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Human Plasma Dopamine-Beta-Hydroxylase: Variation in Thermal Stability

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SUMMARY

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There are wide individual variations in the thermal stability of human plasma dopamine-β-hydroxylase (DBH) during incubation at 55°. When the ratio of enzyme activity after heating at 55° for 20 minutes to that before heating (a heated-to-control, or H/C ratio) was used as a measure of thermostability, 10.2% of frozen serum samples from 362 randomly selected subjects had relatively thermolabile DBH (H/C < 0.86). These subjects also had a significantly lower average basal DBH activity (471 ± 49 units/ml, mean ± SEM, N = 37) than that of the randomly selected subjects with more thermostable enzyme (829 ± 25 units/ml, N = 325, $p < 0.001$). However, there was not a significant correlation of the trait of thermolabile DBH (H/C < 0.86) with the presence of the previously described allele for very low basal DBH enzymatic activity (< 50 units/ml). The results of experiments in which plasma from subjects with thermolabile and thermostable DBH were mixed and experiments in which DBH was partially purified from plasma by gel filtration chromatography were compatible with the conclusion that variations in the structural properties of DBH itself were probably related to variations in relative thermostability.

INTRODUCTION

Dopamine-β-hydroxylase (E.C. 1.14.17.1) catalyzes the conversion of dopamine to norepinephrine (1), is localized to catecholamine-containing vesicles in the adrenal medulla and sympathetic nerves (2, 3), is released with catecholamines in response to stimulation of these structures (4-8), and is found circulating in blood (9). Inheritance plays an important role in the determination of human plasma DBH[1] levels. An allele (DBH^L) for very low enzymatic and immunoreactive plasma DBH in man is inherited as an autosomal recessive trait (10-12). The gene frequency of this allele is approximately 20%, and about one-third of a randomly selected population is heterozygous for the trait of very low enzyme activity (11, 12). Even though the allele for very low DBH enzymatic activity is associated with a decrease in the quantity of circulating DBH protein (12), it is possible that it might result in the presence of a variant biochemical form of DBH in plasma.

Thermal stability is one of the most sensitive indicators of differences in structure of proteins (13). If there are individual differences in thermal stability of DBH in human plasma, these differences might rep-

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[1] The abbreviation used is: DBH, dopamine-β-hydroxylase.

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resent another type of biochemical variation in this circulating enzyme—variation that might be correlated with genetically-determined individual differences in enzyme activities. The purpose of the present series of experiments was to study the thermal stability of DBH in human blood to determine whether there are significant individual variations in this biochemical property of DBH.

METHODS

Subjects. Blood samples were obtained by venipuncture from consecutive, randomly-selected subjects. Serum samples were obtained from 401 subjects aged 6–18, and were stored frozen for three years. The school children from whom these samples were obtained included only those subjects whose parents had given written informed consent. The characteristics of this population have been described in detail elsewhere (11). Heparinized plasma samples were also obtained from 133 consecutive, randomly-selected, unrelated blood donors at the Mayo Clinic Blood Bank. All of these subjects were white. Multiple blood samples for repeated biochemical tests were obtained from selected individuals with either thermolabile or thermostable DBH. Written informed consent was obtained before blood samples were withdrawn.

Dopamine-β-hydroxylase assay procedure. DBH enzymatic activity was measured by the method of Molinoff *et al.* (14) as modified to measure plasma or serum enzyme activity (9, 11). β-Phenylethylamine was used as substrate for the reaction, and β-phenylethanolamine was used as an internal standard for the portion of the reaction catalyzed by phenylethanolamine-N-methyltransferase. In some experiments tyramine was used as substrate and octopamine was used as an internal standard for the phenylethanolamine-N-methyltransferase catalyzed step. Plasma was diluted 1:50 with ice-cold water prior to enzyme assay. $CuSO_4$ at a final concentration of 3.3 μM was added to the reaction mixture to inhibit endogenous DBH inhibitors, and 1 M acetate buffer, pH 4.9, was substituted for the Tris buffer used in the original procedure (14). All assays were performed in duplicate except as noted. Undiluted fractions from gel filtration chromatography were assayed with tyramine as substrate and with no added $CuSO_4$. One unit of the enzyme activity represented the production of 1 nmole of β-phenylethanolamine per hour of incubation at 37°.

Thermal inactivation. Serum or plasma samples were diluted 1:50 with water, and 200 μl aliquots were placed in reaction tubes. Thermal stability was determined by incubation at 55° for variable periods of time prior to the determination of DBH activity. Samples kept in ice served as controls. Samples with very low DBH were diluted with water 1:20, and were assayed with tyramine as substrate. Fractions obtained by gel filtration chromatography were subjected to the 55° treatment without further dilution.

