Strain-differences of Sensitivity to Volatile Anesthetics and Their Genetic Character in Mice

Tomoko TANAKA, Kenji OGLI, Hisao KOMATSU, Junko NOGAYA and Satoshi YOKONO

Using loss of the righting reflex, we determined the ED_{50} values for enflurane, isoflurane, sevoflurane and halothane in white-haired ddN mice and black-haired C57BL mice. The ED_{50}s (Mean ± SEM) in ddN and C57BL mice for enflurane were 1.65 ± 0.01 and 1.19 ± 0.01% atm, for isoflurane 1.02 ± 0.01 and 0.74 ± 0.01% atm, for sevoflurane 2.29 ± 0.03 and 1.95 ± 0.03% atm, and for halothane 0.97 ± 0.01 and 0.97 ± 0.01% atm, respectively. The results indicate that the ddN strain is more resistant to enflurane, isoflurane and sevoflurane than the C57BL strain. The sensitivities to enflurane and isoflurane in F1 progeny of reciprocal crosses between ddN and C57BL mice revealed that in the ddN strain enflurane resistance is an incompletely dominant or polygenic character, isoflurane resistance in ddN strain is an autosomal recessive character and both are controlled by genes on the sex (X) chromosome. Enflurane and isoflurane resistances are controlled by at least 2 genes, one on the X chromosome, and each resistance is controlled by a different genetic mode. (Key words: enflurane, halothane, isoflurane, sevoflurane, anesthetic sensitivity, genetic character)

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We have been investigating opisthotonus as an episode during anesthesia in mice, and found that the incidences of opisthotonus differed among strains[1]. The differences of incidence are likely to reflect distinctive characteristics of the central nervous system (CNS) mechanisms and anesthetic sensitivity among the strains. We therefore attempted to measure the sensitivity to anesthetic agents in different strains of mice. Anesthetic requirements vary slightly among species or classes[2]. If we were able to obtain strains of mice which have obviously different sensitivities to anesthetic agents, we may be able to find a clue to clarify the neural mechanism of anesthesia by analyzing the differences in components and neurotransmitters in the CNS between the strains[3,4].

We studied EC_{50} values for enflurane, isoflurane, sevoflurane and halothane by measuring loss of the righting reflex in white-haired ddN and black-haired C57BL mice, which show

Department of Anesthesiology and Emergency Medicine, Kagawa Medical School, Kagawa, Japan

Address reprint requests to Dr. Tanaka: Department of Anesthesiology and Emergency Medicine, Kagawa Medical School, 1750-1, Ikenobe, Miki-cho, Kagawa, 761-07 Japan

Table 1. Righting-reflex ED_{50}s of Inhaled Anesthetics in Mice (% atm)

		ddN	C57BL
Enflurane		1.65 ± 0.01 (26)	1.19 ± 0.01 (16)[a]
	male	1.65 ± 0.01 (14)	1.17 ± 0.02 (9)
	female	1.66 ± 0.02 (12)	1.21 ± 0.01 (7)
Isoflurane		1.02 ± 0.01 (26)	0.74 ± 0.01 (16)[a]
	male	1.02 ± 0.01 (14)	0.73 ± 0.01 (9)
	female	1.01 ± 0.02 (12)	0.74 ± 0.01 (7)
Sevoflurane		2.29 ± 0.03 (23)	1.95 ± 0.03 (24)[a]
	male	2.26 ± 0.06 (11)	1.96 ± 0.05 (12)
	female	2.31 ± 0.03 (12)	1.95 ± 0.03 (12)
Halothane		0.97 ± 0.01 (24)	0.97 ± 0.01 (24)
	male	0.98 ± 0.01 (12)	0.96 ± 0.01 (12)
	female	0.95 ± 0.01 (12)	0.98 ± 0.01 (12)

