Hum Genet (1997) 99 : 52–55

ORIGINAL INVESTIGATION

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Possible control of dopamine β-hydroxylase via a codominant mechanism associated with the polymorphic $(GT)_n$ repeat at its gene locus in healthy individuals

Received: 30 July 1996 / Revised: 23 September 1996

Abstract Six allelic fragments were typed by a PCR-based process with a pair of primers specific for a sequence containing the polymorphic $(GT)_n$ repeat at the human dopamine β-hydroxylase (DBH) locus in 125 unrelated healthy individuals. Their frequencies among these individuals were 0.012 (A1), 0.08 (A2), 0.344 (A3), 0.548 (A4), 0.004 (A5) and 0.012 (A6); the two major alleles, A3 and A4, made up nearly 90% of the alleles. These individuals were divided into four groups according to the genotype they possessed, i.e. A3/A3, A4/A4, A3/A4 and others (mixed group). Kruskal-Wallis analysis revealed a significant difference in serum DBH activity among these four genetic groups ($H = 32.7$, $P < 0.0001$). The homozygotic genotypes, A3/A3 and A4/A4, were associated with low and high DBH activity, respectively, and the heterozygotic genotype, A3/A4, seemed to play a role in keeping the DBH activity at a moderate level. The present work suggests that the human DBH is likely to be controlled via a codominant mechanism associated with the dinucleotide repeat polymorphism at its gene locus.

Introduction

Both dopamine and noradrenaline are catecholaminergic neurotransmitters which play a crucial role in regulating a variety of physiological functions and activities. Dopamine β-hydroxylase (DBH; EC 1.14.17.1) is the enzyme which catalyses the conversion of dopamine to noradrenaline, so that it may be as important as dopamine and noradrenaline themselves in the development of various diseases related to biochemical disturbances or dysfunctions of the catecholamine pathway, especially those involved in nervous and mental disorders. DBH activity has long been established as being genetically regulated and there are genetic variations in serum DBH activity among individuals (Weinshilboum et al. 1973, 1975; Goldin et al. 1982). However, the mechanism of genetic control of DBH activity does not seem to have been elucidated at a more basic level, although both a model of autosomal codominant inheritance and a genetic polymorphism have been proposed (Dunnette and Weinshilboum 1982; Weinshilboum 1983).

The human DBH gene has been localised to chromosome 9q34 (Craig et al. 1988). A polymorphic region due to a $(GT)_n$ dinucleotide repeat, a microsatellite repeat, at the human DBH locus has been sequenced (EMBL accession number X63418) and five alleles have been identified in a population of randomly selected individuals (Porter et al. 1992). The present study, therefore, was designed to examine if the polymorphic dinucleotide repeat would be associated with the genetic control of DBH activity in humans.

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Materials and methods

Subjects

One hundred and twenty-five unrelated healthy subjects, 58 males aged 47.3 ± 14.9 years and 67 females aged 48.1 ± 13.9 years, came voluntarily from all parts of the United Kingdom. They all gave informed consent for blood samples to be taken. Overnight fasting blood was drawn from the ante-cubital vein between 8.00 a.m. and 9.00 a.m. and cooled to 2–4° C immediately. The serum for measurements of DBH activity and noradrenaline levels was separated by centrifugation at 4° C. Sodium metabisulphate at a final concentration of 1 mM was added to the serum for the measurement of noradrenaline. All the samples of serum were kept in a freezer at –45° C until they were used within 10 weeks.

Biochemical analysis

DBH activity in the serum was photometrically assayed following the method reported by Nagatsu and Udenfriend (1972). Tyramine was used as substrate and DBH activity in the serum

was expressed in nmoles per minute per millilitre. Noradrenaline in the serum was extracted with WA-4 alumina (Sigma) by following a procedure previously described (Bouloux et al. 1985), and was then analysed using reversed-phase high performance liquid chromatography with electrochemical detection (Wei et al. 1992).

