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Genetic Analysis of Serum Dopamine-*Beta*-Hydroxylase Activity in Rats

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SUMMARY

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The inheritance of serum dopamine-β-hydroxylase activity was studied using Mendelian crosses derived from four inbred rat strains. Serum enzyme activity is inherited as an autosomal co-dominant trait in female rats. In males, however, serum dopamine-β-hydroxylase activity is inherited as if it were an autosomal dominant trait, with low activity being dominant over high activity, indicating that sex-related modifiers of serum enzyme activity are present in rats. The contribution of sex-linked inheritance to serum dopamine-β-hydroxylase activity is suggested by the data.

INTRODUCTION

Dopamine-β-hydroxylase (EC 1.14.2.1)[2] is the enzyme responsible for converting dopamine to norepinephrine in noradrenergic cells. The presence of DBH activity in the circulatory system, first described by Weinshilboum and Axelrod (1), was thought to reflect the rate of sympathetic neuronal discharge, since DBH and several other large proteins stored in noradrenergic vesicles are discharged from the cell by exocytosis, along with the catecholamines, in response to nerve stimulation (2-4). Although initial reports appeared to substantiate a relationship between circulating DBH activity and sympathetic nervous system activity, recent investigations seriously question the intimacy of such a relationship. To wit, circulating DBH activity in rats did not parallel plasma norepinephrine concentration in response to sympathetically active drugs (5), and serum enzyme activity was not altered in response to environmental stessors causing well documented changes in sympathoadrenal function (6, Cooper and Stolk, unpublished data). Similarly, in man serum DBH activity is not significantly altered in circumstances associated with increased sympathetic nervous system function such as hypertension (7-9), anxiety (Stolk and Friedman, manuscript in preparation), and insulin-induced hypoglycemia (10). The above considerations, coupled with the finding that human serum DBH is under genetic regulation (see below), suggest strongly that the circulating enzyme activity is a stable trait variable rather than a reflective index of phasic physiologic func-physiologic function.

Weinshilboum *et al.* (11) reported that

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[2] The abbreviations used are: DBH, dopamine-β-hydroxylase; WF, Wistar/Furth; LEW, Wistar/Lewis; F344, Fischer F344; and BUF, Buffalo.

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very low serum DBH activity in humans is inherited as an autosomal recessive trait. Low enzyme activity levels subsequently were shown to correlate with low immunoreactive serum DBH protein concentrations (12). Although inheritable, the wide range of DBH activity and protein concentrations in human population (cf., 9, 11, 12) would not realistically appear to be accounted for by gross differences in enzyme protein synthesis within sympathoadrenal cells. The presence of active dimeric and polymeric forms of plasma DBH (13, 14) also is insufficient to account for the wide range of enzyme values in man, since the native tetrameric form of this enzyme (15, 16) is by far the major moiety in plasma. In an effort to determine the inherited factors regulating serum DBH activity we adopted the rat as the most appropriate experimental subject. The data presented herein document the descriptive genetics of serum DBH activity in rats.

METHODS

Parental stock of WF, LEW, F344 and BUF inbred rat strains propagated by rigid brother-sister mating were obtained from Microbiological Associates, Inc. (Walkersville, Md.). The minimum number of generations inbred at the time of delivery from the supplier was F48 for WF, F66 for LEW, F100 for F344, and F65 for BUF strains. Colonies of each inbred strain were established in our facility by brother-sister mating through at least two generations prior to hybridization. One male and three females were housed together in a large breeding cage for 2 weeks after reaching sexual maturity. Cage mates were then isolated until parturition. Litters remained intact until weaning at 28 days of age; thereafter, litter-mates of the same sex were housed in groups of from 3 to 5 until sacrifice. The serum DBH activity values reported in this paper were obtained from 72 to 84 day old rats. Serum enzyme activity, which is high in neonatal rats, stabilizes by 70 days (6) and remains constant until at least 180 days of age.

