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Lack of inhibition of glucuronidation in isolated rat hepatocytes by diethyl ether anesthesia

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Diethyl ether anesthesia has been shown to decrease the glucuronidation of both exogenous and endogenous compounds in rats [1, 2]. The inhibition of glucuronidation by diethyl ether is caused by a depletion of uridine diphosphate glucuronic acid (UDPGA), an essential cofactor for glucuronidation [3–5]. A 10-min exposure to diethyl ether results in a decrease in UDPGA levels of 90–95% [5, 6]. However, when animals are allowed to recover from the anesthesia, the UDPGA concentration returns to a control value within 1 hr after exposure. All the previous studies [1, 3–5] determined UDPGA levels in tissue homogenates. Therefore, it was of interest to determine if diethyl ether anesthesia decreases UDPGA levels and thus glucuronidation in another hepatic preparation, isolated hepatocytes, used to study this conjugation pathway. Diethyl ether anesthesia was compared to urethane anesthesia, as urethane has been shown to decrease UDPGA levels only slightly in rats [4, 5].

Male Sprague–Dawley rats were anesthetized with urethane (approximately 1.5 g/kg) i.p. or diethyl ether by continuous inhalation. Hepatocytes were isolated by a modification of the procedure described by Eacho and Weiner [7]. The abdominal cavity was opened, the inferior vena cava was ligated above the renal vein, and the chest cavity was opened. An incision was made in the right atrium through which the inferior vena cava was canulated. A retrograde perfusion of the liver was initiated with a Ca^{2+}-free Krebs–Ringer bicarbonate buffer containing 5.5 mM glucose and 0.5 mM ethyleneglycolbis(aminoethyl-ether)tetra-acetate (EGTA). Livers were perfused until relatively cleared of blood and then perfused with a Krebs–Ringer bicarbonate buffer containing 4.75 mM Ca^{2+} and 0.5 mg/ml collagenase at a flow rate of 17–20 ml/min for 20 min. Cells were diluted to a final concentration of 2×10^6 cells/ml with Krebs–Ringer bicarbonate buffer containing 1% bovine serum albumin.

Glucuronide formation of *p*-nitrophenol (*p*-NP) was determined as previously described [8]. The level of UDPGA in hepatocytes was determined enzymatically by the method of Ullrich and Bock [9]. Aliquots (2 ml) of cell suspension containing approximately 2×10^6 cells/ml were removed at 0, 15 and 30 min after the addition of *p*-NP and then boiled for 2 min. After heating, the suspensions were cooled on ice and centrifuged for 15 min at 10,000 *g*. UDPGA was determined in the supernatant fluid by means of the 4-methylumbelliferone-glucuronyl transferase assay [10].

A comparison of the effects of urethane and diethyl ether anesthesia, used in the preparation of isolated hepatocytes, on glucuronide formation is shown in Fig. 1. No significant difference was found between urethane and diethyl ether in the amount of *p*-NP glucuronide formed. Similarly, UDPGA levels were not found to be significantly different between the two anesthetics at each time interval (Table 1). UDPGA concentrations of 3.10 ± 0.24 and 3.20 ± 0.21 nmoles/10^6 cells were found at 30 min in hepatocytes of rats anesthetized with urethane or diethyl ether respectively. These levels are within the range of previous studies [9, 11, 12]. When calculated on a basis of 127×10^6 cells/g liver (wet wt) [13], a level of 0.41 μmole/g liver was obtained. This level is in agreement with that found in the studies *in vivo* [14, 15] and in perfused livers [14, 16].

