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FINAL EXAMINATION FOR THE DEGREE

MASTER OF SCIENCE

Student: John Affronti

Date: Aug. 17, 1981

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ABSTRACT

Title of Dissertation: Plasma Dopamine-Beta-Hydroxylase as an Index of Peripheral Noradrenergic Activity

John P. Affronti, Master of Science, 1981

Dissertation directed by: C. Raymond Lake, M.D., Ph.D., Assoc. Prof. of Pharmacology and Psychiatry, Department of Pharmacology

Dopamine-Beta-Hydroxylase (DBH) (E.C.1.14.17.1) is the biosynthetic enzyme for norepinephrine and is released with the neurotransmitter during nerve depolarization. The enzyme can be measured in plasma, and such measurements may give a chemical estimation for peripheral noradrenergic neurotransmission. The literature was reviewed to assess the validity of using plasma DBH as an index of peripheral noradrenergic activity. Conclusions indicate that although serum DBH reflects sympathetic activity in some situations, many factors limit its use to estimate acute sympatho-adrenalmedullary function. Despite these limitations its measurement in patients with essential hypertension, psychiatric disease or under psychological stress has provided interesting results and prompted new avenues of research. Future uses of DBH are discussed and the use of cerebrospinal fluid DBH as an index of central non-adrenergic activity is addressed.

PLASMA DOPAMINE-BETA-HYDROXYLASE ACTIVITY AS AN INDEX OF PERIPHERAL NORADRENERGIC ACTIVITY

by

John Affronti

Thesis submitted to the Faculty of the Department of Pharmacology Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Master of Science 1981

To my Father and Mother.

I would like to thank the members of my student advisory committee and foremost, my primary advisor, Dr. Lake for their suggestions and comments which were extremely helpful during the writing of this paper. I am also deeply grateful to Dr. Aronow and the members of the Pharmacology Department as well as Dr. Bullard and the Graduate Education Committee for their encouragement and support while studying at the Uniformed Services University of the Health Sciences. In addition, I would like to express my appreciation to Annette Stirba for her editorial comments and a special thanks to Cathy Cameron for providing her word processing expertise.

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Abbreviations

CA - Catecholamines

NE - Norepinephrine

EPI - Epinephrine

DA - Dopamine

DBH - Dopamine-Beta-Hydroxylase

CSF - Cerebrospinal Fluid

6-OHDA - 6-Hydroxydopamine

DOPA - Dihydroxyphenylalanine

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modifications of the spectrophotometric assay have also been described (112, 61, 73). These employ the use of high pressure liquid chromatography and ^{14}C-tyramine as a substrate. These methods can measure DBH levels in rat serum but are used less.

B. The Radioenzymatic Assay

The coupled radioenzymatic assay (254) has been the most sensitive technique for measuring plasma DBH activity in animal studies. Although the spectrophotometric method is adequate for measuring most human samples, the enzymatic assay is used when doing experimental work with animals. A simplified diagram of the general procedure appears in figure 6.

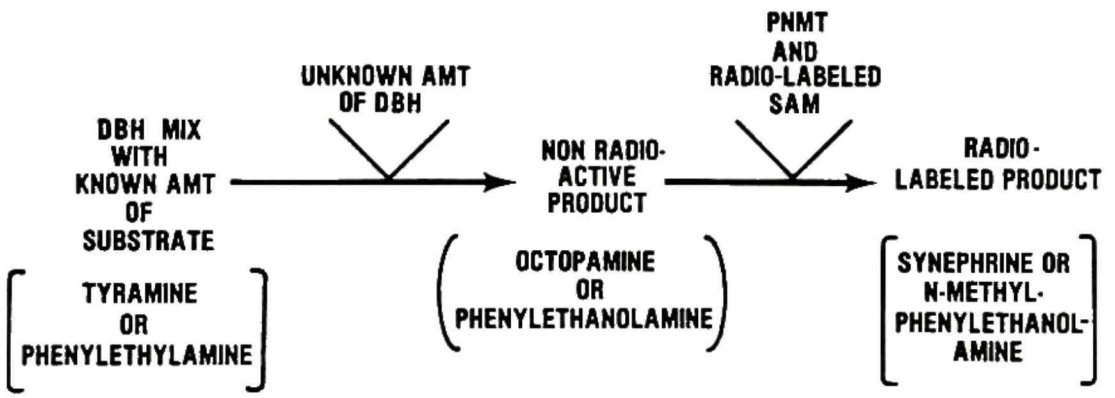

FIG. 6 A SIMPLIFIED DIAGRAM OF THE RADIOENZYMATIC ASSAY FOR DBH

(AMOUNT OF PRODUCT ANALYZED BY SCINTILLATION COUNTER)

The procedure calls for the addition of a "DBH mix", which contains a known amount of substrate, to a plasma sample containing an unknown amount of DBH. Sodium fumarate, catalase, ascorbic acid, and an acidic buffer are included in the DBH mix along with the substrate. In addition, a monoamine oxidase inhibitor is added to the mix to prevent metabolism of the substrate by monoamine oxidase present in the sample.

Two substrates are frequently used in the plasma assay. Even though tyramine has a higher specificity for the DBH reaction, phenylethylamine is used more often because it is not necessary to dry the samples overnight to remove a volatile radioactive contaminant that is present in the final organic solvent extract when tyramine is used (178). This volatile contaminant may be due to the N-methylation of tyramine by phenylethanolamine-N-methyltransferase (PNMT) (254). Phenylethylamine is also N-methylated by PNMT especially when the substrate concentration is high (148, 178). When tyramine is used, nonsaturating levels are necessary because it can inhibit the subsequent PNMT reaction (254, 129).

The samples to which DBH mix is added may contain varying amounts of endogenous DBH inhibitors (42). To reduce the effect of these inhibitors, low concentrations of $CuSO_4$ and N-ethylmaleimide are added to the samples (70, 42). Since each sample may have varying amounts of endogenous inhibitors, it has been suggested that duplicates of every sample be analyzed with varying amounts of copper or N-ethylmaleimide. In this way, a concentration yielding optimal enzyme activity can be determined (148).

The DBH mix is added to the plasma sample containing DBH; the mixture is allowed to incubate at 37°C for 30 to 90 minutes in a shaking water bath, after which the reaction is stopped by the addition of a second mix. The PNMT mix contains the enzyme PNMT and a buffer at a pH of 8.6, which effectively terminates the DBH reaction. The PNMT mix also contains EDTA, which acts to reduce copper's inhibitory effect on PNMT as well as to further inhibit DBH (28).

PNMT catalyzes the conversion of the DBH reaction products into radioactive compounds by combining them with the radiolabeled methyl group from S-adenosyl-methionine (SAM). The quantity of these radioactive compounds is then estimated by liquid scintillation spectrometry after the excess radiolabeled SAM is removed by extraction with an organic solvent.