Sera were obtained from neck stump blood samples collected after sacrifice by guillotine. Samples were distributed in aliquots immediately and stored at 62°C until assayed. Serum DBH activity was determined using the coupled enzyme procedure described initially by Molinoff *et al.* (17). Briefly, sera are incubated at pH 5.0 with substrate (tyramine) during the first step of the assay; the second step in the procedure involves *N*-methylation of the DBH reaction product (octopamine) using a partially purified bovine phenylethanolamine-*N*-methyltransferase (PNMT) preparation (18) and [^{14}C]methyl S-adenosyl-L-methionine (45–57 mCi/nMol; New England Nuclear) as methyl donor. The product of the second step in the assay procedure, *N*-[^{14}C]methyl octopamine, is isolated by solvent extraction and measured by liquid scintillation spectrometry. Known amounts of DL-octopamine added to boiled sera samples served as external standards in quantifying the amount of product formed. One unit of DBH activity is defined as the formation of 1 mμmol product per hour of incubation at 37°.

Sera were diluted with three volumes of distilled water immediately prior to assay. Cupric ion is necessary to neutralize endogenous serum factors inhibitory to DBH (1, 6). Results obtained in our laboratory reveal maximal serum DBH activity over a range of [Cu^{2+}] from 25 to 60 μM (final concentration in the first step of the coupled assay). Optimal [Cu^{2+}] range was independent of rat strain, and a concentration of 36 μM was used routinely for all studies described herein.

The large number of individual subjects measured, and the time required for breeding all appropriate groups of rats, necessitated that a common group of serum samples be measured in each assay to serve as an interassay standard. Aliquots of serum obtained from 8 individual LEW males served as the comparator group over the 18 months required to obtain the study data. Data from each individual assay were standardized to the initial absolute serum DBH activity of the 8 LEW males. Statistics for this comparator group over the 12 separate assays that comprise the present data base are presented in Table 1. These data indicate the reproducibility of the assay. Comparison of the mean absolute value with the mean standardized value for each subject reveals that DBH activity tended

to decrease slightly over the 18 months samples were stored; however, loss of activity in the comparator group averaged less than 1.3%, and in no case was greater than 1.8% (animal #4; Table 1). Potential distortion due to standardizing serum DBH values to the comparator group of LEW males, therefore, was insignificant.

Statistical analyses were performed on the standardized data. Bartlett's test for homogeneity of variance (19) was used in analyses of pedigree data. This procedure can simultaneously test for normality in distributions of the serum DBH values. Significant differences between group means were assessed by two-tailed Student's *t*-tests corrected for groups of unequal variance. In view of the large number of subjects involved in the *t*-test analyses, criterion for rejection of the null hypothesis was set at the 2% level.

RESULTS

Strain and sex dependent differences in serum DBH activity

Serum DBH activity was measured in adult male and female rats of four inbred strains. Significant between-strain differences were obtained in both male and female rats (Table 2). The rank-order of serum DBH activity in the 4 strains differed for male and female subjects, however. Although the WF strain exhibited the lowest values for each sex, LEW males and the BUF and F344 females had the highest serum enzyme activity for each sex.

Within-strain comparisons revealed that males have significantly higher serum DBH activity than females in the LEW and WF strains, whereas males have significantly lower enzyme activity in the BUF and F344 strains. These sex-dependent differences have been replicated at least twice in independent studies for the LEW, WF and F344 strains (data not shown).

Breeding studies

Two complete studies (assessing F_1 and F_2 generations and backcrosses of F_1 to parental strains) and one partial study (assessing F_1 offspring only) were conducted.

TABLE 2

Serum DBH activity in four inbred rat strains

Strain	Serum DBH activity	
	Males (N)	Female (N)
	(*units/ml* ± *S.D.*)	
LEW	12.47 ± 1.17[a] (37)	11.10 ± 0.73[b] (11)
BUF	10.32 ± 0.63[c] (13)	13.07 ± 0.63[d, e] (6)
F344	9.99 ± 0.83[c] (60)	12.90 ± 1.24[d, e] (39)
WF	7.62 ± 1.00[a] (35)	6.94 ± 0.91[b, e] (30)

[a] Significantly different ($p < 0.02$) from males of all other strains.

