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L-Tyrosine-3-hydroxylase regulation in the brain: genetic aspects

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Summary. L-tyrosine-3-hydroxylase (TH) is the first and rate limiting enzyme in the biosynthetic pathway of catecholamine neurotransmitters (dopamine, noradrenaline, adrenaline). Implication of dopamine (DA) in various psychopathological phenomena, such as schizophrenia, has considerably contributed to the intensity of investigation of basic biochemical regulation of TH by activation and induction. Here we consider a third, constitutional (genotypic) aspect of regulation and present evidence that differences in mesencephalic (TH/SN), striatal (TH/CS), and hypothalamic (TH/HT) TH activity between virtually isogeneic strains of mice can be explained by segregating genetic factors. Biometrical genetic analysis of progenitor strains and their crosses indicated significant additive gene effects for TH/SN, TH/CS, and TH/HT, whereas dominance effects were statistically non-significant. A monogenic model of inheritance for TH/SN and TH/CS could not be rejected, while more than one gene was indicated for TH/HT. Significant positive phenotypic correlations were found in genetically segregating populations among mesencephalic, striatal and hypothalamic TH activities. This would suggest that some common genetic factors (or linked genes) are involved in the genetic variation of all three traits. A genetic selection experiment to elucidate the cellular and biochemical mechanisms underlying these variations is in progress.

Keywords: Amino acids – Tyrosine hydroxylase – Brain – Genetics – Mouse

L-tyrosine-3-hydroxylase (TH)[1], EC 1.14.16.2, tyrosine 3-monooxygenase (L-tyrosine, tetrahydropteridine: oxygen oxireductase, 3-hydroxylating) is a pteridine requiring monooxigenase, or mixed function oxidase, which catalyzes the conversion of tyrosine to DOPA, the first step in the biosynthesis of catecholamines (dopamine, noradrenaline, and adrenaline):

[1] TH, L-tyrosine-3-hydroxylase;

$$\text{L-tyrosine} + O_2 + (BH_4)^2 + \rightarrow \text{L-DOPA} + (BH_2)^3 + H_2O$$

The reduced cofactor is regenerated by another enzyme, dihydropteridine reductase.

Extensive work has been done on acute regulation of TH activity, which includes activation and induction. Activation refers to rapid increase in TH activity, whereas induction causes a somewhat slower response via increase in the amount of TH mRNA. Previous reports demonstrated that genetic differences in brain TH activity can be attributed to differences in the regional amounts of the TH enzyme protein due to heritable cellular or regulatory differences [1, 3, 10, 11, 12]. Such alterations can also play a significant role in the etiology of hypertension, Parkinson's disease, depression, and schizophrenia. In this work we have chosen to focus on the long-term, constitutional, genetic regulation of TH activity in the brain of a mammalian species, and to apply biometrical genetic methods to analyze data obtained in a series of experiments from highly inbred mouse strains, their hybrids, and segregating populations [11, 13, 14].

Experimental procedures

BALB/cJ (C), C57BL/6ByJ (B6), and CXBI/ByJ (I) mice were purchased from the Jackson Laboratory and bred in our Animal Facility. B6 females were crossed with C or I males and the F2 descendants of the hybrids were replicated by equal division of each corresponding F2 litter, resulting in (B6C)F2-alpha, (B6C)F2-beta, (B6I)F2-alpha, and (B6I)F2-beta generations. For the TH assays, 234 mice were used, with a mean subject age ($x \pm$ S.E.M.) of 20.3 ± 0.5 weeks. Animals were killed by decapitation, their brains were quickly removed, the whole hypothalamus (HT), (excluding the preoptic and mammillary regions), samples of corpus striatum (CS), and the A-8-substantia nigra-A10 region (SN) as a block of mesencephalic tissue, were dissected, frozen on dry ice, and stored at $-70°C$. After homogenization of striatal and hypothalamic samples, aliquots were taken for protein determination. TH activity (TH/HT, TH/CS, TH/SN) was determined by radioenzyme assay. Estimation of genetic parameters was based on the quantitative genetic additive-dominance model [9] and nonparametric methods [5, 6, 7]. The additive--dominance model assumes that the departure [d] of two homozygous strains from their mid-point reflects the simple additive effects of all genes which affect the trait by which the strains differ. The additive-dominance model was tested by Least Square Analysis of Mean (Cavalli's joint scaling test, for a more detailed description see Ref. [9]). Minimum number of genes (effective factors) involved was estimated as suggested by Bruell [2] and Falconer [8]. The estimations are based on the following relationships:

$$n = \frac{R^2}{8(V_{F2} - V_{F1})} \text{ and}$$

$$k = (2a^2 + d^2)/4\,(V_{F2} - V_E),$$

where R is the difference between the parental strains, V_{F2} and V_{F1} are the variances of the F2 and F1 generations, respectively, a is half of the difference between parental strains, d is the deviation of the F1 hydrid from the midpoint between the parental strains, and V_E is the environmental variance, estimated as the average variance of the genetically

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[2] BH_4, tetrahydrobiopterine;
[3] BH_2, dihydrobiopterine.

homogeneous parental strains and the F1 hybrids. These estimates are based on a number of assumptions which are difficult to meet and fullfilment of the criteria cannot always be tested. Therefore, such estimates should be viewed as debatable indicators of the complexity of the genetic system.

Results and discussion

Multiple comparison of all pairs of means (Bonferroni test) for TH/SN indicated that each non-segregating population was significantly different from the others: C $\gg$ (B6C)F1 > B6 > (B6I)F1 > I. For TH/CS we found that C > (B6C)F1, B6, (B6I)F1 $\gg$ I, and for TH/HT the rank order was C $\gg$ (B6C)F1 > B6 > I, (B6I)F1. The F2 generations for all three brain areas had a similar rank order: (B6C)F2-alpha, B6C)F2-beta $\gg$ (B6I)F2-alpha, (B6I)F2-beta. Additive and dominance effects were assessed by Cavalli's joint scaling test ([9]; Table 1).

Significant additive effects, [d], were found in TH/SN and TH/CS for both types of crosses (B6XC and B6XI), whereas in TH/HT [d] was highly significant in the B6XC cross only. For B6XC, however, a goodness of fit test of the additive-dominance model indicated a significant deviation (Chi square test, $p < 0.05$), rendering this [d] value questionable. No significant dominance effect, [h], was found for any regional TH activity. The complexity of the genetic control was also assessed for each variable and cross. For TH/SN and TH/CS the involvement of at least one major gene in the B6XC cross was indicated, whereas in the B6XI cross no significant genetic variability was found [13, 14]. In the TH/HT experiments, significant genetic differences and variability were detected only in the B6XC cross, and estimation of the minimum number of genes involved yielded 1.9. This result was supported by Elston's non-parametric one-locus test, leading to the rejection of the monogenic model of inheritance [11].

Table 1. Estimates of additive and dominance effects in mesencephalic and striatal tyrosine hydroxylase activity (X $\pm$ S.E.)

		BXC Cross	BXI Cross
ln (TH/SN + 1)	m	1.53 $\pm$ 0.02	1.28 $\pm$ 0.02
	[d]	0.15 $\pm$ 0.02	0.10 $\pm$ 0.02
	[h]	−0.02 $\pm$ 0.02	0.01 $\pm$ 0.03
	Chi-Square	0.60 ($p > 0.05$)	0.54 ($p > 0.05$)
ln (TH/CS)	m	2.03 $\pm$ 0.02	1.77 $\pm$ 0.02
	[d]	0.12 $\pm$ 0.02	0.14 $\pm$ 0.02
	[h]	−0.03 $\pm$ 0.03	0.06 $\pm$ 0.03
	Chi-Square	1.05 ($p > 0.05$)	0.27 ($p > 0.05$)
log (TH/HT)	m	0.29 $\pm$ 0.01	0.24 $\pm$ 0.01
	[d]	0.05 $\pm$ 0.01	−0.01 $\pm$ 0.01
	[h]	−0.01 $\pm$ 0.01	−0.02 $\pm$ 8.99
	Chi-Square	7.81 ($p < 0.05$)	0.84 ($p > 0.05$)