Gel filtration chromatography. Gel filtration chromatography of plasma was performed at 4° with a 1.6 × 95 cm column that contained Bio-Gel A 1.5 M (200–400 mesh, Bio-Rad Laboratories). The eluant was 0.15 M sodium chloride that contained 10 mM potassium phosphate buffer, pH 7.4. Forty drop (2.6 ml) fractions were collected. The flow rate of the column was approximately 4.8 ml per hour. A sample of 0.4 ml of plasma mixed with 0.4 ml of the elution buffer was applied to the column. Standards for the calibration of molecular weight were passed through the column separately. These standards included: ovalbumin, human albumin, human gamma globulin, catalase, and β-galactosidase. The concentrations of protein in column fractions were estimated by the measurement of absorbance at 280 nm.

Statistics. Tests of statistical significance between group means were performed using Student's *t*-test. Tests of significance between group frequencies were performed by Chi square analysis with the Yates correction for continuity (15).

Materials. Tyramine hydrochloride and octopamine hydrochloride were obtained from Calbiochem, La Jolla, California. S-[methyl-^{14}C]-adenosyl-L-methionine (specific activity 55–58 mCi/mM) was purchased from New England Nuclear Corporation, Boston, Massachusetts. Triton X-

100 was obtained from Packard Instrument Company, Inc., Downers Grove, Illinois. Pargyline hydrochloride was purchased from Abbott Laboratories, North Chicago, Illinois. Sigma Chemical Company, St. Louis, Missouri, supplied Tris(hydroxymethyl)aminomethane base, purified human gamma globulin, ovalbumin. β-Galactosidase was purchased from Worthington Biochemical Company, Freehold, New Jersey. Catalase was purchased from Boehringer Biochemical, Mannheim, West Germany. β-Phenylethylamine and β-phenylethanolamine were obtained from K & K Chemical Corporation, Plainview, New York. Bio-Gel A 1.5 M (200–400 mesh) was purchased from Bio-Rad Corporation, Richmond, California.

RESULTS

Initial Observation of Thermolability of Plasma DBH

Introduction. Preliminary studies were performed to determine whether individual variations in the thermostability of plasma DBH exist. Striking individual differences in the thermal stability of the enzyme were observed. The DBH activity in most samples changed little after incubation at 55° for up to 45 min. However, the enzyme activity in samples from a few individuals was quite thermolabile under these conditions (Fig. 1). To determine how common such thermolability was in a randomly selected population, a survey was performed of the thermal stability of plasma DBH in a large number of samples that had been frozen for up to four years.

Frequency distribution histogram of DBH "H/C ratios." When serum samples from 362 unrelated school children aged 6–18 were studied, there was little change in the DBH activity of most samples after 20 min of incubation at 55°. However, a small number of subjects had thermolabile DBH. A heated-to-control ratio, the ratio of DBH activity in a sample heated at 55° for 20 min to the basal (unheated) enzyme activity was calculated for each sample. The frequency distribution histogram of H/C ratios was quite skewed (Fig. 2), with values as low as 0.1—indicating 90% inactivation

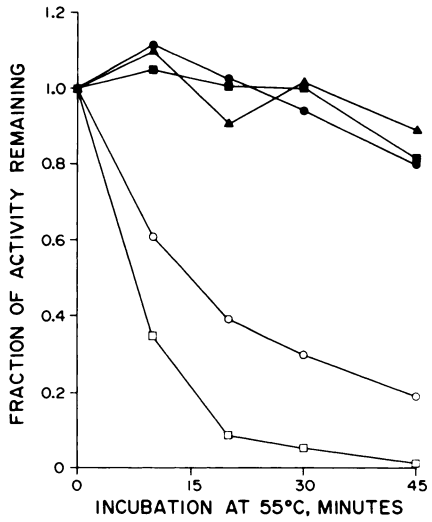

FIG. 1. *Human plasma DBH thermal inactivation at 55°*

Plasma was diluted 1:50 with water and heated at 55° prior to DBH assay. Samples had been stored at −20° for one month. Representative data for three plasma samples with thermostable DBH (closed symbols) and two plasma samples with thermolabile DBH (open symbols) are shown. Each point is the average of two determinations.

during heating. Although serum from most subjects changed little in DBH activity after heating, it was difficult to clearly separate thermolabile from thermostable samples just by inspection of the frequency distribution histogram. Therefore, a "normal" curve was modeled to the downslope of H/C ratios with values of 1.02 or above (the peak of the distribution in Fig. 2). When this was done, a standard deviation of 0.054 was calculated and an H/C ratio of 0.86, 3 standard deviations below the peak, was chosen as the dividing line between subjects with labile ($H/C < 0.86$) and subjects with thermostable ($H/C > 0.86$) DBH. This procedure was used to make possible classification of subjects for biochemical and population studies. Of this particular population, 10.2% (37/362) fell into the thermolabile group.

