Relationship between Gene Polymorphism and Plasma Dopamine β-Hydroxylase Activity in Hypertensive and Normotensive Subjects

Koichi Higashimori, Jitsuo Higaki, Atsushi Kamitani, Yi Zhao, Tetsuro Miki, Hiroshi Mikami, and Toshio Ogihara

The association between restriction fragment length polymorphism (RFLP) in the 3'-untranslated region of the dopamine β-hydroxylase (DBH) gene and essential hypertension, and the relationship between this RFLP and plasma DBH activity were investigated. A 322 bp segment of the 3'-untranslated region of the human DBH gene was amplified by polymerase chain reaction (PCR). The restriction enzyme SphI cleaved the 322 bp PCR product into 201 and 121 bp fragments for that allele which contained the SphI cutting site. This allele was designated S. The allele that lacked the SphI restriction site was designated L. Plasma DBH activity was determined by a spectrophotometric method. For this polymorphic restriction site, the frequency of the minor allele of the polymorphism was similar in the hypertensive and normotensive groups (0.39 vs. 0.35, respectively). In the normotensive group, there was a significant (p < 0.01) relationship between plasma DBH activities and genotypes: 65 ± 24, 55 ± 20 and 45 ± 14 IU/l for homozygotes (LL), heterozygotes (LS) and homozygotes (SS), respectively. In the hypertensive group, no significant relationship was observed. These results demonstrate that a SphI RFLP in the 3'-untranslated region of the DBH gene is not associated with essential hypertension in the group studied. However, in only normotensive subjects, this RFLP may be responsible in part for the variance of plasma DBH activity. (Hypertens Res 1994; 17: 49-53)

Key Words: dopamine β-hydroxylase, essential hypertension, polymerase chain reaction, restriction fragment length polymorphism

Essential hypertension is a heterogeneous disorder which is the result of interactions among numerous environmental and genetic factors. Despite extensive research on its etiology, the primary cause of essential hypertension has not yet been elucidated. It has been estimated that 20-40% of the interindividual blood pressure (BP) variance is genetically determined (1). The unimodal distribution of BP in the general population as well as in the offspring of hypertensive parents suggests that BP behaves as a polygenic trait (2). Thus, genetic approaches may provide new insights into the pathogenesis of hypertension. The role of a single gene can be investigated by case-controlled studies designed to find an association between essential hypertension and a marker genotype at the selected gene locus. Recently, several candidate gene probes have become available, and many association or linkage analyses have been reported (3-14).

The autonomic nervous system plays an important role in the regulation of the cardiovascular system, and hyperactivity of the sympathetic nervous system has been suggested to play an important role in the development of essential hypertension. Dopamine β-hydroxylase (DBH), the enzyme that catalyzes the conversion of dopamine to norepinephrine, belongs with tyrosine hydroxylase and phenylethanolamine-N-methyl transferase to a family of enzymes involved in the synthesis of catecholamines from tyrosine. DBH occurs in both a soluble and a membrane-bound form (15), and is localized in synaptic vesicles in noradrenaline and adrenaline neurons and in chromaffin granules in adrenomedullary cells (16). The soluble enzyme is released into the synaptic cleft at the time of vesicular exocytosis and is presumably the source of the enzyme present in blood (17). Some of the variations in plasma DBH activities among individuals have been reported to be genetically determined (18-21). A major gene polymorphism that regulates plasma DBH activity has been described, and this polymorphism has been estimated to be responsible for between 50-75% of the population variance of serum DBH activity in humans (18).

DBH has been considered to have a possible relationship to the development of psychiatric disorders (22), congestive heart failure (23) and hypertension (24). However, there are still no convincing...
data that relate the level of plasma DBH activity to the pathophysiology of essential hypertension. Recently, the sequence of human DBH cDNA has been reported by Lamouroux et al. (25). Kobayashi et al. (26) subsequently demonstrated that there is a single DBH gene of approximately 23 kb and that it is composed of 12 exons. They also isolated two kinds of cDNA (types A and B) encoding human DBH from a pheochromocytoma cDNA library. Type A (2.7 kb) and B (2.4 kb) encoded the same amino acid sequence and differed only in the 3'-untranslated region. The difference at nucleotide 1912 causes a SphI restriction fragment length polymorphism (RFLP).

