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Citation Classics

Simple Photometric Assay of Dopamine-β-Hydroxylase Activity in Human Blood: Useful in Clinical Chemistry

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Featured Article: Nagatsu T and Udenfriend S. Photometric assay of dopamine-β-hydroxylase in human blood. Clin Chem 1972;18:980–3.[2]

This report described a simple assay for measuring the activity of dopamine-β-hydroxylase (DBH[3]) in human blood. DBH is a copper-containing, ascorbate-requiring glycoprotein enzyme that catalyzes the hydroxylation of dopamine to norepinephrine (NE), the precursor of epinephrine (E). DBH is localized in the synaptic vesicles in the central NE and E neurons and peripheral sympathetic NE neurons and in the chromaffin granules in E and NE cells of the adrenal medulla; DBH is released by exocytosis together with NE or E, and appears in both blood and cerebrospinal fluid (CSF).

Since I was a medical student at Nagoya University, Japan, I have been interested in the chemical mechanisms of neuropsychiatric diseases and, for that reason, in the biochemistry of catecholamine neurotransmitters that are assumed to be closely related to various brain diseases. I first worked with Sidney Udenfriend in 1964 at NIH, where we discovered tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamines. Studies on DBH occupied my attention during a sabbatical year in 1972, during which I again worked with Sidney Udenfriend, who was then at the Roche Institute of Molecular Biology. Our project was to examine the changes in the DBH molecule in humans in neural diseases. For this purpose we needed a quick assay for DBH activity in human blood.

DBH activity was difficult to assay in crude preparations, including blood, owing to the presence of natural inhibitors that bind to copper at the active center of the enzyme. These natural inhibitors are various sulfhydryl compounds such as glutathione and cysteine. We found that the action of these inhibitors could be completely removed by treating the crude preparations with an SH-blocking agent, N-ethylmaleimide *(1)*. In 1971, with a 2-step radio-enzymatic assay using phenylethanolamine-N-methyltransferase (PNMT), DBH activity in human blood was detected for the first time *(2)*. Copper was used to remove the inhibition by natural inhibitors. Although this method is sensitive (approximately 10 pmol), a saturating substrate concentration cannot be used to obtain the *V*max, and the optimal concentration of copper must be determined, owing to inhibition of PNMT by the copper.

We therefore developed the photometric activity assay described in the report featured here. N-ethylmaleimide was used to completely remove the inhibition by natural inhibitors in human serum at a saturating concentration of the substrate, tyramine. Octopamine formed by the DBH reaction was oxidized to p-hydroxybenzaldehyde by periodate, and measured by photometry. The *V*max value in human blood, which is genetically determined, is highly variable [approximately 1–100 U (μmol/min/L serum), with the mean value being approximately 40 U] but is very constant in each healthy individual. A few healthy persons have very low activity. Only human blood has activity high enough to be measured by this method (sensitivity, approximately 2 nmol), and the activities in various other mammals, including monkeys, were <0.1 U. Human CSF also has very low activity (approximately 1–100 mU), but the activity in CSF can be measured by HPLC with electrochemical detection with dopamine as the substrate, affording a higher sensitivity of approximately 0.5 pmol. We also developed a sensitive ELISA to measure human DBH protein, and found that the DBH activity:DBH protein ratio is the same in human serum and CSF and does not change in patients with Parkinson disease, whose CSF has very low DBH activity and protein levels compared with CSF from healthy individuals. These patients also have moderate but significantly decreased activity in their serum.

In 1989 we cloned the human dopamine β-hydroxylase (dopamine β-monooxygenase) (*DBH*) gene *(3)*. This gene resides on chromosome 9p34 and

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[2] This paper has been cited 563 times since its publication.
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[3] Nonstandard abbreviations: DBH, dopamine-β-hydroxylase; NE, norepinephrine; E, epinephrine; CSF, cerebrospinal fluid; PNMT, phenylethanolamine-N-methyltransferase.

spans a stretch of approximately 23 kb. A functional −1021C→T polymorphism in the *DBH* gene was demonstrated to regulate plasma DBH activity, and individuals with the genotype T/T have genetically determined low DBH activity in their plasma *(4)*.

The reason for the frequent citation of our report may be the simplicity of the photometric method in clinical chemistry for determining the activity in human blood, which parallels the protein level, and the high interest in DBH activity in the blood in various diseases that affect central and peripheral catecholamine systems, such as DBH deficiency and pheochromocytoma.

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References

1. Nagatsu T, Kuzuya H, Hidaka H. Inhibition of dopamine β-hydroxylase by sulfhydryl compounds and the nature of the natural inhibitors. Biochim Biophys Acta 1967;139:319–27.
2. Weinshilboum R, Axelrod J. Serum dopamine-β-hydroxylase activity. Circ Res 1971;28:307–15.
3. Kobayashi K, Kurosawa Y, Fujita K, Nagatsu T. Human dopamine β-hydroxylase gene: two mRNA types having different 3′-terminal regions are produced through alternative polyadenylation. Nucleic Acids Res 1989;17:1089–120.
4. Zabetian CP, Anderson GM, Buxbaum SG, Elston RC, Ichinose H, Nagatsu T, et al. A quantitative-trait analysis of human plasma-dopamine β-hydroxylase activity: evidence for a major functional polymorphism at the *DBH* locus. Am J Hum Genet 2001;68:515–22.