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EFFECT OF INHALATION ANESTHETICS ON ANTIPYRINE PHARMACOKINETICS OF MICE

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Abstract—The effects of the volatile anesthetics, enflurane, isoflurane and halothane, on the pharmacokinetics of antipyrine were examined in mice. The administration of 0.75% isoflurane or 1.0% enflurane in air resulted in a 173 and a 206% increase, respectively, in antipyrine plasma half-life and a 29.1 and a 41.2% decrease in antipyrine total body clearance. There was also an almost 2-fold increase in the volume of distribution of antipyrine. Halothane, at 0.5% in air, had no significant effect upon antipyrine plasma half-life or its volume of distribution. There was no significant change in antipyrine total body clearance and volume of distribution 4 hr after exposure to the volatile agents, but there was a small increase in half-life. The exposures to the volatile anesthetics were also carried out in an atmosphere of 8% oxygen. Antipyrine plasma half-life was increased significantly by 48% in mice breathing 8% oxygen, compared to mice breathing air. Isoflurane in 8% oxygen increased the plasma half-life of antipyrine by 296% compared to mice breathing 8% oxygen. This increase was greater than the effect of isoflurane seen in mice breathing air. Mice breathing halothane in 8% oxygen exhibited a 21% increase in antipyrine plasma half-life and mice breathing enflurane in 8% oxygen, a 117% increase in antipyrine plasma half-life, although the changes were not markedly different from those seen in mice breathing air. Enflurane and isoflurane produced a significant increase in the volume of distribution for antipyrine in the mice breathing 8% oxygen. Total body clearance of antipyrine was decreased markedly in mice breathing isoflurane and enflurane but showed a lesser decrease in mice breathing halothane in 8% oxygen. *In vitro* in mouse microsomes, halothane, enflurane and isoflurane were all inhibitors of aminopyrine metabolism. Possible mechanisms for these results are discussed.

The quest for volatile anesthetics which undergo little or no metabolism has been so successful that scant attention has been directed toward the possible effects of volatile anesthetics on the metabolism of other drugs. Anesthetics are noted for their effects on membranes and, since cytochrome P-450, the primary drug-metabolizing enzyme system, is membrane-bound, it is not unreasonable that anesthetics might affect cytochrome P-450 drug-metabolizing activity. Previous studies from this laboratory have revealed that halothane, a volatile anesthetic agent, has a profound effect on the function of cytochrome P-450 [1]. This effect is a composite of two mechanisms of interaction. One, which is irreversible, involves the destruction of a portion of the cytochrome P-450 molecule by reactive halothane metabolites formed under hypoxic conditions *in vivo* or *in vitro*. The second mechanism of interaction is reversible and involves masking of the enzymatic site of cytochrome P-450 by a strong enzyme–halothane complex or a reversible alteration in the enzyme structure by halothane. While halothane probably exhibits both types of interaction with cytochrome P-450 when metabolism occurs anaerobically, the other two widely used volatile anesthetic agents, enflurane and isoflurane, are not metabolized by way of reactive intermediates and do not irreversibly inhibit P-450, anaerobically or aerobically [1], nor do they form strong complexes with this enzyme as does halothane [2].

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There have been a number of reports that general anesthetics can decrease significantly the rate of elimination of other drugs administered during anesthesia. Thus, the clearance of ketamine, lidocaine, pancuronium and fentanyl have all been reported to be decreased by volatile anesthetics in laboratory animals [3–7]. The mechanism for the decrease in the clearance of these drugs has not been elucidated. Ketamine, lidocaine and pancuronium are all metabolized extensively by the liver [4–6], and a decreased elimination is likely to result from a decrease in hepatic metabolism. A change in the capacity of the liver to metabolize a drug can be brought about, for a slowly eliminated drug, by a change in the intrinsic ability of the liver to metabolize the drug or, for a rapidly eliminated drug, by a change in blood flow to the liver [8]. Alternatively, a decrease in oxygen and nutrient supply to the liver brought about by a decreased blood flow can affect the metabolism of both rapidly and slowly eliminated drugs [9, 10].

In this study we examined, in mice, the effects of the volatile anesthetic agents, halothane, enflurane and isoflurane, and hypoxia on the elimination of antipyrine, an agent whose elimination is thought to reflect the activity of hepatic cytochrome P-450 [11].