Number of mice is shown in parentheses.
Values are mean ± SEM.
a; C57BL mice differ from ddN mice at significance levels of $P < 0.01$

high and low incidences of anesthetic-induced opisthotonus, respectively. We also discussed the genetic characteristics of the sensitivity to anesthetic agents.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee. All mice employed in this experiment were fed Oriental Yeast Co. laboratory chow and water at libitum, and were maintained on a 12 hour light: 12 hour dark cycle. Fifty ddN mice and forty C57BL mice ranging in age from 8 to 12 weeks and in weight from 25 to 35 grams were used. The C57BL was an inbred strain. The ddN mice used in these experiments were considered an inbred strain, as these closed colony mice were inbred for more than 20 generations and the nineteen marker genes of the six mice selected at random were equal, as determined by Dr. Hideki Kato, Central Institute for Experimental Animals. Twenty-six ddN (fourteen males and twelve females) and sixteen C57BL (nine males and seven females) mice were studied with enflurane and isoflurane, and twenty-four (twelve males and twelve females) of each strain were studied with halothane and sevoflurane. One ddN male mouse died during this study. Individual mice were placed in a rolling drum partitioned into five chambers which was placed in a 25 liter (l) closed transparent container with a carbon dioxide (CO_2) absorber. Four liters per minute ($l \cdot min^{-1}$) of anesthetic-containing fresh gas in air flowed into the container through a vaporizer. The anesthetic concentration in the container was equalized with a fan and continuously monitored using an infrared detector (Normac, Datex). The temperature in the container was monitored and maintained at between 26 and 28°C by a circulating-water heat exchanger. Based on a pilot study, the initial concentration of each anesthetic agent was set at a subanesthetic level. An initial 30-min period of equilibration, at approximate levels of 1.0% for enflurane, 0.65% for isoflurane, 1.5% for sevoflurane, and 0.8% for halothane, was imposed before testing the righting reflex. The concen-

Table 2. Righting-reflex ED_{50}s for Enflurane and Isoflurane in F1 hybrids (% atm)

		Total population	C57BL female × ddN male	ddN female × C57BL male
Enflurane		1.36 ± 0.02 (22)		
	male	1.33 ± 0.02 (10)	1.30 ± 0.01 (5)	1.37 ± 0.03 (5)[a]
	female	1.38 ± 0.01 (12)	1.39 ± 0.01 (5)[b]	1.37 ± 0.01 (7)
Isoflurane		0.79 ± 0.01 (22)		
	male	0.77 ± 0.02 (10)	0.73 ± 0.01 (5)	0.82 ± 0.01 (5)[a]
	female	0.80 ± 0.01 (12)	0.80 ± 0.01 (5)[b]	0.80 ± 0.01 (7)

Number of mice is shown in parentheses. Values are mean ± SEM.

a; Significantly different from the male ED_{50}s of hybrids from C57BL female ×ddN male, $P < 0.05$.

b; Female differ from male at significance levels of $P < 0.05$.

tration of anesthetic agent was increased in 0.05% steps. The predetermined concentration was obtained and held constant for at least 15 minutes. The assumption that end-tidal, alveolar, arterial, and brain anesthetic partial pressures are equal after 15 minutes of equilibration may be incorrect, but any disequilibrium would usually be small[2,5]. After each period of equilibration, the rolling device was turned by hand and the mice were rolled over. The mice which did not right themselves during a one minute observation failed the test and were considered anesthetized. Each mouse was tested two or three times at few-day intervals. Although some ddN mice exhibited opisthotonus during exposure to anesthetics, opisthotonus which interfered with the righting reflex measurement was not observed during the ED_{50} test. The ED_{50} for each mouse was determined by averaging the concentrations that just abolished or just allowed the righting reflex. The ED_{50} and standard error for each strain of mice were calculated from the individual values. Significance was calculated with an unpaired Wilcoxon test. After determination of the ED_{50} for one anesthetic agent, that for the next agent was examined after an interval of five days or more.

The same experimental procedure was followed for enflurane and isoflurane in F1 hybrid mice produced by cross-mating ddN and C57BL mice, and in second generation (F2) mice produced by mating F1 mice with one another. Two sets of parents were used to breed F1 and F2 progeny.

Results

The ED_{50} values in ddN mice for enflurane, isoflurane, and sevoflurane were significantly higher than those in C57BL mice (table 1). Strain differences were found between ddN and C57BL mice, especially in enflurane and isoflurane. No significant differences were observed between ED_{50}s for halothane in both strains. This indicates that the ddN strain is more resistant to enflurane, isoflurane and sevoflurane than the C57BL strain. Sex differences in ED_{50}s for each strain were not significant for the anesthetics examined (table 1).

The ED_{50}s for enflurane and isoflurane in F1 hybrids of the reciprocal crosses between ddN and C57BL strains are shown in table 2.

The ED_{50} values in F2 offspring are presented in table 3.

See "Discussion" for further explanation.

