Age-Dependence of Hypertensive-Normotensive Differences in Plasma Norepinephrine

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SUMMARY We compared venous plasma norepinephrine (NE) concentrations in 191 resting, supine patients with essential hypertension and 129 normotensive controls. Among normotensives, plasma NE increased significantly with age, but among hypertensives, no age-related increase occurred, due to relatively high NE values among young hypertensives. When patients and controls less than 40 years old were considered, hypertensives showed significantly higher plasma NE than the controls (317 vs 245 pg/ml, t = 3.15, p < 0.01); but above the age of 40 years, no significant hypertensive-normotensive difference was obtained. These results, predicted by recent literature reviews, help to resolve the persistent controversy about sympathetic neural activity in essential hypertension, since such activity appears to be abnormal mainly in young patients. The data are consistent with increased sympathetic nervous system activity in the early stages of essential hypertension. (Hypertension 5: 100-104, 1983)

KEY WORDS • norepinephrine • hypertension • blood pressure • age • catecholamines • epinephrine

THE possible role of increased sympathetic nervous system activity in the etiology of essential hypertension has been a persistently controversial topic in hypertension research. The introduction of adequately sensitive and specific techniques for measuring plasma norepinephrine (NE)1 and indications that plasma NE provides an index of sympathetic neural activity in humans2 promised to resolve this issue, but instead led to conflicting results. Recently, literature reviews of the large number of comparative studies of NE in patients with essential hypertension and in normotensive controls used a statistical approach in which each study provided single data points for the hypertensive and normotensive groups, so that factors could be identified that differentiated positive studies — those reporting significant hypertensive-normotensive differences in plasma NE — from negative studies — those reporting no significant differences.3.4 One such factor was the age of the patient population: studies of relatively young patients (40 years old or less) were usually positive, while studies of older patients were usually negative. One suggestion from these reviews, then, was that sympathetic neural activity, as indicated by circulating NE levels, may be abnormal mainly in young patients with essential hypertension. This in turn would implicate a pathogenetic role for enhanced sympathetic neural activity in the development of essential hypertension in some patients. We considered this hypothesis to be sufficiently important that we tested it by comparing NE levels in a large number of individuals of various ages with essential hypertension and a large number of normotensive controls.

Methods

Subjects
Normotensive outpatient controls were mainly from the Clinical Center at the National Institutes of Health (NIH), but also from the Holt-Krock Clinic, Fort...
Smith, Arkansas. All had normal medical histories and physical examinations and had a mean upright (sitting or standing) systolic and diastolic pressure less than 140/90 mm Hg on at least three separate occasions. The vast majority of the controls were neither medical personnel nor laboratory coworkers, and were Caucasian. The inpatient controls were NIH normal volunteers, recruited mainly through the NIH normal volunteer program. There were 101 outpatient and 28 inpatient normotensive controls.

The outpatients with essential hypertension had been referred to Dr. Michael Coleman at the Holt Krock Clinic, Fort Smith, Arkansas. All had had secondary forms of hypertension excluded by blood and urinary testing, and, when appropriate, intravenous pyelography, renal scanning, computerized tomography, and arteriography. In these patients, the diagnosis of hypertension was confirmed by upright (sitting or standing) blood pressure averaging greater than 140 mm Hg systolic or 90 mm Hg diastolic on at least three separate occasions. However, on the day of blood sampling for NE determination, supine blood pressure occasionally was less than these values. Therefore, for the purposes of this analysis, separate hypertensive-normotensive comparisons were conducted, based on the diagnostic history of the individuals and the blood pressure at the time of blood sampling. Hypertensive criteria for this analysis were a resting, supine systolic pressure more than or equal to 140 mm Hg, or else diastolic pressure more than or equal to 90 mm Hg. All the hypertensive patients were Caucasians either previously untreated for hypertension, or else they had discontinued any antihypertensive medication at least 1 week prior to blood sampling. Inpatients with essential hypertension were referred to and evaluated in the NIH Clinical Center. There were 136 outpatients and 55 inpatients with essential hypertension.

### Conditions of Blood Sampling

Blood was drawn according to a standard procedure. With the patient supine, an intravenous catheter or needle was inserted into a forearm vein and a heparin lock attached. After 30 minutes to 1 hour, blood was drawn through the heparin lock for NE determination. The patients had been instructed not to smoke cigarettes or drink coffee for 24 hours prior to venipuncture. Blood sampling occurred at various uncontrolled times of day, and the patients were not fasting.

