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Genetic Influences on GABA-Related Seizures[1]

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MARLEY, R J , D GAFFNEY AND J M WEHNER *Genetic influences on GABA-related seizures* PHARMACOL BIOCHEM BEHAV **24**(3) 665–672, 1986 —Genetic differences in susceptibility to chemically induced seizures were examined in various populations of mice Three inbred strains C57BL, DBA, and C3H and a heterogenous stock (HS) of mice were tested for sensitivity to seizures induced by 3-mercaptopropionic acid (MP) and flurothyl Dose response curves were constructed for each population of mice with each agent by quantitating latencies to specific stages of seizures Significant strain and sex differences were observed in sensitivity to MP-induced seizures Rank order from least sensitive to most sensitive was C57BL, HS, DBA, and C3H Sensitivity to flurothyl-induced seizures was also strain dependent, but the rank order of sensitivity was different than for MP The least sensitive strain was C57BL followed by HS, C3H, and DBA Analysis of GABA receptors in seven brain regions obtained from C57BL and DBA using ^{3}H-muscimol to measure high affinity GABA binding did not reveal significant differences in receptor number between these two strains It thus appears that different genetic factors influence susceptibility to MP-induced seizures than to flurothyl-induced seizures Furthermore, there is probably little correlation between the number of high affinity GABA receptors and sensitivity to seizures

Seizures GABA receptors Genetics

IT is well documented that seizures are controlled to some extent by genetic factors [12,19] Genetic influences on seizure activity can be assessed by examining the responses of inbred strains of mice to various seizure-inducing agents Previous studies have demonstrated that certain inbred strains of mice differ radically in their response to seizure-inducing environmental stimuli, such as loud sound [2] However, audiogenic seizures are dependent on particular stages of development, such that older mice do not demonstrate the sensitivity to audiogenic seizures that is exhibited in 20–30 day old mice [16] The results of strain comparisons of susceptibility to audiogenic seizures lead to the conclusion that DBA mice are very seizure-sensitive, while C57BL are a more resistant strain The sensitivity of a strain to seizures should be dependent on the stimuli inducing seizures, but there appears to be some correlation between sensitivity to audiogenic seizures at a particular developmental stage and sensitivity to electroconvulsive seizures [3]

Since the majority of seizure literature using the mouse as a model has dealt with audiogenic seizures, less is known about genetic influences on the susceptibility of adult mice to other seizure-producing agents In order to investigate genetic influences on seizure activity in adult mice and to examine ultimately the influence of various agents on latency to seizure, we have screened a series of inbred mouse strains (C57BL, DBA, and C3H) and a heterogenous stock of mice (HS) for sensitivity to two types of chemically-induced seizures

The inhibitory neurotransmitter, GABA (gamma-aminobutyric acid), has been implicated in the control of seizure activity [27] because a variety of convulsant agents alter GABA levels In the present study, two convulsant agents which have been shown to affect, either directly or indirectly, the GABAergic system, were utilized to induce seizures The first agent, 3-mercaptopropionic acid (MP), is a potent inhibitor of glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA formation The inhibition of GAD by MP is believed to be a critical factor in the development of seizures, since such treatment results in a decrease in the amount of GABA released into the synaptic cleft [9]

Another agent which induces seizure activity in rodents is flurothyl (bis[2,2,2-trifluroethyl] ether) Flurothyl has been used as an indicator of CNS excitability with the stages of seizure activity representing interactions between neural excitatory and inhibitory systems [6,25] Myoclonus is thought to reflect the beginning of a clonic-tonic discharge, which is usually extinguished by inhibitory mechanisms The

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latency to flurothyl-induced myoclonus, thus, should reflect overall CNS excitability Clonus, in contrast, appears to represent a breakdown of the inhibitory system and reflects the efficiency of neuronal inhibitory mechanisms It has been proposed that the clonic phase of flurothyl-induced seizure activity is produced by alterations in the GABAergic system because the potent GABA antagonist, bicuculline, reduces the latency to seizures produced by flurothyl [7] Therefore, flurothyl-induced seizures were used here as a second measure of CNS excitability

Biochemical differences in the GABAergic system among the various inbred strains of mice should lead to differences in seizure sensitivity Most biochemical studies have shown no differences in the GAD activity [23], or GABA uptake in mice differing in seizure sensitivity [22] However, Tiku [24] has reported that C57BL and DBA mice differ significantly in GABA postsynaptic receptors Using ^{3}H-GABA binding to characterize these receptors, it was observed that DBA mice had significantly higher binding affinities and lower receptor densities at both the high and low affinity sites of the GABA receptor than C57BL mice Regional brain analysis of these receptors revealed significantly less ^{3}H-GABA binding in hippocampus, striatum, diencephalon and cerebral cortex in DBA mice These results would suggest that differences in GABA receptors should result in differential seizure sensitivity to GABA altering agents Therefore, in our study, we examined whether the most seizure-sensitive and least seizure-sensitive inbred strains differed in their number of GABA receptors by measuring ^{3}H-muscimol binding in seven brain regions