The above procedure is relatively easy to perform and gives reproducible results when assaying one sample on 6 different occasions with a standard error of 1.8% of the mean (254). Various precautions should be taken to avoid erroneous interpretation of the results. Laduron (148) discusses a number of problems which may be encountered when performing the assay and gives suggestions on how to avoid them.

A major concern already addressed is the possibility that substrates other than phenylethylamine or tyramine will be converted to radioactive products and produce data that misrepresent the true amount of DBH in the sample. Substantial amounts of "false substrate" could be present in the sample. Blood samples may contain varying amounts of these false substrates depending on the kind of experimental conditions experienced by the donor. It is a useful practice to separate the reaction products by thin layer chromatography and measure their radioactivity to insure that only the proper product is radioactive (254).

Choice of "blanks" in an enzymatic assay is crucial. The use of a "blank" sample with an omitted substrate might produce abnormally high levels of radioactivity, thus warning of "false substrates". A "blank" consisting of a boiled sample would not reflect such problems. Other enzymes in the blood sample able to convert various substrates

into radioactive compounds under assay conditions may distort results (25). Boiling samples for "blanks" to denature DBH also denatures these additional enzymes and artifactually increases the blank-to-standard ratio. Blank samples can be made in several ways (148). A specific DBH inhibitor such as fusaric acid could be added. Alternatives to omitting substrates from the reaction mixture include omitting essential cofactors such as fumarate or ascorbic acid. Since some of these methods reduce DBH activity more than others and each checks for different sources of error in the interpretation of results, the use of more than one type of blank is advisable (148).

A problem in the coupled enzymatic assay is the existence of substances in the blood which affect the characteristics of DBH activity. As previously mentioned, endogenous inhibitors are known to be present and various compounds such as copper are added to assay samples in order to block these inhibitory effects. Since high concentrations of copper can also inhibit DBH (176), a concentration must be found which provides optimal enzymatic activity. Furthermore, it is possible for levels of these endogenous inhibitors to vary among subjects as well as within the same subject as a result of changing experimental conditions. Laduron suggests that each sample be assayed in the presence of different concentrations of copper to determine which concentration gives the highest DBH activity (148).

PNMT may be influenced by inhibitors and activators. NE as well as EPI inhibits PNMT (178). The levels of these amines in the blood can vary under many different conditions (280, 137, 34). To insure that fluctuations in PNMT activity are not the cause of fluctuations in the rate of radioactive product formation, an internal standard

is used. A known amount of PNMT substrate is added to the sample and the DBH reaction not allowed to take place. By comparing these internal standards from sample to sample, variation in results due to variation in PNMT activity is monitored.

After all checks have been made and the optimal copper concentrations are determined, the linearity of the DBH reaction must be demonstrated. This is accomplished by adding purified bovine enzyme in concentrations across the range of the experimental samples to boiled experimental sample aliquots (178). A linear increase in the concentration of enzyme in a sample with an optimal concentration of copper should produce a linear increase in the amount of substrate converted to radioactive product. It is essential that the coupled assay be proven linear because tyramine used in high concentrations will inhibit the PNMT reaction (see page 10). The inhibition will cause a non-linear increase in the amount of products formed in response to a linear increase in DBH concentration, making the determination of DBH concentrations difficult.

C. The Radioimmuno Assays

The activity of DBH and the quantity of DBH protein in a blood sample have been shown to be significantly correlated when performing both radioenzymatic and radioimmunoassays on the same sample (272, 125, 44, 220). Although earlier comparisons between activity and quantity showed a low coefficient of correlation (219), it was later determined that the DBH antibody made from bovine adrenal DBH was inappropriate for measuring human DBH (220). The composition of the human enzyme is different from the bovine species. Rush et al. used

both human and bovine enzyme as antigen for antibody production, and used the different antibodies in their radioimmunoassay for DBH in human serum (219, 220). They speculated that differences in their assay results were due to the inability of antibody against bovine DBH to crossreact with the human enzyme.

The immunoassays provide an advantage over the radioenzymatic and spectrophotometric assays in that the results of the former are not subject to the effects of endogenous inhibitors or other complicating factors inherent in the enzymatic assays. The drawback of the immunoassay is the necessity of production and storage of antibody made with enzyme from the species to be studied. While this is relatively easy for the laboratory that limits its studies to one specie, those wishing to study a variety of animals must make antibody specific for each species studied.

V. Location of DBH

A. Location of DBH in the Periphery

In addition to techniques that give quantitative estimates of DBH, qualitative immunofluorescent methods have been developed which can localize DBH in sections prepared for light microscopy (74, 100). Rabbit antibody to DBH is allowed to complex with the enzyme in tissue slices and a fluorescein labelled anti-rabbit immunoglobulin is then applied. On completion of this procedure, the areas of fluorescence correspond to the sites of the enzyme. DBH has been visualized by immunofluorescence in the adrenal medulla, acinar ducts of salivary and sublingual glands and in peripheral noradrenergic neurons.

The plasma contains DBH but the cellular elements do not (254).

DBH is found within the adrenal medulla (99, 100) but not in the adrenal cortex (128). In the rat, most medullary cells have a relatively weak fluorescence; however, several cell islands having a strong fluorescence are observed (74).

DBH is found in the saliva and the acinar ducts of the sublingual glands. Noradrenergic neurons are not totally responsible for the saliva DBH because thirty days after these glands are denervated a substantial amount remains (29, 275). DBH's presence in the ducts suggests that it is transported by the salivary gland from the circulation into the saliva.

DBH has been found in the vast majority of ganglia cells known to store NE (74). Noradrenergic axons and nerve terminals of the superior cervical ganglion did not stain. The authors believed the lack of staining was due to the low penetration of antibodies into the presynaptic boutons. The presence of DBH in adrenergic neuronal processes which innervate the vasculature and other organs has been verified through the use of morphological and biochemical techniques (232).
(See page 18)

B. Location of DBH in the Cell

The early studies of the intracellular location of DBH focused on the medullary cells of the adrenal gland because of the relative abundance of enzyme in their cytoplasm. Existence of granular elements in the medullary cells was suggested at the turn of the century, when they were observed after fixation with bichromate (40, 110, 166). They

were thought to differ from mitochondria and proposed to be clumps of oxidized CA (15). Blaschko and Welch presented the first evidence for their occurence in vivo as structural elements (16). Subsequently these granules were observed by phase contrast and dark field microscopy (103). Electron microscopy revealed that a delicate membrane or sac invested these granules (153). These observations led to the general consensus that the structures are vesicles and the granular appearance is a staining/fixation artifact representing the precipitation and condensation of oxidized CA (121). These vesicles contain the majority of intracellular DBH (123).

