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Classical Genetic Analysis of GABA-Related Seizures

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MARTIN, B J , R J MARLEY, L L MINER AND J M WEHNER *Classical genetic analysis of GABA-related seizures* PHARMACOL BIOCHEM BEHAV 29(3) 501–507, 1988 —Differences in resistance to 3-mercaptopropionic acid (MP)-induced seizures exist between DBA/2Ibg and C57BL/6Ibg inbred strains of mice, C57BL/6Ibg mice are more resistant to MP-induced seizures To determine the mode of inheritance for seizure resistance, a classical genetic analysis was conducted using these two parental strains, their F1, F2, and backcross generations Latencies to seizure onset and tonus after intraperitoneal (IP) injections of MP (25–40 mg/kg) were quantified For all populations mean latencies to onset and tonus decreased in a dose dependent manner with the hybrid generations exhibiting a seizure resistant phenotype resembling the C57BL/6Ibg strain In general, female mice were less resistant to MP-induced seizures than males, however, a significant degree of resistance was retained by the C57BL/6Ibg females and their female progeny A quantitative assessment of the pattern of inheritance for seizure resistance using a weighted least-squares regression approach indicated that an additive-dominance model explained latency to seizure onset data at 25, 35 and 40 mg/kg However, at 30 mg/kg, the model required the addition of an epistatic parameter to best describe mean scores at this dose The results of these analyses suggest that resistance to MP-induced seizures is transmitted in a dominant manner

Classical genetic cross Inbred mouse strains 3-Mercaptopropionic acid Seizures

DIFFERENTIAL susceptibility to seizure onset in rodents has been shown to reflect a substantial genetic component Strains of laboratory animals have been used to characterize the heterogeneous expressions of convulsive disorders within several seizure-initiation paradigms In a majority of the animal models, the genetic predisposition to seizure onset is manifested as an altered threshold to seizure inducing stimuli which may be experimentally manipulated to trigger convulsive activity These include sensory stimuli such as loud sound [4, 6, 31], a burst of air [14], postural stimulation [11], or chemicals [17, 21, 29]

The involvement of various neurotransmitters in the pathogenesis of seizure disorders is complex Attempts to determine an etiological role for a neurotransmitter defect have implicated several excitatory neurotransmitters in the regulation of seizure activity [9] However, the evidence for a unilateral excitatory transmitter defect has been inconsistent. It has been proposed that seizure production and propagation involve inhibitory pathways and that disinhibition of these inhibitory pathways results in seizure activity [5, 8, 24, 37]. GABA (gamma-aminobutyric acid) is the major inhibitory neurotransmitter in brain Pharmacological manipulations of GABA activity at pre- and postsynapses result in changes in seizure thresholds [1, 7, 13, 26] The chemoconvulsant, 3-mercaptopropionic acid (MP), is a reversible, competitive inhibitor of GAD (glutamate decarboxylase), the rate-limiting enzyme that determines steady-state levels of brain GABA. A decrease of GAD activity due to MP treatment results in a deficit in the amount of GABA available for release into the synaptic cleft Karlsson *et al* [10] have shown that a time course of GAD activity after MP injection is related to GABA levels and seizure onset. The duration of seizure susceptibility corresponds to the time course of enzyme activity At 15 min the threshold of seizure susceptibility begins to increase, reflecting the reversal of GAD activity to normal levels.

Recently, Marley *et al* [17] demonstrated significant differences in latencies to seizure onset and tonus among inbred strains of mice, C57BL/6Ibg, C3H/2Ibg, DBA/2Ibg, and a heterogeneous stock of mice, HS, when challenged with convulsant doses of MP. The results of this investigation indicated that the C57BL/6Ibg strain was unique among the four populations tested Both male and female C57BL/6Ibg mice displayed an increased resistance to MP-induced seizures as compared to the other strains In order to determine the pattern of inheritance of seizure resistance a classical genetic analysis was conducted using as parental populations two strains demonstrating maximum differences in their seizure responses The latencies to seizure onset and tonus after MP treatment were evaluated in C57BL/6Ibg (most resistant) and DBA/2Ibg (least resistant) inbreds, their F1, F2, and backcross generations.