Relationship of thermolability to DBH activity. A wide range of basal DBH activity (activity in unheated samples) was present in subjects with thermostable DBH (Fig. 3), while somewhat lower basal DBH

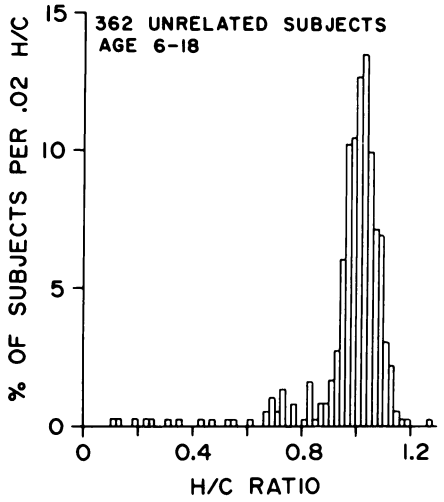

FIG. 2. *Human serum DBH thermolability*

Serum samples from 362 unrelated subjects aged 6–18 were incubated for 20 minutes at 55°, and the fraction of baseline activity remaining, or H/C ratio, was calculated for each sample. The samples had previously been stored frozen for over three years.

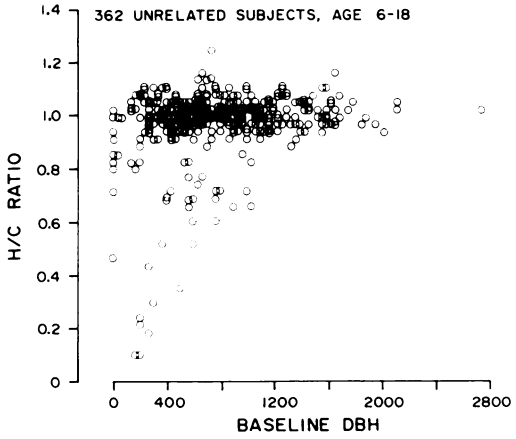

FIG. 3. *Correlation of human serum DBH activity with thermolability*

The H/C ratio is plotted versus baseline DBH activity for the same 362 subjects shown in Fig. 2. Thermolabile DBH is defined as having an H/C ratio less than 0.86. DBH activity is expressed in nmoles phenylethanolamine produced/hour/ml.

activity was found in subjects with thermolabile DBH (H/C < 0.86). The average DBH activity of the 37 subjects in the thermolabile group was 471 ± 49 units/ml (mean ± SEM), significantly lower than that of the 325 subjects with thermostable enzyme, 829 ± 25 units/ml ($p < 0.001$).

Since decreased basal serum DBH levels are also associated with the presence of the allele DBH^L, thermal stability of DBH was also measured in blood from homozygotes and heterozygotes for this allele. These samples had been obtained in the course of previous family studies of the inheritance of the trait of very low enzymatic activity (11, 12) and had been frozen for three years. Only 5 of 27 (18.5%) unrelated subjects homozygous for the allele for low enzyme activity (enzyme activity <50 units/ml) had thermolabile DBH. Of obligate heterozygotes for the allele for low DBH activity, 13.5% (5/37) had thermolabile enzyme. The frequency of occurrence of the trait of thermolabile DBH in these two groups was not significantly different from the randomly selected population ($\chi^2 = 0.73, p > 0.30$ and $\chi^2 = 0.08, p > 0.70$, respectively). Therefore, the trait of thermolabile DBH does not appear to segregate with the allele for very low basal DBH activity. If thermolability of DBH eventually proves to be genetically determined, it is probably not due to the allele for very low enzymatic activity.

Familial aggregation of thermolabile DBH. Of the 37 subjects with labile DBH (H/C < 0.86) identified during the study of the 362 unrelated school children, blood was available from 21 siblings in 21 separate families. Eight of these twenty-one subjects (38.1%) had thermolabile enzyme. This is a significant familial aggregation of the trait of thermolabile DBH ($\chi^2 = 7.78, p < 0.01$) since only 10.2% of the randomly selected unrelated population had thermolabile enzyme.

Characteristics of the Development of DBH Thermolability

Introduction. Serum samples from three subjects determined previously to have thermolabile DBH (H/C < 0.86) were treated in a variety of fashions prior to heating at 55° to determine whether the treatment of the sample prior to thermal inactivation might influence the thermal stability of the enzyme. Fresh serum samples were obtained and were tested immediately, stored at room temperature for 5–6 days, stored at 4° for 1 month or stored at

−20° for 1 month. There was no change in basal DBH activity in response to any of these treatments. The enzyme was not clearly thermolabile in fresh serum samples, with "borderline" H/C ratios of 0.76, 0.79 and 0.90, but became clearly labile after 5–6 days at room temperature, or after 1 month at 4° or at −20°. For example, the H/C ratios were 0.37, 0.18, and 0.40 after 1 month at −20°. Repetitive freezing and thawing of samples resulted in the development of thermolability more rapidly than if the sample was thawed only once. These results indicated that thermolability developed in these three samples after storage at −20° for 1 month, and increased the possibility that the results from the samples frozen for several years used in the population survey might reflect the eventual thermal lability of DBH in the samples. However, the results also demonstrated the need for a procedure to bring about the reproducible and rapid development of DBH thermolability in fresh samples.

