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Halogenated Anesthetics Increase Oxygen Consumption in Isolated Hepatocytes from Phenobarbital-treated Rats

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Using suspensions of hepatocytes isolated from phenobarbital-treated and untreated rats (+PB cells and −PB cells, respectively), the authors examined the effects of halothane, enflurane, and isoflurane on O_2 consumption (VO_2), and on extracellular P_{O_2} and energy status at steady states of O_2 and energy metabolism. In +PB cells, all three agents produced increases in VO_2 which were largest at 1 MAC and progressively smaller at 2 and 3 MAC. At all three doses, VO_2 increases were largest with enflurane (48% at 1 MAC), intermediate with halothane (24%), and smallest with isoflurane (11%). These anesthetic-induced VO_2 increases were abolished by prior addition of a cytochrome P450 inhibitor (metyrapone) to the incubations. In −PB cells, all three agents produced slight and comparable decreases in VO_2 at 1 MAC, with further decreases at 2 and 3 MAC. In +PB cell suspensions at steady states of O_2 and energy metabolism, 1 MAC enflurane or halothane, but not isoflurane, produced significant declines in steady state P_{O_2} (from initial values of 24 mmHg to values < 10 mmHg) and reductions in adenosine triphosphate/adenosine diphosphate ratio (ATP/ADP). These changes were absent in −PB cells exposed to the same conditions or in +PB cells not exposed to anesthetic. The authors conclude that clinical doses of enflurane and, to a lesser extent, halothane produce statistically significant increases in O_2 consumption, reflecting enhanced cytochrome P450 activity, in liver cells isolated from phenobarbital-treated rats. Such increases in O_2 demand represent a mechanism by which anesthetic metabolism could contribute to intrahepatic hypoxia. Anesthetic-induced VO_2 increases by themselves are unlikely to be an important mechanism of cellular injury in the phenobarbital-hypoxia model of anesthetic hepatotoxicity, since enflurane showed larger VO_2 increases than halothane but is considerably less hepatotoxic. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Complications: hepatotoxicity. Metabolism: adenosine diphosphate; adenosine triphosphate; enzyme induction; hypoxia; lactate formation; oxygen consumption.)

THE OCCURRENCE OF FOCAL hepatic necrosis in phenobarbital-pre-treated adult male rats exposed to halothane in 14% O_2 is a widely studied animal model of halothane hepatitis.[1–3] The essential role of enhanced halothane biotransformation in this form of hepatotoxicity is indicated by the requirement for chronic phenobarbital treatment, which elevates cytochrome P450 activity, and by the ability of acutely administered P450-specific inhibitors, such as metyrapone or cimetidine, to prevent or ameliorate liver damage.[4–6]

The specific biochemical mechanism by which increased halothane metabolism contributes to hepatocellular injury remains unsettled. One current hypothesis proposes that halothane metabolism itself causes injury by generating halothane metabolites that react with and damage essential cellular components.[1] The major elements of this mechanism are consistent with existing experimental evidence: P450-mediated halothane metabolism gives rise to reactive metabolites *via* an alternative (reductive) pathway[7,8]; and phenobarbital pretreatment[9] and hypoxia[10,11] have both been shown to increase the rate of halothane reductive metabolism.

Alternative pathogenetic schemes have proposed that hypoxia is the direct cause of hepatocellular injury.[12–14] The existence of substantial intrahepatic hypoxia with 14% O_2 is confirmed by the accumulation of reductive metabolites of halothane in the liver[15]; such moieties are not formed to any substantial extent unless P_{O_2} at the subcellular locus of P450 activity is less than 20 mmHg.[11,16] However, the combination of hypoxia plus halothane is not sufficient to produce hepatic injury in this model when halothane metabolism is reduced or absent[4–6]; and hypoxia-centered proposals have so far failed to provide evidence that halothane metabolism could be making its essential contribution to liver cell damage by intensifying hypoxia.