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METHOD

Animals

Male and female mice from two inbred strains (C57BL/6Ibg and DBA/2Ibg) were used in this analysis Mating pairs produced the following genetic crosses C57BL × DBA=F1 and the reciprocal cross, DBA × C57BL, F1 × F1=F2, F1 × C57BL=CDXC (backcross 1), and F1 × DBA=CDXD (backcross 2) All mice were weaned at 25 days of age and housed with two to six like-sex littermates Animals were maintained on a 12-hour light/dark cycle (lights on at 0700 to 1900) and were permitted free access to food (Wayne Lab Blox) and water The bedding used in housing the animals was aspen hardwood chips (Beta-chips, Northeastern Products, Warrensberg, NY) All testing was done when the mice were 60–90 days old

Drug

Three-mercaptopropionic acid (Sigma Chemical Co , St Louis, MO) was dissolved in 0 9% saline and was administered by intraperitoneal (IP) injection in a volume of 0 02 ml/g body weight The doses used were 25, 30, 35 and 40 mg/kg All solutions were prepared fresh daily

Seizure Testing

Animals were placed in an air-conditioned testing room with 34 watt overhead fluorescent lighting at least one hour before seizure testing began After IP injection, mice were individually placed in a 1 5 liter glass jar for observation Latencies from injection to seizure onset and tonus were recorded to the nearest 5 sec

MP-induced seizures are characterized by distinct stages of motor behavior (1) clonus is identified as a loss of righting reflex with convulsive movements in all extremities, (2) wild running is purposeless, random running and jumping, and (3) tonus is characterized by full caudal limb extension and respiratory arrest that often results in death It was observed that tonus was always preceded by a bout of wild running For the purposes of this study, seizure onset was defined as clonus or wild running, whichever occurred first Observation time was limited to 900 sec after IP injection, any animal not seizing within this time period was given a score of 900

Data Analysis

Initially latencies from IP injection to seizure onset and tonus were analyzed separately for all groups using 3-way analyses of variance (ANOVA) to determine sex, dose, and generation effects Reciprocal F1 data were tested using a 3-way ANOVA to detect the presence of maternal effects Subsequent 2-way ANOVAs were then conducted separately for male and female onset and tonus latency scores For those analyses in which significant differences were observed, the results were subjected to Tukey's B *post-hoc* test to determine the rank order of generation responses

To assess the extent to which strain differences at different doses are due to the same genetic influences, Pearson's correlations among doses were computed for both males and females Mean latencies at each dose were correlated across the generations, resulting in a 4×4 correlation matrix for each sex

Genetic Analysis

Latencies to seizure onset and tonus were measured in the two parental strains, C57BL/6Ibg and DBA/2Ibg, their F1, F2 and backcross generations to determine the mode of inheritance for seizure resistance Mean latencies to tonus resulted in the maximum latency (900 sec) for at least one population per dose This lack of variance within generations for tonus prevented genetic assessment of tonus data Therefore, genetic analyses were undertaken for latency to onset scores only Latency scores were transformed by a natural log scale transformation to meet the assumptions of normality and homoscedasticity The transformed data were then subjected to a means analysis proposed by Mather and Jinks [18] This model utilizes a weighted least-squares regression approach to estimate genetic parameters as shown in Table 1 Weights are calculated from the reciprocals of the variances of the observed means The estimated parameters include midparent value (m), an additive genetic effect [d], and dominance deviations [h] In addition, three parameters accounting for epistasis, or non-allelic interactions, can also be estimated interaction between homozygous combinations at different loci [i], between homozygous and heterozygous combinations [j]; and between heterozygous combinations [l] The fit of the model is evaluated statistically by means of a Chi-square test applied to the observed and expected population means First a simple additive-dominance model (estimating m, [d], and [h]) is fitted to the data This model can be rejected by a significant Chi-square, indicating a significant difference between the expected and observed means More complex models can be formulated with the addition of the epistatic parameters The interaction parameters may be added one or two at a time in any combination to best explain the data This method leads to the selection of the best fitting, most parsimonious model