Table 3. Righting-reflex ED_{50}s for Enflurane and Isoflurane in F2 male offsprings (% atm) and their expected genotypes

F2 males from C57BL female × ddN male				
No.	ED_{50}s for Enflurane		ED_{50}s for Isoflurane	
1	1.775		1.375	
2	1.750		1.100	
3	1.725		1.275	
4	1.675	(RY;RR)	1.375	
5	1.625	(RY;RR)	1.350	
6	1.525	(SY;RR)	1.225	
7	1.525	(SY;RR)	1.125	
8	1.500	(SY;RR)	0.975	(RY;RR) or (SY;RR)
9	1.475	(SY;RR) or (RY;RS)	0.975	(RY;RR) or (SY;RR)
10	1.450	(RY;RS)	1.050	(RY;RR)
11	1.450	(RY;RS)	1.025	(RY;RR)
12	1.300	(SY;RS)	0.900	(SY;RR)
13	1.225	(RY;SS)	0.775	(RY;SS) or (RY;RS) or (SY;RS) or (SY;SS)
F2 males from ddN female × C57BL male				
No.	ED_{50}s for Enflurane		ED_{50}s for Isoflurane	
1	1.650	(RY;RR)	0.950	(SY;RR)
2	1.625	(RY;RR)	0.950	(SY;RR)
3	1.575	(SY;RR)	0.925	(SY;RR)
4	1.375	(RY;SR)	0.850	(RY;SS) (RY;RS)
5	1.325	(SY;SR)	0.800	(RY;SS) (RY;RS)
6	1.300	(SY;SR)	0.875	(RY;SS) (RY;RS)
7	1.300	(SY;SR)	0.775	(RY;SS) (RY;RS)
8	1.250	(RY;SS)	0.775	(RY;SS) (RY;RS)
9	1.225	(RY;SS)	0.725	(SY;RS) (SY;SS)

Discussion

The minimum alveolar concentration of anesthetic (MAC) has been supposed to be fairly constant in a given species[5]. If there was any variation in MAC for intraspecies, it has been said to be less than 10 per cent. However, a previous report on strain differences of ether susceptibility in C57BL/6 mice and C3H/He mice showed an ED_{50} difference of about 30 per cent[6]. Our results also demonstrate that the anesthetic requirements to abolish the righting reflex in mice significantly differ among strains. In this study, ddN mice were resistant and C57BL mice were susceptible to enflurane, isoflurane and sevoflurane, especially in enflurane the ED_{50}s differ about 40 per cent (table 1). In our previous study, we found that ddN mice had a higher incidence of opisthotonus than C57BL mice during exposure to volatile anesthetics and hypothesized that the central nervous system (CNS) of ddN mice is more excitable than that of C57BL mice[1]. Our present data, at least in part, may support this hypothesis. Halothane, which seldom causes opisthotonus, showed no difference in ED_{50}s between both strains. Although the order of the incidence of opisthotonus is sevoflurane > isoflurane > enflurane > halothane[7], the order of the strain differences in ED_{50}s

is enflurane > isoflurane > sevoflurane > halothane. This indicates that the CNS excitability to anesthetic sensitivity and opisthotonus seem to have different factors. Although the reason for the degree of strain difference in ED_{50} in each anesthetic is not clear, it is possible that the mechanism of anesthesia is different for each anesthetic. This speculation is also supported by the fact that, in our results, the slope of the hill plot (considered to represent the number of active sites of anesthesia[8]) obtained from our dose-response curve (figure not shown) is different in each anesthetic: 53, 18, 15, and 27 for enflurane, isoflurane, sevoflurane, and halothane, respectively.

In F1 hybrids of the reciprocal crosses between ddN and C57BL strains, the ED_{50}s for enflurane and isoflurane were estimated to reflect the genetic bases of their strain differences (table 2). The ED_{50}s for enflurane in F1 hybrids were intermediate between those of their parents and significantly different from both of them. This suggests that enflurane resistance in the ddN strain is an incompletely dominant autosomal or polygenic character, which coincides with a previous report that anesthetic sensitivity to nitrous oxide in mice was determined by polygenes[9]. Isoflurane resistance in the ddN strain seems to be a recessive autosomal character as the ED_{50} in F1 males from C57BL females × ddN males is the same as that in C57BL males. The F1 males from ddN females × C57BL males had a significantly higher ED_{50} than the F1 males from the reciprocal crosses for both enflurane and isoflurane, indicating that enflurane resistance and isoflurane resistance are controlled by genes on the sex (X) chromosome. The genetic character of sensitivity to anesthetic agents in Drosophila melanogaster was previously investigated, and it was indicated that the resistance to halothane and chloroform anesthesia was a sex-linked recessive trait and an incompletely dominant autosomal character, respectively[10]. Since the ED_{50}s were not different from each other in the females between crosses, there do not seem to be any maternal or cytosomal effects on enflurane or isoflurane resistance.

