PATTERN OF DISTRIBUTION OF DOPAMINE-ß-HYDROXYLASE ACTIVITY IN NORMAL SUBJECTS AND IN PATIENTS WITH MITRAL STENOSIS OR LEFT TO RIGHT SHUNTS

KOUICHI OGAWA, TAKAYUKI ITO, MASA-AKI BAN, HATSUTOSHI SHIOZU, KEIJI MIZUTANI* and TATSUO SATAKE

Eighteen patients with mitral stenosis and twelve patients with left-to-right shunts heart disease and six normal subjects were catheterized and plasma dopamine-ß-hydroxylase (DBH) activities in various parts of cardiovascular system were measured in order to assess the role of sympathetic activity in pulmonary hypertension.

Arteriovenous differences of DBH activities were positive in normal subjects and patients with normal pulmonary artery pressure. A negative difference was found in patients with pulmonary hypertension. DBH activity in patients with pulmonary hypertension was significantly elevated compared with that of normal subjects. There was a weak correlation between mean pulmonary artery pressure and DBH activity in pulmonary artery. There were significant differences between normal, mitral stenosis with pulmonary hypertension and left-to-right shunts heart diseases about the pattern of distribution of DBH activity among these groups. A significant difference of the distribution between mitral stenosis with normal pulmonary artery pressure and that with pulmonary hypertension was also found. These findings suggested that a prolonged, increased level of sympathetic nervous system activity among patients with mitral stenosis and left-to-right shunt heart disease developed pulmonary hypertension. Thus, a significant contribution of sympathetic nervous activity to the establishment of pulmonary hypertension was suggested.

Key Words:
Dopamine-ß-hydroxylase
Pulmonary hypertension
Congestive heart failure
Mitrall stenosis
Left-to-right shunt heart disease

Dopamine-ß-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine is located in the storage vesicles of the sympathetic nerves and the chromaffine cells in the adrenal medulla and is released from these sites together with norepinephrine. 1-3 The concentra-

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Second Department of Internal Medicine, Nagoya University School of Medicine, Nagoya and Department of Cardiology, Meijo Hospital*, Nagoya, Japan
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Mailing address; Kouchi Ogawa, M.D. Second Department of Internal Medicine, Nagoya University School of Medicine, 65, Tsuruma-cho, Showa-ku, Nagoya, Japan

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<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>DBH Levels (I, U.)</th>
<th>PAP mmHg</th>
<th>AP mmHg</th>
<th>Shunt</th>
<th>NYHA</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>VIC</td>
<td>VSC</td>
<td>RA</td>
<td>RV</td>
<td>PA</td>
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<tr>
<td>1. Normal</td>
<td>6</td>
<td>39.2</td>
<td>±4.0</td>
<td>20.9</td>
<td>±2.1</td>
<td>18.7</td>
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<tr>
<td>2. MS</td>
<td>12</td>
<td>43.5</td>
<td>±2.2</td>
<td>20.0</td>
<td>±4.2</td>
<td>22.2</td>
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<tr>
<td>3. MS + PH</td>
<td>6</td>
<td>40.3</td>
<td>±3.2</td>
<td>71.8</td>
<td>±23.6*</td>
<td>70.9</td>
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<td>4. L-R Shunt</td>
<td>9</td>
<td>30.8</td>
<td>±3.4</td>
<td>43.7</td>
<td>±10.3*</td>
<td>43.1</td>
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<tr>
<td>5. VSD + PH</td>
<td>3</td>
<td>31.7</td>
<td>±7.9</td>
<td>67.9</td>
<td>±38.4</td>
<td>72.4</td>
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</tbody>
</table>

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(5): shows values after the addition of 2 patients of 3 years old.

*P < 0.05, **P < 0.01 compared with Normal, Mann-Whitney U test was used for statistical significance.
tion of DBH in plasma has been reported to be a useful index of the sympathetic function\(^4,5\). We reported here the pattern of distribution of DBH activity in normal subjects, in patients with mitral stenosis or left-to-right shunt heart diseases. In this study we measured DBH activity in vasoactive pulmonary hypertension. Two types of pulmonary hypertension were selected for study: hypertension caused by pulmonary arteriolar constriction in response to a high left atrial pressure in patients with mitral stenosis, and hyperkinetic pulmonary hypertension due to excess volume flow caused by a large left-to-right shunt.