Differences in male and female seizure onset responses were indicated by a significant main effect in the initial 3-way ANOVA However, no 2-way or 3-way interactions that included sex as a factor were significant This suggests, that although males and females differ in the degree of susceptibility to MP-induced seizures, they do not demonstrate different neurochemical mechanisms in their regulation of sei-

TABLE 1

GENETIC MODELS FOR MEAN ANALYSIS

Generation	Genetic Models: Additive-Dominance	Genetic Models: Full Model With Epistasis
P_1 (C57BL)	m + [d]	m + [d] + [i]
P_2 (DBA)	m − [d]	m − [d] + [i]
F_1	m + [h]	m + [h] + [l]
F_2	m + 1/2[h]	m + 1/2[h]+ 1/4[l]
B_1 (CD × C)	m + 1/2[d] + 1/2[h]	m + 1/2[d] + 1/2[h] + 1/4[i] + 1/4[j] + 1/4[l]
B_2 (CD × D)	m − 1/2[d] + 1/2[h]	m − 1/2[d] + 1/2[h] + 1/4[i] − 1/4[j] + 1/4[l]

Genetic models used to estimate genetic parameters m, midparent value, [d], summation of additive genetic effects, [h], summation of dominance deviations, [i], epistatic interactions between homozygous combinations of alleles at different loci, [j], epistatic interactions between homozygous and heterozygous combinations of alleles at different loci, and [l], epistatic interactions between heterozygous combination of alleles at different loci

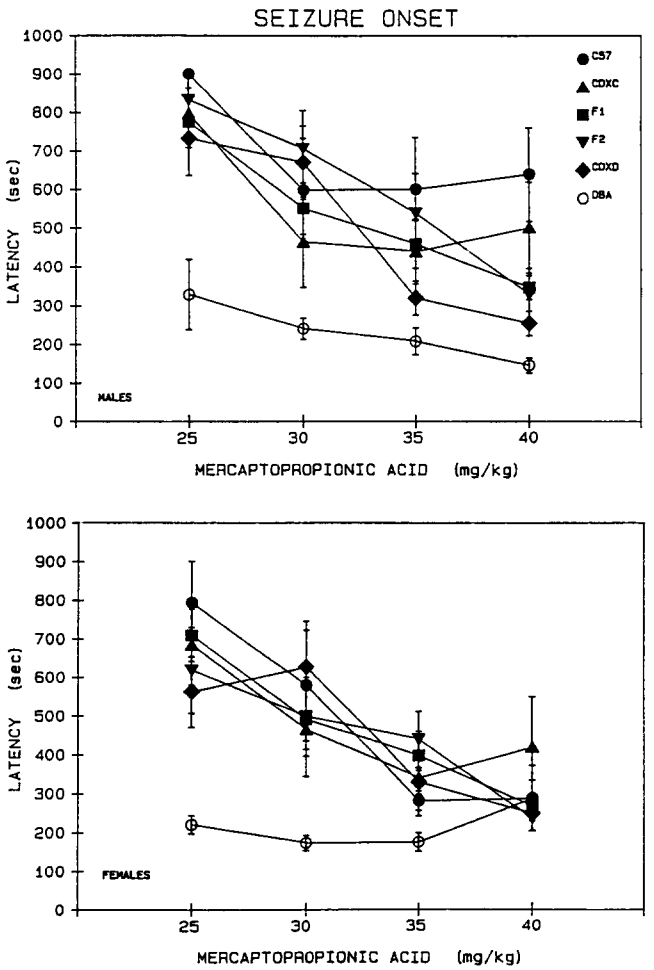

FIG 1 Latency to seizure onset induced by IP injections of 3-mercaptopropionic acid in two parental inbred strains of mice, their F1, F2 and backcross generations Latencies were scored as described under the Method section (mean±SEM, n=6–13)

zure activity across doses and/or generations A similar mechanism of action between the sexes implies that the pattern of inheritance for seizure resistance is the same for both sexes. For this reason, transformed male and female means and variances were averaged at each dose to create one mean and one variance for each generation to be used in the means analysis. An average of male and female variances was used instead of a pooled population variance at each dose in order to restrict the variance to within sex variation, excluding any between sex variation

