

Halothane Inhibits Metabolism of Enflurane in Fischer 344 Rats

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The authors investigated the effect of prior administration of halothane upon the metabolism of enflurane. Twenty-four, one-year-old male, Fischer 344 rats were assigned randomly to four anesthetic exposure groups. Groups 1 and 2 were controls exposed only to halothane and enflurane, respectively. Group 3 was exposed for 1 h to 0.3% halothane, followed by 2 h of 1% enflurane. Group 4 was exposed for 1 h to 1% halothane and then to 2 h of 1% enflurane. Blood samples were taken prior to, immediately following, and 4, 24, and 48 h after anesthetic exposure. Serum was assayed for inorganic fluoride (F^-), SGOT and SGPT. Twenty-four-hour urinary collections were assayed for F^- excretion. Group 1 rats exposed to halothane alone had the lowest peak mean serum F^- ($5.0 \mu M$). Group 2 rats exposed to enflurane alone had the highest serum F^- concentration 4 h after anesthesia ($18.7 \mu M$). Peak serum F^- in Group 3 rats ($9.5 \mu M$) was significantly lower than in Group 2 rats (enflurane control). In Group 4 rats, serum F^- was not significantly different from Group 1 rats (halothane control) at any time. In the first 24 h after anesthetic exposure, urinary F^- excretion in Groups 2 and 3 was significantly higher than in Groups 1 and 4. This study demonstrated that prior exposure to halothane reduced the metabolism of enflurane; previous work suggested that this was due to an interaction of halothane with hepatic cytochrome P-450. The author's results have implications for other drugs that are administered during anesthesia and are metabolized by the hepatic mixed function oxidase system. (Key words: Anesthetics, volatile; enflurane; halothane. Biotransformation: inhibition; fluorometabolites. Ions: fluoride. Liver: metabolism.)

IN A RECENT clinical study, the occurrence of an arrhythmia in a patient after two MAC hours of halothane anesthesia led to the substitution of enflurane for halothane as the anesthetic agent. Measurement of the serum inorganic fluoride (F^-) level following approximately three MAC hours of enflurane exposure, revealed a peak value of only $5 \mu M$. Based on previous reports, this serum F^- level was considerably below what we expected after such an exposure to enflurane. For example, Cousins *et al.*¹ reported a mean peak serum F^- of $22 \mu M$ after an average exposure to enflurane of 2.7 MAC hours.

The present study was designed to investigate the influence of prior administration of halothane on the *in vivo* biotransformation of enflurane to its potentially nephrotoxic metabolite, F^- . The Fischer 344 rat was chosen for this investigation because it is an experimental animal model that frequently has been used to study anesthetic defluorination and nephrotoxicity.^{2,3}

Methods

Twenty-four 1-year-old male Fischer 344 rats‡ were bedded on ground corn cob,§ four to a cage, and quarantined for 1 week. Room temperature was maintained at $21 \pm 1^\circ C$, and artificial light provided from 6 o'clock AM to 7 o'clock PM each day. Rat chow¶ containing 21 mg/kg of F^- and tap water containing 1 ppm of F^- ($52.6 \mu M$) were allowed *ad libitum* except as noted below. At the start of the experiment, rats were placed in individual metabolic cages. After 7 days of equilibration, daily 24-h urine collections were obtained for two weeks. Body weight was measured daily.

Rats then were allocated randomly to four anesthetic exposure groups: 1) 1% halothane for 3 h; 2) compressed air for 1 h, followed by 1% enflurane for 2 h; 3) 0.3% halothane for 1 h, followed by 1% enflurane for 2 h; 4) 1% halothane for 1 h, followed by 1% enflurane for 2 h.

On the day of anesthetic exposure, each group was placed in separate plexiglass chambers; anesthetics were vaporized in compressed air. Inspired anesthetic and oxygen concentrations were monitored with a Hewlett-Packard model 5830A gas chromatograph and with an Instrumentation Laboratories #401 oxygen analyzer, respectively. Rectal temperature of representative rats was monitored with a multichannel Yellow Springs Tele-Thermometer; body temperature was maintained in the normothermic range, with heated water mattresses placed under the floor of the chambers. After recovery from anesthesia, rats were returned to their metabolic cages. One-milliliter samples of blood were drawn from the tail before, immediately after, 4 and 24 h after anesthetic exposure. The 48 h blood samples were obtained by decapitation. Serum and urinary samples were analyzed for F^- with an Orion Ion Specific F^- electrode and Corning

