

Effect of Streptozotocin-induced Diabetes in the Rat on the Metabolism of Fluorinated Volatile Anesthetics

Eugene J. Pantuck, M.D.,* Carol B. Pantuck, B.A.,† Allan H. Conney, Ph.D.‡

Three weeks after dosing male Fischer 344 rats with streptozotocin to induce diabetes, enflurane was administered ip, and 1 h later, fluoride levels were measured in plasma and livers were removed. Hepatic microsomes were prepared, and the oxidative defluorination of enflurane, isoflurane, and methoxyflurane and the reductive defluorination of halothane were measured *in vitro*. In diabetic rats the defluorination of enflurane was increased 3.4-fold over control levels *in vivo* and 2.7-fold *in vitro*. Insulin treatment prevented these effects. *In vitro* metabolism of isoflurane by livers from diabetic rats was 2.5-fold greater than by livers from control rats, but defluorination of methoxyflurane and of halothane was not altered. The results show that streptozotocin-induced diabetes in rats enhances the defluorination of enflurane and of isoflurane but not of methoxyflurane or halothane. (Key words: Anesthetics, volatile; enflurane; halothane; isoflurane; methoxyflurane. Biotransformation, drug; defluorination; metabolites. Diabetes. Ions: fluoride. Metabolism: cytochrome P-450; defluorination; enzyme induction; metabolites; microsomes.)

OXIDATIVE BIOTRANSFORMATION OF the fluorinated ether anesthetics, methoxyflurane, enflurane, and isoflurane, by the cytochrome P-450 enzyme system results in the release of inorganic fluoride.¹⁻⁶ This metabolite is the cause of the nephrotoxicity that occurs as a dose-related injury in patients administered methoxyflurane.⁷⁻⁹ In rats, conventional phenobarbital-type or polycyclic hydrocarbon-type inducers of cytochrome P-450 produce little or no enhancement of enflurane or isoflurane metabolism,^{4,10-16} whereas treatment of rats with isoniazid or with ethanol enhances the metabolism of methoxyflurane, enflurane, and isoflurane.¹⁴⁻¹⁸ Substantially greater than usual levels of inorganic fluoride have been observed following enflurane anesthesia in some patients who have been treated with isoniazid.¹⁹ Changes in hepatic microsomal enflurane metabolizing activity following ethanol administration to rats has been shown to be paralleled by changes in the amount of a microsomal protein that electrophoreses in the molecular weight region of the cytochrome P-450 isozymes.¹⁸ Treatment of rats with isoniazid

but not with Aroclor® 1254, a mixed inducer of phenobarbital- and polycyclic hydrocarbon-inducible isozymes of cytochrome P-450, causes the formation of a microsomal protein of identical molecular weight.¹⁸ Diabetes has been reported to induce in rats the formation of a microsomal protein of similar molecular weight to that induced by ethanol and isoniazid.^{18,20-23} Diabetes, ethanol, and isoniazid all induce the hydroxylation of aniline^{16,20,21,24-26} and the demethylation (at low substrate concentrations) of N-nitrosodimethylamine,^{22,23,26,27} an activity that is not inducible by phenobarbital or 3-methylcholanthrene.²⁸ These observations suggest that diabetes, ethanol, and isoniazid may induce the same isozyme of cytochrome P-450.

We have carried out studies in rats to determine whether diabetes increases the oxidative defluorination of the fluorinated ether anesthetics. In addition, we have examined the effects of diabetes on the defluorination of halothane, a reaction that is catalyzed by cytochrome P-450 but is reductive rather than oxidative, occurs only under hypoxic conditions, and is phenobarbital-inducible.^{29,30}

Materials and Methods

Fifty-three male Fischer 344 rats (Charles River Laboratories, Cambridge, MA), 4-5 weeks old, were given Purina® Rat Chow (Ralston Purina, St. Louis, MO) and deionized water *ad libitum*. We elected to carry out our study in young animals because we found that young animals withstood treatment with streptozotocin better than did adult animals. The rats were administered streptozotocin (Sigma Chemical Company, St. Louis, MO) (50 mg/kg, in 0.1 M sodium citrate, pH 4.5, ip) or vehicle, once daily, for 2 days. This drug, which causes pancreatic beta cell destruction, has been widely used to induce diabetes in experimental animals. The concentration of glucose in urine was monitored with Keto-Diastix® reagent strips (Ames Company, Elkhart, IN). The development of diabetes, defined as urine glucose concentration reaching 250 mg/dl, was confirmed in all streptozotocin-treated animals used in the study. Eight control and eight streptozotocin-treated rats were housed individually in metabolic cages, and their consumption of food and water was measured. These rats were used only for this purpose. The remaining rats were housed in wire-mesh cages without bedding in treatment groups as follows: 1—control (n = 9); 2—treated with streptozotocin (n = 6); 3—treated

* Professor of Anesthesiology.