RESULTS

Seizure Onset

Latencies to seizure onset after IP injection decreased in a dose dependent manner for all generations (Fig 1) Initially, tests of significance using a 3-way ANOVA for the full complement of data resulted in highly significant group, sex, and dose effects, $F(6,474)=22\ 9$, $p<0\ 001$, $F(1,474)=13\ 4$, $p<0.001$, $F(3,474)=44.6$, $p<0\ 001$, respectively. The significant sex differences in the original parental data and hybrid generations resulted in separate analyses for males and females In addition, a 3-way ANOVA of the reciprocal cross latencies to seizure onset and tonus indicated no maternal effects Consequently, the F1 data were combined in subsequent analyses

TABLE 2

CORRELATIONS OF LATENCY TO SEIZURE ONSET AMONG DOSES

	25 mg/kg	30 mg/kg	35 mg/kg	40 mg/kg
		Males		
25 mg/kg	1 00	83*	92*	83*
30 mg/kg		1 00	67	41
35 mg/kg			1 00	87*
40 mg/kg				1 00
		Females		
25 mg/kg	1 00	85*	69	26
30 mg/kg		1 00	73*	− 06
35 mg/kg			1 00	− 19
40 mg/kg				1 00

Pearson's correlation matrices for latency to seizure onset scores among doses of 3-mercaptopropionic acid common to two parental inbred strains of mice and their F1, F2 and backcross generations (n=6, *$p<0\ 05$)

Figure 1 presents the mean latencies to seizure onset as a function of MP doses common to all male populations The mean score for C57BL/6Ibg males at 25 mg/kg is at ceiling, with the hybrid generations clustering between 830 and 730 sec Group and dose effects were both highly significant, $F(5,239)=9\ 8$, $p<0\ 001$, and, $F(3,239)=25\ 2$, $p<0\ 001$

The mean latencies for the female populations presented in Fig 1 demonstrate a similar dose dependent pattern of seizure activity, however, females seized below the 900 sec time limit at all doses, indicating that on average, females display a decreased resistance to MP-induced seizures A 2-way ANOVA also resulted in significant group and dose effects, $F(5,234)=4\ 5$, $p<0\ 001$, and, $F(3,234)=19\ 6$, $p<0\ 001$, respectively

For both males and females the mean responses to seizure onset of the derived generations are similar to those exhibited by the C57BL/6Ibg strain *Post-hoc* analyses confirmed no differences among these five generations, in contrast, male and female DBA/2Ibg mice were significantly less resistant to MP-induced seizures (Tukey's B, $p<0\ 05$)

The correlations presented in Table 2 indicate that strain differences are positively correlated among doses, suggesting a common genetic mechanism operating for all doses of MP For females, the correlations are substantial for doses in the low to middle range (doses 25 and 30 mg/kg, 25 and 35 mg/kg, and 30 and 35 mg/kg). However, the correlations involving 40 mg/kg are low because of a maximal response at which the female generations are no longer differentiated

Tonus

The mean latencies to tonus shown in Fig 2 indicate that across all doses at least one generation of males is at ceiling of 900 sec, and with the exception of the substantial decrease exhibited by the DBA/2Ibg inbreds at 40 mg/kg, all other groups clustered between 725 and 900 sec As with seizure

