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SHORT COMMUNICATIONS

Decreased glucuronidation of bilirubin by diethyl ether anesthesia

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Diethyl ether (DE) anesthesia has been shown to dramatically reduce hepatic uridine diphosphoglucuronic acid (UDPGA) levels in rats [1–3]. Within 10 min of the onset of DE-induced narcosis, UDPGA levels are reduced to 10% of controls and remain low while the animal is anesthetized [4]. However, liver UDPGA levels return to control values within 1 hr after cessation of ether exposure [3]. This drastic decrease in UDPGA levels is not a general characteristic of anesthetics as narcotic doses of urethane and pentobarbital produce only a slight decrease in levels [2, 3].

UDPGA is necessary for glucuronidation, an important biotransformation pathway in the elimination of endogenous and exogenous chemicals. We have shown recently that decreasing the hepatic UDPGA level lowers the biliary excretion of a number of exogenous compounds [4]. Bilirubin is an endogenous compound that normally is glucuronidated in liver prior to its excretion as the monoglucuronide (BMG) and the diglucuronide (BDG) into bile. Therefore, it was of interest to determine if the DE-induced decrease in hepatic UDPGA levels altered the glucuronidation of bilirubin.

Male Sprague–Dawley rats were anesthetized with urethane (1 g/kg, i.p.) or DE by continuous inhalation. The bile duct of each rat was cannulated with PE-10 tubing [5]. Body temperature was maintained at 37° to prevent hypothermic alterations in biliary excretion [6]. Bile was collected in the dark at 4° for eight consecutive 30-min periods following surgery.

Bilirubin, BMG and BDG in bile were separated by high-pressure liquid chromatography (HPLC) [7] immediately after the end of each collection and detected spectrophotometrically at 436 nm. Quantitation was based on bilirubin standards, as bilirubin, BMG and BDG have the same extinction coefficients [8]. The validity of this was established in our system: [^{14}C]-bilirubin conjugates were prepared by administration of 4-[^{14}C]-delta-aminolevulinic acid to rats [9, 10] and were isolated by preparative HPLC. Glucuronides and bilirubin formed from the hydrolysis of the glucuronides had the same specific activity to peak height ratio. More than one isomer of bilirubin exists in bile and the hydrolysates. The IXα isomer is predominant over the XIVα and IIIα isomers. So, for this reason, the IXα isomer was used for quantitation. The standards did not contain appreciable amounts of the XIVα and IIIα isomers by HPLC analysis [11].

Comparisons of the effects of urethane and DE anesthesia on bile production and biliary concentration of BMG and BDG are shown in Fig. 1. Bile flow was higher (13–54%) in DE- than in urethane-anesthetized rats. A pronounced difference existed in the concentration of BDG in the bile of the two groups. The biliary concentration of BDG in DE-induced anesthesia was one-third of that in urethane anesthesia. However, the concentration of BMG in the two groups was essentially the same. The concentration ratios of BDG to BMG were significantly different ($P < 0.05$) at all time points; urethane gave an average ratio of 1.7 while DE gave a ratio of 0.7.

To assess the effect of recovery from DE anesthesia on biliary excretion of bilirubin products, rats in a second group were administered DE for the duration of the surgical procedure (5–8 min) and then were placed in restraining cages and their temperature regulated. Bile was collected for 1 min prior to and for eight consecutive 15-min periods following the cessation of exposure. Analysis was again done by HPLC. Figure 2 depicts the concentration of BMG and BDG in bile at various times during recovery from DE anesthesia. Immediately after removal of DE, the concentration of BDG in bile was 15 μM. The concentration increased during recovery to a plateau of about 55 μM 90 min later. In contrast to BDG, the concentration of BMG was relatively constant during the recovery from DE anesthesia. It increased only 15 μM during the same period and achieved a level comparable to that observed during continuous DE or urethane anesthesia.

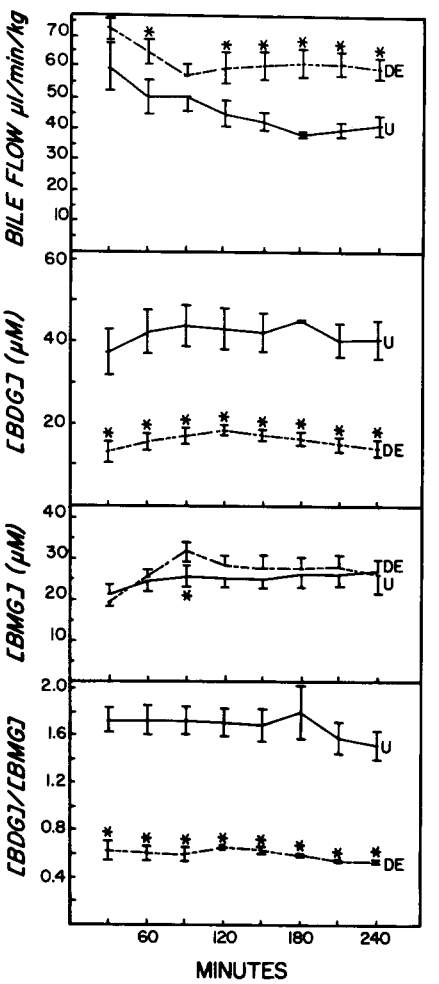

Fig. 1. Effects of continuous DE or urethane (U) anesthesia on bile flow and bilirubin conjugate composition in rats. Values are means ± S.E. (N = 4). Key: (*) statistically different ($P < 0.05$) by Student's *t*-test.

