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Effects of Inhalation Anesthetics on Hepatic Glucuronide Conjugation:

A Study of the Rat in Vitro

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The effects of four inhalation anesthetics on rat-liver microsomal glucuronyltransferase activity have been examined *in vitro*. Anesthetics depressed this reaction in a dose-related, noncompetitive manner. Equal MAC values inhibited in a quantitatively similar manner. Formation of the glucuronic acid donor, UDPGA (uridine diphosphoglucuronic acid) was depressed by only one of the anesthetics studied, diethyl ether. (Key words: Diethyl ether, Halothane, Methoxyflurane, Chloroform, Microsomal glucuronyltransferase.)

LIVER microsomal glucuronyltransferases (EC 2.4.1.17) metabolize a variety of drugs in addition to many endogenous compounds. Substrates for this enzyme system are conjugated by transfer of glucuronic acid from uridine diphosphoglucuronic acid (UDPGA).[1] The glucuronic acid donor, UDPGA, is produced by oxidation of UDP-α-D-glucose in the soluble fraction of the hepatic cell in an enzymatic reaction employing nicotinamide adenine dinucleotide (NAD) as hydrogen acceptor.[2] Many drugs, foreign compounds, and naturally-occurring substances such as bilirubin and certain hormones are excreted in the urine of man and animals as glucuronides. Some compounds (*e.g.*, morphine) are directly esterified, whereas others are conjugated after prior hydroxylation (*e.g.*, barbiturates). The effects of inhalation anesthetics on glucuronide conjugation reactions have not been described previously. Such effects, determined in an *in-vitro* study, are reported below.

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Methods

Male Sprague-Dawley rats weighing 160–190 g were used. Care was taken to insure that the animals had no contact with environmental microsomal enzyme-inducing agents. Each animal was stunned with a blow to the head and the liver was excised, weighed, and placed in ice-cold 50 mM tris–HCl (tris [hydroxymethyl] aminoethane) buffer, *p*H 7.4. Livers were minced, then homogenized with a Teflon pestle in cold tris–HCl buffer (2 ml buffer/g liver). The homogenate was centrifuged at 9,500 × *g* in a refrigerated Sorvall-centrifuge for 20 minutes and the supernatant fluid decanted. This fraction, used for most experiments in the study, is referred to as "9,500 × *g* supernatant." For kinetic experiments, pure liver microsomes, obtained by centrifuging the 9,500 × *g* supernatant fraction at 105,000 × *g* for an hour in a refrigerated International preparative ultracentrifuge, were used. The soluble fraction was discarded and the microsomal pellet resuspended in tris–HCl buffer. Protein concentrations were determined by the biuret method,[3] using bovine albumin as standard.

Measurement of Glucuronide Conjugation

Glucuronyltransferase activity was studied by measuring the rate of glucuronide conjugation of p-nitrophenol, using an analytic technique modified after Henderson and Dewaide.[4] Incubation beakers contained UDPGA (2 μmol), saccharo-1,4-lactone (1 μmol), $MgCl_2$ (10 μmol), and either 0.5 ml 9,500 × *g* supernatant (25 mg protein) or 0.5 ml microsomes

TABLE 1. Anesthetic Concentrations (±SE) in Incubation Mixtures with Respect to Vapor Phase Concentrations*

	Anesthetic in Vapor Phase		
	3 MAC	6 MAC	9 MAC
Diethyl ether	15.3 ± 0.5 mM	20.3 ± 0.8 mM	30.3 ± 1.1 mM
Chloroform	1.2 ± 0.14 mM	1.8 ± 0.1 mM	3.6 ± 0.35 mM
Methoxyflurane	0.7 ± 0.1 mM	2.05 ± 0.12 mM	3.0 ± .15 mM
Halothane	0.96 ± 0.12 mM	1.32 ± 0.1 mM	2.2 ± 0.16 mM

* n = 6 for each value reported.

(15 mg protein). A combination of UDPG (2 μmol) and NAD, 2.5 μmol, was added instead of UDPGA when a UDPGA-generating system was desired. Final beaker volumes were brought to 2.5 ml with 50 mM tris–HCl buffer. p-Nitrophenol concentrations ranged from 2×10^{-5} to 2×10^{-4} M. Incubations were carried out in a Dubnoff metabolic incubator at 37 C for 15 minutes. Reactions were terminated by adding 3 ml of 0.3 N trichloroacetic acid to each beaker. Samples were then centrifuged at 5,000 × g for 10 minutes and 3 ml of supernatant were removed. Each fraction was brought to *p*H 11–12 by addition of 2 N NaOH, then diluted to 10 ml with distilled water. The rate of glucuronide formation was determined spectrophotometrically by the ΔE at 398 nm between incubated and zero-time samples. Results were expressed as nmol p-nitrophenol conjugated per minute per mg protein. Statistical differences were computed by Student's t test and an analysis of variance.

