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VECTORIAL RELEASE OF SULFOCONJUGATES IN THE VASCULARLY PERFUSED MOUSE SMALL INTESTINE

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Abstract—Sulfoconjugates are formed from various xenobiotics and drugs in the vascularly perfused mouse small intestine. They can be grouped according to the sidedness of their release from the epithelium. Conjugates of paracetamol and salicylamide, like 1-naphthol-sulfate, are released exclusively into the vascular medium, whereas those of diethylstilbestrol, ethinyl-estradiol and isoprenaline appear only in the luminal perfusion medium. It is concluded from these results that selective anion transport systems for sulfoconjugates exist in the brush-border membrane as well as in the basolateral membrane of the enterocyte.

The intestinal mucosa of several species, including mouse, is capable of forming sulfoconjugates of phenolic foreign compounds [1, 2]. The parent drugs are lipophilic and therefore enter the cell by diffusion. In contrast, the conjugates are hydrophilic anions at physiological pH values, and therefore do not easily permeate the cell membrane. Hence the conjugates require transport mechanisms to exit from the cell. Transport mechanisms for these anions have been described in the liver. In hepatocytes, drug conjugates may preferentially leave the cell via the biliary or the venous cell-pole and thus the conjugates appear either in the bile or in the systemic circulation. The enterocyte shows an even more pronounced morphological and functional polarity than the hepatocyte, and one may expect a sidedness of conjugate release in this tissue in analogy to the liver. Indeed, absorption from the lumen to the blood of bile acids, e.g. taurocholic acid, as well as secretion from the blood to the lumen of sulfonic acids, e.g. 2-naphthol-orange, has been observed in the small intestine [3].

Recently, it was shown that 1-naphthol is sulfoconjugated in the vascularly perfused mouse small intestine, and that its conjugate is released to the basolateral side only [2]. In this paper further evidence will be given for the existence of a basolateral, as well as a brush-border membrane transport system for drug sulfoconjugates. Based on the specificity of these transport systems, two groups of sulfoconjugates can be discerned: one group which is exclusively released to the luminal side and one group which is released to the blood side of the epithelium.

MATERIALS AND METHODS

Chemicals. $H_2{}^{35}SO_4$, carrier-free, 37 MBq/ml, was purchased from New England Nuclear (Dreieich, F.R.G.) and was diluted 1:10 with distilled water to give a stock solution. Isoprenaline, DES†, salicylamide, and paracetamol were from Serva (Heidelberg, F.R.G.). BSA was from Behringwerke (Marburg, F.R.G.). EE was a generous gift from Schering AG (Berlin, F.R.G.). All other chemicals were obtained from Merck (Darmstadt, F.R.G.). All substances were of the highest analytical grade available.

Animals. Male NMRI-SPF-mice (25–30 g) were purchased from Wiga (Sulzfeld, F.R.G.). The animals had free access to tap water and to a commercial pelleted diet (Altromin®). Food, but not water, was removed 12 hr before the experiment.

Vascular perfusion. Vascular perfusion was performed as described earlier [2]. Briefly, under pentobarbital anaesthesia, an open perfusion of the small intestine from the ligament of Treitz to the ileocecal valve was established. Vascular perfusion medium was infused at a rate of 2.5–3 ml/min into the superior mesenteric artery and was collected at the portal vein. The vascular perfusion medium consisted of human erythrocytes in a modified Krebs–Ringer buffer and the luminal medium of Dulbecco's phosphate-buffered saline. No inorganic sulfate was incorporated into the media, but the vascular perfusion medium contained a tracer amount of $^{35}SO_4$. The lumen was perfused simultaneously at a rate of 1 ml/min. Drugs were added to the luminal perfusion media, which were supplemented with albumin (1 mg/ml) in the DES and EE experiments in order to enhance the solubility of these compounds.

Detection of conjugates. Conjugates were separated from free radiolabelled sulfate by the addition of 100 μl 0.25 M Na_2SO_4 and the subsequent addition of 100 μl 0.5 M $BaCl_2$ to 400 μl of medium. The precipitate was separated by centrifugation and 400 μl of the supernatant was counted in 10 ml of Bray scintillator. In control experiments it was confirmed that the radioactivity in the supernatant was not further decreased when the precipitation procedure was repeated. Furthermore, no decrease in optical density exceeding the dilution factor was seen when solutions of the sulfate ester of 1-naphthol

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† Abbreviations: DES, diethyl-stilbestrol; EE, ethinyl-estradiol; PAPS, 3′-phosphoadenosine-5′-phosphosulfate.

