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Mutagenicity of Halogenated Ether Anesthetics

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An *in vitro* microbial assay system employing two histidine-dependent strains of *Salmonella typhimurium*, TA1535 and TA100, was used to test the mutagenicities of enflurane, methoxyflurane, isoflurane and fluroxene. Enflurane, isoflurane and fluroxene in concentrations ranging from 0.01 to 30 per cent and methoxyflurane in concentrations ranging from 0.01 to 7 per cent were incubated with bacteria in the presence or absence of homogenates of liver prepared from rats pretreated with the enzyme inducer, Aroclor 1254. Enflurane, methoxyflurane, isoflurane, and urines from patients anesthetized with these agents were not mutagenic. Fluroxene, however, was highly mutagenic, and therefore poses a possible hazard for operating room personnel and patients. (Key words: Anesthetics, volatile, enflurane; Anesthetics, volatile, methoxyflurane; Anesthetics, volatile, isoflurane; Anesthetics, volatile, fluroxene; Biotransformation (drug), liver homogenate; Bacteria, mutagenicity; Toxicity, mutagenicity.)

WE HAVE REPORTED that halothane was not mutagenic in the *Salmonella* assay system developed by Ames.[1,2] That study was prompted by epidemiologic surveys that suggested an increased incidence of malignancies in operating room personnel and by the known correlation between the mutagenicity and carcinogenicity of chemicals.[3–6] In the present study, we examined the mutagenic properties of four halogenated ether anesthetic agents using a system similar to that previously described.[1,2] The anesthetic agents tested were enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether), methoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether), isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether), and fluroxene (2,2,2-trifluoroethyl vinyl ether).

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Methods and Materials

The methods and materials used for testing anesthetic agents in the *Salmonella* assay system have recently been described. They are summarized below. All anesthetics tested were commercially available preparations.

Bacterial Preparation

Two histidine-dependent strains of *Salmonella typhimurium*, TA1535 and TA100, were employed.[2] For each experiment, inocula from stock cultures were grown overnight at 37 C in a nutrient broth and used the next morning.

Metabolic System

A mammalian metabolic system was prepared from a homogenate of livers from male Sprague-Dawley rats treated with polychlorinated biphenyl, 1,500 mg/kg (PCB; Aroclor 1254)[1,2] five days prior to sacrifice. Aroclor is a potent inducer of the mixed-function oxidase system. Each drug was tested with and without the metabolic system.

Desiccator Incubation Experiments

Plates containing freshly grown bacteria, agar, and metabolic system (when required) were placed in 9-liter, airtight desiccator jars. The volumes of liquid anesthetic necessary to give the desired vapor concentrations were added to the desiccators. In other experiments performed under identical conditions, anesthetic concentrations were verified by gas chromatographic analysis of desiccator atmosphere and were found not to vary from predicted values by more than 10 per cent, nor to vary significantly with time. The vapor concentrations of anesthetic gases ranged from 0.01 to 30 per cent for enflurane, isoflurane and fluroxene, and from 0.01 to 7 per cent for methoxyflurane. Vinylidene chloride, 3 per cent, was used as the positive control for both bacterial strains. After adding the test compound, the sealed desiccator jars were incubated at 37 C for eight hours. The plates were removed from the desiccators and allowed to incubate at 37 C for a further 40 hours. Colonies on each plate were then counted. Triplicate plates were prepared at each drug concentration, and each experiment was performed at least twice.

Liquid Incubation Experiments

Enflurane, methoxyflurane, isoflurane, and fluroxene were tested at the same concentrations used in the desiccator incubation experiments. Freshly grown bacteria, rat liver homogenate, and various amounts of liquid anesthetic or vinylidene chloride were added to sterile, air-tight screw-top test tubes. The tubes were incubated at 37 C on a rotator for two hours, after which molten top agar was added and the contents poured onto glucose minimal medium plates. The plates were incubated at 37 C in air for two days and revertant colonies counted. Triplicate plates were prepared at each anesthetic concentration, and each experiment was performed at least twice.