METHOD

Animals

Equal numbers of male and female mice (60–90 days of age) from three inbred strains (C57/BL/6Ibg, DBA/2Ibg and C3H/2Ibg) and one outbred line (HS) were tested Mice which have been maintained in the breeding colony at the Institute for Behavioral Genetics for at least 20 generations were used in this study All mice were weaned at 25 days and housed in groups of like-sex littermates A 12-hr light/12-hr dark cycle (lights on 7 a m until 7 p m) was maintained and the animals were allowed free access to food (Wayne Lab Blox) and water

Chemicals

Flurothyl (Indoklon), bis[2,2,2-trifluroethyl] ether was a generous gift from Ohio Anesthetics, Murray Hill, NJ Three-mercaptopropionic acid and muscimol were purchased from Sigma Chemical Co ^{3}H-muscimol was purchased from NEN (specific activity = 11–20 Ci/mmol)

Procedure for 3-Mercaptopropionic Acid (MP)-Induced Seizures

Prior to measurement of seizure susceptibility, all animals were placed in an isolated, air conditioned, testing room with 34 Watt overhead fluorescent lighting for at least one hour

MP was administered intraperitoneally in 0 9% saline at a dose between 15 mg/kg and 45 mg/kg in a volume of 0 02 ml/g All solutions were prepared fresh daily Mice were placed individually in a 1 5 liter Pyrex jar for observation of seizure activity Latencies from injection of MP to clonus, wild running, tonus, and death were recorded to the nearest

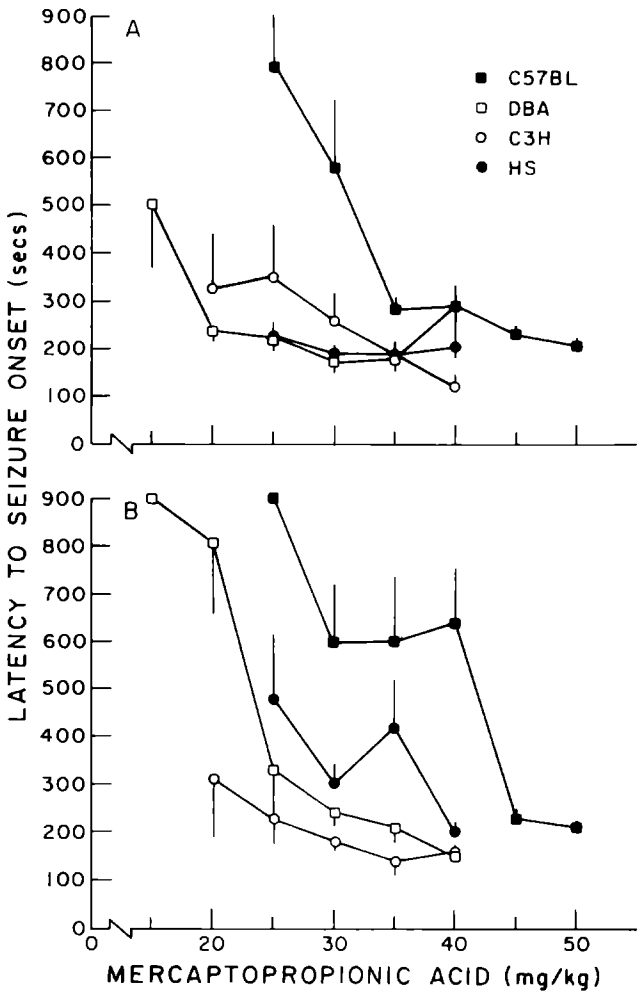

FIG 1 Latency to onset of seizures induced by 3-mercaptopropionic acid in various populations of mice Seizures were chemically-induced and measured as described under the Method section (mean±SEM, n=6) (A) Females, (B) males

5 seconds These stages of seizure activity were identified as follows (1) clonus was the loss of body posture, with convulsive movements in all extremities, (2) wild running consisted of a bout of uninhibited running and jumping, distinct from the running and jumping associated with a fear response, and (3) tonus was characterized by full caudal limb extension and respiratory arrest Multiple clonic seizures and bouts of wild running were often observed during the test periods, however, as tonus was often fatal, multiple occurrences of this stage were uncommon Tonus was always preceded by a bout of wild running

Observation periods were limited to 900 seconds and any animal not displaying seizure activity within this period was given a score of 900 seconds