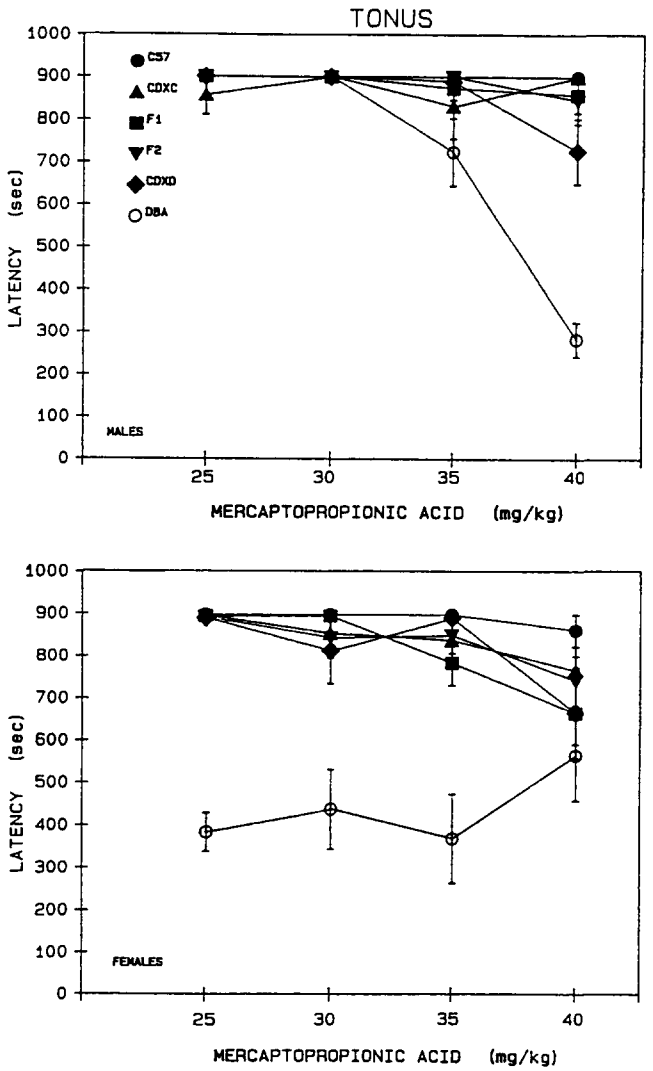

FIG 2 Latency to onset of tonus induced by IP injections of 3-mercaptopropionic acid in two parental inbred strains of mice, their F1, F2 and backcross generations Latencies were scored as described under the Method section (mean±SEM, n=6–13)

onset, the effects of group and dose are highly significant, $F(5,239)=16\ 2$, $p<0\ 001$, and, $F(3,234)=15\ 0$, $p<0\ 001$

Figure 2 presents the significant group and dose effects for the female generations, $F(5,234)=27\ 9$, $p<0\ 001$, and, $F(3,234)=6\ 8$, $p<0\ 001$ Like the male populations, female C57BL/6Ibg and hybrid generations cluster above 675 sec, however, by 40 mg/kg no group is at ceiling The high proportion of maximum mean latencies (900 sec) precludes a *post-hoc* analysis of tonus scores

Genetic Analysis

Transformed mean latency scores for seizure onset were fit to the simple additive-dominance model shown in Table 1 Parameter estimates and tests of significance for each dose are presented in Table 3 The nonsignificant Chi-square estimates at doses 25, 35 and 40 mg/kg suggest that the additive-dominance model adequately explains the data at these doses However, at 30 mg/kg the model required the addition of an epistatic parameter, [l], indicating the presence of interactions between heterozygous pairs of alleles

DISCUSSION

The results of a classical genetic cross between a seizure susceptible strain, DBA/2Ibg, and a seizure resistant strain, C57BL/6Ibg, indicate that the hybrid populations display a phenotype that resembles the C57BL/6Ibg strain This is the case regardless of whether the measure is latency to seizure onset or latency to tonus This is interesting in light of our previous work which showed that the level of seizure susceptibility in three other genetic stocks of mice more closely resembled the DBA/2Ibg strain [17] This investigation demonstrates that the genotype responsible for seizure resistance within the GABAergic system is inherited in a dominant fashion