This study examines the possibility that P450-mediated halothane biotransformation *via* the O_2-dependent ("oxidative") pathway[10] is a mechanism by which halothane metabolism could contribute directly to intrahepatic hypoxia. Halothane oxidative metabolism consumes two oxygen atoms for every molecule of halothane metabolized, and is thought to be considerably more rapid than halothane reductive metabolism at all P_{O_2} levels above virtual anoxia.[11,17] An acute increase in cellular O_2 demand due to halothane oxidative metabolism might precipitate overt hypoxia in marginally oxygenated liver of hypoxemic animals. Halothane oxidative metabolism would be enhanced by phenobarbital pretreatment and diminished by P450-specific inhibitors, just as would halothane reductive metabolism.

The goal of this study was, therefore, to ascertain the occurrence and extent of increased O_2 consumption arising from halothane metabolism by liver cells, and to examine the potential of that extra O_2 demand to pro-

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mote hepatocellular hypoxia and energy insufficiency under conditions relevant to the animal model. Parallel studies were also carried out with enflurane and isoflurane to ascertain whether the effects of halothane on O_2 consumption were sufficiently distinctive to account for the latter's selective hepatotoxicity.[13,18]

Methods

Adult male F344 rats, with or without prior phenobarbital treatment, were used for preparation of isolated hepatocytes exactly as described in a previous paper from this laboratory.[19] For experimental studies, hepatocyte suspensions in Krebs HCO_3 buffer + 5 mM glucose were preincubated for 10 min in sealed round-bottom flasks gassed with O_2/CO_2 (95/5) in a rotating water bath at 37° C, then incubated at the same temperature with rapid magnetic stirring in a specially designed 1.8 ml all-glass incubation chamber with built-in O_2 electrode (Oxygraph; Gilson Medical Electronics, Middleton, WI). Ambient extracellular P_{O_2} was registered directly from the suspension and continuously recorded.

With the chamber completely filled ($2-4 \times 10^6$ cells/ml) and sealed (= closed system), the rate of hepatocyte O_2 consumption (VO_2) was directly measureable as the linear slope of the P_{O_2} *versus* time recorder tracing. Anesthetics were diluted in absolute ethanol as described below, and injected into the suspension through an addition port. The amounts of the three anesthetics to be injected in order to achieve specific and equianesthetic doses were determined in preliminary experiments: hepatocyte suspensions were equilibrated in sealed flasks with O_2/CO_2 (95%/5%) containing the desired percentage of anesthetic vapor, and the concentration of dissolved anesthetic in aliquots of the suspension was then measured by gas chromatography.[20] A 1% halothane mixture gave rise to 10 mg% halothane in the cell suspension; 2% enflurane gave 15 mg%, and 1.5% isoflurane 8 mg%. Since volumes of anesthetic to be injected were considerably less than one microliter, liquid anesthetics were diluted 50-fold in absolute ethanol to minimize errors of (volume) measurement. Changes in VO_2 resulting from injection of ethanol-diluted anesthetic were corrected for the slight decrease (averaging 2% per addition) observed in control incubations carried out with injections of ethanol alone. Each anesthetic type was tested against a fresh aliquot of a given hepatocyte preparation by stepwise injection of three 1-MAC increments of that agent. Intervals of 30–45 s between injections were sufficient to allow VO_2 to stabilize. Complete testing of all concentrations of all three agents plus an ethanol blank, a total of four separate incubations, was thus easily completed in 15–20 min. This design permitted assessment of VO_2 dependence on anesthetic type and on anesthetic dose over the 1–3 MAC range by use of two-way ANOVA with repeated measures on both type and concentration.[21] Within-group differences in VO_2 between any individual type/concentration of anesthetic and its corresponding zero-MAC control (= rate measured in that same incubation prior to any anesthetic addition) were determined by the Student's *t* test for paired data. Comparison of differences between +PB and −PB groups for the same type/concentration of anesthetic were done with the *t* test for unpaired data.