† Senior Staff Associate in Anesthesiology.

‡ Laboratory Head.

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Address reprint requests to Dr. Pantuck: Department of Anesthesiology, Columbia-Presbyterian Medical Center, 622 West 168th Street, New York, New York 10032.

with streptozotocin and, beginning when the development of diabetes was confirmed (day 3 or 4 after the initial dose of streptozotocin), injected daily with insulin (protamine zinc insulin suspension, Eli Lilly and Company, Indianapolis, IN) (1.25 units/100 g sc) (n = 12); and 4—treated as Group 3 but with insulin discontinued on the 19th day after the initial dose of streptozotocin (n = 10).

On the 21st day after the rats were administered the initial dose of streptozotocin or the vehicle, enflurane metabolism *in vivo* was measured, as described previously.¹⁸ At 7–8 weeks of age, the activities of hepatic microsomal oxidative drug metabolizing enzymes in rats have essentially reached adult levels. Following administration of enflurane (Anaquest, Madison, WI) (0.6 µl/g ip), the rats were permitted no further food or water. One hour after they were dosed with enflurane, the rats were anesthetized with diethyl ether for 20–30 s to facilitate withdrawal by vena caval puncture of the largest possible volume of blood. The heparinized blood was centrifuged and the plasma used immediately for measurement of fluoride and glucose content.

As soon as withdrawal of blood was completed, livers were excised and microsomes prepared from them, as described by Levin *et al.*³¹ The microsomes were stored at -90° C until used for measurements of protein content, cytochrome P-450 content, and anesthetic defluorinating activity. Preliminary experiments established that the enzymes measured in microsomes were stable under the conditions of freezing and storage.

Glucose concentration in plasma was measured with a Beckman Glucose Analyzer 2® (Beckman, Fullerton, CA).

Fluoride concentration in plasma was determined using an ion-specific electrode (Orion Research, Inc., Cambridge, MA), as described by Fry and Taves.³² Standard curves were prepared by adding known amounts of fluoride to plasma from rats maintained for 3 weeks on Purina® Rat Chow and deionized water *ad libitum*.

The protein concentration in rat liver microsomes was determined by the method of Lowry *et al.*,³³ using bovine serum albumin as a standard.

Cytochrome P-450 content in rat liver microsomes was determined spectrally from the carbon monoxide difference spectrum of the reduced hemoprotein by the method of Omura and Sato.³⁴

The rates of enflurane, isoflurane, and methoxyflurane defluorination by rat liver microsomes were determined by incubation of 10 µl of anesthetic with 5 mg of microsomal protein as described by Rice *et al.*^{15,17} The reductive defluorination of halothane was determined by incubation of 10 µl of halothane with 5 mg of microsomal protein under hypoxic conditions as described by Van Dyke and Gandolfi.²⁹ Inorganic fluoride released during a 30-min incubation was measured with an ion-specific electrode as

TABLE 1. Effect of Prior Administration of Enflurane to Rats on the Defluorination of Fluorinated Volatile Anesthetics *In Vitro* by Hepatic Microsomes

Anesthetic	<i>In Vitro</i> Defluorination (nmol F ⁻ · mg protein ⁻¹ · min ⁻¹)	
	Administered Saline	Administered Enflurane
None	NQ	NQ
Enflurane	0.062 ± 0.005	0.064 ± 0.007
Isoflurane	0.043 ± 0.003	0.043 ± 0.002
Methoxyflurane	0.090 ± 0.004	0.101 ± 0.003
Halothane	0.029 ± 0.007	0.030 ± 0.008

Twelve male Fischer 344 rats weighing 120–130 g were given Purina® Rat Chow and deionized water *ad libitum*. Six were administered enflurane, 0.6 µl/g ip, and six were administered an equivalent volume of saline, ip. One hour later the rats were killed. Hepatic microsomes were prepared and were assayed for defluorination activity as described in "Methods." Values are mean ± SE. Data were analyzed statistically as described in "Methods." There were no statistically significant differences between control animals and those administered enflurane.

NQ = values too low to be quantified.

described earlier. Incubations using heat-inactivated microsomes were used as blanks. Standard curves were prepared by adding known amounts of fluoride to blanks. Preliminary experiments established that the administration of enflurane (0.6 µl/g ip) to rats 1 h prior to preparation of microsomes did not influence either the levels of fluoride in blanks or the rates of defluorination of the anesthetics (table 1).