**PATIENTS STUDIED AND METHODS**

Eighteen patients with mitral stenosis (7 men and 11 women, 28 to 50 years of age, mean age 42.4 ± 1.8) and 12 patients with congenital left-to-right shunt heart diseases (3 men and 9 women, 19 to 51 years of age, mean age 31.0 ± 3.4) were selected for cardiac catheterization. Six with mitral stenosis had pulmonary hypertension with mean pulmonary artery pressure greater than 25 mmHg. Of the 12 patients with left-to-right shunt heart diseases (2 patent ductus arteriosus; 4 atrial septal defects; 6 ventricular septal defects), three had ventricular septal defect associated with pulmonary artery hypertension. Six patients with a functional systolic murmur (4 men and 2 women, 29 to 52 years of age, mean age 39.2 ± 4.0) and neither shunt nor high pulmonary pressure were served as normal subjects. Thus the final groups studied were twelve patients with mitral stenosis and normal pulmonary pressure, nine patients with left-to-right shunt and normal pulmonary pressure, six patients with mitral stenosis and pulmonary hypertension, three patients with left-to-right shunt and pulmonary hypertension, and six normal subjects. Two patients of 3 years old with ventricular septal defect and pulmonary hypertension were also studied. None of patients had overt signs of congestive heart failure at the time of cardiac catheterization.

Written consents were given by all patients and cardiac catheterization was carried out with an intracardiac catheter in the morning after fasting over night. Inferior vena cava (VCI), superior vena cava (VCS), right atrial (RA), right ventricular (RV), pulmonary arterial (PA) and pulmonary capillary wedge (PCW) pressure were measured with a Courmand catheter. Monitoring and recording of the pressure were done on

Nihon Koden multipurpose polygraph with Mingograph 800 recorder utilizing T.I.K. pressure transducers. Mean pressures were obtained by electrical integration of the pulse contours.

Heparinized blood samples were obtained from PA, RV, RA, VCS, VCI, and the peripheral artery in that order during right cardiac catheterization. Blood was centrifuged within 30 min. after sampling and plasma was stored at −20°C until assayed. The plasma DBH activity was measured using the procedure of Nagatsu and Udenfriend\(^7\) in duplicated on 10μl aliquots of plasma. Results were expressed as international units, 1 μmol of octopamine formed per min. per liter of plasma at 37°C.

All values were expressed as mean ± standard error of mean (SEM). Correlations were computed by the least squares method. Wilcoxon matched pair signed-rank test for related samples and Mann-Whitney U test for independent samples were used for statistical analysis. The \( \chi^2 \) test was used to determine the significance of two independent group\(^8\).

**RESULTS**

*The Pattern of Distribution of DBH Activity in Normal Subjects*

Line 1 of Table I shows mean plasma DBH activity of six normal subjects. DBH activity in the peripheral arteries was highest and the lowest was in the pulmonary artery. The mean difference of DBH activity between the peripheral artery and the pulmonary artery was 15.4 ± 6.2 I.U. and mean arteriovenous (A-V) (V: average of DBH activity in VCS and VCI) difference of normal subjects group was 6.7 ± 5.2 I.U. However, the difference in DBH activity between the peripheral and the pulmonary arteries was not statistically significant.

*DBH Activity in Patients with Mitral Stenosis without Pulmonary Hypertension*

Line 2 of Table I shows mean plasma DBH activity of twelve patients with mitral stenosis with mean pulmonary arterial pressure less than 25 mmHg. DBH activity in the peripheral arteries was highest and the lowest in the pulmonary artery (mean difference: 12.8 ± 3.7 I.U. \( P < 0.01 \)). The mean A-V difference of this group was 7.8 ± 5.1 I.U. DBH activity in patients with mitral stenosis without pulmonary hypertension was slightly higher compared with normal subjects (NS).
DBH Activity in Patients with Mitral Stenosis with Pulmonary Hypertension

Line 3 of Table I shows mean plasma DBH activity of six patients with mitral stenosis with pulmonary hypertension. The highest DBH activity was in the inferior vena cava; lowest activity was in the pulmonary artery (mean difference: $22.2 \pm 9.1$ I.U.). The mean A-V difference of this group was $-6.1 \pm 2.5$ I.U. ($P < 0.05$). Arterial DBH activity was lower than peripheral venous DBH activity and the activity was highest in the inferior vena cava in this pulmonary hypertension group. There was no significant difference between peripheral arterial and pulmonary artery DBH levels. However, DBH activity in patients with mitral stenosis and pulmonary hypertension was increased over that found in both the normal group ($P < 0.05$) and the group with mitral stenosis without pulmonary hypertension ($P < 0.05$).