Urinary Metabolites

Twenty-four-hour urine collections were obtained both before and immediately after operation from patients anesthetized with enflurane, methoxyflurane, or isoflurane. Urine was added to test tubes containing freshly grown bacteria, metabolic system and beta-glucuronidase, the latter to hydrolyze glucuronides, thereby freeing possibly mutagenic, conjugated metabolites. Molten top agar was added to each tube and the entire contents poured onto

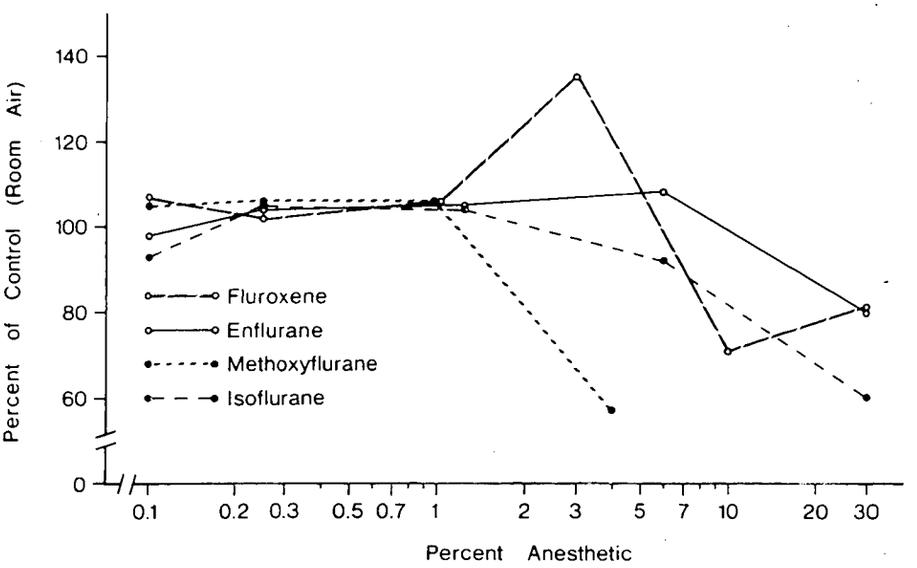

FIG. 1. Revertant colonies of *Salmonella typhimurium*, TA100, after desiccator incubation with four halogenated ether anesthetics in the presence of the liver homogenate metabolic activation system. These agents did not significantly increase the number of revertant colonies above control but were toxic to bacteria at the highest vapor concentrations tested.

