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Age-dependent Alterations in Nitrous Oxide Requirement of Mice

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To determine the relationship of nitrous oxide requirement to age in mice, the authors repeatedly tested the righting-reflex response in stock CD-1 mice at 50 to 703 days of age. Over this age range, nitrous oxide requirement (±SE) progressively decreased from 1.48 ± 0.02 atm to 1.09 ± 0.06 atm. A second set of experiments measured changes in nitrous oxide requirement with age in mice selectively bred for resistance (HI mice) and susceptibility (LO mice) to nitrous oxide anesthesia. When tested at two months of age, selected HI and LO mice had nitrous oxide ED_{50} values of approximately 2.0 and 1.1 atm, respectively. At 11 to 14 months, the nitrous oxide ED_{50} of the HI mice had decreased to approximately 1.5 atm. In contrast, the nitrous oxide ED_{50} of the LO mice showed a much smaller decrease over this age range. Thus, the separation in nitrous oxide requirement between the HI and LO lines tended to disappear with age. By correlating the difference in anesthetic requirement between the HI and LO mice with biochemical and biophysical alterations in the central nervous system, studies on aging that use selectively bred lines may be helpful in investigating the mechanism of anesthetic action and the mechanism by which aging affects anesthetic action. (Key words: Age factors. Anesthetics, gases: nitrous oxide. Genetic factors. Potency, anesthetic: age factors; ED_{50}; righting reflex. Theories of anesthesia.)

ALTHOUGH OLDER PATIENTS are more sensitive to inhaled anesthetics, only a few studies have quantitated the relationship of age to anesthetic requirement for inhaled agents. In humans, halothane MAC decreases from 1.1% atm in the neonate to 0.63% atm in elderly patients with a mean age of 81 years.[1] Isoflurane MAC decreases from 1.28 ± 0.01% atm in 19- to 30-year-old patients to 1.05 ± 0.05 atm in those older than 55 years of age. For these same age groups, nitrous oxide MAC is estimated to decrease from 1.16 to 1.00 atm.[2] MACs for halothane and isoflurane are approximately 20% lower in rats that are about eight months old compared with those three months old.[3] This is the only quantitative study in animals that has measured the alteration in anesthetic requirement with age. Since the relative potencies of inhaled agents as a function of age must be determined before other comparative studies relating the cardiovascular, respiratory, or neuromuscular effects with age are performed, we examined anesthetic requirement in mice over a broader age range.

We measured nitrous oxide requirement in normal CD-1 mice for approximately two years. In addition, we measured the age-dependent alterations in anesthetic requirement for two lines of mice selectively bred for resistance and for susceptibility to nitrous oxide anesthesia.[4,5] At the end of ten generations of selective breeding, the mean nitrous oxide requirements for these two lines were separated by more than 0.7 atm when animals were tested at two months of age. We reasoned that if an age-dependent alteration in nitrous oxide potency occurred in one line but not in the other, this might provide us with a model to study not only the mechanism by which anesthetic requirement decreases with age, but also the mechanism of anesthetic action itself.

Materials and Methods

EXPERIMENT 1

Twenty male, stock CD-1 mice, obtained from Charles River, were allowed to adapt to laboratory conditions for one week before being tested at 50 days of age for nitrous oxide requirement. Mice were housed three or four to a cage and were located in a room that contained only CD-1 mice. Mice were identified by ear marks. Each cage had a filter top cover. Room temperature was kept near 22°C, and a light:dark cycle of 12 hours:12 hours was imposed. Purina® Chow and tap water were provided *ad libitum*. For testing of nitrous oxide ED_{50}, as many as eight unrestrained mice were placed in individual wire-mesh cages that could be rotated at 4 rpm in a 20-l hyperbaric chamber. Two additional mice (restrained) were placed in the chamber. Chamber temperature was kept between 33° C and 35° C with circulating water heat exchangers to keep the rectal temperature of the restrained mice between 36.5° C and 38.0° C. A fan in the chamber circulated gases through a soda-lime container to remove carbon dioxide. The hyperbaric chamber was flushed with pure oxygen for 10 min, and 1.22 atm of nitrous oxide were added. Following a 30-min period of equilibration, animals were given the righting-reflex test; those rolling over two time or more during five complete turns of the rotator failed the test and were considered anesthetized. Nitrous oxide then was added or removed in 0.11-atm

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steps, and the mice were retested after 15-min periods of equilibration. The partial pressure of oxygen remained between 0.6 and 1.0 atm during the testing procedure. Nitrous oxide requirement was calculated for each mouse by averaging the partial pressure that just permitted and just prevented the animal from righting itself. The ED_{50} and standard errors for mice in a given age group were calculated from these individual cross-over values. If an animal died during the ED_{50} determinations, its responses were not included in the ED_{50} calculations. Significance between the youngest and oldest animals was calculated using an unpaired or paired *t* test (two-tailed tests). Following the initial testing of nitrous oxide ED_{50}, mice were returned to their cages and retested at 30- to 100-day intervals. Testing procedures were not separated by more than four hours from each other in the diurnal cycle.