METHODS

In vivo *studies*. Five-week-old male BDF_1 mice (Harlan Sprague–Dawley, Madison, WI), weighing 16–20 g and allowed free access to food and water, were injected intraperitoneally with 50 mg/kg antipyrine (Aldrich Chemical Co., Milwaukee, WI) dis-

solved in 0.2 ml of 0.9% NaCl. Immediately following injection, animals were placed in a large Plexiglas box with a fan to circulate the gas mixture and were exposed to either air, 0.5% halothane in air, 0.75% isoflurane in air or 1% enflurane in air. The concentrations of anesthetic agents were approximately two-thirds the minimum alveolar concentration (MAC*) for each agent [12]. In other studies, halothane concentrations of 1.0% and 1.5% in air were employed or the air was replaced by a mixture of 8% oxygen in nitrogen. Oxygen levels were monitored by means of an oxygen analyzer (Salter Instrument Co., Arvin, CA). Halothane (Fluothane; Ayerst Laboratories, New York, NY), isoflurane (Forane, Anaquest; Madison, WI) and enflurane (Ethrane, Anaquest; Madison, WI) were delivered by means of the appropriate vaporizer at a gas flow rate of 4 l/min. Some mice were exposed to the anesthetic agent for 2 hr and then removed to room air for 4 hr before administering antipyrine. Mice were killed by exsanguination by orbital sinus puncture [13]. Blood was collected into heparinzed tubes, and plasma was separated rapidly using a microcentrifuge (Microfuge-B, Beckman Instruments, Fullerton, CA) and then stored at −20° until assay of antipyrine. There were at least six mice at each time point.

Antipyrine was assayed by a modification of the gas chromatographic method of Prescott *et al.* [14]. To 0.2 ml plasma was added 2 μg/ml aminopyrine internal standard, 0.2 ml of 5 M NaOH, and the mixture was extracted with 8 ml chloroform. The chloroform layer was removed, dried with anhydrous Na_2SO_4, and evaporated to dryness under a stream of N_2 at 30°. The residue was dissolved in 0.2 ml ethyl acetate, and 2 μl was taken for gas chromatography (GC) on a 25 m SE-54 capillary GC column with a temperature gradient of 75–100° over 10 min with a nitrogen-phosphorus detector.

Pharmacokinetic analysis of antipyrine plasma concentration data was performed using the NONLIN least squares regression analysis computer program [15] with a 1/y weighting factor and pharmacokinetic parameters for a one-compartment open model calculated according to Wagner [16]. Clearance, steady-state volume of distribution, and half-life together with their standard errors were determined [17]. Data were fit directly to clearance and volume of distribution terms. Because of the inaccuracy of extrapolation, the half-life was fit directly in a separate determination.

In vitro *studies*. Microsomes from male mice were prepared from the livers of male BDF_1 mice. Animals were killed by cervical dislocation and their livers were rapidly removed and washed with ice-cold aqueous 1.15% KCl, minced, and homogenized in 3 vol. of aqueous 1.15% KCl. The homogenate was centrifuged at 1,000 *g* for 15 min; the supernatant fraction was centrifuged at 9,000 *g* for 20 min and then at 105,000 *g* for 1 hr. The resulting microsomal pellet was washed by suspension in aqueous 1.15% KCl and centrifuged as above. For assay, the microsomal pellet was resuspended in 0.05 M Tris buffer, pH 7.4.

Aminopyrine N-demethylation was measured by formaldehyde production according to the method of Nash [18]. The standard reaction mixture contained (in a volume of 3 ml) 150 μmoles Tris buffer, pH 7.4, microsomes (3 mg protein), 6 μmoles aminopyrine, and an NADPH-generating system. The latter consisted of 20 μmoles glucose-6-phosphate, 1 μmole $NADP^+$ and 1 unit of glucose-6-phosphatedehydrogenase. Incubations were carried out for 10 min at 37° in unstoppered test tubes. The volatile anesthetic was added, undiluted as liquid or diluted in ethanol, directly to the incubation mixture. At the conclusion of the incubation, 1 ml of the incubation mixture was added to 1 ml of hexane for halothane extraction. Following extraction, the hexane layer was sampled for GC analysis using a Hewlett–Packard model 5880 gas chromatograph equipped with an electron capture detector and a 6-ft carbowax column maintained at 100°.