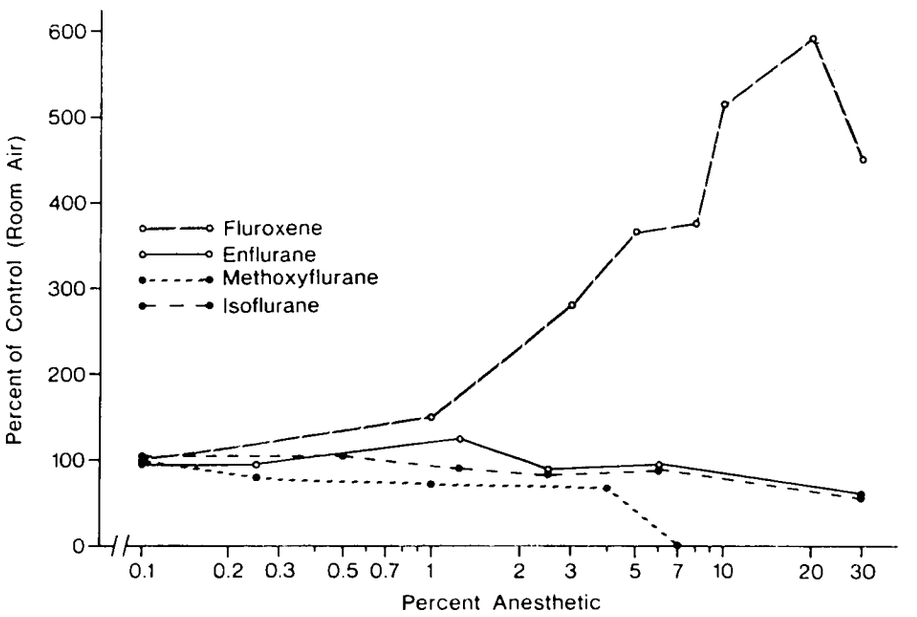

FIG. 2. Revertant colonies of *Salmonella typhimurium*, TA 1535, expressed as percentage of control (room air) after liquid incubation with four halogenated ether anesthetics in the presence of the metabolic activation system. Enflurane, methoxyflurane and isoflurane did not increase the number of revertant colonies above control; methoxyflurane was toxic to bacteria at the highest vapor concentration tested. Fluroxene was mutagenic in a dose-related fashion at vapor concentrations above 1 per cent. Peak mutagenicity was seen at 20 per cent.

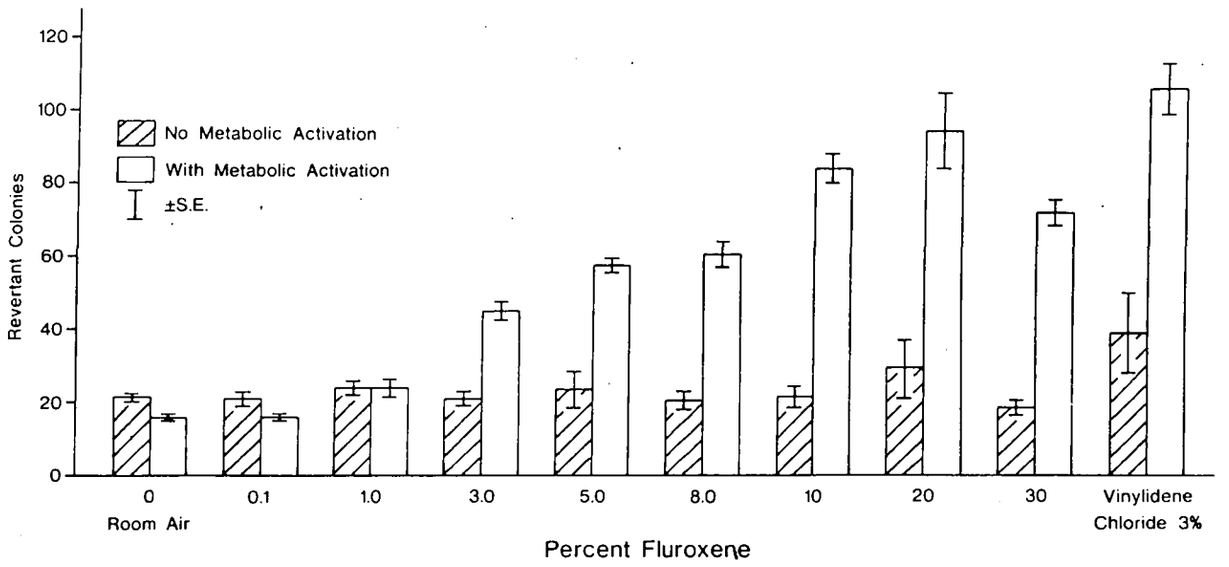

FIG. 3. Number of revertant colonies of *Salmonella typhimurium*, TA1535, after liquid incubation with fluroxene. There was a dose-related increase in the number of revertant colonies at vapor concentrations above 1 per cent only when the metabolic activation system was present. Similarly, the greatest increase in the number of revertant colonies with vinylidene chloride occurred in the presence of the metabolic activation system.

glucose minimal medium plates. Plates were incubated at 37 C for two days and revertant colonies counted.

ANALYSIS OF DATA

The numbers of revertant colonies in treated plates were compared with the numbers of spontaneous revertant colonies in plates exposed to room air. t tests were used for statistical analysis; $P < 0.05$ was considered significant.

Results

None of the anesthetic agents was mutagenic when assayed by desiccator incubation employing strain TA100 and the liver homogenate metabolic-activation system (fig. 1). By contrast, the positive control, vinylidene chloride, was mutagenic when activated by the liver homogenate. When the liver homogenate was omitted, neither vinylidene chloride nor the anesthetic agents were mutagenic. Similar results were obtained with strain TA1535.

