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Potencies of Convulsant Drugs in Mice Selectively Bred for Resistance and Susceptibility to Nitrous Oxide Anesthesia

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To test the possibility that mice selectively bred for resistance (HI mice) and susceptibility (LO mice) to nitrous oxide anesthesia have general differences in central nervous system excitability, we examined the effects of six convulsant agents in these two lines of mice. Using high-pressure helium, flurothyl, pentylenetetrazol, strychnine, (+)-bicuculline, and picrotoxin, we induced convulsions in HI and LO mice. For all of the agents tested, HI mice were significantly more susceptible to convulsions than LO mice. LO mice convulsed at a 13 to 25 per cent higher dose of convulsant agent than did HI mice. In contrast, nitrous oxide requirements (as measured by the partial pressure of nitrous oxide required to abolish the righting reflex), were 49 to 65 per cent higher in HI mice. Therefore, the higher nitrous oxide requirement in HI mice is probably due, at least in part, to a generalized increase in central nervous system excitability. (Key words: Anesthetics, gases: nitrous oxide. Brain: convulsions; high pressure; flurothyl; pentylenetetrazol; picrotoxin; strychnine; bicuculline. Genetic factors. Potency: ED_{50}.)

MICE from a normal population were sorted into two groups according to their resistance (HI mice) or susceptibility (LO mice) to nitrous oxide anesthesia.[1,2] By breeding HI males with HI females and LO males with LO females, the difference in nitrous oxide requirement was enhanced in the offspring.[1,2] At the tenth generation of this selective breeding process, the difference between the nitrous oxide requirements of the two lines was more than 0.7 atm.[2]

One possible explanation for this difference is that the breeding process selected for general differences in central nervous system excitability. That is, a generalized increase in excitability in the HI mice might antagonize anesthesia in this line. If so, HI mice should be more sensitive to convulsants than LO mice. Therefore, using the two lines of mice, we induced convulsions in several ways. We applied 1) high-pressure helium, which produces convulsions in mice when pressures reach approximately 100 atm[3]; 2) flurothyl ($CF_3CH_2OCH_2CF_3$), which has been used clinically as a substitute for electroshock therapy[4]; 3) the commonly used convulsant pentylenetetrazol[5]; 4) strychnine, which acts as a competitive antagonist of the inhibitory transmitter glycine[5]; 5) (+)-bicuculline, which displaces the inhibitory transmitter gamma-aminobutyric acid (GABA) from neuronal binding sites[6,7]; and 6) picrotoxin, which also acts on the GABA system, but is thought to bind at a site distinct from the GABA recognition site.[8]

Materials and Methods

The procedure for selecting two lines of mice, one resistant (HI mice) and one susceptible (LO mice) to nitrous oxide anesthesia, has been described in an earlier report.[1] For the present experiments, we first determined for each mouse the nitrous oxide ED_{50} for the righting reflex, *i.e.*, the average of the partial pressures of nitrous oxide that just permits and just prevents the righting reflex. Our technique for determining the nitrous oxide ED_{50} also has been described previously.[1,2] We studied HI and LO mice (3 to 5 months of age) of the seventh through eleventh generations.

We used a 20-l hyperbaric chamber to study the high-pressure nervous syndrome.[9] For each high-pressure run, four mice (two HI and two LO) were placed in individual wire-mesh cages and were observed through clear plastic windows. Rectal probes were inserted into two additional restrained mice whose temperatures were maintained between 36.5° and 38.0° C by adjusting the chamber temperature through circulating-water heat exchangers. Chamber temperature was usually near 35° C. The hyperbaric chamber was initially flushed with oxygen for 10 min. After sealing the chamber, helium was added at a rate of one atm/min. Chamber gases were mixed by a motor-driven induction fan that circulated the gases through a soda-lime container to remove carbon dioxide. The effects of pressure were quantitated by measuring in each mouse the threshold pressure required to produce four behavioral end points: mild tremors, coarse tremors, convulsions, and death. Mild tremors were defined as being intermittent twitching of the neck and back muscles, during which the mouse usually assumed a hunched posture. Coarse tremors were characterized by shivering of the whole body and difficulty in coordinating movement. Convulsions consisted of seizures of such magnitude as to prevent the animal from righting itself, and were often immediately followed by a brief period of respiratory failure. The mouse was considered dead after complete absence of movement for one minute.

