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Differences in Activity in Cerebral Methyltransferases and Monoamine Oxidases Between Audiogenic Seizure Susceptible and Resistant Mice and Deermice

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DOYLE, R. L. AND O. Z. SELLINGER. *Differences in activity in cerebral methyltransferases and monoamine oxidases between audiogenic seizure susceptible and resistant mice and deermice.* PHARMAC. BIOCHEM. BEHAV. 13(4) 589–591, 1980.—The specific activity of cerebral histamine N-methyltransferase (HMT) was significantly lower in the audiogenic seizure-susceptible (SS) 21-day old DBA/2J mouse when compared to the non-susceptible 70-day old DBA/2J mouse but not when compared to the seizure-resistant (SR) C57Bl/6J mouse at 21 days of age. There was no significant difference between the two strains at 70 days of age. The lower HMT specific activity was also observed in a SS mutant of the deermouse *Peromyscus maniculatus*, relative to the SR, wild-type animal. The activity of cerebral catechol-O-methyltransferase (COMT) was significantly lower in the DBA/2J mice relative to the C57Bl/6J at 21 and 70 days while, in *Peromyscus*, it was higher in the SS mutant than in the SR animal. The activity of MAO, B was lower in the 21-day old, relative to the 70-day old DBA/2J and the 21-day old C57Bl/6J mice. There were no differences in MAO A or B between SS and SR *Peromyscus*. The findings raise the possibility that cerebral methylation may operate at characteristic rates in SS animals.

Brain methyltransferases MAO Audiogenic seizures *Mus* *Peromyscus*

RECENT evidence obtained in our laboratory points to the involvement of methylation in the cellular response of brain tissue to the convulsant agent L-methionine-dl-sulfoximine (MSO) [14,15]. Both the *in vivo* methylation of cerebral histamine by histamine N-methyltransferase (HMT) [16] and the methylation of transfer ribonucleic acids (tRNA) by cerebral tRNA-methyltransferases [3,13] appear to be significantly elevated [13,16] and the substrate specificity of some of the indiviudal tRNA methyltransferases [3] altered in the epileptogenic MSO brain. These observations have raised the possibility that methyl group transfer reactions operate in the MSO epileptogenic brain cells at characteristic levels, possibly "marking" the preconvulsant state biochemically.

In an attempt to ascertain the involvement of methyl group transfer reactions in an animal model with a genetically determined, rather than a chemically elicited, seizure response, two inbred strains of *Mus* with varying degrees of audiogenic seizure susceptibility and an autosomal recessive audiogenic seizure-susceptible mutant of the deermouse *Peromyscus maniculatus bairdii* [1,4] were compared by determining the levels of their cerebral HMT activity. In view of the dissimilar distribution of HMT and of another methyltransferase, catechol-O-methyltransferase, (COMT) throughout a number of (rat) brain structures [11], the activity of cerebral COMT was also compared between seizure-susceptible (SS) and -resistant (SR) animals. Furthermore, since the methylated products formed via HMT and COMT action undergo oxidative deamination by monoamine oxidases (MAO), the A and B forms of this enzyme activity were also determined in the *Mus* and *Peromyscus* brains.

Animals

Males of the DBA/2J and C57Bl/6J inbred strains and the mutant epileptic (epep) *Peromyscus* used throughout the study were obtained from stocks maintained at the Mammalian Genetics Center of the University of Michigan, Ann Arbor, Michigan. The wild-type, SR (EpEp) deermice were wild-conceived animals obtained from Dr. L. Masters, Museum of Zoology, University of Michigan, Ann Arbor, MI. The animals received "Teklan 4% mouse and rat diet" and water ad lib and were housed in stainless steel cages, 1–6/cage.

