Genetic Variation in Activity of Enzymes Involved in Synthesis of Catecholamines
(mice/phenylethanolamine N-methyltransferase/tyrosine hydroxylase)

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ABSTRACT A genetic analysis of differences in adrenal phenylethanolamine N-methyltransferase (EC 2.1.1.X) and tyrosine hydroxylase (EC 1.99.1.X.) and brain tyrosine hydroxylase was performed in three inbred mouse strains. In adrenal glands, the pattern of inheritance of both enzymes was similar; genes carried by the CBA/J strain appeared to be dominant to those of the other strains. The pattern of inheritance of tyrosine hydroxylase activity in adrenals differed from that in the brain. In both tissues, dominant genes appeared to determine intermediate, rather than extreme, levels of enzyme activities.

Several gene variants affecting the synthesis of the adrenocortical (1) and thyroid (2) hormones have been identified, and their mode of inheritance has been elucidated. Inherited variations in the enzymes involved in the synthesis of the catecholamines, however, are not known. The extent of enzyme polymorphism reported in animal and human populations (3), the important roles played by the catecholamines in the production and maintenance of normal and, possibly, psychopathological behavior (4), and the growing evidence for the involvement of genetic factors in the etiology of human psychopathology (5) all suggest that a search for genetic variation in the enzymes involved in catecholamine synthesis would be worthwhile.

The two enzymes we investigated were phenylethanolamine N-methyltransferase (EC 2.1.1.X) and tyrosine hydroxylase (EC 1.99.1.X). Phenylethanolamine N-methyltransferase is the enzyme that converts norepinephrine to epinephrine, the principal hormone secreted by the adrenal medulla. Norepinephrine functions as a neurotransmitter in the adrenergic nervous system and as a neuroregulatory agent in brain. Tyrosine hydroxylase is the enzyme involved in the first and rate-limiting step in catecholamine biosynthesis, converting tyrosine to dihydroxyphenylalanine. The availability of multiple inbred mouse strains made possible the initial screening needed for detection of genetic variation in these enzymes. Marked strain differences in these two enzymes were found (6). In the present study, these differences are shown to have a genetic basis. We present results of a preliminary genetic analysis of differences between adrenal phenylethanolamine N-methyltransferase and tyrosine hydroxylase and brain tyrosine hydroxylase in three strains of mice.

MATERIALS AND METHODS

Animals. All animals were descendents of mouse strains purchased either from the Jackson Laboratory (BALB/cJ, CBA/J) or from the Department of Radiobiology, Stanford University School of Medicine (C57BL/Ka).

Two female mice were housed together with one male under standard conditions and were provided with free access to food and water. Before parturition, males were removed from the cage and the females were housed individually. After parturition, the cages were left undisturbed until the pups were weaned at about 25-28 days of age, at which time males were housed in groups of from four to eight animals per cage. Cages were changed once weekly thereafter. At the time of the study, all mice were from 7 to 9 weeks of age.