Latencies from the time of MP injection to the onset of seizure activity and tonus were analysed initially using a three-way analysis of variance (ANOVA) to assess effects of population, sex, and dose of MP Sex differences within each population and population differences for each sex were further analyzed using two-way ANOVAs The rank ordering of the various populations was determined by employing Tukey's B post hoc tests following ANOVAs in which a

significant population effect was observed The analyses of population differences were conducted only on the doses of MP common to the four populations tested (25 mg/kg through 40 mg/kg) Analyses of sex differences within each population were conducted on all doses tested at which at least one of the sexes seized Differences between populations in the type of activity marking the onset of seizure activity and in the frequency of death immediately following tonic seizures were tested using the Chi-square test [20]

Procedure for Flurothyl-Induced Seizures

Susceptibility to seizures induced by inhalation of flurothyl (bis[2,2,2-trifluroethyl] ether) was determined by the method described by Smolen *et al* [21] Mice were placed individually into a modified 435 ml ointment jar with a screw cap lid which had a rubber septum in the middle A Plexiglas support located 15 mm below the septum contained a 1-cm square piece of Whatman No 1 filter paper Flurothyl was injected through the septum in doses ranging from 3 μl to 9 μl During the test sessions the chamber was kept air tight At the end of the session a water aspirator was used to flush the chamber with air through two glass tubes mounted in the lid Latencies to the first instance of myoclonus and clonus were recorded to the nearest second In this experiment, myoclonus was defined by the first spasm of the neck and head musculature, while clonus was characterized by the total loss of the animal's body posture Except at the highest doses of flurothyl tested, the components of seizure activity were easily distinguished Tonic seizures were not observed at any of the doses tested, however, this is probably reflective of the protocol which we employed to measure susceptibility to flurothyl-induced seizures Truitt *et al* [25] have reported induction of tonus with flurothyl, but only if the animal remained in the jar long enough to recover from the initial clonic convulsions To study the tonic phase of flurothyl-induced seizure activity, the agent must be delivered to the animal in a rapid flow of a high vapor concentration or administered intravenously As our primary interest was in the myoclonic and clonic phases of flurothyl-induced seizure activity, no attempt was made to specifically induce tonic seizures

To minimize the effects of circadian rhythms associated with the effects of flurothyl [25], all testing was done between 1000 and 1500 hr The mice remained in the test apparatus until the onset of clonus or for a maximum period of five min after flurothyl administration Any animal not displaying myoclonus and/or clonus within this time period was given a score of 300 sec

As in the MP-induced seizure tests, two separate three-way ANOVAs were used to assess the effects of population, sex, and dose of flurothyl on the latencies to myoclonus and clonus Two-way ANOVAs and Tukey's B post hoc tests were then employed to analyze further sex and population differences These analyses were conducted only on the doses of flurothyl (4 μl to 7 μl) common to the four populations tested

Procedure for GABA Receptor Analysis

GABA receptors were measured using the potent agonist muscimol ^{3}H-muscimol has been shown to bind to high affinity GABA postsynaptic receptors [4] Mice were placed in the laboratory for a three hour time period before they were sacrificed Animals were sacrificed by cervical dislocation and brains quickly removed and placed on ice Brains were

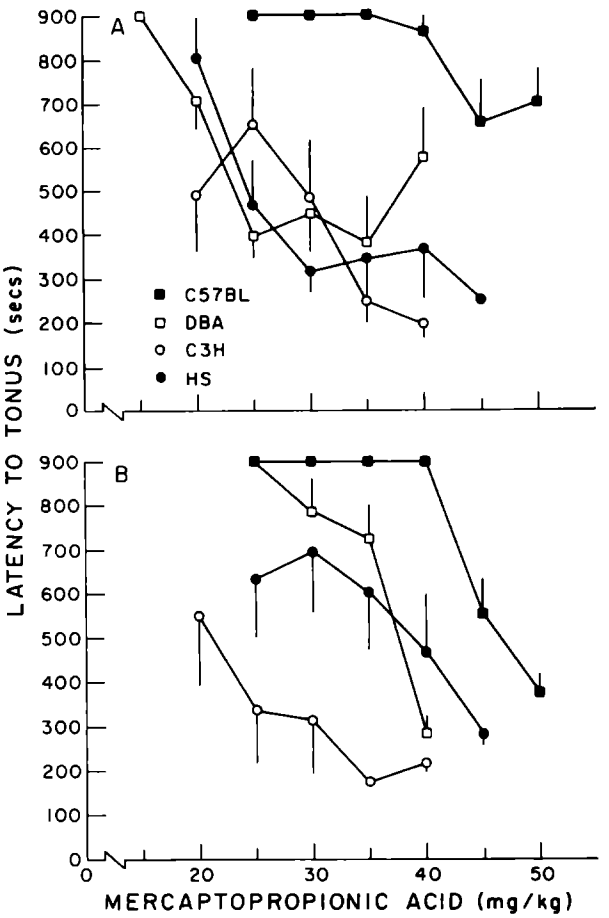

FIG 2 Latency to onset of tonus induced by 3-mercaptopropionic acid in various populations of mice Seizures were chemically-induced and measured as described under the Method section (mean±SEM, n=6) (A) Females, (B) males

dissected into seven parts cerebral cortex, midbrain, hindbrain, hippocampus, cerebellum, hypothalamus, and striatum Brain parts were weighed and homogenized in ice cold 0 32 M sucrose The remaining steps in tissue preparation were performed at 4°C The homogenate was centrifuged at 1,000×g for 10 min The supernatant was removed and centrifuged at 20,000×g for 20 min The resulting pellet was saved and frozen at −70°C for at least 18 hr to destroy GABA uptake sites