The present study describes the results of an association study in which we used a polymerase chain reaction (PCR)-RFLP technique to detect the SphI RFLP located at the 3'-untranslated region in exon 12 of the DBH gene in groups of essential hypertensive and normotensive subjects. We also analyzed the relationship between this polymorphism and the plasma DBH activity in the two groups.

Materials and Methods

Patients Selection
The study population were all Japanese and consisted of 108 hypertensive outpatients at Osaka University Hospital and 85 normotensive subjects. Subjects in the hypertensive group had been diagnosed as having essential hypertension according to conventional criteria, which included a supine systolic blood pressure (SBP) of more than 160 mmHg and/or a diastolic blood pressure (DBP) of more than 95 mmHg on 3 occasions spanning 2 months. A detailed family history was taken for each subject. All patients had a family history of essential hypertension in at least one parent and one sibling. The normotensive subjects were selected according to the criteria that they had a SBP and DBP of less than 140 and 90 mmHg, respectively, without antihypertensive treatment, had no family history of hypertension in first degree relatives, and were matched with the hypertensive subjects for age and sex (Table 1). In both groups, patients with congestive heart failure, psychiatric disorders or thyroid disease, which may alter DBH activity (18), were excluded.

PCR-RFLP Analyses
DNA was extracted from peripheral blood leukocytes using a sodium dodecyl sulfate/proteinase K and phenol extraction method (27). Oligonucleotide primers of 20 nucleotides in length were synthesized on a DNA synthesizer (Applied Biosystems Japan, model 391, Tokyo, Japan). The sequence of the sense primer and the antisense primer were 5'-CATCTCCACACTGGAAGAGC-3' and 5'-GGTCCAGGGTAGGTCACAG-3', respectively, which are complementary to the sequences from nucleotide 1713 to 1732 and 2015 to 2034 of human DBH cDNA (26). PCR was performed in a final volume of 50 µl which contained 1 µg of genomic DNA, 1 µg each of the oligonucleotides and 200 µM each of the four dNTP. The reaction buffer used was one recommended by the manufacturer (10mM Tris, pH 8.4, 50 mM KCl, 1.5mM MgCl2). After heating for 5 min at 95°C and immediate cooling for 5 min at 55°C, 3 units of thermostable Taq polymerase (Perkin Elmer Cetus, Norwalk, CT) were added. Amplification was carried out in a Program Temp Control System (ASTEC PC-700, Fukuoka, Japan) for 30 cycles with steps of denaturation at 93°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min. The PCR products (5 µl) were digested overnight at 37°C with 2 units of restriction enzyme SphI (Takara Shuzo Co., LTD, Kyoto, Japan) in the recommended restriction buffer (total volume: 20 µl), and were applied to 2% agarose gels and subjected to electrophoresis at 80 V for 1 h. The amplified DNA was visualized directly by ethidium bromide staining.

Plasma DBH Measurement
Plasma DBH activity was determined by a spectrophotometric method (28).

Statistical Analysis
Values were expressed as mean ±SD. For each biallelic RFLP, allele frequencies were deduced from genotype frequencies, and deviation from Hardy-Weinberg equilibrium was tested by chi-squared analysis with 2 degrees of freedom. One-way analysis of variance followed by Duncan’s multiple range test was used to compare group means for the different parameters studied.

Results
The PCR amplified a 322 bp segment at the 3'-untranslated region of the human DBH gene. The restriction enzyme SphI cleaved the 322 bp PCR product into 201 and 121 bp fragments for that allele which contained the SphI cutting site. This allele was designated S. The allele that lacked the SphI restriction site was designated L. Thus, each DNA sample yielded one of three possible genotypes, either LL, LS or SS (Fig. 1). Frequencies for LL, LS and SS genotypes were respectively 45, 42 and 21 in the hypertensive subjects, and 35, 40 and 10 in the normotensives. Derived allele frequencies for L and S alleles were 0.61 and 0.39 in the hypertensive, and 0.65 and 0.35 in the normotensive subjects, respectively, indicating that there was no sig-
significant difference between these two groups (Table 2). Plasma DBH activity did not differ significantly between hypertensive and normotensive subjects (62±28 and 58±22 IU/l). Mean plasma DBH activity for each genotype in each population was then compared. In the normotensive group, plasma DBH activity was 65±24, 55±20 and 45±14 IU/l for LL, LS and SS, respectively, (p<0.01) (Fig.2-a). In the hypertensive group, no significant relationship was observed (60±31 for LL, 60±26 for LS and 69±26 for SS) (Fig. 2-b).

