Genetic Control of α -Glycerolphosphate Dehydrogenase in Mouse Brain

(age/autosomal/cerebellum/heat denaturation)

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ABSTRACT Activity of α -glycerolphosphate dehydrogenase (EC 1.1.1.8), linked to cytoplasmic nicotinamide adenine dinucleotide, is higher in brains of BALB/cJ mice than in brains of C57BL/6J mice. The magnitude of the difference is dependent on both the age and brain region of the mice. Maximal differences are observed at 20 days of age and are detected in the cerebellum, with only marginal differences occurring in other brain regions. Kidney and liver of BALB/cJ mice have higher enzyme activity than those of C57BL/6J mice, whereas muscle-enzyme activity is lower in BALB/cJ mice than in C57BL/6J mice.

In addition to differences in activity, α -glycerolphosphate dehydrogenase in BALB/cJ mice is more sensitive to heat denaturation. The half-life for the enzyme at 50° in BALB/cJ, (BALB/cJ $\times$ C57BL/6J) F_1 hybrid, and C57BL/6J mice is 1, 1.7, and 3 min, respectively. Genetic analysis of the mode of inheritance of the heat-sensitive enzyme reveals that the trait is determined by a single autosomal gene. Genetic analysis of the activity of α -glycerolphosphate dehydrogenase in the cerebellum indicates that the mechanism of inheritance for this trait involves two or more genes and that one of these genes also confers heat sensitivity on the enzyme. The affect of these genes on enzyme activity is codominant and additive.

α -Glycerolphosphate dehydrogenase (EC 1.1.1.8; GPDH) linked to cytoplasmic nicotinamide adenine dinucleotide catalyzes the interconversion of α -glycerolphosphate and dihydroxyacetone phosphate (1). In the central nervous system, the specific activity of GPDH increases during the period of accelerated brain growth and maturation (2). A similar increase in specific activity occurs for several brain enzymes linked to energy metabolism, myelin synthesis, and neurotransmitter metabolism (3). Some enzymes develop at different times in different regions of the brain (4, 5). Cellular mechanisms that initiate and control the development of individual enzymes are undoubtedly varied and complex in nature, and it is unknown at the moment whether the enzymes of a pathway are developed in coordination. It would, therefore, be advantageous if selected enzymes could be found that respond to environmental stimuli, such as changes in circulating hormone concentrations, and could also be studied by tissue-culture methods so that the experimental approach to the study of development could be simplified.

Several interesting observations have been made on the responsiveness of GPDH to hormonal stimulation both *in vivo* and *in vitro*. Adrenalectomy and hypophysectomy of rats result in a decline in GPDH activity in the cerebellum and brainstem that can be partially restored by injections of cortisol (6). Cortisol also effects the activity of GPDH in cultures of

glial cells but not the activity in cultures from other tissue sources (7, 8). These results suggest that the regulation of GPDH by cortisol is specific for neural tissue.

It is evident that GPDH represents a valuable system for investigation of mechanisms controlling enzyme development in the brain. In this report, we present evidence that the activity of GPDH in the cerebellum of inbred mice is controlled by at least 2 loci. One locus, *Gdc-1*, has been identified as the structural locus, while the function of the other loci is unknown.

MATERIALS AND METHODS

BALB/cJ and C57BL/6J mice, the F_1 hybrid, and the reciprocal backcross generations were produced in the research colonies at The Jackson Laboratory. The age of mice was determined by both the conceptual date, as determined by the presence of a vaginal plug, and the birth date.

Preparation of Tissue Extracts. Total enzyme activity was measured in a tissue that was homogenized in 9 volumes of 0.25 M sucrose-0.1 mM EDTA for 2 min in a Teflon-glass, Potter-Elvehjem-type homogenizer. For heat denaturation studies, tissues were homogenized for 2 min in 9 volumes of 50 mM Tris-HCl (pH 7.5). The homogenate was centrifuged at $100,000 \times g$ for 1 hr; the supernatant solution was the source of enzyme.

Protein was determined by the method of Lowry *et al.* (9).

