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Potencies of Inhaled Anesthetics and Alcohol in Mice Selectively Bred for Resistance and Susceptibility to Nitrous Oxide Anesthesia

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A selective breeding process designed to produce mice resistant (HI mice) and susceptible (LO mice) to nitrous oxide anesthesia was continued through 10 generations. At the tenth generation, the nitrous oxide requirements of the HI and LO mice (as measured by the partial pressure of nitrous oxide required to abolish the righting reflex) were separated by more than 0.7 atm. The HI mice also had a higher anesthetic requirement for cyclopropane, enflurane, isoflurane, halothane, and methoxyflurane, as measured by response to a tail-clamp stimulus. HI mice given an intraperitoneal injection of ethanol (4 g/kg) had 44 per cent shorter sleep times and 12 per cent higher blood alcohol levels upon awakening than did LO mice. For nitrogen, argon, cyclopropane, isoflurane, enflurane, halothane, and methoxyflurane, we determined the doses at which the righting reflex was abolished in HI and LO mice. The separation in righting-reflex ED_{50}s between these two lines was inversely related to the lipid solubility of the anesthetic. For the most lipid-soluble anesthetic, methoxyflurane, no significant differences in potency, as measured by the righting-reflex ED_{50}, could be detected between the HI and LO mice. In contrast, the separation in anesthetic requirements, as measured by the tail-clamp ED_{50}, was approximately the same for each of the anesthetics tested. (Key words: Alcohol. Anesthetics, gases: argon; cyclopropane; nitrogen; nitrous oxide. Anesthetics, volatile: enflurane; halothane; isoflurane; methoxyflurane. Genetic factors. Potency anesthetic: ED_{50}; LD_{50}; righting reflex; tail-clamp ED_{50}. Theories of anesthesia: lipid solubility.)

WE PREVIOUSLY REPORTED that mice from a normal population could be separated into two groups with reproducibly high or reproducibly low nitrous oxide requirements.[1] Nitrous oxide potencies were determined by measuring the partial pressure of nitrous oxide needed to abolish the righting reflex. By breeding mice having high anesthetic requirements with one another, and those having low anesthetic requirements with one another, the separation in nitrous oxide requirement was enhanced in the offspring.[1] We continued this selective breeding for resistance and susceptibility to nitrous oxide through ten generations, and have measured the potencies of other anesthetics in these mice using both the righting reflex and the response to tail-clamp stimulus.

Materials and Methods

Selective breeding of mice for resistance (HI mice) and susceptibility (LO mice) to nitrous oxide and the procedure for determining nitrous oxide ED_{50}, (effective doses in 50 per cent of the subjects) have been described in an earlier report.[1] We continued this selective breeding through ten generations by mating mice having the lowest nitrous oxide requirements with one another and those having the highest requirements with one another. We then tested the righting-reflex ED_{50}s for nitrous oxide in the offspring. As in our earlier study, we avoided brother-sister matings for mice in the sixth through tenth generations; one male was mated with two or three females. Mice were 7 to 12 weeks of age when tested for righting-reflex ED_{50}s for nitrous oxide.

The anesthetic potencies of methoxyflurane, halothane, isoflurane, enflurane, cyclopropane, argon, and nitrogen also were measured in HI and LO mice. When anesthetic potency was determined by assessing the ability to pass a righting-reflex test, unrestrained mice (as many as eight for each determination) were placed in individual wire-mesh cages that could be rotated at 4 rpm in a 20-l hyperbaric chamber.[2] In two additional restrained mice, rectal temperatures were monitored and maintained between 36.5° and 38.0° C by adjusting the chamber temperature using circulating-water heat exchangers. Chamber temperature was usually between 33° and 35° C. Carbon dioxide was removed by circulating the chamber gases through a soda-lime container.