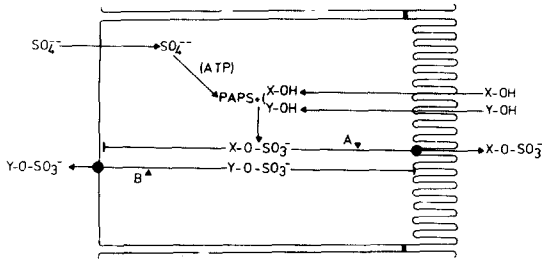

Fig. 1. Schematic representation of co-substrate, substrate and metabolite flow through an enterocyte in the vascularly perfused mouse small intestine. [^{35}S]Sulfate enters the cell from the blood and is converted to [^{35}S]PAPS. Phenolic drugs (*X*-OH and *Y*-OH) diffuse through the brush-border membrane and are conjugated with sulfate. Their metabolites leave the cell via specific transport systems in the basolateral or brush-border membrane.

were treated in the same manner. Free sulfate, however, was removed below the detection limit.

All experiments were done at least in triplicate. With respect to the varying isotope dilution by the non-labelled sulfate pool it would be unreasonable to calculate mean values. Therefore representative experiments are given in the figures. The mean increase in conjugate release was consistently in the range 8- to 12-fold in the case of isoprenaline and diethyl-stilbestrol, and in the range 2- to 3-fold in the case of salicylamide, paracetamol and ethinyl-estradiol.

RESULTS

A schematic representation of the experimental conditions described here is given in Fig. 1. Radioactive sulfate from the vascular perfusion medium was taken up into the enterocytes over the basolateral membrane. It was incorporated into the endogenous PAPS pool and from there transferred to substrates. Phenolic drugs entered the cells by diffusion from the luminal perfusion medium. They were sulfoconjugated and their anionic metabolites were released through a gated pore or by a carrier

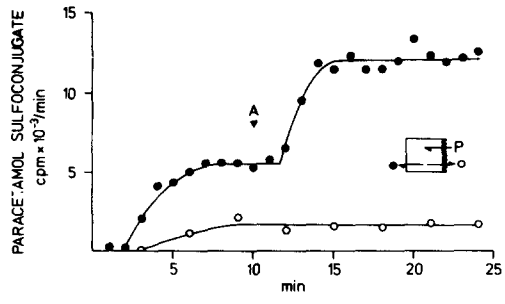

Fig. 2. Release of paracetamol sulfoconjugate. Upon addition of [^{35}S]sulfate to the vascular medium at zero time, an endogenous sulfoconjugate was formed. At time A, when a steady-state was reached, 100 μM paracetamol was added to the luminal medium. The conjugate was released to the blood side exclusively (open circles: lumen; closed circles: blood).

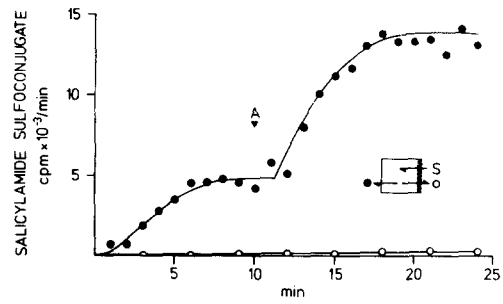

Fig. 3. Release of salicylamide sulfoconjugate. Salicylamide (100 μM) was added at time A (open circles: lumen; closed circles: blood).

mechanism in the brush-border (A) or basolateral cell membrane (B). Free radioactive sulfate in the effluent media was precipitated with $Ba(OH)_2$ and the residual activity, which represents total conjugates, was quantitated.

One or more ^{35}S-containing metabolites was released to both the luminal and blood side of the epithelium to a varying extent even in the absence of phenolic drugs. The chemical nature of these endogenous metabolites is not yet clear. Appropriate controls were carried out to exclude the possibility that any constituent of the perfusion media was responsible for their formation.

Upon addition of a phenol to the luminal medium conjugate release was considerably increased. In the case of paracetamol (Fig. 2) and salicylamide (Fig. 3), which were both given at a concentration of 100 μM, the increase was observed in the vascular perfusate exclusively. There was no change of conjugate release into the luminal perfusate from baseline values. These two substances behave in the same manner as 1-naphthol-sulfate, a sulfoconjugate which is exclusively released into the vascular medium [2].

Quite the opposite was seen when EE was used as the substrate at a concentration of 10 μM. A sulfated metabolite appeared in the luminal, but not in the vascular, perfusate (Fig. 4). A steep increase was observed immediately after the control medium was changed to EE-containing medium, the delay being in the range of the transit time of the perfusion

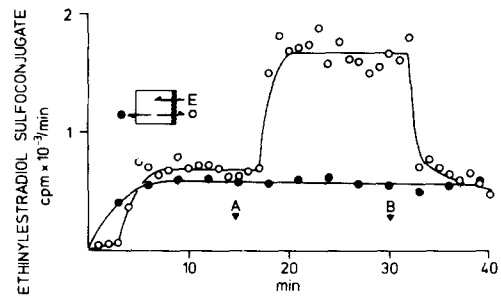

Fig. 4. Release of ethinyl-estradiol (EE) sulfoconjugate. The drug was present at a concentration of 10 μM together with 1 mg/ml bovine serum albumin between A and B (open circles: lumen; closed circles: blood).