Incubations were also carried out in an open-system configuration to investigate the effects of changes in O_2 consumption on ambient P_{O_2} in the context of steady states of O_2 metabolism approximating the situation in intact tissue. Details of this approach were also described previously[19]; in brief, the incubation chamber was partially filled (1.2 ml) and left unstoppered so that fresh O_2-containing gas mixture could be circulated continuously across the surface of the suspension. Under such conditions, O_2 utilization by the suspended hepatocytes depletes liquid-phase O_2 faster than it can be replaced by O_2 diffusion from the gas phase, so that a P_{O_2} gradient forms across the gas-liquid interface. A steady state of O_2 metabolism is reached when liquid-phase P_{O_2} drops far enough, and the P_{O_2} gradient thus becomes large enough, to drive O_2 into the suspension exactly as fast as the hepatocytes remove it. A subsequent change in O_2 demand alters liquid-phase P_{O_2}, and, thus, the magnitude of the P_{O_2} gradient. O_2 supply thus changes in the same direction as O_2 demand, so that, eventually, a new steady state supervenes, but at a new value of P_{O_2}.

The status of energy metabolism in hepatocytes under such steady-state conditions was evaluated by acid quenching after 10 min of incubation, followed by analysis of the extracts for lactate and adenine nucleotides, as carried out previously.[19] Lactate metabolism was expressed as Δlactate, the difference between lactate concentrations at the beginning and end of the incubation period. Adenine nucleotide metabolism was characterized as the ratio of adenosine tri- and diphosphate concentrations (ATP/ADP). For such steady-state experiments measuring changes in P_{O_2}, ATP, ADP, and lactate, each separate concentration of a specific anesthetic agent required a separate incubation, so that only one or two agents could be studied at all three concentrations in any given preparation. Specific incubation sequences were always run in duplicate on successive days, the second time in reverse order, to compensate for possible changes in baseline metabolic status in cells stored for as long as 2–3 h, the period required to complete all incubations. The dependence of ATP/ADP and Δlactate on anesthetic concentration over the range 1–3 MAC and on the presence/ absence of phe-

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TABLE 1. Rates of O_2 Consumption (VO_2) in Isolated Hepatocytes*

Treatment/Anesthetic	Anesthetic Dose		
	1 MAC	2 MAC	3 MAC
+PB/enflurane†	148 ± 6¶**	134 ± 11¶**	125 ± 8¶**
+PB/halothane‡	124 ± 7¶**	112 ± 10**	101 ± 6**
+PB/isoflurane‡§	111 ± 7	105 ± 7	95 ± 8
−PB/enflurane§	84 ± 8	73 ± 8¶	75 ± 8¶
−PB/halothane§	89 ± 2	80 ± 4¶	73 ± 5¶
−PB/isoflurane§	89 ± 6	83 ± 11	81 ± 12

† +PB = phenobarbital-treated; −PB = untreated.
* Values given are means ± SEM for N = 3 preparations, expressed as percent of control value (no anesthetic).
†‡§ Significantly different groups of 1–3 MAC values are designated by different symbols.
¶ Significantly different from no-anesthetic control value (100).
** Significantly different from corresponding untreated (−PB) value.

nobarbital treatment were examined separately for each anesthetic agent using two-way ANOVA with repeated measures on concentration only. For any given agent, differences in steady state P_{O_2}, ATP/ADP, or Δlactate values between the 1-, 2-, or 3-MAC incubation and the 0-MAC (control) incubation run with each preparation were compared using the Student's *t* test for paired data. The Bonferroni correction was applied when multiple comparisons were made using the *t* test.[21]

All statistical evaluations performed in this study used $P < .05$ or better as grounds for rejecting the null hypothesis and inferring statistical significance.

