The meaning of dopamine β-hydroxylase in essential hypertension

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(Received 4 August 1975)

Summary

1. Resting plasma dopamine β-hydroxylase (DBH) activity and haemodynamic parameters were studied in untreated borderline (twenty-nine) and permanent (twenty-seven) essential hypertensive patients. DBH was also measured in sixty-three apparently healthy subjects.

2. Mean DBH values were not significantly different between the groups.

3. Cardiac output, cardiopulmonary blood volume and the cardiopulmonary blood volume/total blood volume ratio (CPBV/TBV) were significantly higher in borderline than in permanent hypertensive patients.

4. In borderline hypertensive patients, plasma DBH activity was directly correlated with diastolic arterial pressure and with values of cardiac output, cardiopulmonary blood volume and CPBV/TBV ratio. No such correlations could be observed in the permanent hypertensive group.

5. These results suggest that plasma DBH activities in borderline hypertension mainly depend on the sympathetic activity responsible for the haemodynamic variations. Contrariwise, plasma DBH activities in permanent essential hypertensive patients appear to reflect other factors.

Key words: cardiac output, cardiopulmonary blood volume, dopamine β-hydroxylase, essential hypertension, sympathetic tone.

Introduction

Dopamine β-hydroxylase (EC 1.14.17.1), the enzyme that catalyses the hydroxylation of dopamine on the beta carbon atom to form noradrenaline (Kaufman & Friedman, 1965), is localized in the chromaffin granules of the adrenal medulla (Kirshner, 1957) and the vesicular structures of the sympathetic nerves (Potter & Axelrod, 1963; Stjärne & Lishajko, 1967; Hörttagl, Hörttagl & Winkler, 1969). In response to nerve stimulation (Gewirtz & Kopin, 1970; Weinshilboum, Thoa, Johnson, Kopin & Axelrod, 1971b), the content of the sympathetic vesicles including noradrenaline and the soluble form of DBH(1) is released by exocytosis (Viveros, Arqueros & Kirshner, 1968).

In contrast with catecholamines, which immediately undergo axonal reuptake, DBH persists in the circulation with a much longer half-life (Rush & Geffen, 1972). For this reason, it has been suggested that DBH may serve as an index of sympathetic activity. This theory is now supported by a growing number of pharmacological and clinical studies. The proportional release of noradrenaline and DBH is enhanced by phenoxybenzamine (De Potter, Chubb, Post & De Schepdryver, 1971; Johnson, Thoa, Weinsilboum, Axelrod & Kopin, 1971), calcium (Johnson et al., 1971), swimming (Roffman, Freedman & Goldstein, 1973) or immobilization stress (Weinsilboum, Kvetnansky, Axelrod & Kopin, 1971a) and reduced by prostaglandins (Johnson et al., 1971), colchicine, vinblastin, cytotoxic B (Thoa, Wooten, Axelrod & Kopin, 1972)

(1) Abbreviation: DBH, dopamine β-hydroxylase.
and chemical sympathectomy (Weinshilboum & Axelrod, 1971c). Increase in human plasma activity also occurs during brief physical exercise (Planz & Palm, 1973), cold pressor test (Wooten & Cardon, 1973); inversely, a reduction related to impaired sympathetic function is observed in some familial dysautonomia patients (Weinshilboum, Alexander, Lovenberg, 1973; Palm, Axelrod, 1971b). However, the view that plasma DBH may be useful in measuring sympathetic activity in human hypertension, is not accepted by all investigators (Geffen, Rush, Louis & Doyle, 1973; Horwitz, Alexander, Lovenberg & Keiser, 1973; Louis, Doyle, Anavekar, Johnston, Geffen & Rush, 1974; Schanberg, Stone, Kirshner, Gannels & Robinson, 1974; Stone, Gannels, Robinson, Schanberg & Kirshner, 1974).

