Plasma Norepinephrine in Essential Hypertension

A Study of the Studies

DAVID S. GOLDSTEIN, M.D., PH.D.

SUMMARY  Of 32 studies comparing plasma norepinephrine concentrations in hypertensive and normotensive groups, 28 (88%) reported higher levels in the hypertensive group. However, only 13 (41%) of the studies reported statistically significant hypertensive-normotensive differences in norepinephrine, leading the present attempt to identify factors differentiating “positive” studies (those reporting significant hypertensive-normotensive differences) from “negative” studies (those reporting nonsignificant differences). Hypertensive norepinephrine levels were similar in positive and negative studies (281 vs 288 pg/ml), but normotensive levels were lower in the positive studies (177 vs 269 pg/ml). When compared with the fluorimetric technique, the radioenzymatic type of assay was associated both with a lower frequency of positive results (25% vs 100%) and greater intrastudy standard deviations (152 vs 72 pg/ml). Hypertensive-normotensive differences varied inversely with age ($r = -0.37$). Resolution of the persisting controversy about norepinephrine levels in essential hypertension will require more attention to the causes of variability associated with the assay technique, to the sources, characteristics, and treatment of the normotensive controls, and to the age of the patient population. (Hypertension 3: 48-52, 1981)

KEY WORDS  • norepinephrine  • catecholamines  • hypertension

THE introduction about a decade ago of sensitive techniques for measuring plasma norepinephrine, and indications that those levels reflect sympathetic neural activity, promised to help answer a perennial question in hypertension research: Does sympathetic hyperactivity cause essential hypertension? Despite the subsequent publication of more than 30 comparative studies in hypertensives and normotensive controls, controversy persists about whether patients with hypertension exhibit elevated levels of norepinephrine. This report reexamines these studies in an attempt to identify the causes of the inconsistencies in results.

Methods

The studies considered in this review satisfied the following criteria: 1) they were published since 1973, in English; 2) they were not merely abstracts, but were primary data sources independent of data from other sources; 3) they specifically involved plasma norepinephrine, not total catecholamines; 4) they used a sensitive and reliable fluorimetric technique, such as that of Renzini, et al.; a radioenzymatic assay; or high-pressure liquid chromatography with electrochemical detection; and 5) they reported resting, supine plasma norepinephrine levels in both a normotensive control group and an entire hypertensive group (as opposed to a subgroup selected for certain characteristics).

The large number of studies culled using these criteria — 32 — allowed a statistical approach in which the mean hypertensive and normotensive values from each study provided single data points. When mean group values had not been reported, they were derived from the weighted contributions of the subgroups in the study; and when standard deviations (SDS) had not been reported, they were calculated from standard errors of the mean (SEMs) by multiplying the SEMS by the square root of the number of observations. Statistical analyses used two-tailed independent-means and dependent-means t tests, chi-squared tests, and Pearson correlation coefficients. All studies, despite their probably variable reliability, were accorded equal weight.
Results

Table 1 displays hypertensive and normotensive mean norepinephrine levels from the 32 studies. Twenty-eight (88%) reported higher levels in the hypertensives, by an average of 54 pg/ml ($t = 4.06, p < 0.001$). Only 13 reported statistically significant hypertensive-normotensive differences in norepinephrine. Since about two-fifths of the studies were "positive" (i.e., reporting a significant hypertensive-normotensive difference) and about three-fifths were "negative" (reporting no significant difference), one can appreciate that any generalizations from the literature might be questioned.

What made the "positive" studies positive, and the "negative" negative? If the cause were a variably represented "hyperadrenergic" subgroup of hypertensives, since studies with a preponderance of these patients should have been positive due to the excessive mean norepinephrine levels in the hypertensive groups. However, mean hypertensive levels were not significantly higher in the positive than the negative studies.

