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Metabolic Clearance Rate of Dopamine β-Hydroxylase in the Rat

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SUMMARY

The metabolic clearance rate (MCR) of both bovine and rat dopamine β-hydroxylase (DBH) preparations was measured using two complementary procedures, pulse-dose injection and constant infusion of enzyme into the rat circulation. Rats that received injections of DBH activity had plasma DBH activity levels similar to those of controls by 24 hr after a pulse dose of rat DBH. The DBH MCR computed by stochastic analysis of the disappearance curve of injected DBH activity was about 1.0 ml/hr/100 g body weight; the mean transit time of DBH was about 8 hr. The disappearance curve of heterologous enzyme (bovine DBH) was more rapid than that of the rat, yielding an MCR of about 8 ml/hr/100 g body weight. MCR values obtained with the constant-infusion technique were similar to those obtained with the pulse-dose technique. These kinetic parameters are consistent with the time frame for altered plasma DBH activity observed with pharmacological and endocrinological factors. These data support the conclusion that plasma DBH turnover time is measured in hours, not days.

INTRODUCTION

DBH[3] (EC 1.14.17.1), the enzyme that converts dopamine to norepinephrine in sympathetic neurons, enters the circulatory compartment presumably after exocytotic release from nerve cells with the sympathetic neurotransmitter (1, 2). Circulating DBH is found in many species, including man. Since DBH is a relatively stable molecule that derives from a defined population of neurons, measurements of the circulating DBH activity level offer the potential for assessing sympathetic neurotransmitter enzyme metabolism under differing environmental or pathophysiological conditions. The latter rationale has provided the impetus for many studies on serum DBH activity and its relationship to sympathetic nervous system function in a variety of species (3). Although sympathetic neuronal activity does not correlate reliably with circulating DBH levels (3), genetic factors in man (4) and in rat (5), several inherited diseases (see ref. 6), thyroid disease (7), and experimental diabetes (8) are associated with predictable changes in serum DBH activity. A given level of DBH activity in the circulatory compartment reflects the balance between the enzyme entry rate (presumably deriving from the exocytotic release of DBH from sympathoadrenal cells) and peripheral disposal pathways (collectively characterized by the MCR of DBH). We previously reported evidence that modifications in peripheral DBH disposal pathways are major factors accounting for the altered circulating DBH activity accompanying both experimental diabetes (8) and thyroid dysfunction in rats (7). In view of the importance of disposal pathways for determining circulating DBH levels, it is not surprising that attempts to correlate DBH activity with sympathoadrenal function without taking into account alterations in disposal pathways have been largely unsuccessful.

Efforts to assess the circulating DBH disposal pathways have been few, and the published data are not consistent. Our initial studies (7) employed a pulse-dose injection of heterologous bDBH and quantified the disappearance of injected bDBH activity from serum as a reflection of the function of the DBH disposal pathways in the rat. Later, we used homologous rDBH in a similar experimental paradigm (8). With both bDBH and rDBH preparations, we obtained results which suggest that the turnover of DBH in the rat is relatively robust. In contrast, Grzanna and Coyle (9), Geyer *et al.* (10), and Geyer and Schanberg (11) concluded that that the "half-life" of DBH in the rat is relatively long (3–5 days). The disparity

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[3] The abbreviations used are: DBH, dopamine β-hydroxylase (bDBH and rDBH are bovine and rat DBH, respectively); MCR, metabolic clearance rate; hCG, human chorionic gonadotropin.

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between these reports in the kinetic picture of DBH disposal compromises understanding of the potential physiological consequences reflected in measurements of circulating DBH activity. Recognizing that resolution of the disparity can best be achieved by actually quantifying the kinetic parameters governing DBH metabolism in the rat, we have devised two different experimental procedures that give quantitative estimates of MCR of DBH in the rat. Our analysis of the *in vivo* kinetics of DBH disposal shows that the MCR of the DBH enzymes in the rat is far more rapid than heretofore suspected. Indeed, the mean turnover time of DBH is measured in hours, not in days.

METHODS

Animals

The experimental animals were WKY and F344 rats bred in our own colonies. The initial breeding stock, obtained from Taconic Farms (WKY strain) or from Microbiological Associates (F344 strain), was maintained by brother-sister mating in our colony for at least three generations. Offspring were weaned at 28 days of age, housed in groups of two to five littermates by sex in an environmentally controlled room (lights on from 6 a.m. to 6 p.m.), and had free access to food and water until the time of surgery. All animals were 11–13 weeks old at the time of experimentation.

Surgical Procedures and Blood Sampling

Cannulae were implanted bilaterally into the external jugular veins under ketamine anesthesia at least 2 days prior to determining the DBH MCR. Construction of cannulae and surgical implantation techniques were similar to those described by Weeks (12). Cannulae were kept patent by daily flushing with heparinized saline [0.5 mg of crystalline porcine heparin (Sigma Chemical Company) per milliliter of 0.9% NaCl solution]. For any given rat, one cannula was selected for blood sampling at the start of the experiment; the other cannula was used exclusively for administration of the DBH preparation. Blood samples (approximately 125 μl) were withdrawn from the cannula by syringe and processed immediately. Plasma was separated using a Beckman Microfuge, and a 50-μl aliquot was stored frozen at −10° until assay (within 3 days). The volume of blood collected for each sample was minimized to avoid excessive depletion of the rat's circulatory compartment during the course of each experiment.

DBH Activity

DBH activity was measured using the radioenzymatic assay procedure of Molinoff *et al.* (13). Serum was diluted 1:5 (v/v) with ice-cold distilled water, and 100-μl aliquots were assayed in duplicate. Ten microliters of aqueous cupric sulfate were added to the reaction mixture (final concentration 33 μM) to neutralize serum DBH inhibitors. Boiled serum served as the reaction blank. Tyramine was the DBH substrate, [^{14}C-*methyl*]*S*-adenosyl-L-methionine was the methyl donor in the phenylethanolamine *N*-methyltransferase reaction, and known amounts of octopamine added to boiled serum served as standards for calculating enzyme activity. One unit of DBH activity was defined as that resulting in the formation of 1 nmole of [^{14}C]synephrine per hour of incubation at 37°.

hCG Radioimmunoassay

Serum hCG levels were determined by radioimmunoassay as described previously (14). Radioiodination of hCG to a specific activity of 70–100 μCi/μg with ^{125}I was performed by the chloramine T method. The highly purified hCG (CR121) preparation, obtained from the Center for Population Research, National Institute of Child Health and Human Development, National Institutes of Health, served as the infused material, reference preparation, and radioligand.

Determination of the DBH MCR

Two different procedures based on the principles of *in vivo* tracer kinetics (15) were devised to obtain estimates of the MCR of a given DBH preparation: (a) the pulse-dose procedure and (b) the constant-infusion procedure. We employed DBH enzymatic activity as the quantitative variable rather than DBH immunoreactive protein since the accumulation of partially degraded DBH moieties with immunoreactivity, but lacking enzymatic activity, could give a misleading picture of the kinetics of the metabolism of authentic enzyme.