PCR amplification and DNA analysis

Genomic DNA was extracted from whole blood by using the InstaGene matrix (Bio-Rad) and the amplification of fragments containing the dinucleotide repeat was performed by the PCR process with a pair of primers, 5′TATGGAGAAAAGGAGAAGCAGG3′ (GT strand) and 5′TCTGGGCTCATGCTCACATA3′ (CA strand). The AmpliWax gem-facilitated hot start technique was applied to the PCR amplification with a DNA Thermal Cycler 480 (Perkin Elmer). A 25-µl reaction volume contained 10 mM TRIS-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 µM of each dNTP, 0.4 µM of each primer, 1.0 U *Taq* DNA polymerase and 20–40 ng genomic DNA. The conditions needed for the PCR process included initial denaturation at 96° C for 3 min, ten cycles of 96° C for 45 s, 60° C for 30 s and 72° C for 10 s, followed by 30 cycles of 94° C for 30 s, 60° C for 30 s and 72° C for 10 s and final elongation at 72° C for 5 min. The PCR products were digested with *Hae*III at 37° C for at least 3 h. The digested PCR products were electrophoresed in 12% native polyacrylamide gels (Bio-Rad) followed by ethidium bromide staining. *Hae*III digestion produced four fragments. The predicted length of the digested PCR fragment containing the $(GT)_n$ dinucleotide repeat should be 71 bp according to the sequence data retrieved from the EMBL nucleic acid databases (accession number X63418) and the other three were 9 bp, 20 bp and 57 bp.

All the samples were run randomly. The Hardy-Weinberg equilibrium for genotypic frequencies was determined with the chi-square goodness-of-fit test and Kruskal-Wallis one-way ANOVA was also used for analysing data.

Table 1 Allelic frequencies of a $(GT)_n$ dinucleotide repeat at the human dopamine-β-hydroxylase (DBH) gene among unrelated healthy individuals

Allele	Size (bp)	Frequency
A1	75	0.012
A2	73	0.080
A3[a]	71	0.344
A4	69	0.548
A5	67	0.004
A6	65	0.012

[a] Corresponding to the sequence retrieved from the EMBL nucleic acid databases (accession number X63418)

Table 2 Genotypic frequencies of the $(GT)_n$ dinucleotide repeat at the human DBH gene among unrelated healthy individuals

Genotype	Frequency
A1/A3	0.008
A1/A4	0.016
A2/A3	0.056
A2/A4	0.096
A2/A6	0.008
A3/A3	0.112
A3/A4	0.400
A4/A4	0.280
A4/A5	0.008
A4/A6	0.016

Results

Six alleles were typed by the PCR-based process among these unrelated individuals. As shown in Table 1, the frequencies of two major alleles, A3 and A4, were found to make up nearly 90% of the alleles. This finding is quite similar to that reported by Porter et al. (1992), although they did not find the very rare one, A6.

Theoretically, there should be 21 individual genotypes that could be parentally matched with the six alleles in the general population, six homozygotic and 15 heterozygotic. In the present study, however, we have only found ten genotypes in the randomly selected population, two homozygotic and eight heterozygotic, due to the low frequency of some of the alleles (Table 2). The chi-square goodness-of-fit test showed that genotypic frequencies were in Hardy-Weinberg equilibrium among these individuals ($\chi^2 = 0.43$, $df = 15$, $P > 0.995$).

Kruskal-Wallis one-way ANOVA analysis revealed a significant difference in serum DBH activity among these four groups classified according to the genotype possessed by these individuals ($H = 32.7$, $P < 0.0001$), and males and females showed a corresponding alteration ($H = 23.4$, $P < 0.0001$ in males and $H = 9.9$, $P < 0.02$ in females). Of these four genetic groups, the individuals with A4/A4 genotype had the highest activity of serum DBH and those with A3/A3 genotype had the lowest. The individuals with A3/A4 genotype had a moderate level of serum DBH activity compared with the above two groups (Table 3).

No significant difference in serum noradrenaline levels was found among these four genetic groups. There was no significant correlation between serum noradrenaline levels and DBH activity.