### Table 1. Plasma Norepinephrine Levels in Patients with Essential Hypertension (H) and in Normotensive Controls (N)

<table>
<thead>
<tr>
<th>Author</th>
<th>H/N (no.)</th>
<th>Age H/N (yrs)</th>
<th>Assay</th>
<th>MAP H/N (mm Hg)</th>
<th>NE H/N</th>
<th>SD H/N</th>
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<td>112/93</td>
<td>263/250</td>
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<td>?</td>
<td>F</td>
<td>?</td>
<td>257/135*</td>
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<td>?</td>
<td>201/128*</td>
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<td>41/39</td>
<td>R</td>
<td>?</td>
<td>332/226*</td>
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<td>F</td>
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<td>20/17</td>
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<td>F</td>
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<td>21/11</td>
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<td>F</td>
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<td>?</td>
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*Significant hypertensive-normotensive difference with $p < 0.05$.
†Taken from figure.
*Approximate ages.
R = radioenzymatic; F = fluorimetric; H = high-pressure liquid chromatography with electrochemical detection; MAP = group mean blood pressure; NE = group mean norepinephrine level; SD = standard deviation.
studies (281 vs 288 pg/ml). In contrast, the normotensive control levels were significantly lower in the positive studies (177 vs 269 pg/ml, \( t = 4.27, p < 0.001 \)). Standard deviations of normotensive levels were also smaller in the positive studies (76 vs 152 pg/ml, \( t = 3.03, p < 0.005 \)).

These results emphasize the need for close attention to factors of variability obtained norepinephrine levels, and to characteristics of the normotensive controls. Several of these factors are considered below.

**Number of Subjects**

It would be inappropriate to weight studies with different numbers of patients equally, if small studies, perhaps involving selected series of patients and therefore smaller standard deviations of results, tended to be positive while larger studies, involving more generalized patient populations and larger standard deviations, tended to be negative. In fact, however, seven of 15 (47%) studies with less than 50 subjects were positive, and six of 17 (35%) with more than 50 subjects were positive, a nonsignificant difference in proportions. Average standard deviations of norepinephrine values were also similar in the two types of studies (134 vs 138 pg/ml respectively). The size of the study was therefore unrelated to the frequency of positive results.

**Type of Assay**

Of seven studies using a fluorimetric assay, all seven were positive, while only six of 24 (25%) using a radioenzymatic assay were positive (\( \chi^2 = 3.42, p < 0.001 \)), despite roughly comparable hypertensive-normotensive mean differences (68 and 49 pg/ml respectively). The average standard deviation of norepinephrine levels was significantly greater with the radioenzymatic technique, for both hypertensive (160 vs 97 pg/ml, \( t = 2.46, p < 0.05 \)) and normotensive (144 vs 46 pg/ml, \( t = 3.12, p < 0.001 \)) groups.

The definition of a "normal" norepinephrine level depended on the type of assay. For the fluorimetric type, the mean normotensive level was 147 pg/ml, compared with 251 pg/ml for the radioenzymatic (\( t = 4.10, p < 0.001 \)). Of the 22 studies using a radioenzymatic technique and specifying the enzyme, nine used phenylethanolamine-N-methyl-transferase (PNMT) and 13 used catechol-O-methyl-transferase (COMT). For the PNMT type of radioenzymatic assay, the mean normotensive level was 283 pg/ml, compared with 222 pg/ml for the COMT type (\( t = 2.32, p < 0.05 \)).

**Patient Age**

Lake, et al.\(^{22}\) have suggested that systematic differences in group mean age introduced artifactual effects in prior positive studies, because plasma norepinephrine varied with age and because hypertensives were older than normotensives. Across the 27 studies that listed mean group ages between 20 and 50 years, mean group norepinephrine levels did correlate significantly with mean group age for the normotensives (\( r = 0.42, p < 0.05 \)) but not the hypertensives (\( r = 0.28 \)). The hypertensives were also older than the normotensives, by an average of 3.4 years (\( t = 3.74, p < 0.001 \)).

Nevertheless, this artifactual effect of age would require that positive studies include older hypertensives than normotensives, and negative studies include groups of similar age. The correlation between the difference in group mean ages and the difference in group mean norepinephrine levels, 0.15, was negligible.

In contrast, hypertensive-normotensive differences correlated negatively with mean patient age (\( r = -0.37, p < 0.05 \)). Seven of 11 (64%) studies with a mean patient age less than 40 years were positive, while three of 17 (18%) with a mean patient age greater than or equal to 40 years were positive (\( \chi^2 = 4.26, p < 0.05 \)).

**Severity of Hypertension**

If norepinephrine levels varied with the severity of hypertension, then positive results could have derived from the inclusion of hypertensives with excessively high blood pressures. Across the 44 groups in which mean pressures were reported or calculable, a significant correlation of 0.36 (\( p < 0.05 \)) was obtained between mean pressures and mean norepinephrine levels. However, the mean pressures of the hypertensives in the negative studies were actually higher than those in the positive studies (116 vs 109 mm Hg), though not significantly so.

**Constitution of Control Groups**

The available literature provides little information about whether the controls were inpatients or outpatients, laboratory workers or newcomers to a hospital setting, whether they had previously undergone phlebotomy, smoked, drank alcohol or coffee recently, whether they were paid, or how they were recruited.