On the day of the assay, the pellet was thawed and resuspended in 75 volumes of 50 mM Tris Citrate (pH=7 1 at 4°C) by sonication at setting No 1 on a Branson model sonicator for 15 sec To this solution was added 10% Triton X-100 to give a final concentration of 0 05% (v/v) and the samples were incubated at 37°C for 30 min The extracted membranes were spun at 40,000×g for 10 min The resulting pellet was homogenized in Tris Citrate buffer immediately before addition to the receptor assay

^{3}H-Muscimol Assay

^{3}H-muscimol binding was done essentially as described by Frere *et al* [4] For larger brain parts including cortex, midbrain, and hindbrain both B_{max} and K_d were determined by competition experiments with varying concentrations of

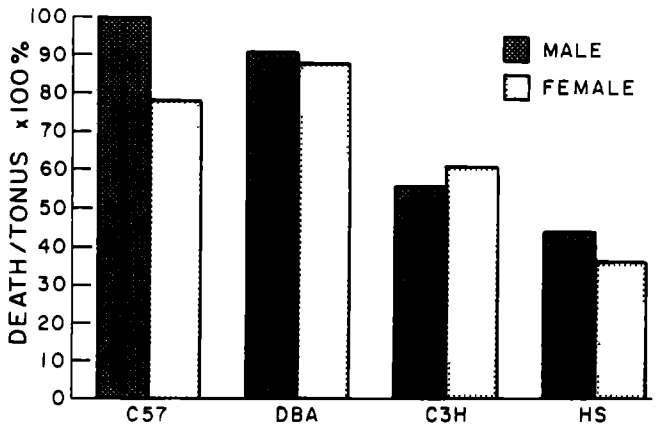

FIG 3 Percent lethality induced by 3-mercaptopropionic acid Seizures were chemically-induced and measured as described under the Method section Percent lethality is expressed as the number of deaths per number of tonic seizures

TABLE 1

CONVULSANT AND LETHAL ACTIVITY OF 3-MERCAPTOPROPIONIC ACID IN MICE

Population	CD100 (mg/kg)	% Death
C57 male	45	83
HS male	30	0
DBA male	25	0
C3H male	25	17
C57 female	35	0
C3H female	30	33
HS female	25	33
DBA female	20	50

unlabelled muscimol from 6–120 nM and 5 nM ^{3}H-muscimol For smaller brain parts determinations were performed at two concentrations of ^{3}H-muscimol, 2 5 nM and 50 nM Binding data was analyzed by Scatchard analysis using the EBDA program of McPherson modified for the IBM P C [11]

RESULTS

MP-Induced Seizures

Intraperitoneal injections of MP produced clonic and tonic seizures in each population of mice, but the sensitivity to MP varied among populations and between sexes The C57 strain was unique among the populations studied, in that both males and females of this strain were much more resistant to MP-induced seizures than were the other populations tested It was also observed that, in general, females were more sensitive to the effects of MP than were the males

The mean latencies to onset of seizure activity after administration of MP are presented in Fig 1A and B as a function of dose of MP The latency to onset of MP-induced seizure activity varied among the inbred strains and the HS stock The effects of dose, sex, and population were all highly significant, $F(3,160)=11\ 0$, $p<0\ 001$, $F(1,160)=9\ 4$, and $F(3,160)=51\ 0$, $p<0\ 001$, respectively Comparison of the latencies to seizure onset for the individual populations showed that while the C57 strain displayed the longest latency to seizure onset (Tukey's B, $p<0\ 01$), the DBA, C3H, and HS populations tested did not differ on this measure of seizure sensitivity Additionally, there were significant sex by population, $F(3,160)=5\ 5$, $p<0\ 01$, and population by dose, $F(9,160)=2\ 3$, $p<0\ 05$, interactions Separate analyses on data from the different populations and sexes were conducted to further elucidate the meaning of these interactions

The rank order of seizure sensitivity for the females was the same as that observed in the overall analysis, that is, the female C57 mice showed a greater latency to seizure onset than females from the other three populations, but there was no difference within these three, $F(3,80)=18\ 8$, $p<0\ 001$, Tukey's B, $p<0\ 01$ The HS males, however, were more resistant to the onset of MP-induced seizures than either the C3H or DBA males, but still less resistant than the C57 males, $F(3,80)=34\ 3$, $p<0\ 001$, Tukey's B, $p<0\ 05$

