An Epidemiological Study of the Relationship between Serum Dopamine-Beta-Hydroxylase Activity and Urinary Noradrenaline, and Their Relationship to Blood Pressure in Women Living in a Rural Area

Shingo Katsuno, Kunihiro Sakamoto, Sachiko Yoshimoto and Fumiko Takao

Department of Hygiene, Hyogo College of Medicine, Nishinomiya

Tomikoo Tanaka

Sasayama Health Center, Hyogo

SUMMARY

The relationship between sympathetic neural activity and blood pressure was examined for 167 women living in a rural area in Hyogo Prefecture, under ordinary conditions. In this study, serum Dopamine-beta-hydroxylase (DBH) activity and urinary noradrenaline/creatinine (NA/cr) ratio were used as the indices of sympathetic neural activity.

The results obtained are as follows:

1. No significant correlation was observed between serum DBH activity and urinary NA/cr. However, several different subgroups were included in the subjects examined, particularly among the age group of 40-49, one whose urinary NA/cr correlated positively to serum DBH activity and the others without this correlation.

2. The relation of serum DBH activity or urinary NA/cr to blood pressure was not clear when the factors were examined independently for the subjects as a whole.

3. However, when the same subjects were subdivided based on the 33 and 66 percentile values of serum DBH activity and urinary NA/cr and respective blood pressure levels were compared, blood pressure tended to increase with urinary NA/cr in the group with a moderate DBH activity level (20-40 IU).

4. In the subgroup selected from a scatter diagram of serum DBH activity and urinary NA/cr, which fell within ±1 standard deviation from the regression line, systolic and diastolic blood pressure increased as serum DBH activity or urinary NA/cr increased. These relations were observed for all age groups.

The results suggest that sympathetic neural activity causally affects blood pressure level in some subjects.

INTRODUCTION

There have been many reports which suggest that the sympathetic nervous system plays an important role in the pathogenesis of essential hypertension. Clinical and biochemical signs of increased sympathetic activity are observed in patients with essential hypertension, and particularly in those with labile hypertension\(^1\text{-}^3\). This increased activity is also observed in the early stages of hypertension in spontaneously hypertensive rats, which have been used as a model of human essential hypertension.\(^4\)

The above suggest that measurement of sympathetic activity may be useful to predict the development of hypertension.

The most commonly used indices to measure sympathetic activity are the excretion of urinary catecholamine, concentration of plasma catecholamine and serum Dopamine-beta-hydroxylase activity (DBH is the enzyme which catalyzes the final stage of noradrenaline biosynthesis).\(^5\) Studies of the
relationship between these indices and hypertension are usually based on findings from selected hospital inpatients. However, in order to demonstrate the relation between sympathetic activity and hypertension, it is necessary to conduct epidemiological studies of the general population as well.

Since 1974, the present authors have surveyed the relation between serum DBH activity and hypertension in a rural of Japan, i.e. Taki County, Hyogo Prefecture, where the cerebrovascular disease mortality rates and the prevalence of hypertension are higher than the average for Japan as a whole. It was previously reported from this district that serum DBH activity tended to increase with an increase in blood pressure within normal ranges (i.e. below 150/90 mmHg) and that those with higher serum DBH activity (DBH ≥ 70 international units, IU) had higher blood pressure than those with low DBH activity (DBH < 15 IU).

In the present study, the amounts of urinary noradrenaline (NA) were measured along with serum DBH activity and the relationship between sympathetic activity and blood pressure was examined in women residing in Taki County, under ordinary conditions.

MATERIALS AND METHODS

The subjects were 167 women from N district, Taki County, Hyogo Prefecture. They were grouped by age as follows: 27-aged 30 to 39, 60-aged 40 to 49, 52-aged 50 to 59, 28-aged 60 to 69, and for a total of 167.

