Dopamine Beta-Hydroxylase and Plasma Renin Activity in Patients with Low-, Normal-, and High-Renin Essential Hypertension

WILLIAM J. LAWTON, M.D., ANNETTE FITZ, M.D., CHRISTINE GRANT, M.S., AND DAVID L. WITTE, PH.D.

SUMMARY The relationship of serum dopamine-β-hydroxylase (DBH), plasma renin activity (PRA) and urinary catecholamines (IU catechol) in various forms of essential hypertension (EHT) (low, normal and high renin) was evaluated. Eighty-four predominantly white, young (37 ± 8 years (SD)), mildly hypertensive patients (diastolic pressure 93 ± 4 mm Hg (SD)) continued their regular diet and received no medications. Thirteen patients had low-renin, 64 had normal-renin, and seven had high-renin EHT. DBH, total IU catechols and urinary norepinephrine were not different between these renin subgroups. DBH was significantly lower in all hypertensives (55.6 ± 36 IU) and in the low-renin subgroup (46 ± 30 IU) compared with normal subjects (68 ± 35 IU) (p < 0.01). However, the DBH range was so broad that an individual DBH value did not distinguish EHT from normals. After a baseline period, patients were randomly assigned to receive chlorthalidone 50 mg q.d.m. or placebo in a double-blind study. In the chlorthalidone group 1 month after therapy, the diastolic pressure decreased, PRA increased, and total IU catechols and urinary norepinephrine increased. Serum DBH did not change during diuretic therapy. A significant correlation could not be shown between pretreatment DBH and the changes in PRA and IU catechols before and after diuretics for all treated EHT patients. However, within the normal PRA EHT subgroup receiving chlorthalidone, the one-third of patients with lowest pretreatment DBH levels (n = 10) were compared with the one-third of patients with the highest pretreatment DBH values (n = 10). The lower DBH patients showed significantly less change in PRA (ΔPRA = 2.9 ± 1.8 ng/ml/hr) compared with the higher DBH patients (ΔPRA = 8.2 ± 1.6; p < 0.05). In some EHT patients, DBH levels may be related to PRA response to diuretic therapy.

HUMAN ESSENTIAL HYPERTENSION is the result of complex interrelationships between renal, hormonal, cardiovascular and neuronal factors. Alterations in activity of the sympathetic nervous system, and in the renin-angiotensin system, occur in some patients with essential hypertension. Stimulation of the sympathetic nervous system results in release of the enzyme dopamine β-hydroxylase (DBH) as well as norepinephrine. Some investigators, therefore, have suggested that circulating plasma concentrations of DBH might be a marker for sympathetic activity.

The sympathetic nervous system is a factor in the regulation of renin and aldosterone. Plasma renin activity (PRA) is stimulated by the activation of β-adrenergic receptors and can be blocked by β-adrenergic blocking agents. Suppression of sympathetic activity has been suggested as a mechanism for suppressed PRA in hypertensive patients.

We have studied PRA and the sympathetic nervous system in essential hypertension and the relationship of PRA and DBH. DBH values are known to overlap widely between hypertensives and normals, and the absolute DBH level in a person appears to be genetically determined. Absolute levels of DBH may therefore not be indicative of the level of sympathetic nervous system activity. We felt it was reasonable, however, to follow DBH values longitudinally in the same patients before and after an intervention which should alter sympathetic nervous system activity to determine if DBH reflects change in sympathetic activity in the same person. We studied a group of patients before and after diuretic therapy, a treatment known to stimulate renin activity and reduce plasma volume. Plasma volume reduction is also known to stimulate sympathetic nervous system activity. By looking at baseline and serial changes in DBH, and comparing these with another standard of sympathetic nervous system activity, we attempted to answer several questions: 1) Is PRA in the ambulant, untreated patient with low-normal high-renin essential hypertension related to DBH? 2) Since diuretic therapy alters plasma volume and sympathetic nervous system activity, is DBH an indicator of this change in the same individuals followed longitudinally? 3) Are the changes in PRA during diuretic therapy related to either changes in DBH or to pretreatment levels of DBH?