Female mice from the C57, DBA, and HS groups displayed a significantly shorter latency to the onset of MP-induced seizures than their male counterparts, $F(1,60)=6\ 2$, $p<0\ 02$, $F(1,60)=23\ 0$, $p<0\ 001$, and $F(1,40)=10\ 6$, $p<0\ 01$, respectively, while no sex differences were observed for the C3H population on this measure

The mean latencies to tonus after administration of MP are presented in Fig 2A and B As with the latency to seizure onset, significant main effects of dose, sex, and population were observed, $F(3,160)=6\ 1$, $p<0\ 001$, $F(1,160)=7\ 2$, $p<0\ 01$, and $F(3,160)=64\ 8$, $p<0\ 001$, respectively The C57 strain again exhibited the greatest resistance to MP, but in contrast to the similarity in the onset of seizures observed among the DBA, C3H, and HS groups, significant differences in latency to MP-induced tonus were observed for these three populations The overall rank-order for resistance to MP-induced tonic seizures was C57 > DBA > HS > C3H (Tukey's B, $p<0.01$) Significant population by sex interactions were again observed, $F(3,160)=8\ 9$, $p<0\ 001$

Separate ANOVAs of population differences for each of the sexes and sex differences for each of the populations were conducted These analyses revealed that both C57 females and C57 males were significantly more resistant to MP-induced tonic seizures than individuals of the same sex from the other populations and that DBA, C3H, and HS females and DBA and HS males did not significantly differ on this measure, $F(3,80)=34\ 0$, $p<0\ 001$, for the females and $F(3,80)=39\ 7$, $p<0\ 001$, for the males, Tukey's B, $p<0\ 01$ C3H males, however, were much less resistant than males from the other groups

Both DBA and HS males were more resistant to tonus than were their female counterparts, $F(1,50)=22\ 8$, $p<0\ 001$, and $F(1,40)=7\ 4$, $p<0\ 01$, respectively, while C57 females were found to be more resistant than C57 males, $F(1,60)=7\ 8$, $p<0\ 01$ C3H males and females did not differ in their latencies to MP-induced tonus

In addition to these differences in seizure threshold sensitivity, certain qualitative differences in seizure activity were also observed Most notable among these were differences in the type of behavior marking the onset of seizure activity and differences in the frequency of deaths immediately following MP-induced tonus

During audiogenic seizures and seizures induced by maxi-

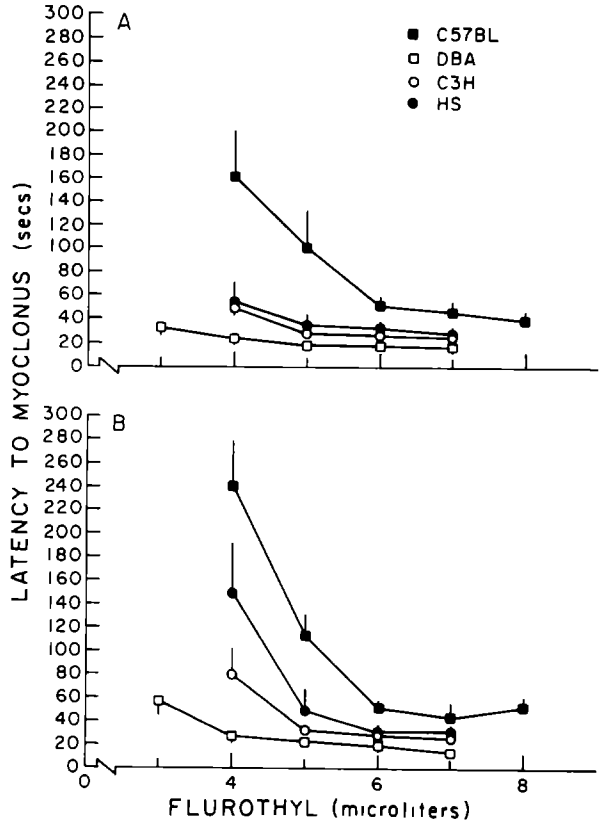

FIG 4 Latency to myoclonus induced by flurothyl in various populations of mice Seizures were chemically-induced and measured as described under the Method section (mean±SEM, n=8) (A) Females, (B) males

