MOLECULAR PHARMACOLOGY, 19:444–450

Human Dopamine-β-Hydroxylase

Comparison of the Enzyme from Plasma, Adrenal Medulla, and Pheochromocytoma by Radioimmunoassay

RONALD P. FRIGON, DANIEL T. O'CONNOR, AND GAIL L. LEVINE

Departments of Medicine, Veterans Administration Medical Center and the University of California, San Diego, California 92161

Received October 28, 1980; Accepted January 9, 1981

SUMMARY

FRIGON, R. P., D. T. O'CONNOR, AND G. L. LEVINE. Human dopamine-β-hydroxylase: comparison of the enzyme from plasma, adrenal medulla, and pheochromocytoma by radioimmunoassay. *Mol. Pharmacol.* **19**:444–450 (1981).

A homologous radioimmunoassay for human plasma dopamine-β-hydroxylase (DBH) is described. An antiserum specific for the purified plasma enzyme was found to cross-react fully with soluble DBH isolated from human pheochromocytoma and normal adrenal medulla. Similarly, an assay developed using pheochromocytoma DBH as an antigen gave equivalent results, and the two methods were interchangeable for the determination of immunoreactive DBH in human plasma. Bovine adrenal medullary DBH, on the other hand, cross-reacted poorly in these assays. The homospecific activity of DBH in pooled, normal human plasma, expressed as units of enzymatic activity per milligram of immunoreactive protein, ranged from 3.1 to 5.8 units/mg. This compares favorably with DBH in a chromaffin granule lysate from pheochromocytoma, 3.8 units/mg, as well as normal human adrenal medulla, 4.1 units/ mg. Plasma DBH was further separated into enzymatically active tetrameric and dimeric fractions. The homospecific activity of the predominant tetrameric fraction from pooled plasma was about 20% greater than the dimeric enzyme. The results of this study are compatible with the conclusion that various forms of soluble human DBH are immunologically indistinguishable by homologous radioimmunoassay. Comparison of the enzymatic and immunoreactive levels of circulating DBH, on the other hand, may be altered by structural differences between the multiple forms of the plasma enzyme.

INTRODUCTION

The discovery of DBH[1] (EC 1.14.17.1) activity in serum (1) suggested the possibility that the circulating level of DBH reflects catecholamine neurotransmitter secretion of sympathetic nerve and the adrenal medulla (2, 3). The advent of a direct RIA for DBH as an estimator of circulating enzyme activity (4–6) revealed difficulties in using heterologous antibovine adrenal DBH antisera to bind the human serum enzyme. Poor correlation between DBH immunoreactive protein and DBH enzymatic activity, however, has been attributed to weak or variable interspecies cross-reactivity, and investigations using homologous antihuman DBH antisera have since demonstrated excellent correlation between enzymatic and immunoreactive DBH in human plasma (5–9). According to availability, human DBH preparations from autopsy adrenal glands or from pheochromocytoma, a tumor of the adrenal medulla, have been used interchangeably to raise homologous antibodies. One study appears to have demonstrated the equivalence of antiadrenal DBH or antipheochromocytoma DBH in a plasma DBH RIA (7). However, plasma DBH may arise from sympathetic nerve (1, 3) rather than from the adrenal medulla, and circulating enzymatically active DBH occurs in multiple forms (10, 11). Furthermore, levels of circulating DBH may be determined genetically or may be altered in the pathological state (12).

The aim of the present study was to devise a direct, specific RIA for human plasma DBH. This study has addressed the issue of the immunological cross-reactivity of various forms of human DBH by using this RIA. Direct evidence is presented supporting the immunological equivalence of human plasma DBH and the pheochromocytoma-derived enzyme. The ratio of enzymatic activity to units of immunoreactive protein, the homo-

The abbreviations used are: DBH, dopamine-β-hydroxylase; RIA, radioimmunoassay; PAGE, polyacrylamide gel electrophoresis.

0026-895X/81/030444-07$02.00/0

specific activity (13), of circulating DBH has been further compared between the multimeric forms of this enzyme in plasma.

EXPERIMENTAL PROCEDURE

Materials. Human plasma was separated from blood treated with sodium citrate or heparin and stored at 5° until use. Surgical specimens of human pheochromocytoma and adrenal glands were placed immediately following surgery in 0.3 M sucrose at 0°, then maintained at 0° for 1–6 hr (depending on location received) until enzyme preparation. Human adrenal glands were obtained secondary to surgery for pheochromocytoma or hypernephroma and were otherwise normal. Bovine adrenal glands were generously provided by a local slaughterhouse and were placed, immediately following dissection, in 0.3 M sucrose at 0° for about 1 hr until use. $Na^{125}I$, 100 mCi/ml, supplied by Amersham/Searle, Arlington Heights, Ill., was diluted to 40 mCi/ml with water before use. Other chemicals were reagent grade unless specifically indicated.