Subjects and Procedures

We studied patients from the hypertension clinics at the University Hospital and Veterans Administration Medical Center, and most were participants of the Veterans Administration-National Institutes of Health Study of Mild Hypertension. Sustained hypertension was documented by successive diastolic blood pressures of 90–105 mm Hg. Triplicate blood pressures were recorded in the right arm sitting position in the outpatient clinic and mean pressures were

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calculated, on three separate occasions, at least 1 month apart. There were no recognizable causes of secondary hypertension as determined by medical history, physical examination, urinalysis, blood urea nitrogen, serum creatinine, creatinine clearance, serum electrolytes and urinary catecholamines. Patients were free of cardiovascular disease as determined by history and physical examination, chest x-ray and ECG. All patients were white except one black subject, and were ages 21-50 years (37 ± 8 years, mean ± sd). There were 60 men and 24 women in the study.

Normal subjects were obtained from a population with a similar age range (32 ± 12 years) and similar race, with normal sitting blood pressure obtained on a single determination (diastolic pressure 76 ± 8 mm Hg, p < 0.001, compared with all hypertensives). Fifty-four male and 62 female normal subjects comprised the normal group for DBH determination.

All hypertensive subjects had mild-to-moderate sustained elevations in blood pressure, were untreated at the time of the study, and were asked to continue their regular diet. The subjects collected outpatient urine samples for 24 hours before the third clinic visit for determination of urinary sodium, creatinine, total catecholamines and norepinephrine. At the time of the third clinic visit, PRA and serum DBH levels were drawn simultaneously while the patients were ambulant and upright. Blood pressures were recorded as described above and blood pressures averaged at the third visit are subsequently referred to as “before treatment” pressures. At the end of the third visit the patients were randomly assigned to receive either active treatment with chlorthalidone 50 mg q.a.m. or placebo. The study was conducted in a double-blind manner. One month later, the patients again collected urine 24 hours before the clinic visit for determination of sodium, creatinine, total catecholamines and norepinephrine. Again, PRA and serum DBH were drawn simultaneously at the fourth clinic visit, and blood pressures recorded and referred to as post-treatment pressures.

Chemical Methods

PRA was measured by radioimmunoassay of angiotensin I, a modification of the method of Haber.22 Sodium concentration was measured by flame photometry. Twenty-four-hour urine collections were assessed for completeness by measurement of total creatinine content. Urine collections that contained less than 15 mg/kg creatinine/24 hours were considered incomplete and discarded. The hypertensive subjects were compared with our previous studies of normotensive control subjects in whom the normal hyperbolic relation of PRA and urinary sodium was established. For the hypertensives before treatment, 24-hour urine sodium values were plotted against PRA to classify the study subjects into high, normal, and low PRA groups.22

Twenty-four-hour urine collections for catecholamines were collected in bottles containing 20 ml concentrated hydrochloric acid (pH 2.0 or less). Total and fractionated catecholamines were determined fluorometrically using prepacked columns by Bio-Rad.24 DBH activity was assayed by the method of Nagatsu and Udenfriend25 in which DBH catalyzes the conversion of tyramine to octopamine. Octopamine is then oxidized to p-hydroxybenzaldehyde and measured spectrophotometrically.

Data were analyzed statistically using the paired t test for comparison of data in the same patient before and after treatment. The unpaired t test was used to compare normals to hypertensive subjects and to compare hypertensive subgroups. Spearman correlation coefficients were determined for selected parameters and significance defined as p < 0.05 using a two-tailed test. Values are mean ± sd unless otherwise indicated.