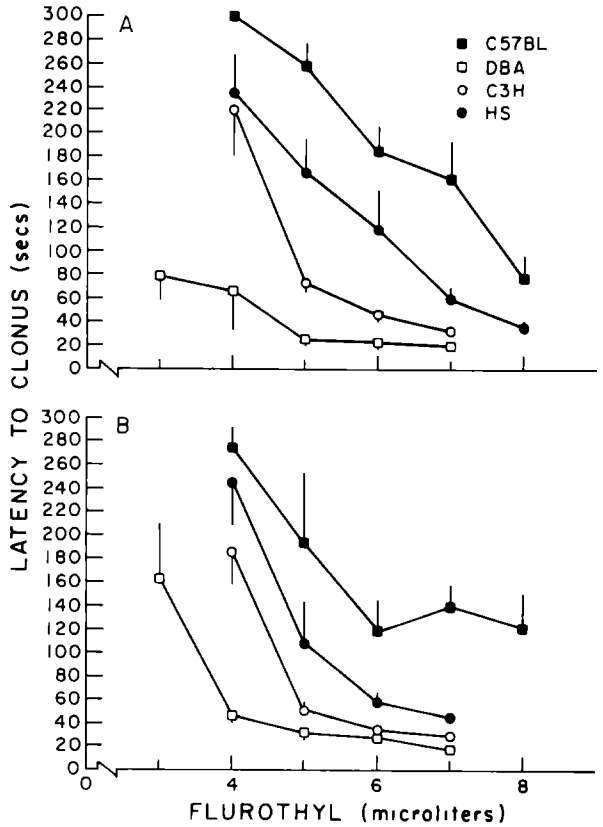

FIG 5 Latency to clonus induced by flurothyl in various populations of mice Seizures were chemically-induced and measured as described under the Method section (mean±SEM, n=8) (A) Females, (B) males

mal electroshock treatment, the onset of seizure activity is usually marked by a bout of wild running Among DBA and C57 mice, however, the onset of MP-induced seizure activity was always marked by clonic seizures, followed by one or more bouts of wild running For a significant number of the C3H and HS mice (16%) onset of seizure activity was characterized by a bout of wild running, rather than clonic seizures, $\chi^2(7)=18\ 6$, $p<0\ 05$ Within the various lines, no sex differences were observed on this parameter of MP-induced seizures

Differences between populations and sexes were even greater for the frequency of tonus-induced death associated with MP treatment In this study, almost all instances of lethality due to MP treatment immediately followed a tonic seizure, though all tonic seizures did not necessarily lead to death An analysis of the overall frequency of tonic seizure-induced death, without regard to dose, revealed that the various lines differ significantly on this measure, $\chi^2(3)=28\ 5$, $p<0\ 001$ The results of this analysis are presented graphically in Fig 3 This difference between populations did not seem to be directly related to the observed differential susceptibility to MP-induced seizures The minimum dose at which all animals in a particular population seized (CD100), as well as the percentage of animals dying at this dose (% Death) are presented in Table 1 No significant correlation was observed between these two parameters of seizure activity (r=0 40) Interestingly, when the two sexes were analyzed separately on these two parameters, substantial, but nonsignificant, correlations were observed The males showed a positive correlation (r= 92) and the females a negative correlation (r=− 93) between seizure sensitivity and lethality

Flurothyl-Induced Seizures

Inhalation of flurothyl resulted in myoclonic and clonic seizures in all populations of mice tested As with MP-induced seizures, there are definite genetic differences in sensitivity to flurothyl, with the C57 strain again being the most resistant to chemically induced seizures However, when flurothyl was the seizure-inducing agent, sex differences in seizure sensitivity within the various populations were observed in only the HS

The mean latencies to flurothyl-induced myoclonus as a function of flurothyl dose are presented in Fig 4A and B The main effects on myoclonus for dose, sex and population were all significant, $F(3,224)=31\ 9$, $p<0\ 001$, $F(1,224)=7\ 1$, $p<0\ 01$, and $F(3,224)=38.6$, $p<0\ 001$ The C57 strain was the most resistant, overall, to flurothyl-induced myoclonus, while the DBA strain was the least resistant (Tukey's B, $p<0\ 01$) No significant difference was observed between the HS line and the C3H strain on this measure of seizure sensitivity There were significant dose by population and dose

TABLE 2
MUSCIMOL RECEPTOR BINDING IN INBRED STRAINS OF MICE

A Affinities and Binding Capacities

Region	K_D (nM)		B_{max} (pmol/mg protein)	
	C57	DBA	C57	DBA
Cerebral Cortex	73 1 ± 14	23 1 ± 5	4 27 ± 0 6	4 8 ± 0 9
Midbrain	37 4 ± 8	49 0 ± 14	2 93 ± 0 4	2 8 ± 0 1
Hindbrain	16 5 ± 4	21 3 ± 4	1 80 ± 0 4	1 7 ± 0 1
Cerebellum	5 7 ± 1	4 6 ± 2	6 03 ± 1 2	5 9 ± 1 3

B Specific muscimol binding (pmol/mg protein)

