Proliferation of Smooth Endoplasmic Reticulum and Induction of Microsomal Drug-metabolizing Enzymes after Ether or Halothane

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Hepatic drug-metabolizing enzymes and hepatic ultrastructure were studied in rats after two hours of anesthesia with 1 MAC halothane or diethyl ether. Twelve hours after cessation of either anesthetic smooth endoplasmic reticulum was increased in centrilobular but not in periportal hepatocytes. This change persisted at 24- and 36-hour sampling times. Microsomal cytochrome P₄₅₀ and cytochrome b₅ decreased after halothane anesthesia (by 7 to 20 per cent of control). Diethyl ether caused increased cytochrome P₄₅₀ and cytochrome b₅ (27 and 18 per cent, respectively) at the 36-hour sampling time. NADPH cytochrome c reductase did not change significantly after ether agent. The authors interpret these results to mean that both agents promote conversion of rough endoplasmic reticulum to smooth endoplasmic reticulum or, alternatively, that the anesthetics decrease degradation of smooth endoplasmic membranes. Since only ether caused an increase in the microsomal content of enzymes of the drug-metabolizing enzyme system, it is concluded that these two anesthetics act on hepatic cells by dissimilar mechanisms. (Key words: Anesthetics, volatile, halothane; Anesthetics, volatile, diethyl ether; Metabolism, anesthetic; Metabolism, liver; Enzymes, cytochrome P₄₅₀; Enzymes, cytochrome b₅; Enzymes, NADPH cytochrome c reductase; Induction, enzymes; Biotransformation, enzyme induction; Biotransformation, microsomes.)

The potent inhalational anesthetics are metabolically degraded by the hepatic microsomal drug-metabolizing enzyme system (cytochrome P₄₅₀ system) of an exposed animal. The liver responds to many such lipid-soluble foreign compounds by increasing the capacity of the hepatic drug-metabolizing enzyme system. This process of enzyme induction, most extensively studied following phenobarbital administration, is associated with alterations in hepatic ultrastructure. The most prominent of these changes is a proliferation of the smooth endoplasmic reticulum. Ernster and Orrenius demonstrated that, following phenobarbital, hepatic drug-metabolizing enzymatic activity increased first in membranes of the rough endoplasmic reticulum and increased later in membranes of smooth endoplasmic reticulum. They also found a lack of strict parallelism between membrane formation and demonstrable enzymatic synthesis. We have examined the relationship of enzyme induction and proliferation of membranes of the smooth endoplasmic reticulum following a single exposure to either of two anesthetics.

Materials and Methods

Young male Wistar rats were fed Purina Rat Chow ad lib. for five days prior to study, at the end of which time their average weight was 130 g. The animals were kept in air-conditioned quarters with a regulated diurnal cycle (lighted from 7 a.m. to 7 p.m.) in metal cages with wire mesh floors. The use of insecticides was avoided. A total of 90 animals was used in this study.

At the beginning of the study the animals were removed from food and three groups established at random. One group was exposed to diethyl ether, 1 MAC (3.2 per cent), vaporized in air with sufficient oxygen added to maintain 21 per cent concentration for two hours. The second group was exposed to halothane, 1 MAC (1.3 per cent), in air for two hours. The anesthetics were administered in a 9.4-l plastic enclosure at a flow rate of 5 l/min, and anesthetic concentrations were checked periodically by gas chromatography. Rectal temperatures were monitored and maintained at 37°C with surface heating. The remaining group served as unanesthetized controls and, except for the omission of the anesthetic exposure, was handled in a manner similar to the two anesthetized groups, including exposure to air in a similar plastic enclosure.

In order to control diurnal variation, the experiments were timed so that all animals were sacrificed at approximately 9 a.m. Twelve, 24 and 36 hours after cessation of anesthetic administration (14, 26 and 38 hours after the initiation of fasting) animals from each group were sacrificed by cervical dislocation. The livers were perfused via the portal vein with iced saline solution (0.9 per cent) to remove blood.