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TABLE 1. Mean (±SE) Pressure Thresholds (Atm) for End Points of the High-pressure Nervous Syndrome in Mice Bred for Resistance (HI) and Susceptibility (LO) to Nitrous Oxide

End Point	HI Mice (n = 6)	LO Mice (n = 6)
Mild tremors	54 ± 6.7	69 ± 0.9
Coarse tremors	73 ± 0.6	80 ± 1.0*
Convulsions	77 ± 1.7	89 ± 3.1†
Death	108 ± 3.4	125 ± 3.4†

We tested female mice of the seventh generation. Nitrous oxide ED_{50}s for the same groups of mice brought to high pressure were 2.03 ± 0.06 atm for the HI mice, and 1.36 ± 0.04 atm for the LO mice. LO mice differed from HI mice at significance levels of $*P < 0.005$ and $\dagger P < 0.01$.

TABLE 3. Mean (±SE) ED_{50}s for Convulsions and LD_{50}s (mg/kg) after Intraperitoneal Injections of Strychnine in Mice Bred for Resistance (HI) and Susceptibility (LO) to Nitrous Oxide

End Point	HI Mice (n = 58)	LO Mice (n = 53)
ED_{50} for clonic convulsions	1.02 ± 0.07	1.04 ± 0.08
ED_{50} for tonic convulsions	1.19 ± 0.05	1.38 ± 0.07*
Death	1.25 ± 0.06	1.43 ± 0.06*

Doses of strychnine ranged from 0.75 to 1.75 mg/kg. LO mice (21 females, 32 males) and HI mice (19 females, 39 males) of the tenth and eleventh generations were studied in these experiments. Nitrous oxide ED_{50}s for these same animals were 1.84 ± 0.02 atm (HI mice) and 1.14 ± 0.02 atm (LO mice). LO mice differed from HI mice at a significance level of $*P < 0.05$.

To study flurothyl-induced convulsions, we placed mice in groups of five into a 2000-ml Erlenmeyer flask. Mice were marked with different colors for identification. The flask was flushed with oxygen for 2 min, stoppered, and slowly evacuated to a pressure of about 0.75 atm. Known amounts of flurothyl were aspirated into the flask from microliter pipets, and the pressure in the flask was allowed to reach ambient pressure. Mice were observed for 15 min for clonic convulsions, which were defined as being seizures of such magnitude as to prevent the mouse from righting itself. At 15 min, using a glass syringe, we removed a sample of gas from the flask and determined the concentration of flurothyl by gas chromatography.

For measurement of convulsions induced by pentylenetetrazol, strychnine, (+)-bicuculline, and picrotoxin, mice were given intraperitoneal injections of each of these agents. For injections of pentylenetetrazol, strychnine hydrochloride, and picrotoxin, agents were dissolved in saline solution at concentrations of 10 mg/ml, 0.1 mg/ml, and 0.5 mg/ml, respectively. For injection of (+)-bicuculline, a solution (containing 0.5 mg/ml) was prepared by dissolving 25 mg of the agent in 5.0 ml 0.5 N HCl and 45 ml saline after adjusting the *p*H to 3.0 with 1 N NaOH. For 45 min after injection, mice were observed for clonic convulsions (loss of righting reflex), tonic convulsions (tonic extension of hind limbs), or death.

Each mouse was tested with one or two convulsant agents. If a mouse was examined twice, the testing periods were separated by at least 10 days. Since the incidence of convulsions may be affected by circadian rhythms,[10] approximately equal numbers of HI and LO mice were tested at the same time of day.

Using the method of Waud,[11] we calculated ED_{50}s for convulsions, LD_{50}s, and standard errors. Significance was assessed using an unpaired *t* test. All measurements were performed by a person who was unaware of the identity (HI or LO) of the mice and the dose given.

Results

HI mice were more sensitive to the excitation caused by high pressure than LO mice (table 1). Pressure thresholds for coarse tremors, convulsions, and death were significantly greater (10 to 16 per cent) in LO mice than in HI mice. The same HI animals had a 49 per cent higher N_2O ED_{50} than LO mice (table 1).

