and arterial plasma NE level or total body NE spillover.

Baseline arterial and venous plasma EPI concentrations were significantly higher in the hypertensive (P<.01) than the normotensive group (Table 2, Fig 2). Among all subjects, there were weak but significant correlations between arterial plasma EPI level and systolic pressure (r=.29; P<.01), diastolic pressure (r=.31; P<.01), and heart rate (r=.25; P<.05). The total body spillover of EPI was significantly increased in the hypertensive subjects (Fig 3), whereas total body and forearm clearances of EPI were similar in both the hypertensive and normotensive groups. In both groups, there were extremely low forearm spillovers of EPI that were both significantly different from zero (P<.01), but there was no difference between values in normotensive and hypertensive subjects (Table 2).

Lower Body Negative Pressure

During LBNP at −15 mm Hg, both systolic and diastolic pressures did not change, but heart rate increased slightly and significantly in both groups by +1±1 and +1±1 beats per minute. Pulse pressure decreased in the normotensive subjects by 2±1 mm Hg (P<.01) and by 3±1 in the hypertensive subjects (P<.01), but these decrements were not significantly different. Forearm vascular resistance increased similarly in both groups by +17±3 and +22±3 AU.

Venous and arterial plasma NE concentrations increased significantly by 31±3% and 31±4% in the normotensive subjects and by 34±5% and 24±4% in the hypertensive subjects (Table 2). The increments of

TABLE 2. Baseline Values and Responses of Catecholamine Kinetic Variables to Lower Body Negative Pressure at −15 mm Hg in Hypertensive and Normotensive Subjects

<table>
<thead>
<tr>
<th>Kinetic Variable</th>
<th>Baseline Level</th>
<th>Change at −15 mm Hg LBNP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotensive</td>
<td>Hypertensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous plasma, nmol · L⁻¹</td>
<td>1.13±0.09</td>
<td>1.29±0.09</td>
</tr>
<tr>
<td>Arterial plasma, nmol · L⁻¹</td>
<td>0.83±0.05</td>
<td>0.95±0.05</td>
</tr>
<tr>
<td>Total body spillover, nmol · min⁻¹· m⁻²</td>
<td>0.85±0.06</td>
<td>0.87±0.05</td>
</tr>
<tr>
<td>Total body clearance, L · min⁻¹</td>
<td>1.03±0.03</td>
<td>0.95±0.03</td>
</tr>
<tr>
<td>Forearm spillover, pmol · 100 mL⁻¹· min⁻¹</td>
<td>0.77±0.07</td>
<td>0.90±0.06</td>
</tr>
<tr>
<td>Forearm clearance, mL · 100 mL⁻¹· min⁻¹</td>
<td>0.84±0.04</td>
<td>0.69±0.04</td>
</tr>
<tr>
<td>Epinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous plasma, nmol · L⁻¹</td>
<td>0.04±0.01</td>
<td>0.06±0.01†</td>
</tr>
<tr>
<td>Arterial plasma, nmol · L⁻¹</td>
<td>0.15±0.01</td>
<td>0.20±0.01†</td>
</tr>
<tr>
<td>Total body spillover, nmol · min⁻¹· m⁻²</td>
<td>0.18±0.01</td>
<td>0.23±0.02*</td>
</tr>
<tr>
<td>Total body clearance, L · min⁻¹</td>
<td>1.22±0.04</td>
<td>1.14±0.04</td>
</tr>
<tr>
<td>Forearm spillover, pmol · 100 mL⁻¹· min⁻¹</td>
<td>0.008±0.002</td>
<td>0.009±0.004</td>
</tr>
<tr>
<td>Forearm clearance, mL · 100 mL⁻¹· min⁻¹</td>
<td>0.69±0.04</td>
<td>0.74±0.05</td>
</tr>
</tbody>
</table>

LBNP indicates lower body negative pressure. Values are mean±SE. *P<.05, †P<.01 vs normotensive subjects; ‡P<.05, §P<.001, ||P<.0001 vs baseline.
forearm and total body NE spillovers reached significance only in the hypertensive group and not in the normotensive group, but these increments were not significantly different between the groups (Table 2). Total body NE clearance decreased significantly in both groups.

Venous plasma EPI concentration did not change during LBNP at −15 mm Hg, but arterial plasma EPI level increased by 36±5% in the normotensive subjects and 38±7% in the hypertensive subjects (P=NS) (Table 2). The increments in arterial plasma EPI levels during LBNP appeared to be due to both an increase in total body EPI spillover (normotensive subjects, +13±5%; hypertensive subjects, +19±6%) and a reduction in total body EPI clearance (−17±2% and −12±3%, respectively). The forearm spillovers of EPI did not increase significantly during LBNP in both groups (Table 2).

During LBNP at −40 mm Hg, pulse pressure decreased significantly more in the hypertensive group (−13±1 mm Hg) than the normotensive group (−9±1 (P<.05), whereas heart rate increased similarly in both groups by 10±1 and 9±1 beats per minute, respectively (P=NS).