Enflurane, methoxyflurane, and isoflurane were not mutagenic with liquid incubation of the anesthetic agents with strain TA1535 and the liver homogenate (fig. 2). Fluroxene, however, was mutagenic, in a dose-related fashion, at concentrations above 1 per cent when the liver homogenate was present. None of the anesthetic agents, including fluroxene, was mutagenic in the absence of the liver homogenate. Fluroxene, in the presence of the liver homogenate, was the only anesthetic agent that induced mutagenesis of strain TA100 in the liquid incubation system.

Figure 3 details the results of the fluroxene liquid incubation experiments using bacterial strain TA1535, with and without the liver homogenate metabolic-activation system. In the presence of the liver homogenate, the number of revertant colonies increased from 17 to a maximum of 97 at 20 per cent fluroxene vapor concentration. The maximum increase was not significantly different from that seen with the positive control, vinylidene chloride. There was no increase in the number of revertant colonies in the absence of the metabolic activation system.

The results of studies performed on the urines of patients anesthetized with enflurane, methoxyflurane, or isoflurane showed no increase in the numbers of revertant colonies for these agents with either strain TA1535 or strain TA100 (table 1).

Discussion

Recent epidemiologic and laboratory data suggested that inhalation anesthetics may have carcinogenic potential.[3-8] The *Salmonella* assay provides a simple test system for the detection of chemical carcinogens as mutagens. Approximately 85 per cent of known animal and human carcinogens examined in this system are mutagenic, and nearly all of the mutagens are carcinogenic. We previously used this assay to demonstrate that halothane, trifluoroacetic acid, a major halothane metabolite, and urine of patients anesthetized with halothane were not mutagenic.[1]

TABLE 1. Urinary Mutagenesis

	Strain	Metabolic Activation	β-Glucuronidase	Preanesthetic Mean ± SE	Postanesthetic Mean ± SE*
Enflurane	TA1535	−	−	28.4 ± 2.1	30.6 ± 5.8
		−	+	71.8 ± 10.4	25.6 ± 3.9
		+	−	30.6 ± 7.9	27.8 ± 5.6
		+	+	64.4 ± 15.7	47.0 ± 12.3
	TA100	−	−	221.0 ± 19.7	207.0 ± 38.2
		−	+	257.0 ± 17.4	258.0 ± 20.8
		+	−	206.8 ± 17.9	208.2 ± 34.9
		+	+	232.8 ± 20.0	265.1 ± 21.2
Methoxyflurane	TA1535	−	−	108.3 ± 12.3	109.3 ± 20.5
		−	+	128.3 ± 18.2	117.0 ± 11.3
		+	−	104.3 ± 14.9	102.0 ± 13.9
		+	+	112.0 ± 14.0	105.7 ± 14.0
	TA100	−	−	219.6 ± 14.9	224.6 ± 14.9
		−	+	192.6 ± 12.5	212.1 ± 13.8
		+	−	212.6 ± 12.4	226.1 ± 15.7
		+	+	179.3 ± 7.0	193.3 ± 9.6
Isoflurane	TA1535	−	−	34.5 ± 2.3	44.2 ± 3.9
		−	+	31.7 ± 3.0	31.0 ± 3.9
		+	−	34.5 ± 2.8	43.0 ± 5.2
		+	+	32.0 ± 1.9	29.8 ± 3.5
	TA100	−	−	223.0 ± 22.1	200.0 ± 12.4
		−	+	229.0 ± 17.9	185.0 ± 12.6
		+	−	235.5 ± 18.9	193.3 ± 15.0
		+	+	202.3 ± 12.8	204.8 ± 11.7

* No significant increase, preanesthetic *vs.* postanesthetic, all groups.