GPDH was Assayed at pH 7.5 in a 1.0-ml reaction mixture containing the following components in μ mol: triethanolamine-HCl, 50; EDTA, 5; NADH, 0.15, and dihydroxyacetone phosphate, 0.8. The reaction rates at saturating-substrate concentrations were linear with respect to time and enzyme concentration. NADH oxidation in the absence of dihydroxyacetone phosphate was negligible, except in mice younger than 15 days of age, when the oxidation due to GPDH was low. In all cases, the reported values are corrected for oxidation of NADH in the absence of dihydroxyacetone phosphate.

RESULTS

Development of GPDH

The development of GPDH in three brain regions of C57BL/6J, BALB/cJ, and (C57BL/6J $\times$ BALB/cJ) F_1 mice is illustrated in Fig. 1. A clear difference between C57BL/6J and BALB/cJ mice is observed in the cerebellum, whereas smaller and less consistent differences are found in the brainstem and cerebral cortex. GPDH activity in the cerebellum of the F_1 hybrid is intermediate, compared to the parental strains (Fig.

Abbreviation: GPDH, glycerolphosphate dehydrogenase.

1B); this suggests that the activity of GPDH in the cerebellum is genetic in origin and codominant in expression. Because of the smaller differences in enzyme activity in the two other brain regions, it was not possible to observe codominance in the F_1 hybrid generation (Fig. 1A and C), or alternatively no genetic differences exist in these regions.

Distribution of GPDH in extraneural tissues of BALB/cJ and C57BL/6J mice

Examination of GPDH activity of several extraneural tissues does not produce a consistent pattern (Table 1). The kidney and, to a lesser degree, the liver were like the cerebellum in that the GPDH activity was higher in BALB/cJ mice than in C57BL/6J mice. On the other hand, the GPDH activity in muscle was higher in C57BL/6J mice than in BALB/cJ mice. The results in Table 1 are expressed as units of activity per ml of supernatant solution; however no difference in protein concentration between strains for any tissue was observed.

Heat denaturation of GPDH

The nonuniform variation in tissue GPDH concentration initially suggested that the genetic effect was not uniform. In

FIG. 1. Patterns of GPDH development in three brain regions of mice. Each point and its vertical bar represents the mean and standard deviation of values obtained from three to seven mice. Assays were run at 37° and specific activity equals μmol of NADH oxidized per min per g of tissue (wet weight). For each brain region data is presented for BALB/cJ (●), (BALB/cJ x C57BL/6J) F_1 hybrid (□), and C57BL/6J (○) mice.

FIG. 2. Heat denaturation of GPDH. Kidneys from eight mice of each genotype were removed and pooled to obtain a $100,000 \times g$ fraction as described in the text. Aliquots of 0.1 ml were removed and heat-denatured at 50° for the indicated times.

order to obtain some evidence as to whether a structural mutation in one of the strains of mice was responsible for the variation in activity, $100,000 \times g$ supernatant solutions from cerebellum, cerebral cortex, kidney, muscle, and liver of BALB/cJ, C57BL/6J, and the F_1 hybrid were subjected to heat denaturation at 50°. Fig. 2 illustrates the data from kidney, which is also representative of the results from other tissues. A remarkable difference in heat sensitivity is observed. The half-lives for GPDH in BALB/cJ, F_1 hybrid, and C57BL/6J are about 1, 1.7, and 3 min, respectively. The non-linear shape of the decay of GPDH in the F_1 hybrid suggests that it is composed of molecules with different half-lives, probably due to a random combination of polypeptide chains derived from the C57BL/6J and BALB/cJ alleles. This would be expected if GPDH is a dimer in mice as it is in other species (10).

The structural mutation that occurred in BALB/cJ mice to change the sensitivity of GPDH to heat denaturation did not correspondingly change its electrophoretic mobility, as observed when GPDH was analyzed by polyacrylamide gel electrophoresis at pH values of 7 and 9.

