Biochemical Pharmacology, Vol. 33, No. 21, pp. 3353–3356, 1984.
Printed in Great Britain.

0006–2952/84 $3.00 + 0.00

SULFATION IN ISOLATED ENTEROCYTES OF GUINEA PIG: DEPENDENCE ON INORGANIC SULFATE

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(*Received* 20 *February* 1984; *accepted* 2 *May* 1984)

Abstract—Isolated intestinal epithelial cells of the guinea-pig were used to study uptake and metabolism of inorganic sulfate in the jejunum (proximal cells) and ileum (distal cells). Proximal enterocytes accumulated sulfate 1.5-fold and distal enterocytes 3.1-fold. Accumulation was almost linearly related to substrate concentrations up to 5 mM. In proximal cells, despite their lower intracellular sulfate levels, sulfate incorporation into acid-precipitable material and sulfate conjugation of 1-naphthol were faster than in distal cells. Formation of 1-naphthyl sulfate increased with extracellular sulfate concentrations up to 1 and 3 mM sulfate in distal and proximal enterocytes, respectively. The data suggest that the extent of intestinal sulfation of phenolic compounds may be enhanced by oral administration of sulfate.

The intestinal mucosa plays a major role in the disposition of inorganic sulfate. It absorbs dietary sulfate [1, 2], utilizes sulfate for the synthesis of glucosaminoglycans [3] and converts potentially harmful phenolic compounds into harmless water-soluble conjugates [4, 5].

Sulfation of orally administered tyramine or salicylamide is increased when they are co-administered with sodium sulfate or L-cysteine [6, 7], a precursor of sulfate [8]. This suggests that the endogenous sulfate concentration is below saturation for the sulfation reaction. The relationship between sulfate levels and sulfation has been studied in the liver [9, 10] and in isolated hepatocytes [11, 12]. In small intestinal cells it has been recently shown that sulfate is a good precursor for the sulfation of 7-hydroxycoumarin [13]. However, the effect of extracellular sulfate concentrations on intracellular sulfate levels and on sulfate metabolism in different parts of the small intestine is not known.

Using isolated enterocytes from jejunum and ileum of guinea-pig the dependence of cellular sulfate uptake and sulfate metabolism on different sulfate concentrations has been studied. Guinea-pig mucosa cells were used, because these cells exhibit a high viability [14, 15], are suitable for transport studies [16] and efficiently conjugate phenolic drugs with sulfate [15, 17, 18]. 1-Naphthol was chosen as substrate for sulfate conjugation, since there are only two pathways for its metabolism resulting in the formation of glucuronic acid or sulfate conjugates [19]. In addition sulfation of macromolecules has been measured to see whether its distribution along the intestine parallels that of 1-naphthol sulfation.

MATERIALS AND METHODS

Chemicals. The radiochemical 1-[^{14}C]-naphthol (20.1 mCi/mmole) was from Amersham Buchler (Braunschweig, F.R.G.) and $Na_2[^{35}S]O_4$ (543 mCi/mmole) was from NEN Chemicals (Dreieich, F.R.G.); silicone oils AR 20 and AR 200 for centrifugal filtration were from Wacker Chemie (München, F.R.G.); thin layer foils and silica gel G were from Merck (Darmstadt, F.R.G.). All other chemicals were commercially obtained, and of analytical grade.

Preparation of intestinal cells. Adult male guinea-pigs (Pbw) weighing 650 g were obtained from Ivanovas (Kisslegg). Enterocytes were isolated as previously described [15]. Enterocytes isolated from the proximal quarter of the small intestine (jejunum) are termed 'proximal cells', those obtained from the distal quarter of the small intestine (ileum) are termed 'distal cells'. The cells were washed and kept at 4° as suspensions (20 mg protein/ml) in standard medium: 130 mM NaCl, 5.2 mM KCl, 0.9 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose buffered with 3 mM sodium phosphate and 15 mM sodium HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.4, plus a mixture of non-essential amino acids and L-glutamine in concentrations as they occur in Minimal Essential Medium (supplied by Seromed, München, F.R.G.). This amino acid mixture was free of cysteine and methionine.

The cells were largely single, retaining their polarity, and were morphologically intact. Cells were only used for experiments when the oxygen consumption was inhibited by more than 70% by oligomycin and subsequently stimulated by the uncoupler carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) by more than 6-fold. Cells from both parts of the small intestine had the same viabilities.

Sulfate uptake. To study uptake of inorganic sulfate, enterocytes (1–2 mg cellular protein/ml) were incubated in 2 ml standard medium with various concentrations of sulfate and 4 μCi/ml of [^{35}S]-sulfate at 37°. The reactions were terminated by withdrawing 200 μl of the suspension at various times and separating cells from the medium by centrifugal filtration [20].

Sulfate incorporation. The incorporation of sulfate into trichloroacetic acid-precipitable material was measured by incubating enterocytes (1–2 mg cellular protein/ml) with 4 μCi/ml of [^{35}S]-sulfate at 37°. Samples (200 μl) were withdrawn at the times indicated and transferred into 5 ml of ice-cold 5% trichloroacetic acid solution. The samples were then sucked through a membrane filter (nitrocellulose, pore size 0.45 μm). The retained material was washed three times with 5 ml trichloroacetic acid solution. The filters were dried and counted in Bray scintillation fluid. The value obtained after 5 sec of incubation was taken as blank and subtracted from the sample values.