RESULTS

In vivo *studies*. Studies of mice exposed to 0.75% isoflurane in air or 1% enflurane in air showed a markedly slower plasma elimination of antipyrine compared to control animals (Fig. 1). The pharmacokinetic parameters for antipyrine elimination are shown in Table 1. With isoflurane there was a 173% increase in antipyrine plasma half-life, with enflurane a 206% increase, and an almost 2-fold increase but no significant change in the volume of distribution of antipyrine with both anesthetic agents. Halothane, 0.5% in air, had no significant effect upon antipyrine plasma half-life or upon its volume of distribution. Isoflurane and enflurane, but not halothane, significantly decreased total body clearance of antipyrine. There was no significant change in antipyrine volume of distribution or total body clearance produced by volatile anesthetic agents 4 hr after exposure to the agent. There was a significant, small increase in antipyrine plasma half-life with all three agents.

Mice were also exposed to anesthetic agents in low (8%) oxygen to see if the effects of the anesthetic agents on the elimination of antipyrine were potentiated under these conditions. This appeared to be the case (Fig. 2 and Table 1). Antipyrine plasma half-life was increased significantly by 48% in mice breathing 8% oxygen, compared to mice breathing air. Isoflurane in 8% oxygen increased the plasma half-life of antipyrine by 296% compared to mice breathing 8% oxygen. This increase was greater than the effect of isoflurane seen in mice breathing air. Mice breathing halothane in 8% oxygen exhibited a small (21%) increase in antipyrine plasma half-life and mice breathing enflurane in 8% oxygen, a 117% increased antipyrine plasma half-life compared to mice breathing 8% oxygen, although the changes were not markedly different from those seen in mice breathing air. Enflurane and isoflurane in 8% oxygen produced a small, but significant increase in the volume of distribution for antipyrine compared to mice breathing 8% oxygen alone. Total body clear-

* MAC: minimum alveolar concentration, that amount of anesthetic inspired to produce anesthesia in 50% of the animals (anesthetic ED_{50}).

Table 1. Effect of inhalation anesthetics on antipyrine pharmacokinetics

	Cl (ml/min/kg)	Vd (l/kg)	$T^{\frac{1}{2}}$ (min)
Control	28.9 ± 1.9	0.55 ± 0.06	13.28 ± 0.55
Halothane	35.7 ± 2.8	0.79 ± 0.11	15.42 ± 0.97
Isoflurane	20.5 ± 0.5*	1.07 ± 0.06*	36.31 ± 1.93*
Enflurane	17.0 ± 0.3*	1.00 ± 0.06*	40.62 ± 2.20*
After 4-hr delay			
Halothane	22.2 ± 1.1	0.54 ± 0.05	16.91 ± 0.73*
Isoflurane	22.1 ± 1.5	0.65 ± 0.05	20.46 ± 0.95*
Enflurane	29.9 ± 1.4	0.78 ± 0.04	17.98 ± 0.58*
Low oxygen (8%)			
Control	28.6 ± 1.1	0.81 ± 0.06	19.71 ± 0.80*
Halothane	24.3 ± 0.5†	0.83 ± 0.04	23.75 ± 0.65*†
Isoflurane	11.0 ± 0.7*†	1.25 ± 0.09*†	78.22 ± 8.87*†
Enflurane	21.6 ± 0.3*†	1.33 ± 0.05*†	42.85 ± 1.66*†

Values were calculated as described in the text and are mean ± SE with at least six mice at each time point. $T^{\frac{1}{2}}$ is the plasma elimination half-life; Vd is the steady-state volume of distribution; and Cl represents clearance. Halothane (0.5%), isoflurane (0.75%) and enflurance (1%) were administered as described in the text. In some studies animals were exposed to a volatile anesthetic for 2 hr and then removed to room air for 4 hr before administration of antipyrine. In other studies air was replaced by an 8% oxygen:92% nitrogen gas mixture.

* $P < 0.05$ when compared with controls in air, using a Bonferroni correction [19].

† $P < 0.05$ when compared with low oxygen control using a Bonferroni correction [19].

ance of antipyrine was decreased significantly in mice breathing isoflurane, enflurane, or halothane in 8% oxygen compared to mice breathing 8% oxygen alone. Studies in which the concentration of halothane in air was varied showed a concentration-dependent increase in antipyrine plasma half-life, a decrease in antipyrine total body clearance but no change in antipyrine volume of distribution except for a decrease at the highest concentration of halothane (Table 2).