For the years 1969-1974, in N district the standardized mortality ratio of cerebral apoplexy is 128.8 (significant for p ≤ 0.005). In 1977, 31.5 percent of the total number of women (310) in N district were examined. After a general medical and biochemical examination*, a selection was made of 110 normotensive and 57 hypertensive subjects (hypertensives: systolic blood pressure 140 mmHg and over and/or diastolic blood pressure 90 mmHg and over) with no other abnormalities. Those who were receiving antihypertensive therapy at that time were excluded.

Blood and urine samples for DBH and NA assay were collected in August 1977. Serum was removed by centrifugation after placing blood on ice for 10 hrs. Urine specimens (100 ml) were collected in polyethylene bottles containing 1 ml of 6 N HCl. Sera and urine were frozen at −20°C until the measurements were carried out. Serum DBH activity was assayed by the method of Nagatsu et al.7 Urinary NA was measured by the method of Imaizumi.8a Catecholamines were removed from 20 ml of urine by absorption on alumina at a pH of 8.4. After elution with 0.2 M acetic acid, they were oxidized with ferricyanide. To determine NA, the difference in fluorescent excitation and emission spectra of the corresponding lutins were used. The unconjugated form of NA was measured in this study and the excreted amounts in casual urine specimens are expressed as NA/creatinine ratio (μg/g). From a survey of 29 of the subjects, the NA/cr ratio was found to reflect adequately the total level of NA excreted over 24 hrs (Fig. 1). Blood pressure was determined twice with the subjects seated and the mean values were recorded.

RESULTS

1. Serum DBH activity and urinary NA/creatinine distributions

* The biochemical examination included the following tests: serum urea nitrogen, blood glucose, serum enzymes including alkaline phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and protein, glucose and urobilinogen urine analyses.
The median values of serum DBH activity and urinary NA/creatinine (NA/cr) in normotensive and hypertensive subjects are shown in Fig. 2, for each age group. Serum DBH activity in normotensives tended to increase with age up to age 59 and then to decrease. Urinary NA/cr in normotensives tended to increase with age. These changes were, however, not significant. Serum DBH activity in hypertensives was similar to that in normotensives, while urinary NA/cr in hypertensives was slightly higher (8μg/g) than in normotensives.

Figure 3 shows serum DBH activity and urinary NA/cr distributions for normotensives and hypertensives. As changes based on age were small, the subjects were examined as a whole. The distributions of serum DBH activity and urinary NA/cr in normotensives skewed right and the 95% range fell between 8-105 IU for serum DBH activity, and between 11-105μg/g for urinary NA/cr, respectively. Distribution
patterns and the ranges were similar for both hypertensives and normotensives.

2. The relationship between serum DBH activity and urinary NA/cr.

Figure 4 is a scatter diagram of serum DBH activity and urinary NA/cr for each age group. No significant correlation was observed between the two indices for individual age groups of normotensives, hypertensives, or of the subject population as a whole. However, several different subgroups were included in the subjects examined, particularly among the group of 40-49 years of age, one whose urinary

40-49 group

50-59 group

p<0.05

* p<0.05, ** p<0.01

systolic blood pressure, ++ diastolic blood pressure

Table 1 Correlation coefficients between serum DBH activity and blood pressure, and between urinary

NA/cr and blood pressure

<table>
<thead>
<tr>
<th>Age group</th>
<th>Serum DBH activity</th>
<th>Urinary NA/cr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP*</td>
<td>DBP**</td>
</tr>
<tr>
<td>30-39</td>
<td>0.15</td>
<td>0.22</td>
</tr>
<tr>
<td>40-49</td>
<td>0.38**</td>
<td>0.30*</td>
</tr>
<tr>
<td>50-59</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>60-69</td>
<td>0.11</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Fig. 5 Median values of blood pressure in subdivided groups based on serum DBH activity and urinary NA/cr levels
NA/cr correlated positively to serum DBH activity and the others without this correlation.