[b] Significantly different ($p < 0.02$) from females of all other strains.

[c] Significantly different ($p < 0.02$) from LEW and WF males.

[d] Significantly different ($p < 0.02$) from LEW and WF females.

[e] Significantly different ($p < 0.02$) from males of same strain.

TABLE 1

Statistics on the 8 LEW male rats used to standardize serum DBH activity in the study population

#	Absolute Activity[a]				Standardized Activity[a]			
	Min[b]	Mean[b]	Max[b]	C.V.[b]	Min[b]	Mean[b]	Max[b]	C.V.[b]
1	11.83	12.57	13.72	0.056	12.04	12.71	13.35	0.033
2	10.70	11.82	12.73	0.071	11.50	11.77	12.34	0.024
3	10.82	12.29	14.20	0.082	11.62	12.32	13.14	0.040
4	9.91	11.10	12.01	0.070	10.67	11.30	11.85	0.032
5	12.41	13.33	14.21	0.045	12.24	13.53	14.19	0.046
6	12.33	13.36	14.30	0.053	12.69	13.48	14.45	0.039
7	10.86	11.39	12.89	0.058	10.88	11.51	12.33	0.046
8	10.16	11.47	13.34	0.109	10.67	11.62	12.72	0.059

[a] Serum DBH activity expressed as units/ml.

[b] Values are the minimum (min), mean and maximum (max) serum DBH activities measured for the indicated subject in the 12 separate assays comprising the present study. C.V. is the coefficient of variation obtained for the 12 individual serum DBH values for each subject.

The complete studies analyzed serum DBH activity in crosses of WF with F344 and LEW with F344. The partial study assessed hybrid offspring of WF and BUF strains. Data for these studies are summarized as follows: (a) WF-F344 study, male offspring (Table 3), female offspring (Table 4); (b) LEW-F344 study, male offspring only (Table 5); (c) WF-BUF study, male and female offspring (Table 6). Detailed data for females in the LEW-F344 study are not presented because the difference in serum DBH activity between strains was small and few LEW female rats were available for analysis. Portions of these data are found in Table 2 and Figure 1.

F_1 Offspring. Female F_1 offspring had serum DBH activity values intermediate to those of parental females in each of the three studies conducted (Tables 4 and 6; data for LEW-F344 study not tabulated). Individual serum DBH activity values for the four parental strains and the three hybrid populations are plotted in Figure 1 to illustrate the separation of female F_1 and parental values. Hybrid and parental groups have similar variance, indicating that each group is homogeneous with respect to serum DBH activity and suggesting that values are distributed normally in each population (group statistics for the F_1 female populations are compiled in Tables 4 and 6).

In contrast to F_1 females, serum DBH activity in hybrid male offspring tends to approximate that of the parental strain

TABLE 3

Analysis of serum DBH activity in male WF, F344, F_1, F_1 backcross and F_2 rats

Group	N	Serum DBH Activity (*units/ml ± S.D.*)		Variance	Bartlett's χ^2
F344	60	9.99 ± 0.83			
WF	35	7.62 ± 1.00[b]	(pooled)	0.925[a]	0.18[a]
F_1	35	7.83 ± 0.95[b]			
F_2	26	8.64 ± 2.14		4.562	20.55[c]
F_1:WF	20	7.81 ± 1.31		1.712	3.33[d]
F_1:F344	21	8.78 ± 1.28		1.634	3.31[d]

[a] Bartlett's test for homogeneity of variances indicates that F344, WF and F_1 populations are similar. Pooled variance for these 3 groups is indicated.

[b] Denotes a significant difference ($p < 0.02$) from serum DBH activity in F344 male rats, but not from each other. Significant differences between DBH activity in other groups are indicated in the text.

[c] Indicates that variance in this population is significantly greater ($p < 0.05$) than pooled variance of F_1 and parental strains.

[d] The difference in variance between the indicated population and pooled F_1 and parental strains approaches statistical significance ($0.05 < p < 0.10$).