Pulse-dose procedure. The pulse-dose procedure employs an experimenal design in which the disappearance of enzyme activity from plasma is measured following a rapidly injected dose of DBH. This procedure utilizes a form of stochastic analysis that has been employed to estimate the MCR of a variety of proteins, including asialofetuin (16), β-glucuronidase (17), and hCG subunits (14). The general formula for calculating the MCR from these data is as follows:

$$\text{MCR} = \text{dose}/[{}_0\textstyle\int^{\infty} C\,dt] \qquad (1)$$

where C is the concentration of the test substance and t is time. Experimentally, to calculate the area under the disappearance curve, we fit the DBH activity level as a function of time to an equation of the following form:

$$\text{DBH}_i = \text{DBH}_A e^{-\alpha t} + \text{DBH}_B e^{-\beta t} \ldots . + \text{DBH}_N e^{-\eta t} \qquad (2)$$

where DBH_i is the increment in plasma enzyme activity above preinjection baseline at time t after injection, DBH_A and α are the parameters of the fastest component of the plasma disappearance curve, and DBH_N and η are the parameters of the slowest component. Using the parameters obtained for the multiexponential fit of the data (Eq. 2), quantitative estimates of the DBH MCR were ascertained from the relationship:

$$\text{DBH MCR} = \frac{\text{DBH dose}}{(\text{DBH}_A/\alpha + \text{DBH}_B/\beta \ldots . + \text{DBH}_N/\eta)} \qquad (3)$$

An estimate of the mean transit time of DBH is calculated by using the parameters of the multiexponential plasma disappearance curve according to the following formula:

$$\textit{Mean transit time} = \frac{\frac{DBH_A}{\alpha^2} + \frac{DBH_B}{\beta^2} \ldots + \frac{DBH_N}{\eta^2}}{\frac{DBH_A}{\alpha} + \frac{DBH_B}{\beta} \ldots + \frac{DBH_N}{\eta}} \quad (4)$$

The reliability of estimates for the parameters of each component of the plasma DBH disappearance curve is dependent on sampling frequency during the time period where total plasma DBH activity is elevated above preinjection levels. The WKY strain was selected for detailed study because it exhibits very low plasma DBH activity (approximately 2.0 units/ml), thus facilitating the discrimination between residual injected and baseline plasma DBH activity levels (i.e., DBH_i). Although the average baseline plasma DBH activity in the WKY strain shows variability somewhat greater than that in inbred strains with higher baseline activity (i.e., the coefficient of variation for mean baseline DBH activity in WKY rats is 0.20, compared with a value of 0.09 for F344 rats, which have baseline activity of approximately 9 units/ml), the magnitude of an increment in total plasma DBH activity (in units per milliliter) after injection of exogenous DBH is greatest relative to baseline in this strain. This consideration is important for arriving at estimates of the parameters of the DBH disappearance curve components. Thus, while quantitative characterization of the parameters of the slowest component is the least precise, extensive use of the WKY strain was aimed at maximizing the demonstration of this component.

Constant-infusion procedure. The constant-infusion procedure for estimating the MCR involves infusing DBH activity at a constant rate for a sufficient period of time to attain a stable level of plasma enzyme activity. The preinfusion DBH activity level is subtracted from the DBH activity level during the infusion, thus giving the increment in DBH activity (i.e., DBH_i) brought about by the infusion. An estimate of the DBH MCR can be derived from the following relationship:

$$\text{DBH MCR} = \frac{\text{DBH infusion rate}}{DBH_i} \quad (5)$$

To calculate the DBH infusion rate, the DBH activity of the infusate is measured directly, and the rate of volume delivery is controlled precisely by using a Sage (Model 355) constant-infusion pump. To facilitate the achievement of steady state, we routinely administer a priming dose of the DBH preparation at the outset. For each experimental determination, evidence that circulating DBH activity closely approximates steady state is based upon a statistical evaluation of plasma enzyme activity levels obtained during the infusion period. Lack of significant regression or stepwise progression in circulating DBH activity in a minimum of three sequential samples obtained over a 3-hr time period during the infusion constitutes our minimal criteria for the steady state.

Purification of bDBH and rDBH

The procedure used to obtain purified bDBH (from fresh bovine adrenal medulla) was similar to that which we described previously (8); the sole modification is that adsorption of the enzyme to concanavalin A (18) was the final step in all preparations used to assess bDH MCR in these studies. Purification of rDBH from a transplantable rate pheochromocytoma is identical with that described previously (8).

Statistical Procedures

The exponential fit of the pulse-dose DBH disappearance curve data was conducted using the computer program of Faden and Rodbard (19).

Regression analysis by the method of least squares was used to ascertain linearity and significance of deviation of calculated regression slope from the horizontal. The criterion for rejection of the null hypothesis was set at the 5% level for the calculated regression parameters.

RESULTS

Studies with rDBH

Determination of rDBH MCR using the pulse-dose procedure. The serum disappearance curve for a pulse dose of rDBH given to WKY rats (Fig. 1) is multiexponential in nature. Following a dose of 208 units/100 g body weight, the plasma DBH activity level declined from about 55 units/ml to about 8 units/ml over a period of 14 hr. By 24 hr the circulating DBH activity level was

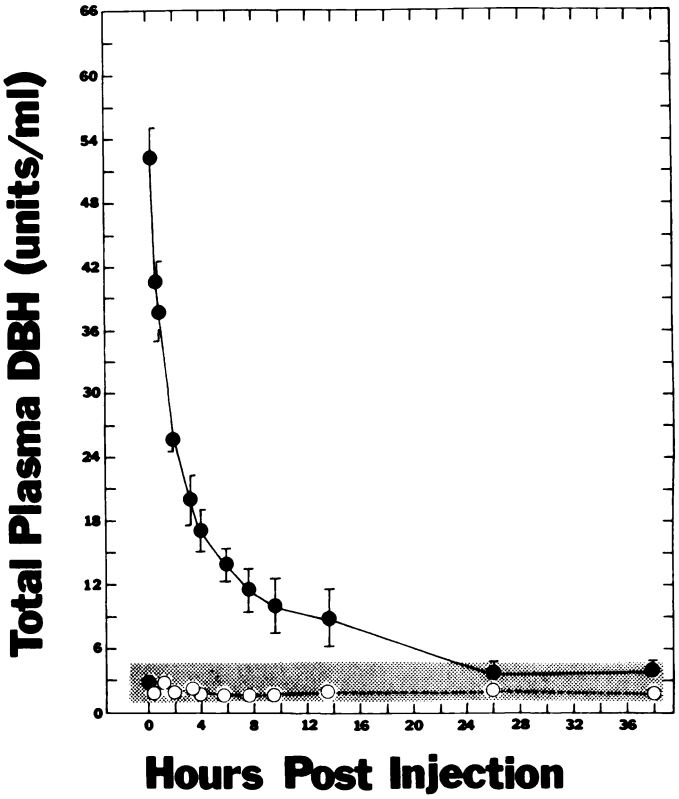

FIG. 1. *Plasma DBH activity as a function of time after a pulse dose (208 units/100 g body weight) in WKY female rats*

Total plasma DBH activity levels (mean ± standard deviation) for three rats receiving rDBH (●) are compared with the mean circulating DBH activity in two rats receiving vehicle (○). The value at time 0 represents the mean baseline DBH activity ascertained from two plasma samples from each of the five rats prior to the injection; the *shaded area* outlines the boundaries (± 2 SD) of the preinjection baseline DBH activity.

indistinguishable from that in vehicle-injected rats and remained so through 38 hr.