For the volatile agents, controlled amounts of anesthetic were delivered by temperature-compensated vaporizers in a flow of oxygen (4 l/min), and were passed continuously through the chamber during the entire experimental period. An initial 1-h period of equilibration at approximate levels of 1.80 per cent atm for enflurane, 0.80 per cent atm for halothane, and 0.90 per cent atm for isoflurane was imposed before testing the righting reflex. For methoxyflurane, the initial equilibration period consisted of 0.30 per cent atm methoxyflurane for 30 min, followed by 0.20 per cent atm methoxyflurane for 1 h. After the period of equilibration, the mice were subjected to five complete turns of the rotator. Animals

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TABLE 1. Righting-reflex ED_{50}s of Inhaled Agents in Mice Selectively Bred for Resistance (HI) and Susceptibility (LO) to Nitrous Oxide Anesthesia

Anesthetic	Generation	Sex	Righting-reflex ED_{50} (atm)		N_2O Righting-reflex ED_{50} for Same Group of Mice (atm)		n	
			HI Mice	LO Mice	HI Mice	LO Mice	HI	LO
Nitrogen	10	F	53.1 ± 1.7	32.0 ± 1.5*	1.83 ± 0.02	1.24 ± 0.02*	16	17
		M	48.5 ± 1.1	29.7 ± 0.9*	1.84 ± 0.02	1.16 ± 0.01*	21	35
		TOTAL	50.5 ± 1.0	30.4 ± 0.8*	1.84 ± 0.02	1.19 ± 0.01*	37	52
Argon	9	F	26.7 ± 0.7	16.4 ± 0.3*	1.73 ± 0.04	1.24 ± 0.02*	16	15
Cyclopropane	8	F	0.168 ± 0.005	0.115 ± 0.004*	2.09 ± 0.03	1.23 ± 0.06*	15	31
		M	0.167 ± 0.005	0.112 ± 0.003*	1.88 ± 0.04	1.21 ± 0.02*	15	26
		TOTAL	0.168 ± 0.004	0.113 ± 0.002*	1.99 ± 0.03	1.22 ± 0.02*	30	57
Isoflurane	9	F	0.00720 ± 0.00030	0.00625 ± 0.00019†	1.75 ± 0.02	1.24 ± 0.02*	25	21
Enflurane	6	F	0.0140 ± 0.0004	0.0118 ± 0.0004*	1.96 ± 0.02	1.37 ± 0.02*	33	23
Halothane	8	F	0.00827 ± 0.00072	0.00611 ± 0.00022*	2.18 ± 0.02	1.25 ± 0.02*	5	18
		M	0.00689 ± 0.00043	0.00650 ± 0.00026	1.78 ± 0.02	1.23 ± 0.03*	8	23
		TOTAL	0.00742 ± 0.00041	0.00636 ± 0.00017‡	1.94 ± 0.06	1.24 ± 0.02*	13	41
Methoxyflurane	10	F	0.00233 ± 0.00006	0.00219 ± 0.00013	1.84 ± 0.02	1.24 ± 0.02*	20	19
		M	0.00223 ± 0.00007	0.00225 ± 0.00008	1.82 ± 0.02	1.16 ± 0.01*	25	37
		TOTAL	0.00227 ± 0.00005	0.00222 ± 0.00007	1.83 ± 0.02	1.19 ± 0.01*	45	56

Values are means ± SE; LO mice differ from HI mice at significance levels of $*P < 0.001$, $\dagger P < 0.025$, and $\ddagger P < 0.01$.

that rolled over two or more times were considered anesthetized. The partial pressure of the anesthetizing gas was then increased or decreased in 20 per cent steps from the initial concentration tested, and the mice were tested again for their ability to right themselves after a 30-min period of equilibration. These changes and the periods of equilibration were continued until a high concentration was obtained at which all animals failed the righting-reflex test, and until a low concentration was obtained at which all animals passed the test.

Righting-reflex ED_{50}s were determined for cyclopropane, argon, and nitrogen using essentially the same procedure as for the volatile agents, except that the experiments were performed in a sealed chamber in the presence of one atmosphere of oxygen. Mice were initially exposed to about 0.17 atm cyclopropane, 16 atm argon, or 30 atm nitrogen, and were exposed to this partial pressure for 30 min before the righting reflex was tested. Partial pressures were thereafter altered in 20 per cent increments or decrements followed by 15-min periods of equilibration before the righting reflex was tested.