The sources of control subjects varied from patients with "various neurological disorders"\(^{19}\) to patients recovering from uterine hemorrhage, respiratory infection, or malnutrition,\(^{12}\) to laboratory workers,\(^{13}\) to patients detected on population screening,\(^{17}\) to clinic frequenters with minor complaints.\(^{18}\) One study reported lower norepinephrine levels in normotensive laboratory staff than in other normotensive controls.\(^{19}\) Another found higher levels in normotensive outpatients than inpatients.\(^{22}\)

The majority of studies did not specify blood pressure — or any other — criteria for inclusion in the normotensive group. Across the restricted interstudy range of mean normotensive pressure, plasma norepinephrine correlated 0.34 with mean norepinephrine pressure — a suggestive but not statistically significant relationship.
Drug Treatment Status

Twenty-seven of the 32 studies described the drug treatment status of the hypertensives. Five studies included only patients who had not previously been treated with an antihypertensive medication. In the remaining 22 studies, medication had been discontinued 1 to 6 weeks prior to phlebotomy. Across these 22 studies, there was no relationship between mean hypertensive norepinephrine levels and amount of time off medication. The mean norepinephrine level of the previously untreated hypertensives (288 pg/ml) was similar to that of the previously treated hypertensives (263 pg/ml).

Sample Handling and Collection

Among the 24 studies reporting their phlebotomy technique, the proportion of positive studies using venepuncture was not significantly different from that using an indwelling catheter. To the extent data were available, the time of day when sampling was done and the length of time the patients were supine prior to sampling were unrelated to the frequency of significant hypertensive-normotensive differences.

Only five studies included information about the kinds of collection tubes used, additives, and length and temperature of storage prior to performing the assay. Yet these mundane points may crucially influence the outcome of a study. For instance, since catecholamine levels may decrease with time in storage, obtained hypertensive-normotensive differences may be artifactualy decreased when the investigator samples a preponderance of elderly normotensives. Hypertensive-normotensive differences occurred when the patients — and controls — were relatively young. Further, the age-norepinephrine relationship was statistically significant across the normotensive but not the hypertensive groups. These two findings are consistent with the hypothesis that elevated resting plasma norepinephrine levels characterize a proportion of young patients with essential hypertension.

The available literature has paid inadequate attention to the interactions among several potential intervening variables that may mediate the relationship between plasma norepinephrine and blood pressure. These include thyroid function, the renin-angiotensin-aldosterone axis, plasma volume, sodium balance, alpha- and beta-receptor sensitivity, and the psychological stress associated with hospitalization, diagnostic maneuvers, and the patient's awareness of the disease. A comprehensive understanding of norepinephrine in essential hypertension will require measurement of or control for these factors.

In summary, three factors differentiated positive from negative studies: 1) normotensive control values; 2) type of assay; and 3) age of the patient population. How and why these factors exert their effects are matters for future research.

Discussion

The vast majority of studies of norepinephrine in patients with essential hypertension and in normotenive controls have reported higher mean levels in the hypertensives. To the extent plasma norepinephrine reflects sympathetic activity, this finding supports the hypothesis that sympathetic activity is increased in essential hypertension. Only about two-fifths of the studies, however, reported statistically significant hypertensive-normotensive differences, leading to the effort, in the present study, to identify factors differentiating positive from negative studies.

Hypertensive norepinephrine levels in the positive studies did not differ from those in the negative studies. This argues against the influence of a variably represented hyperadrenergic subgroup. In contrast, normotensive levels were lower and less variable in the positive studies. Unfortunately, the published literature has virtually ignored the sources, characteristics, and treatment of the normotensive controls.

The likelihood of positive results depended on the type of assay used to measure plasma norepinephrine. The radioenzymatic assay was associated both with a lower frequency of positive results and greater intrastudy standard deviations than the fluorimetric. "Normal" norepinephrine levels varied by up to 100% across assay types. The available literature provides no clues about why the results should have been so assay-dependent.

The age factor has obscured the role of plasma norepinephrine, since norepinephrine tends to increase with age and since hypertensives have been older than normotensives. Hypertensive-normotensive differences in norepinephrine did not derive from the artifactual effects of poor age matching. On the other hand, the largest hypertensive-normotensive differences occurred when the patients — and controls — were relatively young. Further, the age-norepinephrine relationship was statistically significant across the normotensive but not the hypertensive groups. These two findings are consistent with the hypothesis that elevated resting plasma norepinephrine levels characterize a proportion of young patients with essential hypertension.

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