The rDBH disappearance curve data (total DBH activity versus time) illustrated in Fig. 1 were replotted after conversion to DBH_i (expressed as percentage dose per milliliter per 100 g versus time) to facilitate conceptualization of the analysis of the data (Fig. 2). The DBH_i denotes the contribution of injected DBH to the total circulating enzyme activity and was obtained by subtracting the preinjection baseline DBH levels from the total enzyme activity at any time. The preinjection baseline DBH activity for each rat was determined from two plasma samples obtained prior to rDBH injection; the first sample was obtained 12 hr before, and the second sample immediately before, the rDBH pulse dose. To control for possible effects of circulatory compartment size reduction due to the rate of blood removal, circulating DBH activity was assessed in vehicle-treated rats sampled at the same time as rDBH-injected rats. As can be discerned from Fig. 1, baseline DBH activity remained constant in vehicle-injected rats. Accordingly, we assume that subtracting the preinjection DBH activity level from the postinjection level provides a reasonable estimate of the actual increment in enzyme activity attributable to the rDBH injection. Expressing the DBH_i (in units per milliliter of plasma) as a percentage of the dose injected per 100 g body weight provides graphic representation of

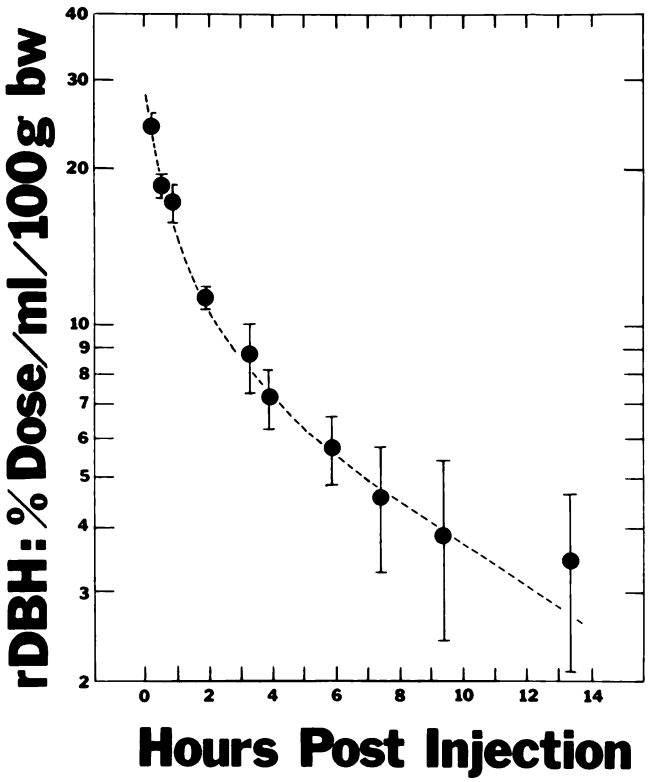

FIG. 2. *Graphic representation of the rDBH disappearance curve for the data illustrated in Fig. 1*

The portion of the total plasma DBH activity attributable to the rDBH pulse dose was calculated for each animal by subtracting the preinjection baseline DBH activity; the resultant difference, the increment of DBH activity (rDBH), is expressed as a percentage of the pulse dosage (208 units/100 g body weight) and plotted versus time postinjection.

TABLE 1

Disappearance curve parameters for rDBH purified from pheochromocytoma (dosage: 207.6 units/100 g body weight) in three WKY female rats

Parameter	Mean value ± SD
A	15.6 ± 1.5% dose/ml/100 g body wt
α	1.91 ± 1.53 hr^{-1}
B	12.9 ± 4.5% dose/ml/100 g body wt
β	0.134 ± 0.074 hr^{-1}
V_D	3.52 ± 0.39 ml/100 g body wt
MCR	0.968 ± 0.261 ml/hr/100 g body wt
Transit Time	7.84 ± 3.04 hr

the data base for calculating the rDBH MCR (Table 1). We obtained a sufficient number of plasma samples in which total DBH activity was discernible from preinjection activity for each individual rat to permit fitting the disappearance curve data to a two-component equation. The rDBH MCR in the three WKY rats estimated by the pulse-dose procedure was 0.97 ± 0.26 ml/hr/100 g body weight (mean ± standard deviation), and the mean transit time for the rDBH was 7.84 ± 3.04 hr.

Determination of rDBH MCR using the constant-infusion procedure. We routinely employed priming doses of rDBH at the start of the constant infusion to reduce the time required to reach apparent steady-state plasma DBH levels. Separate studies confirmed, as would be predicted from the pulse-dose kinetics, that in the absence of a priming dose a stable circulating DBH activity level was not attained until 12–18 hr of rDBH infusion. The addition of a priming dose, in contrast, reliably afforded stable circulating DBH activity levels within 5 hr of infusion. Data from a representative rDBH constant infusion given to a WKY rat after a priming dose are illustrated in Fig. 3. The circulating DBH activity level determined from 5.5 to 9.5 hr of the infusion period

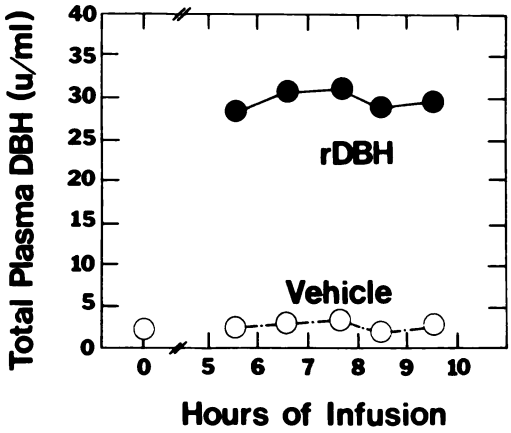

FIG. 3. *Total plasma DBH activity in single WKY rats receiving a constant infusion of rDBH (●) or vehicle (○)*

Each rat received a 0.25-ml priming dose of infusate immediately prior to the start of the constant infusion. The rDBH infusion rate was 41.5 units/hr/100 g body weight (volume rate: 82 μl/hr) following a pulse dose of 128 units/100 g body weight; the volume rate of vehicle infusion was 100 μl/hr. The increment in circulating DBH activity for the rat receiving rDBH was 28.1 ± 1.19 units/ml (mean ± standard deviation for the five values shown).

showed no progressive changes; regression analysis of the plasma DBH activity during this interval yielded a line with a slope indistinguishable from zero. These results indicate that steady-state plasma DBH activity levels can be achieved within an experimentally practical time period. The rDBH MCR estimated from the data shown in Fig. 3 was 1.48 ml/hr/100 g body weight; the mean rDBH MCR for a population of WKY rats as determined by the constant infusion procedure was 1.39 ± 0.19 ml/hr/100 g body weight (mean ± standard deviation; N = 18).

