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Glucuronide synthesis in the isolated perfused rat lung

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We reported earlier [1] that pulmonary microsomes from different animal species are capable of glucuronide synthesis. The present paper shows that not only homogenated preparations of lungs, fortified with high concentrations of the activated glucuronic acid, uridine diphosphate glucuronic acid (UDPGA), show this capability, but also that isolated perfused rat lung conjugates 4-methylumbelliferone in the perfusate with endogenous UDPGA and excretes the formed 4-methylumbelliferylglucuronide (MUG) in the perfusate. The lung, which is the site of entry [2] and accumulation [3–6] of many pharmacologically active agents, is thus capable of the quantitatively most important conjugative biotransformation reaction of xenobiotics in the mammalian organism, yielding a water-soluble, non-toxic product to be excreted from the body.

The perfusions were performed using a method developed in our laboratory for pulmonary steroid metabolism studies [7, 8]. In brief, the rats were anesthetized with intraperitoneal pentobarbitone (Nembutal®, 50 mg/kg) and the trachea was cannulated and connected to a pressure-regulated respirator (Bird®; max. pressure 18 cm H_2O). The pulmonary artery was cannulated and the lungs were excised and connected to the perfusion apparatus. A Harvard pulsatile blood pump (systolic and diastolic pressures 15–18 mmHg and 10–12 mmHg, respectively, flow 10 ml/min) was used. The perfusion medium consisted of heparinized rat blood diluted with Tyrode solution to give a hematocrit of 0.30, fortified with 4-methylumbelliferone (0.29 m-mole/l), and gassed with 6% carbon dioxide in air to keep the pH, pCO_2, and pO_2 within the normal values of arterial blood.

The formation of MUG was measured with slight modifications of the method used previously [9, 10]: the proteins of consecutive 1 ml fractions of the perfusate were denaturated with trichloroacetic acid (TCA). TCA, and the unreacted aglycone were extracted from the supernatant by three successive extractions with diethylether and MUG in the water phase was hydrolyzed with β-glucuronidase. The fluorescence of the liberated aglycone was measured at an alkaline pH. Parallel determinations were carried out without β-glucuronidase in the hydrolysis medium and with the hydrolysis specifically inhibited with 10 m-mole/l D-glucaro-1,4-lactone [11]; no liberation of the aglycone could be detected in these controls. Hydrolysis by β-glucuronidase, and inhibition of the hydrolysis by glucarolactone positively identify the formed conjugate as glucuronide. In addition, paper chromatography of the TCA supernatant fraction in *n*-butanol–pyridine–water, 2:1:1, v/v, resulted in a single spot (in addition to aglycone) with an R_F value similar to that of the authentic MUG. This spot yielded fluorescence similar to that of methylumbelliferone after β-glucuronidase hydrolysis.

All three rat lung preparations studied produced MUG at a constant rate after a lag period of about one hour (Fig. 1). No significant synthesis of glucuronide could be detected in blood perfused without lungs. The lag period is probably due to accumulation of the substrate, and of the reaction product in the lungs before the latter begins to appear in the perfusate. The equilibrium rate of glucuronide formation was 40 to 80 nmole/hr, corresponding to about 0.5–2 nmole/min/g lung weight. Lung homogenates have been reported to synthesize about 30 nmole MUG/min/g wet wt. [12]. MUG formation under the conditions of enzyme assay is rather hard to compare with the glucuronidation rate in lung tissue. In earlier studies with rat liver slices [13] and with perfused rat liver [14] it has been demonstrated that the glucuronidation rate is slightly higher or equal compared with liver homogenates, but much less than the total enzyme capacity in optimally activated preparations. According to the present study perfused rat lung, however, forms considerably less glucuronide than pulmonary broken cell preparations. This could

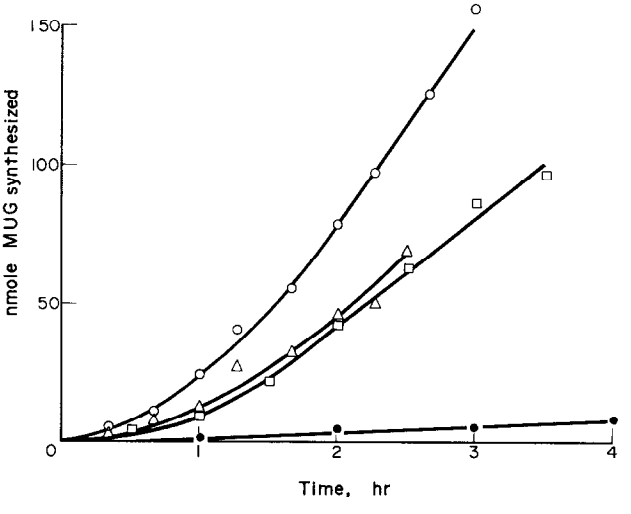

Fig. 1. Synthesis of 4-methylumbelliferylglucuronide in isolated perfused rat lungs. Open symbols represent three separate lung preparations, closed symbols glucuronide formation in the blood (perfusion without lung).

be due to a rate limiting step other than the UDPglucuronosyltransferase activity, e.g. amount of UDPGA available, or permeation of the aglycone or glucuronide into or out of the lung.

Since lungs, in addition to the significant glucuronide synthesis reported here, are also capable of various hydroxylation, acetylation, methylation, and sulfate and glutathione conjugation reactions of xenobiotics [15], their role in xenobiotic biotransformation, especially in the case of drugs entering the body via the lungs, or accumulating in the lungs, should be reconsidered.

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