DBH Activity in Patients with Left to Right Shunt Heart Diseases

Line 4 of Table I shows mean plasma DBH activity of nine patients with congenital left-to-right shunt heart disease with normal pulmonary arterial pressure. Mean DBH activity in the peripheral arterial system was highest and that in the pulmonary artery was lowest in this group and the mean difference between them was $19.5 \pm 6.8$ I.U. The mean A-V difference of this group was $6.2 \pm 3.0$ I.U. The concentration of DBH (i.e. the mean plasma DBH) was significantly elevated compared with that of normal subjects in the inferior vena cava, the right atrium and the right ventricle ($P < 0.05$).

DBH Activity in Patients with Ventricular Septal Defect with Pulmonary Hypertension

Line 5 of Table I shows mean plasma DBH activity of three patients with ventricular septal defect and pulmonary hypertension. DBH activity was in the inferior vena cava; lowest activity was in the pulmonary artery (mean difference: $22.2 \pm 9.1$ I.U.). The mean A-V difference of this group was $-6.1 \pm 2.5$ I.U. ($P < 0.05$). Arterial DBH activity was lower than peripheral venous DBH activity and the activity was highest in the inferior vena cava in this pulmonary hypertension group. There was no significant difference between peripheral arterial and pulmonary artery DBH levels. However, DBH activity in patients with mitral stenosis and pulmonary hypertension was increased over that found in both the normal group ($P < 0.05$) and the group with mitral stenosis without pulmonary hypertension ($P < 0.05$).

**TABLE II**

<table>
<thead>
<tr>
<th></th>
<th>Low DBH (0–25 I.U.)</th>
<th>High DBH (25–50 I.U.)</th>
<th>Very High DBH (50–100 I.U.)</th>
<th>Total</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2. MS</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>3. MS + PH</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>$&lt;0.02$</td>
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<tr>
<td>4. L–R shunt</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>5. VSD + PH</td>
<td>1</td>
<td>1(2)</td>
<td>1</td>
<td>3 (5)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>17</td>
<td>12 (14)</td>
<td>7</td>
<td>36 (38)</td>
<td></td>
</tr>
</tbody>
</table>

( ) shows values after the addition of 2 patients of 3 years old. X² test was used for statistical significance. P values were compared with normal. *This P value was compared between MS and MS + PH.

NS: no significance.
vity was highest in the right ventricle and the lowest was in the pulmonary artery in this group of patients (mean difference: 14.7 ± 13.1 I.U.). The mean A-V difference of this group was -2.9 ± 7.2 I.U.. DBH activity was significantly elevated compared with normal control group in the right atrium, the right ventricle and the pulmonary artery (P < 0.05). Values in parenthesis in the line shows DBH activities after the addition of 2 patients of 3 years old.

**A Correlation between Mean Pulmonary Artery Pressure and DBH Activity in Pulmonary Artery Blood**

There was a weak association between mean pulmonary artery pressure and DBH activity in pulmonary artery blood (γ = 0.41, N = 36). When we omitted left-to-right shunt group with normal pulmonary artery pressure, the relationship between mean pulmonary artery pressure and DBH activity was slightly strengthened (γ = 0.48, N = 27). A significant linear correlation between mean pulmonary artery pressure and DBH activity from the pulmonary artery was also demonstrated among normal, mitral stenosis with or without pulmonary hypertension (γ = 0.41, N = 24). Figure 1 shows mean pulmonary artery pressure and plasma DBH activity from the pulmonary artery of five groups.

**Correlation of Systemic Blood Pressure and Arterial DBH Activity**

No correlation was found between systemic blood pressure and arterial DBH activity.

**The Pattern of Distribution of DBH Activity Among Normal Subjects, Patients with Mitral Stenosis and Left to Right Heart Diseases**

Table II shows the pattern of distribution of DBH activities. DBH activity was divided into three classes: low DBH; from 0 to 25 I.U., high DBH; 25 to 50 I.U., very high DBH; above 50 I.U. DBH activity of the inferior vena cava was used as reference value and χ² test was used to determine the significance. There was a significant difference between normal group and mitral stenosis with pulmonary hypertension (P < 0.02), left to right shunt heart disease groups (P < 0.01). There was also a significant difference between mitral stenosis without pulmonary hypertension and mitral stenosis with pulmonary hypertension (P < 0.05).