Three-gram portions of the livers were homog-

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enized in 20 ml of KCl solution (1.15 per cent) buffered with 0.05 M tris buffer (pH 7.4). The homogenates were centrifuged for an hour at 10,000 × g at 4 C. The supernatants were then centrifuged at 100,000 × g for an hour to sediment the microsomal pellets. The pellets were resuspended in 0.05 M phosphate buffer (pH 7.4). The resulting microsomal suspensions were assayed for protein, cytochrome P₄₅₀, cytochrome b₅, and NADPH cytochrome c reductase.

The methods used for the three enzyme assays were those described by Mazel with modifications described by Brown. Cytochrome b₅ was determined by recording a difference spectrum between oxidized microsomes and microsomes reduced with dithionite. The absorbance difference between the peak at 426 nm and the trough at 412 nm was used with an extinction coefficient of 150/cm/mM to calculate the amount of cytochrome b₅ present in the microsomal suspension.

Cytochrome P₄₅₀ was determined by recording a difference spectrum between reduced microsomes and reduced microsomes plus carbon monoxide. The absorbance difference between the baseline at 490 nm and the peak at 450 nm was used with an extinction coefficient of 91/cm/mM to calculate the amount of cytochrome P₄₅₀ present. NADPH cytochrome c reductase activity was determined by assay of the reduction of cytochrome c by the microsomal suspension in the presence of oxygen and excess NADPH.

The protein concentrations of the microsomal suspensions were determined by the biuret method. All assays were carried out in a Beckman Model 25 dual-beam recording spectrophotometer. Significances of differences in enzyme values between control means at each sampling time and means after exposure to either anesthetic at the corresponding sampling time were tested using the t test for unpaired data. Differences were considered significant when P was less than or equal to 0.05.

Portions of the left lateral lobes of the livers were removed and placed in glutaraldehyde (3 per cent), where they were diced into small cubes approximately 1 mm on a side. The tissue was further fixed in glutaraldehyde for as long as two weeks at 4 C. The tissue was rinsed with sucrose, 10 per cent, dehydrated with acetone, stained with potassium permanganate, and embedded in the plastic Epon 812. Details of this procedure have been described elsewhere. Sections of each specimen were cut .5 to 1.0 μm thick and stained with toluidine blue, 0.5 per cent, in sodium borate, 0.5 per cent. Central veins and portal triads in these sections were identified by light microscopy and the blocks further trimmed in such a way that at least one portal triad or central vein was represented in each block face. Ultrathin sections were then cut from these oriented blocks and mounted on 200-mesh grids. After staining of the ultrathin sections with uranyl acetate and lead citrate, they were examined in a Phillips 300 electron microscope. Cells within five cell diameters of the central veins were designated centrilobular hepatocytes and cells within five cell diameters of the portal triads were designated periportal hepatocytes.

Results

Electron micrographs of approximately 1,000 hepatocytes were available for examination of hepatic ultrastructure. Little or no glycogen was present in the hepatocytes. In the fastest control animals, periportal (fig. 1) and centrilobular (fig. 2) cells showed differences in morphology of mitochondria, organization of rough endoplasmic reticulum, and amount of smooth endoplasmic reticulum. Centrilobular cells had mitochondria with elongated profiles that stained somewhat more intensely and had smaller cross-sectional areas than did mitochondria from periportal cells. Centrilobular cells from the fastest control animals consistently showed (i.e., at each of the three sampling times) cisternae of rough endoplasmic reticulum arranged in typical parallel stacks and collections of smooth endoplasmic reticulum in localized regions of the cytosome. In the periportal cells from fasted animals cisternae of rough endoplasmic reticulum were more isolated and less numerous, and profiles of smooth endoplasmic reticulum were sparse.

