Enflurane, Isoflurane, and Methoxyflurane Metabolism in Rat Hepatic Microsomes from Ethanol-treated Animals

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The effects of ethanol on the metabolism of enflurane, isoflurane, and methoxyflurane were investigated to determine if alterations in biotransformation of these agents occur as a result of this treatment. In vitro incubations of hepatic microsomes from rats pretreated with 10 days' ethanol vapor inhalation revealed a fourfold increase in inorganic fluoride from enflurane when compared with microsomes from untreated rats and from phenobarbital-pretreated rats. Isoflurane, while metabolized to a lesser extent than enflurane, showed a similar stimulation of metabolism. Methoxyflurane, while metabolized to a greater extent than either enflurane or isoflurane, had lesser fluoride production by the microsomes from ethanol-pretreated rats than microsomes from phenobarbital-pretreated rats, but greater fluoride production than that found in microsomes from untreated rats. Ethanol pretreatment did not alter the levels of cytochrome P-450 which is the enzyme responsible for such metabolism. This suggests that the altered metabolism involves either a specific P-450 isozyme or an unidentified enzyme. (Key words: Anesthetics, volatile; enflurane; isoflurane; methoxyflurane. Biotransformation: inorganic fluoride. Liver: function; enzymatic activity. Metabolism: metabolites. Pharmacology: ethanol.)

The release of inorganic fluoride during metabolism of methoxyflurane is sufficient to cause renal toxicity.1 Because of certain similarities in chemical structure, enflurane and isoflurane also release inorganic fluoride2,3 as a result of metabolism by cytochrome P-450.4

Inorganic fluoride is released enzymatically from methoxyflurane by the oxidative cleavage of the ether, while metabolism of the dichloromethyl carbon (β carbon) releases inorganic chloride and produces the acid-labile product, methoxydifluoroacetic acid.5 This latter product may break down nonenzymatically in an acid medium, releasing inorganic fluoride. In contrast, enflurane is metabolized only at the β carbon and undergoes little or no metabolism by the oxidative cleavage of the ether,6 while isoflurane undergoes a very limited metabolism,7 although the pathway of its biotransformation has not been established.

Cytochrome P-450 is not a single enzymatic entity but is a group of closely related enzymatic activities.7 These forms also may differ slightly in their structure and in their ability to be induced. Therefore, in addition to the induction of cytochrome P-450 content, certain of its enzymatic activities can be altered by chronic chemical pretreatments. Treatment of rats with phenobarbital increases the defluorination of methoxyflurane, enflurane, and isoflurane—that of methoxyflurane is most noticeable.8 However, pretreatment of rats with 3-methylcholanthrene, which induces a different form of cytochrome P-450, does not affect the rate of defluorination of methoxyflurane, enflurane, or isoflurane. Treatment with isoniazid has been found to enhance the defluorination of enflurane by rat liver microsomes fourfold or more.9 In contrast, isoniazid pretreatment does not increase the level of cytochrome P-450 and therefore represents an example of activity induction rather than of cytochrome P-450 content.

The present report contains data relating to similar studies on the effects of chronic ethanol treatment on the defluorination of enflurane, isoflurane, and methoxyflurane. The results are compared with phenobarbital-pretreatment values, as well as untreated control values.

**METHODS**

**ANIMAL PREPARATION**

Male Sprague-Dawley rats weighing 250–300 g (obtained from Hilltop Farms, Scottdale, Pennsylvania) were used throughout the study. Ethanol was administered by inhalation in a 50-l Plexiglas® chamber, with the rats housed in separate cages over paper within the chamber. During exposure, the rats were allowed free access to food and water. No other treatment was attempted before or during exposure.

Ethanol exposure was accomplished by pumping ethanol at a fixed rate into a heated glass vessel, where it quickly vaporized. The vaporized ethanol was mixed with room air at a flow rate of approximately 6 l/min through the chamber. The ethanol vaporization was adjusted so that the flow of ethanol was 13.7 mg/l. This exposure was maintained continuously for 10 days.