Results

Table 1 shows the effects of halothane, enflurane, and isoflurane on VO_2 in hepatocytes isolated from phenobarbital treated and untreated rats (+PB cells and −PB cells, respectively). There was significant dependence of VO_2 on all three of the major independent variables—PB treatment, anesthetic type, and anesthetic dose. In the +PB cells, eight of nine mean VO_2 values in the presence of anesthetic were equal to or greater than VO_2 in the absence of anesthetic (=100%), whereas none of the nine VO_2 values in −PB cells exposed to anesthetic met this criterion ($P < .05$ by Fisher's exact test). Decreases in VO_2 over the 1–3-MAC anesthetic dose range were statistically significant for all three anesthetic types in both +PB and −PB cells. Dependence on anesthetic type was significant in +PB cells, but not in −PB cells, as shown in table 1. Differences in VO_2 values between any individual incubation and the corresponding no-anesthetic control, or between corresponding +PB and −PB incubations, are also indicated in table 1.

The potential for such anesthetic-induced changes in hepatocyte O_2 demand to control ambient extracellular

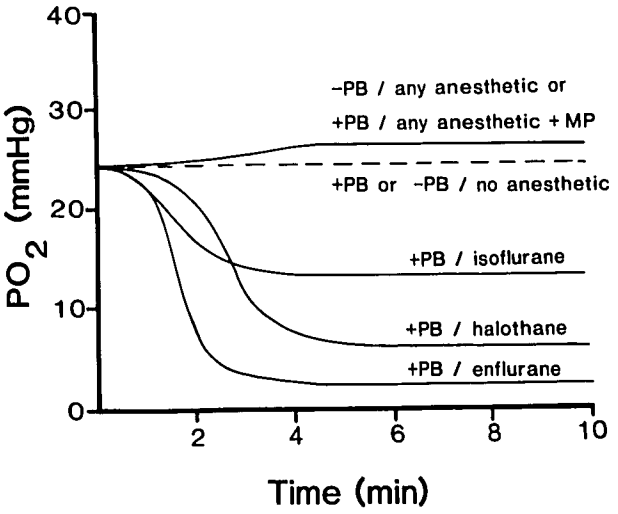

FIG. 1. Continuous recordings of extracellular P_{O_2} in hepatocyte suspensions following introduction of 1 MAC halothane, enflurane, or isoflurane into the gas phase of the open incubation system at zero time. Tracings are representative of those obtained from five different preparations. +PB = cells from phenobarbital-treated rats; −PB = cells from untreated rats; +MP = metyrapone added to the suspension 30–60 s prior to anesthetic introduction.

P_{O_2} under simulated tissue conditions was examined at steady states of O_2 metabolism. Figure 1 shows representative O_2 electrode tracings from steady-state incubations of +PB and −PB cells (N = 5 for each). With O_2 supply held constant, increases in O_2 demand produced by exposing +PB cells to 1-MAC anesthetic led to new steady states that stabilized at lower values of ambient extracellular P_{O_2}. As expected, the magnitude of P_{O_2} decreases in the open system roughly paralleled the extent of VO_2 increases in the closed system shown in table 1. Individual P_{O_2} values attained after anesthetic addition to the steady-state system are shown in table 2. In all five preparations of +PB cells, 1-MAC enflurane produced the lowest values of steady state ambient P_{O_2}, which consistently reached frankly hypoxic levels (<10 mm Hg). Isoflurane produced the smallest changes in

TABLE 2. Individual Values of Steady-State P_{O_2} (mmHg) Attained After 10-Min Incubation

Treatment/Anesthetic	Preparation					Mean ± SEM
	1	2	3	4	5	
−PB/isoflurane*	32	28	24	27	19	26.0 ± 2.2
−PB/halothane*	31	22	27	32	24	27.2 ± 1.9
−PB/enflurane*	29	31	30	22	21	27.4 ± 2.4
+PB/enflurane +MP*	26	31	30	22	21	26.0 ± 2.0
+PB/isoflurane†	14	18	16	9	14	14.2 ± 1.5
+PB/halothane‡	8	5	10	3	6	6.4 ± 1.2
+PB/enflurane§	3	4	7	2	2	3.6 ± 0.9

−PB = untreated; +PB = phenobarbital; MP = metyrapone.
*†‡§ Significantly different groups of values are designated by different symbols.