The anesthetic potencies of cyclopropane, enflurane, isoflurane, halothane, and methoxyflurane also were determined in HI and LO mice by assessing their response to a tail clamp. Mice were placed in individual Plexiglas chambers. Groups of eight mice were given one of the anesthetic agents in oxygen (4 l/min). Rectal temperature was monitored in each mouse and kept between 36.5° and 38.0° C with heat lamps. Mice were initially equilibrated with approximately 0.20 atm cyclopropane for 30 min, or with 2.3 per cent atm enflurane, 1.5 per cent atm isoflurane, or 1.4 per cent atm halothane for 1 h. For methoxyflurane, the initial equilibration consisted of 0.45 per cent atm methoxyflurane for 30 min, followed by 0.30 per cent atm methoxyflurane for 1 h. A tail clamp (alligator clip) was applied to the base of the tail and was oscillated for 60 s. Mice were observed for movement in response to the stimulation. Anesthetic requirement was determined for each mouse by altering anesthetic concentration in 20 per cent steps (*i.e.*, by changing the concentration 20 per cent from the preceding one); by permitting a subsequent period of equilibration at each step (15 min for cyclopropane and 30 min for the volatile anesthetics); and then by examining for response to tail-clamp stimulation.

Concentrations of nitrous oxide were measured using a gas chromatograph with a thermal conductivity detector; concentrations of volatile agents were analyzed using a gas chromatograph with a flame ionization detector. Cyclopropane concentration was measured with a Beckman® LB-1 infrared analyzer. Partial pressures of argon and nitrogen were calculated by measuring the partial pressure of oxygen in the chamber with a Beckman® E2 oxygen analyzer, and by subtracting this value from the total pressure. Anesthetic requirement (ED_{50}) was calculated for each mouse by averaging the concentrations that just abolished or just allowed the righting reflex, or that just prevented and permitted movement in response to tail-clamp stimuli. The ED_{50} and standard error for a group of mice were calculated from the individual crossover values. If a mouse died during the measurement of

TABLE 2. Tail-clamp ED_{50}s of Cyclopropane, Isoflurane, Enflurane, Halothane, and Methoxyflurane in Mice Selectively Bred for Resistance (HI Mice) and Susceptibility (LO Mice) to Nitrous Oxide Anesthesia

Anesthetic	Generation	Sex	Tail-clamp ED_{50} (atm)		N_2O Righting-reflex ED_{50} for Same Group of Mice (atm)		n		$\frac{\left(\frac{\text{HI Tail-clamp } ED_{50}}{\text{LO Tail-clamp } ED_{50}}\right) - 1}{\left(\frac{\text{HI } N_2O\ ED_{50}}{\text{LO } N_2O\ ED_{50}}\right) - 1}$
			HI Mice	LO Mice	HI Mice	LO Mice	HI	LO	
Cyclopropane	8	F	0.266 ± 0.007	0.197 ± 0.008*	2.10 ± 0.03	1.22 ± 0.02*	13	26	
		M	0.266 ± 0.008	0.218 ± 0.006*	1.88 ± 0.04	1.24 ± 0.03*	14	25	
		TOTAL	0.266 ± 0.005	0.208 ± 0.005*	1.98 ± 0.03	1.23 ± 0.02*	27	51	0.46
Isoflurane	5	F	0.0157 ± 0.0006	0.0144 ± 0.0008	1.62 ± 0.05	1.24 ± 0.03*	14	24	
		M	0.0167 ± 0.0004	0.0146 ± 0.0006†	1.57 ± 0.04	1.23 ± 0.02*	21	30	
		TOTAL	0.0163 ± 0.0004	0.0145 ± 0.0004‡	1.59 ± 0.04	1.23 ± 0.02*	35	54	0.42
Enflurane	6	F	0.0252 ± 0.0006	0.0217 ± 0.0008*	1.96 ± 0.02	1.37 ± 0.03*	24	18	0.37
Halothane	11	F	0.0124 ± 0.0007	0.0105 ± 0.0006§	1.89 ± 0.02	1.14 ± 0.02*	16	15	
		M	0.0122 ± 0.0004	0.0099 ± 0.0005*	1.87 ± 0.02	1.15 ± 0.02*	29	24	
		TOTAL	0.0122 ± 0.0004	0.0101 ± 0.0004*	1.88 ± 0.02	1.15 ± 0.01*	45	39	0.33
Methoxyflurane	10	F	0.00513 ± 0.00020	0.00367 ± 0.00045¶	1.84 ± 0.04	1.24 ± 0.02*	11	15	
		M	0.00433 ± 0.00043	0.00359 ± 0.00023	1.83 ± 0.03	1.18 ± 0.02*	12	25	
		TOTAL	0.00471 ± 0.00030	0.00362 ± 0.00022‡	1.84 ± 0.02	1.20 ± 0.01*	23	40	0.56

Values are means ± SE; LO mice differ from HI mice at significance levels of $*P < 0.001$, $\dagger P < 0.01$, $\ddagger P < 0.005$, $\S P < 0.05$, and $\P P < 0.025$.