TABLE 4

Analysis of serum DBH activity in female WF, F344, F_1, F_1 backcross and F_2 rats

Group	N	Serum DBH Activity (*units/ml ± S.D.*)		Variance	Bartlett's χ^2
F344	39	12.90 ± 1.24[b]			
WF	30	6.94 ± 0.91[b]	(pooled)	1.002[a]	4.33[a]
F_1	33	9.54 ± 0.73[b]			
F_2	24	9.86 ± 2.36		5.585	16.42[c]
F_1:WF	18	7.99 ± 1.75		3.069	5.75[c]
F_1:F344	17	10.91 ± 2.10		4.415	9.96[c]

[a] Bartlett's test for homogeneity of variances indicates that F344, WF and F_1 populations are similar. Pooled variance for these three groups is indicated.

[b] Denotes significant differences ($p < 0.02$) between serum DBH activity in each of the indicated populations. Significant differences between values in other groups are indicated in the text.

[c] Indicates that variance in the indicated populations is significantly greater ($p < 0.05$) than pooled variance of F_1 and parental strains.

TABLE 5

Analysis of serum DBH activity in male LEW, F344, F_1, F_1 backcross and F_2 rats

Group	N	Serum DBH Activity		Variance	Bartlett's χ^2
		(*units/ml* ± *S.D.*)			
F344	60	9.99 ± 0.83[b]			
LEW	37	12.47 ± 1.17	(pooled)	1.052[a]	3.63[a]
F_1	21	9.94 ± 1.29[b]			
F_2	45	9.97 ± 1.60		2.554	6.81[c]
F_1:LEW	18	10.85 ± 1.60		2.573	4.16[c]
F_1:F344	60	9.83 ± 1.04		1.092	——

[a] Bartlett's test for homogeneity of variances indicates that F344, LEW and F_1 populations are similar. Pooled variance for these three groups is indicated.

[b] Denotes a significant difference ($p < 0.02$) from serum DBH activity in LEW male rats, but not from each other. Significant differences between DBH activity in other groups are indicated in the text.

[c] Indicates that variance in this population is significantly greater ($p < 0.05$) than pooled variance of F_1 and parental strains.

TABLE 6

Analysis of serum DBH activity in male and female WF, BUF and F_1 rats

Sex	Group	N	Serum DBH Activity
			(*units/ml* ± *S.D.*)
Male	WF	35	7.62 ± 1.00[a]
	BUF	13	10.32 ± 0.63[a]
	F_1	37	8.27 ± 0.69[a]
Female	WF	30	6.94 ± 0.91[a]
	BUF	6	13.07 ± 0.63[a]
	F_1	33	9.54 ± 0.73[a]

[a] Mean serum DBH activity is significantly different ($p < 0.02$) between all groups of the same sex.

having the lower serum DBH activity. As illustrated in Figure 2, hybrid male subjects from WF-F344 (*cf.*, Table 3) and LEW-F344 studies (*cf.*, Table 5) are indistinguishable from inbred WF and F344 males, respectively. Similar results have been obtained for independent experiments using crosses of LEW and WF strains; F_1 male hybrids have the same serum DBH activity as WF males, and both populations have significantly lower enzyme activity than LEW males (data not shown). Only in the cross between WF and BUF rats is serum DBH activity in male F_1 subjects significantly different from males in both parental strains (Fig. 2; Table 6).

Additional experiments were conducted to examine several potential sources of the observed relative differences between serum DBH activity in male and female F_1 offspring and their parents. Copper titration curves for all groups were identical.

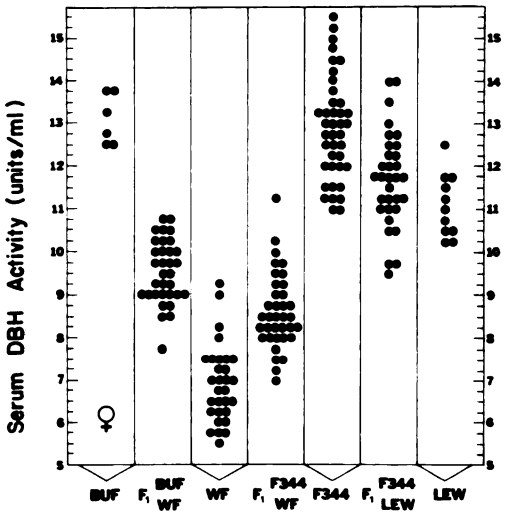

FIG. 1. *Distribution of individual serum DBH activity values in female parental strains and F_1 hybrid rats*

Values plotted are those used to compute the group statistics summarized in Tables 2, 4 and 6. The headings under panels displaying DBH values from F_1 hybrid female offspring indicate parentage.