In general, the quantitative assessment of the pattern of inheritance for resistance to seizure onset supports an additive-dominance hypothesis However, the addition of one epistatic parameter at 30 mg/kg suggests that more than one gene may be responsible for the degree of resistance to MP-induced seizures observed in this study The lack of interallelic interaction parameters at the other doses does not necessarily indicate a single gene effect The activity of several genes may be expressed concurrently in an additive and/or dominant manner representing a sum of independent effects In addition, Mather and Jinks [18] suggest that scale transformations that satisfy linearity may dilute non-independent gene effects A fit of the additive-dominance model to transformed data will permit an interpretation of average independent gene effects and will also retain predictive value, particularly when the results can be legitimately tested against results generated from other statistical analyses The *post-hoc* analyses of the untransformed data show a clear pattern of dominance for seizure resistance, the C57BL/6Ibg mice appear to confer a degree of seizure protection to their progeny

Sex differences observed in the present study show that female mice are less resistant to MP-induced seizures than males, however, a comparative degree of resistance is retained by the C57BL/6Ibg females and their female hybrid progeny The greater degree of variation among the female generations for seizure onset at 25 mg/kg as compared to the male generations at this dose, and the opposite pattern of generation responses at 40 mg/kg, suggests that the dose response curve for females is shifted to the left These results are consistent with recent investigations demonstrating increased sensitivity in female mice [17] and female rats [23] to the effects of drugs that affect GABAergic transmission Decreases in GAD activity due to estradiol benzoate treatment have been reported in discrete brain areas in rat [36] including substantia nigra [20,22], a region which has been implicated in seizure propagation [5,8] In addition, sex differences observed in seizure sensitivity to picrotoxin are age dependent, occurring in sexually mature mice, but not in immature or old animals [15]

Others have attempted to characterize the mode of seizure inheritance using other models of seizure induction Sensory stimuli will trigger seizure onset in some inbred strains of mice (e g , 21-day-old DBA/2J mice) [4, 6, 31] and selected lines such as the GEPR (genetically epilepsy-prone rat) [12] and AUZ (University of Arizona) rats [2] These animals are primarily sensitive to acoustic stimuli, however,

TABLE 3

GENETIC ANALYSIS OF TRANSFORMED LATENCY TO SEIZURE ONSET SCORES

	25 mg/kg			30 mg/kg		
Generation	Observed	Expected	(O−E)	Observed	Expected	(O−E)
P_1 (C57BL)	6 69	6 58	11	6 20	5 97	23
P_2 (DBA)	5 51	5 71	− 20	5 29	5 32	− 03
F_1	6 54	6 57	− 03	6 13	6 13	00
F_2	6 45	6 36	09	6 23	6 25	− 02
B_1 (CD × C)	6 45	6 58	− 13	6 05	6 41	− 36
B_2 (CD × D)	6 38	6 14	24	6 36	6 08	28
	$\chi^2(3)=4\ 34,\ p>0\ 20$			$\chi^2(2)=4\ 78,\ p>0\ 05$		
	35 mg/kg			40 mg/kg		
	Observed	Expected	(O−E)	Observed	Expected	(O−E)
P_1 (C57BL)	5 93	5 91	02	5 97	5 94	03
P_2 (DBA)	5 20	5 29	− 09	5 22	5 23	− 01
F_1	5 95	6 02	− 07	5 68	5 68	00
F_2	6 04	5 81	23	5 53	5 63	− 10
B_1 (CD × C)	5 83	5 96	− 14	5 90	5 80	10
B_2 (CD × D)	5 74	5 66	08	5 49	5 45	04
	$\chi^2(3)=3\ 34,\ p>0\ 10$			$\chi^2(3)=0\ 83,\ p>0\ 80$		

Genetic Parameter Estimates

25 mg/kg	30 mg/kg	35 mg/kg	40 mg/kg
m=6 14 ± 0 12	m=5 65 ± 0 14	m=5 60 ± 0 11	m=5 58 ± 0 12
[d]=0 43 ± 0 12	[d]=0 32 ± 0 13	[d]=0 31 ± 0 10	[d]=0 35 ± 0 12
[h]=0 42 ± 0 19	[h]=1 92 ± 0 64	[h]=0 42 ± 0 17	[h]=−0 09 ± 0 17
	[l] =−1 44 ± 0 62		