3. Relations of serum DBH activity and urinary NA/cr to blood pressure.

Table 1 shows correlation coefficients between serum DBH activity and blood pressure, and between urinary NA/cr and blood pressure. Except for the 40-49 years group, no significant correlation was observed.

The relationship to blood pressure was further analyzed using the following two methods. 1) Subjects aged 40-49 and 50-59 were subdivided into nine groups based on their serum DBH activity and urinary NA/cr levels, and respective blood pressures were compared (Fig. 5). The 33 and 66 percentiles were used as respective criteria. At age 50-59, systolic blood pressure tended to increase with increasing urinary NA/cr in all groups regardless of the level of serum DBH activity. Particularly in the group with a moderate DBH activity level, the subjects with high urinary NA/cr had significantly higher blood pressures than those with low urinary NA/cr. The level of diastolic blood pressure also increased with urinary NA/cr in the groups with moderate and low levels of serum DBH activity but no such correlation was observed in the group with a high level of serum DBH activity. For those aged 40-49, blood pressure did not show a clear correlation with urinary NA/cr as for those aged 50-59. Only in the moderate DBH group did systolic and diastolic blood pressure tend to increase with urinary NA/cr. On the other hand, when blood pressure were compared within groups with the same level of urinary NA/cr, subjects with higher serum DBH activity had higher systolic blood pressure in the group with a moderate urinary NA/cr.

Figure 4 shows several patterns representing the relationship between serum DBH activity and urinary NA/cr. Therefore, 2) The group from the scatter diagram (Fig. 4) whose urinary NA/cr correlated positively to serum DBH activity was selected, and the relation of either serum DBH activity
or urinary NA/cr to blood pressure was then analyzed. A regression line, $y = x$, was postulated, because both levels and ranges of the values were nearly equal. The subjects were subdivided into two groups, one falling within $\pm 1$ standard deviation (S.D.) from the regression line and the other falling outside (Fig. 6). Figure 7 shows the relationship of serum DBH activity and blood pressure for the group within $\pm 1$ S.D. Blood pressure correlated positively with serum DBH activity, and the correlation coefficients were particularly significant for ages 40-49, 50-59 and 60-69. In contrast, no significant correlation was observed between blood pressure and serum DBH activity for the remaining subgroup (Fig. 8).

Urinary NA/cr also correlated positively with blood pressure only for the group within $\pm 1$ S.D. (Fig. 9).

**DISCUSSION**

Plasma noradrenaline level is the most sensitive and specific index of sympathetic activity, but it is not adequate for field surveys because it requires a large quantity of blood and complex measurements. Serum DBH activity and urinary NA also have been used as indices, but studies of the correlation between plasma NA and serum (or plasma) DBH activity, or urinary NA have given conflicting
The DBH level in blood is dependent not only on the rate of its release from sympathetic nerve endings but also on its turnover rate in blood. As DBH in blood has a much longer half-life than NA, serum DBH activity is a rather insensitive index of changes in the sympathetic nerves. On the other hand, urinary NA appears to be derived in part from the kidneys although it originates mainly from plasma NA. These factors may have caused the conflicting results, and they indicate that serum DBH activity and urinary NA do not always reflect sympathetic neural activity. Nevertheless, when sympathetic nerves are stimulated over a long period of time, the synthesis of DBH and NA may increase and this synthesis may be accompanied by an increase in serum DBH and urinary NA levels in most people. The simplicity of serum DBH activity assay and the facility of sample collection for NA measurement are advantageous for epidemiological studies. Therefore, in this study, serum DBH activity and NA in casual urine specimen were used as indices of sympathetic activity. As the NA/cr ratio in casual urine correlated positively with the total NA excreted over 24 hrs, it appears to give an adequate measure of individual level of urinary NA.

In normotensives, urinary NA/cr and serum DBH activity increased slightly with age and the same tendency has been found for plasma NA, suggesting that the indices of sympathetic activity increase with age in normotensives although urinary NA/cr can also be affected by changes with age in creatinine.