Further, mixing equal portions of male F_1 and parental sera yielded DBH activities comparable to the arithmetic mean of the individual sera (Table 7). These results indicate that endogenous serum factors inhibitory to DBH activity are comparable in all groups and cannot account for the male-female differences in the F_1 populations.

The possibility of sex-linkage of serum DBH activity was investigated by comparing F_1 offspring of crosses between strain A females and strain B males with those be-

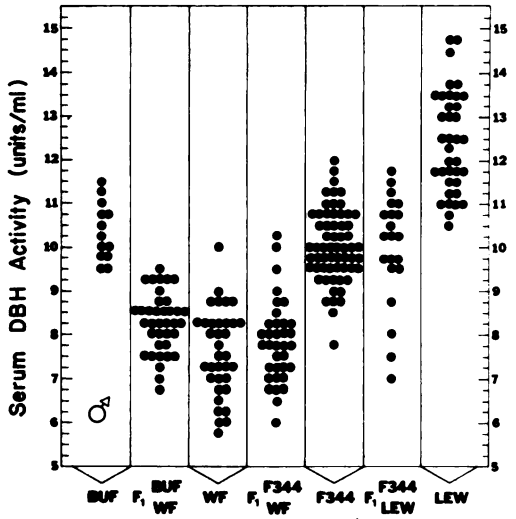

FIG. 2. *Distribution of individual serum DBH activity values in male parental strains and F_1 hybrid rats*

Values plotted are those used to compute the group statistics summarized in Tables 2, 3 and 5. The headings under panels displaying DBH values from F_1 hybrid male offspring indicate parentage.

TABLE 7

Serum DBH activity in pooled samples from WF, F344, and F_1 hybrid male offspring, and the effects of mixing pooled sera on enzyme activity

Sample[a]	Serum Enzyme Activity	
	Observed	Calculated
	(*cpm/ml*)	
WF males	49,950	——
F344 males	65,350	——
Mixed WF & F344 males	54,590	57,650
F_1 males	40,200	——
Mixed F_1 & F344 males	51,520	52,775

[a] Equal aliquots of serum from 5 individual rats of the indicated strain were pooled; mixing experiments used equal aliquots of pooled sera from the indicated single strain pools. Serum DBH activity was assayed in the usual manner (see METHODS).

tween strain B females and Strain A males (reciprocal cross breeding). As expected, serum DBH activity in female reciprocal cross hybrids was identical (data not shown). Male F_1 offspring, however, gave evidence for sex-linkage when one of the parents was a WF rat. Values from experiments where reciprocal crosses were compared directly are summarized in Table 8. Crosses between LEW and F344 strains had comparable serum DBH activity. In the two comparisons where WF rats were one of the parental strains, however, substantial differences in serum DBH activity are observed in the reciprocal cross male offspring. Hybrid male offspring from WF sires and F344 dams had lower DBH activity levels than males from F344 sires and WF dams ($p = 0.068$; Exp. B in Table 8). Hybrid males from WF sires and BUF dams had significantly lower serum DBH activity than F_1 males from the reciprocal cross (Exp. D: Table 8). Despite these differences in mean serum DBH activity, variance analysis of the pooled F_1 males reveals homogeneity comparable to that in the parental strains.