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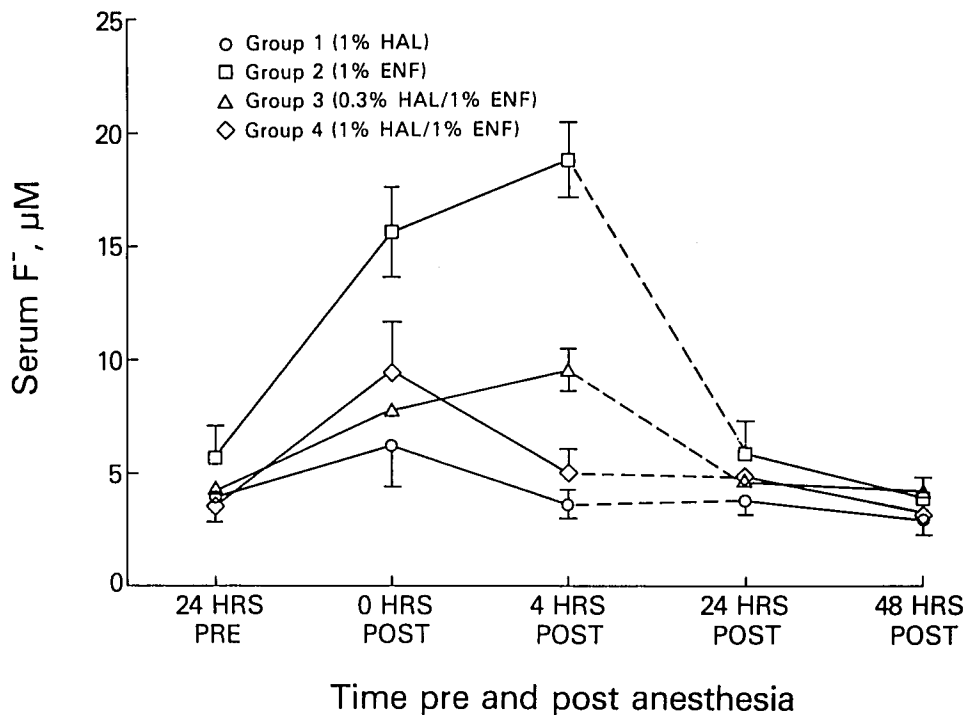


FIG. 1. Serum F⁻ concentrations (±SD) during the experiment. At the end of anesthetic exposure, serum F⁻ in Group 2 was significantly higher than the other three groups. Four hours after anesthesia, serum F⁻ in Group 4 was not significantly different from Group 1. Serum F⁻ in Group 3 was significantly lower than in Group 2, but higher than the other groups. No differences among the groups were present 24 h after anesthetic exposure.

Ion Meter #135.⁴ In addition, SGOT and SGPT were determined before and 24 and 48 h after anesthesia.**

Group mean ± SD for the variables were determined. Differences among the groups were established by one-way analysis of variance and Newman-Keuls contrast test; $P < 0.05$ was considered significant.

Results

There were no important differences among groups prior to anesthetic exposure. Immediately following anesthetic exposure, mean serum F⁻ in Group 2 rats exposed to air and 2 h of 1% enflurane was 15.6 μM (fig. 1). This was significantly higher than that for any of the other three groups, which were not significantly different from each other.

Four hours after anesthesia, three statistically different subsets could be identified. Mean serum F⁻ of Group 2 rats (18.7 μM) still was highest, and different from all other groups. Mean serum F⁻ of Group 3 rats (9.5 μM; 0.3% halothane and 1% enflurane) remained significantly lower than serum F⁻ of rats in Group 2 but was significantly greater than in Groups 1 and 4. In contrast, mean serum F⁻ in rats of Group 4 (5.0 μM; 1% halothane and 1% enflurane) was not significantly different from rats of Group 1, exposed to 1% halothane alone.

During the 24-h period immediately following anesthesia, urinary F⁻ excretion in Groups 2 and 3 was sig-

nificantly higher than in Groups 1 and 4 (table 1). After the first 24 h following anesthesia, there were no significant differences in urinary F⁻ excretion among the four groups. No significant difference attributable to anesthetic effect was seen in body weight (not shown) or urinary volume (fig. 2). Twenty-four hours after anesthesia there were no significant differences from the control period in SGOT or SGPT levels (table 2). The increased levels of SGOT or SGPT 48 h after anesthesia as compared with the control and 24-h levels were attributable to the method of blood collection (*i.e.*, tail blood *vs.* decapitation blood sample). There were no differences among groups, however, at 48 h.