In the present study, four halogenated ethers, enflurane, methoxyflurane, isoflurane, and fluroxene, were tested for mutagenic activity; only fluroxene was positive. It was mutagenic only in the liquid incubation experiments with strains TA1535 and TA100 and only in the presence of the liver homogenate. These strains are sensitive to reversion by base-pair mutagens. Base-pair mutation occurs when one DNA base is substituted for another. A second type of mutation, frame-shift mutation, occurs with an insertion or deletion in the DNA molecule that shifts the normal reading frame for translation.[9] In general, it was not considered necessary to test the inhalation anesthetics for frame-shift mutation, since their simple molecular structure makes this type of mutation improbable. However, commercially available fluroxene contains 0.01 per cent (w/w) of the nonvolatile stabilizer, N-phenyl-1-naphthylamine, a cyclic aromatic amine, and therefore, a possible frame-shift mutagen. Gas chromatography confirmed the presence of this compound and the absence of other contaminants in commercial fluroxene. Thus, further liquid incubation studies were performed with *Salmonella* strains TA1537 and TA98, which are sensitive to frame-shift mutation[2]; fluroxene did not cause mutation in these strains.††

Fluroxene was mutagenic only in the presence of the liver homogenate metabolic-activation system. This suggests that a metabolite of fluroxene or N-phenyl-1-naphthylamine and not molecular fluroxene is the mutagenic compound. Further, fluroxene was mutagenic when tested in the liquid incubation system but not in desiccators. Some carcinogens (*e.g.*, dimethylnitrosamine) are not mutagenic unless the assay is conducted in a liquid incubation system.[10] Thus, our findings suggest either that a nonvolatile component of fluroxene (*i.e.*, its stabilizer) is metabolized to a mutagen, or that fluroxene or its stabilizer is metabolized to a mutagenic product only in a liquid incubation system. Studies aimed at better defining the mutagenicity of the fluroxene mixture are in progress.

In any case, the finding that commercially available fluroxene is mutagenic is cause for concern. This anesthetic was initially marketed in 1954; its production was discontinued in 1975, probably

†† Enflurane and isoflurane do not contain stabilizers; methoxyflurane contains 0.01 per cent (w/w) butylated hydroxytoluene.

because of its flammability. It is now used clinically only by those individuals who have remaining supplies. Because of its lack of commercial potential, it is highly unlikely that the expensive and time-consuming animal studies necessary to determine the carcinogenicity of fluroxene ever will be undertaken. Therefore, its carcinogenic potential and its possible hazard to man will remain a matter of speculation. Since its introduction into clinical practice, several million patients have been anesthetized with fluroxene. Thus, it is possible that fluroxene may have contributed to the increased rate of malignancy found in operating room personnel.

ADDENDUM

Purified N-phenyl-1-naphthylamine has now been tested for mutagenicity in the liquid incubation system at concentrations identical to those in the fluroxene mixture that were mutagenic. The stabilizer was not mutagenic at any concentration, either in the presence or in the absence of the liver homogenate metabolic activation system.

References

1. Baden JM, Brinkenhoff M, Wharton RS, et al: Mutagenicity of volatile anesthetics: Halothane. ANESTHESIOLOGY 45: 311–318, 1976
2. Ames BN, McCann J, Yamasaki E: Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Res 31: 347–364, 1975
3. Cohen EN, Brown BW, Bruce DL, et al: Occupational disease among operating room personnel: A national study. Report of an Ad Hoc Committee on the Effects of Trace Anesthetics on the Health of Operating Room Personnel, American Society of Anesthesiologists. ANESTHESIOLOGY 41:321–340, 1974
4. Corbett TH, Cornell RG, Lieding K, et al: Incidence of cancer among Michigan nurse-anesthetists. ANESTHESIOLOGY 38:260–263, 1973
5. Bruce DL, Eide KA, Linde HW, et al: Causes of death among anesthesiologists: A 20-year survey. ANESTHESIOLOGY 29:565–569, 1968
6. McCann J, Choi E, Yamasaki E, et al: Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Part 1, Assay of 300 chemicals. Proc Natl Acad Sci USA 72:5135–5139, 1975
7. Saffioti U: Memorandum of Alert. National Cancer Institute, March 21, 1975
8. Corbett TH: Cancer and congenital anomalies associated with anesthesia. Ann NY Acad Sci 271:58–66, 1976
9. Watson JD: Molecular Biology of the Gene. Menlo Park, Calif., Benjamin, 1976, pp 275–278
10. Malling HV: Dimethylnitrosamine: Formation of mutagenic compounds by interaction with mouse liver microsomes. Mutation Res 13:425–429, 1971