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EFFECT OF VOLATILE ANESTHETICS ON THE HEPATIC UDP-GLUCURONIC ACID PATHWAY IN MICE*

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Abstract—Large, rapid decreases in hepatic UDP-glucuronic acid concentrations occur in rats following exposure to myriad chemicals. In fact, 80% reductions in UDP-glucuronic acid occur within minutes after exposure to inhalation anesthetics. The present study was designed to determine whether this decrease in hepatic UDP-glucuronic acid may be due to (a) a decrease in the precursor UDP-glucose; (b) decreased activity of UDP-glucose dehydrogenase, which oxidizes UDP-glucose to UDP-glucuronic acid; (c) increased activity of UDP-glucuronosyltransferases; or (d) increased activity of nucleotide pyrophosphatase, which degrades UDP-glucuronic acid to glucuronic acid-1-phosphate. Exposure to halothane, isoflurane and sevoflurane decreased UDP-glucuronic acid concentrations by 40–52% as compared to that in unanesthetized control mice. No sex-dependent or anesthetic-induced effects of UDP-glucose levels and the activities of UDP-glucose dehydrogenase and UDP-glucuronosyltransferase were observed. Nucleotide pyrophosphatase activity was increased by 47–65% in female mice after inhalation of halothane, isoflurane and sevoflurane. The apparent V_{max} for hydrolysis of 4-nitrophenol thymidine 5′-monophosphate ester by nucleotide pyrophosphatase was increased by 56–80% in female mice, whereas the apparent K_m was unchanged. These alterations in nucleotide pyrophosphate kinetics may be responsible, in part, for the marked decrease of hepatic UDP-glucuronic acid concentrations by the volatile anesthetics.

Glucuronidation is responsible for conjugating potentially toxic lipophilic compounds with glucuronic acid, thereby producing molecules with greater aqueous solubility that are excreted more readily into urine and bile [1]. The rate at which any compound may be glucuronidated depends on the concentration and activity of the UDP-glucuronosyltransferases as well as the concentration of the cofactor UDP-glucuronic acid.

Hepatic UDP-glucuronic acid concentrations in experimental animals can be altered by treatment with numerous chemicals. Acute administration of galactosamine [2–4], diethyl ether [5, 6], divinyl ether [6], enflurane, ethanol, halothane, methoxyflurane, pentobarbital, and urethane [7–9] decreases UDP-glucuronic acid levels in rat liver. Unlike galactosamine, which competes with glucose for free UTP [2, 3], the anesthetics reduce hepatic UDP-glucuronic acid concentrations by mechanisms which have yet to be elucidated.

The biochemical pathways that regulate UDP-glucuronic acid concentrations are outlined in Fig. 1. UDP-glucuronic acid is formed after oxidation of UDP-glucose by UDP-glucose dehydrogenase with NAD^+ as the electron acceptor. UDP-glucuronic acid may then be either used as the glucuronic acid donor for xenobiotic conjugation reactions by UDP-glucuronosyltransferases, or degraded to glucuronic acid 1-monophosphate after the phosphodiester bond is cleaved by nucleotide pyrophosphatase. This same enzyme further reduces the glucuronic acid 1-phosphate to free D-glucuronic acid. Decreases in UDP-glucuronic acid concentration may be due to reduced availability of UDP-glucose or decreased UDP-glucose dehydrogenase activity or to increased activities of glucuronosyltransferase or nucleotide pyrophosphatase.

Thus, the present study was designed to evaluate whether exposure of mice to volatile anesthetics and urethane affects one or more aspects of the UDP-glucuronic acid pathway. Both male and female mice were used because preliminary experiments indicated that there may be sex-dependent differences in hepatic UDP-glucuronic acid metabolism.

MATERIALS AND METHODS

Chemicals. Brij 58 (polyoxyethylene 20-cetyl ether), diethylstilbestrol, glycine, magnesium chloride, nicotaminide adenine dinucleotide, 4-nitrophenol, 4-nitrophenol-thymidine 5′-monosphosphate ester, Triton X-100, Trizma base, UDP-glucose dehydrogenase, UDP-glucose, UDP-glucuronic acid and urethane were purchased from the Sigma Chemical Co. (St. Louis, MO). [*monoethyl*-^{3}H]Diethylstilbestrol (105 mCi/mmol), 97% pure by thin-layer chromatography, was obtained from Amersham International (Arlington Heights, IL). All other chemicals were the highest quality available from the Fisher Scientific Co. (Cincinnati, OH). Deionized water was used throughout. Isoflurane (Forane®, Anaquest, Madison, WI), and halothane (Fluothane®, Ayerst Laboratories, New York, NY) were obtained from the Bloomington Hospital Phar-