Vesicles have been isolated from the bovine adrenal gland (15, 16, 96) and biochemical analysis revealed that CA as well as other proteins and lipids were present, but that DBH was the only NE synthesizing enzyme in the vesicle (184). In the adrenal medullary cell, varied amounts of DBH are attached to the vesicle membrane and contained in the liquid or soluble portion of the granule (232, 11). Different degrees of incorporation of the enzyme into the vesicle membrane may reflect different stages in the maturation of the vesicles (232). The composition and enzymatic characteristics of the two forms are similar (109, 131). The purified protein fraction of the vesicle was found to consist of two primary components with molecular weights of 39,000 and 19,400 Daltons (230). In the same study, tryptic digests of both components gave similar "fingerprints", which suggested that the heavier protein may be a dimer of the lighter proteins. Both proteins are enzymatically active (213).

Similar vesicles have also been isolated from other tissues receiving noradrenergic innervation, including the vascular system (54,

55, 56, 111, 205, 204, 223, 224). Histological evidence indicates that these granules are in the noradrenergic nerve terminals of the vas deferens, carotid body, and cardiac muscle (209). The only notable difference in DBH between the adrenal granules and those of the presynaptic neurons in sympathetic tissue is the ratio of soluble to membrane-bound form of the enzyme. In the neuronal vesicles there is a small quantity of soluble DBH. However, the percentage of the soluble form still varies (232, 108, 122).

VI. DBH as an Indication of Noradrenergic Activity

A. Extraneuronal DBH as an Index of Noradrenergic Activity

The storage vesicles containing DBH and NE play an important role in the transmission of neuronal impulses. When a neuron is depolarized, the storage vesicles fuse to the cellular membrane in the nerve terminal which is close to a second neuron (232). This process, called exocytosis, results in the extrusion of vesicle contents into the area between the two neurons called the synaptic cleft or junction. The difference in composition between the extracellular and intravesicular fluids probably results in a rapid disaggregation of the storage complex and its components (128). The membrane of the storage vesicle is recycled into the cytoplasm, perhaps by micropinocytosis, where it seems to be rapidly degraded (247). Some of the NE released by the depolarized noradrenergic neuron (the presynaptic neuron) binds to receptors on the second neuron (the postsynaptic neuron). NE bound to these receptors, causes a conductance change in the membrane of the post synaptic neuron.

DBH in the catecholamine storage vesicles is also released into the synaptic cleft when a noradrenergic neuron is depolarized (37, 77, 242, 261). Although the "soluble DBH" previously described has not been proven to be the type of DBH released, the amount of DBH ejected is proportional to the amount of NE released (261). This fact is significant because the metabolic fate of DBH differs from NE (6). Based on the current understanding of synaptic function, one can assume that levels of NE in the cleft are a result of 4 variables (218):

1. rate of NE release by exocytosis (rate of neuron depolarization)
2. content of NE in the storage vesicle (rate of NE synthesis)
3. rate of NE reuptake
4. metabolic inactivation of NE

DBH levels, however, are believed to be determined only by two variables (218):

1. rate of DBH release by exocytosis (rate of neuron depolarization)
2. content of DBH in storage vesicles (rate of DBH synthesis)

To date no evidence supports the existence of a reuptake mechanism for DBH, nor is there any reason to believe that a process exists for the enzymatic metabolism of the enzyme in the synaptic cleft. This reasoning led many people (37, 77, 95, 261) to believe that if plasma DBH levels reflected levels of enzyme in the synaptic clefts of noradrenergic tracts, then levels of DBH in the blood would be a good indication of noradrenergic activity.

B. Plasma DBH as an Index of Sympathetic Activity

1. Source of Plasma DBH

As shown in a previous section, DBH is found in the adrenal medullary cells as well as in noradrenergic nerve terminals. DBH is released from these cells and appears in the blood (186). To determine whether the quantity of DBH in the blood is influenced by the release of enzyme from the adrenal medulla or from the nerve terminals, two sets of experiments were performed. In the first, the adrenals of rats were surgically removed and plasma DBH levels measured before and after the surgical procedure (256, 257). In the second set of experiments, noradrenergic neurons were destroyed with 6-hydroxydopamine (6-OHDA) which left the adrenal gland unharmed (256). Plasma DBH levels were measured before and after injection of the drug. Adrenalectomy did not significantly change plasma DBH levels. From these results the authors concluded that very little plasma DBH in the rat originates from the adrenal. In the second set of experiments, in which noradrenergic neurons were destroyed with 6-OHDA a significant decrease in plasma DBH resulted. Although the decrease in plasma DBH activity was only 20%, the authors nevertheless contend that neuronal release accounts for a major portion of the plasma DBH. They suggest that the changes in blood volume promoted by 6-OHDA resulted in an underestimation of the decrease in plasma DBH, and that all of the noradrenergic neurons may not have been destroyed. Moreover, another group of investigators who used a higher dose of 6-OHDA reported a larger (40%) decrease in plasma DBH (29). Based on this information, most investigators believe the majority of the DBH in the

circulation comes from the sympathetic nerve endings of the vasculature (184, 75, 253). However, in cases of extreme sympathetic activity such as with prolonged blood loss the adrenals may contribute a substantial portion of the DBH found in the blood of animals. (See page 34)

The path by which DBH enters the blood from the nerve endings may have complicated the results of these experiments and therefore the entrance of the protein into the circulation should be considered when using plasma DBH levels to estimate sympathetic activity. Proteins as large as DBH have a difficult time traversing the capillary wall. As a result, the enzyme probably has direct access to the blood only in special organs where capillaries have large gaps in their endothelial walls (such as the adrenal gland and the spleen) (128). Another indirect means by which large proteins can enter the blood is through the lymphatic system. Lymphatic ducts anchored in the intercellular spaces also have gaps in their endothelium that allow large proteins to enter relatively easily. Once in the lymphatic system, these proteins are carried along with the lymph and released into the circulation at a point just below the jugular vein. DBH has been found in human lymph (2), and lymph DBH levels have been observed to rise with increased sympathetic activity (188). These studies suggest that a major portion of DBH released from adrenergic nerve terminals is transported in the lymph before it enters the circulation. This means that the rate at which DBH enters the circulation is influenced not only by the activity of the sympathetic nervous system, but also by the rate at which lymph is released into the blood. The indirect path followed by DBH delays the enzyme's entrance into the blood. In addition, the lymphatic system is capable of acting as a reservoir for DBH before its release into the

circulation. This may explain why a residual amount of DBH was observed in the blood of rats even after noradrenergic neurons were destroyed with 6-OHDA (29, 256).

Sympathetic nerve terminals seem to be a major source of DBH, but experimental data do not entirely rule out an extraneuronal contribution to circulating DBH activity, nor do they prove that the release of DBH with CA is the only method by which DBH escapes from nerves into the blood. Variable amounts of DBH may be released from granules as a result of lysis (11, 108). It has also been suggested that the extrusion of membrane-bound DBH by a mechanism unrelated to the secretory process may occur in addition to exocytotic release, thus maintaining plasma DBH levels in the absence of sympathetic activity (128).