ANESTHETIC ADMINISTRATION

The effects of four volatile anesthetics, diethyl ether, halothane (Fluothane), chloroform, and methoxyflurane (Penthrane), were studied. A calibrated anesthesia machine (Vernitrol) was used to administer the agents. Anesthetic vapors in a total oxygen flow of 2 l/min were administered to the incubation mixtures under the water-sealed hood of the incubator. Incubator mixtures were allowed to equilibrate with the anesthetic for 5 minutes before substrate and UDPGA (or UDPG + NAD) were added. Mixing was facilitated by placing a glass marble 5 mm in diameter in each beaker. Anesthetic administration was continued and the reactions terminated 15 minutes later.

Part of the study was designed to compare the effects on glucuronide conjugation of equipotent vapor phase concentrations of the four anesthetics. For this purpose, the MAC values of Eger *et al.*[5, 6] were selected; these were given in multiples of 3, 6, and 9 MAC. These vapor phase concentrations would appear to be unusually high, but the anesthetic partition coefficients of dilute suspensions of microsomal protein in buffer are lower than those of blood and liver. For this reason anesthetic concentrations in the beakers were determined 72 times. Halothane, chloroform, and diethyl ether concentrations of these mixtures were determined by gas chromatography after n-heptane extraction. Methoxyflurane concentrations were determined by a low-temperature programmed analysis using peroxide-free diethyl ether as the extraction agent. All gas chromatographic analyses were performed with a Varian 1740 instrument utilizing a 3 per cent SE-30 column and flame ionization detector. Anesthetic concentrations with respect to multiples of MAC are shown in table 1. It will be noted from table 1 that beaker anesthetic concentrations were not strictly multiples of MAC values, particularly at higher concentrations. Undoubtedly the beakers were not at complete anesthetic equilibrium. However, the increases in anesthetic concentrations in the beakers after 10 minutes of exposure were not linear. This indicated that no great increase in concentration occurred during the major portion of the 15-minute reactions.

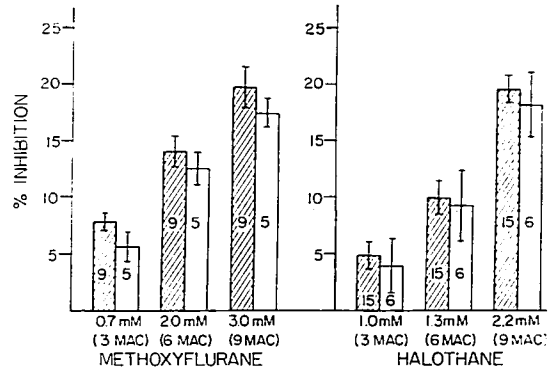

FIG. 1. Per cent inhibition of glucuronide conjugation of p-nitrophenol by methoxyflurane and halothane in 9,500 × *g* supernatant liver fractions. Shaded histograms represent reactions in which the glucuronic acid donor was UDPGA added to the incubations. Histograms not shaded represent reactions in which the UDPGA-generating system (UDPG + NAD) was employed. The number of animals studied is shown on each histogram. Bars represent SE. No significant difference between these two reactions at equal MAC values was observed with either methoxyflurane or halothane. MAC multiples and beaker concentrations in mM are given.

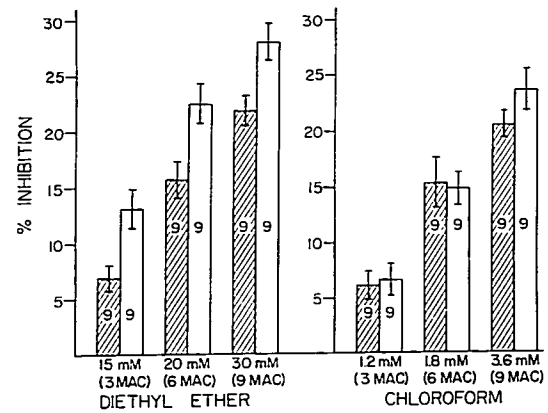

FIG. 2. Per cent inhibition of glucuronide conjugation of p-nitrophenol by diethyl ether and chloroform in 9,500 × *g* supernatant liver fractions. As in figure 1, shaded histograms represent reactions with UDPGA; those not shaded represent reactions with UDPG + NAD. In the case of chloroform, neither of the two reactions differed at equal MAC values. The UDPG + NAD reaction was inhibited more by diethyl ether than was the UDPGA reaction at every concentration of diethyl ether. The levels of significance for this difference with diethyl ether were: 3 MAC, $P < 0.025$; 6 MAC, $P < 0.05$; 9 MAC, $P < 0.05$. MAC multiples and beaker concentrations of anesthetics in mM are given.