Phenobarbital pretreatment was accomplished by mixing the phenobarbital with the drinking water (0.2%) and allowing the rats access to the water for five
Microsomal Incubation

Immediately after ethanol exposure (day 10), the rats were killed and the livers were removed and homogenized in three volumes of 0.15 mm Tris buffer, pH 7.4 at 4°C. The homogenate was centrifuged at 10,000 x g, and microsomal pellets were prepared by centrifuging the supernatant fraction at 105,000 x g. Hepatic microsomal concentration was determined by the method of Lowry,10 and cytochrome P-450 level was assayed by the method of Omura and Sato.11 Microsomes from untreated and phenobarbital-treated animals were prepared by the same method.

The microsomal pellet was resuspended in 0.02 m Tris buffer, pH 7.4, and diluted to a final concentration of 5 mg/ml. The incubations were carried out for 30 min at 37°C using 3-ml mixtures containing approximately 15 mg of microsomal protein, 5 μmol of NADP+, 10 μmol of glucose-6-phosphate, and one unit of glucose-6-phosphate dehydrogenase. The incubations contained 1 mm: enflurane, isoflurane, or methoxyflurane. Incubations containing microsomes and substrate only were used as blanks. Incubations were stopped and fluoride ion was determined using an ion electrode (Orion) as described previously.12 All incubations were done in duplicate.

Results

Throughout the exposure to ethanol, the rats gained weight at the rate of 2 g per day while unexposed rats gained approximately 3 g per day. The present study reflects only data on rats with 10 days of ethanol exposure. Concentrations of ethanol higher than the 13.7 mg/l used in the present study resulted in poor growth characteristics and sometimes death. Therefore, the results presented herein are limited to a single concentration with exposure at this level resulting in an apparent accumulation of ethanol in vivo such that serum levels were less than 10 mg/dl at days 2 and 5; 35 mg/dl at day 7, and 248 mg/dl at day 10. Table 1 contains the inorganic fluoride and cytochrome P-450 data. This table is a composite of a number of experiments and n refers to number of animals, not number of incubations. Each incubation was carried out in duplicate and comparisons made between anesthetics using microsomes from the same animals. The most common comparison was to enflurane, thus, the n values are higher for this agent.

The cytochrome P-450 content of microsomes from phenobarbital-pretreated rats was significantly higher than that from the uninduced rats (table 1). However, ethanol pretreatment did not significantly alter the content of cytochrome P-450 from that found in microsomes from untreated rats. Furthermore, cytochrome P-450 assay did not reveal any shift in maximum absorption which might indicate the formation of an altered form of the P-450.

In contrast to the P-450 levels, the inorganic fluoride released from enflurane and isoflurane was approximately fourfold greater in the microsomes from rats exposed to ethanol than in those from uninduced animals. The fluoride released as a result of the metabolism of enflurane and isoflurane by microsomes from phe-
nobarbital-pretreated rats was less than that seen in the ethanol-induced microsomes but not significantly greater than that in the uninduced system.

Methoxyflurane metabolism, as measured by the release of inorganic fluoride, was greatest in the microsomes from phenobarbital-pretreated rats with metabolism occurring to a lesser extent in microsomes from ethanol-pretreated rats and even lower in microsomes from uninduced rats.

The table contains the data from microsomes obtained from animals killed immediately at the conclusion of exposure. If the animals were killed 24 h after the exposure was stopped, the fluoride released from enflurane was $0.91 \pm 0.28$ nmol/mg protein, indicating a relatively rapid loss of enhanced activity.

**Discussion**

The administration of ethanol by inhalation has been used successfully by others to study the effects of ethanol on the metabolism of various substrates by cytochrome P-450. The exposure procedure used in this study resulted in lower final concentrations of blood alcohol than those reported in other studies; this difference may have been the reason that cytochrome P-450 content was not elevated in the present study, whereas a previous study indicated a slight but significant induction. The induction noted in that study may have been the result of a mild starvation since their animals lost weight during exposure. Care was taken in this study to ensure an increase in body weight during the exposure which required a somewhat reduced exposure concentration.