The present work was designed to explore further the possibility that plasma DBH activities might reflect the peripheral sympathetic activity in essential hypertension. The study appeared to be of particular interest in borderline hypertension, where a hyperkinetic state with high cardiac output (Bello, Sevy & Harakal, 1965; Finkelman, Worcel & Agrest, 1965; Kuramoto, Murata, Yasaki, Ikeda & Nakao, 1968; Lund-Johansen, 1967; Safar, Weiss, Levenson, London & Milliez, 1973; Widimsky, Fejfarova & Fejfar, 1957) has generally been ascribed to sympathetic hyperactivity (Doyle & Smirk, 1955; Goldenberg, Pines, Baldwin, Green & Roh, 1948; Tarazi & Dustan, 1973; McCubbin & Page, 1963).

Plasma DBH activities and haemodynamic parameters including cardiac output, total and cardiopulmonary blood volumes, were therefore studied in permanent and borderline hypertensive patients.

Methods

Patients

Haemodynamic and DBH determinations were performed in fifty-six male hypertensive patients. The type of the hypertension was based on outpatient blood pressure recordings. The abolition of the Korotkoff sounds was taken to indicate diastolic pressure. Patients were considered as borderline (twenty-nine cases) when, in the past 12 months, at least three casual blood pressure readings showed one diastolic pressure of 90 mmHg or more and one diastolic pressure of less than 120 mmHg (Julius & Conway, 1968). Age ranged from 20 to 43 years (mean $27 \pm 2$). Patients (twenty-seven cases) were considered to have permanent hypertension when diastolic pressure stayed constantly above 100 mmHg during the past year. Age ranged from 25 to 45 years (mean $35 \pm 2$). All these patients were hospitalized for 6 days. The borderline and eighteen permanent hypertensive patients were untreated. The remaining nine permanent hypertensive patients discontinued their therapy ($\alpha$-methyl-dopa with diuretics) at least 4 weeks before the study. No patient had been taking reserpine or guanethidine.

All subjects were considered to be essential hypertensive patients only after extensive negative investigation including blood and urinary electrolytes, urinary catecholamines, endogenous creatinine clearance, timed intravenous urography with washout test and/or renal arteriography. None had cardiac or neurological involvement. Optic fundi were either normal (thirty-one patients) or showed some arteriovenous nicking (twenty-five permanent hypertensive patients).

DBH activity was also measured in sixty-three apparently normal subjects. Age ranged from 18 to 67 years, with a mean of $42 \pm 3$ years. Their mean diastolic arterial pressure was $72.9 \pm 1$ mmHg. By reason of ethical considerations, they were not submitted to the haemodynamic investigation.

Six days before the explorations, all subjects were placed on a sodium intake of 110 mmol/day because of the heavy repercussion of sodium intake on the haemodynamic parameters and its possible influence on DBH.

Biochemical assay

Plasma DBH activity was measured according to the method of Nagatsu & Udenfriend (1972). Samples of peripheral venous blood were collected during the morning hours (generally at 08.00 hours) just before the haemodynamic measurement.

Haemodynamic determinations

Haemodynamic study was performed according to previously detailed methods (Safar, Weiss, London, Frackowiak & Milliez, 1974) after informed consent had been given by the patients.

Cardiac output was measured at least twice on subjects in the supine position. Water's cuvette was used. Cardiac output was expressed in ml min$^{-1}$.
TABLE 1. Age, haemodynamic parameters and dopamine β-hydroxylase activities of the hypertensive patients

SAP = systolic arterial pressure; DAP = diastolic arterial pressure; MAP = mean arterial pressure; CPBV = cardiopulmonary blood volume; TBV = total blood volume; DBH = dopamine β-hydroxylase; NS = not significant.