Region	[^{3}H-Muscimol]	C57	DBA
Hippocampus	(2 5 nM)	0 03 ± 0 01	0 06 ± 0 03
	(50 0 nM)	0 37 ± 0 10	0 75 ± 0 38
Striatum	(2 5 nM)	0 07 ± 0 02	0 05 ± 0 01
	(50 0 nM)	0 69 ± 0 14	0 67 ± 0 24
Hypothalamus	(2 5 nM)	0 06 ± 0 03	0 05 ± 0 01
	(50 0 nM)	0 72 ± 0 29	0 82 ± 0 34

^{3}H-muscimol binding was measured as described under the Method section

A Saturation curves were generated using final concentrations of 6–120 nM muscimol B_{max} and K_D were determined by Scatchard analysis Data are expressed as mean ± S E M (n=4)

B ^{3}H-muscimol was assayed at 2 5 nM and 50 nM in various brain regions Data are expressed as the mean ± S E M (n=4)

by sex interactions, $F(3,224)=6\ 9$, $p<0\ 001$ and $F(3,224)=4\ 7$, $p<0\ 01$, respectively

Separate analysis of population differences for the two sexes revealed significant population differences within the individual sexes, $F(3,112)=20\ 3$, $p<0\ 001$, for the males, and $F(3,112)=19\ 2$, $p<0\ 001$, for the females The rank order of these populations, however, differed from that observed in the overall analysis for the females, but not the males Among the females, there was no difference between the C3H, HS, and DBA populations in their latencies to myoclonus, but the C57 females were significantly more resistant (Tukey's B, $p<0\ 01$)

While the overall analysis suggested the presence of significant sex differences for this measure, analysis of sex differences within the individual populations found males and females differing in their myoclonic latencies only within the HS population, where the males were observed to be much more resistant to flurothyl-induced myoclonus than the females, $F(1,56)=4\ 4$, $p<0\ 05$

The latency to clonus after administration of flurothyl is shown in Fig 5A and B. The main effects for dose, sex, and population were all highly significant, $F(3,224)=70\ 7$, $p<0\ 001$, $F(1,224)=12\ 0$, $p<0\ 001$, and $F(3,224)=104\ 5$, $p<0\ 001$ The overall rank order of the groups in latency to clonus was very similar to that observed for myoclonic latency The C57 strain again displayed a longer latency and the DBA a shorter latency to clonus However, in contrast to the latency to myoclonic seizures, C3H and HS populations were significantly different in their susceptibility to clonic seizures (Tukey s B, $p<0\ 01$), with the HS line being more resistant This rank order was the same for both males and females Significant dose by population interactions were also observed in this comparison, $F(9,224)=5\ 4$, $p<0\ 001$

Separate analyses for males and females revealed significant population effects for both sexes, $F(3,112)=45\ 9$, $p<0\ 001$, for the males, and $F(3,112)=58\ 7$, $p<0\ 001$, for the females With the exception of the C57 strain, no sex differences were observed in the various populations C57 females were more resistant to flurothyl-induced clonus than were C57 males, $F(1,56)=8\ 9$, $p<0\ 01$

Lethality was not observed in any of the populations tested after exposure to flurothyl

^{3}H-Muscimol Binding

High affinity GABA receptors can be measured in brain using the ligand ^{3}H-muscimol Seven brain regions were removed from DBA and C57BL mice and analyzed for receptor number and the affinity of these receptors for muscimol DBA and C57BL mice were selected for analysis because in three out of the four measures of seizure sensitivity using MP and flurothyl, DBA were the most seizure sensitive and C57BL were the most resistant The results of the GABA receptor analysis are shown in Table 2 There were no significant differences in B_{max} or K_d between DBA and C57BL in any brain region except in cerebral cortex, where DBA had significantly higher affinity for muscimol than C57BL ($p<0\ 05$)

DISCUSSION

It is well established that genetic factors influence the susceptibility of animals to seizures [12,19] Most often these studies in mice have been performed by inducing seizures with audiogenic stimuli However, audiogenic seizures are not only influenced by the genotype of mice, but also by developmental factors [5,16] Usually, only two inbred strains of mice have been compared for seizure sensitivity and these are most commonly C57BL and DBA [15–18] The results of these studies have indicated that C57BL are a resistant strain and DBA a sensitive strain The results of our studies also indicate that C57BL mice are highly resistant to the induction of seizures, deviating substantially from the other groups in their high threshold of seizure sensitivity DBA mice, in contrast, appear to be about equally sensitive compared to the C3H strain and the HS mice

The overall similarity of the DBA and C3H strains and the HS mice in their reponse to these chemically-induced seizures suggests that these groups reflect a more common level of seizure sensitivity in the mouse Adult DBA mice, thus, do not appear to be particularly seizure-sensitive to some commonly used chemical agents as might be predicted from their known developmental sensitivity to audiogenic stimuli Moreover, Deckard *et al* [3] have provided evidence that the developmental period of sensitivity to audiogenic and electroconvulsive seizures observed for DBA mice is also observed in other strains of mice The only strain not exhibiting a developmental period of high seizure sensitivity was the C57 strain, again indicating that it is the C57, not the DBA strain, that may be quite different in its ability to withstand seizure promoting activity These results suggest that some aspect of the functioning of the central nervous system in C57 mice is unique in its lack of response to seizure-inducing agents