ED_{50}, data for that animal were not included in the calculations. Significance was calculated with an unpaired t test. Measurements of anesthetic ED_{50} were performed by an observer who was unaware of whether HI or LO mice were being tested.

The LD_{50}s for nitrous oxide also were determined in several of the HI and LO animals. Mice were initially exposed to 1.45 atm of nitrous oxide for 30 min in the hyperbaric chamber. All mice survived exposure to nitrous oxide at this partial pressure. The partial pressure was then raised in 0.11-atm increments; the mice were examined after a 15-min period of equilibration at each step. For each mouse, an LD_{50} was calculated by averaging the partial pressure of nitrous oxide at death and the highest partial pressure of nitrous oxide at which the mouse survived. Measurements of LD_{50} were performed in the presence of one atmosphere oxygen under controlled temperature conditions. These experiments also were carried out in a blind fashion, *i.e.*, the observer did not know if animals were HI or LO mice.

HI and LO mice also were tested for their susceptibility to alcohol. Mice were given intraperitoneal injections of ethanol [20 per cent (w/v) in saline] at a dose of 4 g ethanol/kg. The time between injection and loss of the righting reflex was designated as the sleep onset time. The time between the loss and recovery of the righting reflex was designated the sleep time. Mice were considered to have regained the righting reflex if they were able to right themselves completely three times within 30 s. Rectal temperatures were monitored and maintained between 36.5° and 38.0° C. On awakening, mice were killed by cervical dislocation, and blood was removed from the inferior vena cava. Blood alcohol levels were determined using an enzymatic procedure.[3]

In another set of studies, rectal temperatures of awake HI and LO mice were measured by inserting a thermistor probe (lubricated in glycerol) to a depth of 2.5 cm; the temperature measurement was taken 30 s after insertion.

Results

Progressively greater separations in nitrous oxide ED_{50}s were obtained between HI and LO mice with each generation of selective breeding. At the tenth generation, nitrous oxide righting-reflex ED_{50}s (± SE) were 1.93 ± 0.02 and 1.18 ± 0.02 atm for the HI (n = 33) and LO (n = 31) female offspring, respectively. In males of the tenth generation, nitrous oxide righting-reflex ED_{50}s were 1.86 ± 0.02 atm (n = 32) for the HI mice and 1.15 ± 0.02 atm (n = 43) for the LO mice. The nitrous oxide righting-reflex ED_{50}s for the population extremes of the HI and LO mice (those selected as breeders to produce the following generations) were separated

TABLE 3. Sleep Onset Times, Sleep Times, and Blood Alcohol Levels on Awakening for HI and LO Mice Given an Intraperitoneal Injection of 4 g Ethanol/kg*

	HI Mice (n = 25)	LO Mice (n = 32)
Sleep onset time(s)	137 ± 13	144 ± 24
Sleep time (min)	56.6 ± 3.3	100.5 ± 6.2†
Blood ethanol level upon awakening (mg/ml)	4.16 ± 0.06	3.71 ± 0.06†
Nitrous oxide righting-reflex ED_{50} (atm)	1.74 ± 0.03	1.30 ± 0.02†

Values are means ± SE.
* We used male mice from the sixth generation bred for resistance (HI mice) and susceptibility (LO mice) to nitrous oxide anesthesia.
† LO mice differed from HI mice at a significance level of $P < 0.001$.

by approximately 1.0 atm in nitrous oxide requirement for animals of the seventh through tenth generations.

After nitrous oxide righting-reflex ED_{50}s were measured in all of the offspring of a given generation, the mice with the highest and lowest nitrous oxide requirements were selected to produce the following generation. The remaining mice were tested (before 5 months of age) to determine the potencies of other anesthetics for the HI and LO lines. Righting-reflex ED_{50}s in HI and LO mice for seven different inhaled agents are presented in table 1. Also presented in this table are the nitrous oxide ED_{50}s for the same group of mice tested with a given anesthetic. For each of the anesthetics tested except methoxyflurane, the HI mice had a significantly greater righting-reflex ED_{50} than did LO mice (table 1). In addition, when tested using the tail-clamp procedure, the HI mice had significantly greater requirements for cyclopropane, isoflurane, enflurane, halothane, and methoxyflurane (table 2).