Enzyme preparation. Human plasma DBH was purified by using a modification of an earlier protocol (11). Enzyme samples following gel filtration on Bio-Gel A-0.5 m (9) were concentrated by ultrafiltration on Amicon PM-30 membranes then subjected to PAGE. Gels were pre-electrophoresed at 2 mA for 2 hr in 0.42 M Tris-HCl, pH 8.9, then loaded with 0.05–0.1 mg of protein and run at 3 mA for 2–4 hr at 5° according to the method of Davis (14). After electrophoresis, the zone containing DBH enzyme activity was cut and eluted into 0.1 M sodium phosphate at pH 7. Polyacrylamide fragments were removed by 0.8-μm membrane filtration and the filtrate was further concentrated by ultrafiltration if necessary. Human pheochromocytoma DBH and bovine adrenal medulla DBH were prepared according to reported procedures (15, 16). DBH activity was measured by using the spectrophotometric assay of Nagatsu and Udenfriend (17) with tyramine as substrate. Units of activity were expressed as micromoles of octopamine formed per minute per liter at 37° (units per liter). Total protein was determined by using the method of Lowry *et al.* (18) with bovine serum albumin as standard. Tetrameric and dimeric human plasma DBH fractions were separated by gel filtration of a concanavalin A-purified sample of plasma DBH as previously described (11).

Immunochemical methods. New Zealand White rabbits were immunized with purified antigens as previously described (11). Ouchterlony double-immunodiffusion was performed on Hyland immunodiffusion plates in 0.1 M sodium chloride-0.02 M sodium phosphate (pH 7.5) at 4° for 18–24 hr. DBH was iodinated by using an immobilized, coupled glucose oxidase-lactoperoxidase method (19), supplied as Enzymobeads by Bio-Rad Laboratories, Richmond, Calif. A 0.05-ml sample containing 0.01 mg of purified protein was combined with 0.025 ml of 0.02 M sodium phosphate at pH 7.4, 0.05 ml of Enzymobead suspension, 1 mCi of $Na^{125}I$ in 0.025 ml, and 0.025 ml of 1% β-D-glucose. The mixture was agitated at room temperature for 30 min, then the Enzymobeads were separated by low-speed centrifugation. The supernatant was applied to a column (1.6 × 86 cm) of Ultrogel ACA-22. The [^{125}I]DBH was eluted at 5 ml/hr with a buffer containing 0.15 M NaCl; ovalbumin, 1 mg/ml; sodium azide, 1 mg/ml; and 0.01 M sodium phosphate at pH 7.4. [^{125}I]DBH was localized by counting radioactivity and by comparing the elution profile with that of enzymatically active, unlabeled DBH.

Antibody titration. Antiserum titrations of [^{125}I]DBH were performed by incubating a mixture containing 0.01 ml of [^{125}I]DBH (4000–6000 cpm), 0.01 ml of antiserum or normal rabbit serum dilutions, and 0.08 ml of RIA buffer (0.15 M sodium chloride; 0.01 M sodium phosphate at pH 7.4; ovalbumin, 10 mg/ml; and sodium azide, 1 mg/ml) at 37° for 1 hr then further at 4° for 18 hr. To each sample, 0.1 ml of immobilized goat antirabbit immunoglobulin G suspension (Immunobeads, Bio-Rad) was then added, and the samples were again incubated at 37° for 2 hr. The immobilized DBH-anti-DBH complex was collected by centrifugation and the pellets were washed with 0.2 ml of water. Pellets were then counted for radioactivity.

RIA. RIA was performed in a manner identical with the antibody titration experiments, except that reagents were initially combined in the following order: 0.07 ml of RIA buffer, 0.01 ml of unlabeled DBH standards [concentrations estimated by using the method of Lowry *et al.* (18) with a bovine albumin reference] or dilutions of unknowns, 0.01 ml of [^{125}I]DBH, and 0.01 ml of diluted antiserum. The competitive binding standard curves were obtained at an antiserum dilution adjusted to produce 50% binding of precipitable [^{125}I]DBH. Unknown samples were diluted with RIA buffer to target values within the working range of assay. Appropriate multiples of this dilution were also assayed, and all unknowns were measured at least in duplicate at each of two dilutions. Blanks were estimated by replacing antiserum with normal rabbit serum, and the maximal binding for each assay was determined at the zero dose level of unlabeled DBH standard. Competitive binding data reduction was accomplished by using a method of nonlinear regression analysis of the dose-response curve (20). Statistical analysis of results was performed according to the method of Rodbard (21) to determine the coefficient of variation.