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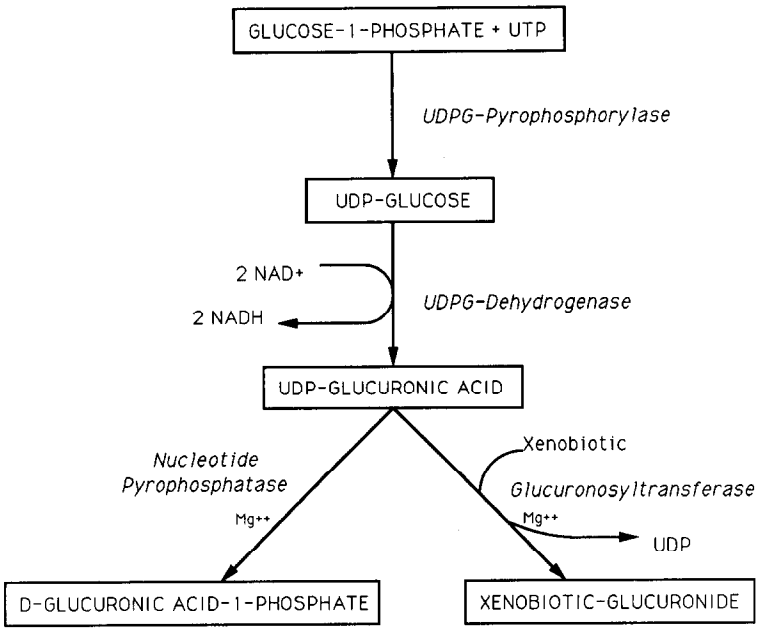

Fig. 1. UDP-glucuronic acid pathway.

macy (Bloomington, IN). Sevoflurane was provided by the Maruishi Pharmaceutical Co. (Osaka, Japan).

Animals. Male (19–24 g) and female (17–20 g) Swiss–Webster mice were purchased from Harlan Sprague–Dawley Inc. (Indianapolis, IN). All mice were housed in stainless steel cages in temperature controlled (20–27°) quarters with a 12-hr light/dark cycle and had free access to Purina Laboratory Mouse Chow No. 5015 and water. Animal husbandry was consistent with the Public Health Service Guide to the Care and Use of Laboratory Animals. Mice were exposed to the anesthetics in an inhalation chamber with continuous flow of anesthetic and air and removal of carbon dioxide. The anesthetics were volatilized using a calibrated Fluotek Mark II vaporizer and an air flow rate of 1–2 L/min. The animals were maintained at normal atmospheric concentrations of 21% oxygen. Anesthetic concentrations (3.5% halothane, 3.5% isoflurane, and 3.5% sevoflurane) were adequate to induce and maintain narcosis. Urethane (1.2 g/kg) was administered i.p. All mice were exsanguinated and their livers removed 15 min after onset of narcosis. Control mice were exposed to air in the inhalation chamber and then treated similarly.

Preparation of subcellular fractions. After treatment and exsanguination of the mice, 0.5 g of liver was added to 4.5 mL of distilled water in a test tube and placed into a boiling water bath for 4 min, homogenized and centrifuged at 3500 *g* for 10 min. An aliquot of the resulting heat-treated supernatant fraction of the liver homogenate was used for determination of UDP-glucuronic acid and UDP-glucose. Comparison of concentrations of UDP-glucose and UDP-glucuronic acid determined in liver frozen in liquid nitrogen with liver subjected to boiling water bath temperatures indicated that UDP-glucuronic acid and UDP-glucose are stable to the conditions of this procedure [7] (data not shown). Another 1.0 g of liver tissue from a different mouse was homogenized for 15 sec in 3.0 mL of ice-cold 1.15% KCl using a Brinkmann polytron with a 10 mm probe generator at setting 5.5. An aliquot of this homogenate was used for measurement of UDP-glucuronosyltransferase activity. The homogenate was centrifuged at 10,000 *g* for 20 min at 5° to remove mitochondrial and cellular debris. The supernatant fraction was then centrifuged at 105,000 *g* for 60 min at 5°. The resulting supernatant fraction and pellet were the sources of cytosolic (glucose dehydrogenase) and microsomal (nucleotide pyrophosphatase) enzymes respectively.