Development of thermolability by pre-treatment at 37°. Incubation at 37° prior to heating at 55° was the procedure used in an attempt to speed up and standardize conditions for the development of DBH thermolability. Incubation at 37° was used because storage of samples at room temperature resulted in the development of thermolability more rapidly than storage at 4° or −20°. H/C ratios for a fresh plasma sample from an individual with thermolabile DBH incubated for up to 44 hours at 37° are shown in Fig. 4A. No change in basal DBH activity occurred as a result of incubation at 37°. H/C ratios after "pre-treatment" at 37° were determined in the usual fashion after heating at 55° for 20 min. Thermolability developed rapidly during the first day of incubation at 37° (Fig. 4A).

Fresh plasma samples from three individuals with thermostable and three individuals with thermolabile enzyme were then incubated at 37° for up to six days. The thermal stability of the three labile samples decreased dramatically after one day of pre-treatment at 37° while no change in the thermal stability of the other three samples occurred (Fig. 4B). After six days of incubation at 37° there was no change in the basal enzyme activity of any of the samples (average ratio of activity on day 6 to activity in fresh sample of 1.03 ± .05). However, by that time the H/C ratios of all samples were in the thermolabile range. Because of these results, a procedure which involved the incubation of fresh plasma samples at 37° for 18 hours was chosen as a convenient method of pre-treatment to bring about the development of thermolability under con-

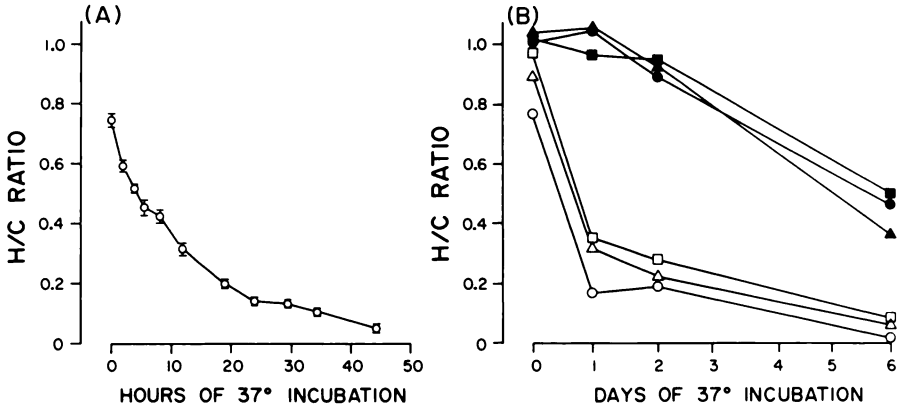

FIG. 4. *Development of thermolability during pre-treatment at 37°*

Fresh plasma samples were obtained from individuals with thermostable DBH (closed symbols) or thermolabile DBH (open symbols) and treated at 37° prior to determination of thermolability. A) One thermolabile sample was assayed after incubation at 37° for up to 44 hours. The values represent the mean ± SEM of three determinations. B) Three samples of each type were assayed after incubation at 37° for up to six days. Each value represents the mean of two determinations.

trolled conditions. Henceforth the phrase "development of thermolability" will refer to the process of "pre-treatment" of a sample at 37° for 18 hours, and "expression of thermolability" will refer to the process which occurs during incubation at 55°. The trait of thermolabile DBH appears to depend upon both appropriate development and expression of thermolability.

Fresh plasma samples from one individual with thermolabile and one individual with thermostable DBH were heated to 37° for 18 hours, and were subsequently heated at various temperatures for 10 min (Fig. 5). There was about a 3.5° difference between the two samples in the temperature at which 50% inactivation of DBH occurred. The difference in thermal stability between the two samples was approximately as great at 55° as at any other temperature. Fresh samples from these same two subjects were also used to study the time course of inactivation of DBH at 55° after pre-treatment at 37° for 18 hours (Fig. 6). After one hour of incubation at 55° the thermostable sample was 20% inactivated while the thermolabile sample was over 90% inactivated. When the data from this experiment were plotted in semilogarithmic fashion (Fig. 6B) the time course of the enzyme inactivation was linear for at least 30 min.