HI mice were less sensitive to an intraperitoneal injection of alcohol (4 g ethanol/kg) than LO mice (table 3). The HI mice had 44 per cent shorter sleep times and 12 per cent higher blood alcohol levels upon awakening. Sleep onset times of the HI and LO mice were not significantly different (table 3).

TABLE 4. Nitrous Oxide LD_{50}s in Mice Selectively Bred for Resistance (HI) and Susceptibility (LO) to Nitrous Oxide Anesthesia*

	N_2O LD_{50} (atm)			
	HI Mice	n	LO Mice	n
Females	2.23 ± 0.12	5	1.96 ± 0.04†	17
Males	2.16 ± 0.08	8	2.08 ± 0.08	21
Total population	2.19 ± 0.08	13	2.03 ± 0.04	38

Values are means ± SE.
* We used mice from the eighth generation.
† LO mice differed from HI mice at a significance level of $P < 0.025$.

Nitrous oxide LD_{50}s were determined in 5-month-old mice of the eighth generation (table 4). LD_{50}s tended to be greater for the HI mice, and a significant difference was found for the females. However, for the number of mice tested, no significant difference in LD_{50} could be detected between the HI and LO male mice or the combined LD_{50}s for the males and females.

Average (± SE) rectal temperatures of awake HI mice (n = 27) and LO mice (n = 31) of the eighth generation did not differ significantly: HI, 37.8° ± 0.1° C; LO, 37.7° ± 0.1° C.

Discussion

The difference in nitrous oxide requirements between the HI and LO mice has tended to increase with the number of generations. One factor that may limit the enhancement of this separation in future generations is that in HI mice, the ED_{50} of nitrous oxide is close to its LD_{50} (table 4). Indeed, several of the HI offspring died during the testing of nitrous oxide ED_{50}. For most generations, fewer than 7 per cent of the HI mice died during this testing. However, in the ninth generation, 30 per cent of the HI offspring died during the testing procedure. Thus, further increases in the nitrous oxide ED_{50} of the HI mice would have to be accompanied by a parallel increase in the LD_{50}.

TABLE 5. Relative Potencies of Inhaled Anesthetics, as Measured by the Righting Reflex, in Mice Selectively Bred for Resistance (HI) and Susceptibility (LO) to Nitrous Oxide Anesthesia

Anesthetic	$\left(\frac{\text{HI anesthetic } ED_{50}}{\text{LO anesthetic } ED_{50}}\right) - 1$	$\left(\frac{\text{HI } N_2O\ ED_{50}}{\text{LO } N_2O\ ED_{50}}\right) - 1$	$\frac{\left(\frac{\text{HI anesthetic } ED_{50}}{\text{LO anesthetic } ED_{50}}\right) - 1}{\left(\frac{\text{HI } N_2O\ ED_{50}}{\text{LO } N_2O\ ED_{50}}\right) - 1}$
Nitrogen	0.66	0.55	1.20
Argon	0.63	0.40	1.58
Nitrous oxide	NA*	NA*	1.00
Cyclopropane	0.49	0.63	0.78
Isoflurane	0.15	0.41	0.37
Enflurane	0.19	0.43	0.44
Halothane	0.17	0.56	0.30
Methoxyflurane	0.02	0.54	0.04

* Not applicable.