RESULTS

The major, tetrameric form of human plasma DBH (10) was purified by substituting a micropreparative method of PAGE for an earlier reported procedure of preparative isoelectric focusing (11). Figure 1 (*right*) shows a PAGE pattern of the enzyme as occasionally isolated according to the original protocol (11) which produced variable quality and low yields. DBH enzymatic activity was found exclusively associated with a single component (*middle band* of Fig. 1, *right*). The gel in Fig. 1 (*left*) shows a sample of PAGE-eluted enzyme after re-electrophoresis. As a drawback to the method, about 50% of the enzymatic activity was often lost, and we have observed the appearance of minor, faster-migrating bands thought to be degradation products, as seen in Fig. 1 (*left*). The purified human plasma enzyme

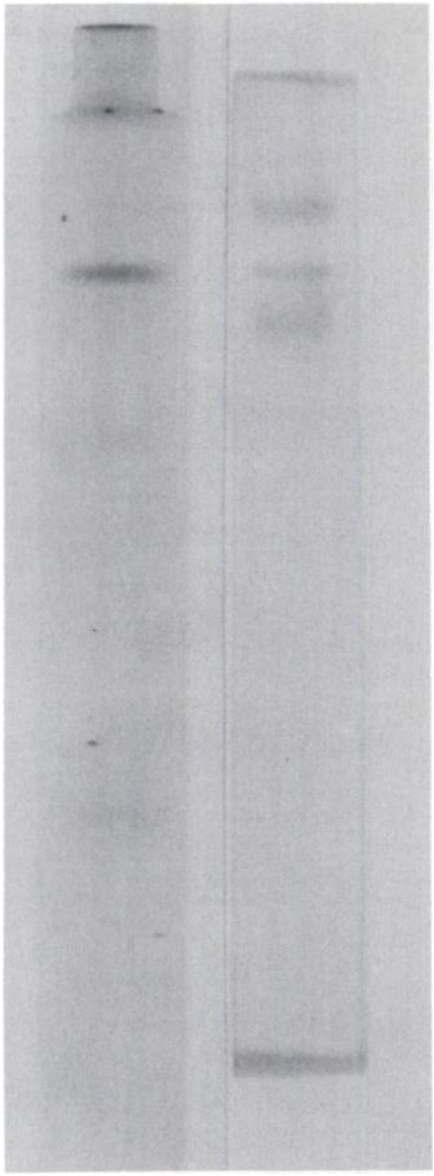

FIG. 1. *PAGE of human plasma DBH*

Electrophoresis was performed using Tris-glycine buffer, pH 8.9, on 6% acrylamide gels (13), and protein was stained with Coomassie blue G250. *Right*, gel was loaded with 20–30 μg of purified human plasma DBH obtained by using a preparation method described earlier, including preparative sucrose gradient isoelectric focusing (11). *Left*, 10 μg of purified human plasma DBH obtained following elution of enzyme from PAGE (see Experimental Procedure).

comigrated on PAGE with purified DBH from human pheochromocytoma (15).

The purified plasma protein was radioiodinated by using a solid-phase lactoperoxidase method (19). The [^{125}I]DBH had a specific radioactivity of 5–10 μCi/μg of protein, and comigrated on gel filtration with the native plasma enzyme activity, which we identified as a 300,000 mol wt tetrameric form of this protein (10, 11, 15). Radioiodination of DBH from human pheochromocytoma produced similar results, and homogeneous preparations of this enzyme were more easily obtained.

The immunoprecipitability of [^{125}I]DBH was typically 70–80%. This observation contrasts the 90–100% precipitability of unlabeled DBH which was demonstrated by immunotitration of the native enzymatic activity (11) and suggests that the [^{125}I]DBH preparations may contain residual fragments of damaged, iodinated protein or some free ^{125}I. We have also observed a gradual decrease in [^{125}I]DBH precipitability with time of storage at 4°. Comparative radioiodination of the enzyme with the Bolton-Hunter reagent (7, 22) yielded a qualitatively similar isolate. Furthermore, the competitive binding curves produced with unlabeled enzyme and [^{125}I]DBH by using either radiolabeling method were not different.

The titration of [^{125}I]DBH with various antisera is shown in Fig. 2. Anti-DBH antisera specific for the human plasma (tetrameric form), the human pheochromocytoma, and the bovine adrenal enzymes are represented. The percentage binding of [^{125}I]DBH in Fig. 2 has been recalculated so that the value at 100% represents the maximal amount of [^{125}I]DBH precipitable by specific, homologous antiserum. In each case, this amount was 70–80% of the total radioactivity and is specified as immunotitratable DBH. By estimating the titer as the dilution of antiserum binding 50% of the titratable enzyme, we observed a variation in titer between different antisera. We also found, as shown in Fig. 2, that antisera specific for the plasma DBH or the pheochromocytoma enzyme were similarly able to titrate [^{125}I]DBH from either source, thus supporting structural similarity and immunological identity between these enzymes. On the other hand, one antiserum directed against bovine adrenal DBH, which showed a strong precipitin band against the purified bovine enzyme in an Ouchter-

