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THE EFFECTIVENESS OF DIFFERENT SULFATE PRECURSORS IN SUPPORTING EXTRAHEPATIC SULFATE CONJUGATION

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Abstract—The effectiveness of different sulfur-containing compounds in supplying inorganic sulfate for sulfate conjugation was studied in isolated cells from rat small intestine, kidney and lung. With cells isolated from the small intestine and kidney, inorganic sulfate was by far the most effective source for intracellular active sulfate as judged by the ability to support sulfate conjugation of 7-hydroxycoumarin. Kidney cells could also use cysteine, *N*-acetylcysteine and glutathione as a sulfate source, whereas isolated small intestinal cells did not seem to break down and use these sulfur-containing compounds. With isolated lung cells cysteine was the most efficient sulfate precursor. Of the other precursors *N*-acetylcysteine and inorganic sulfate were used for sulfate conjugation to some extent.

Conjugation with inorganic sulfate is one of the most common of the so-called phase II reactions of drug metabolism [1]. Compounds which contain one or more hydroxyl group(s) are particularly good substrates for sulfate conjugation, especially those with a phenolic hydroxyl group [1, 2]. The liver is the tissue which generally has the largest capacity for drug metabolic reactions, but extrahepatic tissues can have important qualitative roles to play. It has been shown that the small intestine has the ability to conjugate a number of xenobiotics [3, 4] and could readily cope with the conjugation of ingested phenolic material [5]. Sulfate conjugation in the liver is readily saturated [2, 6], and even though glucuronidation in the liver generally takes over, at high substrate concentration it is possible that the kidney could play an important role in metabolizing circulating xenobiotics. Although, in general, the lung has a relatively small capacity for drug metabolism, small quantities of inhaled compounds could be effectively metabolized at the site of absorption.

Sulfate conjugation is regulated by a number of factors including substrate concentration [2, 6], availability of inorganic sulfate [2, 7, 8] and the synthesis of adenosine 3′-phosphate 5′-sulfatophosphate (PAPS), which is the 'active form' in which sulfate is transferred to the substrate. The availability of inorganic sulfate has been studied almost exclusively in connection with hepatic availability [2, 7, 8]. Extrahepatic tissues, however, are also active in sulfate conjugation and could have important qualitative differences in the various control processes. In the present investigation the effectiveness of different sulfur-containing compounds as precursors of PAPS in extrahepatic tissues was studied. These studies were performed using isolated cells from the relevant tissues. Use of isolated cells facilitates changes in extracellular fluid composition, eliminates any hepatic influence and allows easy collection of metabolites.

MATERIALS AND METHODS

Reduced glutathione, L-cysteine hydrochloride, *N*-acetylcysteine, protease type VII, sulfatase type H-1 and Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) were purchased from the Sigma Chemical Co. (St. Louis, MO). Collagenase was purchased from Boehringer Mannheim (F.R.G.). 7-Hydroxycoumarin (umbelliferone) was purchased from Fluka AG. (Switzerland). [U-^{14}C]phenol (71.1 mCi/mmole) was purchased from Amsterdam, U.K. Lumagel, scintillation fluid and scintillation vials (6 ml volume) were purchased from Lumac B.V. (Schaesberg, The Netherlands). All other chemicals were of analytical or reagent grade and purchased through local chemical suppliers.

Animals. Male Sprague–Dawley rats, 180–220 g, and allowed free access to food and water, were used throughout.

Cell preparations. Kidney cells were isolated by a collagenase perfusion method as described by Jones *et al.* [9], and were used throughout at a concentration of 1–2 × 10^6 cells/ml.

Lung cells were isolated by a recently developed method involving perfusion of a protease solution via the trachea [10]. Lung cells were used at a concentration of 3 or 4 × 10^6 cells/ml.

Cells were isolated from the small intestine by the method of Dawson and Bridges [11], slightly modified, as follows. The exerted pieces of intestine were incubated (20 min at 37°) in a Ca^{2+}- and Mg^{2+}-free Hanks buffer (50 ml) containing protease type VII (1 mg/ml) and EDTA (1 mM). The portions of intestine were then transferred to (50 ml) Krebs–Hepes buffer containing 1% bovine serum albumin (w/v) and glucose (5 mM), and incubated for a further 10 min at 37°. The cells from both flasks were pooled and gave a cell yield of 350–400 × 10^6 cells from 60 cm of rat small intestine. The intestinal cells were used at a concentration of 2 × 10^6 cells/ml buffer.

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Metabolism experiments. The buffer used throughout these experiments was a sulfate-free Krebs–Hepes buffer, pH 7.4, with the $MgSO_4$ in the buffer replaced by $MgCl_2$. Cell incubations were performed in rotating, round-bottomed flasks at 37°. Kidney and small intestinal cells were incubated under a carbogen (95% O_2, 5% CO_2) atmosphere and lung cells were incubated under air.