Results

All four anesthetics inhibited the rate of p-nitrophenol conjugation in both 9,500 × *g* supernatant and microsomes in a dose-dependent fashion. Inhibition occurred both when UDPGA was used as glucuronic acid donor and when the UDPGA-generating system (UDPG + NAD) was employed. There was considerable variation in the absolute rates of p-nitrophenol conjugated by each rat liver

preparation. The control rates with UDPGA ranged from 0.6 to 1.4 nmol p-nitrophenol conjugated per minute per mg protein.

For this reason, results with anesthetics are expressed in terms of per cent inhibition compared with control values. Data obtained with 9,500 × *g* supernatant incubations are illustrated in figures 1 and 2. Only in the case of diethyl ether is metabolism with UDPGA inhibited significantly less than metabolism with UDPG + NAD. When the effects of the four anesthetics at each MAC multiple on the UDPGA reaction were compared by analysis of variance, no significant differences were found. This would indicate that equipotent concentrations of anesthetics produce equal degrees of inhibition of microsomal glucuronyltransferase. Caution must be exercised in this interpretation, however, as the variance is somewhat high.

Kinetic studies of anesthetic effects on UDPGA-glucuronyltransferase were performed using microsomes. The Lineweaver-Burk plot of the effect of 9 MAC diethyl ether on the reaction is illustrated in figure 3. The similar K_m values (6.25×10^{-5}M) and different V_{max} values (control, 2.2 nmol/min/mg protein; ether, 1.75 nmol/min/mg protein) indicate that inhibition is noncompetitive. Calculated depression of V_{max} by 9 MAC diethyl ether is 20.5 per cent.

Discussion

UDP glucuronyltransferase is a hepatic microsomal enzyme(s) catalyzing the transfer of glucuronic acid from UDPGA to a variety of phenolic, alcoholic, carboxylic, and amine substrates.[7] In addition, glucuronides of naturally-occurring compounds like bilirubin, steroids, and thyroxine appear to be formed in the same pathway as conjugation of xenobiotics.[5, 9] Figure 4 schematically illustrates the sequence of these reactions for the substrate used in this study, p-nitrophenol. UDPG dehydrogenase catalyzes synthesis of UDPGA. UDPGA has the α configuration at the glucuronic acid–phosphate link. However, glucuronides formed by glucuronyltransferase invariably have the β configuration. Thus, transfer proceeds by a Walden inversion mechanism. No factors other than the donor UDPGA are needed, although the divalent cations Mg^{++}, Mn^{++}, and Co^{++} enhance the activity of many glucuronide conjugation reactions.[10]

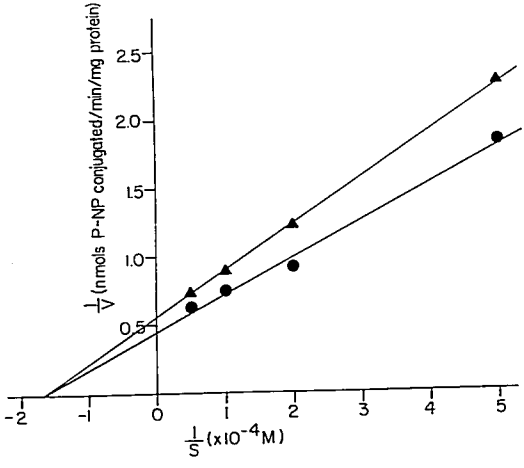

FIG. 3. Lineweaver-Burk double reciprocal plot illustrating the nature of inhibition of UDPGA glucuronide conjugation by 9 MAC diethyl ether. Circles represent control reaction; triangles, reaction in the presence of diethyl ether. Since the K_m values are similar (reciprocal of the x intercept) and the V_{max} values are different (reciprocal of the y intercept), the inhibition is noncompetitive. Values represent the means for six animals.