Discussion

The results of this *in vivo* animal study demonstrated that prior administration of halothane significantly reduced the elevation of serum F⁻ produced from the *in vivo* metabolic degradation of enflurane. These results

TABLE 1. Urinary F⁻ excretion ± SD (mmol/day)

	Control Preexposure	0-24 h Postexposure	24-48 h Postexposure
Group 1	4.0 ± 1.4	4.9 ± 0.65	4.2 ± 1.2
Group 2	5.2 ± 1.8	21.6* ± 0.87	5.0 ± 2.3
Group 3	4.2 ± 1.0	17.6* ± 3.3	7.6 ± 2.7
Group 4	3.8 ± 0.6	8.9 ± 3.2	5.8 ± 1.2

* Groups 2 and 3 were significantly different from Groups 1 and 2.

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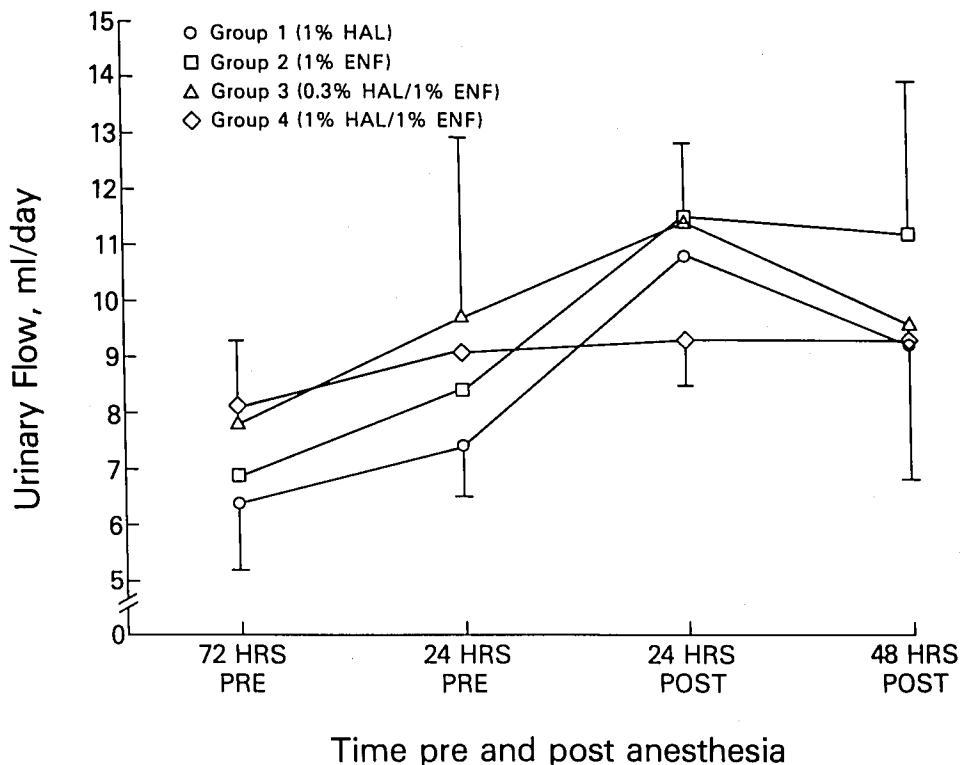


FIG. 2. Urinary flow (\pm SD) during the experiment. No significant differences were seen among the groups.

confirmed our single observation in a human that exposure to halothane in some way resulted in an elevation of serum F^- that was less than expected after subsequent exposure to enflurane.

A number of explanations are possible for our results. These include 1) competition between the two anesthetic agents for the binding sites of hepatic cytochrome P-450; 2) inactivation or destruction of cytochrome P-450 by halothane; 3) reduction in delivery of enflurane to the enzymatic site of degradation, possibly via a halothane-induced reduction in liver blood flow; 4) reduction in uptake of enflurane in those animals exposed to both agents, secondary to increased myocardial depression and lowered cardiac output; and 5) reduction in activity of the cytochrome P-450 enzyme complex due to reduced availability of oxygen.