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TABLE 3. ATP/ADP in Isolated Hepatocytes after 10-Min Steady-State Incubation*

Anesthetic	Treatment	Anesthetic Dose 1 MAC	2 MAC	3 MAC
Enflurane	Phenobarbital	69 ± 2†‡	54 ± 6†	63 ± 6†
	Untreated	86 ± 6	62 ± 5†	61 ± 8†
Halothane	Phenobarbital	67 ± 8†‡	53 ± 5†	47 ± 5†‡
	Untreated	88 ± 2	61 ± 2†	64 ± 2†
Isoflurane	Phenobarbital	82 ± 13	53 ± 3†	50 ± 8†
	Untreated	80 ± 10	62 ± 8†	63 ± 3†

* Values given are means ± SEM for N = 5 preparations, expressed as % of control value (no anesthetic).
† Significantly different from no-anesthetic control value (100).
‡ Significantly different from corresponding untreated value.

P_{O_2}, while the effect of halothane was intermediate in all five preparations. In −PB cell suspensions, all three anesthetics at 1 MAC produced slight, essentially identical increases in extracellular P_{O_2} under steady-state conditions, consistent with the modest inhibition of VO_2 produced in the closed system by those agents. Note that, in the presence of P450-specific inhibitor (0.25 mM metyrapone), +PB cells exposed to anesthetic showed responses in O_2 metabolism similar to those of −PB cells.

The effects on hepatocellular energy status that developed during the steady-state incubations described above are shown in tables 3 and 4, for halothane, enflurane, and isoflurane. With all three agents, increases in administered dose from 1 to 3 MAC were associated with statistically significant decreases in ATP/ADP (table 3) and shifts toward lactate formation (table 4). In addition to anesthetic dose, the other variable studied for its effects on metabolite levels in incubated hepatocytes was the presence/absence of prior phenobarbital treatment. As a group, steady-state incubations of +PB cells with halothane at 1, 2, and 3 MAC showed significantly lower ATP/ADP values than did those of −PB cells, whereas this difference between +PB and −PB cells did not achieve statistical significance for incubations with enflurane or isoflurane. The same comparison with regard to lactate metabolism shows that +PB cells demonstrated significantly less lactate production, for the 1-, 2-, and 3-MAC doses as a group, than did −PB cells when the agent used was either halothane or enflurane (table 4). For individual incubations, differences between +PB and −PB cells with regard to metabolite levels were significant only for ATP/ADP, and then only with halothane at 1 or 3 MAC or enflurane at 1 MAC.

TABLE 4. Δlactate in Isolated Hepatocytes after 10-Min Steady-state Incubation*

Anesthetic	Treatment	Anesthetic Dose 1 MAC	2 MAC	3 MAC
Enflurane	Phenobarbital†	−33 ± 18	−12 ± 41	97 ± 51
	Untreated	25 ± 27	124 ± 50	214 ± 47
Halothane	Phenobarbital†	34 ± 22	34 ± 25	104 ± 39
	Untreated	113 ± 58	189 ± 93	216 ± 35
Isoflurane	Phenobarbital	8 ± 65	68 ± 63	68 ± 65
	Untreated	11 ± 63	97 ± 37	145 ± 35

* Values given are means ± SEM for N = 5 preparations, expressed as difference from control value (no anesthetic).
† Significantly different from corresponding untreated group of 1, 2, and 3 MAC values.

Discussion

As in a previous study from this laboratory,[19] work reported here has used isolated hepatocytes to investigate hepatic actions of volatile anesthetics which are mediated directly at the (sub)cellular level. The use of clinically meaningful anesthetic concentrations and incubation conditions enhances the relevance of observed *in vitro* effects to the responses of tissue *in vivo*.