Experiment 2

ED_{50} values also were measured, as a function of age, in mice selectively bred for resistance (HI mice) and susceptibility (LO mice) to nitrous oxide anesthesia.[4,5] Breeder animals (those in each line having the highest and lowest requirements) were examined from generations 6 through 13. These animals initially were tested for righting-reflex ED_{50} at approximately two months of age, and then were mated (one male mated with two or three females) to produce the succeeding generations of HI and LO mice. These parents were later retested for nitrous oxide requirement at approximately 10-month intervals. The procedures used for testing the nitrous oxide righting-reflex ED_{50} in HI and LO mice were the same as described above. Housing conditions were also the same, except that male breeders were placed in individual cages after mating. For statistical comparison, the separation in nitrous oxide ED_{50}s between the HI and LO lines were calculated for three different age groups: at approximately two months of age, at 11 to 14 months of age, and at 19 to 21 months of age. Significance between the three different age groups was calculated by an analysis of variance employing the Newman-Keuls test for multiple comparisons. Since it was shown in a previous study[5] that nitrous oxide ED_{50} values are essentially the same for male and female mice in a given line, nitrous oxide ED_{50}s were calculated using the combined populations of male and female animals.

Results

Experiment 1

The mean (±SE) nitrous oxide righting-reflex ED_{50} for 19 50-day-old male mice was 1.48 ± 0.02 atm. Nitrous oxide requirement progressively decreased with age, the lowest value being 1.09 ± 0.06 atm at 703 days (fig. 1). The values at these two ages were significantly different from each other ($P < 0.001$), both when examined as independent groups and by self-pairing the values in those six animals which survived the full 703 days with the initial 50-day values. Of the deaths that occurred, two were caused by fighting, and two occurred during the testing of nitrous oxide ED_{50} at 141 days of age. The other deaths had unknown causes. Average weights increased from 31 ± 1 g for 50-day-old mice to 47.2 ± 2 g for 703-day-old mice.

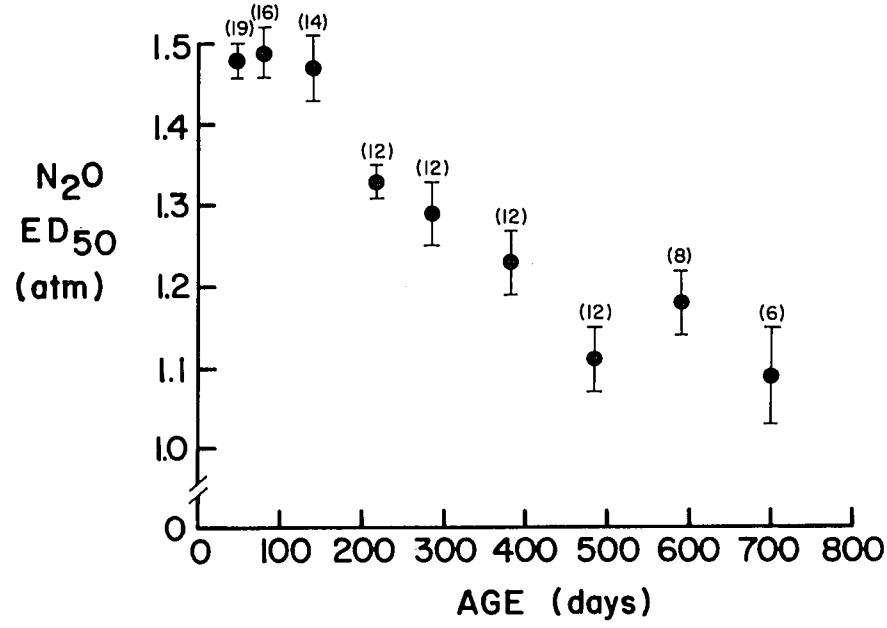

FIG. 1. Nitrous oxide requirement in stock CD-1 male mice as a function of age. The same animals were tested repeatedly for approximately two years. Bars represent ±1 SE. Numbers of animals are given in parentheses.