2. Fate of Plasma DBH

The amount of DBH present in the blood is influenced by the rate and method of exit as well as the rate of entry. Although very little is known about the mechanism of clearance for plasma DBH, it has been suggested that the enzymatic removal of a terminal sialic group (186) exposes a galactose residue which may bind to a specific site on the membrane of a hepatocyte, thus effectively removing the protein from circulation (181, 245). Another possible route of exit is by excretion into the saliva via the sublingual and submaxilary glands (29) (see page 17). DBH is found in the acinar ducts of these glands even after the glands are denervated (29, 275). Researchers infusing radiolabelled DBH into sheep report that after 24 to 48 hours the highest levels of radioactivity were found in the liver, kidney and lungs and suggest that these organs may play a role in the removal of the enzyme from

the circulation (218); however, investigators failed to detect DBH activity in the urine (186). This is not surprising, because the intact DBH protein with its molecular weight being above 80,000 Daltons would not be expected to pass into the glomerular filtrate (206). No evidence of pulmonary inactivation of human DBH was found in a study of pulmonary artery and left ventricular DBH levels in 14 subjects (234).

The limited amount of information on the metabolism of plasma DBH has complicated efforts to determine the half-life of the enzyme in the blood. A few investigators who have nevertheless attempted to determine the rate of clearance obtained differing results (82, 218, 93). In one study, purified bovine adrenal DBH was injected into rats. Then blood samples were taken at different times and assayed for DBH activity (82). The results of these experiments revealed a biphasic decline in DBH activity. An initial rapid phase with a half-life of 2.2 to 3.0 hours was observed, followed by a second phase with a half-life of 4.5 days. In another study, ^{125}I-labelled purified bovine adrenal DBH was injected into sheep (218). By monitoring the decline of radioactivity in the blood over a period of 8 hours, a half-life of about 3 hours was calculated. Similar results were observed in swim-stressed rats (212). A different method of calculating the half-life of DBH was employed in another study (93). An antirat DBH antibody which reduced DBH plasma activity was injected into rats. By monitoring the rate by which DBH returned to the blood and assuming that the rate of return is equal to the rate of enzyme clearance, a half life of 4.2 days was calculated.

After considering all the animal data, a possible explanation for the results is an increased rate of clearance for high concentrations of

DBH, followed by a slower rate when levels reach a lower concentration. It must be remembered that the above data were obtained from animal models which may not be representative of the human state. The only study on the fate of human plasma DBH reported a half-life of 8 to 12 hours, which is not consistent with any of the results above (128).

3. Factors Influencing Plasma DBH

a. Normal Levels in Human Plasma

When it became apparent that some, if not most, of the plasma DBH originated from peripheral sympathetic neurons, many investigators started to study the relationship between plasma DBH levels and sympathetic activity. A first objective for many researchers was the description of "normal" values for plasma DBH activity in man (186, 105, 260). A review of normal plasma DBH activity was done by Weinshilboum (253). Despite the different values produced by different assay techniques, large groups of human samples assayed with the same substrate and under similar conditions gave a wide range of values for plasma DBH activity (3, 260, 105, 111). Some normal individuals have plasma enzyme levels below the sensitivity of the radioenzymatic assay (about 50 nmole/ml/hr). Three percent of 227 normals ages 9 to 64 had very low activity (below detectible levels) in one study (260), and ten percent of 106 patients had low levels in another (263). This suggests the existence of a subgroup of normals with low DBH levels. Other normal patients had very high enzyme activity (2500 nmole/ml/hr) (260). This wide range of enzyme values in the normal population limits the information to be gathered from comparative studies. Weinshilboum notes the

distribution of plasma DBH values is not statistically normal, but skewed to the left of the mean (253, 259, 258). A comparison of the distribution of DBH values among different age groups suggests changes in plasma DBH during growth and development (65, 263) (see page 29). No significant differences have been reported between male and female subjects' values in large studies (105, 259, 258). A large survey of black and white subjects in the United States (173) found no interracial differences in serum DBH activity, which conflicts with results from a smaller study (105).

Although differences between normal individuals can be large, variation of plasma DBH activity in a single individual is minimal (254, 91, 139). In many studies (263, 194, 84, 186), day-to-day variation in individuals plasma DBH levels was statistically non-significant. Similar results were observed in the month-to-month variation (263, 217), and one study showed that an adult's plasma enzyme activity remained the same over a period of seven years (139). While comparisons of plasma DBH levels between subjects may yield a limited amount of information, studies which monitor changes in an individual's plasma enzyme level under various conditions may better elucidate the relationship between sympathetic activity and plasma DBH concentration.

b. Genetic Regulation

Much evidence suggests that the large differences in plasma DBH activity between individuals are due more to effects of inheritance than differences in exocytosis (253). Genetic influence was implicated when low plasma DBH levels were detected in patients with the autosomal dominant disease familial dysautonomia.

If heritability were totally responsible for a trait, the correlation for that trait in monozygotic twins would be expected to be 1.0, and the correlation for the trait in siblings, dizygotic twins or parent child pairs would be expected to be 0.5 (20). The actual correlations of DBH values among different relatives are listed in Table 1.

Table 1: The Comparison of Plasma DBH Activity Among Relatives

Pair Studied	Correlation	Ref.
monozygotic twins	.96	217
dizygotic twins	.75	217
siblings	.50	194
Father/child	.51	194
Mother/child	.48	194

There are no differences between father/child, mother/child, father/son, or father/daughter pairs, indicating a lack of specific influence by the X choromosome (211).

Very low plasma DBH activity was observed in 3 to 10% of a randomly selected sample of the population (263, 260). Based on RIA assay results, the low DBH activity has been tentatively attributed to low quantities of enzyme in the blood (44, 45). Results from family studies (260) are compatible with the monogenic or mendelian inheritance of the low plasma DBH trait, and the trait was suggested as autosomal recessive (50). In a subsequent study, however, the same families described previously (50) were reanalyzed using a more powerful genetic analysis (81). With the results of a pedigree segregation analysis, the

"environmental" mode of transmission was rejected along with the dominant and recessive modes of inheritance. The tests did not consider the possibility of polygenic inheritance, but within single gene alternatives, codominant inheritance was found to be most likely (81). Codominant inheritance is thought to be responsible for variations in the activity of other proteins. The hemoglobin protein, for example, is known to exist in different forms. Individuals with a homozygous genotype for the sickle cell trait synthesize only the "S" form of the molecule. Heterozygous individuals produce both the normal and "S" forms. If a similar situation exists with DBH and both alleles are expressed, then perhaps variant forms of DBH (membrane-bound vs. nonmembrane-bound?) are expressed by each allele. These different forms of enzyme may determine the amount of DBH released by exocytosis, thereby influencing the levels of the enzyme in circulation. Enzyme activity in the blood of presumed heterozygotes (i.e., parents of children with low serum DBH levels) was intermediate between values from homozygotes and a randomly selected sample of the population (260). In matings of two heterozygotes, 22% of the siblings had very low plasma DBH activity (260). This is much higher than the 3 to 4% found in the randomly selected sample of the population, and very close to the 25% predicted for monogenic inheritance (260). The fact that presumed heterozygotes had intermediate plasma DBH levels further suggests that the inheritance is codominant.