### Norepinephrine Assay

Blood obtained through the heparin lock was collected in tubes containing acid-citrate-dextrose anticoagulant and stored in ice until the plasma was separated. The plasma was stored at −70°C or in a tank of liquid nitrogen until the time of assay. Plasma NE was measured using the phenylethanolamine-N-methyltransferase radioenzymatic technique.

### Data Analysis

Patient data, including blood pressures, pulse rates, ages, sexes, and plasma NE values, were stored using the NIH WYLBUR computer system. For the purposes of this study, the patients were classified by diagnostic history (essential hypertension or normotensive control), as well as by blood pressure at the time of blood sampling, patient age, sex, and inpatient or outpatient status. Analyses of variance (ANOVAs) were conducted for factors of interest using the NIH’s SAS statistical program package. Independent-means t tests also were conducted using the mean patient values and standard errors.

### Results

When NE levels were plotted as a function of age, the normotensives showed a significant age-norepinephrine relationship (r = 0.29, p < 0.01), but the hypertensives did not (r = 0.04, fig. 1). The difference in hypertensive and normotensive trends in NE levels with age, assessed by the age-diagnosis interaction effect in the ANOVA, was highly significant (F = 4.05, p < 0.001).

Across all patients, plasma NE levels were slightly but statistically significantly higher in those with a diagnosis of essential hypertension than the normotensive controls (323 vs 268 pg/ml, t = 3.20, p < 0.01). In considering the slope for the line of best fit (25.3 pg/ml/10 years) relating NE to age across normotensives, adjustment of the mean hypertensive-normotensive difference for the observed age mismatch of 10 years between the hypertensive and normotensive groups resulted in a loss of statistical significance for the overall difference in NE between the groups.

To evaluate further the age-dependence of hypertensive-normotensive differences in NE, we studied the hypertensive and normotensive groups according to age (table 1). When the subjects were 40 years or older, no significant hypertensive-normotensive difference in plasma NE was obtained. When the patients were younger than 40, the hypertensive-normotensive difference in plasma NE was highly significant (317 vs 245 pg/ml, t = 3.15, p < 0.01). After adjustment for the observed mismatch in mean age of 7 years, the hypertensive-normotensive difference among these relatively young patients remained statistically significant (t = 2.37, p < 0.05).

For both hypertensives and normotensives, outpatient plasma NE levels exceeded inpatient levels (table 1), although the difference was statistically significant only in the hypertensives.

When hypertensive-normotensive differences were considered as a function of age in the outpatient and inpatient groups separately, once again the hypertensives showed higher plasma NE levels only among the group less than 40 years old. For inpatients, the mean difference was not statistically significant, most likely due to high inter-individual standard deviations and small numbers of subjects, rather than to the absence of a mean difference.

When the data were analyzed in terms of the actual blood pressure at the time of blood sampling, and not
FIGURE 1. Hypertensive and normotensive individual norepinephrine levels as a function of age. M = male; F = female. Plasma norepinephrine increased with age in the normotensive but not in the hypertensives (16 data points among the hypertensives and 8 among the normotensives were obscured).

TABLE 1. Plasma Norepinephrine (NE) in Patients with Essential Hypertension (EH) and in Normotensive Controls (NT), Classified by Age, Sex, and Inpatient or Outpatient Status