When multiple comparisons were made, results were analyzed using analysis of variance followed by the Bonferroni Method. All other data were analyzed using the unpaired Student's *t* test. Differences in mean values were considered significant if *P* was less than 0.05 using a two-tailed test.

Results

Food and water consumption were measured in eight control and eight diabetic rats housed individually in metabolic cages and used only for this purpose. Food consumption did not differ significantly between the two groups. The mean ± SE quantity of Purina® Rat Chow ingested per day was 19.1 ± 1 g by the control rats and 21 ± 1 g by the diabetic rats. Water consumption was 2.7 times greater in the diabetic than the control rats. The mean volume of water consumed per day was 46 ± 2 ml by the control rats and 123 ± 2 ml by the diabetic rats (*P* < 0.001).

At the time that drug metabolism studies were initiated, diabetic rats that had not been treated with insulin (Group 2) weighed 49% less than control rats (Group 1) (*P* < 0.01) (table 2). There was no significant difference in weight between diabetic rats treated with insulin until the initiation of drug metabolism studies (Group 3) and control rats. Diabetic rats in whom insulin treatment was discon-

TABLE 2. Effect of Streptozotocin-induced Diabetes and Treatment with Insulin on Hepatic Microsomal Cytochrome P-450 Content

Treatment Group	Number	Weight (g)	Ratio of Liver Weight to Body Weight	Blood Glucose (mg/dl)	Hepatic Microsomal Cytochrome P-450 Content (nmol/mg protein)
1. Control	9	133.1 ± 2.9	0.042 ± 0.001	170 ± 4	0.54 ± 0.04
2. Diabetic	6	68.2 ± 3.5*	0.056 ± 0.001*	513 ± 33*	0.49 ± 0.04
3. Diabetic treated with insulin until day 22	12	126.7 ± 2.6	0.041 ± 0.001	245 ± 35	0.47 ± 0.02
4. Diabetic treated with insulin until day 19	10	105.8 ± 2.6*	0.045 ± 0.000*	450 ± 16*	0.47 ± 0.02

Values are mean ± SE.

* Significantly different from control ($P < 0.01$).

tinued 3 days before the initiation of drug metabolism studies (Group 4) weighed 20% less than control rats ($P < 0.01$). Compared with the control rats, plasma glucose was increased three-fold in untreated diabetic rats ($P < 0.01$), was not significantly changed in Group 3 insulin-treated diabetic rats, and was increased 2.6-fold in Group 4 insulin-treated diabetic rats ($P < 0.01$) (table 2). Compared with control rats, the ratio of liver weight to body weight was increased 33% in untreated diabetic rats ($P < 0.01$), but was not different in either of the groups of diabetic rats that had been treated with insulin (table 2). Compared with control rats, hepatic microsomal P-450 content was not significantly different in any of the groups of diabetic rats (table 2).

In vivo defluorination of enflurane was 3.5 times greater ($P < 0.01$) (table 3) and *in vitro* defluorination was 2.7 times faster ($P < 0.01$) (tables 3 and 4) in untreated diabetic rats than in control rats. The *in vivo* and *in vitro* defluorination of enflurane by Group 3 insulin-treated diabetic rats was not significantly different from control rats (table 3). The defluorination of enflurane was 2.1 times greater *in vivo* ($P < 0.01$) and 1.6 times faster *in vitro* ($P < 0.01$) in Group 4 insulin-treated diabetic rats than in control rats. Diabetic rats not treated with insulin metabolized isoflurane *in vitro* 2.5 times faster than control rats ($P < 0.01$) (table 4). There was no significant difference

between diabetic rats and control rats in the *in vitro* defluorination of methoxyflurane or in the *in vitro* reductive defluorination of halothane.

Discussion

The results of these studies show that streptozotocin-induced diabetes in the rat produces an increase in hepatic microsomal enflurane defluorinating enzyme activity and enhances enflurane defluorination *in vivo* and that these effects are prevented by treatment with insulin. The results further show that in the rat, diabetes produces an increase in the *in vitro* hepatic microsomal defluorination of isoflurane but not of methoxyflurane or of halothane. In addition, the results show that in the rat, loss of adequate control of diabetes for just a few days prior to anesthetic administration, a situation encountered clinically, results in an appreciable increase in enflurane defluorination.