Frequency distribution histogram of thermolability after pre-treatment at 37°. The frequency distribution of H/C ratios was determined in fresh plasma samples after pre-treatment at 37°. Blood was obtained from 133 unrelated adult blood donors. These fresh samples were heated at 37° for 18 hours prior to the determination of H/C ratios by incubation at 55° for 20

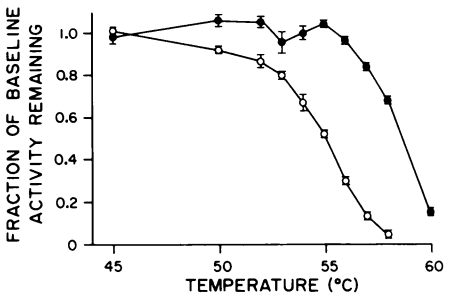

FIG. 5. *Temperature dependence of human plasma DBH heat inactivation*

Plasma samples from a subject with thermolabile (open circles) and a subject with thermostable (closed circles) DBH were pre-treated at 37° for 18 hours, and then were diluted 1:50 with water and subjected to heat inactivation for 10 minutes at the temperature indicated. All values are expressed as a fraction of the activity in a sample not subjected to the second thermal treatment. Each point represents the mean ± SEM of three determinations.

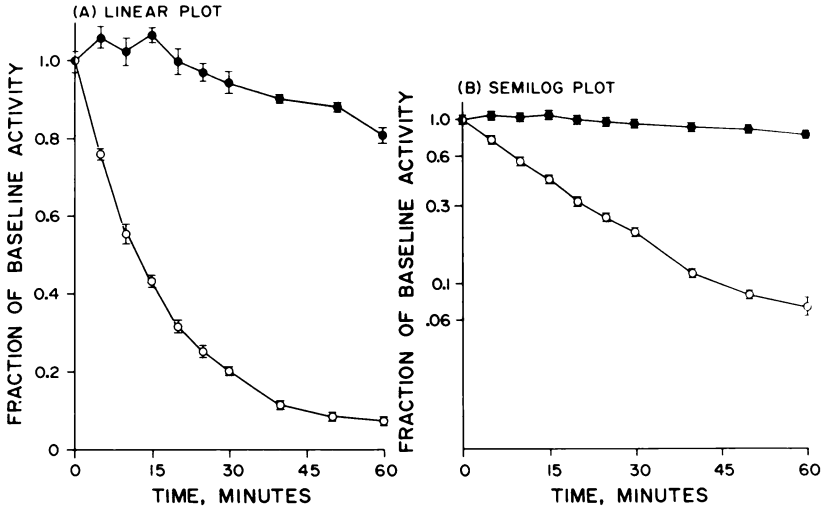

FIG. 6. *Time course of thermal inactivation of human plasma DBH*

Plasma samples pre-treated at 37° were diluted 1:50 and were subjected to thermal inactivation at 55° for the times indicated. Each point represents the mean ± SEM of four determinations. The sample with thermostable DBH is indicated by closed circles and the sample with thermolabile DBH by open circles.

min. The frequency distribution histogram of H/C ratios for these 133 samples (Fig. 7) was similar to that determined for the frozen samples from 362 unrelated subjects aged 6–18 (Fig. 2). When the same method was used for choosing a line of demarcation between thermolabile and thermostable samples as that described for the data shown in Fig. 2, a value of 0.80 was chosen. Of the fresh samples 13.5% (18/133) were thermolabile (H/C < 0.80)—a value very similar to the 10.2% found for frozen samples. The slight difference in the average H/C ratio for subjects with thermostable enzyme when compared with the data in Fig. 2 was almost certainly due to a slight but consistent elevation of DBH thermostability in serum samples as compared with that determined in heparinized plasma samples. This slight difference between serum and plasma did not obscure the thermolabile group which had greatly decreased H/C ratios.

Biochemical Characteristics of Thermolability

Introduction. It was not clear whether individual variations in the thermolability of DBH were dependent on characteristics of the enzyme itself, of other plasma constituents, or of both DBH and other plasma "factors." Therefore, a series of experiments was performed in an initial attempt to determine whether the differences in thermolability might reflect biochemical differences in DBH or whether individual variations in other plasma constituents might play a role in the development and/or expression of thermolability.

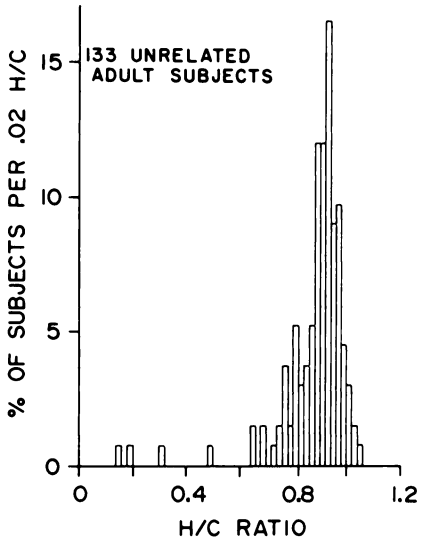

FIG. 7. *Fresh human plasma DBH thermolability following 37° pre-treatment*

Fresh plasma samples from 133 subjects aged 19–61 were pre-treated by incubation at 37° for 18 hours prior to incubation at 55° for 20 minutes and the determination of H/C ratios.