Results

Eighty-four patients collected complete 24-hour urine specimens based on 24-hour creatinine excretion at the time of both the third and fourth clinic visits. For each of these 84 patients, PRA was plotted against 24-hour urine sodium concentration at the time of the last pretreatment visit. Thirteen patients in whom renin values were below the normal curve were considered to have low-renin essential hypertension, 64 had normal PRA levels, and seven patients were considered to have high renin levels (table 1). The urinary sodium excretion was similar in the high-renin essential hypertensives (UNa+ = 211 ± 89 mEq/24 hr) and the normal-renin essential hypertensives (UNa+ = 214 ± 86 mEq/24 hr). As the low-renin subgroup was defined during normal dietary intake and in the ambulant state, low-renin patients could only be defined if 24-hour urine sodium excretion was less than 150 mEq/24 hr. The UNa+ in the low-renin group = 110 ± 30 mEq/24 hr. The low-renin patients were slightly older than the normal-renin and high-renin patients. All patients were white except for one black in the low-renin group. The diastolic blood pressures were similar in all groups (table 1).

Before Medication

Figure 1 shows the DBH levels and PRA for all patients before receiving medication. The DBH levels in renin subgroups were compared to each other, and there were no significant differences (fig. 1).

| TABLE 1. Characteristics of Patients With Essential Hypertension, Before Treatment |
|-----------------------------------------------|----------------|----------------|
| Low renin | Normal renin | High renin |
| n | 13 | 64 | 7 |
| Age (years) | 40 ± 6 | 37 ± 7 | 34 ± 8 |
| Males | 7 | 46 | 7 |
| Females | 6 | 18 | 0 |
| Diastolic blood pressure (mm Hg) | 93 ± 3 | 93 ± 4 | 91 ± 1 |

Values are mean = sd.
DBH in the normal subjects was 68 ± 35 IU (n = 116). The renin subgroups were also compared to DBH values in the normal group and significant differences were not found between the normals and the high-renin or normal-renin subgroups. The DBH values in low-renin hypertensives were significantly lower than normal subjects (p < 0.05). DBH values in all hypertensives (n = 84) (DBH = 55.6 ± 36 IU) were significantly lower when compared with DBH values in the normals (p < 0.01).

Table 2 compares DBH, PRA, total urinary catecholamines and urinary norepinephrine for each of the renin subgroups before treatment. Total and fractionated urinary catecholamines, as reported in μg/24 hours, and also calculated in μg/g creatinine, were not significantly different between the groups.

In further analyzing DBH values, our normal subjects displayed a bimodal distribution (fig. 2), as reported by others. In our normals, 20 persons had DBH levels of less than 35 IU, 34 had levels of 36–59 IU, and 62 had values greater than 60 IU (fig. 2.)

The 84 hypertensive subjects in this study are part of a larger group (n = 135). In the additional 51 subjects, simultaneous DBH, PRA and blood pressure data are available from the last pretreatment visit.

Table 2. Plasma Renin Activity (PRA), Dopamine β-hydroxylase (DBH), and Urinary Catecholamines Before Treatment in Patients With Essential Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Low renin</th>
<th>Normal renin</th>
<th>High renin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>64</td>
<td>7</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>0.8 ± 0.4</td>
<td>1.9 ± 1.2</td>
<td>6.8 ± 1.1</td>
</tr>
<tr>
<td>DBH (IU)</td>
<td>46 ± 30</td>
<td>59 ± 39</td>
<td>43 ± 22</td>
</tr>
<tr>
<td>Total urinary catecholamines (μg/24 hr)</td>
<td>41.4 ± 20</td>
<td>49.1 ± 25</td>
<td>48.2 ± 32</td>
</tr>
<tr>
<td>Urinary norepinephrine (μg/24 hr)</td>
<td>33.0 ± 19.9</td>
<td>34.2 ± 15.5</td>
<td>24.8</td>
</tr>
</tbody>
</table>

Values are mean ± sd.