<table>
<thead>
<tr>
<th>Hypertensive state</th>
<th>Age (years)</th>
<th>Arterial pressure (mmHg)</th>
<th>Cardiac output (ml min⁻¹kg⁻¹)</th>
<th>CPBV (ml/kg)</th>
<th>TBV (ml/kg)</th>
<th>CPBV/TBV (%)</th>
<th>DBH (i.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borderline</td>
<td>27</td>
<td>140 85 105</td>
<td>98 ± 4</td>
<td>14.5 ± 0.5</td>
<td>73.4 ± 1.5</td>
<td>20.02 ± 0.67</td>
<td>40 ± 4</td>
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<td>(n = 29)</td>
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<tr>
<td>Permanent</td>
<td>35</td>
<td>180 112 138</td>
<td>81 ± 4</td>
<td>12.5 ± 0.5</td>
<td>71.1 ± 1.3</td>
<td>17.69 ± 0.63</td>
<td>43 ± 5</td>
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<tr>
<td>(n = 27)</td>
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<td>P</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001 &lt; 0.001 &lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.015</td>
<td>NS</td>
<td>&lt; 0.02</td>
<td>NS</td>
</tr>
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</table>

Mean arterial pressure (MAP) was measured with a Thomson–Telco apparatus.

Cardiopulmonary blood volume (CPBV) was defined as the volume between the main pulmonary artery and the tip of the arterial catheter placed into the aortic root. It was estimated by the Hamilton method (Hamilton, Moore, Kinsman & Spurling, 1932) as follows:

\[
CPBV (\text{ml/kg}) = \frac{CO (\text{ml s}^{-1} \text{kg}^{-1}) \times Tm (PA-Ar) (s)}{}
\]

where Tm (PA–Ar) is the mean transit time (s) from the pulmonary artery (PA) to the tip of the arterial catheter (Ar). A correction factor for the sampling system was subtracted from observed Tm.

Total blood volume (TBV) was measured, as previously detailed (Safar et al., 1974), by the isotope-dilution technique on patients in the recumbent position, and was expressed in ml/kg. The CPBV/TBV ratio was defined as the fraction of TBV in the heart and lungs.

Classical methods (difference of mean values, correlations, stepwise regression) were used (Croxton & Cowden, 1944) for statistical analysis. All mean values are given ± SEM.

Results

Plasma DBH activities in apparently normal males ranged from near 0 to 100 i.u. with a mean value of 41.2 ± 3 i.u.; 80% had DBH activities below 60 i.u. In borderline and permanent hypertensive patients, mean values were respectively 40 ± 4 and 43 ± 5 i.u. and these were not significantly different from values

Fig. 1. Relation between plasma DBH activity and diastolic arterial pressure in borderline hypertensive patients.

Fig. 2. Relation between plasma DBH activity and diastolic arterial pressure in normal subjects.
in healthy subjects. 86% of borderline and 77% of permanent hypertensive patients had plasma DBH activities below 60 i.u. Plasma DBH was not related to age in the hypertensive patients as a whole or when they were separated into two groups. Results of haemodynamic investigations are given in Table 1.

Plasma DBH activities were directly related with diastolic arterial pressure in borderline hypertensive patients ($r =+0.59; P<0.001$) (Fig. 1), but not in control subjects ($r =-0.01$) (Fig. 2) or in permanent hypertensive patients ($r =-0.35$) (Fig. 3).

In borderline hypertensive patients there was also a positive correlation between DBH and cardiac output ($P<0.005$) (Fig. 4), and between DBH and the CPBV/TBV ratio ($P<0.005$) (Fig. 5).

**Discussion**

Plasma DBH activity is fairly constant for a given individual over extended periods (Horwitz et al., 1973; Rush, Nagatsu, Geffen & Udenfriend, 1973). The values which we have found in healthy persons are similar to those reported by others (Nagatsu & Udenfriend, 1972).