7-Hydroxycoumarin sulfation was determined as described by Dawson and Bridges [4]. In brief, samples (2 ml) were withdrawn from the incubation at the times required, and the free, unreacted substrate was removed by repeated extractions with ether containing 1.5% isoamyl alcohol (3 × 7 ml). The aqueous layer was then acidified and deconjugated overnight with sulfatase (1 mg/ml) in the presence of saccarolactone (1 mM). The 7-hydroxycoumarin released was then determined fluorimetrically.

[^{14}C]Phenol sulfate was determined by the thin layer chromatographic method described by Shirkey *et al.* [3].

RESULTS

With cells isolated from rat small intestine, inorganic sulfate appeared to be the only effective source for intracellular active sulfate. The amino acids cysteine and *N*-acetylcysteine, and the tripeptide glutathione did not appear to be broken down and used for sulfate conjugation of 7-hydroxycoumarin in the time studied (Fig. 1). A small amount of sulfate conjugates is produced, even in the absence of an extracellular sulfate source. This probably represents the intracellular sulfate store.

Although again inorganic sulfate was by far the most effective source for PAPS, kidney cells, however, did effectively catabolize the amino acids and the glutathione to inorganic sulfate (Fig. 2). Cysteine, as may be expected, was the most effective of the three, whilst *N*-acetylcysteine and glutathione were equally efficient precursors (Fig. 2). This reflects the need for one (*N*-acetylcysteine) or two (glutathione) additional steps in the catabolic route to sulfate.

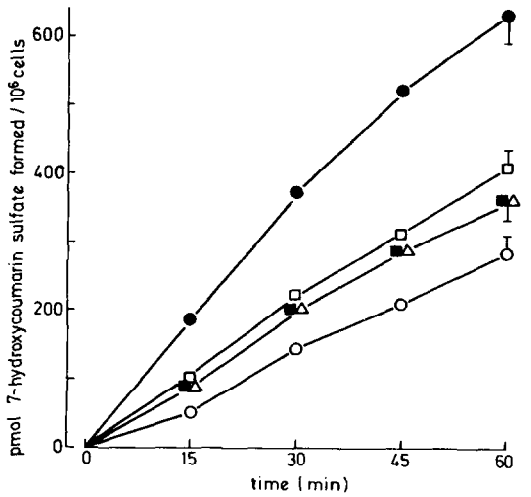

Fig. 2. Sulfate conjugation of 7-hydroxycoumarin by isolated kidney cells. Cells isolated from rat kidney were incubated at a concentration of 1–2 × 10^6 cells/ml in Krebs–Hepes buffer with 10 μM 7-hydroxycoumarin. Present in the incubation were: no sulfur source, ○; 1 mM Na_2SO_4, ●; 1 mM cysteine, □; 1 mM *N*-acetylcysteine, ■; 1 mM reduced glutathione, △.

With isolated lung cells, however, the picture was quite different. In this instance, using both 7-hydroxycoumarin and phenol as substrates, cysteine was by far the most efficient precursor for active sulfate (Fig. 3 and Table 1). Of the other precursors, *N*-acetylcysteine and inorganic sulfate were used for sulfate conjugation to some extent whereas the involvement of glutathione seems unlikely to be of any significance.

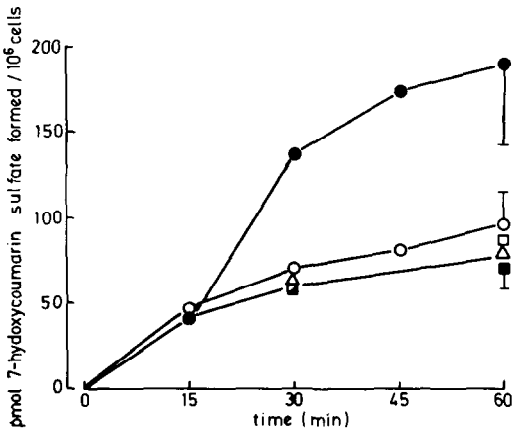

Fig. 1. Sulfate conjugation of 7-hydroxycoumarin by isolated small intestinal cells. Cells isolated from rat small intestine were incubated at a concentration of 2 × 10^6 cells/ml in Krebs–Hepes buffer with 100 μM 7-hydroxycoumarin. Present in the incubation were: no sulfur source, ○; 1 mM Na_2SO_4, ●; 1 mM cysteine, □; 1 mM *N*-acetylcysteine, ■; 1 mM reduced glutathione, △.