These 51 subjects had inaccurate 24-hour urine collections and are not included in the catecholamine analysis or in the longitudinal aspects of this study. The frequency distribution of DBH for the entire hypertensive group (n = 135) is shown in fig. 3.

A non-normal, although non-bimodal, distribution of DBH is present. A possible relationship between DBH and both PRA and catecholamine excretion at the last pretreatment visit was analyzed by determining Spearman correlation coefficients. Using the larger hypertensive population of 135 persons, the following values were obtained: DBH and PRA (r = −0.04), DBH and systolic blood pressure (r = 0.06), DBH and diastolic blood pressure (r = −0.02). None of these correlations were significant.

With the hypertensives (n = 84) in whom complete urine collections were available, correlation coefficients were also determined for pretreatment DBH and each of the following pretreatment values: PRA, 24-hour urinary catecholamines, 24-hour urinary norepinephrine, systolic and diastolic blood pressure. These correlation coefficients were determined within each subgroup, i.e., normal-renin.
(n = 64), high-renin (n = 7), and low-renin essential hypertensives (n = 13) and for the entire hypertensive group (n = 84), and no significant correlations were detected.

We compared baseline data in the patients destined to receive chlorthalidone with those who would receive placebo. The patients were compared to each other within normal-renin, low-renin and high-renin hypertensive subgroups. This analysis was done to assure adequate randomization. The following variables, obtained at the last visit before medication, were compared: age, sex, systolic and diastolic blood pressure, PRA, DBH, 24-hour urine sodium excretion and total urinary catecholamines. There were no differences between groups for any of these parameters except for the ages in the normal-renin essential hypertensives. Patients who would receive chlorthalidone were 34.5 ± 9.9 years old, and those in the placebo group were 38.9 ± 8.2 years old (p < 0.05). Although this difference is statistically significant, the actual age difference is small and probably not clinically significant, particularly since other factors such as PRA, DBH and blood pressure and catecholamines were the same.

After Chlorthalidone or Placebo Treatment

One month after patients were randomly assigned to active treatment or placebo groups, the effect of chlorthalidone therapy was assessed in all patients. In the patients (low-, normal- and high-renin combined) treated with chlorthalidone, systolic pressure (pretreatment: 135 ± 10 mm Hg, and posttreatment 126 ± 10 mm Hg, n = 38; p < 0.001) and diastolic pressure decreased as expected (table 3).

PRA increased as expected. DBH did not change after 1 month of chlorthalidone therapy. The total urinary catecholamine excretion and urinary noradrenaline increased after treatment.

The subjects receiving placebo showed minimal decreases in systolic pressure (pretreatment 137 ± 13 mm Hg; posttreatment 134 ± 15; n = 42), diastolic pressure and renin activity. In addition, in the placebo group, the DBH was not different 1 month after placebo therapy when compared with control values.

<table>
<thead>
<tr>
<th>Table 3. Effect of Treatment on Plasma Renin Activity (PRA), Dopamine β-Hydroxylase (DBH), and Urinary Catecholamines in Patients With Essential Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients receiving chlorthalidone 50 mg/day</td>
</tr>
<tr>
<td>Pretreatment</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
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<tr>
<td>DBH (IU)</td>
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<tr>
<td>Total urinary catecholamines (µg/24 hr)</td>
</tr>
<tr>
<td>Urinary norepinephrine (µg/24 hr)</td>
</tr>
</tbody>
</table>

Values are mean ± sd.
Comparison of pre- and posttreatment values:
* p < 0.001.
† p < 0.01.
‡ p < 0.02.
The changes in DBH from pretreatment to posttreatment were not significantly different between the chlorthalidone-treated group (1.7 ± 6.7 IU) and the placebo group (−1.30 ± 9.95 IU). Also in the placebo group, the total urinary catecholamines and urinary norepinephrine values were not different after placebo therapy (table 3).