<table>
<thead>
<tr>
<th>Subject group</th>
<th>No. of subjects</th>
<th>Age</th>
<th>NE (pg/ml)</th>
<th>NE (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EH  NT</td>
<td>H</td>
<td>N</td>
<td>H</td>
</tr>
<tr>
<td>Overall</td>
<td>191 129</td>
<td>43 33</td>
<td>323 ± 169</td>
<td>268 ± 137*</td>
</tr>
<tr>
<td>Under 40 yrs</td>
<td>83 85</td>
<td>30 23</td>
<td>317 ± 164</td>
<td>245 ± 130*</td>
</tr>
<tr>
<td>40 yrs or over</td>
<td>108 44</td>
<td>52 52</td>
<td>327 ± 174</td>
<td>314 ± 138</td>
</tr>
<tr>
<td>Women</td>
<td>93 70</td>
<td>43 32</td>
<td>310 ± 155</td>
<td>271 ± 142</td>
</tr>
<tr>
<td>Under 40 yrs</td>
<td>38 48</td>
<td>31 24</td>
<td>307 ± 142</td>
<td>252 ± 148</td>
</tr>
<tr>
<td>40 yrs or over</td>
<td>55 22</td>
<td>51 51</td>
<td>312 ± 166</td>
<td>311 ± 122</td>
</tr>
<tr>
<td>Men</td>
<td>98 59</td>
<td>42 34</td>
<td>335 ± 182</td>
<td>269 ± 131*</td>
</tr>
<tr>
<td>Under 40 yrs</td>
<td>45 37</td>
<td>30 22</td>
<td>326 ± 181</td>
<td>235 ± 106*</td>
</tr>
<tr>
<td>40 yrs or over</td>
<td>53 22</td>
<td>53 54</td>
<td>344 ± 183</td>
<td>316 ± 156</td>
</tr>
<tr>
<td>Outpatients</td>
<td>136 101</td>
<td>42 31</td>
<td>354 ± 177</td>
<td>274 ± 139*</td>
</tr>
<tr>
<td>Under 40 yrs</td>
<td>67 72</td>
<td>30 22</td>
<td>334 ± 163</td>
<td>255 ± 133*</td>
</tr>
<tr>
<td>40 yrs or over</td>
<td>69 29</td>
<td>52 52</td>
<td>374 ± 190</td>
<td>321 ± 144</td>
</tr>
<tr>
<td>Inpatients</td>
<td>55 28</td>
<td>45 40</td>
<td>249 ± 121</td>
<td>247 ± 129</td>
</tr>
<tr>
<td>Under 40 yrs</td>
<td>16 13</td>
<td>31 25</td>
<td>247 ± 152</td>
<td>186 ± 103</td>
</tr>
<tr>
<td>40 yrs or over</td>
<td>39 15</td>
<td>52 53</td>
<td>250 ± 107</td>
<td>299 ± 130</td>
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</tbody>
</table>

Age decade (yrs)

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<tbody>
<tr>
<td>15-24</td>
<td>20</td>
<td>42</td>
<td>316 ± 150</td>
<td>199 ± 96*</td>
</tr>
<tr>
<td>25-34</td>
<td>32</td>
<td>31</td>
<td>301 ± 149</td>
<td>322 ± 143</td>
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<tr>
<td>35-44</td>
<td>53</td>
<td>11</td>
<td>343 ± 189</td>
<td>306 ± 168</td>
</tr>
<tr>
<td>45-54</td>
<td>49</td>
<td>20</td>
<td>309 ± 170</td>
<td>270 ± 115</td>
</tr>
<tr>
<td>55-64</td>
<td>27</td>
<td>12</td>
<td>321 ± 171</td>
<td>334 ± 112</td>
</tr>
<tr>
<td>65-74</td>
<td>8</td>
<td>4</td>
<td>365 ± 177</td>
<td>393 ± 218</td>
</tr>
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</table>

Norepinephrine mean values are expressed as ± 1 sd.

*Hypertensive-normotensive difference significant, with p < 0.01.
just in terms of the diagnostic history, no important changes in the pattern of hypertensive-normotensive differences emerged. When outpatients with a diagnosis of essential hypertension and with blood pressures satisfying hypertensive criteria at the time of venipuncture were compared with subjects who were normotensive by history and at the time of phlebotomy, the consistently hypertensive patients had clearly higher NE levels (372 vs 276 pg/ml, t = 3.47, p < 0.001). Patients with a history of hypertension but who were normotensive at the time of the study showed intermediate NE values (mean, 338 pg/ml).

Across all patients, no significant correlation was obtained between plasma NE and systolic blood pressure, and no significant pressure-NE relationship occurred even among hypertensives less than 40 years old.

When the data were analyzed separately by sex, no important differences in plasma NE appeared between the hypertensive men and women and between the normotensive men and women, but the overall hypertensive-normotensive difference in NE was statistically significant only among men. In the decade from 25 to 34 years old, the group of normotensive females included some with high plasma NE, so that the obtained mean NE value was significantly higher among the women in this decade than among the normotensive men (t = 2.67, p < 0.05). Most of the 25- to 34-year-old normotensives with NE levels greater than 375 pg/ml were women. Menstrual dates were recorded in seven women during this decade. The mean NE level of the women apparently menstruating at the time of blood sampling was significantly higher than for the remaining nonmenstruating women (t = 3.31, p < 0.05).