Other variant forms of the enzyme have been described (45, 47 47). A rare variant of the enzyme which possesses low activity (45) as well as a thermolabile variant found in 10% of the randomly selected population (47) have been observed. The use of this thermolabile

variant as a marker in future genetic studies of DBH will further elucidate the effect of inheritance on plasma DBH activity. At this point, however, much evidence suggests that heritability is responsible for most differences between individuals (50, 157). It is assumed that the genetic variable is expressed through variations in enzyme characteristics, synthesis, or the amount of enzyme attached to the vesicle wall. However, genetic influence on adrenergic function cannot be ruled out.

c. Growth and Development

Based on the information given in the previous two sections, most researchers believe that monitoring a single individual's plasma DBH activity under various conditions will show more clearly any relationship of enzyme levels to sympathetic activity than an experimental design comparing control and experimental groups. Knowledge of "normal" variations in plasma DBH levels which occur in humans is essential if one is to extract meaningful information from such studies. One factor that causes changes in enzyme levels is the developmental process.

Neurocrest tissue appears early in morphogenesis, giving rise to sympathetic ganglia and adrenal medullary tissue (107). This tissue migrates from the neural tube early in embryonic life and also gives rise to melanophores (cells that specialize in tyrosine metabolism) (262, 266). In frog whole embryos and neuralcrest tissue, DA appears earlier than NE (19). Investigators injecting ^{3}H DOPA into chicks less than 30 hours old observed the formation of DA, but not NE (18). The formation of NE did not occur until the third day of the chick's life. This and

similar studies (121) suggest that enzymes of the CA pathway appear during development in the same sequence as in the NE biosynthetic pathway.

DBH activity in the sympathetically innervated heart and salivary glands of the rat rose over the first two weeks of life and then remained constant (144, 199). This pattern contrasts with changes in rat plasma DBH activity. Plasma DBH values in the rat peak at 3 to 7 times adult levels 14-18 days after birth, and then decline to adult levels by day 50 to 60 (9, 130, 144, 199). This inconsistancy might be due to a rapidly developing sympathetic nervous system which produces the initial peak, and a continuing increase in blood volume after 14 to 18 days that causes a decline in plasma enzyme concentrations. Other developmental changes which may influence plasma DBH include:

1. Changes in the functional activity of the sympathetic nervous system
2. Changes in the accessibility of DBH to the blood
3. Changes in the removal of DBH from the circulation

There are interspecies differences. The Japanese monkey experiences a 10-fold increase in plasma DBH activity between 3 months and 10 years of age, without a dramatic decline in activity over time (117). Even with the 10-fold increase over the first 10 years of life, the adult monkey's plasma DBH activity is only 1/100th that of humans.

Results of studies on human subjects conflict in many respects, but all show a rise in plasma DBH activity from birth to about age 6 (65, 255, 128, 263, 258, 217). While one study demonstrated a rise through the sixth decade of life (65), another showed no significant

changes in plasma enzyme activity after age six (255). However, a study of 367 subjects ranging in age from birth to 73 years showed plasma DBH activity reaching a maximum at age 15 followed by a progressive decrease to levels significantly lower than the maximum levels by the fifth decade (128). This agrees with two other studies (258, 194). An increase was observed in one study after 50 years of age (128).

An attractive explanation for the changes in plasma DBH activity is that they result from changes in the functional state of the sympathetic nervous system in transitional periods of infancy and adolescence. This is only speculation, however, and until more thorough investigations are performed, the physiological basis for the changes will remain unknown.

d. Diurnal Changes in Plasma DBH

Short-term 24-hour rhythms of plasma DBH activity exist in many species, including man (167, 169). Investigators have observed a circadian rhythm in plasma DBH levels only in some laboratory rat strains (8). Plasma DBH levels at 4 a.m. were observed to be twice as high as any other time of day in the Holtzman rat (8). No rhythm was observed in another rat strain (8).

A 24-hour rhythm has been described in normal and blind men (58) as being a decrease in plasma enzyme activity between the hours of 12 p.m. and 4 a.m. (58, 196, 244). A 10 to 15% change was seen in the above studies, and the rhythm could be disrupted by sleep deprivation or keeping the subjects in a horizontal position for 24 hours (196). These results have prompted some to believe that daily changes in plasma DBH activity are partly due to changes in posture and physical activity (253, 128, 184); others disagree (244).

e. Hormonal Regulation

Except for pathological states and situations where endocrine organs are removed, most changes in plasma DBH activity corresponding with hormonal fluctuations are small and often hard to reproduce. Conflicting results have been reported about human plasma DBH activity during the menstrual cycle. In one study, enyzme activity approximately 10% above average monthly levels was seen soon after ovulation, and decreases reaching 10% below monthly averages were observed during the premenstrual period (141). Despite these findings, two later studies could not demonstrate a relationship between plasma DBH levels and the menstrual cycle in humans (277, 251). Although data from animal experiments support the concept of catecholamine mediated pituitary hormone secretion (116, 208, 248), the above studies involving humans suggest that the peripheral sympathetic system is only minimally involved. However, women who experience increased blood pressure or overt hypertension while taking oral contraceptives consisting of progesterone/estrogen combinations do have increased plasma DBH levels (210). (See page 41)

More striking changes in plasma DBH activity occur in some human diseases and in animal studies involving excision of vital endocrine organs. Decreased plasma DBH activity is observed in patients with hyperthroid disease, and changes in enzyme levels are inversely related to changes in thyroxin levels induced by pharmacological therapy (190, 192). More information concerning plasma DBH and thyroid disease appears in the section on plasma DBH levels in endocrine disease. Although there were no differences between the mean DBH activity of adrenalectomized patients and control subjects (191), animal studies in

which the pituitary gland was removed from rats reported an increase of 100 to 150% over control values (67, 143). A moderate decrease in the elevation of plasma DBH activity was observed after long-term administration of adrenocorticotrophic hormone at a supraphysiological dosage (63). It was suggested that this decrease resulted from a sympathetic response to the expanded extracelular fluid caused by excessive secretion of mineralocorticoids from the adrenal cortex (143). Dexamethazone, an adrenocorticosteroid with no sodium retaining qualities and therefore no mechanism of expanding extracellular fluid volume, produced no decrease in plasma DBH levels in the hypophysectomized rats (143). However, pitressin, possessing the antidiuretic qualities of the hypophyseal hormone vasopressin, significantly reduced plasma DBH activity even when values were corrected for extracellular fluid variation by using hematocrit comparisons (67). The changes in plasma DBH following hypophysectomy reflect the compensatory adjustments of the sympathetic nervous system. These adjustments attempt to maintain blood pressure in the face of changes in the vascular volume. The belief is further supported by a report that pitressin reverses high DBH levels in rats with hereditary diabetes insipidus, a condition characterized by low vasopressin levels (274).