Effect of the circulating DBH increment on the estimate of rDBH MCR. One aspect of the pulse-dose and the constant-infusion procedures relevant to our picture of DBH kinetics in the rat is the extent to which the rDBH MCR is dependent on the circulating DBH activity level. To evaluate the relationship between the estimated rDBH MCR and the steady-state increment in circulating DBH activity attained during constant infusion, we performed a regression analysis of the data for the 13 WKY rats studied. As shown in Fig. 4, the estimated MCR values obtained in WKY rats failed to show a significant relationship with the DBH_i effected by differing rDBH infusion rates in different experiments. Thus, the metabolic pathways for DBH in the rat appeared to behave in a reasonably linear kinetic fashion over a wide range of DBH_i. However, although there was no prominent relationship between infused rDBH dosage and estimated MCR in these experiments, a potential subtle interaction might be revealed by a systematic study of differing rDBH infusion rates using wider dosages of DBH and a design that minimizes interassay variation.

Comparison of rDBH MCR values obtained in two inbred rat strains. Parallel studies were conducted in F344 rats to ascertain whether the DBH metabolic parameters obtained in WKY rats represented a special circumstance. F344 rats had approximately 5-fold higher mean plasma DBH activity levels than WKY rats [8.21 ± 0.31 units/ml versus 1.89 ± 0.20 units/ml, mean ± standard error of the mean for F344 (N = 17) and WKY rats (N = 18), respectively]. Nonetheless, F344 rats had rDBH MCR values (as estimated by the rDBH constant infusion method) quite similar to those of WKY rats: F344 rDBH MCR = 1.40 ± 0.18 ml/hr/100 g body weight (mean ± standard deviation); WKY rDBH MCR = 1.39 ± 0.19 ml/hr/100 g body weight. Thus, the rDBH MCR values obtained in WKY rats are not idiosyncratic to this strain.

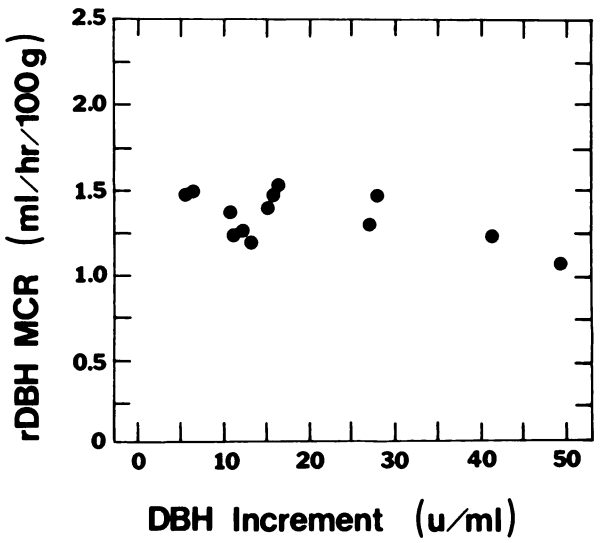

FIG. 4. *Relationship between rDBH MCR determined by constant rDBH infusion to 11 WKY female rats and the plasma DBH increment* Each point represents the data obtained in an individual rat.

Studies with bDBH

Estimation of the bDBH MCR using the pulse-dose procedure. Casual inspection of bDBH pulse-dose disappearance curves obtained in our laboratory with F344 rats (7, 8) reveals (a) that the curves are complex, as noted by other investigators (cf. ref. 10), and (b) that the level of total circulating DBH activity returns to the preinjection baseline level within 12 hr. The latter observation is at variance with other reports (10), but has remained consistent in our laboratory across major experiments (cf. refs. 7 and 8). In consideraton of these observations, we employed a frequent blood-sampling schedule during the initial 12-hr period following bDBH injection to allow a more precise analysis of the disappearance curve than previously reported. Furthermore, we conducted the pulse-dose studies in WKY rats to maximize the probability of discerning small residual increments in circulating bDBH activity for the longest possible time after injection. A detailed disappearance curve following the pulse-dose injection of 1353 units of bDBH per 100 g body weight is presented in Fig. 5. There was a progressive decline in the plasma DBH activity from initial values of about 300 units/ml to about 3 units/ml (99% decrement) within 8 hr. Thereafter, the circulating DBH activity became indistinguishable from preinjection activity levels, or from circulating DBH activity levels in vehicle-treated rats, and remained so through 48 hr after the pulse dose.

Conversion of the raw data (total plasma DBH activity) detailed in Fig. 5 to the DBH_i, expressed as a percentage of the injected bDBH dosage (percentage dose/ml/100 g body weight) with respect to time yielded the disappearance curve shown in Fig. 6. We fitted the disappearance curve data to a two-component exponential equation. The analysis yielded an estimated MCR of 7.73 ± 1.17 ml/hr/100 g body weight (mean ± standard deviation); the mean transit time of bDBH in the rat is about 1.5 hr. Thus, although the MCR of bDBH is significantly more rapid than that of rDBH, both estimates give a kinetic picture of DBH transit times measured in hours, rather than days.