Male and female latency to seizure onset scores from the dose response curves shown in Fig 1 were transformed by a natural log scale transformation Male and female means and variances averaged at each dose were fit to the genetic models presented in Table 1 The nonsignificant χ^2 estimates at 25, 35 and 40 mg/kg indicates a good fit of the simple additive-dominance model The addition of one epistatic parameter was required at 30 mg/kg Genetic parameters estimated are m, midparent value ±SE, [d], summation of additive genetic effects ±SE, [h], summation of dominance deviations ±SE, and [l], epistatic interactions between heterozygous pairs of alleles ±SE

they also exhibit a generalized predisposition to electroconvulsive shock, kindling [25] and chemical convulsants [12]

The specific mode of inheritance responsible for the seizure prone and/or seizure resistant phenotype has been determined in relatively few animal models Quantitative assessments of genetic transmission of audiogenic seizure susceptibility in mice have produced a variety of hypotheses ranging from single autosomal dominant to single autosomal recessive to a multifactorial model of inheritance [30] Recently, a genetic analysis using C57BL/6J × DBA/2J recombinant inbreds has refuted the single locus hypothesis and supports a polygenic system, albeit with a small number of loci involved [31]. Similar multifactorial hypotheses have been proposed for high pressure neurologic syndrome (HPNS) type 2 seizures in mice [19] and isoniazid-induced seizures in 8-week-old mice [34]

In contrast to the pattern of dominance for seizure resistance observed in our investigation, other classical genetic models using chemoconvulsants such as nicotine [21] and caffeine [28,29] have indicated a single gene effect with dominance for seizure susceptibility In addition, a recent classical genetic analysis investigating the coincident sensitivity to seizures induced by two agents, caffeine and the benzodiazepine inverse agonist, methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM), has indicated that hyperresponsiveness to the proconvulsant actions of DMCM is inherited in a dominant manner, determined by a single autosomal gene [27] The direction of dominance in our results may also represent mechanisms with the GABA/benzodiazepine (BZ) system reflecting the indirect actions of MP on GABA synthesis, rather than a direct influence at the receptor level

Extensive characterization of the GABAergic system in seizure susceptible and seizure resistant mice has been performed Early studies on GAD activity and GABA uptake did not reveal significant differences between seizure susceptible and resistant strains [32,33] Studies measuring high affinity GABA binding using [^{3}H]-muscimol in C57BL/6Ibg and DBA/2Ibg mice resulted in no receptor differences between these two strains [17] However, low affinity GABA binding parameters as measured by [^{3}H]-GABA did differ between these strains in both affinity and receptor number

[35] Other strain differences similar to ours have been demonstrated for bicuculline (Bic)-induced seizures ($GABA_A$ receptor antagonist), C57BL/6Ibg mice were significantly more resistant than C3H/2Ibg mice [3] This relative resistance to Bic-induced seizures paralleled the resistance to Bic-induced epileptiform activity observed in hippocampal slice preparations from these inbred strains [3] In addition, a recent investigation that examined coupling between the GABA receptor and the benzodiazepine receptor in five genetic stocks of mice [16], including C57BL/6Ibg and DBA/2Ibg, determined that enhancement of [^{3}H]-flunitrazepam (FNZ)-binding by GABA was positively correlated with the degree of resistance to MP-induced seizures Mice that exhibited a greater degree of seizure resistance (C57BL/6Ibg) also demonstrated a greater degree of enhancement of [^{3}H]-FNZ binding Whether these differences at the GABA/BZ barbiturate receptor complex reflect the differences observed by others in low affinity GABA binding [35], a variation in the molecular properties of the BZ receptor, or some fundamental difference in membrane microenvironment remains to be determined, as well as how these differences function in genotypic regulation of seizure thresholds

In summary, the results of a classical genetic cross between a seizure susceptible strain and a seizure resistant strain indicate that the genotype responsible for resistance to MP-induced seizures is transmitted in a dominant fashion A quantitative assessment of the data suggests that an additive-dominance model adequately explains the pattern of generation responses

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