TABLE 1. Distribution of GPDH in tissues of 20-day-old mice

	Activity*	
	BALB/cJ	C57BL/6J
Liver	0.654 ± 0.055	$0.532 \pm 0.063^\dagger$
Kidney	0.842 ± 0.053	0.481 ± 0.045
Muscle	0.934 ± 0.033	1.024 ± 0.095

* Activity is μmol of NADH oxidized per min per mg of protein at room temperature (26°).

† Differences between strains were significant at the 1% level for liver and kidney and at the 5% level for muscle. Eight mice were assayed to obtain each value.

FIG. 3. Frequency distribution of GPDH in the cerebellum in parental strains, B6CF₁, and in the progeny of the backcross generation.

The frequency of occurrence of a heat-sensitive GPDH in selected inbred strains of mice

In addition to BALB/cJ and C57BL/6J, 13 other strains of mice were analyzed for sensitivity of the enzyme to heat denaturation and activity in the cerebellum (Table 2). Only CBA/J strain of mice possessed a heat-sensitive form of GPDH. CBA/J mice also had the same high activity of GPDH in the cerebellum as did BALB/cJ mice; however, no correlation between GPDH activity in the kidney and its sensitivity to heat denaturation was observed.

The infrequent occurrence of the heat-sensitive form of the enzyme indicates that the gene coding for GPDH probably mutated in BALB/c or its ancestors sometime before the year 1918 when BALB/c and DBA mice were crossed to derive, among others, the CBA strain (11).

Genetic analysis

Enzyme Activity in the Cerebellum. Differences in activity of GPDH in cerebellums of (BALB/cJ x C57BL/6J)_F₁ hybrid and BALB/cJ parental strain permitted an analysis of the backcross generation. A frequency distribution for the activity of the enzyme in the backcross generation is presented in Fig. 3. A large proportion of mice possessed a GPDH activity that is intermediate between that observed in _F₁ hybrid and BALB/cJ mice, which indicates that more than one gene determines the activity of the enzyme in the cerebellum.

Sensitivity to Heat Denaturation. It was stated earlier that cytoplasmic GPDH in BALB/cJ mice was more sensitive to heat denaturation than the enzyme from C57BL/6J mice. This observation permitted us to determine whether the

FIG. 4. Relationship between GPDH activity in the cerebellum and the sensitivity of GPDH to heat denaturation in BALB/cJ x (BALB/cJ x C57BL/6J)_F₁ (O) and in C57BL/6J x (BALB/cJ x C57BL/6J)_F₁ (●) backcross generations. Specific activity is the same as defined in Fig. 1. The correlation coefficient r is -0.787 ($P < 0.001$).

structural gene was one of the genes involved in determining the difference in enzyme activity between the two strains of mice. GPDH from the kidneys of BALB/cJ x F₁ and C57BL/6J x F₁ mice as well as their reciprocal backcross generations were analyzed with respect to their heat-denaturation characteristics. For a single allelic pair in a segregating backcross generation, the two phenotypes would theoretically appear in a 1:1 proportion. In the C57BL/6J x F₁ backcross, 44 determinations were made: 21 mice had C57BL/6J-type heat denaturation and 23 mice had the F₁ hybrid-type. When tested for inheritance by a single allelic pair, χ^2 was 0.091 ($0.8 > P > 0.7$). In the BALB/cJ x F₁ backcross, 110 determinations were made: 65 mice had the BALB/cJ phenotype and 45 mice had the F₁ hybrid phenotype. χ^2 was 3.6 ($0.1 > P > 0.05$) for a single allelic pair. The results considered together are consistent with the hypothesis that heat-denaturation characteristics are determined by a single pair of alleles.