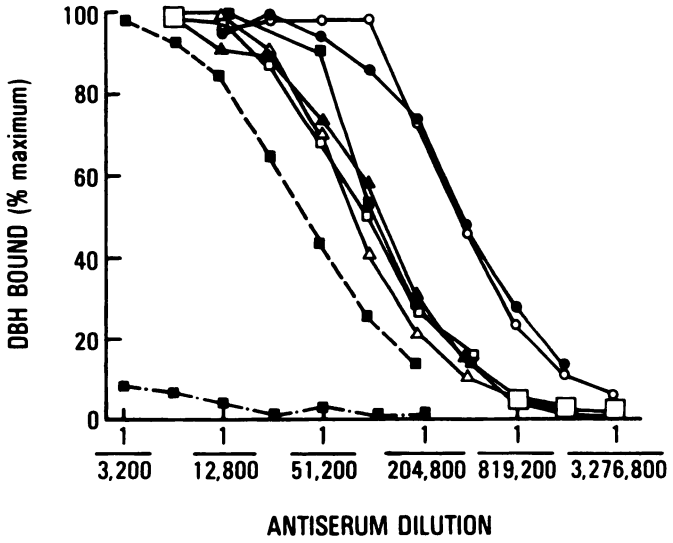

FIG. 2. *Antiserum titration of human DBH*

DBH bound represents the percentage of [^{125}I]DBH maximally precipitated by homologous antiserum titrated by various dilutions of test antisera. In each case, the maximal precipitability was 70–80% of the total radioactivity in the samples. ○—○, ●—●, Pheochromocytoma and plasma DBH, respectively, incubated with antipheochromocytoma DBH antiserum; ■—■, ▲—▲, plasma DBH preparation, △—△, □—□, pheochromycotoma DBH preparation, each incubated with antiplasma DBH antiserum. ■- - -■, □-·-□, Plasma DBH incubated with a second antiplasma DBH antiserum and antibovine adrenal DBH antiserum, respectively. *Large squares* represent clusters of several symbols omitted for clarity.

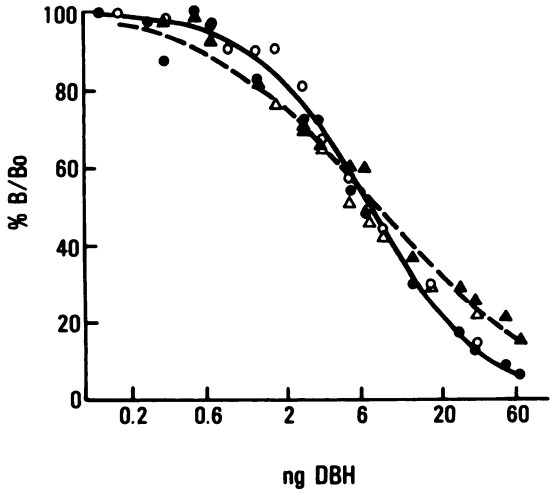

FIG. 3. *Human DBH RIA standard curve*

The curves represent the dose-response displacement of [^{125}I]DBH by unlabeled enzyme as a function of unlabeled enzyme shown on a logarithmic scale in nanograms. The response variable $\%B/B_0$ is calculated from the binding of [^{125}I]DBH, where B = precipitated radioactivity (sample dose) − blank, and B_0 = precipitated radioactivity (zero dose) − blank. Samples contained varying amounts of unlabeled purified DBH standards or unknowns. The zero-dose value was measured with no unlabeled DBH present. Blank radioactivity represents the amount of [^{125}I]DBH nonspecifically precipitated by the assay with appropriate dilutions of nonimmune rabbit serum. Other conditions are described under Experimental Procedure. ▲, ○, Human plasma and pheochromocytoma DBH standards in the assay with [^{125}I]plasma DBH and antiplasma DBH antiserum (– – –) or [^{125}I]pheochromocytoma DBH and antipheochromocytoma DBH antiserum (——), respectively. Cross-reactivity of enzymes is indicated by including the response to plasma DBH (●) and pheochromocytoma DBH (△) along the standard curves of crossed specificity (——) and (– – –), respectively. The standard curves drawn were calculated by using a nonlinear least-squares approximation method (20).

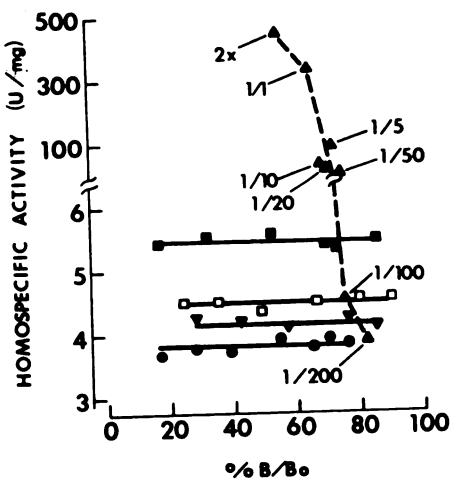

FIG. 4. *The "dosing-out" response of DBH in the human DBH RIA*

Parallelism of the competitive displacement of [^{125}I]DBH by various samples of enzyme relative to the response of purified standard DBH preparation was tested by expressing the observed enzyme homospecific activity, in units per milligram of immunoreactive protein, as a function of the extent of [^{125}I]DBH displacement, $\%B/B_0$ (see Fig. 3). Each point was corrected for sample dilution in the assay, and a one-to-one correspondence of unknown antigen to standard antigen in the assay would be indicated as a horizontal line. Deviation from this response would indicate immunological differences between unknown and standard antigens. ●, Pooled human plasma; ▼, a human adrenal chromaffin vesicle lysate; ▲, a bovine adrenal chromaffin vesicle lysate. The dilutions of bovine chromaffin vesicle lysate are indicated for each point (▲). The 1:1 dilution had a DBH activity of 135 units/liter. ■, □, Isolated fractions of human plasma DBH tetramer and dimer, respectively. The *solid horizontal lines* through the points indicate the mean values of the respective sets of homospecific activities.