The morphologic differences between centrilobular and periportal cells were consistently found at each of the three sampling times, and in the fastest controls there was little morphologic difference in cells of animals fasted for 14, 26 or 38 hours.

Hepatocytes from animals that received ether or halothane showed increased amounts of smooth endoplasmic reticulum compared with hepatocytes from the fastest unanesthetized control animals. The increase in smooth endoplasmic reticulum occurred predominantly in the centrilobular cells and was seen by the 12-hour sampling time after discontinuation of either agent (figs. 3 and 4). The morphologic appearances and frequencies of occurrence of mitochondria, microbodies, lipid droplets, and lysosomes were no different from those of the control hepatocytes. Similarly, bile canaliculi were unaltered. In central hepatocytes after the use of either agent the cisternae of rough endoplasmic reticulum were
Fig. 1 (above). Periportal hepatocyte from an unanesthetized (control) rat fasted for 14 hours. Comparison with centrilobular hepatocytes shows larger, more rounded mitochondria (M), isolated cisternae of rough endoplasmic reticulum (RER) and a few dispersed profiles of smooth endoplasmic reticulum (SER). The nucleus (N) is at the margin of the micrograph. ×24,900.

Fig. 2 (below). Centrilobular hepatocyte from an unanesthetized (control) rat fasted for 14 hours. Comparison with periportal hepatocytes shows smaller, more elongated mitochondria (M), cisternae of rough endoplasmic reticulum (RER) occur in parallel stacks, and small collections of vesicles of smooth endoplasmic reticulum (SER) are found throughout the cytosome. A microbody (Mi) and portions of the nucleus (N) and a bile canaliculus (BC) are shown. ×24,900.
FIG. 3 (above). Centrilobular hepatocyte 12 hours after a 2-MAC-hour exposure to diethyl ether. Comparison with control hepatocytes shows increased numbers of vesicles of smooth endoplasmic reticulum and stacks of rough endoplasmic reticulum composed of fewer, shortened cisternae. Note mitochondria characteristic of centrilobular cells. ×24,900.

FIG. 4 (below). Centrilobular hepatocyte 12 hours after a 2-MAC-hour exposure to halothane. Comparison with control hepatocytes shows increased numbers of vesicles of smooth endoplasmic reticulum and stacks of rough endoplasmic reticulum composed of fewer, shortened cisternae. ×24,900.
shortened and there were fewer cisternae in each stack. After ether, the increase in smooth endoplasmic reticulum seen at 12 hours persisted to the 24- and 36-hour sampling times. A similar result was seen after halothane, although hepatocytes from some animals showed even more smooth endoplasmic reticulum at 24 and 36 hours than at 12 hours.

Examination of periportal cells at all sampling times after either agent showed slight increases in smooth endoplasmic reticulum but little other morphologic alteration (fig. 5).

In unanesthetized animals, fasting for 14 hours resulted in an increase in microsomal cytochrome P₄₅₀ to .55 ± 0.04 nm/mg, compared with animals fed ad lib., whose microsomes contained .33 ± 0.03 nm/mg (P < 0.05). There was no further change in cytochrome P₄₅₀ values after 26 or 38 hours of fasting. There was no significant effect of fasting on the values of microsomal cytochrome b₅ or NADPH cytochrome c reductase activity per mg microsomal protein.

After the 2-MAC-hour exposure to diethyl ether there was no significant change in any of the enzymes studied until the 36-hour sampling time. At that time, cytochrome P₄₅₀ and cytochrome b₅ had increased by 27 and 18 per cent, respectively (table 1).

With halothane, there was a 20 per cent decrease in cytochrome b₅ 12 hours after completion of the anesthesia (table 2). The remaining values of cytochrome P₄₅₀ and cytochrome b₅ after halothane were all approximately 10 per cent below the values obtained in control animals, although none of these values was significantly different from the corresponding control mean.