Mixing experiments. Fresh plasma samples were obtained from the same three individuals with labile and three individuals with stable DBH for whom data are shown in Fig. 4B. Plasma was pre-treated for 18 hours at 37° both separately and in the nine possible combinations of equal volume mixtures of labile and stable samples, i.e., a mixture was made of each individual labile sample with each individual stable sample. The samples and mixtures were diluted 1:50 with water, a portion of each was heated at 55° for 20 min and the activities of the six initial samples and the nine mixtures were measured before and after 55° treatment. The control and 55° heated DBH activities as well as the H/C ratios for each of the six initial samples are shown in Table 1. The control and 55° heated activities as well as the H/C ratios for each of the nine mixtures are shown in Table 2.

If the differences in thermolability of plasma DBH result from differences in the

TABLE 1

Characteristics of DBH in plasma samples used for mixing experiments

Fresh plasma samples were pre-treated for 18 hours at 37° prior to determination of DBH activity. Control DBH was determined with no further thermal treatment. Heated DBH was determined following a 20 minute treatment at 55° of a portion of the plasma diluted 1:50 with water.

DBH Type	Sample	DBH (*units/ml*)		H/C ratio
		Control	Heated	
Labile	A	212	37	0.174
	B	394	141	0.358
	C	235	75	0.319
Stable	D	602	630	1.047
	E	944	915	0.969
	F	190	201	1.058

TABLE 2

Mixing experiment

Each mixture consisted of equal volumes of one fresh plasma sample with thermolabile DBH and one fresh plasma sample with thermostable DBH. Characteristics of the individual plasma samples are given in Table 1. Each mixture was pre-treated for 18 hours at 37°, then diluted 1:50 with water and a portion of each heated to 55° for 20 minutes. DBH enzymatic activity was measured in both the control (not 55° treated) and heated (55° treated) portions. See "mixing experiments" in the text for details. Predicted H/C ratios for the "DBH structure" model were calculated from the equation $(H_1 + H_2)/(C_1 + C_2)$ while those for the "plasma factor" model were calculated from the equation $[(H_1/C_1) + (H_2/C_2)] + 2$.

Mixture	DBH Activity				H/C Ratios		
	Control		Heated		Predicted		Observed
	Observed	Arithmetic mean	Observed	Arithmetic mean	"Plasma factor" model	"DBH structure" model	
A + D	436	407	310	333	.611	.819	.712
A + E	617	578	595	476	.572	.824	.965
A + F	171	201	106	119	.616	.592	.620
B + D	521	498	340	385	.703	.774	.653
B + E	710	669	569	528	.664	.789	.802
B + F	286	292	144	171	.708	.586	.504
C + D	432	419	317	353	.683	.842	.734
C + E	605	590	503	495	.644	.840	.832
C + F	226	213	136	138	.689	.649	.600

DBH molecule itself, it would be expected that the ratio of inactivation of each DBH molecule in the mixture would depend on its own intrinsic characteristics. Although it might seem that the H/C ratios of the mixtures would be the arithmetic means of those in the initial samples under these circumstances, this is not the case because the baseline enzyme activities in the initial samples differ. Because of that fact, the H/C ratios of mixtures would be related to the relative contribution of DBH from each of the two initial samples. If a model based on a difference in "DBH structure" is used to analyze the results of the mixing experiments, the expected H/C ratio for each mixture can be calculated from the formula $(H_1 + H_2)/(C_1 + C_2)$. When the actual H/C ratios measured for the nine mixtures are plotted against the expected results calculated with this equation (Fig. 8B), the correlation of the observed with the expected values is 0.79 and the slope is 1.03, i.e., the results are similar to the predictions made on the basis of the model.

The other extreme alternative is that the expression of thermolability is due entirely to a plasma factor. In that case the baseline DBH values of the initial samples are not important, and the H/C ratios of the mixtures would be the arithmetic means of those of the two samples used to make the mixture if a linear relationship between the plasma factor and the thermolability is assumed. When the actual H/C ratios are plotted against those predicted by this "plasma factor" model (Fig. 8A), the slope of the data points is −1.99, far from the line of identity. The sum of the squares of the difference between the predicted and observed H/C ratios is 0.068 for the "DBH structure" model and is 0.274 for the "plasma factor" model. These observations are most compatible with the conclusion that differences in the expression of the trait of DBH thermolability are most likely due to differences in the DBH molecules themselves.