lony double-immunodiffusion experiment, bound little ^{125}I-labeled human DBH at moderate dilutions. Although variability in antibody production in rabbits may, in part, account for this observation, we made no attempt to screen a number of antibovine DBH antisera for cross-reactivity. The difference in avidity for two antihuman plasma DBH antisera is also shown in Fig. 2. Regardless, however, each human antiserum was, at some dilution, able to precipitate quantitatively various ^{125}I-labeled human DBH preparations. The highest-titer antisera were used in subsequent experiments.

The RIA standard curve for human DBH is shown in Fig. 3. The *solid line* represents the response to pheochromocytoma DBH standards in the assay using an antipheochromocytoma DBH antiserum and respective [^{125}I]DBH. The *dashed line* shows a similar response to purified plasma DBH in an ^{125}I-labeled plasma DBH-antiplasma DBH assay. The cross-comparison of plasma DBH with the pheochromocytoma-derived RIA standards and with pheochromocytoma DBH in the plasma assay is shown by the superposition of the respective data. An immunological equivalence between plasma and pheochromocytoma DBH is apparent, and either assay system produced equivalent results. Most experiments were conducted using the pheochromocytoma-antipheochromocytoma DBH RIA. The type of dose-response curve shown in Fig. 3 was used as a metric for the RIA of human DBH, and unknown sample responses were maintained within the region of the curve where relative binding of [^{125}I]DBH was between 20% and 75% for optimal results. Within this region of the standard curve, the within-assay coefficient of variation was 10% and the between-assay coefficient of variation was 12%, both of which were estimated from the response of multiple samples repeated in several assays (21).

We further tested the cross-reactivity of various forms of DBH at different doses, and the results are expressed in Fig. 4. These modified "dosing" curves demonstrate a parallelism of the dose response of pooled human plasma and human adrenal chromaffin vesicle lysate in the pheochromocytoma-antipheochromocytoma assay system. Bovine adrenal DBH, on the other hand, produced a nonparallel response in this human DBH assay, thus identifying a limited cross-reactivity of this species with these antisera. The homospecific activity (units per milligram) (22) was calculated as the ratio of DBH activity in units per liter to the measured immunoreactive protein in micrograms per milliliter.

Table 1 contains values of homospecific activity for several fractions of human DBH. Of particular interest is the similarity in homospecific activity between DBH in pooled human plasma, and the chromaffin vesicle lysates of normal human adrenal and pheochromocy-

TABLE 1

Homospecific Activity of Fractions of Human DBH

The homospecific activity of various human DBH fractions was calculated as the ratio of enzymatic activity to the concentration of immunoreactive protein measured by RIA. RIA was performed using pheochromocytoma DBH standards and antipheochromocytoma DBH antiserum. Other protein concentrations were estimated by using the method of Lowry *et al.* (18). Specific activity was calculated as the ratio between enzymatic activity and total protein. Further details are given under Experimental Procedure.

Fraction	Total protein	Enzyme activity	Specific activity	Immunoreactivity	
				Protein	Homospecific activity
	μg/ml	*units/liter*	*units/mg*	*μg/ml*	*units/mg*
Pooled plasma	72,000	43.2	0.0006	13.5	3.2
Plasma fractions[a]					
ConA-affinity	7,520	172	0.023	46.5	3.7
Gel filtration (2×)	698	156	0.22	40.0	3.9
EDTA-inhibited plasma[b]		0 (35)		7.8 (8.5)	0 (4.1)
Adrenal lysate[c]	3,000	36.5	0.012	8.7	4.2
Pheochromocytoma lysate[c]	4,200	172	0.04	45.2	3.8
Pheochromocytoma DBH[d]	200	743	3.7	191	3.9

[a] Plasma fractions were obtained during the isolation of human plasma DBH according to the reported procedure (11). The samples represent pooled eluates from chromatographic separations on concanavalin A-agarose (ConA-affinity) and Bio-Gel A-0.5m gel filtration (twice), respectively. The gel filtration fraction represents the major DBH fraction.

[b] Heparinized human plasma was treated with EDTA, 1 mg/ml, to inhibit DBH enzymatic activity. Values in parentheses were obtained on the same plasma without EDTA.

[c] Lysates were obtained from purified chromaffin granules isolated by discontinuous sucrose gradient centrifugation (16).

[d] A fraction of highly purified DBH obtained from a separate pheochromocytoma different from the preparation of enzyme used in the RIA system.

toma. We found that over a wide range of DBH enzyme activities and relative degrees of purity in plasma and chromaffin granule lysates the homospecific activity of DBH was about 4 units/mg. The equal measurability of DBH immunoreactive protein in heparinized plasma before and after total inhibition of enzyme activity with the Cu^{2+} chelator, EDTA, shown in Table 1, indicates that variations in activity must reflect changes in over-all specific enzymatic activity, that is, relative to total enzyme protein, or changes in the degree of immunological cross-reactivity of the enzymes. The homospecific activity should thus be insensitive to differences in the apparent specific activity observed during protein purification, for example, and ideally should serve as an indicator of enzyme damage during isolation and the presence of a fraction of less active or inactive enzyme in any isolated sample (13).