By comparing the responses of the tested mouse populations on two different seizure-inducing agents that are thought to involve the GABAergic system, several conclusions relating to the genetic influences regulating sensitivity are evident First, in overall sensitivity the rank order comparison demonstrates that the same genetic factors are not regulating the two types of seizures While C57BL are always the more resistant strain, the rank order of the other strains varies according to seizure-inducing agent Furthermore, while females were found to be generally more sensitive to MP than males, only limited sex differences were observed when the seizure-inducing agent was flurothyl These dissimilarities in responses to the two agents provide support for the conclusion that the mechanisms by which the two agents operate may be quite different The GABAergic system has been shown to be involved in both types of seizures [7,9], but other neurotransmitter systems may also be altered by either, or both, of the chemical agents Alterations in other neurotransmitter systems may be a direct effect of the chemical agents, or may be the result of an imbalance created by a reduction in GABA For example, glutamate levels may be changed in response to the GAD inhibitor MP, whereas flurothyl working through a different mechanism may alter another neurotransmitter system Others have demonstrated that the cholinergic system, as well as the GABAergic system, may be involved in flurothyl-induced seizures [7]

In terms of lethality, the two agents are also different MP produced death in a high percentage of animals, but there was no significant correlation between seizure sensitivity and lethality This suggests that the seizures and death are independent aspects of MP's actions Mice appear to be more sensitive to the lethal effects of MP since previous studies with rats indicated only a 30% mortality rate after exposure to MP [10] Flurothyl, on the other hand, is much less lethal While other investigators have observed the infrequent occurrence of tonus-induced death, in mice, following flurothyl treatment (A Smolen, personal communication), no instances of tonus or death were observed in the present study

Some investigators have explored potential differences in the GABAergic system between seizure-prone mice such as the DBA strain and seizure-resistant mice such as C57BL Syles and Horton [23] analyzed GABA levels and GAD activity in mice which are either susceptible or resistant to audiogenic seizures They observed no differences between the two types of mice in the ability of these strains to synthesize or store GABA Spyrou *et al* [22] detected no differences between DBA and C57BL in synaptosomal ^{3}H-GABA uptake or in the binding of the uptake inhibitor, ^{3}H-nipecotic acid

Differences have been reported for Na^+-independent binding at the postsynaptic GABA receptor between DBA and C57BL mice Ticku [24] examined the binding of ^{3}H-GABA to high and low affinity binding sites in membranes prepared from C57BL with membranes prepared from DBA mice He observed significant differences between these two inbred lines in both numbers of receptors and their binding affinities These studies suggest that agents that alter GABA levels may produce differential seizure effects in various inbred strains because these lines differ in their number of postsynaptic receptors

The failure of our studies using ^{3}H-muscimol to measure differences in GABA receptor density and affinity in all brain regions except for the K_D difference in cortex may be reflective of a number of considerations, any of which could be relevant toward understanding the relationship between the GABAergic system and seizure susceptibility ^{3}H-Muscimol is believed to reflect primarily the high affinity state of the GABA receptor and is relatively insensitive to the lower affinity states [4]

A more important difference may be the preferential binding of muscimol to the bicuculline-sensitive, chloride-dependent, $GABA_A$ receptor subtype [1,13] ^{3}H-GABA binds to both the $GABA_A$ subtype and to the bicuculline-insensitive, baclofen-specific $GABA_B$ receptor subtype It is possible that the greater differences in the number of receptors observed using ^{3}H-GABA, as opposed to ^{3}H-muscimol, may reflect differences in $GABA_B$, but not $GABA_A$ receptor subtypes The evidence presented here would suggest that it may be important to distinguish between the "A" and "B" subtypes of GABA receptors in future analyses

Robertson [15] has examined DBA and C57BL for differences in benzodiazepine receptors and has observed increased ^{3}H-flunitrazepam binding in DBA during the time period which these mice are sensitive to audiogenic seizures, but they did not observe this difference in adult mice More recently, the opposite trend was observed by Olsen *et al* [14] They used receptor autoradiographic techniques to evaluate ^{3}H-flunitrazepam binding in brain slices from DBA, C57BL, and a seizure-resistant recombinant inbred line and demonstrated reduced binding in the midbrain region in the DBA brain The role of the GABA/BZ system in the genetic regulation of seizure sensitivity may be understood with further examination of GABA receptors subtypes, the endogenous factors which regulate the GABA/benzodiazepine receptor complex, and the coupled Cl^- channel Such studies would be facilitated by the use of multiple inbred strains that exhibit differential seizure sensitivity or by classical genetic analysis of crosses generated from a seizure-sensitive and seizure-resistant strain

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