Fig. 9 The relationship between urinary NA/cr and blood pressure in the subgroups within ±1 S. D. (see Fig. 6)
clearance.

No significant correlation was observed here between serum DBH activity and urinary NA/cr. A scatter diagram (Fig. 4) of the two indices was similar to the one for plasma DBH activity and NA, reported by Lake et al.11)

In this study, the subjects chosen were ordinary people living in a rural area in Hyogo. Urinary NA/cr levels in hypertensives were slightly higher than in normotensives, while serum DBH activity levels in hypertensives and normotensives were similar. The relationship between these indices and blood pressure was not clear when the factors were examined independently among the subjects as a whole. However, when the subjects were subdivided into nine groups based on the 33 and 66 percentile values of the two indices and the levels of blood pressure were compared, blood pressure tended to increase with urinary NA/cr in the group with a moderate serum DBH activity level.

Furthermore, in the subgroup selected from a scatter diagram of serum DBH activity and urinary NA/cr, which fell within ±1 S. D. from the regression line, systolic and diastolic blood pressure increased as serum DBH activity or urinary NA/cr increased. These relations were observed for all age groups.

Thus, the effects of sympathetic activity on blood pressure were clarified by a study of the relationship of serum DBH activity and urinary NA/cr. Although the results were taken from a cross-sectional analysis, they suggest that sympathetic activity causally affects the level of blood pressure in some people and that it is effective in field surveys to measure multiple indices of sympathetic activity in addition to casual blood pressure. Nevertheless, it is preferable to follow blood pressure and sympathetic activity longitudinally and to estimate individual basal levels.8,21) Further prospective studies are needed to give a complete picture of the role of sympathetic activity in the incidence of hypertension in the area which was the object of this study.

REFERENCES

16) Kuchel, O., Cuche, J. L., Buu, N. T. and Genest, J.: An increase in urinary catecholamines of renal origin in
兵庫県農村地区の女性住民における
血清 Dopamine-β-hydroxylase 活性と
尿中 Noradrenaline の相互関連性、
およびその血圧との関係についての疫学的研究

兵庫医科大学衛生学教室

勝 野 眞 吾・阪 本 州 弘

吉 本 佐 雅 子・高 尾 文 子

兵庫県篠山保健所

中 登 美 子

日常の生活状態における交感神経活性と血圧の関係を明らかにするため、兵庫県下の農村地区婦人 167 名を対象に交感神経活性の指標として血清 Dopamine-β-hydroxylase（DBH）活性と尿中 Noradrenaline/creatinine (NA/cr) 比を用いて両指標間の相互関連性およびその血圧との関係を検討した。

1. 血清 DBH 活性と尿中 NA/cr の間には全体として有意の関連性は認められなかった。しかし、調査した対象者の中には尿中 NA/cr が血清 DBH 活性と正の相関を示す群とこの関係がみられない群の異なった Subgroup の存在が認められた。

2. 全対象者について血清 DBH 活性と血圧、あるいは尿中 NA/cr と血圧の関係をそれぞれ別個にみるとその関連性はいずれもあまり顕著ではなかった。

3. しかし、血清 DBH 活性、尿中 NA/cr のそれぞれ 33、66 パーセントイル値で対象者を 9 群に分け、各群の血圧値を比較すると中程度の血清 DBH 活性 (20–40 単位) を示す群では血圧値は尿中 NA/cr が高い程高い値を示す傾向が認められた。

4. 一方、対象者のうち、その血清 DBH 活性と尿中 NA/cr が両指標間の回帰直線から±1 標準偏差の限界内に存在する者を選ぶとこの群では最大血圧、最小血圧は血清 DBH 活性あるいは尿中 NA/cr が高い程高い値を示した。この関係は 30、40、50、60 歳代の各年齢階層でみられた。

これらの結果から、同地区住民のうちある者では交感神経活性が血圧の維持に重要な役割をはたしていると考えられる。