A possible explanation for the failure of diethyl ether to decrease UDPGA levels in hepatocytes as it does in tissue homogenates may relate to the differences in metabolic capabilities and thus the capacity for synthesizing UDPGA in these preparations. Once hepatic UDPGA levels have been depleted by diethyl ether, the hepatocyte is disrupted in the preparation of liver homogenates and therefore is incapable of subsequent formation of UDPGA. Conversely, hepatocytes retain their full spectrum of biochemical capabilities during isolation and incubation. Therefore, because glucose, a precursor of UDPGA, is present at physiological levels in most incubation buffers [17] and all the enzymes necessary for the synthesis of UDPGA are available, hepatocytes can resynthesize UDPGA and thus prevent depletion. Furthermore, since the liver must be perfused and the cells then incubated, filtered, centrifuged, and diluted to the appropriate concentration, there is sufficient time (approximately 1.5 hr) after cessation of exposure to diethyl ether for UDPGA levels to return to normal values. This conclusion is supported by the previously mentioned study in which animals anesthetized with diethyl ether were allowed to recover, and UDPGA levels rapidly returned to control concentrations by 1 hr after exposure [5]. Similarly, Aune *et al.* [18] examined the direct effects of ether on the metabolism of paracetamol in isolated hepatocytes and found that, when the concentration of ether is maintained throughout the incubation, the formation of glucuronide is reduced 70%.

Although not significantly different, there was a trend for UDPGA levels from rats anesthetized with ether to be slightly higher than those from animals anesthetized with

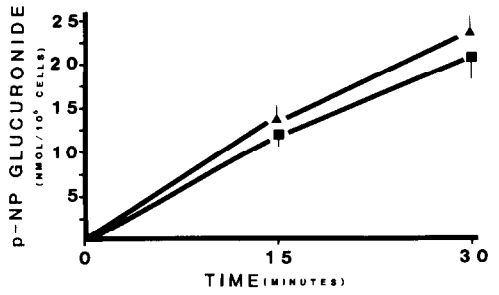

Fig. 1. Effects of urethane (■) and diethyl ether (▲) on the glucuronidation of *p*-NP in isolated hepatocytes. Data represent the mean ± S.E.M. of four determinations. Student's *t*-test was performed to determine statistical significance.

Table 1. Effects of urethane and diethyl ether on UDPGA levels in isolated hepatocytes

Treatment	UDPGA (nmoles/10^6 cells) 0 min	15 min	30 min
Urethane	2.14 ± 0.13	2.25 ± 0.18	3.10 ± 0.24
Ether	2.17 ± 0.24	1.99 ± 0.04	3.20 ± 0.21

Values represent the mean ± S.E.M. of four experiments.

urethane. Because ether anesthesia drastically reduces hepatic UDPGA levels while urethane does not [3, 5], this trend may be due to "overshoot" of the synthetic enzymes attempting to restore control levels while UDPGA is also being utilized to glucuronidate *p*-NP. Watkins and Klaassen [5] also observed a small increase in UDPGA levels over control values in animals allowed to recover from ether anesthesia.

Inasmuch as the concentration of UDPGA can be an important determinant of the rates of glucuronidation and xenobiotic elimination, the use of diethyl ether as an anesthetic must be carefully considered in some experiments. However, when diethyl ether anesthesia is used in the preparation of isolated hepatocytes, its effects on glucuronidation are not of consequence.

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In vitro phosphorylation and the identification of multiple protein changes in membranes of Chinese hamster lung cells resistant to adriamycin

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Previous studies have shown that plasma membranes of cells resistant to the antitumor agent adriamycin contain a phosphorylated glycoprotein (P180) which is not detected in drug sensitive cells [1, 2]. It has also been observed that certain isolates of resistant cells revert to drug sensitivity during growth in culture and that during this reversion process there is a loss of P180 from the cell surface [3]. These results suggest that this protein may play an important role in the cellular changes which contribute to drug resistance. Recent studies also show that, under conditions in which the level of phosphorylation of this protein is enhanced, there is a rapid conversion of the resistant cell to a drug sensitive phenotype [3, 4]. Thus, phosphorylation of protein P180 may be a means of regulating cell resistance to adriamycin. Additional studies also suggest that adriamycin resistance is related to changes occurring in the plasma membrane. Thus, it has been shown that resistance is due to a membrane restriction to drug uptake [5, 6] and/