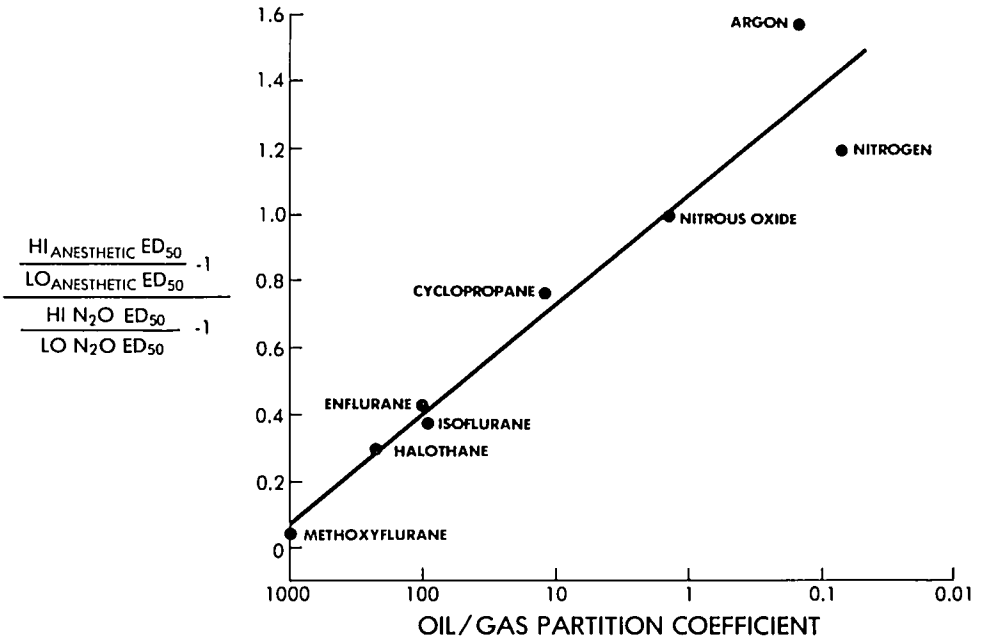

FIG. 1. The relationship of the separation in righting-reflex ED_{50} between HI mice (resistant to nitrous oxide anesthesia) and LO mice (susceptible to nitrous oxide anesthesia) and the anesthetic oil/gas partition coefficient. A value of zero for the ratio plotted on the ordinate indicates that the HI and LO mice are equally sensitive to an anesthetic. A ratio greater than 1.0 indicates that the separation in righting-reflex ED_{50}s between the HI and LO mice is greater than that for nitrous oxide (see text for details). The line was drawn through the points using linear regression analysis. The correlation coefficient of this line is 0.966. Oil/gas partition coefficients were taken from reference 7.

The nitrous oxide LD_{50} of the HI mice was only slightly (approximately 8 per cent) greater than the LD_{50} of the LO mice (table 4), and both of these values are similar to the nitrous oxide LD_{50} (2.23 ± 0.09 atm) for stock CD-1 mice.[4] This contrasts with the increase of about 50 to 60 per cent for the righting-reflex nitrous oxide ED_{50} in the HI compared with LO mice. Therefore, the breeding procedure that produces differences in anesthetic sensitivities does not produce a general resistance or susceptibility to nitrous oxide toxicity that is of the same magnitude.

The shorter sleep times induced by ethanol in the HI mice and the increased blood alcohol levels upon awakening in the HI mice (table 3) demonstrate that other hypnotic agents besides inhaled anesthetics may exhibit a differential sensitivity in these two lines. However, the magnitude of the separation in anesthetic requirements appears to be greater for nitrous oxide than for ethanol, since the HI mice awaken at only a 12 per cent higher blood alcohol than the LO mice, but had a 34 per cent higher nitrous oxide requirement (table 3). Similarly, we have shown that mice bred for resistance (short-sleep mice) and sensitivity (long-sleep mice) to alcohol also are more resistant and sensitive, respectively, to inhaled anesthetics. Also, the differential effect of alcohol between the short-sleep and long-sleep mice is greater than that of inhaled anesthetics.[5] These data suggest that the mechanism(s) responsible for the separation in nitrous oxide ED_{50}s does not equally affect the potency of alcohol. This suggests that the basic mechanisms underlying the actions of these two groups of anesthetics overlap but are not identical.

The larger tail-clamp ED_{50}s for several inhaled agents for HI compared with LO mice indicate that these lines are resistant and susceptible to anesthesia, and that the experiments did not singularly select for mice with different righting-response capabilities. The HI mice had tail-clamp ED_{50}s that were 28, 16, 12, 21, and 30 per cent higher for cyclopropane, enflurane, isoflurane, halothane, and methoxyflurane, respectively (table 2; data from males and females combined). These same mice were tested prior to tail-clamp measurements for their nitrous oxide righting-reflex ED_{50}s. For the above five groups, the nitrous oxide ED_{50}s were 61, 43, 29, 64, and 53 per cent greater, respectively, for HI compared with LO mice (table 2). Thus, the separation in anesthetic requirements between the HI and LO mice for cyclopropane, enflurane, isoflurane, halothane, and methoxyflurane, as measured by the tail-clamp procedure, is less than half of the separation in nitrous oxide righting-reflex potencies. Tail-clamp measurements for argon and nitrogen were not performed, since this would have required exposing the observer to excessively high pressures. Tail-clamp measurements for nitrous oxide also would be difficult, but possible, if a walk-in hyperbaric chamber were available. However, we have found that for stock CD-1 mice, the tail-clamp ED_{50} for nitrous oxide is actually greater than its LD_{50}.[4] We therefore did not attempt to measure nitrous oxide tail-clamp ED_{50}s in the HI and LO mice.