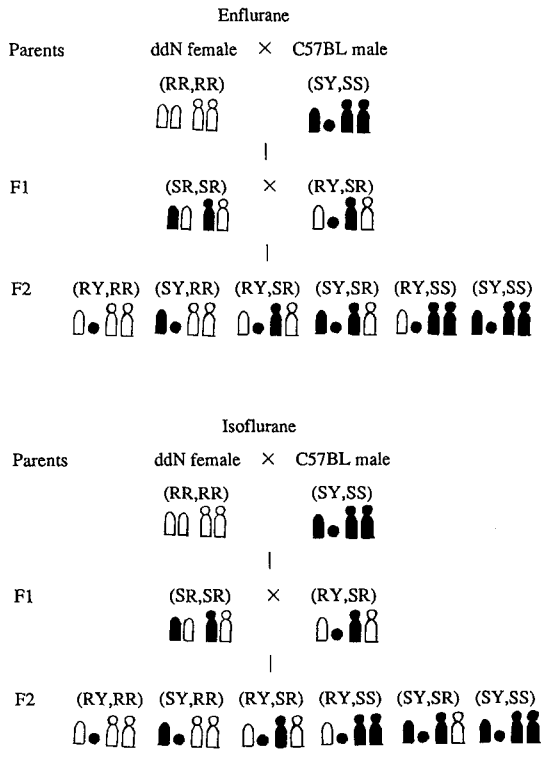

Fig. 1. The related genotype model of each generation only male in F2 with enflurane and isoflurane sensitivity.

(aY,bc); a = genes on the X chromosome
bc = genes on the autosomal chromosome

Further genetic studies were carried out in the F2 progeny. We hypothesized that one gene was on the X chromosome and another gene was on the autosomes since anesthetic resistance is controlled by at least 2 genes[9,10]. The genotypes of the F2 male progeny are shown in figure

1; the sensitive gene, resistant gene and Y chromosome are represented as S, R, and Y, respectively. The observed ratio of genotypes for enflurane sensitivity in F2 males from the parents between ddN females and C57BL males was (RY;RR) : (SY;RR) : (RY;SR) : (SY;SR) : (RY;SS) : (SY;SS) = 2 : 1 : 1 : 3 : 2 : 0 in nine mice, while the expected ratio is 1 : 1 : 2 : 2 : 1 : 1. The observed ratio for isoflurane sensitivity was {(RY;RR) + (SY;RR)} : {(RY;SR) + (RY;SS)} : {(SY;SR) + (SY;SS)} = 3 : 5 : 1, while the expected ratio is 2 : 3 : 3 since the phenotype of SR and SS is the same in a recessive character. These ratios are agreeable to the expected ones. The observed ratios suggest that enflurane resistance and isoflurane resistance are controlled by at least 2 genes, one on the sex (X) chromosome and the other on the autosomes, but each resistance is controlled by a different genetic mode. The ratio in the F2 males from the parents between C57BL females and ddN males was more complicated. It may indicate the existence of active genes or factors for both anesthetic resistances or it may indicate the selective occurrence of resistances in the process of the crosses. Incidentally the genetic character of hair color obeyed Mendel's Law. That is, F1 hybrids produced from ddN (white) and C57BL (black) mice were all black and F2 progeny were white and black-haired at a ratio of approximately 1:3, indicating no genetic imbalances in F2 offspring between ddN and C57BL strains. F2 female progeny were not discussed as only a small umber of them were produced. This may be due to some genetic difficulty in producing female between ddN and C57BL strains.

In summary, each volatile anesthetic agent may have a different mechanism for anesthesia since the ratio of ED_{50} values of ddN mice to those of C57BL mice were different for all four anesthetics. Furthermore, in mice, anesthetic sensitivity, at least to enflurane and isoflurane, is controlled by different genetic modes.

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