Determination of bDBH MCR using the constant-infusion procedure. Total circulating DBH activity levels during the constant-infusion procedure reached a stable level within several hours after the start of constant bDBH infusion (Fig. 7). Inspection of the values obtained between 2 and 8 hr during the infusion failed to reveal any systematic change in DBH activity over time, and regression analysis of plasma enzyme activity during this interval yielded a line with a slope indistinguishable from zero. Infusion of the vehicle failed to alter endogenous

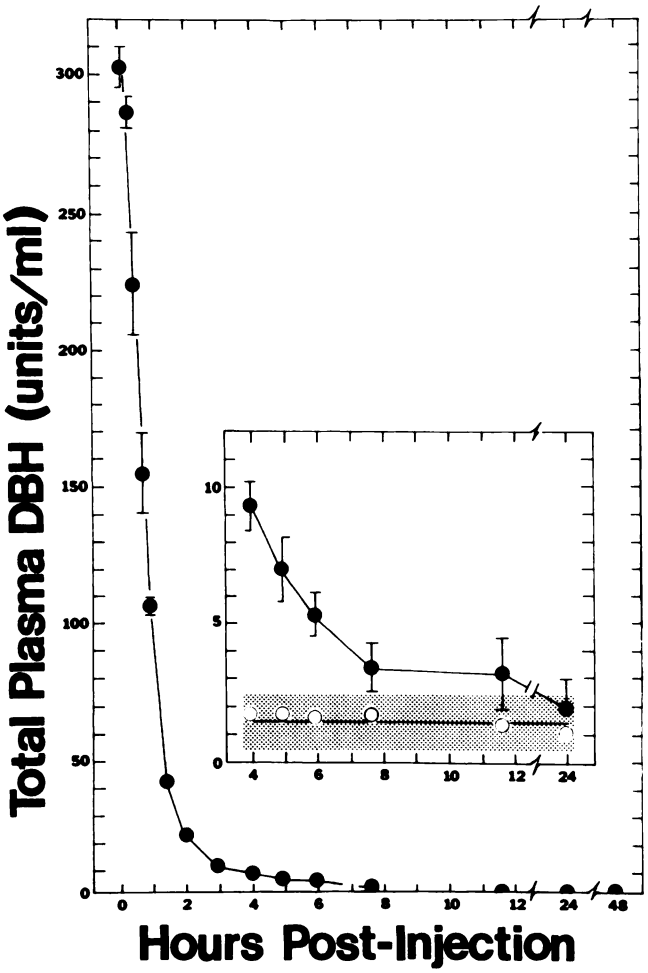

FIG. 5. *Plasma DBH activity as a function of time after a pulse dose of bDBH (1343 units/100 g body weight) in WKY rats*

Total plasma DBH activity levels (mean ± standard deviation) for three rats receiving bDBH (●) and for two rats receiving enzyme vehicle (○) are plotted. The *shaded area* outlines the boundaries (± 2 SD) of the mean baseline circulating DBH activity ascertained from two plasma samples from each of the six rats prior to the injection.

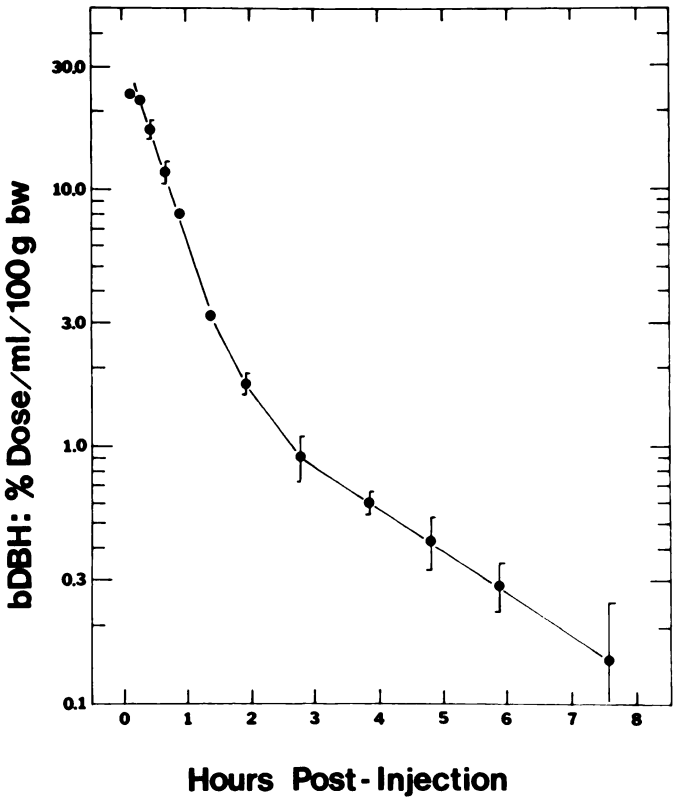

FIG. 6. *Graphic representation of the bDBH disappearance curve for the data illustrated in Fig. 5*

The portion of the total plasma DBH activity attributable to the bDBH pulse dose was calculated for each animal by subtracting the preinjection baseline DBH activity; the resultant difference, the increment of DBH activity (bDBH), is expressed as a percentage of the pulse dosage (1343 units/100 g body weight) and plotted versus time postinjection.

plasma DBH activity. These observations support the logic for calculating the bDBH MCR from the plasma DBH_i (total DBH activity level minus preinjection activity level) and the bDBH infusion rate (Eq. 4, Methods). The bDBH MCR calculated given the data obtained by the constant-infusion procedure was 8.69 ± 2.04 ml/hr/100 g body weight (mean ± standard deviation for three rats infused with the same bDBH preparation used for the pulse-dose studies summarized in Figs. 5 and 6). This esimate compares favorably with the bDBH MCR obtained with the pulse-dose procedure (7.73 ± 1.17 ml/hr/100 g body weight).

Studies with hCG

The ability of the constant-infusion and pulse-dose procedures to yield similar estimates of the MCR for other glycoproteins in the rat circulatory compartment was evaluated with hCG. Previous investigations have documented the similarity of hCG MCR estimates obtained using pulse-dose and constant-infusion procedures in humans (14, 20). The hCG MCR in rats estimated by

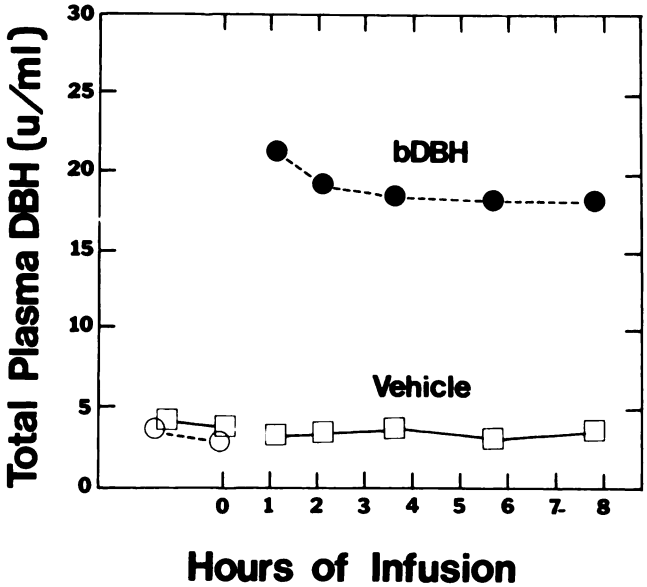

FIG. 7. *Total plasma DBH activity in individual WKY rats receiving a constant infusion of bDBH (●) or vehicle (□)*

The bDBH infusion rate was 136 units/100 g body weight (volume 96 μl/hr); the volume of vehicle infused in the control rat was 98 μl/hr.

the pulse-dose procedure was 0.80 ± 0.11 ml/hr/100 g body weight (mean ± standard deviation; $N = 3$), and by the constant-infusion procedure was 0.88 ± 0.15 ml/hr/100 g body weight (mean ± standard deviation; $N = 3$). We conclude that the ability of these two procedures to yield comparable estimates of the MCR is not idiosyncratic to DBH.