F_2 offspring. Mean serum DBH activity in male F_2 subjects is not significantly different from that in the parental male with the lower serum enzyme values (Tables 4 and 6). Population variance in F_2 males is significantly higher than that in males from pooled F_1 and parental strains. Mean serum DBH activity in the female F_2 offspring from the WF-F344 study is intermediate to that of the parent females and comparable to values in the female F_1 subjects (Table 5). Female F_2 population variance was significantly greater than that in pooled pa-

TABLE 8

Serum DBH activity in male F_1 offspring obtained by reciprocal cross breeding

Exp.	Offspring	N	Serum DBH Activity[a]
	(*Dam* × *Sire*)		(*units/ml* ± *S.D.*)
A	F344 × LEW	8	10.43 ± 0.60
	LEW × F344	6	10.26 ± 0.77
B	WF × F344	13	8.30 ± 1.17[b]
	F344 × WF	13	7.37 ± 1.29[b]
C[c]	WF × F344	6	7.91 ± 0.59[d]
	F344 × WF	9	7.39 ± 0.42[d]
D	WF × BUF	23	8.58 ± 0.58[d]
	BUF × WF	14	7.76 ± 0.52[d]

[a] All values tabulated represent raw data on serum DBH, and not converted data. Except where indicated, the data for experiments contained in this table are included in major breeding studies presented in Tables 2, 4 and 5.

[b] $p = 0.068$ for difference between groups mean.

[c] Independent experiment not included in the primary data presented elsewhere in this study.

[d] Group means are significantly different ($p < 0.02$).

rental and F_1 populations (Table 5). Similar results were obtained for female F_2 progeny in the LEW-F344 study (data not shown).

Backcross of F_1 offspring to parental strains. In accord with previously discussed data (Fig. 2; Tables 3, 5, and 6), data compiled for F_1 male hybrids predicts that crossing the latter subjects with the parental strain having the lower serum DBH activity would yield a population whose mean enzyme activity and variance were indistinguishable from F_1 and appropriate parent males. This prediction was substantiated in both the F_1 backcross to WF (F344-WF study; Table 3) and the F_1 backcross to F344 (LEW-F344 study; Table 5). Backcrossing the F_1 to parental strain having the higher serum DBH activity yielded offspring whose mean enzyme values were intermediate to the F_1 and parental males (Tables 3 and 5). Variance in the F_1 backcross to LEW was significantly different from that in pooled F_1, LEW and F344 males (Table 5). Mean serum DBH activity in the backcross populations was significantly different in both studies.

Female offspring from the F_1 backcross to parental strains in the WF-F344 study yielded populations with serum DBH values intermediate to those of the F_1 and the respective parental strain (Table 4). Variance in the female backcross offspring was significantly greater than that in pooled F_1 and parental females. Mean serum DBH activity in the 2 backcross populations was significantly different (Table 4).

DISCUSSION

The genetic determination of serum DBH activity in rats is complex. Results obtained in the present study suggest that circulating enzyme activity is determined at a single gene locus in females, while in males either multiple loci are involved or DBH activity is modified by (an) independent factor(s). Data consistent with the latter alternatives regarding serum DBH activity in males include: (a) possible sex-linkage of enzyme activity, and (b) qualitative differences in activity between male and female rats in a given pedigree. Data obtained in female rats will be discussed first.

Inheritance of serum DBH activity in female rats. Serum DBH activity in female offspring appears to be inherited as an autosomal co-dominant trait; the data are compatible with monogenic inheritance (although they do not preclude segregation at 2 major loci). Results supporting these conclusions are as follows. First, enzyme activity in F_1 hybrid females is intermediate to that in the appropriate parent strains (Fig. 1). Group variance in hybrid and parental strains is comparable (Tables 4 and 6), indicating that each of these groups of offspring derive from populations of comparable homogeneity. Second, F_1 and F_2 populations have similar serum activity, but variance estimates in the F_2 group are significantly greater than those in pooled F_1 and parental groups (Table 4); these differences in variance would be predicted if serum DBH activity is transmitted at a single gene locus. The data summarized in Table 4 have been reanalyzed in Table 9 using both autosomal co-dominant and autosomal dominant models of inheritance; analysis for both models assumes inheritance at a single gene locus. The model for autosomal dominant inheritance is not predictive of the observed serum DBH activity distribution in female F_2 and F_1 backcross offspring in the WF/F344 study; the model assuming autosomal co-dominant inheritance at a single locus, however, closely approximates the observed distribution.