Discussion

DBH is unique among the catecholamine-synthesizing enzymes in that a large amount of enzyme is found in blood, which suggests that serum DBH activity may be used as an index of sympathetic nervous system activity (29). However, there is wide variation in enzyme activity between different individuals, and evidence suggests that this is mostly due to genetic factors (18). Thus, enthusiasm for the use of serum DBH as a measure of acute alterations in adrenergic function in humans has been damped.

DBH is of interest because of its possible relationship with congestive heart failure and hypertension. Patients with congestive heart failure have lower plasma DBH concentrations than normal controls. This may be due to decreased sympathetic nerve synthesis or reduced release of DBH (23). It is of interest that BP was stable from day to day in patients with low DBH activities, whereas patients with high DBH activity exhibited greater lability of BP (24). There have been many reports about the relationship between serum DBH activity and essential hypertension (24, 30-32), but in several large studies, no significant differences were found between serum DBH activity of normotensive control subjects and that of hypertensive patients (33, 34). In the present study, we also were unable to demonstrate any significant difference in plasma DBH activities between hypertensive and normotensive subjects.

The present study demonstrated no association between a polymorphism in the 3'-untranslated region of the DBH gene and essential hypertension. The lack of association of this RFLP with essential hypertension does not mean that the DBH gene is not implicated in the etiology of the disease. The possibility that other RFLPs in or near the DBH gene are responsible, at least in part, for essential hypertension could not be excluded.

The most interesting finding in the present study is that the DBH gene polymorphism studied showed a significant relationship to plasma DBH activity in normotensive subjects. It is now clear that in man

Table 2. Frequencies of DBH RFLP in hypertensive and normotensive subjects

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>RFLP</th>
<th>HBP (42%)</th>
<th>NBP (41%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL</td>
<td>45</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>42</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>21</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td>L</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.39</td>
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</tr>
</tbody>
</table>

Fig. 1. Examples of the 3 patterns of SphI RFLP of the DBH gene. 10 samples before (upper panel) and after (lower panel) digestion with SphI: lane 1 homozygotes SS; lanes 2, 3, 8, 10 homozygotes LL; lanes 4, 5, 6, 7, 9 heterozygotes LS; lane 11 negative control; lane 12 molecular size marker (λ X174/Hae III).
most of the wide variation in DBH activity among individuals is due to the effect of inheritance and that a single locus is responsible for variation in enzyme activity in at least half of the total population (I8). Although the polymorphism within the 3'-untranslated region of the DBH gene that we have studied does not represent such a major gene polymorphism, it may be responsible in part for the variance of DBH activity. The functional significance of the 3'-untranslated region is unknown. Several reports suggest that the 3'-untranslated region of other genes may be involved in mRNA stability and translational efficiency (16,35-37).

Furthermore, we did not observe a significant relationship between DBH gene polymorphism and the plasma DBH activity in essential hypertensive patients, which tempts us to speculate that (I) hypertension may have some effects on the regulation of synthesis or release of DBH and that (2) antihypertensive drugs may alter DBH activity. While contradictory results have been reported concerning the effects of beta-blockers on DBH activity (23,38,39), most changes in human serum DBH activity in response to this kind of drug have been quantitatively small (I8). However, little is known about the effects of other kinds of antihypertensive drugs on DBH activity.

In conclusion, the present study demonstrated that a SphI RFLP in the 3'-untranslated region of the DBH gene is not implicated in essential hypertension, and suggests that this polymorphism may be responsible in part for the variance of plasma DBH activity in normotensive subjects, but not in hypertensives.

Fig. 2. Plasma DBH activities (IU/l) in individuals with the LL, LS and SS genotypes in (a) normotensive and (b) hypertensive groups. Solid vertical bars indicate mean±SD for each group (*p<0.01).

References

9. Zee RYL, Griffiths LR, Morris BJ: Marked associa-