Gel filtration chromatography experiments. To examine its thermal stability properties more directly, DBH from plasma was partially purified by gel filtration chromatography on Bio-Gel A 1.5 M. Gel filtration chromatography was performed with fresh plasma samples from three subjects with thermolabile and two subjects with

thermostable enzyme activity. Representative elution patterns are shown in Fig. 9 for plasma from one individual with thermolabile DBH (Fig. 9A) and from one subject with thermostable enzyme (Fig. 9B). Elution patterns from the other subjects studied were not significantly different from those shown in the figure. The elution patterns for subjects with thermostable DBH were similar to those previously described by Rosenberg *et al.* (16). A high molecular weight DBH (apparent "tetramer") accounts for approximately 75% of the total enzyme activity with the remainder of the activity in a lower molecular weight constituent (apparent "dimer"). Calibration of our column with proteins of known molecular weight allowed us to estimate a molecular weight of approximately 530,000 for the apparent "tetramer" and approximately 240,000 for the apparent "dimer," results which agree well with the estimates of Rosenberg *et al.* (16). Although both tetramer and dimer were present in plasma samples with labile DBH, the tetramer made up approximately 90% of the total DBH activity, a larger proportion than was the case with plasma samples containing thermostable enzyme. The dimer made up approximately 8%, and a third peak of DBH enzymatic activity with an apparent molecular weight of 70,000 contained about 2% of the total enzyme. These elution patterns were repeatedly obtained with either fresh samples or samples of plasma pre-treated at 37°. The plasma samples with thermostable DBH showed ratios of tetramer to dimer that ranged from 2.4 to 3.6 (four

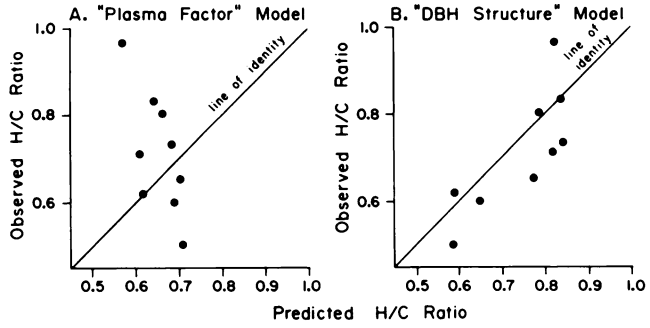

FIG. 8. *Thermolability in mixtures of plasma with thermolabile and thermostable DBH*
The observed H/C ratios are plotted against the predicted H/C ratios calculated on the basis of two different models. See text and Table 2 for details.

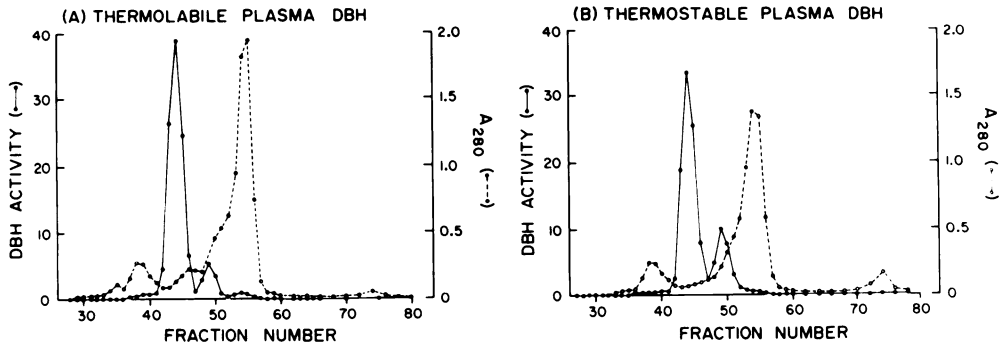

FIG. 9. *Gel filtration chromatography of human plasma*
Fresh human plasma diluted 1:1 with 0.15 M NaCl and 10 mM potassium phosphate, pH 7.4, was applied to a column of Bio-Gel A 1.5 M and was eluted with the same buffer. DBH activity (nmoles octopamine/ml/hr, closed circles) and protein concentration (A_{280}, open circles) were determined. A) Plasma from a subject with thermolabile DBH. B) Plasma from a subject with thermostable DBH.

separate determinations) and never showed a clear third peak of DBH enzymatic activity. Plasma with labile DBH had ratios of tetramer to dimer of 7.3 to 10.8 (six separate determinations), and always had a low molecular weight third peak of enzyme activity which contained 2–3% of the total DBH activity. These data raise the possibility that an enzymatically active "monomer" of DBH may be present in the blood of individuals with thermolabile enzyme activity and that differences in the aggregation behavior of the enzyme may correlate with the thermal stability of DBH.

The apparent DBH dimer isolated by gel filtration chromatography was always rapidly inactivated at 55°, whether it was isolated from plasma with thermolabile or thermostable enzyme. The apparent monomer was also rapidly inactivated by 55° treatment. However, the apparent tetramer was more thermostable. H/C ratios for the tetramer freshly isolated from plasma with thermostable DBH ranged from 0.87 to 0.95, while the tetramer freshly isolated from samples of plasma with thermolabile DBH had H/C ratios that initially ranged from 0.65 to 0.86. When freshly isolated DBH tetramer from two plasma samples with thermostable enzyme was stored at 4° for 5 days, the H/C ratios changed little, from 0.87 and 0.90 initially to 0.90 and 0.77. However, the H/C ratios for tetramer from two plasma samples from subjects with thermolabile DBH decreased from 0.69 and 0.65 to values of 0.21 and 0.27 during storage at 4° for 5 days. Therefore, the major form of DBH partially purified by gel filtration chromatography from whole plasma with thermolabile enzyme was more thermolabile than that isolated from plasma with stable enzyme. These data, like the results of the "mixing" experiments, support the conclusion that differences in the DBH molecule are essential for individual differences in its thermal stability to occur. Once again, however, the results do not rule out the possibility that in addition to the requirement for a specific type of DBH molecule, other plasma factors may also be involved in the development of the thermolability of the enzyme.