**DISCUSSION**

In a single individual, plasma levels of DBH are remarkably constant from day to day and month to month and have been found to remain stable for up to seven years. The present investigation showed that in each individual plasma DBH activity in the peripheral arteries was highest and that of the pulmonary artery was lowest in normal individual. This pattern of distribution was the same in patients with mitral stenosis or left to right shunt heart disease without pulmonary hypertension. The A-V difference in DBH levels was similar in the normal group and in patients with mitral stenosis and left-to-right shunt heart disease without pulmonary hypertension.

There was a question as to how DBH, which is released into the extracellular synaptic cleft, reaches the blood circulation. DBH is a large protein with a molecular weight of 290,000 and should have difficulty in passing through the membranes of the blood vessels. Åberg et al. proposed the lymphatic system as a possible route for the transport of DBH. However, their observations showed that DBH activity in the lymph samples was in all cases lower than that of in the corresponding arterial plasma samples. Our findings are consistent with their findings: arterial plasma DBH activity was always higher than that from the inferior vena cava. Direct release of DBH into arterial blood, and no mediation through the lymph system, seems to us to be the main source of serum DBH. It is interesting that plasma DBH activities from the pulmonary artery were always lowest.

In patients with mitral stenosis and pulmonary hypertension, DBH activity was highest in the inferior vena cava and the lowest in the pulmonary artery and the A-V difference was negative in this patient group. The difference distinguished the group with mitral stenosis and pulmonary hypertension from the others studied. In certain patients with mitral stenosis of such severity as to elevate the left atrial pressure to 25 mmHg or higher, the pulmonary arterial pressure may rise out of proportion of the left arterial pressure. Some investigations consider this an active form of pulmonary hypertension mediated by a reflex response to a critical level of pulmonary venous pressure. The studies of Wood also suggest a vasoconstrictive element; he reported a fall in pulmonary arterial pressure and resistance and a rise in cardiac output and left atrial pressure following acetylcholine injec-
tion into the pulmonary artery of patients with mitral stenosis and marked pulmonary hypertension.15

In previous study we reported that there was a tendency of increase of DBH activity in patients with congestive heart failure in the peripheral venous blood.16 The mechanism of the increase of DBH activity seems to be same in that of the present study among patients with pulmonary hypertension. It is known that the level of noradrenaline in the heart of patients in congestive heart failure is depleted. This depletion of noradrenaline is present in both the right and left ventricles regardless of which ventricle is subject to the pulmonary hemodynamic burden. Sympathetic activity in these patients is supported by circulating catecholamines released from peripheral sympathetic endings.16 Vogel and Chisdey demonstrated increases in plasma levels of norepinephrine after propranolol were given to calves in heart failure, these increases were over and above those noted to occur in heart failure alone. They suggested that generalized adrenergic stimulation has been initiated from extracardiac sources, primarily the adrenal medulla.17 Our findings in patients with pulmonary hypertension may be related to the possible reflection of norepinephrine release in patients with congestive heart failure.

The wide variation of plasma DBH activity found from patient to patient appears to be primarily genetically determined. Nevertheless, environmental and disease processes may induce changes in enzymatic activity that could be used as an index of sympathetic function particularly in individuals studied over a period of time.18 The plasma DBH values were not distributed parametrically, but were skewed to the right. There were at least two groups in the distribution pattern of DBH activity, namely low DBH subgroup (0–25 I.U.) and high DBH subgroup (25–50 I.U.).19 In the low DBH subgroup, there were individuals showed their DBH activities were nearly 0. This very low subgroup was reported about 3–4% of the total population. In our another study, 2% of total normal population was found to be very low DBH subgroup and two patients of very low DBH activity (one in mitral stenosis and normal pulmonary artery pressure group and the other in ventricular septal defect and normal pulmonary artery pressure) were found and included in this study.

In conclusion, there were significant differences of distribution pattern of DBH activities in patients with left-to-right shunt heart disease with or without pulmonary hypertension and patients with mitral stenosis and pulmonary hypertension. DBH activities in patients with mitral stenosis and with left-to-right shunt heart diseases without pulmonary hypertension were already higher than that in normal subjects. Thus, it was suggested that an increased level of sympathetic nervous system activity among patients who presumable had it over a period years developed pulmonary hypertension.

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REFERENCES

DBH and Pulmonary Hypertension