f. Blood Volume Changes

Further evidence suggests that changes in serum DBH activity reflect the sympathetic compensation accompanying variations in vascular volume. An overview of these studies reveals that changes in plasma DBH activity are observed primarily with drastic and/or chronic blood volume alterations. In one study, experimenters measured a significant increase in NE after a 40-60% blood loss in dogs. Hemoconcentration is

a possible explanation. A sustained and controlled hypotension was not maintained, and the rise in plasma enzyme activity did not reach levels significantly different from control values (200). In another dog study, blood pressure was reduced and maintained at 35 mm Hg for 1 hour resulting in a significant increase in plasma NE and DBH (31). These changes were abolished by adrenalectomy suggesting that in periods of extreme sympathetic activation, the adrenal medulla may be a major source of plasma DBH. Variation of the extracellular fluid volume using dietary sodium restriction (145) and Desoxycorticosteriod Acetate (DOCA - salt) infusion (142) changes plasma DBH in the expected direction.

Variations in human DBH values accompanying less drastic changes in extracellular volume are not always significant, but support conclusions made with animal data. Two groups examining plasma DBH activity in patients undergoing mild or short-term extracellular volume manipulation failed to show significant changes from control levels (182, 134), but one found a 10% increase in patients on 10 meq sodium diet for 4 days (182). In other human studies, a significant 30% increase in plasma DBH was observed after sodium depletion for 4 days (241), and a 20% decrease with a volume expansion induced by intravenous infusion of saline (2400 ml) over a 2½ hour period (4).

The long half-life of DBH in the blood (128) may result in a large pool of the enzyme in the circulation. In order to see variations in this large circulating pool, drastic or long-term changes in vascular volume would be required to alter the amount of DBH entering the circulation.

g. Posture and Physical Activity

Posture influences the activity of the sympathetic nervous system (133). Although plasma CA levels more closely reflect the acute increase in sympathetic activity seen with a change from the supine to the erect state (133), small increases in plasma DBH activity also occur (241, 196). These increases are not observed after brief (30 minutes) changes in posture, however (271, 182). DBH's route of entrance into the circulation and its relatively long half-life of 8 to 12 hours in the blood may explain the lesser DBH elevation contrasted to the increases seen in plasma NE resulting from sympathetic activation. Some plasma DBH may enter the circulation via the lymphatic system (2), explaining the delay and diminished DBH increase with acute sympathetic activity. In longer periods of supine and erect posture, a 22% increase in plasma DBH activity was observed (241).

Small but significant rises in plasma DBH can be observed after 5 to 15 minutes of exercise (271, 13, 202, 203), and the degree of enzyme elevation is related to the amount of work done (202). Changes are seen more acutely after exercise on a stationary bicycle than in tilting (271) or blood volume variation (241). This may relate to increased physical activity promoting faster flow of the DBH carrying lymph into the circulation (2, 215).

The experimental results of this section are important to consider when designing experiments involving bedridden patients. Of a more phylogenetic interest is the observation that man's plasma DBH levels are much higher than those of other species which stand less erect and closer to the ground (196). A comparison of plasma DBH in humans and giraffes would be interesting.

h. Stress and Behavior

Laboratory rats experience an almost immediate increase in plasma DBH after forced immobilization and handling (257, 132). The excess DBH causing this initial elevation might originate in the adrenal, from which it has direct access to the circulation (132), but adrenalectomized and sham operated rats exposed to the same stressful situation also had elevations in DBH (257).

Rats forced to swim for 2 hours increased their plasma DBH levels after one trial and after swimming 2 trials a day for 3 days (212). The chronic but not the acute stress resulted in elevated enzyme activity in the adrenal and cervical ganglia possibly secondary to de novo enzyme-protein synthesis induced by chronic stress.

Human subjects have volunteered for such stresses as exercise, sustained hand grip, hypoglycemic shock and submersion of one hand in ice water (cold pressor test) while their plasma DBH activity was monitored. The effects of exercise on plasma DBH were discussed above. Sustained hand grip and hypoglycemic shock produced minimal changes in plasma DBH activity despite large increases in blood pressure and NE during both tests (195).

The most widely used stress model employed in human plasma DBH studies is the cold pressor test. In most investigations, the painful 3 to 5 minute stress period caused little or no rise in plasma DBH (64, 237, 269), despite reports that it causes significant increases in plasma CA levels (269). In one study, a small decrease in serum enzyme was observed (237). In the studies where significant increases were demonstrated (271), factors other than neuronal release of DBH such as plasma volume changes or nonspecific changes in plasma proteins

may have been important in altering enzyme activity (231). Moreover, variations in other high molecular weight plasma proteins suggest that changes in plasma volume may have caused alterations in plasma DBH activity purely on a dilutional basis (231).

The disparity between animal and human stress studies in inducing DBH changes may be explained by differences in the severity of the stress used in the two types of experiments. In addition, humans have a much larger circulating pool of enzyme which make small changes secondary to variations in neuronal and adrenal exocytosis difficult to detect. The results of these studies are consistent with others in finding that serum DBH is not as good an indicator of acute changes in human sympathetic activity as NE.

In prolonged periods of psychological stress, significant elevations in plasma DBH have been observed. A mean increase of 24 ± 6% in plasma DBH activity was demonstrated in a pilot study where blood samples were analyzed before and after an 8-hour anxiety producing psychotherapy session (229). Increases in tissue DBH have been associated with premortem stress (228). Low correlations between plasma DBH and infant irritability have been reported (207).

Although many of these preliminary studies do not address the variables of subject activity and posture as well as other pertinant factors, they nevertheless encourage further examination of long-term psychological stress and its influence on plasma DBH activity.

Although investigators who exposed humans to 10°C temperatures reported an increase in plasma DBH activity (68), another research group which submersed its subjects to the neck in 10°C water for one hour saw no change in the enzyme level over the 90 minute study

period (113). Despite the differing results, variations in experimental design, including the effect of partial buoyancy in liquid, restricts one from describing the data as conflicting.

Plasma DBH has been monitored during sexual activity. In addition to physiological indications of increased sympathetic activity during sexual arousal (114, 187, 7), serum DBH and NE levels increased significantly (268, 267). No variation in plasma DBH activity was seen after experiencing 11 weeks of a normal pregnancy (184), and a 68% increase in plasma DBH was seen during the normal delivery of a first born (101).

C. Plasma DBH in Clinical Medicine

1. Cardiovascular Disease

a. Hypertension

i. Primary Hypertension

Interest in the relationship of plasma DBH and hypertension stems from the role of the sympathetic nervous system in blood pressure regulation (33). The etiology of almost 90% of the patients with high blood pressure is unknown. This large group is said to have "primary" or "essential" hypertension and may consist of several subgroups with differing pathologies (226), one of which appears to be neurogenic in nature (115, 33).