In vitro *studies*. The *in vitro* effects of the anesthetic agents upon the N-demethylation of aminopyrine by the hepatic microsomal fraction were studied (Table 3). Halothane, enflurane and isoflurane were all inhibitors of aminopyrine metabolism.

DISCUSSION

Antipyrine elimination is used widely in humans and animals to measure the intrinsic metabolizing capacity of hepatic cytochrome P-450 [11, 20]. Isoflurane and enflurane, but not halothane, at equipotent anesthetic concentrations (two-thirds MAC) decreased the total body clearance of antipyrine in mice, thus prolonging its plasma half-life. The decreased total body clearance of antipyrine was reversible by breathing air for 4 hr. At high concentrations of halothane there was a decrease in antipyrine elimination but halothane was less potent in this regard than isoflurane or enflurane. Wood and Wood [7] previously reported a dose-dependent

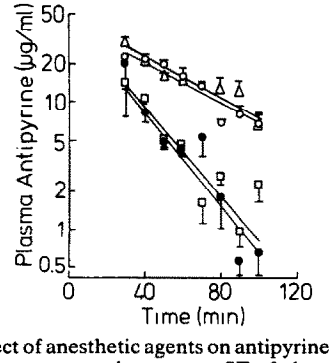

Fig. 1. Effect of anesthetic agents on antipyrine elimination. Each point represents the mean ± SE of plasma antipyrine concentration in six to eight mice exposed to air (●), halothane (□), isoflurane (○), or enflurane (△). Continuous lines are computer-derived fits to the data.

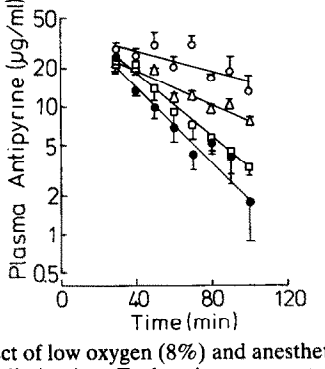

Fig. 2. Effect of low oxygen (8%) and anesthetic agents on antipyrine elimination. Each point represents mean ± SE of plasma antipyrine concentration in six to eight mice exposed to low oxygen (●), halothane and low oxygen (□), isoflurane and low oxygen (○), or enflurane and low oxygen (△). Continuous lines are computer-derived fits to the data.

Table 2. Effect of halothane on antipyrine pharmacokinetics

	Cl (ml/min/kg)	Vd (l/kg)	$T^{\frac{1}{2}}$ (min)
Control	42.9 ± 2.3	0.80 ± 0.07	12.97 ± 0.46
Halothane (0.5%)	29.5 ± 1.1*	0.76 ± 0.05	17.94 ± 0.62*
Halothane (1.0%)	3.49 ± 1.6	1.24 ± 0.12	24.70 ± 1.53*
Halothane (1.5%)	12.1 ± 0.8*	0.43 ± 0.07*	24.94 ± 2.53*

Values were calculated as described in the text and are mean ± SE with six animals per time point.

*$P < 0.05$ when compared with controls using a Bonferroni correction [19].

inhibition of aminopyrine metabolism by halothane in the rat.

Previous studies *in vitro* have yielded a confusing picture of the effects of the volatile anesthetics on hepatic drug metabolism. These effects range from a stimulation of the microsomal oxidation of aminopyrine by methoxyflurane [21] to an apparent destruction of microsomal cytochrome P-450 by halothane under anaerobic conditions [1]. Previous studies have demonstrated an inhibition of the oxidative metabolism of aminopyrine by rat microsomes with volatile anesthetics [22]. This has been confirmed with mouse microsomes in the present study. Inhibition of cytochrome P-450-dependent metabolism by halothane is only partially reversible, particularly when the metabolism is carried out under hypoxic conditions [1]. Inhibition of the enzymatic activity of cytochrome P-450 by enflurane and isoflurane, although as great as that by halothane, is completely reversible under normoxic or hypoxic conditions [1].