A Correlation between GPDH Activity in the Cerebellum and Its Sensitivity to Heat Denaturation. During the course of analyzing enzyme activities in the cerebellum, it became apparent that a correlation existed between enzyme activity in the cerebellum and its sensitivity to heat denaturation. These data are presented in Fig. 4. The correlation coefficient, r , is -0.787 ($P < 0.001$, $df = 123$). Mice in group A (Fig. 4) are homozygous for the BALB/cJ structural allele, and in groups B and C are heterozygous and homozygous, respectively, for the C57BL/6J allele at the structural locus. However, at any other locus or loci, mice will be heterozygous and homozygous for the BALB/cJ alleles in group A, and heterozygous and homozygous for the C57BL/6J alleles in group C. At loci other than the structural locus, mice in group B designated by the *open circles* will have the genotype of mice in group A, and those designated by *closed circles* will have the genotype of mice in group C. The progressive increase of enzyme activity in the cerebellum reflects the number of BALB/cJ alleles present at these loci.

DISCUSSION

The evidence presented in this paper indicates that genetic differences exist in the regulation of GPDH in the cerebellums of inbred strains of mice. Genetic analysis demonstrates that the trait is under polygenic control; however, the fact that GPDH from BALB/cJ mice is more heat-sensitive than GPDH from C57BL/6J mice has enabled us to determine that the structural locus is one of the loci that control the enzyme activity in the cerebellum. We propose that the symbols *Gdc-1^b* and *Gdc-1^c* representing cytoplasmic GPDH with C57BL/6J- and BALB/cJ-type heat-denaturation characteristics, respectively, be used for the alleles at the structural locus.

GPDH has been purified and its structure has been studied in rabbit muscle (12), in rat muscle (13) and liver (14), in honey bees (15), and in chicken liver and breast muscle (16). The structure of GPDH emerging from these studies shows that the enzyme has a molecular weight between 60,000 and 70,000 and is composed of two subunits. Genetic studies on GPDH from *Drosophila* show that the two subunits are identical in structure (10). A similar conclusion that GPDH in mice is composed of two identical subunits can also be derived from the results of heat denaturation studies on GPDH from the two parental inbred strains of mice and their F₁ hybrid.

TABLE 2. Heat denaturation and cerebellar activity of GPDH in several inbred strains of mice

Strain*	N	% Activity remaining after 4 min at 50°		Cerebellar activity	
		Mean	SD	Mean	SD
A/J	4	35.27	2.58	19.97	0.85
AKR/J	4	31.94	2.56	19.33	0.92
BALB/cJ	4	5.90	1.77	27.58	2.60
BUB/BnJ	4	34.97	2.90	21.14	1.96
CBA/J	4	3.97	1.50	29.50	0.84
C3HeB/FeJ	4	33.90	0.79	20.61	0.81
C3H/HeJ	4	35.32	0.98	18.81	1.24
C57BL/KsJ	4	45.84	2.05	23.31	1.00
C57BL/6J	4	37.70	1.53	15.12	0.83
C57Br/cdJ	4	41.27	0.87	22.02	1.26
DBA/2J	3	38.73	2.39	26.79	0.46
LP/J	4	48.87	2.64	26.52	0.56
RF/J	3	49.26	2.31	21.38	3.26
SJL/J	4	47.00	1.43	18.40	1.11
SWR/J	4	34.90	0.77	22.54	2.25

* Mice were females, 35 ± 3 days of age.

The enzyme from both parental strains exhibits a linear rate of heat denaturation indicating that one molecular species is disappearing. On the other hand, when GPDH from the F₁ hybrid mice is heat denatured, a nonlinear curve is obtained indicating the presence of more than one molecular species. It is probable that the decay curve in F₁ hybrid mice is the resultant of decay curves of three molecular species that arise by the random combination of polypeptides from the *Gdc-1^b* and *Gdc-1^c* alleles. This is analogous to the generation of 3 electrophoretic forms, in a 1:2:1 ratio, in F₁ hybrid offspring from *Drosophila* produced by matings of parents with electrophoretic variant forms of the enzyme. The heat-denaturation data and the genetic data that heat-denaturation of the enzyme is determined by a single pair of alleles can most simply be interpreted by postulating that GPDH in inbred mice is composed of two identical subunits both of which are coded for by the *Gdc-1* locus. Definitive proof for this postulate must come however from detailed structural studies of the purified enzyme from BALB/cJ and C57BL/6J mice.

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