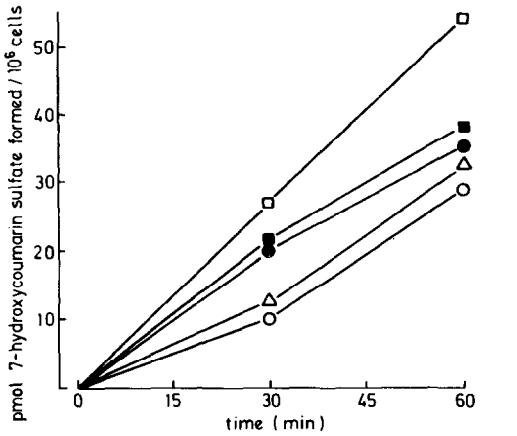

Fig. 3. Sulfate conjugation of 7-hydroxycoumarin by isolated lung cells. Cells isolated from rat lung were incubated at a concentration of 3 × 10^6 cells/ml in Krebs–Hepes buffer with 100 μM 7-hydroxycoumarin. Present in the incubation were: no sulfur source, ○; 1 mM Na_2SO_4, ●; 1 mM cysteine, □; 1 mM *N*-acetylcysteine, ■; 1 mM reduced glutathione, △.

Table 1. Sulfate conjugation of phenol by isolated lung cells

Sulfur source	Phenol sulfate formed (pmole/10^6 cells per hr)
$NaSO_4$ (1 mM)	266
Cys (1 mM)	497
NAC (1 mM)	382
GSH (1 mM)	270

NAC = *N*-acetylcysteine, Cys = cysteine, GSH = reduced glutathione.

DISCUSSION

It has been shown both *in vivo* and *in vitro* that the availability of inorganic sulfate can be limiting for sulfate conjugation in the liver [2, 7, 8]. Experiments performed *in vivo* have also demonstrated that the supply of inorganic sulfate can be maintained by the administration of L-cysteine, D-cysteine or *N*-acetylcysteine [12–16]. It has been shown *in vitro* using isolated hepatocytes that cysteine and *N*-acetylcysteine are fairly good as precursors of PAPS in the liver, but methionine is hardly used in this respect and cystine not at all [7, 12]. It has also been shown that the oxidative route for cysteine metabolism can be decreased when cysteine is required for glutathione synthesis in hepatocytes [12].

The use of sulfur-containing amino acids in the supply of PAPS to extrahepatic tissues has, however, not previously been confirmed. This is mainly due to the difficulty of performing such studies *in vivo*, due to the large hepatic capacity for sulfation. It has been demonstrated that extrahepatic tissues can sulfate a wide range of xenobiotic compounds [2]. The kidney can sulfate 7-hydroxycoumarin and paracetamol, and has been demonstrated using isolated cell preparations [9, 17]. Cells from the rat small intestine have the ability to sulfate phenol, 1-naphthol and 7-hydroxycoumarin [3], and intestinal preparations from the rat and other species have been shown to sulfate a variety of xenobiotics [4, 5, 11, 18, 19]. Isolated perfused lung can sulfate phenol and lung cultures have the ability to sulfate 1-naphthol and 3-hydroxybenzpyrene [20–23]. There is, however, only one previous report of sulfation by isolated lung cells, and that was with 7-hydroxycoumarin (formed from 7-ethoxycoumarin) as the substrate [10].

In the present report, extrahepatic sulfation was investigated using cells isolated from the relevant tissues, and various sources for PAPS were tested for their efficacy. It appears that the kidney is most like the liver with respect to the use of sulfate precursors for PAPS. In isolated kidney cells inorganic sulfate was by far the most active precursor, but cysteine, *N*-acetylcysteine and glutathione could also be used for this purpose [7, 12]. In the small intestinal cells only inorganic sulfate itself was made available for sulfate conjugation. This is in contrast to the lung cells where cysteine was more effective than sodium sulfate at providing PAPS. This appears to indicate that lung cells have limited ability to take up sulfate from the extracellular space. Sulfate uptake has been shown to be a carrier-mediated process, and that, in erythrocytes at least, sodium is taken up at the same time as the sulfate [2].

For liver, kidney and the small intestine an adequate supply of inorganic sulfate (and adenosine triphosphate) should be sufficient to allow sulfate conjugation to occur. The lung, however, requires a supply of cysteine for a maximal rate of sulfate conjugation. This could have important implications in certain instances of pulmonary tumours which lack sulfate-conjugating ability [22]. Also, in instances where there is a large demand for cysteine by the liver, e.g. when glutathione synthesis is urgently required [12], then sulfate conjugation in the lung could be adversely affected. Studies are in progress to determine whether the same lack of sulfate uptake is evident in the isolated perfused lung.

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