DISCUSSION

On the basis of the principles of *in vivo* tracer kinetics, we devised two different, but complementary, noncompartmental approaches to assess the kinetic parameters of DBH metabolism in the rat. Whether homologous rDBH or heterologous bDBH preparations are employed, estimates of the specific DBH MCR in WKY rats obtained by our constant-infusion and pulse-dose injection procedures yield comparable values. Thus, estimates of the MCR obtained by pulse-dose and constant-infusion procedures, respectively, for bDBH were 7.32 ± 1.17 ml/hr/100 g body weight and 8.69 ± 2.04, and for rDBH were 0.97 ± 0.26 ml/hr/100 g body weight and 1.39 ± 0.19 (mean ± standard deviation). Over a 10-fold range in DBH infusion rates, there was no significant dependence of the estimated MCR value on the increment in the plasma DBH level. From this we can deduce that for practical purposes DBH metabolic pathways behave linearly in the physiological range of DBH levels, as well as substantially above it. As judged by rats receiving vehicle alone, the procedures themselves effected no alterations in endogenous circulating DBH activity due to factors attendant to handling, injection, or blood sampling. Finally, the comparability of MCR estimates by the two procedures is not peculiar to DBH preparations, since both procedures yield consistent MCR values for an unrelated glycoprotein, hCG. Given the system's apparent linearity and its stability during the study period, we conclude that the methods employed give reasonable estimates of the MCR for bDBH, rDBH, and hCG, and that each of these glycoproteins has a demonstrably different MCR in the rat.

Our data on the transit time of DBH in the rat's circulation are at variance with previous reports (9–11). Careful attention to the procedural details employed to assess DBH metabolism are necessary to arrive at a valid picture of DBH kinetics in the rat. Grzanna and Coyle (9) employed a nonstandard procedure to assess circulating DBH metabolism. They observed that the injection of anti-rDBH antiserum into rats causes a rapid elimination of DBH activity from the circulation, and used the rate of circulating DBH activity reappearance to estimate DBH turnover in serum. The validity of their approach, although unique, is difficult to appreciate. First, their calculation of the DBH half-life assumes a single exponential model; all published data for the rat (cf. Fig. 1; refs. 7, 8, 10, 11) and other species (cf. ref. 21) clearly require a multicomponent metabolic system. Second, the Grzanna and Coyle model assumes that the first-order rate constant of circulating DBH activity degradation (k_D in their model: ref. 9) remains constant after injection of anti-rDBH antiserum. This assumption is directly contradicted by their own observation that k_D is markedly altered by the introduction of antiserum. Third, Grzanna and Coyle (9) asusme that excess anti-rDBH antibodies injected initially are inconsequential to subsequent DBH molecules entering the circulation. The investigators attempted to evaluate the persistence of anti-rDBH antibodies in the circulation postinjection with an *in vitro* neutralization test (using normal rat serum, containing DBH activity, plus post-antibody injection serum); however, their observation (9) that the enzyme-neutralizing effect of the anti-rDBH antiserum is 1000 times greater *in vivo* than *in vitro* clearly indicates that *in vitro* neutralization of DBH activity by an incomplete antigen-antibody reaction is the least sensitive procedure for evaluating the persistence of injected antibodies. Each of the preceding problems seriously undermines the validity of the Grzanna and Coyle (9) estimate of the kinetics of circulating DBH metabolism.

Geyer and Schanberg (11) attempted to use radioiodinated DBH to assess the metabolism of circulating DBH activity in the rat. However, there are many methodological difficulties associated with the use of radioiodinated glycoprotein preparations as "tracers" that were not accounted for by the latter investigators. To be valid, tracer studies of *in vivo* kinetics must employ radioactive molecules that are chemically and physiologically identical with the naturally occurring protein. Since DBH is not naturally iodinated, one must prove that the procedures for introducing iodine atoms into its structure do not alter its enzymatic or physiological behavior. Although chromatographic identity can be established (21), proof of unaltered physiology is difficult to ascertain. Beyond the issue of whether iodinated DBH is a valid tracer are several troublesome technical problems. First, the purified DBH preparations that are subjected to iodination undoubtedly contain both contaminating proteins and modified DBH molecules that lack enzymatic activity. The tracer preparation thus contains radiolabeled impurities that are coadministered with authentic radiolabeled DBH. Second, as injected radiolabeled DBH undergoes degradation *in vivo*, radioactive metabolic fragments may accumulate in tissues and plasma. Third, after the liberation of radioiodide from the DBH molecule and assorted contaminants, the rat's thyroid gland utilizes the iodine to synthesize and secrete iodothyronines. While one can minimize the contribution of the thyroid gland to recirculation or radioiodide by appropriate pretreatment of the animal, a procedure not employed by Geyer and Schanberg (11), this precaution minimizes the contribution of only one source of irrelevant serum radioiodine-containing moieties. To measure radiolabeled DBH kinetics, all irrelevant forms of radioactivity must be chromatographically separated from enzymatically active radiolabeled DBH in each sample collected. In other words, for valid kinetic assessment of DBH metabolism one must measure the change in radiolabeled DBH specifically [as attempted by Rush and Geffen (21)], since simple measures of radioactivity alone as employed by Geyer and Schanberg (11) generate a composite picture of *radioiodine* metabolism, not a specific analysis of authentic DBH kinetics. Collectively, the above problems persuaded us to develop alternative approaches for measuring DBH metabolism. The techniques developed in the present study circumvent these

problems by employing the key functional property of DBH—its enzymatic activity.

Our studies of the kinetics of bDBH and rDBH metabolism in the rat clearly indicate that the time frame of enzymatically active DBH turnover is measured in hours, not in days as suggested by Geyer *et al.* (10). These workers measured DBH activity in rat plasma after an i.v. injection of bDBH and interpreted their data as indicating that plasma DBH activity has a half-life of about 5 days. Our previously reported experience (7, 8) with pulse-dose injections of rat DBH seemed at variance with this kinetic picture, particularly with regard to the existence of a slow-phase component with parameters measured in terms of days. In addition, it is well recognized that the precision with which the parameters can be measured is much lower for slow-phase than for rapid-phase components. Accordingly, to obtain optimal resolution of the slow-phase components of the DBH disappearance curve, we selected an inbred rat strain with low baseline levels of endogenous plasma DBH activity [which also happened to be the same strain used by Geyer and Schanberg (11)], used pulse dosages of DBH causing nearly a 200-fold elevation in total plasma DBH activity [to duplicate the initial procedure used by Geyer *et al.* (10)], and obtained frequent blood samples throughout the time period required for the DBH activity level to decline to preinjection levels. Nevertheless, we observed no component of the disappearance curve with a half-time measured in days. In fact, within 12 hr of a pulse dose of bDBH (Fig. 1) or within 24 hr of a pulse dose of rDBH (Fig. 2), the injected DBH activity was no longer evident in the circulation as a significant increment of enzyme activity over that in the preinjected or vehicle-injected control rats. While one could reason that the specific rat strain employed in these studies (WKY) has an inordinately high DBH MCR, comparison of MCR values in different inbred rat strains reveals that the DBH MCR value in WKY rats is indistinguishable from that in F344 rats (Results: "Comparison of rDBH MCR values . . . "); therefore, the observed relatively rapid clearance in WKY rats is not a special case. Thus, our kinetic analysis yields a mean transit time for rDBH of 7.8 hr, whereas the data of Geyer *et al.* (10) translate into a mean transit time of about 7 days. We suggest that the explanation for the discrepancy between these estimates lies within the methodological approach for resolving the slower components of the DBH disappearance curve. In the present study, rigorous attention was given to delineating these components.