Plasma dilution experiments. Finally, experiments were performed to test the hypothesis that plasma constituents other than DBH might be required for either the development of thermolability during 37° pre-treatment or for its expression during 55° incubation in samples with appropriate DBH molecules. Fresh plasma was obtained from the two subjects for whom data are shown in Figs. 5 and 6. The plasma was heated at 37° for 18 hours in the usual fashion, undiluted, as well as diluted 1:50 with water or 1:50 with plasma from a subject with thermostable DBH. It was necessary to use plasma with thermostable enzyme (H/C = 0.84) but very low endogenous DBH activity (<50 units/ml) for the dilution so that the endogenous enzyme activity would not completely mask the activity in the sample being diluted. Although the expected differences in thermostability were present after pre-treatment of undiluted samples, the H/C ratio of the thermolabile sample was increased significantly after pre-treatment when diluted with water (Table 3). This finding indicated that some factor found in plasma was necessary during the 37° pre-treatment for thermolability to develop. However, when the same sample was incubated with plasma from a subject with thermostable DBH but low enzyme activity, there was a significant decrease in the H/C ratio of the thermolabile plasma sample. No substantial changes occurred in the thermostable sample diluted either with water or with plasma. No differences were found in the "expression" of the trait of thermolability when undiluted samples were pre-treated at 37° and were then heated at 55° either undiluted or diluted with water. Therefore the presence of plasma was necessary for the development of the thermolability of DBH during 37° pre-treatment, and the plasma from one subject with thermostable enzyme substituted at least partially for plasma from the subject with thermolabile enzyme. Even though individual variations in the DBH molecule itself appear to be involved in differences in the thermostability of the enzyme, development of thermolability in fresh samples during 37° pre-treatment apparently requires the presence of a plasma factor or of plasma factors that

TABLE 3

Development of thermolability during 37° treatment

Plasma samples from one individual with thermolabile and one individual with thermostable DBH were incubated for 18 hours at 37°. During the 37° pre-treatment the samples were either undiluted, diluted 1:50 with water, or diluted 1:50 with plasma from a subject with very low but thermostable plasma DBH activity. The samples were then heated at 55° for 20 minutes at a final dilution of 1:50 and H/C ratios were determined (mean ± SEM, N = 3 determinations).

Enzyme type	H/C Ratios		
	37° Incubation conditions		
	Undiluted	Diluted with water	Diluted with plasma
Thermolabile	.190 ± .008	.776 ± .018**	.495 ± .046*
Thermostable	.943 ± .005	1.089 ± .022	1.006 ± .031

* Significantly different ($p < .005$) from sample diluted with water.

** Significantly different ($p < .001$) from undiluted sample.

are present in the blood of subjects with both labile and stable DBH.

DISCUSSION

Variations in thermal stability have been very useful in the elucidation of the molecular basis of the regulation of many enzyme systems (13, 17, 18). Therefore, the observation that there are wide individual variations in the thermal stability characteristics of DBH may be important in future studies of the regulation of this catecholamine biosynthetic enzyme by either genetic or other factors. Although the enzyme in most subjects is thermostable at 55°, a small percentage of randomly selected subjects, approximately 10–14%, have DBH that is rapidly inactivated by this treatment. There is a significant familial aggregation of the trait of thermolabile DBH, but the possible role of inheritance in this phenomenon can only be determined by twin or family studies. Even though the trait of thermolabile DBH is associated with a significant decrease in basal DBH activity, this trait does not appear to be associated with the allele for very low DBH enzymatic activity, DBH^L (19). Thermolability can be developed in a fresh plasma sample by pre-treatment at 37° for 18–24 hours, and the expression of the trait of thermolability appears to depend on characteristics of the DBH molecule itself. However, proof of this conclusion must await the isolation and biochemical characterization of DBH from the blood of subjects with thermolabile and thermostable enzyme.

Differences in thermal stability have been used as one means of distinguishing between molecular variants of many enzymes (13, 17, 18). Differences in the thermal stability of plasma DBH may reflect structural differences in the enzyme molecule such as alterations of the amino acid sequence of DBH due to variations of the DBH structural gene or alterations resulting from post-translational modification of the enzyme. The observations described here expand our understanding of the biochemical diversity of human DBH, an important catecholamine biosynthetic enzyme, and may eventually help to increase our understanding of the biochemistry and genetic regulation of this enzyme activity in man.

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