Anesthetic potencies of a broader spectrum of inhaled agents were measured in HI and LO mice using the righting-reflex response (table 1). The percentage increase in righting-reflex ED_{50} of HI compared with LO mice [(HI anesthetic ED_{50}/LO anesthetic ED_{50}) − 1], which was calculated from the data in table 1, is listed

in table 5 for seven inhaled agents. [When both males and females were tested to a given anesthetic, only the value for the combined population (males plus females) is given in table 5.] The mice tested with different anesthetics were from different generations; the percentage increase in nitrous oxide righting-reflex ED_{50} of the HI relative to the LO mice varied from 40 to 63 per cent, depending on the group of mice examined (table 5). In order to compare the relative potencies of the inhaled agents in HI and LO mice, a correction factor must be made for these differences in nitrous oxide potencies. Such a correction can be performed by dividing the percentage increase in anesthetic righting-reflex ED_{50}s of HI compared with LO mice by the percentage increase in righting-reflex ED_{50}s for nitrous oxide [(HI nitrous oxide ED_{50}/LO nitrous oxide ED_{50}) − 1] for the same group of mice (table 5). This ratio, listed in the right-hand column of table 5, allows all anesthetics to be compared on a common scale. In using this ratio, we assume only that an alteration in nitrous oxide potencies of the HI and LO mice would be accompanied by a proportional change in anesthetic potency for any other agent. A ratio of 1.0 indicates that the separation in righting-reflex ED_{50}s between the HI and LO mice is the same as that for nitrous oxide, whereas a ratio of zero indicates that the HI and LO mice are equally sensitive to an anesthetic.

The anesthetics in table 5 are listed in order of increasing lipid solubility. The separation in righting-reflex ED_{50} between the HI and LO mice is greatest for the least lipid-soluble anesthetics, nitrogen and argon. As the lipid solubility of an agent increases, the separation of anesthetic potencies progressively decreases. For methoxyflurane, the most lipid-soluble of the anesthetics tested, no significant difference in righting-reflex ED_{50} between HI and LO mice could be detected, despite the large number of animals examined in each group (table 1). The relationship between the separation in HI and LO righting-reflex ED_{50}s and the oil/gas partition coefficient of an agent is presented in figure 1.

Since relatively high pressures are required to anesthetize mice with argon or nitrogen, and since high pressures are known to antagonize anesthesia,[2,6,7] it might be argued that part of the separation in nitrogen and argon requirements (table 1) is due to the higher pressures experienced by the HI mice. However, a rise of 20 atm in pressure over this range is said to increase the nitrogen ED_{50} in mice by only 14 per cent[8] or less.[9] In addition, in several of our experiments with LO mice, we redetermined the nitrogen ED_{50}s in the LO mice at an increased total pressure. The pressure was increased by the addition of helium, which does not add to the anesthetic effect.[10] We found the nitrogen ED_{50} in 11 LO mice to increase from 30.3 ± 1.2 atm when helium was not present, to 35.0 ± 1.0 atm when an average of 18 atm of helium was present. In contrast, the nitrogen ED_{50} in the HI mice was above 50 atm (table 1). Thus, the separation in nitrogen and argon requirements between the HI and LO mice cannot be explained simply by the higher pressures experienced by the HI mice.

Another difficulty is encountered in measuring the anesthetic ED_{50}s of the very lipid-soluble agents (*i.e.*, methoxyflurane and halothane). Because these soluble anesthetics are taken up to a large extent by body tissues, alveolar concentrations will be less than measured inspired concentrations.[11] However, we allowed prolonged periods of equilibration before measuring anesthetic potencies; such precautions should decrease the difference between the alveolar and inspired concentrations.[12] Furthermore, any differences between these concentrations should be found for both the HI and LO mice, since there is no reason to believe that the uptake of anesthetics by one line of mice differs substantially from the uptake by the other line (*e.g.*, no consistent difference in weight between the two lines of mice was observed). Thus, our inability to find a significant difference for methoxyflurane requirement between the HI and LO mice is unlikely to be explained by differences in methoxyflurane uptake, and suggests that the sensitivity of the central nervous system to methoxyflurane, as measured by the righting reflex, is the same in these two lines.

