Lack of Correlation of Plasma Norepinephrine and Dopamine-β-hydroxylase in Hypertensive and Normotensive Subjects

CHARLES R. LAKE, MICHAEL G. ZIEGLER, MICHAEL COLEMAN, AND IRWIN J. KOPIN

SUMMARY Dopamine-β-hydroxylase (DBH) and norepinephrine (NE) have been determined in over 350 plasma samples from 174 subjects while resting supine (basal sample), standing, or exercising. Although increments in both NE and DBH were found with postural change, the further increase in plasma levels of NE during exertion was not attended by any change in levels of DBH. There was no significant correlation between basal levels of DBH and NE nor was there any correlation in their increments after standing or exercising. DBH activity in plasma of subjects with moderate essential hypertension was not different from that of normotensive subjects. It is concluded that plasma DBH is a poor index of acute sympathetic neuronal activity.

THE ENZYME dopamine-β-hydroxylase (DBH), which is responsible for the formation of norepinephrine (NE) from dopamine,1 is released along with the catecholamines from the adrenal medulla2 and from stimulated sympathetic nerves in perfused organs.3-5 The enzyme is present in plasma of man and other animals.6 In animals, the levels of the enzyme are increased with stress7 and decreased after chemical destruction of the sympathetic nerve endings but not after adrenalectomy.8 Thus, DBH in plasma appears to come from sympathetic nerve endings. In man, levels of DBH in plasma are reported to be increased by procedures that increase sympathetic neuronal activity, e.g., exercise,9-11 immersion of the hand in cold water,10 and insulin-induced hypoglycemia.12 Basal levels of DBH vary widely between normal individuals, and the degree of elevation of the enzyme with stress is usually relatively small (10-25%), so increases in enzyme levels are not always observed after stress.13 Furthermore, during the cold pressor test, levels of the other large protein molecules increase in parallel with DBH.14 Thus, some investigators have found DBH to be a useful index of sympathetic function15-20 while others have not.21-27 Because of the controversy over the meaning of small changes in DBH activity, Noth et al.28 have stressed the need for the analysis of NE and DBH from the same plasma samples to determine whether DBH is an index of acute changes in sympathetic neuronal activity. In the present study, changes in plasma levels of DBH, total protein, a representative large protein molecule (prolactin), and NE are examined in normotensive, healthy subjects at rest, while standing, and after a standard exertion known to produce an increase in plasma levels of NE and pulse rate.29 Since some studies have found DBH activity to be elevated in hypertensive patients or correlated with blood pressure15-16-30 but other studies have not,21-24,25,31,32 we also measured DBH of subjects with essential hypertension under basal conditions and while standing.

Methods

Normotensive Caucasian volunteers (68 individuals of both sexes), without significant abnormalities on the basis of medical history and physical examination, and who ranged in age from 10 to 70 years, and 106 outpatients (age range, 16 to 78 years) with mild to moderate essential hypertension were asked not to take medication for 7 days nor tobacco, coffee, or tea for at least 3 hours prior to reporting for the test procedures. After a thorough explanation of the procedure, subjects gave written consent and they were asked to lie supine and relax in a quiet room. The needle of a "heparin lock" with a 3-way stopcock was inserted into an antecubital vein. A solution containing 30 units of sodium heparin (Upjohn) per milliliter sterile saline was used to flush the catheter and
prevent clotting. A blood sample (12-ml) was obtained from all subjects after they appeared to be relaxed and had a stable pulse rate, but no sooner than 20 minutes after venipuncture (basal sample). After the basal sample was obtained, 51 normotensive and 104 hypertensive subjects stood and another blood sample was taken after 10 minutes. Thirty-seven of the standing normotensive subjects were asked to remain standing and to maintain 30% of their maximal grip on a hand dynamometer (Asimow Engineering Corp.) for 5 minutes, when a third blood sample was obtained.29 Blood samples were cooled in iced 20-ml Vacutainer tubes (Becton-Dickinson) which contained 2 ml of acid-citrate-dextrose (ACD) or were heparin-coated. All samples were centrifuged at 4°C within 30 minutes and the plasma was transferred to Falcon no.2006 tubes and frozen at −70°C until assayed for NE,29 DBH,6 protein,33 and prolactin34 from the same plasma samples.

Results
Change from supine to erect posture for 10 minutes significantly increased plasma levels of total protein, prolactin, and DBH by 10-20% (Fig. 1). Levels of NE in plasma, however, were increased by 87%. The increase in levels of DBH (16± 5%) appeared to be somewhat greater than those of either total protein (8 ± 2%) or prolactin (10 ± 4%), and the increase in DBH (expressed in units of activity per milligram of protein) after standing was significant (p < 0.05). After 37 normotensive subjects had sustained an isometric hand grip for 5 minutes while standing, plasma levels of NE increased to 170% of the basal level, but there were no further changes in total protein, prolactin, or DBH (Fig. 1).