HI and LO mice of the eighth generation were exposed to concentrations of flurothyl ranging from 0.073 to 0.164 per cent atm. The HI mice (ten males, seven females)

TABLE 2. Mean (±SE) ED_{50}s for Convulsions and LD_{50}s (mg/kg) after Intraperitoneal Injections of Pentylenetetrazol in HI and LO mice Bred for Resistance (HI) and Susceptibility (LO) to Nitrous Oxide

End Point	HI Mice (n = 40)	LO Mice (n = 70)
ED_{50} for clonic convulsions	53.3 ± 2.2	53.3 ± 2.7
ED_{50} for tonic convulsions	62.5 ± 2.9	73.9 ± 2.0*
Death	64.4 ± 3.3	76.6 ± 1.9†

LO mice (38 females, 32 males) and HI mice (12 females, 28 males) of the eighth generation were used in these experiments. Nitrous oxide ED_{50}s for the same groups of animals were 1.89 ± 0.01 atm for the HI mice, and 1.22 ± 0.01 atm for the LO mice. LO mice differed from HI mice at significance levels of $*P < 0.005$ and $\dagger P < 0.001$.

TABLE 4. Mean (±SE) ED_{50}s for convulsions and LD_{50}s (mg/kg) after Intraperitoneal Injections of (+)-Bicuculline in Mice Bred for Resistance (HI) and Susceptibility (LO) to Nitrous Oxide

End Point	HI Mice (n = 59)	LO Mice (n = 61)
ED_{50} for clonic convulsions	3.46 ± 0.23	3.76 ± 0.24
ED_{50} for tonic convulsions	3.62 ± 0.24	4.51 ± 0.20*
Death	3.62 ± 0.24	4.51 ± 0.20*

Doses of bicuculline ranged from 2.0 to 6.0 mg/kg. LO mice (30 females, 31 males) and HI mice (30 females, 29 males) of the eleventh generation were examined. Nitrous oxide ED_{50}s for these same animals were 1.78 ± 0.01 atm (HI mice) and 1.12 ± 0.01 atm (LO mice). LO mice differed from HI mice at a significance level of $*P < 0.01$.

had a significantly ($P < 0.025$) lower ED_{50} for clonic convulsions (0.103 ± 0.007 per cent atm) than did LO mice (23 males, 17 females; 0.125 ± 0.005 per cent atm). Nitrous oxide requirements for these same mice were 2.02 ± 0.04 atm (HI) and 1.25 ± 0.02 atm (LO). At the doses examined, only one animal—a HI mouse exposed to 0.122 per cent atm flurothyl—had a tonic convulsion.

HI and LO mice given intraperitoneal injections of pentylenetetrazol (at doses ranging from 40 to 90 mg/kg) had identical ED_{50}s for clonic convulsions (table 2). However, the ED_{50} for tonic convulsions and the LD_{50} in LO mice were significantly higher (approximately 18 per cent) than in HI mice (table 2).

Similarly, ED_{50}s for tonic convulsions and LD_{50}s for mice injected with strychnine (table 3), (+)-bicuculline (table 4), and picrotoxin (table 5) were significantly greater (13 to 25 per cent) in LO mice than in HI mice. However, no significant differences in ED_{50}s for clonic convulsions induced by these three agents could be detected between the HI and LO mice (tables 3–5).

At a given dose of convulsant, the time for onset of the seizures also tended to be shorter in the HI than in the LO mice. However, since all of the mice did not convulse at most of the doses examined, this presents a problem in interpreting the latency times in those animals that did convulse. Nevertheless, at the highest doses of each agent examined, almost all of the animals convulsed, and the time for onset of tonic seizures was from 12 to 266 per cent greater for the LO mice than for the HI mice (table 6). However, for the limited number of mice tested at these highest doses, the differences in onset times to tonic convulsions were significantly greater only for picrotoxin (table 6).

TABLE 5. Mean (±SE) ED_{50}s for Convulsions and LD_{50}s (mg/kg) after Intraperitoneal Injections of Picrotoxin in Mice Bred for Resistance (HI) and Susceptibility (LO) to Nitrous Oxide

End Point	HI Mice (n = 103)	LO Mice (n = 98)
ED_{50} for clonic convulsions	3.84 ± 0.45	4.05 ± 0.50
ED_{50} for tonic convulsions	6.62 ± 0.35	7.58 ± 0.32*
Death	7.39 ± 0.33	8.34 ± 0.34*

Doses of picrotoxin ranged from 3.0 to 12.0 mg/kg. LO mice (52 females, 46 males) and HI mice (45 females, 58 males) of the tenth and eleventh generations were examined. Nitrous oxide ED_{50}s for these same animals were 1.86 ± 0.01 atm (HI mice) and 1.13 ± 0.01 atm (LO mice). LO mice differed from HI mice at a significance level of $*P < 0.05$.