**Discussion**

This study was conducted in fasting animals because there is evidence that the initiation of fasting affects both hepatic morphology and the hepatic drug-metabolizing enzyme system. Brown and Sagalyn have reported increased cytochrome P₄₅₀, cytochrome b₅ and NADPH cytochrome c reductase per mg microsomal protein after 48 hours of fasting in male rats. Ichikawa et al. reported an increase in cytochrome P₄₅₀ (but no change in cytochrome b₅) per mg microsomal protein after 48 hours of fasting in male rats. Our control animals showed an increase in cytochrome P₄₅₀ only, manifested 14 hours after the initiation of fasting. The reason for the differences obtained by different investigators is not apparent, although it may be related to selection of sampling times.

Glycogen depletion occurs when previously fed ani-
TABLE 1. Effects of a Single 2-MAC-hour Exposure to Diethyl Ether on Components of the Hepatic Microsomal Drug-metabolizing Enzyme System in Fasted Male Wistar Rats

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Percentage of Control Mean (± SEM) after Ether</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>12 Hours (n = 5) 24 Hours (n = 5) 56 Hours (n = 8)</td>
</tr>
<tr>
<td>Cytochrome P450</td>
<td>106 ± 12 81 ± 7.9 127* ± 8.8</td>
</tr>
<tr>
<td>Cytochrome b$_5$</td>
<td>97 ± 3.3 88 ± 5.7 118* ± 7.9</td>
</tr>
<tr>
<td>NADPH cytochrome c reductase</td>
<td>102 ± 6.6 119 ± 24 105 ± 3.7</td>
</tr>
</tbody>
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* Differs from control mean, P ≤ 0.05.

TABLE 2. Effects of a Single 2-MAC-hour Exposure to Halothane on Components of the Hepatic Microsomal Drug-Metabolizing Enzyme System in Fasted Male Wistar Rats

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Percentage of Control Mean (± SEM) after Halothane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 Hours (n = 6) 24 Hours (n = 8) 56 Hours (n = 6)</td>
</tr>
<tr>
<td>Cytochrome P450</td>
<td>89 ± 7.5 87 ± 8.5 86 ± 9.8</td>
</tr>
<tr>
<td>Cytochrome b$_5$</td>
<td>80* ± 6.3 93 ± 5.6 88 ± 4.7</td>
</tr>
<tr>
<td>NADPH cytochrome c reductase</td>
<td>107 ± 8.1 123 ± 18 98 ± 6.2</td>
</tr>
</tbody>
</table>

* Differs from control mean, P ≤ 0.05.

mals are fasted. Within the first six hours of fasting a proliferation of smooth endoplasmic reticulum occurs. As glycogen depletion progresses and glycogen levels become very low, the smooth endoplasmic reticulum recedes toward basal levels. The control animals in this study were fed ad lib. and presumably had their peak glycogen levels at the beginning of the fasting interval. Little glycogen and similar morphologic patterns were seen after 14, 26, and 38 hours of fasting. We believe this represents a stable morphologic baseline, especially with reference to changes in the endoplasmic reticulum. It was against this baseline that comparisons of ultrastructure were made at the corresponding times following anesthetic exposure.

Ether and halothane caused proliferation of smooth endoplasmic reticulum in liver cells. This effect was more prominent in centrilobular hepatocytes, although periportal cells participated to a lesser extent. Burger and Herndon have reported similar findings in a study of phenobarbital, in which alterations of hepatic smooth endoplasmic reticulum in rats began in the centrilobular region and after ten days of exposure extended from the centrilobular region to involve most, but not all, of the hepatic lobule. That study and the observations of this report emphasize the importance of recognizing morphologic differences in hepatocytes according to their localization within the liver lobule. It is important in studies of drug effects on hepatic ultrastructure to account for this by sampling from known regions of the lobule.