During rest, there was no relationship between plasma levels of NE and DBH activity in normotensive (Fig. 2) or hypertensive subjects, nor were the increases (or percent increases) in NE related to levels, increases, or percent increases of DBH activity (Table 1). Basal DBH activity was not related to blood pressure or pulse rate, but the percent increases in DBH and pulse rate upon standing correlated significantly (r = 0.45; p < 0.003) in the normotensive subjects only.

The levels of DBH in the hypertensive and normotensive subjects did not differ significantly (Table 2). Basal DBH activity ranged from 0 to 1898 units in normotensive subjects and from 7 to 2180 units in the hypertensive subjects.

Discussion
A variety of procedures known to increase sympathetic nervous activity in humans are reported to result in increases in plasma levels of DBH,9-12 but this change has not been observed consistently.13 Stone and associates14 note that increases in DBH activity during the cold pressor test are accompanied by changes in levels of other plasma proteins, suggesting that changes in plasma volume might account for the changes in DBH levels. Ton et al.25 suggest that when people stand, changes in plasma volume might explain changes in levels of cholesterol, triglycerides, and total protein as well as in hematocrit. In this study, the percent increase in plasma levels of DBH after standing erect for 10 minutes was not significantly greater than the percent changes in total plasma protein or in another large protein molecule, prolactin; the ratio of DBH to total protein, however, increased significantly (p
because the small molecules of prolactin can more readily reduce an increase in DBH which exceeds that of prolactin ganglion or after denervation of the carotid sinus. Indeed, DBH has been found in human lymph, and the levels of DBH in lymph of the dog increase with stimulation of the stellate ganglion or after denervation of the carotid sinus. The increase of DBH activity in plasma that attends standing has a long half-life estimated at over 10 hours. Thus, transfer of DBH from extracellular fluids which contain relatively high levels of accumulated DBH. This could provide an explanation for acute elevation of the enzyme during postural changes or exercise. The additional sympathetic activity evoked by isometric exercise with one hand would not then be expected to produce any change in plasma DBH, although the levels of catecholamines are elevated further. The lack of correlation between basal plasma levels of DBH activity and NE suggests that DBH is not a reliable index of the degree of sympathetic neuronal discharge of the catecholamine in individuals. Furthermore, based on the results obtained in normotensive and hypertensive subjects (Table 1), DBH levels do not provide an index of the response of NE release evoked by standing. There was no correlation between the percent increase in DBH levels and the percent increase in NE levels (Table 1), although in a previously reported series of 12 normotensive subjects there did appear to be such a relationship. The positive significant correlation between percent increases in DBH and pulse rate might be explained if both parameters reflect the degree of circulatory adjustment to entry of interstitial fluid into the circulation after postural change. It is not surprising that DBH levels fail to reflect short-term increases in sympathetic nervous activity. DBH has a long half-life estimated at over 10 hours. Thus, transfer of DBH from extracellular fluid to the circulation could provide an explanation for acute elevation of the enzyme during postural changes or exercise. The additional sympathetic activity evoked by isometric exercise with one hand would not then be expected to produce any change in plasma DBH, although the levels of catecholamines are elevated further.

### Table 1: Correlation of Basal Levels and Increments in Plasma Levels of Norepinephrine (NE) and Dopamine-ß-hydroxylase (DBH) in Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Basal NE vs. basal DBH</th>
<th>Increment* NE vs. basal DBH</th>
<th>Increment NE vs. increment DBH</th>
<th>% Increment NE vs. % increment DBH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives</td>
<td>r &lt; 0.04 (68)</td>
<td>-0.09 (51)</td>
<td>-0.04 (34)</td>
<td>-0.26 (33)</td>
</tr>
<tr>
<td></td>
<td>P 0.69 (68)</td>
<td>0.60 (51)</td>
<td>0.81 (34)</td>
<td>0.15 (33)</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>r &lt; 0.05 (106)</td>
<td>0.05 (104)</td>
<td>-0.01 (104)</td>
<td>-0.06 (104)</td>
</tr>
<tr>
<td></td>
<td>P 0.64 (106)</td>
<td>0.59 (104)</td>
<td>0.96 (104)</td>
<td>0.56 (104)</td>
</tr>
</tbody>
</table>

Abbreviations: r = correlation coefficient for the indicated variables; P = level of significance (no significant correlations were noted); n = number of subjects included in calculating the correlation coefficients. In some normotensive subjects, levels of DBH or NE while standing were not determined.

* The increase in levels upon standing (standing level minus basal level).
† Subjects were not included who had DBH activity below 100 units (one normotensive subject).