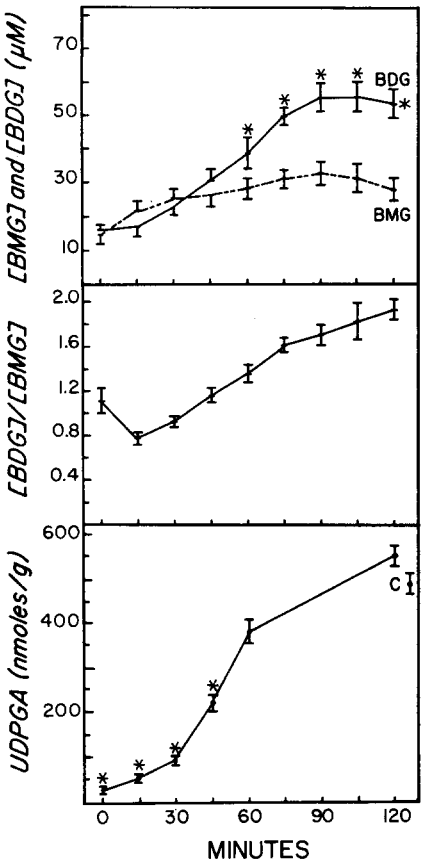

Fig. 2. Rat biliary conjugate composition of bilirubin during recovery from DE anesthesia. Exposure was stopped at 0 min. Values are means ± S.E. (N = 5). Asterisks in the top panel indicate values that are statistically different ($P < 0.05$) by Student's *t*-test. Data in the bottom panel are based on Ref. 3. Asterisks in the bottom panel indicate values that are statistically different ($P < 0.05$) from control level (C) by Dunnett's test.

The bile BDG to BMG ratio (Fig. 2) changed with time during recovery from DE anesthesia. The ratio was as low as 0.8 for the first 15 min of recovery and increased to approximately 2. Thus, the ratio immediately after the cessation of DE exposure was similar to that observed during continuous DE anesthesia (Fig. 1), while 2 hr after the recovery from DE anesthesia the ratio was similar to that with urethane. The increase in the BDG/BMG paralleled the increase in hepatic UDPGA levels during recovery from DE exposure (Fig. 2).

The present data agree with BDG/BMG ratios 2 hr after recovery from DE anesthesia [12] and during pentobarbital anesthesia [8, 13]. Unlike this anesthetic and urethane, DE drastically decreased the concentration of BDG in bile, resulting in a ratio of 0.7.

Bilirubin UDP-glucuronosyltransferase (UDPGT) has a velocity estimated to be 9% of the *in vitro* V_{max} [14] at the hepatic UDPGA concentration found during DE anesthesia. This activity may account for the continued production of low levels of BMG and BDG during DE anesthesia. The much higher capacity of UDPGT for BMG formation than for BDG production [15] may be why the biliary concentration of BMG did not change appreciably during DE exposure while BDG levels dramatically decreased.

The effect of DE anesthesia on the relative decrease of BDG/BMG has been ascribed previously to hypothermia [16]. However, a change of bilirubin conjugate composition in bile has been reported during DE anesthesia when the temperature of the animals was regulated [17]; it was suggested that DE affected UDPGT directly. The present study shows that the mechanism of action of DE on bilirubin glucuronidation is the same mechanism responsible for decreased glucuronidation of exogenous compounds [14]. DE depletes hepatic UDPGA [1, 2], which causes decreased glucuronidation, since glucuronosyltransferase activity is dependent on UDPGA levels [18]. An alternative explanation for the decrease in BDG excretion is that DE may affect BDG transport into bile. There is some evidence against this explanation: DE did not affect BMG excretion; presumably BMG transport occurs in the same lipophilic environment as BDG transport. Glucuronidation has been shown to be the rate-limiting step in BDG transport [19]. Galactosamine, which also depletes the UDPGA, reduces bilirubin excretion [4]. Galactosamine is not lipophilic and is unlikely to interact at the lipophilic transport site. So, two similar agents which deplete UDPGA by different mechanisms, decrease bilirubin excretion. Finally, DE has been shown not to decrease the excretion of phenophthalein glucuronide [4]. The complicating factor that DE alters the glucuronidation of both exogenous and endogenous chemicals needs to be considered in experiments in which DE is used as the anesthetic.

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