The DBA/2J mice were examined at 21 days of age, the peak of audiogenic seizure susceptibility and intensity [17] and at 70 days by which time they have become SR. C57Bl/6J mice, generally SR at all ages [17], were compared to the DBA/2J mice at 21 and 70 days, as "negative controls." The SS deermice were derived from homozygous matings (epep×epep) and were tested individually for clonic or tonic seizure response at least once after weaning using the key-jingling procedure of Barto [1]. The epep animals

were compared to the wild-type EpEp *Peromyscus* who are generally SR at all ages [1,4]

METHOD

The activities of HMT, COMT, and MAO, types A and B were determined by the procedures of Schatz and Sellinger [15], Porcher and Heller [11], and Owen *et al.* [10], respectively. Protein was determined according to Lowry *et al.* [8]. Enzyme activity is expressed as specific activity in nmoles of product/h/mg of protein. Closely analogous results were obtained by expressing the data in nmoles/hr/g of wet tissue (not shown). HMT was determined on whole brain homogenates and on high-speed supernatants prepared by centrifuging 1:10 (w/v) homogenates in 0.25 M sucrose at 104,000×g for 30 min. It has been previously demonstrated [19] that, in Swiss-Webster mice approximately 50% of the homogenate HMT activity is recovered in the high-speed supernatant. It should be noted that all enzyme activities were determined on tissue samples obtained from individual brains.

The statistical significance was determined using Students *t*-test and the 0.05 level of significance was selected using the "two-tailed" test.

RESULTS AND DISCUSSION

Results of a survey of HMT activity in the brains of DBA/2J and C57Bl/6J mice at 21 and 70 days are illustrated in Table 1. The supernatant HMT value of the SS DBA/2J mice at 21 days of age was significantly lower than that of the SR DBA/2J mice at 70 days, but not significantly different from that of the SR C57Bl/6J mice at 21 days. Furthermore, while there was a significant increase in HMT activity levels between the 21- and 70-day old animals within each strain, there was no significant difference between the HMT values of the DBA/2J and C57Bl/6J mice at either 21 or 70 days. There was no significant difference in the homogenate of 21-vs 70-day old DBA/2J mice (data not shown).

As illustrated in Table 2, the specific activity of HMT was consistently lower in the SS *Peromyscus* than in the comparable SR *Peromyscus* animals whether tested in the homogenate or in the high-speed supernatant. Complementary data for *Mus* regarding COMT and MAO, types A and B, are shown in Table 3. The DBA/2J animals exhibited significantly lower COMT values than the C57Bl/6J mice at both 21 and 70 days, confirming the work of Schlesinger *et al.* [18]; there was, however, no significant difference in the DBA/2J mice at 21 vs 70 days. Table 3 shows that MAO, type B (substrate phenylethylamine) was also significantly lower in the brains of the 21-day old SS DBA/2J mice when compared to either 21-day old SR C57Bl/6J or 70-day old SR DBA/2J animals. It should be noted, however, that there was no significant age-related difference in MAO, type B activity in the C57Bl/6J mice. Table 3 also shows a difference in the specific activity of MAO, type A between 21- and 70-day old DBA/2J mice. This observation is to be compared to the findings of Schlesinger *et al.* [18] who reported no difference in the activity of cerebral 5-hydroxytryptamine-preferring (MAO, type A) enzyme between C57Bl/6J and DBA/2J mice and of Kellogg [6] who described significantly higher MAO, type A values in 21-day old SR C57Bl/6J mice relative to DBA/2J SS mice of the same age.

TABLE 1

CEREBRAL HISTAMINE N-METHYLTRANSFERASE IN DBA/2J AND C57Bl/6J MICE*

Mouse Strain	Age, days	
	21	70
DBA/2J	5.66 ± 0.45[1]	6.78 ± 0.10[2]
C57Bl/6J	5.48 ± 0.10[3]	6.33 ± 0.17[4]

*nmoles product/hr/mg protein of mean ± SEM assayed in supernatant fraction in 4–6 animals. The following comparisons were significantly different at $p<0.05$: 1 vs 2, 3 vs 4.