In contrast to the results obtained with the righting-reflex test, the relative separation in anesthetic requirements between the HI and LO mice, as measured by the tail-clamp ED_{50}, was approximately the same for cyclopropane, enflurane, isoflurane, halothane, and methoxyflurane (table 2). To compare the relative tail-clamp ED_{50}s in the HI and LO mice, a correction was made (as described above for the righting-reflex ED_{50}) to normalize for the differences in anesthetic potencies observed between different generations and groups of mice. This normalization was performed by dividing the percentage increase in tail-clamp ED_{50} of HI compared with LO mice, by the percentage increase in nitrous oxide requirement (table 2, right-hand column). These ratios were similar for the five inhaled anesthetics examined and ranged between 0.37 and 0.56 (table 2). No correlation could be found between the separation in the tail-clamp ED_{50}s for HI and LO mice and the oil/gas partition coefficient. Thus, the separation in anesthetic requirements between the two lines of mice depends upon the endpoint used to assess anesthetic potency.

These relative differences between tail-clamp and righting-reflex ED_{50}s in the HI and LO mice suggests that our selective breeding procedure produced, at least in part, mice with different righting-response capabilities. Such alterations in the righting response might arise from changes in any of the neuronal pathways involved

in the balance and orientation system. Components of this system include 1) inputs (vestibular, visual, and skin and joint receptors); 2) processing units (vestibulocerebellum, vestibular nuclei, and the reticular formation); and 3) outputs (oculomotor control for coordinating movement of the eyes and spinal motor control for contraction of neck, limb, and body muscles).[13] We appear to have selected out one or more components in this balance and orientation system that are differentially sensitive to inhaled anesthetics. Furthermore, this differential sensitivity is related to the lipid solubility of the anesthetic.

The only notable difference in appearance between the HI and LO mice was that a fraction of the HI mice had extensive hair loss. This hairlessness first appeared in the fourth generation of the HI mice. In the seventh through tenth generations, between 20 and 40 per cent of the male and female HI mice exhibited this trait of hairlessness. However, these hairless mice showed no detectable differences in nitrous oxide requirements compared with the rest of the HI animals. For example, hairless female mice of the tenth generation had a nitrous oxide ED_{50} of 1.94 atm compared with 1.93 atm for all of the HI female offspring in the tenth generation. These hairless mice are fertile; most, but not all, of the offspring produced by mating a hairless male with a hairless female also are hairless.

We also measured the temperatures of the HI and LO mice, since an alteration in body temperature can significantly influence anesthetic requirement.[14] However, the rectal temperatures of awake HI and LO mice were identical. Moreover, since the ED_{50} measurements were performed under controlled temperature conditions (see Materials and Methods), the differences in anesthetic sensitivity between the HI and LO mice cannot be due to alterations in body temperature.

The differences in anesthetic potencies between the HI and LO mice cannot be explained by differences in health status. Ten mice from each group (at approximately 5 months of age) were subjected to viral and histopathologic examination. Pneumonia virus, reovirus, and mouse hepatitis virus could not be detected in either of the lines. One of ten LO mice and one of ten HI mice had significant titers for Sendai virus. Lung abnormalities (thickening of alveolar septa) were found in three of the ten LO mice and in two of the ten HI mice. In both groups, the liver and kidneys appeared normal.

In summary, we have continued a process for selectively breeding mice for resistance and susceptibility to nitrous oxide anesthesia through ten generations. At the tenth generation, nitrous oxide righting-reflex ED_{50}s in the two lines were separated by more than 0.7 atm. For other inhaled anesthetics, the separation in righting-reflex ED_{50}s between the HI and LO mice is inversely proportional to the lipid solubility of the agent. In contrast, the relative separation in tail-clamp ED_{50}s between the HI and LO mice is approximately the same for all anesthetics. An explanation for these phenomena on a neuronal pathway or molecular level remains to be determined.

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