Plasma DBH activity depends not only on peripheral sympathetic activity but also on other factors as evidenced by the wide range of normal values (Horwitz et al., 1973; Weinshilboum & Axelrod, 1971a), the small changes which are brought about by stress (Planz & Palm, 1973) and familial differences (Weinshilboum et al., 1971b; Weinshilboum, Thoa, Johnson & Weidman, 1973; Wooten, Elridge, Axelrod & Stern, 1973). The question has therefore to be raised whether DBH activity yields valid information about the status of the sympathetic nervous system in human hypertension.

It is generally accepted that borderline hypertensive patients are characterized by a sympathetic activity apparently responsible for some of the clinical symptoms, e.g. modification of the vascular response to noradrenaline (Doyle & Smirk, 1955; Goldenberg et al., 1948; McCubbin & Page, 1963; Tarazi & Dustan, 1973), and a characteristic haemodynamic pattern (Bello et al., 1965; Finkelman et al., 1965; Kuramoto et al., 1968; Lund-Johansen, 1967; Safar et al., 1973; Widimsky et al., 1973).
1957). This is borne out in the present study, in which borderline hypertensive patients exhibited higher cardiac output associated with an increase in CPBV and the CPBV/TBV ratio. Previous findings (Safar et al., 1974) suggest that these increases result from a rise in venous tone, consequent on a sympathetic overactivity. In this study, plasma DBH activities correlated closely with cardiac output and with other haemodynamic parameters (CPBV/TBV ratio and diastolic arterial pressure), which this and other (Safar et al., 1974) studies have shown to be directly related to cardiac output. The positive correlations between those haemodynamic parameters and plasma DBH values suggest a common dependence on sympathetic overactivity. Plasma DBH activities seem therefore to reflect essentially the peripheral sympathetic tone in borderline hypertension.

In permanent hypertensive patients, none of these correlations could be detected. In particular plasma DBH was not related to diastolic arterial pressure, as in normal subjects. The lack in correlation indicates that one or both the two factors are relatively independent of the sympathetic tone. Permanent hypertension appears to be related to a volume mechanism rather than to a neural mechanism (Safar et al., 1974) and to be characterized by a weak sympathetic tone (Finkielman et al., 1965; De Quattro & Miura, 1973; Tarazi & Dustan, 1973). The same wide range and a similar distribution of plasma DBH values are noted as well in established hypertensive patients and control subjects exhibiting low sympathetic tone as in borderline hypertensive patients whose plasma DBH activities seem to reflect sympathetic activity. It may therefore be suggested that, in permanent hypertensive patients and apparently healthy subjects, plasma DBH activity is essentially determined by non-sympathetic factors.

On the basis of the proposed interpretation of plasma DBH values in the two groups of hypertensive patients, and in normal subjects, the finding of similar mean DBH values in the three populations seems rather surprising. It should, however, be remembered that in borderline hypertensive patients, plasma DBH activities increase in proportion to the diastolic arterial pressure and/or the cardiac output. The most likely explanation is that there is a continuous distribution of cardiac output and/or diastolic arterial pressure results in borderline hypertensive patients, which includes low and high values, giving a mean plasma DBH activity close to that in the two other groups. Mean value of plasma DBH activity would have been significantly higher had the study of the borderline hypertensive patients concerned only patients with increased cardiac output.