TABLE 2

CEREBRAL HISTAMINE N-METHYLTRANSFERASE IN THE AUDIOGENIC SEIZURE-SUSCEPTIBLE (SS) *vs* RESISTANT (SR) *Peromyscus*

Fraction	SS	SR
Homogenate	1.66 ± 0.11	1.97 ± 0.08†
Supernatant	5.56 ± 0.38	6.64 ± 0.17†

*nmoles product/hr/mg protein of mean ± SEM in 6–7 sixty day-old animals.

†denotes significance at $p<0.05$, relative to SS animals.

TABLE 3

CEREBRAL CATECHOL-O-METHYLTRANSFERASE (COMT) AND MONOAMINE OXIDASE (MAO), TYPES A AND B IN DBA/2J AND C57Bl/6J MICE*

Mouse strain		Age, days	COMT	MAO, type A	MAO, type B
DBA/2J	(SS)	21	1.72 ± 0.03[1]	1.68 ± 0.51[5]	4.04 ± 0.12[7]
C57Bl/6J	(SR)	21	2.41 ± 0.01[2]	n.d.	4.98 ± 0.37[8]
DBA/2J	(SR)	70	1.60 ± 0.05[3]	13.7 ± 0.50[6]	5.02 ± 0.32[9]
C57Bl/6J	(SR)	70	2.14 ± 0.03[4]	n.d.	4.09 ± 0.31[10]

*nmoles product/hr/mg of protein of mean ± SEM in 4–6 animals per group. The following comparisons were significantly different at $p<0.05$: 1 vs 2; 1 vs 4; 2 vs 4; 3 vs 2; 3 vs 4; 5 vs 6; 7 vs 8; 7 vs 9.

SS: seizure-susceptible.

SR: seizure-resistant.

n.d.: not determined.

TABLE 4

CEREBRAL CATECHOL-O-METHYLTRANSFERASE (COMT) AND MONOAMINE OXIDASE (MAO), TYPES A AND B IN SS AND SR *Peromyscus**

Animal	COMT	MAO, type A	MAO, type B
SR	1.81 ± 0.04 (7)	15.4 ± 0.98 (8)	6.02 ± 0.36 (9)
SS	2.71 ± 0.10 (6)†	16.6 ± 1.23 (7)	5.55 ± 0.45 (8)

*nmoles product/hr/mg of protein of mean ± SEM. Animals were 60 days old.

†Denotes a significant difference at $p<0.05$. The numbers in parenthesis refer to the number of animals.

The activities of COMT, MAO, type A and type B in *Peromyscus* brain (Table 4) indicate a significant elevation of COMT in the SS animal and no evident difference in either type of MAO.

To summarize, we report significantly lower HMT activities in the brains of two different SS animal models and a significantly higher COMT activity in the SS than in the SR *Peromyscus* brain. In addition, we demonstrate that the activity of cerebral MAO, type B, the enzyme responsible for the conversion of cerebral 3-methylhistamine to 3-methylimidazoleacetic acid [5,21] is significantly lower in the 21-day old SS DBA/2J mice than in the 70-day old SR animals.

It should be noted that within *Mus* and *Peromyscus* there are different genetic models of seizure susceptibility [7, 9, 17, 20]. The multiformity of results obtained when data is compared across different seizure models may thus be reflective of the genetic diversity evident in the inherited epilepsies. A similar multiformity of results exists within the literature of non-inherited, electrically or chemically elicited seizures [22]. Our demonstration of abnormalities of cerebral methyl group transfer reactions in both a chemically induced (MSO) and a genetically derived seizure state (audiogenic seizures in *Peromyscus*) should greatly facilitate the search to understand the relationships between biochemical and genetic mechanisms of seizure susceptibility. Work is in progress to further elucidate the nature of cerebral methylation reactions in *Peromyscus* as they may relate to this trait.

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