Chemical and enzyme activity determinations. Concentration of UDP-glucuronic acid in boiled liver homogenates was determined by the radiometric method of Watkins and Klaassen [7] using [^{3}H]diethylstilbestrol as the aglycone and microsomes from guinea pig liver as the source of UDP-glucuronosyltransferase. UDP-glucose was quantitated spectrophotometrically [10]. Activity of UDP-glucose dehydrogenase (EC 1.1.1.22) toward 1.5 mM UDP-glucose was determined at 25° by the method of Strominger *et al.* [11]. Conjugation of 5 mM 4-nitrophenol, 15 mM morphine, and 1 mM diethylstilbestrol by UDP-glucuronosyltransferases (EC 2.4.1.17) was measured at 37° by the methods described by Watkins and Klaassen [12]. Hydrolysis of 4-nitrophenol thymidine 5′-monophosphate ester by nucleotide pyrophosphatase (EC 3.6.1.9) at 37° was quantitated by a modification of the method of Bischoff *et al.* [13]. The modified method consisted of assaying 50–150 μg of microsomal protein in the presence of 300 mM Tris–HCl, pH 9.0, with 10 mM $MgCl_2$ and 1×10^{-5} M 4-nitrophenol thymidine 5′-monophosphate ester in a total volume of 1.0 mL. The reaction at 37° was terminated after 10 min by

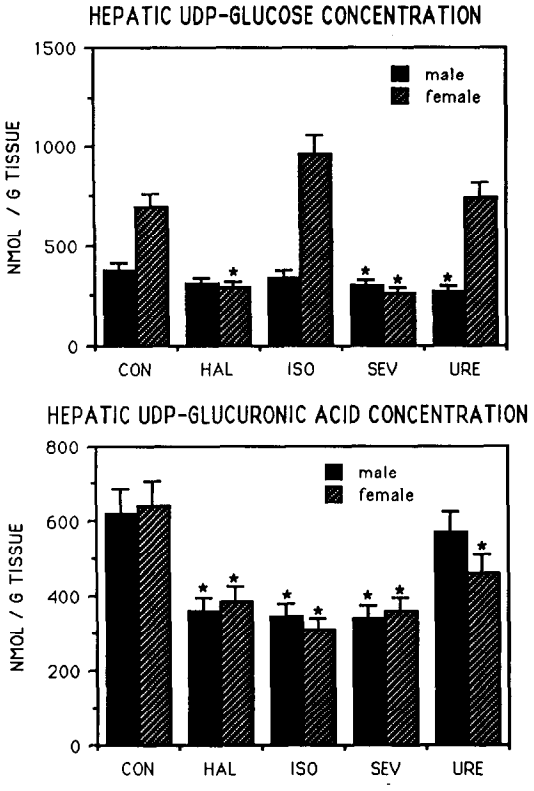

Fig. 2. Effect of inhalational anesthetics on hepatic UDP-glucose and UDP-glucuronic acid concentrations. Mice were exposed for 15 min after onset of narcosis to the anesthetics at the following concentrations: 3.5% halothane, 3.5% isoflurane, 3.5% sevoflurane and 1.2 g urethane/kg. Values are means ± SE for seven mice per group. Asterisks indicate significant differences from unanesthetized controls of the same sex at $P < 0.05$.

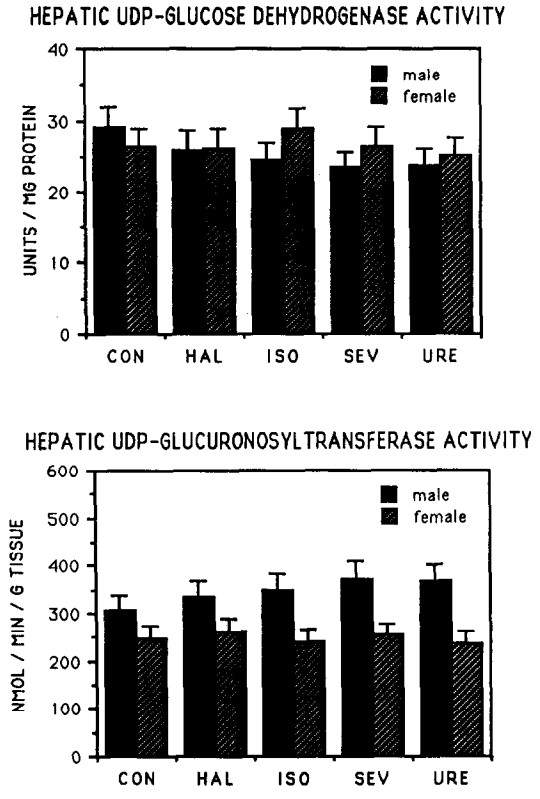

Fig. 3. Activity of hepatic UDP-glucose dehydrogenase and diethystilbestrol UDP-glucuronosyltransferase after exposure of mice to inhalational anesthetics. Experimental conditions are described in the legend of Fig. 2. Values are means ± SE for seven mice.