Table 3 Comparison of serum DBH activity among the healthy individuals classified genetically. The values are expressed as mean ± S.D. (nmol/min/ml) and the number of subjects is shown in parenthesis. Kruskal-Wallis analysis showed a significant difference among the four groups, i.e. [a]$H = 23.4$, $P < 0.0001$; [b]$H = 9.9$, $P < 0.02$; [c]$H = 32.7$, $P < 0.0001$

Subjects	Genotypes			
	A3/A4	A4/A4	A3/A3	Others
Male[a]	18.7 ± 13.6 (20)	33.4 ± 9.4 (16)	9.0 ± 5.4 (9)	19.6 ± 10.2 (11)
Female[b]	23.1 ± 11.7 (27)	27.0 ± 9.5 (18)	9.6 ± 5.6 (5)	22.6 ± 8.7 (14)
Total[c]	21.3 ± 12.6 (47)	29.9 ± 9.9 (34)	9.2 ± 5.2 (14)	21.3 ± 9.3 (25)

Discussion

Microsatellite repeats, the simple sequence repeat (SSR), are ubiquitous and highly polymorphic in mammalian genomes, and at least 30 000 loci are present in the human genome (Charlesworth et al. 1994). They are mostly located on the non-coding region of genes, including the introns and 5′- or 3′-flanking regions (Tripathi and Brahmachari 1991). Although their functional significance is still far from being understood, some preliminary work has demonstrated that the SSRs such as purine-pyrimidine repeats may play a role in regulating gene transcription. For example, the $(TG)_n$ repeat located upstream of the rat prolactin gene exerts a negative effect on gene transcription (Naylor and Clark 1990). Deletion of the $(CA)_n$ repeat at the human cytosolic phospholipase A_2 ($cPLA_2$) gene reveals an inhibitory effect on the $cPLA_2$ promoter activity in a bronchial epithelial cell line (Wu et al. 1994). The present results indicate that the $(GT)_n$ repeat located at the human DBH locus is likely to bear a relationship to the control of such enzyme activity via a codominant mechanism. As shown in Table 3, the individuals with the A4/A4 genotype had the highest activity of serum DBH, those with the A3/A3 genotype had the lowest, and those carrying the heterozygotic genotype, A3/A4, had a moderate level of serum DBH activity.

DBH has been localised in both the central and peripheral noradrenergic nervous systems (Hartman 1973). The circulating DBH is mostly released, together with noradrenaline, from the sympathetic nervous system (Weinshilboum et al. 1971). The levels of circulating noradrenaline, however, are easily altered by many factors which possibly act on the excitability of the sympathetic nervous system, such as stress, whereas DBH activity in the circulation is relatively constant over time (Lamprecht et al. 1975). Perhaps this is the major reason for the failure to find levels of serum noradrenaline to be significantly correlated to serum DBH activity in this study. Lack of correlation between plasma DBH and noradrenaline has also been reported in hypertensive and normotensive subjects (Lake et al. 1977).

It has long been proposed that DBH activity alters in patients with mental illness, especially those with affective disorder or schizophrenia, although the findings to data have been inconsistent (Wise and Stein 1973; Wyatt et al. 1975; Markianos et al. 1976; Melter et al. 1976; Fujita et al. 1978; van Kammen et al. 1983; Wei et al. 1992). DBH deficiency has also been reported in some neurological or physical diseases in humans (Biaggioni et al. 1990). For example, individuals with a history of heart attacks had lower levels of DBH than did others (Asamoah et al. 1987); the subjects with high DBH activity tended to show higher and less stable levels of blood pressure (Schanberg et al. 1974); plasma DBH was significantly decreased in familial dysautonomia (Weinshilboum and Axelrod 1971); and orthostatic hypotension was related to DBH deficiency (Robertson et al. 1992; Gentric et al. 1993). Neonates with DBH deficiency were found to have episodic hypothermia, hypoglycemia and hypotension (Robertson et al. 1991). Taken together, the polymorphic $(GT)_n$ repeat at the human DBH locus may be an important genetic marker which predisposes some individuals to a vulnerability to some mental and neurological diseases involving disturbances of the catecholamine pathway.

Acknowledgements The authors wish to thank Dr. Harold Hillman and Dr. Robert Masters for the collection of blood samples as well as Mrs. Veronica Hanson and Mrs. Lynda Hughes for their help in this study.

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