For the entire hypertensive group (low-, normal- and high-renin combined; n = 84), changes in DBH from pre- to posttreatment were not significantly correlated with changes in PRA (r = 0.04) or changes in total catecholamine excretion (r = −0.10). For the 39 patients treated with diuretics, changes in DBH from pre- to posttreatment were also not significantly correlated with ΔPRA (r = −0.02), Δurinary catecholamines (r = −0.02), or Δurinary norepinephrine (r = 0.06).

To assess the possibility that DBH may be predictive of subsequent changes in the sympathetic nervous system, pretreatment DBH was correlated with the change in urinary catecholamines and PRA in the diuretic group (n = 39). Pretreatment DBH did not significantly correlate with changes in PRA (r = 0.26), changes in total catecholamine excretion (r = −0.09) or changes in urinary norepinephrine (r = −0.03).

To further assess DBH levels in relationship to the renin-angiotensin system and catecholamine excretion in subjects receiving diuretic therapy, the one-third of normal PRA subjects with the lowest pretreatment DBH values (n = 10) were compared to the one-third with the highest pretreatment DBH levels (n = 10). After diuretics, the one-third with lowest pretreatment DBH values had the following values: DBH = 19.6 ± 1.4 IU (SEM); ΔPRA = 2.9 ± 1.8 ng/ml/hr; Δurinary catecholamines = 9.3 ± 6.7 μg/24 hr. The one-third of patients with the highest pretreatment DBH values were as follows: DBH = 89.7 ± 4.1 IU (p < 0.001); ΔPRA = 8.2 ± 1.6 ng/ml/hr (p < 0.05); Δurinary catecholamines = 13.2 ± 3.8 μg/24 hr (NS).

Discussion

Conflicting results have been reported regarding levels of DBH in relation to human essential hypertension. Our finding of lower DBH values in our hypertensives compared with our controls differs from some other studies. Stone et al. reported that plasma DBH was highest in patients with primary hypertension and differed from the lower values in normal subjects and patients with secondary forms of hypertension. The normal group in Stone's study contained many subjects in whom DBH levels were low (DBH < 35 IU = 79% of group; DBH > 60 IU = 14% of group). In our normal group, only 17% of subjects had DBH levels < 35 IU, 29% 36–59 IU, and 53% DBH > 60 IU. The normals in Stone's study included 31% black subjects, while all our normals were white. As blacks reportedly have lower DBH values, racial differences may be one factor in the differences between our normals and Stone's. Aoki et al. also found elevated DBH levels in patients with essential hypertension and low activities of DBH in both normotensive and hypertensive patients in chronic renal failure on prolonged hemodialysis. Schanberg and associates reported elevated serum DBH in labile and fixed essential hypertensives compared with normals.66 Lability of blood pressure was not studied in either our normals or hypertensives. Geffen et al. also reported a positive correlation between the resting diastolic blood pressure and plasma DBH levels.29 Alexandre and associates4 reported that only in their borderline hypertensives did DBH correlate directly with diastolic pressure and cardiac output. In contrast to others, Alexandre found no differences in DBH levels between patients with hypertension and healthy subjects. Horwitz and colleagues, in studying both normals and patients with hypertension, found that the serum DBH activity did not correlate with blood pressure or age. Aberg et al. also failed to show a significant relationship between DBH and blood pressure and did not detect a significant difference in levels of DBH between normal and hypertensive subjects. Lovenberg, in a review moderated by Kopin, also could not find a significant correlation between DBH and blood pressure. Leon et al. concluded that DBH did not appear to be related to blood pressure and is of limited value as an indicator of sympathetic nervous system activity.

Some of the controversy results from the fact that there is an extremely wide range of normal DBH levels and the ranges overlap in normotensive and hypertensive populations. However, DBH levels remain quite constant with repeated measurement over several months.

In our population, the range in both normals and hypertensives is so broad that the modest difference between the groups has little physiologic significance. In our study, an individual DBH value did not distinguish a hypertensive patient from a normal subject.