Assay. Animals were killed by decapitation; adrenals and brains were removed and frozen immediately on Dry Ice. The glands were homogenized in 0.3 ml of ice-cold 0.32 M sucrose. Homogenates for assay of phenylethanolamine N-methyltransferase activity were centrifuged at 30,000 × g for 30 min, and 100-μl portions were assayed. The activity was measured by the method of Axelrod (7); phenylethanolamine was used as substrate and S-[methyl-14C]adenosylmethionine was used as methyl donor. The small amount of radioactive methanol formed enzymatically as an interfering product was removed by the procedure of Deguchi and Bar- chas (8). Samples in which the substrate was not included were used as blanks. Tyrosine hydroxylase activity was measured by the method described by Levitt (personal communication). The sucrose homogenate was centrifuged at 10,000 × g for 20 min and a 50-μl portion of the supernatant was assayed with [14C]tyrosine as the substrate. Boiled enzyme was used in the blanks; the radioactivity in these blanks was slightly less than in blanks in which enzyme was not included. For determination of tyrosine hydroxylase activity, incubation with the following reagents was performed (all concentrations are in micromoles): Potassium phosphate buffer (pH 6), 100; 2-mercaptoethanol, 100; NSD-1055 (4-bromo, 3-hydroxybenzoxoxymine. Brocresine, a generous gift of Lederle Laboratories, New York) 0.1; 2-amino 4-hydroxy-6,7-dimethyltetrahydropteridine, 1.0; and L-[14C] tyrosine, 0.3 μCi/mm (New England Nuclear Corp.). Samples were incubated at 37° for 15 min, after which 0.1 ml of 25% trichloroacetic acid was added. Samples were transferred to tubes containing 50 μg of L-(DOPA) dihydroxyphenylalanine, and centrifuged at 17,000 × g for 10 min. To the resulting supernatant, 5 ml of 0.2 M sodium acetate, 0.2 ml of 0.2 M ethylenediamine tetaacetate (EDTA), and 0.3 g of alumina were added. The sample was titrated to pH 8.6 with 1 N NaOH, stirred for 3 min, and poured as a slurry over a column packed with glass wool. The column was washed with 30 ml of water, and the L-[14C]dihydroxyphenylalanine was eluted with 6 ml of 0.3 M acetic acid.
Brains were homogenized in four volumes of ice-cold sucrose in a Polytron (Brinkman Instrument Co.) generator, a unit that also acts as a low energy sonifier. Of the measurable tyrosine hydroxylase in brain, 96% was liberated into the supernatant. Samples were centrifuged as described above and 0.5-ml portions of the supernatant were assayed.

**Genetic Analysis.** Since in our initial study (6) maximum differences were not detected between any two strains in all assays of enzyme, a diallel analysis of three inbred strains and their hybrids was used for evaluation of the genetic contributions to interstrain enzyme differences. The diallel method consists of the generation of all possible matings between n number of strains; for n strains there are n² matings (9). The diallel method assesses the kinds and magnitudes of the variation contributing to strain differences without the necessity of breeding F₂ and backcross generations.

Analysis of variance of the scores of the parents and their hybrids yields estimates of the genotypic and environmental components of variance. The genotypic variance is partitioned further into two orthogonal components representing the general combining ability and specific combining ability, respectively. The general combining ability represents the average performance of a given strain in all hybrid combinations and gives an estimate of additive gene effects. The specific combining ability provides an estimate of gene effects due to dominance and epistasis (nonallelic interactions), and reflects the departure of hybrid scores from the values one might have expected on the basis of the parental scores. The differences arising as a consequence of A female x B male, as opposed to B female x A male, matings are reflected as reciprocal effects. The presence of significant reciprocal effects is generally due to pre- and/or postnatal differences in maternal environment or X-linkage. Measurements made on eight mice of each inbred strain and of each F₁ hybrid were subjected to analysis.

**RESULTS**

Table 1 shows the results of the diallel analyses for adrenal phenylethanolamine N-methyltransferase and tyrosine hydroxylase and for brain tyrosine hydroxylase. For each character, the general and specific combining abilities were highly significant, indicating that genetic factors play an important role in determination of the differences between the strains studied. Since all reciprocal effects were not significant, pre- and postnatal biological and cultural maternal effects and also X-linkage could be eliminated as important determinants of the observed differences.