The components of the microsomal drug-metabolizing enzyme system include cytochrome P450, which can complex with numerous drugs and molecular oxygen. To such a complex two electrons are transferred from NADPH via NADPH cytochrome c reductase (and under some conditions via cytochrome b$_5$) to yield as products regenerated cytochrome P450, water, and the oxidized drug. In this study we evaluated the responses of hepatic drug-metabolizing enzymes to two anesthetics by measuring amounts of enzyme present per mg protein in the microsomal fraction of hepatocytes. By increasing components of the hepatic drug-metabolizing enzyme system, diethyl ether behaved much like a classic enzyme-inducing agent such as phenobarbital. It differs in that the increase in components of the hepatic drug-metabolizing enzyme system was not as great as is commonly found after repeated phenobarbital administration. This may be related to a limited ability of ether to induce components of the drug-metabolizing system or, as seems more likely to us, to the fact that the livers were briefly exposed to a relatively small dose of this soluble anesthetic. Ether was administered for two hours at a constant inspired concentration and undoubtedly did not achieve an equilibrated tension of 1 MAC in the liver. Brown and Sagalyn have demonstrated that a greater total exposure to diethyl ether (achieved by exposures repeated daily) produced progressive increases in hepatic drug-metabolizing enzymes. We suggest that within the framework of a single-exposure protocol, greater and hence more readily identifiable biochemical and morphologic responses could be achieved by increasing the inspired concentration and/or duration of the anesthetic administration.

Except for the one significantly different value at the 12-hour sampling time, the values for cytochrome P450 and cytochrome b$_5$ following halothane exposure were not statistically different from the control means. However, all were about 10 per cent below the corresponding control values. When the values for all determinations (n = 20) of cytochrome P450 and cytochrome b$_5$ were combined and compared with the corresponding values obtained in control animals, the mean decreases in cytochrome P450 and cytochrome b$_5$ were 13 and 12 per cent, respectively, and were statistically significant. This consistent decrease in microsomal cytochromes after halothane differs from the response seen after most drugs that cause enzyme induction. Van Dyke and co-workers have recently reported strong binding of halothane metabolites to phospholipids and proteins of the
microsomal fraction. Such binding could interfere with the formation of the reduced form of cytochrome $b_5$ or with the formation of the carbon monoxide–P450 complex upon which the assays for these two cytochromes are based.

The morphologic finding of increased smooth endoplasmic reticulum at 12 hours does not parallel the biochemical responses of the microsomal drug-metabolizing enzymes to either of these drugs. The microsomal fraction of liver cells represents the endoplasmic reticulum of the intact cell, which may be subdivided into rough and smooth. The membranes of both subfractions are composed of a phospholipid bilayer, within which occurs a wide variety of proteins, including the hepatic drug-metabolizing enzymes. Rough endoplasmic reticulum consists of membranes bearing ribosomes, whereas smooth endoplasmic reticulum is devoid of ribosomes. Present concepts of the manner in which membranes of the endoplasmic reticulum proliferate$^{14, 19, 20}$ argue that proteins of newly-forming membranes are produced in regions of rough endoplasmic reticulum by the action of attached ribosomes. The membranes are subsequently converted into smooth endoplasmic reticulum as ribosomes are lost from the rough endoplasmic membranes. The finding in this study of increased smooth endoplasmic reticulum prior to a measurable increase in microsomal drug-metabolizing enzymes suggests that an early effect of both anesthetics is to promote conversion of membranes of rough endoplasmic reticulum to membranes of smooth endoplasmic reticulum without an increase in the concentration of drug-metabolizing enzymes in the total endoplasmic reticulum (i.e., in the microsomal fraction). Another possible explanation is that the anesthetics have interfered with the degradation of the smooth endoplasmic membranes.

On the basis of these experiments, we conclude that ether and halothane provoke similar morphologic changes in hepatic ultrastructure. Biochemical data from the same animals suggests, however, that the interactions of these two drugs with hepatocytes are quite different. We believe that further study of anesthetic interactions with hepatocytes needs to consider the details of enzyme production and action within subfractions of the endoplasmic reticulum.

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References