placing the samples in a boiling water bath for 2 min. The resulting suspension was then centrifuged at 1600 *g* for 5 min. Absorbance of the liberated 4-nitrophenol was measured spectrophotometrically at 405 nm. An extinction coefficient of $18{,}000\ M^{-1} \times cm^{-1}$ was used in all calculations. Studies measuring apparent K_m and V_{max} values for the hydrolysis of 4-nitrophenol thymidine 5′-monophosphate by nucleotide pyrophosphatase used the following substrate concentrations: 1×10^{-3} M, 5×10^{-4} M, 1×10^{-4} M, 5×10^{-5} M and 1×10^{-5} M. All other aspects of the aforementioned modified method were kept constant.

All assays were performed in duplicate or triplicate with appropriate blanks. Enzyme reactions were proportional to incubation time and protein concentration. Protein concentration was determined by the method of Lowry *et al.* [14] using bovine serum albumin as the standard.

Statistical analysis. Means and standard errors of the mean were generated for all data in each group. The data were analyzed by a one-way analysis of variance, and significant differences were determined using Duncan's new multiple range test. Bartlett's test indicated that there was no heterogeneity of variance. $P < 0.05$ was the level of significance.

RESULTS

The top panel of Fig. 2 indicates that hepatic UDP-glucose concentrations were decreased in male mice exposed to sevoflurane (20%) and urethane (27%) and in female mice exposed to halothane (58%), and sevoflurane (62%). The bottom panel of Fig. 2 shows that inhalational anesthetics decreased hepatic UDP-glucuronic acid by 40–52% in male and female mice. In addition, female mice anesthetized with urethane exhibited a 28% decrease in hepatic UDP-glucuronic acid.

Administration of either inhalational anesthetics or urethane did not alter significantly the oxidation of UDP-glucose by UDP-glucose dehydrogenase (Fig. 3 top) or the glucuronidation of diethylstilbestrol by UDP-glucuronosyltransferase (Fig. 3 bottom) in either male or female mice.

Figure 4 illustrates that nucleotide pyrophosphatase activity was increased in female mice after exposure to halothane (56%), isoflurane (47%) or sevoflurane (65%). In contrast, the apparent increase of 13–27% for male mice was not statistically significant.

Table 1 indicates that no significant change in apparent K_m was observed during the hydrolysis of 4-nitrophenol thymidine 5′-monophosphate by nucleotide pyrophosphatase in female mice. In contrast, the apparent V_{max} for this reaction was

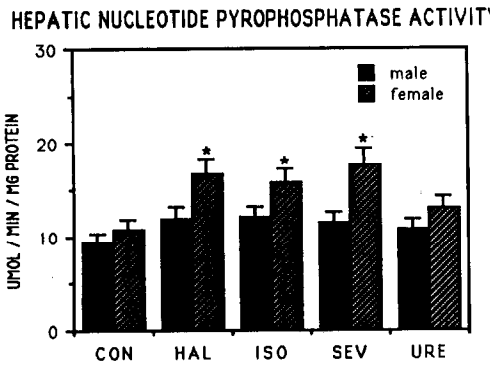

Fig. 4. Hepatic nucleotide pyrophosphatase activity after exposure of mice to inhalational anesthetics. Experimental conditions are described in the legend to Fig. 2. Values are means ± SE for seven mice. Asterisks indicate significant differences from control mice of the same sex at $P < 0.05$.

Table 1. Effect of volatile anesthetics on the apparent kinetic constants of microsomal nucleotide pyrophosphatase from female mouse liver

Treatment	V_{max} (μmol/min/g protein)	K_m ($M \times 10^{-5}$)
Control	14.3 ± 1.8	2.0 ± 0.2
Halothane	22.6 ± 2.3*	2.0 ± 0.1
Isoflurane	23.9 ± 1.0*	2.0 ± 0.1
Sevoflurane	22.3 ± 2.4*	2.0 ± 0.1
Urethane	16.8 ± 1.4	1.7 ± 0.1

Values are means ± SE (N = 4).
* Values are significantly different from control at $P < 0.05$.

increased significantly in female mice exposed to halothane (58%), isoflurane (68%) and sevoflurane (56%).

DISCUSSION

Exposure of rats to volatile anesthetics reduces hepatic UDP-glucuronic acid concentrations, and alters the rate of conjugation of compounds such as acetaminophen, bilirubin, diethylstilbestrol, iopanoic acid and valproic acid [15–20]. The focus of this study was to determine the mechanism by which the volatile anesthetics halothane, isoflurane and sevoflurane reduce hepatic UDP-glucuronic acid concentrations.