Table 2 shows the mean [± standard error of the mean (SE)] activities of the enzymes in the three parental strains of mice and their hybrids. Since reciprocal hybrid combinations were not significantly different (Table 1), they were pooled for the purposes of tabular presentation. BALB/cJ had the highest and C57BL/Ka the lowest activities for both adrenal enzymes. Table 2 shows the pattern of inheritance of both adrenal enzymes to be similar; BALB/cJ x CBA/J and C57BL/Ka x BALB/cJ F₁ hybrids resemble CBA/J and C57BL/Ka, respectively. Thus, the pertinent gene or genes

<table>
<thead>
<tr>
<th>Strains:</th>
<th>n</th>
<th>Phenylethanolamine</th>
<th>Tyrosine hydroxylase†</th>
<th>Tyrosine hydroxylase‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/cJ</td>
<td>8</td>
<td>0.198 ± 0.015</td>
<td>5.28 ± 0.81</td>
<td>14.6 ± 1.14</td>
</tr>
<tr>
<td>CBA/J</td>
<td>8</td>
<td>0.124 ± 0.007</td>
<td>1.99 ± 0.14</td>
<td>9.4 ± 0.66</td>
</tr>
<tr>
<td>C57BL/Ka</td>
<td>8</td>
<td>0.085 ± 0.008</td>
<td>1.40 ± 0.09</td>
<td>11.6 ± 0.99</td>
</tr>
<tr>
<td>F₁ mice:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/cJ x CBA/J</td>
<td>16</td>
<td>0.119 ± 0.008</td>
<td>2.24 ± 0.10</td>
<td>12.5 ± 0.66</td>
</tr>
<tr>
<td>CBA/J x C57BL/Ka</td>
<td>16</td>
<td>0.143 ± 0.010</td>
<td>1.82 ± 0.16</td>
<td>11.7 ± 0.51</td>
</tr>
<tr>
<td>C57BL/Ka x BALB/cJ</td>
<td>16</td>
<td>0.118 ± 0.006</td>
<td>2.17 ± 0.10</td>
<td>10.4 ± 0.70</td>
</tr>
</tbody>
</table>

* nmol of N-[¹⁴C]methylenephenylethanolamine formed per hr per pair of adrenals.
† nmol of [¹⁴C]DOPA formed per hr per pair of adrenals.
‡ nmol of [¹⁴C]DOPA formed per hr per g of brain tissue.
Our findings show that in house mice (Mus musculus) genetic variation exists, within the normal range, that affects the enzymes involved in the synthesis of catecholamines. This variation could result either from differences in the number of enzyme molecules, or from differences in their structure, or from both. Axelrod and Vesell (10) found that adrenal phenylethanolamine N-methyltransferase showed no inraspecific heterogeneity in several species when subjected to starch block electrophoresis or when its heat stability was examined. However, a broader sampling, beginning with the strains studied here, might reveal either or both enzymes to be structurally heterogeneous within a single species.

In adrenal gland, phenylethanolamine N-methyltransferase and tyrosine hydroxylase activities were correlated over all the genotypic groups studied here, suggesting that in these strains, the two enzymes are controlled by the same genetic factor(s), possibly due to close linkage on the same chromosome or to coordinate gene regulation. These possibilities might be tested by the examination of segregating generations (F1 and backcrosses) to see whether the correlation between the enzyme activities is maintained or breaks down (11).

The patterns of inheritance of tyrosine hydroxylase activity are different in adrenal gland and in brain. Therefore there must also be differences at the biochemical level in these tissues. It is possible that different enzymes are present in the two tissues. Alternatively, a single kind of protein molecule with tyrosine hydroxylase activity may be present in both adrenal gland and brain, but genetically controlled differences in the mechanisms regulating the synthesis, activation, and degradation of the enzyme may be operative in the two tissues.

CBA/J and C57BL/Ka appear to carry dominant genes for the adrenal enzymes and for brain tyrosine hydroxylase, respectively. The activities of both adrenal enzymes in the F1 hybrids resembled the activities in the inbred CBA/J strain, which, relative to the other inbred strains studied here, had intermediate enzyme activities. Similarly, the brain tyrosine hydroxylase activity in all the F1 hybrids resembled that of the intermediate inbred strain, in this case C57BL/Ka. Thus, for all three enzyme characters, dominant genes appear to determine intermediate, rather than extreme, activity. This is of considerable theoretical interest to geneticists (12), and will be discussed in greater detail elsewhere.

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