The variability in data reported by different investigators may also be due to different methods of measuring DBH, as reviewed by Nagatsu and Udenfriend.25 The assay used in this study is that of Nagatsu and Udenfriend, and uses optimal pH (5.0) and saturating amounts of the substrate tyramine, producing maximum values of DBH.

Kaneko suggested that abnormalities in the sympathetic nervous system may be a mechanism for suppressed renin activity. The role of the sympathetic nervous system in suppressed PRA has also been examined by Jose, Crout and Kaplan, who concluded that the suppressed renin may be caused by inhibition of renin releasing mechanisms, but does not appear to be caused by an intrinsic defect in the sympathetic nervous system. Conflicting results by Esler suggest that low-renin essential hypertension is caused by suppression of the sympathetic nervous system. Noth and Mulrow also reported low DBH levels in low-renin hypertension.

When our patients were categorized according to renin profile, the DBH values in the low-renin sub-
The finding of low DBH values in low-renin hypertensives is similar to that reported by Noth and Mulrow.11 The significance of this finding, however, is unclear. If renin and DBH both reflected the activity of the sympathetic nervous system, one might expect low DBH values in low-renin essential hypertension, and high DBH values in high-renin essential hypertension. Our high-renin hypertensives, however, had DBH values similar to those in our low-renin patients. In addition, DBH levels were not significantly different between the subgroups nor between the normal-renin and high-renin hypertensives and the controls. Our failure to show a significant correlation between DBH and PRA in the entire hypertensive study group (n = 135) before antihypertensive therapy suggests that DBH and PRA are not related. DBH and renin may be related at the low end of the renin scale due to unknown factors which influence both.

Total and fractionated urinary catecholamines have been used as an accepted index of sympathetic activity.7, 8, 31, 32 We observed no difference in urinary catecholamines between the low-, normal- or high-renin groups before treatment. We did not find significant correlations between total and fractionated urinary catecholamines and either PRA or DBH before diuretic treatment. Jose et al.8 also did not find a significant correlation between urinary norepinephrine excretion and PRA. Lake et al.33 also failed to find a significant correlation between DBH and plasma norepinephrine in resting and exercising subjects.

We wished to determine if DBH was a marker for changes in catecholamine excretion when the same persons received diuretic therapy. After 1 month of chlorthalidone therapy, total urinary catecholamines and norepinephrine increased, but DBH did not change. In addition, DBH levels in patients who took diuretics were not different from DBH levels in the placebo group. Since the total DBH pool is large,34 it is possible that small changes in DBH levels, over 1 month, may not be detected.

DBH may reflect a chronic state of sympathetic nervous system activity; it could predict sympathetic nervous system response to stimuli such as volume depletion induced by diuretic therapy. We therefore determined correlation coefficients between pretreatment DBH or change in DBH and the changes in PRA, total urinary catecholamines, and urinary norepinephrine in the diuretic-treated group. None of the correlations were significant.

We compared one-third of the normal PRA patients with lowest pretreatment DBH values to the third with the highest pretreatment DBH values. Using this arbitrary division, the lower DBH patients showed significantly less change in PRA compared with the higher DBH patients. The change in urinary catecholamines, however, was not different between the two groups.

The physiologic significance of these data is hard to determine. Both the low DBH and low PRA responsiveness in some of these patients may reflect some other abnormality, perhaps in sodium balance. Low PRA activity in hypertensives has also been postulated to reflect a state of chronic sodium excess, perhaps in part related to increased aldosterone sensitivity to the low levels of angiotensin II.24 Lake and Ziegler have reported that acute saline loading in normal and essential hypertensives leads to a small but significant decrease in DBH.8 Grobecker et al. have reported that DOCA-salt hypertensive rats have a tendency toward lower plasma DBH levels.86 It is therefore possible that in some patients with essential hypertension, DBH values may be related to PRA to diuretic therapy, perhaps as a marker of some other abnormality.

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References

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