Discussion

Clinical investigators have argued for years about the possible pathogenetic role of enhanced sympathetic neural activity in essential hypertension. The introduction of adequately sensitive and specific assay techniques for measuring plasma NE in humans, which has been used as an index of sympathetic neural activity, has only heightened the controversy. Over 70 studies have compared NE levels in patients with essential hypertension and in normotensive controls. Although most investigators have reported higher mean levels in the hypertensives, usually the hypertensive-normotensive differences have not been statistically significant.

When investigators have considered the relationship between plasma NE and age, most have reported that in normotensives, plasma NE increases with age. On the other hand, in patients with essential hypertension, no such age-NE relationship has been found. Further, several otherwise negative studies have reported that relatively young hypertensives showed significantly higher mean plasma NE levels than young normotensives.

This series of findings led us to reexamine the raw data from our previously published studies. We consistently had found and reported no statistically significant hypertensive-normotensive difference in plasma NE. We also noted that across hypertensive and normotensive groups combined, plasma NE increased with age. Since hypertensive groups were older than normotensive groups, we suggested that poor age matching could exaggerate obtained hypertensive-normotensive differences in NE.

The present review of our data, based on possibly the largest series of patients and controls so far reported, does not negate our previous findings but clearly calls for a modification of the inferences drawn. The crux of our results was that, overall, normotensives showed a positive relationship between plasma NE and age; hypertensives did not. This difference in trends was highly significant by ANOVA. This means that, even with perfect age matching, relatively young patients with essential hypertension would show higher NE levels than young normotensive controls, while relatively old patients with hypertension would show no difference from older controls. Thus, poor age matching will amplify hypertensive-normotensive differences in NE, but the artifactual effects of poor age matching cannot account for all the differences observed.

Our finding of a dependence of hypertensive-normotensive differences in plasma NE on the age of the patients helps to resolve the controversy about the possible role of sympathetic neural activity in essential hypertension, since our results, and the results of large-scale analytical reviews, point to an involvement of enhanced sympathetic outflow in the early stages of essential hypertension.

The scatter plots in figure 1 demonstrate dramatic interindividual variability in plasma NE even among normotensive controls. Part of this variability probably resulted from incomplete control of such factors as the time of day when sampling occurred, the length of time the subjects had fasted, concurrent illnesses, unreported medication intake, psychological stress, sodium intake, physical training, and obesity.

Considering the large interindividual standard deviations in plasma NE, it is not surprising that the trends we observed required many subjects before statistical significance could be attained.

In the decade from 25 to 34 years old, the normotensive group had somewhat higher mean plasma NE than would be indicated from the regression line of plasma NE and age (fig. 1). In examining the raw data from this group, we found that the subjects with high plasma NE values usually were women. The women in this age group who were menstruating at the time of blood sampling showed significantly higher plasma NE levels than the women who were not. It is possible, then, that variations in plasma NE in the menstrual cycle may also contribute to interindividual standard deviations, and may help to explain why, overall, male hypertensives showed significantly higher plasma NE than male normotensives, while no significant overall
hypertensive-normotensive difference in NE was obtained in women. The medical literature in this area is scanty, and the findings have been discrepant.\textsuperscript{9,10}

When blood pressure at the time of blood sampling — rather than diagnostic history — determined the categorization of hypertensives and normotensives, no important changes in our findings occurred. Interestingly, the group of outpatients with a history of hypertension but normal blood pressure at the time of venipuncture had a somewhat lower mean NE level than the group of similar age with a history of hypertension but who also had high blood pressure at the time of venipuncture. Perhaps hypertensive-normotensive differences in NE in young subjects are more apparent when the patients with hypertension show consistently elevated blood pressure. This would support the suggestion of a recent large-scale literature review.\textsuperscript{4}

In summary, upon review of the data from a large number of patients with essential hypertension and normotensive controls, we found that plasma NE increased with age in the normotensives but not the hypertensives, so that hypertensive-normotensive differences in NE were most apparent among young subjects. Further study is needed of relatively young patients with essential hypertension and age-matched controls, to determine how — or whether — enhanced sympathetic nervous system activity in this age group causes high blood pressure.

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