A major advantage of knowing the DBH MCR is the ability to quantify the metabolic disposal rate of the enzyme based upon the DBH MCR and the concentration of enzyme in the plasma. Provided that the system is in the steady state, the rate of disposal of plasma DBH activity is equivalent to the rate of DBH entry into plasma. The latter presumably occurs by direct release into the system by exocytosis in the case of the adrenal medulla (22), or via one or more additional compartments (i.e., lymphatics) following exocytosis from postganglionic sympathetic fibers (1, 2, 10, 23). The quantitative validity of the degradation rates depends upon the validity of the procedure for estimating the MCR. One important factor is the metabolic similarity of the enzyme administered to the endogenous enzyme. On the basis of the immunological differences between bDBH and native rDBH (see above), one would not be surprised to find that the bovine enzyme is not an appropriate protein for obtaining quantitative estimates of native DBH metabolism. Indeed, its MCR was nearly 8-fold greater than that of rDBH.

While we have reason to suspect that quantitative estimates of the metabolism of rDBH purified from pheochromocytoma closely approximate the characteristics of native rDBH metabolism, the immunological and biochemical similarities between pheochromocytoma rDBH and native rDBH do not by themselves necessarily confer metabolic identity. Nevertheless, it is important to emphasize that *either* the bDBH or the pheochromocytoma rDBH MCR can provide valuable qualitative information as long as the metabolic processes involved in their clearance are shared by the native enzyme. That is, the different DBH preparations each may provide valid relative estimates of the DBH MCR if the major process (or processes) involved in their metabolism reflect a process (or processes) important in native DBH metabolism. We employed this qualitative approach previously to assess the mechanisms responsible for altered circulating DBH activity in thyroid dysfunction (7) and in streptozotocin-induced diabetes mellitus in rats (8). Estimates of the bDBH MCR relative to control were used to demonstrate the importance of altered DBH disposal pathways to observed changes in circulating endogenous DBH activity in thyroid dysfunction. The bDBH MCR was decreased in hypothroidism (where circulating DBH levels are increased), and increased in hyperthyroidism (where circulating DBH levels are reduced); moreover, the extent to which the apparent bDBH MCR was decreased in hypothyroidism reflected the extent to which endogenous circulating DBH was increased. Similarly, in streptozotocin-induced diabetes, estimates of both the bDBH and the rDBH MCR revealed that the extent to which the disposal of each enzyme preparation was decreased closely approximated the extent to which endogenous circulating DBH was increased. Thus, although quantitative estimates of the DBH MCR for either bDBH or pheochromocytoma rDBH may be different from those for native DBH (as well as being different from each other, as documented in the present study), their ability to reflect processes shared by native DBH disposal pathways allows logical but qualitative interpretation of the physiological mechanisms involved.

The validity of using quantitative estimates of the rDBH MCR obtained with rDBH preparations purified from rat pheochromocytoma can be evaluated to some extent by applying the kinetic parameters obtained in the present study to conditions where alterations in circulating DBH activity levels have been documented. The reported increase in circulating DBH activity following streptozotocin treatment is well documented; our finding of a 4- to 8-fold increase in circulating DBH activity within 4 days after streptozotocin treatment of F344 rats (8) is consistent with results obtained in an outbred rat strain by other investigators (24); preliminary results for streptozotocin-treated WKY rats in our labo-

ratory reveal an increment in circulating DBH activity of 9 units/ml by 4 days. Using the kinetic parameters ascertained in the present study, we can calculate whether a DBH_i of 9 units/ml is reasonable to expect within 4 days of streptozotocin treatment given the mechanism involves altered metabolic disposal. At a baseline plasma DBH activity of 1.5 units/ml and a mean transit time of 7.8 hr (the value estimated using rDBH purified from pheochromocytoma; Table 1), the maximal rate of rise in serum DBH activity is 0.19 unit/ml/hr.[4] Thus, a DBH_i of 9 units/ml could be achieved within 2 days without any major change in the rate of DBH entry simply by modifying peripheral disposal pathways (8). In contrast, using the mean transit time of 168 hr derived from the data of Geyer *et al.* (10), the maximal rate of rise of the plasma DBH activity is 0.0089 unit/ml/hr, and a DBH_i of 9 units/ml requires 42 days to achieve. The kinetic picture of plasma DBH metabolism presented in the present study thus is consonant with the alteration in plasma DBH activity induced by streptozotocin treatment.

A second condition in which a rapid alteration in endogenous circulating DBH activity has been observed is during an infusion with asialo-fetuin; results for F344 rats demonstrate that endogenous circulating DBH activity increases at a rate of approximately 0.8 unit/ml/hr during the initial 6 hr of infusion with asialofetuin (25). Quantitative estimates of the rDBH MCR in this strain are indistinguishable from those obtained in WKY rats in the present study (see Results). Given a baseline plasma DBH activity of about 8 units/ml in F344 rats (Results) and a mean transit time of 7.8 hr, the maximal rate of rise of plasma DBH activity will be 1.05 units/ml/hr assuming no change in the rate of DBH entry. We presume that the major effect of asialo-fetuin is to inhibit hepatic disposal of DBH (cf. ref. 16); indeed, the observed rate of rise of plasma DBH activity is consistent with this hypothesis and our kinetic parameters. In contrast, using the transit time from the data of Geyer *et al.* (10), the predicted rate of increase in DBH activity is approximately 0.0595 unit/ml/hr, a value more than 13-fold lower than observed.

The above considerations support the conclusion that the MCR of native DBH activity in the rat's circulatory compartment is considerably greater than that estimated previously and provide support for the reasonable use of employing rDBH purified from pheochromocytoma to estimate the kinetic parameters governing the metabolism of endogenous DBH.