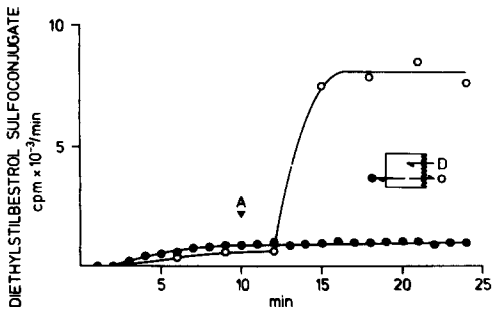

Fig. 5. Release of diethyl-stilbestrol (DES) sulfoconjugate. DES (100 μM) was added at time A to the luminal perfusion medium together with 1 mg/ml bovine serum albumin (open circles: lumen; closed circles: blood).

medium through the gut lumen. This effect was easily reversible after switching back to an EE-free solution at B. The synthetic estrogen-like substances DES, when given at 100 μM, behaved in the same manner as EE (Fig. 5).

The β-sympathomimetic drug isoprenaline has been shown to be conjugated with sulfate in dog small intestine [4]. Similarly, in our preparation, when 10 and 100 μM isoprenaline was given luminally, a stepwise increase of conjugate release to the lumen was observed (Fig. 6), and again, a release of the conjugate to the vascular compartment was not detectable.

DISCUSSION

It is well known that the transfer of solutes by the epithelium of the gut does not take place from the lumen to blood exclusively. Quite in contrast, large quantities of solutes, namely electrolytes, are released into the lumen as part of the normal function of this organ. Furthermore, secretion into the gut lumen of several drugs such as cardiac glycosides, quaternary ammonium compounds and organic anions has been demonstrated for the small intestine [3]. The co-existence of such asymmetrically directed transport systems in the intestinal epithelium resembles that of the tubular epithelium in the kidneys, where chemically related organic anions are either absorbed or secreted.

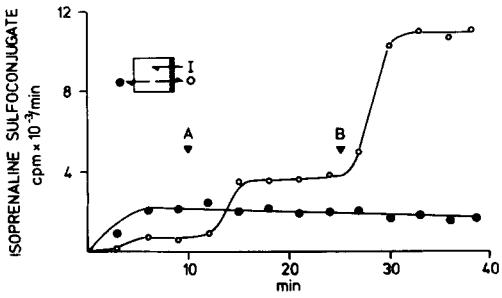

Fig. 6. Release of isoprenaline sulfoconjugate. Isoprenaline (10 μM) was added at time A and 100 μM at time B to the luminal perfusion medium (open circles: lumen; closed circles: blood).

It has been described recently that the sulfoconjugate of 1-naphthol is exclusively released from the murine intestinal epithelium into the blood across the basolateral membrane of the enterocytes [2]. In order to gain more insight into the mechanism of anion transport in the small intestine, other substrates for sulfoconjugation were studied. A large number of small samples were obtained by the use of radiolabelled sulfate together with unlabelled drugs, as is usually done for measuring the sulfotransferase activity *in vitro* [5]. However, since the pool-size of inactive sulfate is unknown, the amount of conjugate released cannot be quantitated. Since our main interest was focused on the sidedness of the sulfoconjugate release, this shortcoming seemed tolerable. In former experiments with 1-naphthol, the identity of the sulfoconjugate had been confirmed by double-tagging and chromatographical identification [2]. No attempt was therefore made to characterize further the chemical nature of the ^{35}S-containing compounds.

Intestinal sulfoconjugation of isoprenalin was described in dogs, whereas in mouse this substrate was not sulfoconjugated in the intestinal cytosol [5]. The lack of isoprenalin conjugation in the *in vitro* system described in that paper, which is in contrast to our findings, might be attributed to the presence of an inhibitor of phenol-sulfotransferase, as described in rat liver cytosol [6, 7], which is latent in the vascularly perfused preparation. The release of conjugates across the two membranes turned out to be mutually exclusive for the group of substrates studied here and revealed a high specificity of the respective transport systems. Two groups of sulfoconjugates can be distinguished. One (the conjugates of 1-naphthol, salicylamide and paracetamol) is released across the basolateral membrane. The other (the conjugates of isoprenaline, EE and DES) leaves the cell across the brush-border membrane. There is one striking chemical difference between the two groups. If one assumes that only one sulfate residue is transferred to each drug molecule, a free hydroxyl group remains at some distance to the esterified site in the members of the second group of conjugates. Thereby, the lipophilicity of this part of the molecule is markedly different and might account for the different affinities of the two groups of conjugates to the respective transport systems. This view is strengthened by the fact that β-naphthol-orange, which is actively secreted into the lumen in guinea-pig ileum [3], shares this physico-chemical property with isoprenaline and its congeners. More work is required, however, to understand the molecular basis of the transport selectivity.

In summary, it may be concluded that transport systems of sulfoconjugates exist in the luminal as well as in the contraluminal membranes of the intestine which show a high specificity for different sulfoconjugates. The properties of these transport systems and their importance in the transport of other organic anions remain to be elucidated.

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