TABLE 1. ED_{50}s as a Function of Age for Mice Resistant (HI Mice) and Susceptible (LO Mice) to Nitrous Oxide Anesthesia

Age (months)	Generation	Nitrous Oxide ED_{50} (atm) HI Mice	LO Mice
2	6	2.04 ± 0.02 (14)*	1.16 ± 0.02 (14)
12	6	1.67 ± 0.03 (14)	1.20 ± 0.04 (13)
19	6	1.44 ± 0.04 (9)	1.06 ± 0.04 (10)
2.5	7	2.13 ± 0.01 (16)	1.14 ± 0.02 (16)
11	7	1.63 ± 0.06 (7)	1.05 ± 0.05 (14)
21	7	1.30 ± 0.09 (4)	0.73 ± 0.12 (11)
2	8	2.06 ± 0.02 (16)	1.07 ± 0.02 (16)
14	8	1.68 ± 0.07 (7)	1.10 ± 0.03 (12)
19.5	8	1.50 ± 0.06 (6)	0.775 ± 0.064 (12)
2.5	9	1.88 ± 0.01 (18)	0.945 ± 0.055 (18)
11	9	1.60 ± 0.04 (15)	1.12 ± 0.02 (14)
20	9	1.11 ± 0.07 (7)	0.600 ± 0.055 (12)
2	10	2.04 ± 0.01 (27)	1.05 ± 0.01 (27)
12	10	1.46 ± 0.04 (25)	0.914 ± 0.034 (22)
21	10	1.21 ± 0.03 (10)	0.847 ± 0.026 (15)
2	11	2.03 ± 0.01 (42)	0.968 ± 0.018 (42)
12	11	1.36 ± 0.03 (31)	0.754 ± 0.031 (37)
21	11	1.32 ± 0.03 (14)	0.869 ± 0.029 (29)
2	12	1.99 ± 0.01 (24)	0.972 ± 0.024 (24)
11.5	12	1.48 ± 0.04 (19)	0.897 ± 0.025 (24)
2	13	2.04 ± 0.01 (24)	0.959 ± 0.024 (24)
11.5	13	1.42 ± 0.04 (16)	0.887 ± 0.031 (22)

* Numbers of animals are given in parentheses and include both female and male mice.

EXPERIMENT 2

When first tested, nitrous oxide requirements of HI and LO mice selected as breeders (approximately two months of age) were separated by 0.9 to 1.1 atm (table 1). Nitrous oxide ED_{50} values in the HI mice decreased with age. ED_{50}s of 11- to 14-month-old and 19- to 21-month-old mice were approximately 20 to 40% lower than ED_{50}s of these same mice at two months of age (table 1). In contrast, little or no decrease was seen in the ED_{50}s of the LO mice at 11 to 14 months of age compared with two months of age. LO mice in the 19- to 21-month age group tended toward lower nitrous oxide ED_{50} values than LO mice at two months of age, but the magnitude of this decrease was less than that observed in the HI animals (table 1). That is, with age, the separation in nitrous oxide requirement between the two lines tended to disappear. The separation in nitrous oxide ED_{50}s between the selected HI and LO mice decreased from 0.98 ± 0.019 atm at two months of age to 0.55 ± 0.018 atm at 11 to 14 months, and to 0.50 ± 0.055 atm at 19 to 21 months of age (fig. 2). The separation in nitrous oxide ED_{50}s between the HI and LO lines at two months of age was significantly different from that obtained at 11 to 14 months ($P < 0.001$) and from that obtained at 19 to 21 months of age ($P < 0.001$). However, no significant difference could be detected in the separation of nitrous oxide ED_{50}s between mice of the 11- to 14-month-old and 19- to 21-month-old groups.

The decrease in the numbers of HI and LO animals tested at the older ages was due to deaths. The HI mice were more likely to die during the testing of nitrous oxide ED_{50} since they were exposed to higher partial pressures of nitrous oxide. Most of the deaths occurring between testing periods were of unknown causes; how-