Many experiments found no significant correlation between plasma DBH and blood pressure (252, 135, 105, 173). This observation was expected since many factors besides sympathetic neuronal activity regulate blood pressure. Also two studies found no plasma enzyme differences between essential hypertensives and controls (see Table 2).

Table 2. Plasma DBH Differences Between Groups of Hypertensives and Controls

Type of Hypertension	Difference in Plasma DBH from Control Group	n	Ref
Essential	None	70	105
Essential	None	68	135
Essential	Increase	29	221
Essential	Increase	20	163
Essential	Increase	28	78
Low Renin Essential	Decrease	7	191
Low Renin Essential	Non Significant Decrease	7	149
Labile Essential Hypertension	Increase	6	221
Labile Essential Hypertension	Increase*	9	236

* Control group consisted of hypertensive subjects.

This could be due to the wide range of plasma DBH activity in normals (3, 260, 105) (see page 25). In addition it is possible that the essential hypertensive groups used in these experiments contained individuals with a nonneurogenic variation of the disease or a form of hypertension associated with low DBH levels (191, 149) (see Table 2). Studies using smaller groups of subjects have found differences in the plasma DBH activity of essential hypertensives and controls (see Table 2). It is possible that these smaller groups had a large proportion of patients with a type of hypertension associated with high plasma DBH values, thus, significant correlations were observed (221, 236).

Subtypes of essential hypertension have been described. Hypertensive patients are classified by their plasma renin concentration (27); low, normal, and high renin hypertension have been described (147). Low renin hypertensives have low levels of plasma DBH in some studies (see Table 2). However one study could not demonstrate the lower plasma DBH values to be significantly different from those in normal renin hypertensives (149). Low plasma DBH activity may reflect the reduced sympathetic activity in response to hypertension produced by some form of renal or adrenal mechanism. This idea is supported by reports of reduced plasma DBH in secondary hypertensive patients with renal disorders and adrenocortical pathology (236). However, reduced enzyme synthesis, dilutional effects and other factors cannot be ruled out.

Labile or borderline hypertensive patients usually have normal blood pressure but are susceptable to periods of blood pressure elevation (115), possibly due to neurogenic mechanisms (115). Labile hypertensives have increased plasma DBH when compared with controls (221) and other types of hypertensives (221, 236) (see Table 2). Although this evidence supports the belief that the sympathetic nervous system is overactive (115) dilutional effects and other factors may be involved. Unfortunately, the wide range of plasma DBH activity in labile hypertensives limits the use of this parameter in the diagnosis of hypertension.

Some studies report an abnormal increase in serum DBH in hypertensive patients during excercise (201) and standing (39) and another found a significant decrease in the plasma DBH of hypertensives receiving pharmacotherapy (79). Another investigation disagrees (1).

Because essential hypertensives are a heterogenous group and different experimental designs were used, these studies are difficult to interpret. A subgroup of essential hypertension may well exist with an overactive sympathetic nervous system reflected by increased plasma DBH levels. However, the wide normal range of DBH activity and the small change in enzyme levels accompanying the disorder may mask its appearance.

ii. Secondary Hypertension

Increases of 50 to 60% in plasma DBH accompany spontaneous or induced hypertensive episodes in quadriplegic patients (170, 183). The sympathetic nervous system may be involved in oral contraceptive related hypertension because parallel increases in DBH activity and blood pressure occur in patients who experience elevated blood pressure while being treated daily with estrogen-progesterone contraceptives (210).

Patients with increased blood pressure resulting from renal parenchymal disease have decreased serum DBH (236), Plasma DBH activity is higher in patients who have hypotension during hemodialysis as opposed to those who do not (160). In a separate study, anephric patients with hypotension had lower plasma DBH levels than normotensives without kidneys (265). Although investigators in the latter study (265) suggest the hypotension is caused by decreased sympathetic activity, in one of the other studies (236) the investigators proposed that changes in serum DBH are a result of sympathetic compensation for renally induced blood pressure deviations.

b. Miscellaneous Cardiovascular Disease

Increases in plasma DBH activity have been observed after myocardial infarction (52, 95, 189, 193), and are thought to reflect increased sympathetic activity, which accompanies this condition (246, 164). These results further support the involvement of the sympathetic nervous system in the etiology of cardiac arrythmias, occurring soon after myocardial infarction (253).

The reason for decreases in serum DBH after congestive failure remains a mystery (106). Decreased DBH synthesis has been proposed as an explanation (106) but it is unclear why the reduced rate of enzyme synthesis would accompany increased sympathetic activity (21, 22).

2. Neurological Disease

Familial dysautonomia is characterized by sensory disturbances and an altered autonomic nervous system (279). It is inherited in an autosomal recessive fashion (17) and occurs in Ashkenazic Jewish children. Measurements of urinary CA metabolites suggest that the children with this disease have an impaired ability to convert DA to NE (231). Since this reaction is catalyzed by DBH, plasma DBH was assayed in patients with this disorder. Patients with familial dysautonomia have plasma DBH levels significantly lower than those of age-matched control groups (255, 279, 63). Plasma DBH and NE did not increase normally in response to standing or excercise (279).

Patients with torsion dystonia have autosomal recessive, autosomal dominant or acquired forms of the disease (278). Plasma DBH activity appears to be almost twice as high in blood from patients with the autosomal dominant form of dystonia as in control subjects or in patients with the autosomal recessive form of the disorder (273) (48).

Down's syndrome (26, 66, 264) and the dysequilibrium syndrome (94) are associated with low plasma DBH. Patients with Huntington's chorea have high or normal plasma DBH activity (158, 171, 227), whereas Lesch-Nyhan patients have exhibited both high (211) and low levels of plasma DBH activity (136). Individuals with migraine vascular headaches had higher plasma DBH activity than a control group (90), and 27 comatose patients had low plasma enzyme levels (159).

3. Psychiatric Disease

In the early 1970's a theory suggested that schizophrenia was caused by a deficiency of DBH in the brain (233). This speculation gained popular recognition by many neuroscientists and prompted investigations of plasma DBH in patients with this disorder. Four studies found normal plasma DBH levels in schizophrenics (see Table 3).

Table 3. Plasma DBH Differences between Groups of Psychiatric Patients and Controls.

Illness	Difference in Plasma DBH from Control Group	n	Ref
Schizophrenia	None	22-35	43, 35 263, 174
Schizophrenia	Decrease	149	72
Unipolar & Bipolar Depression	None	9-86	263, 140 174, 156
Unipolar Psychotic Depression	Decrease	22	174
Autism	Decrease	11	138
Alcoholism	None	6-24	225 239

A study using a high performance liquid chromatography technique and larger groups of schizophrenic and control subjects found decreased plasma DBH activity in the schizophrenic patients (72). The reason for this difference is unclear.