Table 2. Comparison of phenotypic correlation coefficients in pooled (B6C)F_2 (upper triangle) with mean correlation coefficients of nonsegrating generations (lower triangle)

	TH/SN	TH/CS	TH/HT
TH/SN	—	0.49**	0.29**
TH/CS	−0.06	—	0.34**
TH/HT	−0.12	0.11	—

** $p < 0.01$

Results of correlation analysis of untransformed data are summarized in a matrix, where the upper triangle shows the phenotypic correlation coefficients in the pooled (B6C)F2 generation and the lower triangle exhibits the mean correlation coefficients of nonsegregating generations, an estimate of environmental correlation (Table 2).

In factor analysis of the phenotypic correlation matrix of the pooled (B6C)F_2 generation the first two principal components explained 83% of the total variance in data space. TH/SN and TH/CS had their highest correlation with the first principal component (0.79 and 0.82, respectively), whereas TH/HT had its highest correlation (0.73) with the second principal component, and a correlation coefficient of 0.67 with the first principal component. Our earlier studies on several highly inbred strains indicated that BALB/cJ has the highest and CXBI/ByJ the lowest TH/SN [15, 17]. This has been confirmed in these studies. Further investigations of the genetic control suggest that the difference between BALB/cJ and other strains involves a major gene and other factors, such as background polygenes, gene interactions, and random developmental variations. The nature of these effects is largely additive. Presumably, the suggested major gene has pleiotropic effects and this is reflected as significant positive correlation between TH/SN and TH/CS in segregating (B6XC)F2 generations. TH/HT is less closely associated with TH/SN-TH/CS variation, and effects of more than one genetic factor are indicated.

There are several sources of hereditary effects that can be expected to influence TH activity in the brain: Structural differences between TH molecules leading to changes in catalytic activity, variations in regulatory DNA regions of the TH gene affecting TH mRNA production per cell, or variations at cellular level resulting in different densities of axonal innervation of target areas. It has recently been reported that the locus of the structural gene encoding TH, *Th*, has allelic variants, however none of the possible biochemical, or physiological consequences of this structural variability are known. Genetic variation in brain TH activity has been demonstrated by several authors [1, 3, 10, 11, 12, 13, 14, 15, 16, 17], and it was also suggested that strain differences in mouse mesencephalic TH activity are due to variations in number of dopaminergic neurones [1, 10]. Our recent finding that there are strain dependent variations in kinetic properties (V_{max}) of mesencephalic TH activity seems to support the above proposal, however, does not exclude some other explanations [12].

In a summary of previous works, Ciaranello and Boehme [4] described "an association of enzyme phenotypes", i.e., levels of adrenal dopamine beta-hydroxylase, phenylethanolamine N-methyltransferase and TH, and brain TH were higher in C (BALB/cJ) mice than in various other strains, including BALB/cN, one of its sublines. F1 hybrids of the sublines had intermediate enzyme levels, and the association of phenotypes persisted. Further work on adrenal enzyme levels in segregating generations suggested that each enzyme was under control of a single genetic factor, and differences in PNMT levels were due to differences in the rate of enzyme degradation. Our previous studies on TH/SN and TH/CS in BALB/cJ and BALB/cByJ, a subline related to BALB/cN, yielded similar results to those of Ciaranello, i.e., TH activities were higher in BALB/cJ than in its subline, BALB/cBy [17].

In conclusion, the results suggest that there is a robust gene effect that is largely responsible for the observed genetic correlation between TH activity in the midbrain DA neuron cell body area (TH/SN) and TH activity in corpus striatum, the target area. This hypothesis is consistent with earlier reports [1, 10] demonstrating that strain differences in TH/SN are entirely attributable to differences in number of mesencephalic DA neurons, and TH/CS reflects density of axonal arborization of these neurons. However, other explanations of genetic variability in brain TH activity are also conceivable, and genetic selection experiments are needed to elucidate the cellular and biochemical mechanisms underlying these variations. Development of Congenic Recombinant Animal Model (CRAM) lines, to provide an analytical tool for such studies, is in progress.

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