Inheritance of serum DBH activity in male rats. Serum DBH activity in male rats appears to be inherited as an autosomal trait, with low activity being dominant to high. However, independent factors also appear to exist, one of which may involve sex-linkage at least in pedigrees in which WF rats are one of the parental strains.

Data suggesting that low serum DBH activity is inherited like an autosomal dominant trait are best defined in the LEW-F344 study (Table 5), where no evidence for sex-linkage was found in the F_1 generation (Table 8). Serum enzyme activity in F_1 rats is indistinguishable from that in F344 males. Variance in the F_1, LEW and F344 populations is homogeneous, indicating that distribution of values in these three populations is similar and normal. Data obtained in the F_2 generation demonstrate

TABLE 9

Analysis of serum DBH activity data from WF/F344 female offspring using alternative models for autosomal codominant and autosomal dominant inheritance at a single locus

Phenotype[a]	Expected: Co-Dominant	Observed	Expected: Dominant
A. F_2 (N = 24)			
1. WF	6	6	18 (16.1)[b]
2. F_1	12	11	0 (2.5)[b]
3. F344	6	7	6 (5.4)[b]
χ^2	0.250	(35.170)[b]*	
B. Backcrosses (N = 35)			
1. WF	9	13	17.5 (15.7)[b]
2. F_1	17.5	16	0 (3.6)[b]
3. F344	8.5	6	17.5 (15.7)[b]
χ^2	2.642	(46.169)[b]*	
C. Combined Backcrosses & F_2			
χ^2	1.434	(79.268)[b]*	

[a] Phenotypes was assigned as follows (cf. Table 4 for data): (1) the arithmetic mean between WF females and F_1 females (value = 8.24 units/ml) and between F_1 females and F344 females (value = 11.22 units/ml) were calculated; (2) limits for serum DBH activity in each phenotype were established from these arithmetic means (WF: <8.24 units/ml; F_1: 8.25–11.22 units/ml; F344: <11.23 units/ml). Measured serum DBH activity in individual rats from the indicated generations was used to assign phenotype. The use of this procedure for assigning phenotype depends upon normal distribution of serum DBH activity in F344, WF, and F_1 female offspring. Bartlett's test for homogeneity of variance suggests similar variance, and thus, normal distribution in each of these populations (Table 4).

[b] An artificial expected distribution of phenotypes for the autosomal dominant model was constructed to allow computation of the χ^2 statistic. Since the derived limits of the F_1 phenotype overlaps at least the upper and lower 10% of the measured WF and F344 populations, respectively, subjects from both expected parental phenotype groups were moved in the F_1 phenotype cell (values in parenthesis). While the resultant χ^2 statistic is, thus, an approximation, the same conclusions would be reached if the overlap with each parental strain was as high as 35%. The statistics for the WF and F344 groups (Table 4) reveal that the extremes ranges of the derived F_1 phenotype do not come within 1.4 S.D. of the mean for either parental strain, lending credance to the conclusions made from the χ^2 analysis.

* $p < 0.05$ (two-tailed).

that population variance is significantly different from that in the pooled parental and F_1 subjects (Table 5). The distribution of measured serum DBH activity values in individual F_2 offspring closely approximates that predicted by a model assuming autosomal dominant inheritance (Table 10). The same model accurately predicts the distribution of activities in the F_1 backcross populations. We conclude, therefore, that in male rats serum DBH activity is inherited as an autosomal dominant trait, with low activity being dominant to high activity; the data are closely predicted by a model assuming inheritance at a single locus.

Sex-linked modification of serum DBH activity is suggested by data in the F_1 generations obtained by reciprocal cross breeding where WF rats are one of the parental strains (Table 8). Hybrid males derived from a WF dam have consistently higher serum DBH activity than do those from a WF sire. These results suggest that the presence of an X chromosome from the WF strain modifies the apparent pattern of DBH activity inheritance to a co-dominant mode of inheritance in male rats. Analysis of such inheritance beyond the F_1 generation will require systematic breeding of reciprocal cross hybrids as well as the presence of an independent genetic marker that does not show sex-linkage.