Our study shows no increase in total microsomal cytochrome P-450 content in diabetic rats. Enhanced metabolism of a substrate can result from induction of an isozyme of cytochrome P-450 without a measurable increase in total microsomal cytochrome P-450 content if the enhanced metabolism results from the synthesis of a small amount, relative to the total quantity of cytochrome P-450 present, of an isozyme with an extremely high capacity to metabolize the substrate. This can also happen if the increase in cytochrome P-450 resulting from the synthesis of the isozyme is offset by repressed synthesis or enhanced degradation of one or more other isozymes. The metabolism of a number of substrates is decreased in diabetic rats,^{21,22} suggesting the possibility that diabetes may repress the synthesis or enhance the degradation of isozymes of cytochrome P-450. Unlike the metabolism of enflurane, the metabolism of methoxyflurane is quite markedly induced by phenobarbital^{10,12-15} as well as being induced by ethanol^{14,15} and by isoniazid.^{16,17} This suggests that substantial metabolism of methoxyflurane can occur by more than one isozyme of cytochrome P-450. The failure of diabetes to increase significantly the defluorination of methoxyflurane in the rat could be the result

TABLE 3. Effect of Streptozotocin-induced Diabetes and Treatment with Insulin on the Defluorination of Enflurane *In Vivo* and *In Vitro*

Treatment Group	Enflurane Defluorination	
	<i>In Vivo</i> (nmol F ⁻ /ml plasma)	<i>In Vitro</i> (nmol F ⁻ · mg protein ⁻¹ · min ⁻¹)
1. Control	8.58 ± 0.45	0.051 ± 0.005
2. Diabetic	29.56 ± 2.44*	0.140 ± 0.010*
3. Diabetic treated with insulin until day 22	11.64 ± 0.67	0.048 ± 0.002
4. Diabetic treated with insulin until day 19	17.59 ± 0.86*	0.081 ± 0.007*

Values are mean ± SE for 6-12 rats.

* Statistically different from control ($P < 0.01$).

TABLE 4. Effect of Streptozotocin-induced Diabetes on the Defluorination of Fluorinated Volatile Anesthetics

Treatment Group	<i>In Vitro</i> Defluorination (nmol F ⁻ · mg protein ⁻¹ · min ⁻¹)			
	Enflurane	Isoflurane	Methoxyflurane	Halothane
1. Control	0.051 ± 0.005	0.028 ± 0.004	0.059 ± 0.005	0.019 ± 0.007
2. Diabetic	0.140 ± 0.010*	0.069 ± 0.009*	0.065 ± 0.006	0.009 ± 0.001

Values are mean ± SE for 6–9 rats.

* Significantly different from control ($P < 0.01$).

of increased metabolism of methoxyflurane by a diabetes-induced isozyme being offset by decreased metabolism by one or more other isozymes.

Ethanol and isoniazid have been shown to induce in rat liver an identical isozyme of cytochrome P-450, and the purified isozyme has been shown to be capable of hydroxylating aniline and demethylating N-nitrosodimethylamine.²⁶ There is no direct evidence that this isozyme is capable of defluorinating fluorinated volatile ether anesthetics or that it is inducible by diabetes, but the findings of the present study are consistent with these hypotheses.

The mechanism by which diabetes induces the defluorination of enflurane and isoflurane is not known. In the rabbit acetone induces an isozyme homologous to the ethanol- and isoniazid-inducible isozyme of the rat,^{26,35} suggesting that ketosis may be the causative factor. It is of interest, in this regard, that the rabbit isozyme has been shown to hydroxylate acetone to acetol, enabling its conversion to glucose,³⁵ an activity that would serve a physiologic purpose in diabetes. There is substantial, although indirect, evidence that the isozyme of cytochrome P-450 in rats that is inducible by ethanol and isoniazid is inducible by acetone and is capable of hydroxylating acetone to acetol.^{1,26,27,36–38}

If diabetes-induced changes in inhalation anesthetic defluorination comparable to those that we observed in rats occur in humans, they probably do not pose a substantial increase in the risk of nephrotoxicity following the administration of these agents. In the rat the defluorination of methoxyflurane is not enhanced by diabetes. Halothane is not oxidatively defluorinated, and its defluorination is not induced by diabetes. Isoflurane is metabolized to inorganic fluoride to such a small extent that enzyme induction should be of no significance with respect to nephrotoxicity. It is possible that an ordinary clinical exposure to enflurane could result in levels of fluoride in plasma in some diabetic individuals in the nephrotoxic range, but plasma levels of fluoride following enflurane anesthesia decrease at such a rapid rate that renal dysfunction, should it occur, is likely to be transient.^{19,39,40}

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