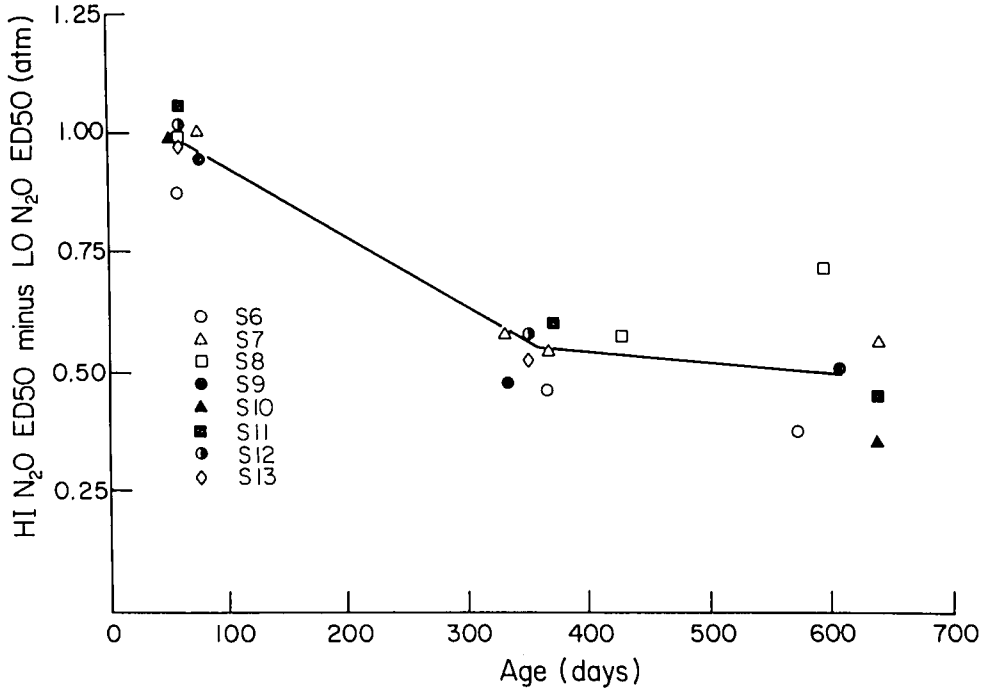

FIG. 2. Separations of HI and LO nitrous oxide ED_{50} values in breeder animals as a function of age. Separations are calculated from the combined population (males plus females). Values are taken from animals of the sixth (○), seventh (△), eighth (□), ninth (●), tenth (▲), eleventh (■), twelfth (◑), and thirteenth (◇) generations. The separations were calculated from the values given in table 1. The lines of the graph connect the average values for the three different age groups.

ever, several older mice developed open sores after being infected with mites and were eliminated from the study.

Discussion

Our studies show that the reduction of nitrous oxide requirement with age in mice parallels the age-dependent decrease in inhaled anesthetic requirement in humans.[1,2] Older mice are also more sensitive to other anesthetics such as alcohol[6,7] and barbiturates.[7,8] However, studies with these agents are complicated by alterations in drug disposition that may occur with age.[6-8] In contrast, nitrous oxide undergoes very little metabolism, and the equilibration between inspired and brain nitrous oxide partial pressures is rapid.[9] Thus, the decrease in nitrous oxide requirement with age must be a result of a greater sensitivity of the central nervous system of the older mice and not to changes in absorption, metabolism, distribution, or excretion.

Nitrous oxide requirement was 28% lower in 703-day-old mice than in 50-day-old mice (fig. 1). However, since the mean life span of mice may vary from nine to more than 28 months,[10,11] depending on the strain of mice and housing conditions, it is difficult to compare these decrements in anesthetic requirement in mice over the same relative age span for humans. Nevertheless, if we use a similar ratio for life span in humans (*i.e.*, 5-year-old and 81-year-old patients *vs.* 50-day-old and 703-day-old mice), halothane requirement decreases from 0.94% atm in the 5-year-old to 0.64% atm in elderly patients with a mean age of 81 years.[1] That is, halothane requirement in humans also decreases by 28% over a similar life span.

The present experiments demonstrate that the difference in nitrous oxide potency between HI and LO mice narrows with age (fig. 2). HI mice exhibit a marked age-related decrease in anesthetic requirement, whereas the magnitude of this age-related decrease is considerably less in the LO mice (table 1). Studies on aging in these selectively bred mice may prove useful in determining the molecular basis for the difference in nitrous oxide requirement between the HI and LO lines and for the decrease in anesthetic requirement with age. Previous attempts to explain the separation in nitrous oxide requirement between HI and LO mice on the basis of alterations in the composition of synaptic membrane lipids revealed no differences in synaptic membrane phospholipid, cholesterol, or fatty acid composition between the two groups.[4] If a biochemical difference is found that might explain the differences in anesthetic potency between young HI and LO mice, this difference should disappear with age. Such a discovery might provide some insight into the mechanism by which anesthetics act.

In summary, in normal mice, nitrous oxide requirement decreases with age, and this decrease parallels that seen clinically in humans. Mice selectively bred for resistance to nitrous oxide anesthesia exhibit a greater age-related decrease in nitrous oxide requirement than mice bred for susceptibility. A further characterization of potencies in aging mice for a variety of inhaled anesthetics eventually should provide a model system appropriate for study of the physiologic effects of inhaled anesthetics in the elderly patient.

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