The present study thus indicates that baseline plasma DBH activity may yield information about the resting activity of the sympathetic system only in borderline hypertension. This result is in disagreement with the previous conflicting reports, which denied or suggested that a single plasma DBH activity constituted a satisfactory index of sympathetic function in human hypertension. Against this concept, Horwitz et al. (1973) failed to observe (in ninety normal subjects and seventy-eight borderline or overt hypertensive patients) either a correlation between plasma enzyme activities and blood pressure, or a reduction of plasma activity after potent anti-adrenergic therapy, or abnormal cardiovascular function in patients with exceptionally low plasma DBH values. However, in favour of this concept, Geffen et al. (1973), studying twenty-eight essential hypertensive patients and eight normotensive or labile subjects, and Louis et al. (1974), studying thirty-seven patients with untreated essential hypertension, observed a positive correlation between resting diastolic pressure and concentrations of serum immunoreactive DBH. Schanberg et al. (1974) claimed that plasma DBH activity was significantly higher in six labile than in six permanent hypertensive patients and that plasma DBH generally paralleled the urinary excretion of noradrenaline. Recently, the same group (Stone et al., 1974) confirmed that plasma DBH activity was significantly higher in nine primary labile than in fifteen primary sustained hypertensive patients. Both categories displayed higher values than twelve primary hypertensive subjects with secondary renal abnormalities, whereas subjects with secondary forms of hypertensive disease (renovascular and adrenocortical hypertension, renal parenchymal disease) had lower values than all the former types of primary hypertension.

The reasons for these discrepancies may be ascribed, at least in part, to methodological differences in the assay procedure. In contrast with us and the other workers who used enzymatic determinations, Geffen et al. (1973) and Louis et al. (1974) employed a solid radioimmunoassay utilizing $^{125}$I-labelled sheep DBH and antibodies to sheep adrenal DBH. With those antibodies directed toward sheep DBH, there is no correlation between amounts of
human immunoreactant protein and enzyme activity, as here measured by the procedure of Nagatsu & Udenfriend (1972) (Rush, Nagatsu, Geffen & Udenfriend, 1973). To the contrary, antibodies directed towards DBH purified from a human phaeochromocytoma lead to a significant correlation between immunoreactant DBH concentrations and enzyme activities (Ebstein, Park, Freedman, Levitz, Ohuchi & Goldstein, 1973). It is therefore clear that the sheep radioimmunoassay does not really measure plasma DBH in man, but detects some immunoreactive fragments. Accordingly, Louis et al. (1974) suggest that their immunoassay may be a more sensitive index of the sympathetic activity than the photometric or the isotopic measurement of the active enzyme. The high sensibility associated to the low specificity of the procedure could therefore account for the close relationship they found between 'DBH' and diastolic arterial pressure in essential hypertension.

However, methodological features cannot explain why the other workers who measured active DBH enzyme arrived at different conclusions about the validity of plasma DBH activity as an index of the sympathetic tone in hypertension (Schanberg et al., 1974; Stone et al., 1974; Horwitz et al., 1973). Differences in the clinical classification, the selection of borderline hypertensive patients with high cardiac output or the study of too small and heterogeneous groups may be responsible for the discrepancies. In order to obtain valid results, we therefore paid special attention to the experimental design. Plasma DBH determinations were performed on two relatively large and homogeneous groups of male patients and it appears reasonable to assume that the slight difference in mean ages (8 years) of the two groups of adult hypertensive patients exerted a negligible influence on results, since human plasma DBH activity increases with age particularly in the first 2–3 years of life, very little increase occurring after 6 years of age (Freedman, Ohuchi, Goldstein, Axelrod, Fish & Dancis, 1972; Weinshilboum et al., 1973).

Under such conditions we found that, in borderline but not in permanent hypertensive patients, plasma DBH activity essentially reflects the resting activity of the sympathetic nervous system. A longitudinal study would be of importance to verify the disappearance of this correlation in patients as they passed from non-established hypertension into permanent hypertension.

The present work also indicates that an isolated DBH determination cannot be useful in establishing the level of sympathetic function in a particular individual. The measurement of sympathetic activity remains a formidable unsolved problem in human hypertension (Koch-Weser, 1973; De Quattro & Miura, 1973).

Acknowledgments

This study was supported by a grant from the Institut National de la Santé et de la Recherche Médicale and from the Fondation de l'Industrie Pharmaceutique pour la Recherche. We thank Mrs Françoise Baldet and Chantal Pilet.

References


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containing vesicles and other cell organelles by density gradient centrifugation. *Journal of Physiology (London)*, 205, 103–114.