Drugs that bind with and are metabolized by cytochrome P-450 have been classified generally into two distinct groups, based on the distinct types of absorption spectra they produced when combined with cytochrome P-450.^{5,6} Halothane, enflurane, and the barbiturates are Type 1 compounds,⁷ characterized by absorption spectra that display a trough in the vicinity of 420–425 $m\mu$, and a peak near 385–390 $m\mu$. It would not be surprising if there was competition for enzymatic binding sites between these spectrally similar substrates, thus limiting the metabolism of each substrate. Our observations could be explained on the basis of competition between the two volatile anesthetic agents for the enzymatic binding sites. There is evidence in the literature, however, to suggest that simple competitive inhibition is not the explanation for our results. Using an *in vitro* rat microsomal prepa-

TABLE 2. SGOT and SGPT \pm SD (IUs)

	Control* Postexposure		24 h* Postexposure		48 h†	
	SGOT	SGPT	SGOT	SGPT	SGOT‡	SGPT‡
Group 1	42.8 \pm 3.9	15.0 \pm 2.0	48.4 \pm 3.9	16.1 \pm 1.4	111.3 \pm 14.8	25.4 \pm 4.9
Group 2	58.3 \pm 8.2	19.0 \pm 2.1	57.1 \pm 10.2	19.4 \pm 1.8	123.0 \pm 14.1	33.4 \pm 5.1
Group 3	42.0 \pm 4.8	16.0 \pm 1.7	45.0 \pm 3.1	16.2 \pm 1.2	101.0 \pm 7.3	28.0 \pm 4.0
Group 4	62.0 \pm 7.4	17.0 \pm 3.3	67.3 \pm 11.0	25.9 \pm 7.7	110.5 \pm 15.2	22.4 \pm 1.0

* Blood collected from tail vein.

† Blood collected by decapitation.

‡ Changes in SGOT and SGPT from the control period were at-

tributable to the method of blood collection. There were no significant differences among groups or significant changes from the control period that could be attributed to the anesthetic regimen.

ration, Brown⁸ showed that halothane would diminish the rate of oxidation of other Type 1 substrates, including hexobarbital, amobarbital, and pentobarbital. Instead of finding the more common competitive inhibition, a dose-dependent noncompetitive inhibition was demonstrated. A further important observation that Brown made in his study was that when halothane was allowed to escape the reaction flasks, the microsomal enzymes recovered their ability to metabolize Type 1 substrates again. If Brown's work can be extrapolated to the present study, it would suggest that at concentrations similar to those used in our study, halothane does not reduce the metabolism of enflurane either by a simple competitive inhibition or by destruction of the cytochrome P-450 system, but rather by a noncompetitive inhibition.

Could reduction of hepatic blood flow secondary to administration of the two anesthetic agents explain our results? We believe not; firstly, kinetic data⁹ suggest that the majority of enflurane metabolism occurs after the termination of its administration, when liver blood flow should be returning to normal. Secondly, a very marked reduction in the hepatic blood flow would be required to reduce metabolism and production of F⁻ to the extent seen in Group 4. Such a reduction in blood flow might be expected to produce significant changes in SGOT and SGPT levels, due to hepatic damage. In our study, 24 h after anesthetic exposure, there were no significant changes in SGOT or SGPT levels that could be attributed to the different anesthetic regimens. Because of the sampling method (*i.e.*, decapitation), however, there was a uniform elevation of SGOT and SGPT levels 48 h after anesthetic exposure. At this time there were still no significant differences among the groups. Additionally, circulatory depression and a resultant reduced uptake of enflurane also would be an unlikely explanation. The administration of halothane prior to enflurane in a clinically low concentration such as was given to Group 3 would not be expected to have a marked effect upon the cardiovascular system. However, to our knowledge, the effect of a combination of volatile anesthetic agents on cardiac output is not known.

What, therefore, is the significance of the observed inhibition? We believe our findings are important because many anesthetic drugs are metabolized by the hepatic cytochrome P-450 system and have the potential to be affected in this manner by halothane. Two other recent reports of halothane interaction are of relevance to our study. In an *in vivo* study in rats, Wood and Wood¹⁰ showed that halothane inhibited the rate of aminopyrine elimination in a dose-dependent fashion for at least 24

h. In dogs anesthetized with halothane, Borel *et al.*¹¹ found that the clearance of fentanyl was prolonged. Can our results be extrapolated to humans? Our study was with an animal model, the Fischer 344 rat, which repeatedly has been demonstrated to be a successful model of human anesthetic biotransformation and toxicity.^{2,3} Perhaps of greater importance was that the initial impetus to perform this study came from the observation of an unusually low serum F⁻ after enflurane anesthesia in a human after previous exposure to halothane. It would appear that halothane inhibits hepatic cytochrome P-450 in humans, but further work is necessary to establish the relevance of these findings to human drug metabolism.

In summary, we have found that prior exposure to halothane will reduce the elevation of serum F⁻ seen after enflurane anesthesia in Fischer 344 rats. This may have further implications for the action of other drugs that may be administered during or immediately after halothane anesthesia.

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