The marked difference between the effects of the volatile anesthetics in inhibiting *in vivo* antipyrine metabolism while having similar inhibitory effects on oxidative metabolism *in vitro* may appear to rule out a direct effect of the volatile anesthetics on cytochrome P-450 in inhibiting *in vivo* antipyrine elimination. The lack of an effect of halothane on antipyrine elimination was particularly surprising in view of its known *in vitro* interaction with cytochrome P-450. However, it is difficult to extrapolate *in vitro* findings to *in vivo* conditions of anesthetics for several reasons. The *in vivo* tissue concentration of the volatile anesthetic will depend upon the agent used. Isoflurane and enflurane are used at higher concentrations than halothane to achieve anesthesia [23, 24]. In fact, although halothane at a 0.6 MAC concentration was without effect on antipyrine elimination, increasing the concentration of halothane up to 2 MAC (the limit of clinically used concentrations) did result in a decrease in the elimination of antipyrine. There may be physiological changes in respiration and hepatic blood flow produced by the anesthetic agents that affect drug elimination. Hepatic clearance of a highly extracted drug is dependent on hepatic blood flow [25, 26]. A decrease in hepatic blood flow will reduce delivery of drug to the liver, resulting in lower drug elimination and higher plasma concentrations of drug. Antipyrine was chosen as a drug whose hepatic elimination in humans and other species is not dependent on hepatic blood flow but is determined by the intrinsic metabolizing capacity of the liver and is dependent on the activity of hepatic cytochrome P-450 [11, 20]. In contrast to some reports [27, 28], a recent study in this laboratory has shown that volatile anesthetics do not alter significantly the perfusion of the liver at the doses used in this study [29]. For all these reasons, the decrease in antipyrine elimination produced by isoflurane and enflurane is unlikely to result from changes in hepatic blood flow.

A decreased oxygen delivery to the liver can result in a decreased ability of the liver to catalyze oxidative metabolism of drugs [9, 30]. The animals used in our study were breathing spontaneously and, while the concentrations of anesthetic were kept low, it is possible that enflurane and isoflurane had an effect on respiration that halothane did not. Enflurane and isoflurane are more potent respiratory depressants than halothane [31–33]. With this in mind, we studied the effects of hypoxia on the delayed elimination of antipyrine produced by the volatile anesthetics. Hypoxia itself produced a small decrease in the elimination of antipyrine but produced no additional effect on antipyrine elimination with halothane or enflurane. However, the effect of isoflurane on antipyrine elimination was potentiated markedly by hypoxia, and the antipyrine half-life was increased to almost 6-fold that in air-breathing control animals.

Table 3. Effects of halothane, enflurane and isoflurane on aminopyrine demethylation *in vitro*

Additions	Anesthetic conc (mM)	Aminopyrine demethylation (nmoles/mg protein/10 min)
Control		96.5 ± 2.1
Halothane	0.4 ± 0.02	48.5 ± 1.4
	1.4 ± 0.12	40.5 ± 1.3
	1.5 ± 0.09	36.2 ± 1.8
Enflurane	0.35 ± 0.03	49.7 ± 1.3
	0.80 ± 0.09	43.8 ± 1.5
	1.10 ± 0.11	39.9 ± 0.9
Isoflurane	0.3 ± 0.02	43.2 ± 1.1
	0.7 ± 0.06	36.2 ± 0.9
	1.1 ± 0.07	33.1 ± 0.7

Volatile anesthetic concentrations were measured in the medium at the end of the 10-min incubation. At the conclusion of the incubation, 1 ml of the incubation mixture was added to 1 ml of hexane for halothane extraction. Following extraction, the hexane layer was sampled for gas chromatographic analysis using a Hewlett–Packard model 5880 gas chromatograph equipped with an electron capture detector and a 6-ft carbowax column maintained at 100°.

It is possible that the mouse can largely compensate for a decreased oxygen delivery to the liver in hypoxia by physiological mechanisms, but that isoflurane inhibits these mechanisms. Finally, it remains a possibility that volatile anesthetics, because of their known membrane-altering properties, could decrease the uptake of antipyrine into hepatocytes, thus decreasing its metabolism.

In summary, the volatile anesthetics, halothane, isoflurane and enflurane, were found to produce a reversible decrease in the elimination of antipyrine in mice. The effect was greater with isoflurane and enflurane than with halothane. There is no simple explanation for the effect of the volatile anesthetics. It is difficult to account for the differential effect of anesthetics by direct inhibition of cytochrome P-450. The effect of anesthetics probably does not involve reduced delivery of antipyrine to the liver but may involve decreased oxygen delivery to the liver, particularly in animals breathing low oxygen exposed to isoflurane, or decreased transport of antipyrine into the hepatocyte.

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