A recent report suggests that schizophrenics may have an abnormally high level of endogenous DBH inhibitors (276). The possibility of elevated endogenous DBH inhibitors in schizophrenia is further supported by increased psychotic symptomology in patients who were given DBH inhibitors (57, 98). Future investigations on endogenous DBH inhibitors in schizophrenic patients are warranted.

Most groups find no differences in plasma activity between controls and patients with various affective disorders such as unipolar and bipolar depression (see Table 3). However, if unipolar depressed patients are divided into those with and without psychotic symptoms, then plasma DBH levels are significantly lower in the unipolar psychotically depressed group (174). Another study reports high plasma DBH values in patients with severe or moderate depression secondary to other disorders (71). This report used a heterogenous sample containing patients with a variety of chronic illnesses which could have influenced plasma DBH activity.

Variations in the diurnal rhythm of DBH in depressed patients have also been observed (244, 168) are more apparent in bipolar patients than unipolar patients but the failure of one (168) to control for physical activity makes the comparison of results difficult. Even though electroconvulsive therapy is thought to increase central noradrenergic activity (177), plasma DBH levels examined before and after treatment showed a small increase (51) or no (140) change.

Decreased plasma DBH levels appear in patients with autism, and patients treated for acute alcoholism had enzyme activities essentially the same as their values before hospitalization (see Table 3).

4. Neoplastic Disease

Plasma DBH has been measured in patients with adrenal medullary tumors in an attempt to determine whether CA release occurs by exocytosis or by some other means (270). Investigators believe that if release occurs by exocytosis, then plasma DBH activity should parallel any changes in plasma CA concentration caused by removal of the pheochromocytoma. Some researchers report no change in plasma DBH after removal of the tumorous adrenal (76, 102), but larger studies report that some patients do experience a decrease in plasma enzyme activity postoperatively (126, 5, 238, 59). Based on these results it has been suggested that the mechanism of catecholamine release may vary from tumor to tumor (6, 126, 238).

Neuroblastoma is the second most common solid malignant tumor that occurs in children (161) and is associated with high urinary excretion of CA. Some patients excrete large quantities of DA and its metabolite homovanillic (vanillymandelic) acid (HVA) while others excrete high levels of DA, NE and their respective metabolites HVA and vanillymandelic acid (VMA) (10). Elevated plasma DBH levels are found primarily in patients with increased urinary excretion of VMA (83). This is consistent with the current understanding of the CA pathway, since DBH is required for the synthesis of NE.

Patients with leukemia and hepatoma also have elevated plasma DBH levels (85). However, the high enzyme activity in these patients

is probably not due to increased sympathetic activity but rather to the impairment of DBH clearing mechanisms because of chemotherapy or by the primary disease process.

5. Endocrine Disease

The metabolism of CA has been investigated in thyroid diseases (41, 155, 97). Patients with hyperthyroidism have significantly lower DBH values than those of controls (190, 192), and patients with hypothyroidism have significantly higher plasma DBH than control subjects (192). Plasma DBH values rose as thyroxin levels fell during the treatment of hyperthyroidism, and plasma DBH activity was inversely related to thyroxin levels during therapy for hypothyroidism. Although this and plasma NE studies (23) suggest decreased sympathetic activity in hyperthyroidism and increased activity in hypothyroidism, the rate of CA metabolism and DBH clearance could vary in the diseased state and contribute to these results.

Patients with orthostatic hypotension and neuropathy caused by diabetes have decreased plasma DBH levels (191) in contrast to animal studies using a pharmacologically induced form of the disease (103) (222). The increased levels in these animals are thought to be caused by impaired clearance (222), whereas the alteration of plasma DBH in the human diabetic is suggested to be a result of abberant sympathetic function (191).

VII. Conclusions

Changes in an individual's plasma DBH activity do reflect long-term changes in peripheral sympathetic activity under certain conditions. However, a number of factors severely limit the use of this enzyme as an index of sympathetic activity in most situations:

1. The wide range of plasma enzyme activity in healthy individuals prevents much meaningful information from being gathered in comparative studies using control and experimental groups.
2. Differences in plasma DBH among individuals are more a result of genetic influence than short-term variations in exocytosis in a given individual.
3. The entry route of DBH into the circulation is questionable. A substantial amount of the enzyme released by nerves may enter the circulation only after it is transported through the lymphatic system, explaining why acute changes in sympathetic activity are rarely reflected by plasma DBH changes.
4. The fate of serum DBH remains unknown. Estimates of the enzyme's half-life in the blood suggest it to be from 8 hours to a few days. This could result in a relatively large pool of enzyme in the circulation. Theoretically, the large circulating pool of DBH would be unaffected by small fluctuations in the rate of enzyme entry into the blood, which may be the reason why large changes in sympathetic activity produce only small alterations in plasma DBH concentration.
5. Physical activity and changes in posture influence plasma DBH and can complicate experimental results.
6. Plasma DBH levels can vary as a result of conditions which have little to do with sympathetic activity: changes in extracellular fluid volume can alter plasma DBH concentration purely on a dilutional basis.

7. The rate of enzyme synthesis can also influence plasma enzyme activity. It is conceivable that a small decrease in the depolarization rate of a neuron coupled with a large increase in the synthesis of DBH could result in increased plasma enzyme activity. In such a situation, the plasma DBH levels would indicate an increase in neuronal depolarization rate when, in fact, the opposite was true.
8. Endogenous inhibitors can affect the results of enzymatic assays. If proper assay techniques are not used, the inhibitors or the agents used to block them may complicate experimental results.
9. The specific activity of the enzyme may vary among individuals. Enzymatic assays which are not verified by radioimmunoassay may give erroneous estimates of DBH protein concentration.
10. Finally, animal studies are at great variance with human studies. The extent of phylogenetic differences between humans and laboratory animals prevents the simple extrapolation of animal data to explain human physiological function.

Despite the constricting situation described above, plasma DBH can yield useful information when used along with other indices of sympathetic activity. Its employment in the study of essential hypertension may help delineate a subgroup of primary hypertensives with a neurogenic form of the disease. Its use as a marker for genetic diseases such as Autism or Torsion Dystonia also looks promising. In addition, the discovery of increased levels of endogenous DBH inhibitors in some schizophrenics has opened a new perspective on the possible causes of that illness.

Although the enthusiasm for DBH as an easy index of peripheral noradrenergic function has passed, recent advances in the detection of cereberospinal fluid (CSF) DBH have generated much interest in the enzyme's use as an indicator of central noradrenergic activity. Since many of the extraneuronal factors which influence the levels of plasma DBH do not affect the concentration of DBH in CSF, levels of the enzyme in the CSF might reflect central noradrenergic activity better than plasma DBH reflects peripheral activity. A number of pilot investigations suggest that DBH in the CSF does reflect central noradrenergic activity accurately (152, 38, 165, 36, 149). More research and improvements in the assay for CSF DBH will be needed to determine the usefulness of this promising new tool.

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