Halothane, enflurane, and isoflurane each significantly increased O_2 consumption in +PB cells compared with −PB cells. That these VO_2 increments in +PB cells were due to enhanced cytochrome P450-mediated anesthetic biotransformation is based on the following evidence: 1) the P450-mediated metabolism of enflurane, isoflurane, and—at P_{O_2} levels above virtual anoxia—halothane consumes both O_2 and anesthetic as co-substrates; 2) increased VO_2 upon exposure to anesthetic was seen only in hepatocytes from rats subjected to prior phenobarbital treatment, which enhances P450 activity; 3) the observed VO_2 increments in +PB cells were abolished by a P450-specific inhibitor (metyrapone); and 4) similar increases in VO_2 have been elicited in this system by other agents (*e.g.*, aminopyrine) which are also known substrates for P450-catalyzed oxidations (GL Becker, unpublished data).

Oxygen consumption in +PB cells appears to consist of a P450-mediated component, which is virtually absent or much reduced in −PB cells, superimposed upon a basal component (presumably due to mitochondrial oxidative phosphorylation), which is similar in −PB and +PB cells. In +PB cells, the increased VO_2 at 1 MAC followed by progressive declines at 2 and 3 MAC can be explained as the sum of P450-mediated O_2 consumption, assumed maximal at 1 MAC and roughly constant thereafter, plus mitochondrial O_2 consumption, the latter showing, in both +PB and −PB cells, a progressive decline over the entire 1–3 MAC anesthetic dose range. Such anesthetic-induced decreases in mitochondrial O_2 consumption have been extensively documented elsewhere.[22]

The results of the closed-system incubations just discussed demonstrate that, in +PB cells exposed to typical

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in vivo doses of halogenated anesthetics, VO_2 increases due to enhanced anesthetic metabolism can outweigh decreases due to reduced mitochondrial oxidative phosphorylation. The open-system results (fig. 1) illustrate the tendency of such net increases in O_2 demand in +PB cells to decrease extracellular P_{O_2} under steady-state conditions approximating those *in vivo,* in which ambient P_{O_2} is dictated by the instantaneous balance between O_2 demand and O_2 supply. Since the anesthetic-induced increases in VO_2 in +PB cells were largest at the lowest doses of anesthetic, the metabolic consequences of those VO_2 increases should have been most apparent at doses used clinically and in the phenobarbital-hypoxia animal model. Indeed, this prediction was borne out in the steady-state system, where statistically significant differences in ATP/ADP between +PB cells and −PB cells occurred at the lowest (1 MAC) doses of halothane and enflurane (table 3). The data also suggest that energy deficits arising from inhibition of mitochondrial ATP production by P450-mediated hypoxia in +PB cells may have been compounded by a diminished glycolytic response. Table 4 shows that +PB cells consistently showed less positive Δlactate values than did −PB cells under the same incubation conditions. Thus, +PB cells showed less lactate production, implying reduced glycolytic ATP production, despite coexisting high energy phosphate deficits, the principal intracellular activator of glycolysis. The mechanism for this inhibitory effect of enhanced anesthetic biotransformation on anaerobic enery metabolism cannot be ascertained from the data of this study.

The ability of phenobarbital pretreatment to promote subcellular hypoxia in the presence of anesthetic substrate for the P450 system was also reported in a study which used preparations of isolated hepatic microsomes (endoplasmic reticulum).[22] Microsomes isolated from phenobarbital-treated animals showed evidence of halothane reductive metabolism at gas phase O_2 concentrations as high as 5%, whereas the critical O_2 level for reductive metabolism in untreated mircosomes was 2%. Although these workers did not directly measure increases in VO_2 arising from phenobarbital-enhanced oxidative metabolism of halothane, their results, like ours, reflect the occurrence of such increases. In their steady-state system, increased VO_2 related to phenobarbital treatment was evidenced by the higher gas phase P_{O_2} necessary to keep liquid phase P_{O_2} constant (at the threshold value for reductive metabolism); in our system, gas phase P_{O_2} was invariant, so increased O_2 consumption was manifest in the form of decreases in liquid phase P_{O_2}.