TABLE 6. Mean (±SE) Onset Time of Tonic Seizures Induced by Four Convulsant Agents

Agent	Dose (mg/kg)	Onset Time of Tonic Seizures (min)	
		HI Mice	LO Mice
Pentylenetetrazol	90	4.5 ± 2.3 (6)*	12.0 ± 3.0 (6)
Strychnine	1.75	2.9 ± 0.2 (6)	4.2 ± 0.9 (5)†
(+)-Bicuculline	6.0	2.5 ± 1.0 (6)	2.8 ± 0.3 (6)
Picrotoxin	12.0	12.8 ± 0.4 (5)	19.2 ± 0.7 (5)‡

* Numbers in parentheses are the number of mice that had tonic convulsions at the dose listed. All those injected had convulsions.

† Only five of six LO mice injected with 1.75 mg strychnine/kg had tonic convulsions.

‡ LO mice significantly different from HI mice at $P < 0.001$.

Discussion

Our results confirm the supposition that HI mice would be more susceptible to the effects of convulsants than LO mice. Several previous studies also have demonstrated that a proneness to convulsions may depend upon genetic factors.[12–14] Examination of the high-pressure nervous syndrome in ten strains of mice showed that the mean convulsion threshold at a compression rate of 40 atm/h varied between 66 and 97 atm, depending upon the strain examined.[15] Pentylenetetrazol induces bursts of high-amplitude spindles in the cortical electroencephalograms of DBA and C3H mice, but not in C57BL/6 and BALB/c mice.[13] In addition, convulsions have been examined in two mouse strains bred for resistance and sensitivity to alcohol.[16] The alcohol-sensitive strain had a much milder alcohol withdrawal reaction than did the alcohol-resistant strain, but the two strains did not differ in vulnerability to tonic convulsions elicited by pentylenetetrazol.[16]

The molecular basis for the differences in convulsion sensitivity between HI and LO mice is unknown. Several mechanisms have been proposed for the effects of each of the agents tested. The application of high pressure is thought to initiate convulsions by compressing critical regions of neuronal membranes,[17] and flurothyl is thought to selectively block inhibitory over excitatory transmission.[18] However, the details of these processes remain speculative.

The pharmacologic bases for the convulsive action of the other agents are perhaps better defined. Pentylenetetrazol exerts a postsynaptic blockade on inhibitory processes mediated by GABA,[19,20] and it may produce excitatory effects by this mechanism. Such a blockade from pentylenetetrazol could result from a reduction in GABA-induced chloride conductance.[21] Bicuculline exerts its effect by directly competing with GABA for its binding site.[6,7,22] Picrotoxin also appears to interfere with GABA transmission,[7,8,22] but noncompetitively inhibits the conductance increase induced by GABA,[22,23] and is thought to act at the ionophore site in the GABA receptor complex.[7] Strychnine acts as a competitive antagonist of the inhibitory transmitter glycine.[5,23]

Thus, the six means by which we induced convulsions probably have quite different mechanistic bases for their

action. Nevertheless, all give approximately the same difference (13 to 25 per cent) in the ED_{50}s for convulsions in the LO *vs.* HI animals. This suggests that the increased susceptibility to convulsions in the HI mice cannot be explained as simply an alteration in a single specific receptor. It may be that the selective breeding process gave rise to many small differences in neurotransmitter systems and in neuronal membrane properties that might account for the differences in excitability in the HI and LO mice.

Although we observed significant differences between the two lines of mice in ED_{50}s for tonic convulsions for pentylenetetrazol, strychnine, bicuculline, and picrotoxin, no significant differences in ED_{50}s for clonic convulsions could be detected for these agents (tables 2–5). At present, we have no explanation for this phenomenon.

In summary, mice selectively bred for resistance to nitrous oxide anesthesia are slightly but significantly more sensitive to convulsant agents than those bred for susceptibility to nitrous oxide anesthesia. Therefore, the higher nitrous oxide requirement might be explained, at least in part, by a generalized alteration in central nervous system excitability.

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