Several points should be emphasized in reviewing the present data. First, ethanol exposure did not enhance the cytochrome P-450 content. Second, release of inorganic fluoride from enflurane and isoflurane is stimulated dramatically by ethanol treatment. Third, release of inorganic fluoride from methoxyflurane is increased only modestly. Fourth, increased metabolism of enflurane and isoflurane is only evident after a prolonged exposure, and then the enhanced activity decreases within 24 h post-exposure, a fact also noted by others. However, the present study used only a single treatment regimen and therefore it remains to be seen what effect other exposure times have on this metabolism.

Undoubtedly, multiple forms of cytochrome P-450 exist. Also, phenobarbital increases a number of forms of P-450, as judged by the increase in enzymatic activity using various substrates. Recent reports indicate that isoniazid, which also enhances the defluorination of enflurane and isoflurane without increasing levels of cytochrome P-450, results in an altered form of the hemoprotein. In this case, the hemoprotein-CO complex absorbs light at 451 nm rather than at 450 nm, as is normally the case. While this slight difference may be important in isoniazid-treated microsomes, no such spectral shift is seen in the ethanol-treated microsomes. In this study, since ethanol pretreatment resulted in a fourfold increase in defluorination of enflurane and isoflurane without increasing the content of P-450, it would be appropriate to speculate that this treatment increases a specific form of P-450. However, the very short half-life of the enhanced enzymatic activity reduces the possibility that this increase in activity is due to a synthesis of new protein but rather to an alteration of an existing enzyme, perhaps by interaction with ethanol. Furthermore, since there is also no spectral evidence to suggest that a different hemoprotein has been formed, as happens with isoniazid treatment, one also may question whether this increased enzymatic activity is due to cytochrome P-450 or to some other as yet unidentified enzyme.

Dooley et al. have reported no apparent induction of enflurane metabolism due to ethanol ingestion by humans. While it is difficult to compare this study in rats with their study in humans, the amount of ethanol ingested and time since last intake may determine the effects on enflurane metabolism. The present data indicate that, at least in the rat, the rate of metabolism drops off very rapidly after exposure. However, the metabolic activity in the rat is greater than in humans, and thus the duration of this effect in humans may be greater than the 12 h noted herein for rats.

Comparison of the defluorination of enflurane and methoxyflurane in the present study raises the interesting possibility that the ethanol pretreatment enhances the enzymatic attack on the $\beta$ carbon of the ether. Burke et al. have reported that enflurane metabolism results in the formation of inorganic fluoride and difluoromethoxydifluoroacetic acid. Thus, any fluoride released from enflurane is the result of the oxidation of the chlorofluoromethyl group ($\beta$ carbon). Methoxyflurane undergoes metabolism by oxidation either of the ether or of the dichloromethyl group ($\beta$ carbon). However, only the oxidation of the ether gives rise directly to inorganic fluoride in microsomal incubation. The oxidation of the dichloromethyl group results in the formation of methoxydifluoro-acetic acid and inorganic fluoride. Therefore, because ethanol treatment enhances the release of fluoride to a greater extent from enflurane than from methoxyflurane, the dehalogenation of terminal carbons probably is the primary enzymatic activity that is induced. Isoflurane defluorination also is enhanced; however, because the exact pathway of metabolism of isoflurane is not known, the comparison of this drug with enflurane and methoxyflurane would be highly speculative.
In summary, while many questions remain, administration of ethanol vapor provides a mechanism introducing ethanol into animals in a constant manner while maintaining body growth. By this technique, it is possible to show that while cytochrome P-450 content does not change, the enzymatic activity of this enzyme system is altered in such a way that the defluorination of enfurane and isoflurane is enhanced while that of methoxyflurane is altered to a more limited extent. The clinical implications of this are not known and may be impossible to determine since this study used inhalation, while human consumption is primarily oral. Nevertheless, the anesthesiologist should recognize the possibility that higher-than-normal levels of serum fluoride may be found following anesthesia in those persons who chronically consume high levels of alcohol.

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References