The extent to which P450-mediated VO_2 increases and their metabolic consequences may contribute to anesthetic hepatotoxicity in any specific *in vivo* situation cannot be ascertained solely from the results of this *in vitro* study, in which only a single biochemical mechanism was monitored; other factors, such as cardiovascular responses, present *in vivo* and capable of altering O_2 supply in accord with O_2 demand, were absent; and accepted measures of hepatocellular necrosis equivalent to those used to quantitate hepatotoxicity *in vivo* were not employed. The possibility that P450-mediated hypoxia by itself could be a dominant mechanism of cellular injury under the specific conditions of the phenobarbital hypoxia model of halothane hepatotoxicity appears to be substantially diminished by one of this study's own findings. The data of table 1, figure 1, and table 2 indicate that +PB cells show significantly greater increases in VO_2 and decreases in steady state P_{O_2} with enflurane than with halothane at comparable anesthetic doses, yet enflurane is not hepatotoxic in the phenobarbital hypoxia model. On the other hand, while unlikely to be a primary determinant of hepatocellular injury, P450-mediated VO_2 increases could enhance the effect of other potentially pathogenic factors. Intracellular O_2 depletion abetted by halothane oxidative metabolism could promote the occurrence of halothane reductive metabolism, which is O_2-inhibited. Alternatively, P450-mediated VO_2 increases could intensify intrahepatic hypoxia created by the combination of hypoxemia plus halothane-induced reduction of splanchnic blood flow.[14] Significant increases in hepatic blood flow in phenobarbital-treated rats have been demonstrated using microsphere injections.[23] However, there were comparable increases in liver mass, so that blood flow per unit mass of liver was not increased. Our data predict that, in phenobarbital-treated (male) rats, exposure to 1 MAC halothane or enflurane should acutely increase O_2 consumption per unit mass of liver by 20–40%. It remains to be determined whether such acute increases in hepatic O_2 consumption are seen *in vivo* and, if so, whether they are countered by proportionate increases in O_2 delivery to avert intrahepatic hypoxia.

The larger increases in VO_2 produced in +PB cells by enflurane compared to halothane at equianesthetic doses were unexpected, since halothane is known to be the more extensively metabolized and the extent of halogenated anesthetic metabolism is presumed to correlate strongly with the rate of hepatic biotransformation. A major concern to be addressed here is that this finding of our study may reflect artifacts in the *in vitro* system. The ability of P450 inhibitor to abolish anesthetic-induced VO_2 increases indicates that the latter do indeed reflect activity of the P450 system. "Uncoupling" of P450-catalyzed oxidations (O_2 utilization in excess of substrate utilization) has been reported for certain fluorinated hydrocarbons[24]; but, even if such uncou-

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pling does occur with enflurane, there is no basis for presuming that intact hepatocytes would display it *in vitro* and not *in vivo*. It should also be mentioned that virtually instantaneous readings of VO_2 in isolated +PB cells and extended collections of anesthetic metabolites during and after anesthesia may be quite different ways of assessing anesthetic metabolism. Although P450-catalyzed anesthetic biotransformation contributes importantly to both, estimates of halothane metabolism relative to enflurane obtained by metabolite collection *in vivo* would be higher than those obtained by VO_2 measurements *in vitro* for two obvious, major reasons: 1) reductive metabolism of halothane would not have registered as changes in VO_2, and could have been substantial at the borderline hypoxic P_{O_2} ranges used in this study; and 2) halothane is eliminated from the body more slowly than enflurane, thereby increasing the opportunity for metabolism of the former relative to the latter.[25] Reconciliation of *in vitro* and *in vivo* measures of anesthetic metabolism clearly will require further detailed study.

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