[4] This calculation was reasoned and performed as follows. The maximal possible rate of rise of plasma DBH activity level with the rate of DBH entry held constant occurs if streptozotocin treatment completely blocks the DBH disposal pathways (i.e., disposal rate = 0). The rate of rise of plasma DBH activity under these conditions is: [(DBH entry rate)/(total DBH pool)] × [baseline plasma DBH activity]. The DBH entry rate = (DBH MCR) × (baseline plasma DBH activity), and the total DBH pool = (DBH entry rate) × (mean transit time). Accordingly, the equation for the maximal rate of rise of plasma DBH activity can be simplified to: [baseline plasma DBH activity)/(mean transit time)].

Parenthetically, it has been shown that streptozotocin treatment markedly inhibits, but does not completely block, the DBH disposal pathways (8). Thus, the rate of rise of plasma DBH activity attributable to attenuated DBH disposal must be somewhat slower that the maximal possible. Naturally, any actual modifications in the DBH entry rate are superimposed upon these effects on disposal.

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REFERENCES

1. Weinshilboum, R. M., N. B. Thoa, D. G. Johnson, I. J. Kopin, and J. Axelrod. Proportional release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves. *Science (Wash. D. C.)* **174**:1349–1351 (1971).
2. Smith, A. D., W. F. DePotter, E. J. Moerman, and A. F. DeSchaepdryver. Release of dopamine-β-hydroxylase and chromogranin A upon stimulation of the splenic nerve. *Tissue Cell* **2**:547–560 (1970).
3. Kopin, I. J., S. Kaufman, H. Viveros, D. Jacobowitz, C. R. Lake, M. G. Ziegler, W. Lovenberg, and F. K. Goodwin. Dopamine-β-hydroxylase: basic and clinical studies. *Ann. Intern. Med.* **85**:211–223 (1976).
4. Weinshilboum, R. M., H. G. Schrott, F. A. Raymond, W. H. Weidman, and L. R. Elveback. Inheritance of very low serum dopamine-β-hydroxylase. *Am. J. Hum. Genet.* **27**:573–585 (1975).
5. Stolk, J. M., J. H. Hurst, D. A. Van Riper, and P. Q. Harris. Genetic analysis of serum dopamine β-hydroxylase activity in rats. *Mol. Pharmacol.* **16**:922–931 (1979).
6. Stolk, J. M., J.H. Hurst, M. J. Friedman, P. Q. Harris, D. A. Van Riper, and B. C. Nisula. Serum dopamine-β-hydroxylase: indicator of what?, in *Enzymes and Neurotransmitters in Mental Disease* (E. Usdin, T. L. Sourkes, and M. B. H. Youdim, eds.). Wiley, New York, 171–192 (1980).
7. Stolk, J. M., J. H. Hurst, and B. C. Nisula. The inverse relationship between serum dopamine-β-hydroxylase activity and thyroid function. *J. Clin. Endocrinol. Metab.* **51**:259–264 (1980).
8. Hurst, J. H., B. C.Nisula, and J. M. Stolk. Circulating dopamine-β-hydroxylase in the rat: importance of altered disposal pathways in experimental diabetes. *J. Pharmacol. Exp. Ther.* **220**:108–114 (1982).
9. Grzanna, R., and J. T. Coyle. Immunochemical studies on the turnover of rat serum dopamine-β-hydroxylase. *Mol. Pharmacol.* **13**:956–964 (1977).
10. Geyer, S. J., S. M. Schanberg, and N. Kirshner. Turnover of dopamine-β-hydroxylase in rat blood and lymph, in *Structure and Function of Monoamine Enzymes* (E. Usdin, N. Weiner, and M. B. H. Youdim, eds.). Marcel Dekker, New York, 423–438 (1977).
11. Geyer, S. J., and S. M. Schanberg. Dopamine-β-hydroxylase: determination of half-life and appearance in lymph fluid after IV injection of ^{125}I-DBH into rats. *Life Sci.* **30**:1087–1100 (1982).
12. Weeks, J. R. Long-term intravenous infusion, in *Methods in Psychobiology* (R. D. Myers, ed.), Vol. II. Academic Press, New York, 155–168 (1975).
13. Molinoff, P. B., R. Weinshilboum, and J. Axelrod. A sensitive enzymatic assay for dopamine-β-hydroxylase. *J. Pharmacol. Exp. Ther.* **178**:425–431 (1971).
14. Wehmann, R. E., and B. C. Nisula. Metabolic clearance rates of the subunits of human chorionic gonadotropin in man. *J. Clin. Endocrinol. Metab.* **48**:753–759 (1979).
15. Shipley, R. A., and R. E. Clark, *Tracer Methods for in Vivo Kinetics.* Academic Press, New York, 77–120 (1972).
16. Regocczy, E., M. T. Debanne, M. W. C. Halton, and A. Koj. Elimination of asialofetuin and asialoorosomucoid by the intact rat. *Biochim. Biophys. Acta* **541**:372–384 (1978).
17. Achord, D. T., F. E. Brot, C. E. Bell, and W. S. Sly. Human β-glucuronidase: *in vivo* clearance and *in vitro* uptake by a glycoprotein recognition system on reticuloendothelial cells. *Cell* **15**:269–278 (1978).
18. Rush, R. A., P. E. Thomas, S. H. Kindler, and S. Udenfriend. The interaction of dopamine-β-hydroxylase with concanavalin A and its use in enzyme purification. *Biochem. Biophys. Res. Commun.* **57**:1301–1305 (1974).
19. Faden, V. B., and D. Rodbard. Operating instructions and listings for new FORTRAN IV-G program, EXPFIT, exponential data processing. National Institute of Child Health and Human Disease, National Institutes of Health, Bethesda, Md. (1975).
20. Wehmann, R. E., and B. C. Nisula. Metabolic and renal clearance rates of purified human chorionic gonadotropin. *J. Clin. Invest.* **68**:184–194 (1981).
21. Rush, R. A., and L. B. Geffen. Radioimmunoassay and clearance of circulating dopamine-beta-hydroxylase. *Circ. Res.* **31**:444–452 (1972).
22. Viveros, O. H., L. Arqueros, and N. Kirshner. Release of catecholamines and dopamine-β-hydroxylase from the adrenal medulla. *Life Sci.* **7**:609–618 (1968).
23. Aberg, H. E., H. E. Hansson, L. Wetterberg, S. B. Ross, and O. Froden. Dopamine-β-hydroxylase in human lymph. *Life Sci.* **14**:65–71 (1974).
24. Berkowitz, B. A., R. Head, T. Joh, and J. Hempstead. Experimental diabetes: alterations in circulating dopamine-β-hydroxylase and norepinephrine. *J. Pharmacol. Exp. Ther.* **213**:18–23 (1980).
25. Stolk, J. M., J.H. Hurst, and B. C. Nisula. Regulation and inheritance of dopamine-β-hydroxylase. *Behav. Genet.* **12**:37–52 (1982).

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