Venous plasma NE concentrations increased similarly by 94±6% and 111±11% in the normotensive and hypertensive subjects, respectively. Arterial NE increased more in the hypertensive (96±8%) than the normotensive (83±8%) group (Table 3), whereas there was no difference between the groups with regard to the responses of forearm and total NE spillovers as well as total body and forearm NE clearances (Table 3).

The responses of arterial plasma EPI concentrations increased by 108±10% in the normotensive subjects and 136±18% in the hypertensive subjects (P=NS) (Table 3). The total body spillover of EPI increased by 69±12% and 81±13%, whereas the total body clearance of EPI decreased by 16±6% and 21±3%. These differences between normotensive and hypertensive individuals were not significant. The forearm spillovers of EPI showed a slight but significant decrease in both groups.

**Discussion**

The present study not only confirms the previously reported elevated plasma EPI levels in individuals with essential hypertension but also demonstrates that this is due to an increased adrenomedullary release of EPI into the bloodstream and not to a decreased clearance of EPI. This is indicated by the increased total body spillover of EPI in combination with an unaltered EPI clearance.
Several previous studies have demonstrated elevated plasma EPI levels in individuals with essential hypertension, suggesting increased adrenomedullary secretion of EPI in hypertensive individuals. Although EPI is released from the adrenal medulla directly into the bloodstream, arterial plasma EPI levels cannot be used as an index of adrenomedullary EPI secretion because most of the circulating EPI is removed rapidly from the circulation. To take into account the removal of EPI from the circulation, adrenomedullary EPI secretion can be assessed by the isotope dilution technique. This technique requires a high analytic sensitivity for measurement of low venous EPI concentrations and venous $[^3]H$EPI activity. With this technique, the present study demonstrates for the first time that the elevated plasma EPI levels in essential hypertensive individuals are due to an increased adrenomedullary secretion of EPI. Since the adrenal medulla serves as a sympathetic ganglion, the increased adrenal EPI release in the mildly hypertensive individuals reflects an increased sympathetic outflow selectively to the adrenal medulla in these individuals.

Increased sympathetic nervous system activity has been implicated as an important pathophysiological mechanism in essential hypertension for a long time. Increased activity of the sympathetic system may not only be manifest at different levels of the sympathoneuronal axis but has also been demonstrated as an increased local NE spillover in certain organs, such as the heart, kidneys, brain, and skeletal muscles. The present observation demonstrates that this increased sympathetic outflow extends also to the adrenal medulla. In contrast, global sympathoneuronal activity, estimated as total body NE spillover, was not elevated in the hypertensive subjects. This is at variance with some but not all previous studies, demonstrating an increased total body NE spillover in hypertensive subjects. It should be noted that reports on NE kinetics or microneurographic data in hypertensive subjects have mainly been based on male subjects. The present study included an approximately equal number of male and female subjects in both groups.

A particular additional finding in the present study is the forearm spillover of EPI in both normotensive and hypertensive individuals. These spillover rates, although extremely low, are significantly different from zero. Under normal conditions, EPI is mainly synthesized in the adrenal medulla and certain brain nuclei. However, in individuals with heart failure but also in healthy subjects during intensive aerobic exercise, EPI can be released from the heart. This released EPI is that is predominantly derived from the circulation, from which it is taken up by sympathetic nerves. Apparently, forearm EPI can recycle because it can be coreleased along with NE from sympathetic nerves. EPI synthesis in the human forearm is facilitated effect on peripheral noradrenergic transmission and that this effect was enhanced in individuals with essential hypertension. Individuals who develop hypertension would be more susceptible to stressful stimuli and exhibit elevated stress-related increments in circulating EPI. Indeed, an abundance of evidence indicates that, in
particular at a young age, individuals with essential hypertension have increased sympathetic responses to psychological stress.15,32 The slightly increased plasma EPI concentrations in the hypertensive subjects fit with the hypothesis of Brown and Macquinn.

A question of particular concern is whether the increased plasma EPI levels may contribute to the deleterious cardiovascular sequelae of hypertension in the long term. The development of these complications may be mediated by well-known adverse effects of catecholamines, such as induction of cardiac arrhythmias, stimulation of vascular and ventricular hypertrophy, and activation of platelets.33-35 Although the arterial plasma EPI levels in the subjects with mild hypertension were only slightly higher than those in the normotensive subjects, it cannot be excluded that chronic exposure of the heart and blood vessels to this circulating EPI may be harmful in the long term.

In conclusion, this study demonstrates that individuals with mild essential hypertension have increased plasma EPI levels and that this is due to an increased basal adrenomedullary secretion of EPI. This indicates that mildly hypertensive subjects have an increased sympathetic outflow to the adrenals. In contrast to some previous studies, we did not find an increased general sympathoneural activity, measured as total body NE spillover. No studies, we did not find an increased general sympathoneural activity, measured as total body NE spillover. No studies, we did not find an increased general sympathoneural activity, measured as total body NE spillover.

Acknowledgments
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References