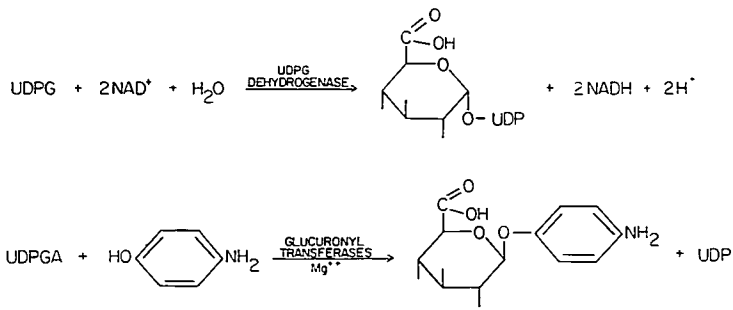

FIG. 4. Pathways of UDPGA synthesis and p-nitrophenol glucuronide formation. The upper panel illustrates the formation of the glucuronic acid donor UDPGA by oxidation of UDPG, with NAD as hydrogen acceptor. The lower panel illustrates the conjugation of p-nitrophenol by microsomal glucuronyltransferases employing UDPGA as glucuronic acid donor.

The quantitative effects of inhalation anesthetics on rat-liver glucuronyltransferase activity are less than effects reported for microsomal NADPH–O_2-dependent mixed-function oxidases.[11] This is not surprising, as different enzymes are involved, and the locations of the two drug-metabolizing systems are different. Glucuronyltransferase activity for substrates like bilirubin, o-aminophenol, and p-nitrophenol is localized predominantly in the rough-surfaced endoplasmic reticulum [12]; NADPH-dependent drug-metabolizing enzymes, on the other hand, are concentrated in the smooth-surfaced endoplasmic reticulum fraction of the liver cell.[13] Quantitative differences between degrees of inhibition by anesthetics in these two groups of drug-metabolizing systems could result from differences in structure and lipid content of either the enzymes or contiguous membranes. Qualitative inhibition seems to be similar, as both systems are inhibited in a noncompetitive manner by anesthetics.

Equal MAC values produced equal degrees of inhibition of glucuronyltransferase; MAC multiples were selected as a rough index of equipotency. Analysis of variance among the four anesthetics at each MAC value showed no significant differences. With the single exception of diethyl ether, the values of the UDPG + NAD reactions were the same as those of the corresponding UDPGA reactions. This points to a major effect of anesthetics on glucuronyltransferases, with little effect on the UDPG dehydrogenase-mediated reaction. The glucuronidation of p-nitrophenol using UDPG + NAD was decreased by diethyl ether at all MAC values significantly more than when UDPGA was used alone. Therefore, diethyl ether must have an additional effect on UDPG dehydrogenase. A postulated explanation for this is predicated on the localization of UDPG dehydrogenase within the liver cell. This enzyme is present in the soluble fraction of the liver cell, and as diethyl ether is the most water-soluble anesthetic employed in the study, concentrations near this enzyme's active site may have been high.

The relevance of these observations to the intact animal or man is at present unknown. If inhibition of glucuronyltransferase activity occurs *in vivo*, measurements of substrates of this reaction (certain drugs, steroids, and hormones) during anesthesia must be interpreted with care. Increased levels of these substances occurring in the anesthetized state could well be at least partially the result of impaired metabolism.

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Neonatology

PREMATURITY AND COMPLICATIONS OF NURSERY ROUTINES 446 infants with birth weights of 1,500 g or less were admitted to the newborn and premature nursery. The 254 (57 per cent) survivors were examined at 10 years of age for abnormalities. From 1947 to 1950, half of the survivors had routinely been given about 60 per cent oxygen. From 1950 to 1953, the other half had been given 40 per cent oxygen or less. The low-oxygen group, in spite of a lower average birth weight of 1,184 g, had a mortality rate of 35 per cent, while the high-oxygen group, with an average birth weight of 1,256 g, had a mortality rate of 46 per cent. However, other factors not controlled may have played a role in this outcome. The low-oxygen group had a 1.5 per cent incidence of blindness due to retrolental fibroplasia, while the high-oxygen group had a 12 per cent incidence. The incidences of milder retrolental fibroplasia were the same in both groups. The incidences of neurologic disorders were similar and high (about 70 per cent) in both groups; these included mental retardation, 43 per cent; spastic diplegia, 32 per cent; learning problems in spite of normal I.Q., 40 per cent. Hearing loss was present in 18 per cent of those examined for this abnormality and was *not* related to the administration of an ototoxic antibiotic. The caloric intakes of all children had been very low in the first week of life and may have contributed to the high incidence of abnormalities. This cannot be evaluated until data obtained from adequately-fed (60 cal/kg/day) newborns of low birth weight are available. (*Lubchenco, L. O., and others: Long-term Follow-up Studies of Prematurely Born Infants. I. Relationship of Handicaps to Nursery Routines. J. Pediat. 80: 501–508, 1972.*)