The isolation of DBH from human tissues proceeds with minimal change in homospecific activity, as shown in Table 1. A 40-fold purification of the pheochromocytoma enzyme to homogeneity by using concanavalin A-affinity chromatography resulted in no loss of homospecific activity. Furthermore, the over-all specific activity of the purified preparation, which was different from that used as tracer and standard in the RIA, was identical with the homospecific activity. In contrast to this rapid purification of DBH, the isolation of the plasma enzyme requires considerably more manipulation (11). As indicated above, the isolation of a homogeneous preparation of plasma DBH by preparative PAGE resulted in a pronounced loss of enzymatic activity with minimal change in immunoreactivity. However, values shown in Table 1 indicate little change in the homospecific activity of plasma DBH during earlier stages of isolation.

The predominant forms of plasma DBH, a 300,000 mol wt tetramer representing 75–80% total enzyme activity, and a 150,000 mol wt dimer, are copurified by using concanavalin A-agarose chromatography (11). Separation of tetrameric plasma DBH subsequently by gel filtration would be expected to reveal differences in homospecific activity of the tetramer relative to whole plasma or to the DBH dimer if these forms differed, provided no other changes were induced by the isolation procedure. Results in Table 1 show that tetrameric DBH has a homospecific activity similar to that of the enzyme in crude plasma, although the over-all degree of purity increased 400-fold. Thus, if differences in specific activity between tetrameric and dimeric forms of plasma DBH occur, they at least must be small in magnitude.

In order to test whether the tetrameric and dimeric forms of plasma DBH were directly distinguishable by RIA specific for the tetrameric enzyme, these fractions were assayed following gel filtration. As shown in Fig. 5,

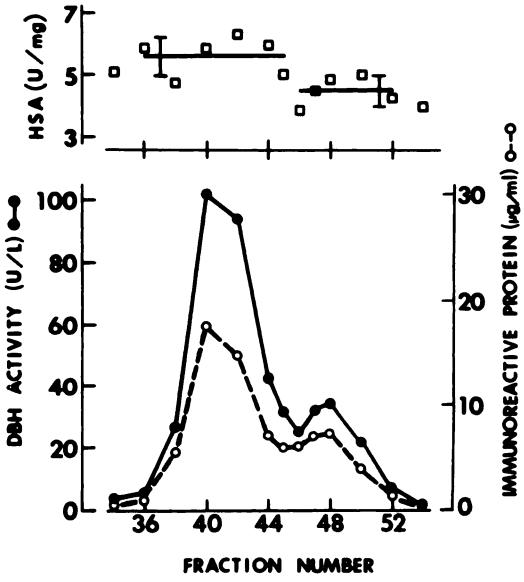

FIG. 5. *The separation of human plasma DBH tetramer and dimer by gel filtration*

DBH, isolated from whole plasma by concanavalin A-affinity chromatography, was further fractionated by gel filtration, and individual fractions were assayed for DBH enzymatic activity (●——●) and DBH immunoreactive protein (○- - -○). RIA was performed using the antipheochromocytoma DBH system (see Fig. 3 and Experimental Procedure). The *lower part* of the figure shows the elution profile, and the *upper part* shows the calculated homospecific activities (*HSA*) of each fraction (□) as the means of duplicate determinations. The *solid lines* through □ indicate the means of inclusive values (limits of line segments); the standard deviations are shown as vertical bars.

a close correspondence between peaks of DBH enzyme activity and peaks of immunoreactive protein was observed. Estimations of homospecific activities across this profile are plotted in the *upper part* of Fig. 5. A decrease in homospecific activity of the dimeric relative to tetrameric peaks is suggested by the results. The mean value of points across the tetramer peak was 5.6 ± 0.6 units/mg (value ± standard deviation), and the mean of points across the dimer peak was 4.5 ± 0.5 units/mg. We further separated the tetrameric and dimeric pools of DBH by repeated gel filtration and assayed the isolated forms. The tetramer-free dimeric fraction was found to have a homospecific activity of 4.5 ± 0.5 units/mg as compared with a value of 5.5 ± 0.4 units/mg for the tetramer fraction, which confirms the results obtained on individual column fractions. As a further check of the relative homospecific activities between tetrameric and dimeric fractions of plasma DBH, the linearity of the RIA dose-response curve for separated tetrameric and dimeric fractions was examined. The results are shown in Fig. 4, and they indicate immunological identity between these forms within the resolution of our methods. The magnitude of the homospecific activities for these fractions is greater than the plasma fraction listed in Table 1. This discrepancy seems to reflect differences between plasma enzyme preparations used for the tetramer-dimer gel filtration experiments and the plasma and fractions represented by the data in Table 1. However, the variation between tetrameric and dimeric DBH was confirmed by similar results from different gel filtration experiments.