It is clear from Fig. 2 that the anesthetics did not affect uniformly the concentration of UDP-glucose, the immediate precursor to UDP-glucuronic acid, because concentrations were either decreased or not changed by some anesthetics in male and female mice. In contrast, all volatile anethetics reduced UDP-glucuronic acid concentrations similarly in a non-sex-dependent fashion.

Figure 3 shows that *in vivo* exposure to the anesthetics did not alter UDP-glucose dehydrogenase activity. Although there were problems with the aqueous solubility of the anesthetics, addition of liquid anesthetic to the *in vitro* assay did not appear to affect dehydrogenase activity (data not presented). Similar *in vitro* experiments measuring the activity of hepatic UDP-glucuronosyltransferase towards diethylstilbestrol and 4-nitrophenol after addition of liquid anesthetic to the reaction mixture failed to demonstrate any effect of the anesthetics. Moreover, *in vivo* exposure to the anesthetics did not change transferase activity to diethylstilbestrol (Fig. 3), morphine or 4-nitrophenol (data not shown). Thus, the reduction in UDP-glucuronic acid concentrations after induction of anesthesia does not appear to be due to a reduction in synthesis or an increase in glucuronidation.

The possibility that the depletion of UDP-glucuronic acid by anesthesia is caused by altered activity of microsomal nucleotide pyrophosphatase is evident from the data in Fig. 4, Table 1, and other experiments from this laboratory [21]. There is a trend toward increased activity of this enzyme in male mice and clear statistically significant increases in nucleotide pyrophosphatase activity in female mice treated with halothane, isoflurane or sevoflurane. Enzyme kinetic studies using microsomes from female mouse liver (Table 1) indicate that the apparent V_{max} for the hydrolysis of 4-nitrophenol thymidine 5′-monophosphate ester was increased significantly by exposure to halothane, isoflurane, and sevoflurane and that apparent K_m was unchanged. Other studies have observed similar effects on the kinetic constants in microsomes from male and female mice after enflurane exposure, and have demonstrated that nucleotide pyrophosphatase activity was increased about 4-fold in enflurane-treated mice when measured toward either 4-nitrophenol thymidine 5′-monophosphate ester or UDP-glucuronic acid [21]. Moreover, to rule out the possibility that a plasma membrane nucleotide pyrophosphatase may play a role in controlling intracellular nucleotide phosphate concentrations [22, 23], the hydrolysis of 4-nitrophenol thymidine 5′-monophosphate ester by liver homogenates was determined. Exposure to the anesthetics did not affect pyrophosphate activity in homogenate samples (data not shown), but increased only microsomal activity (Fig. 4, Table 1), suggesting that microsomal pyrophosphatase probably controls intracellular UDP-glucuronic acid concentrations.

Other workers have observed alterations in enzyme activities after exposure to anesthetics. For example, diethyl ether and methoxyflurane increase microsomal NADPH-dependent cytochrome *c* reductase [24], whereas halothane decreases its activity [25]. *In vivo* exposure to enflurane decreases aminopyrine demethylase activity without affecting aniline hydroxylase [26].

Although general anesthetics clearly affect sensitive membrane proteins [27], the precise mechanisms for these anesthetic-induced effects on enzyme activity are presently unknown. Besides an effect on membrane fluidity [28], alterations in the energy state of the cell after exposure to anesthetics may lead to changes in the concentrations of cofactors needed in the UDP-glucuronic acid pathway [29]. Changes in the $NAD^+/NADH$ ratio could

affect flux through the UDP-glucose dehydrogenase reaction (Fig. 1), or could affect NAD^+ interaction with UDP-glucuronic acid, a substrate for nucleotide pyrophosphatase [30]. However, the present studies, which indicate that exposure to the anesthetics increases nucleotide pyrophosphatase activity, do not rule out these other possible mechanisms for anesthetic-induced reductions in hepatic UDP-glucuronic acid.

In summary, these results show that an immediate early response to voltaile anesthetic exposure includes large decreases in hepatic UDP-glucuronic acid in male and female Swiss–Webster mice, some fluctuation in UDP-glucose levels as well as an increase in nucleotide pyrophosphatase activity in female mice exposed to halothane, isoflurane and sevoflurane. Kinetic studies on nucleotide pyrophosphatase in female mice indicated that V_{max} was increased after exposure to halothane, isoflurane and sevoflurane and that K_m was unchanged. Increases in the activity of nucleotide pyrophosphatase are at least partially responsible for the decrease in hepatic UDP-glucuronic acid metabolism in female Swiss–Webster mice after exposure to volatile anesthetics.

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