The presence of sex-related (as opposed to sex-linked) modifiers is indicated by qualitative differences in serum DBH activity between male and female rats of the parental strains (Table 2). Females have

TABLE 10

Analysis of serum DBH activity data from LEW/F344 male offspring using a model of autosomal dominant inheritance at a single locus

Phenotype[a]	Expected	Observed
A. F_2 (N = 45)		
1. LEW	11.25	10
2. F344	33.75	35
χ^2	0.185	
B. Backcrosses (N = 78)		
1. LEW	9	10
2. F344	69	68
χ^2	0.126	
C. Combined Backcrosses + F_2 χ^2	0.004	

[a] Phenotype was assigned after defining the limit between LEW and F344 phenotypes as the arithmetic mean of the mean serum DBH activities in each population. The limits used to assign phenotype were: LEW, <11.22 units/ml; F344, >11.23 units/ml (cf. Table 5 for data used to calculate limits). The use of this procedure for assigning phenotype depends upon normal distribution of serum DBH activity in F344, LEW and F_1 male offspring. Bartlett's test for homogeneity of variance suggests similar variance and, thus, normal distribution in each of these populations (Table 5).

higher serum enzyme activity than males in F344 and BUF strains, while the converse applied in LEW and WF strains. Systematic evaluation of these modifiers in the breeding studies is complicated by the presence of the inherited factors discussed above. Thus, although autosomal dominant inheritance can closely predict serum DBH activity in males (Table 10), we suggest that either sex-related, or sex-linked, factors are present and modify the autosomal co-dominant inheritance pattern observed in females.

General Comments. Data obtained in the present study were based upon measures of DBH activity, not upon enzyme protein concentration. This limitation was necessitated by the lack of specific immunologic reagents for measuring rat DBH protein concentration. Grzanna and Coyle (20) documented that rat DBH protein reacts poorly, if at all, with heterologous anti-DBH antisera. Experiments conducted in our laboratories using anti-bovine DBH antiserum confirm the complete absence of cross-reactivity with rat protein (unpublished observations). Use of activity values to study the inheritance of serum DBH in humans (11) was subsequently validated by immunochemical measures of protein concentration (12). Whether rats display the same concordance between DBH activity and protein concentration as man must await future experimentation.

Serum DBH activity is an inherited trait in both rats and man, although the pattern of inheritance in these 2 species is different; such differences caution against using the rat as an explicit model for understanding the genetic determinants of human serum DBH metabolism. The general metabolic factors that determine circulating enzyme activity, however, probably are comparable in both species. At a minimum, these metabolic factors include: (1) the steady-state level of DBH in sympathoadrenal cells, (2) the rate of DBH release from cells during the exocytotic process, (3) the accessibility of released enzyme to the circulatory compartment and (4) the rate of serum DBH inactivation. Ample evidence supports the primary role of inheritance in regulating steady-state levels of cellular DBH activity in mice (cf. 21) and rats (Stolk *et al.*, manuscript in preparation). While important, however, cellular DBH activity alone does not account for inherited differences in serum DBH activity in rats; for example, two strains with similar adrenal DBH activity (i.e., WF and F344 females) reveal significant differences in serum enzyme activity (cf. Table 2). The situation in man, if one assumes that cellular DBH levels also are genetically determined in this species, is even more extreme than that in rats, since human serum DBH activity varies over at least a 1000-fold range (cf. 11). Available evidence suggests that the rate of serum DBH degradation in rats is relatively slow (22, 23) in comparison to that in sympathoadrenal cells (cf. 24, 25), thus offering another primary site for regulating circulating enzyme activity. Based upon considerations surrounding the failure of inherited differences in cellular DBH activity to account for serum enzyme activity levels (see above), our working hypothesis is that mechanisms responsible for degrading the serum enzyme are primary in the genetic determination of serum DBH activity in rats.

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