DISCUSSION

A specific, homologous antiserum to human plasma DBH has been shown to cross-react fully with the enzyme from pheochromocytoma in an RIA. The concentration of immunoreactive DBH in human plasma is comparably estimated by using either the assay with specific antiplasma DBH antisera, or by using a similar procedure with a specific antipheochromocytoma DBH antiserum and homologous pheochromocytoma antigen. Also, DBH in the lysate of purified chromaffin granules from normal human adrenal was fully cross-reactive in the RIA. Bovine adrenal DBH and specific antiserum, on the other hand, failed to cross-react with the human enzyme. Our results support the conclusions of other workers (5–9) that a homologous immunoassay for human DBH yields plasma levels of immunoreactive DBH closely corresponding to plasma enzyme activities. Moreover, we have demonstrated that purified human plasma DBH, pheochromocytoma DBH, or normal adrenal DBH serve equally well as homologous antigens, as anticipated from previous studies (5–9). From a practical standpoint, although somewhat restricted by the availability of tissues, the purification of DBH from human pheochromocytoma or normal surgical adrenal glands is technically much simpler, and the quality and yields of a homogeneous product are greater (5–9, 15, 23) than in any attempt to isolate this enzyme from plasma (11, 24, 25).

Human plasma DBH can be isolated as two major, separable, enzymatically active forms (10, 11, 24–26). The predominant species is a 300,000 mol wt tetrameric enzyme which constitutes 75–80% of the total plasma DBH activity (10, 11). The remaining DBH is largely a dimeric form, which differs not only by molecular weight, but perhaps also by increased thermal lability (26) and decreased specific activity (25). Since accurate estimations of specific activity depend on the availability of purified enzyme, we examined the DBH enzymatic activity of these separable fractions relative to immunoreactive protein. Our results have shown that dimeric plasma DBH cross-reacts identically with the tetrameric enzyme in the RIA, but nevertheless exhibits a somewhat lower homospecific activity (22). Park *et al.* (27) reported the isolation of multiple forms of pheochromocytoma DBH with differing specific activity. They suggested that the principal native form of DBH is the tetramer, which they reported as having a value of 30.1 units/mg of total protein, and a homospecific activity of 25.1 units/mg of immunoreactive protein. That study showed that a dimeric form of their pheochromocytoma enzyme was only one-fourth as active per milligram of total protein and had less than one-half the homospecific activity of the predominant tetrameric enzyme. Our results showing decreased homospecific activity in spite of complete immunological cross-reactivity, considered in light of the findings of Park *et al.* (27), support a concept that dimeric human DBH is a dissociation product of the native tetrameric enzyme and that in the process of dissociation either a portion of the enzyme is inactivated or the dimeric enzyme actually has a lower specific activity, i.e., a decreased rate of enzyme catalysis. These alternatives cannot be distinguished by the present studies.

The difference in specific activities between our pheochromocytoma DBH preparations, about 4 units/mg, and the value of 30 units/mg reported by Park *et al.* (27) for the predominant form of DBH is likely due to differences in preparative methodology and techniques of total protein determination. Rush *et al.* (13) have also demonstrated effects of preparative manipulations on the homospecific activity of DBH. The present homospecific activity in the range of 4 units/mg agrees well with our previously reported specific activity 2.3 units/mg (15) and the value of 5.1 units/mg reported by Stone *et al.* (23) for soluble pheochromocytoma DBH.

Pooled normal human plasma in our studies was typically obtained equally from 20 individuals per enzyme preparation. The isolation of a fraction of dimeric plasma DBH with a suggested 20% lower homospecific activity than the tetrameric enzyme thus represents this sort of average observation. However, it has been shown in certain individuals that plasma DBH activity may be thermolabile, and the stability of tetrameric and dimeric forms may be different (26). Furthermore, the possibility remains that the proportion of tetrameric to dimeric DBH varies between some individuals. These factors, as well as the magnitude of plasma DBH levels (6, 7), may be genetically determined or altered in the pathological state and may impinge on the usefulness of plasma DBH as an index of physiological activity in man (12).

ACKNOWLEDGMENTS

The authors wish to thank Dr. Richard A. Stone for the inspiration to initiate this study, Ms. Marta Zekan for her unexcelled typing skills, Michael Alford for assistance in computerizing the data analysis, and Annie Chen for technical assistance.