The 87% increase in plasma levels of NE clearly indicates a significant increase in sympathetic neuronal activity. The further increase (to 170% basal) of NE levels after 5 minutes of hand grip indicates an even greater level of sympathetic neuronal activity during this period of exertion, but there was no further alteration in levels of DBH or in the levels of other proteins measured (Fig. 1). Thus, in a single individual, increases in plasma levels of DBH do not appear to be related to the level of sympathetic neuronal activity even though there is considerable evidence that plasma DBH is derived from sympathetic nerve endings. DBH, however, is a large molecule and, except in organs such as the spleen, may not readily pass directly into the blood stream. Indeed, DBH has been found in human lymph, and the levels of DBH in lymph of the dog increase with stimulation of the stellate ganglion or after denervation of the carotid sinus. The increase of DBH activity in plasma that attends standing or exercise might be due to a transfer to the circulation of the protein from extracellular fluids which contain relatively high levels of accumulated DBH. This could produce an increase in DBH which exceeds that of prolactin because the small molecules of prolactin can more readily leave the circulation. Thus, transfer of DBH from extracellular fluid to the circulation could provide an explanation for acute elevation of the enzyme during postural changes or exercise. The additional sympathetic activity evoked by isometric exercise with one hand would not then be expected to produce any change in plasma DBH, although the levels of catecholamines are elevated further. The lack of correlation between basal plasma levels of DBH activity and NE suggests that DBH is not a reliable index of the degree of sympathetic neuronal discharge of the catecholamine in individuals. Furthermore, based on the results obtained in normotensive and hypertensive subjects (Table 1), DBH levels do not provide an index of the response of NE release evoked by standing. There was no correlation between the percent increase in DBH levels and the percent increase in NE levels (Table 1), although in a previously reported series of 12 normotensive subjects there did appear to be such a relationship. The positive significant correlation between percent increases in DBH and pulse rate might be explained if both parameters reflect the degree of circulatory adjustment to entry of interstitial fluid into the circulation after postural change. It is not surprising that DBH levels fail to reflect short-term increases in sympathetic nervous activity. DBH has a long half-life estimated at over 10 hours. Thus,

### Table 2: Plasma DBH Activity in Normotensive and Hypertensive Clinic Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Basal†</th>
<th>Standing§</th>
<th>Squeezing¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives</td>
<td>(n = 68)</td>
<td>35 ± 2</td>
<td>745 ± 62</td>
<td>858 ± 86</td>
</tr>
<tr>
<td>Essential hypertensives (n = 106)</td>
<td>42 ± 1</td>
<td>656 ± 42</td>
<td>755 ± 48</td>
<td>§</td>
</tr>
<tr>
<td>P (Student's t-test)</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

* Dopamine-ß-hydroxylase (DBH) in units (1 unit is 1 nmol of phenylethylamine converted to phenylethanolamine per milliliter of plasma per hour of incubation) in nonmedicated subjects; values are expressed as means ± SEM. NS = not significant.
† Samples taken at least 20 minutes after venipuncture in supine resting subjects.
‡ Samples taken after standing for 10 minutes (51 normotensive subjects and 104 hypertensive subjects had blood sampled at this time).
¶ Test not conducted in hypertensive subjects.
during periods of normal levels of sympathetic nervous activity in any 10-minute interval, less than 1% of the total amount of plasma DBH is added to the circulation. Since a 10% increase in DBH is the smallest increment that can be determined reliably, it appears unlikely that the amount of DBH released from sympathetic nerves into the blood over a period of a few minutes can be detected easily. Only prolonged changes in sympathetic nervous activity as might occur in chronic diseases should give notable changes in DBH levels.

In Huntington’s disease, familial dystonemia, Shy-Drager syndrome, idiopathic orthostatic hypotension, and Lesch-Nyhan syndrome DBH activity and NE responsivity are both diminished, compared with normal controls of similar age. There is, however, no consistent relation between levels of NE and DBH in the plasma of these individual subjects. In some families with the autosomal dominant form of torsion dystonia, plasma levels of both NE and DBH are significantly elevated. In other diseases, such as primary autism and Trisomy-21 (Lake, Ziegler, and Coleman, unpublished observation), DBH activity is significantly lower than in age-matched control subjects, but NE levels in plasma are normal and respond normally or excessively to the stresses of venipuncture and upright posture.

In contrast to the results reported in some studies but consistent with other reports, DBH activity was not higher in the group of 106 outpatients with essential hypertension than in the 68 normotensive controls of similar age. There is, however, no consistent with other reports, 21-M-25-31-32 DBH activity in any 10-minute interval, less than 1% of the total amount of plasma DBH is added to the circulation. Since a 10% increase in DBH is the smallest increment that can be determined reliably, it appears unlikely that the amount of DBH released from sympathetic nerves into the blood over a period of a few minutes can be detected easily. Only prolonged changes in sympathetic nervous activity as might occur in chronic diseases should give notable changes in DBH levels.

In conclusion it appears that in the same individual, plasma DBH activity is not related to sympathetic neuronal activity as reflected by plasma NE levels. Acute changes in circulating levels of DBH may be the result of redistribution of the enzyme in body fluids rather than alteration in rates of release of the enzyme from sympathetic nerve endings. In some chronic disease states, however, both DBH and NE levels in plasma may be elevated or diminished in parallel. DBH activity in hyperactive subjects does not appear to be different than in normotensive subjects.

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