REFERENCES

1. Weinshilboum, R., and J. Axelrod. Serum dopamine-β-hydroxylase activity. *Circ. Res.* **28**:307–315 (1971).
2. Viveros, O. H., L. Arqueros, and N. Kirshner. Release of catecholamines and dopamine-β-oxidase from the adrenal medulla. *Life Sci* **7**:609–618 (1968).
3. DePotter, W. P., A. F. DeSchaepdryver, E. J. Moerman, and A. D. Smith. Evidence for release of vesicle proteins together with noradrenaline upon stimulation of the splenic nerve. *J. Physiol. (Lond.)* **204**:102–104 (1969).
4. Rush, R. A., P. E. Thomas, T. Nagatsu, and S. Udenfriend. Comparison of human serum dopamine-β-hydroxylase levels by radioimmunoassay and enzymatic assay. *Proc. Natl. Acad. Sci. U. S. A.* **71**:872–874 (1974).
5. Ebstein, R. A., D. H. Park, L. S. Freedman, S. M. Levitz, T. Ohuchi, and M. Goldstein. A radioimmunoassay of human circulatory dopamine-β-hydroxylase. *Life Sci.* **13**:769–774 (1973).
6. Dunnette, J., and R. Weinshilboum. Human serum dopamine-β-hydroxylase: correlation of enzymatic activity with immunoreactive protein in genetically defined samples. *Am. J. Hum. Genet.* **23**:155–156 (1976).
7. Dunnette, J., and R. Weinshilboum. Inheritance of low immunoreactive human plasma dopamine-β-hydroxylase: radioimmunoassay studies. *J. Clin. Invest.* **60**:1080–1087 (1977).
8. Rush, R. A., P. E. Thomas, and S. Udenfriend. Measurement of human dopamine-β-hydroxylase in serum by homologous radioimmunoassay. *Proc. Natl. Acad. Sci. U. S. A.* **72**:750–752 (1975).
9. Kirshner, N., S. M. Schanberg, and H. J. Sage. Homospecific activity of serum and tissue dopamine-β-hydroxylase. *Life Sci.* **17**:423–430 (1977).
10. Rosenberg, R. C., and W. Lovenberg. Active dimers of dopamine-β-hydroxylase in human plasma. *Mol. Pharmacol.* **13**:652–661 (1977).
11. Frigon, R. P., and R. A. Stone. Human plasma dopamine-β-hydroxylase: purification and properties. *J. Biol. Chem.* **253**:6780–6796 (1978).
12. Weinshilboum, R. Serum dopamine-β-hydroxylase. *Pharmacol. Rev.* **30**:133–166 (1979).
13. Rush, R. A., S. H. Kindler, and S. Udenfriend. Homospecific activity, an immunologic index of enzyme homogeneity: changes during the purification of dopamine-β-hydroxylase. *Biochem. Biophys. Res. Commun.* **61**:38–44 (1974).
14. Davis, B. J. Disc electrophoresis. *Ann. N. Y. Acad. Sci.* **12**:404–427 (1964).
15. O'Connor, D. T., R. P. Frigon, and R. A. Stone. Human pheochromocytoma dopamine-*beta*-hydroxylase: purification and molecular parameters of the tetramer. *Mol. Pharmacol.* **16**:529–538 (1979).
16. Smith, A. D., and H. Winkler. A simple method for the isolation of adrenal chromaffin granules on a large scale. *Biochem. J.* **103**:480–482 (1967).
17. Nagatsu, T., and S. Udenfriend. Photometric assay of dopamine-β-hydroxylase activity in human blood. *Clin. Chem.* **18**:980–983 (1972).
18. Lowry, O. H., N. J. Rosebraugh, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–277 (1951).
19. David, G. S., and R. A. Reisfeld. Protein iodination with solid state lactoperoxidase. *Biochemistry* **13**:1014–1021 (1974).
20. Rodbard, D., and D. M. Hutt. Statistical analysis of radioimmunoassays and immunoradiometric (labelled antibody) assays: a generalized, weighted, iterative, least-squares method for logistic curve fitting, in *RIA and Related Procedures in Medicine*. International Atomic Energy Agency, Vienna, 165–192 (1974).
21. Rodbard, D. Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. *Clin. Chem.* **20**:1255–1270 (1974).
22. Bolton, A. E., and W. M. Hunter. The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Application to the radioimmunoassay. *Biochem. J.* **133**:529–539 (1973).
23. Stone, R. A., N. Kirshner, J. Reynolds, and T. C. Vanaman. Purification and properties of dopamine-β-hydroxylase from human pheochromocytoma. *Mol. Pharmacol.* **10**:1009–1015 (1974).
24. Miras-Portugal, M-T., D. Aunis, and P. Mandel. Human serum dopamine-β-hydroxylase. Purification, molecular weight, presence of sugars, and kinetic properties *Biochimie (Paris)* **57**:669–675 (1975).
25. Ikeno, T., S. Hashimoto, H. Kuzuya, and T. Nagatsu. Purification and properties of human serum dopamine-β-hydroxylase. *Mol. Cell. Biochem.* **18**:117–123 (1977).
26. Dunnette, J., and R. Weinshilboum. Human plasma dopamine-β-hydroxylase: variation in thermal stability. *Mol. Pharmacol.* **15**:649–660 (1979).
27. Park, D. H., T. Kashimoto, R. P. Ebstein, and M. Goldstein. Purification and immunochemical characterization of dopamine-β-hydroxylase from human pheochromocytoma. *Mol. Pharmacol.* **12**:73–81 (1976).

Send reprint requests to: Dr. Ronald P. Frigon, Research (151), Veterans Administration Medical Center, San Diego, Calif. 92161.

This work was supported by the Medical Research Service of the Veterans Administration and National Institutes of Health Grant HL18095.