Sulfation of 1-naphthol. To determine sulfation of 1-naphthol, enterocytes (1–2 mg cellular protein/ml) were incubated for 20 min at 37° before the addition of 1.5 μCi/ml of 1-[^{14}C]-naphthol and 100 μM 1-naphthol. Aliquots (200 μl) of the cell suspension were withdrawn at various times, denatured in 400 μl of an ice-cold mixture of methanol/chloroform (1:3) and stirred on a Vortex mixer for 30 sec. After addition of another 600 μl chloroform the samples were stirred again and then centrifuged to separate the organic from the aqueous phase. Samples (50 μl) of the aqueous phase were applied to silica gel 60 thin layer chromatography foils (Merck, Darmstadt, F.R.G.), which were developed in *n*-butanol: 0.01 M tris in water–propionic acid (50:10:0.7) [12, 21]. The R_f-values were 0.67 for 1-naphthyl sulfate, 0.22 for 1-naphthyl glucuronide and 0.92 for 1-naphthol. The radioactive spots containing the conjugates were cut out and placed into vials for liquid scintillation counting.

Cellular protein was determined by a modified Biuret method [22]. The experiments were performed with seven different cell preparations. The data show typical results from a single preparation.

RESULTS

Time course of sulfate disposition

The time courses of sulfate uptake, incorporation into acid-precipitable material and sulfate conjugation were measured in proximal and distal enterocytes at a sulfate concentration of 1 mM (Fig. 1). Proximal and distal enterocytes both took up sulfate over 20–30 min (Fig. 1A); the steady state concentrations of sulfate amounted to about 6.4 nmole sulfate/mg protein in proximal enterocytes, whereas distal cells took up about 13 nmole sulfate/mg protein. Since the aqueous cellular volume of enterocytes amounts to 4.2 μl/mg protein [16], this would correspond to free intracellular sulfate concentrations of about 1.5 and 3.1 mM. Incorporation of sulfate into acid-precipitable material (Fig. 1B), as well as formation of 1-naphthyl sulfate (Fig. 1C), proceeded at almost constant rates for 30 min. Despite the lower sulfate levels in proximal cells, these metabolic reactions were faster in proximal enterocytes than in distal enterocytes.

Effect of sulfate concentration

When sulfate uptake was analysed over a wide range of extracellular sulfate concentrations (0.05–5 mM) a linear dependence of the intracellular steady state concentrations of sulfate on the extracellular levels of the inorganic anion was observed (Fig. 2). At all sulfate concentrations tested, an equilibrium was reached within 30 min and the anion was less accumulated by proximal than by distal cells (Fig. 2).

1-Naphthol sulfation was dependent on the concentration of the inorganic anion, being low in the absence of extracellular sulfate, but markedly stimulated by the addition of sulfate to the medium (Fig. 3). While the formation of 1-naphthyl sulfate was saturated at about 1 mM extracellular sulfate in distal cells, sulfation rates increased up to 3 mM sulfate in proximal enterocytes. At a concentration of 3 mM, rates of 1-naphthol sulfation were more than three times higher in proximal as compared to distal enterocytes.

DISCUSSION

Orally administered sulfate is absorbed by the small intestine, and transiently elevates the serum

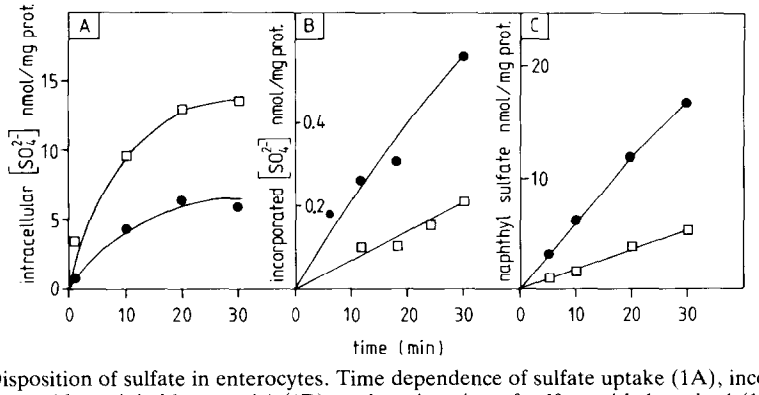

Fig. 1. Disposition of sulfate in enterocytes. Time dependence of sulfate uptake (1A), incorporation of sulfate into acid-precipitable material (1B), and conjugation of sulfate with 1-naphtol (1C). Cells (1–2 mg protein/ml) from proximal (●) or distal (□) small intestine were incubated at 37° in standard medium containing 1 mM Na_2SO_4. Uptake of sulfate and incorporation of sulfate were determined by incubating the cells in the presence of [^{35}S]O_4^{2-}. Formation of 1-naphthyl sulfate was determined after preincubation of the cells for 25 min. 1-[^{14}C]-naphthol was added at a concentration of 100 μM.

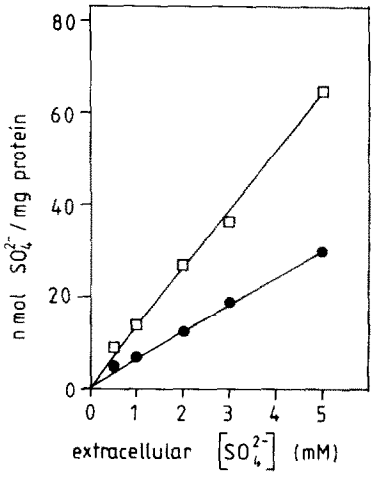

Fig. 2. Effect of extracellular sulfate on intracellular sulfate levels. Cells (1–2 mg protein/ml) from proximal (●) or distal (□) small intestine were incubated together with various concentrations of $[^{35}S]O_4^{2-}$. Intracellular sulfate concentrations were determined at various times. Curves represent the intracellular sulfate levels after 25 min, when intracellular sulfate concentrations were constant.

sulfate levels [2]. Studies performed with intestinal sacs indicate that this transport process is Na^+-dependent [23], proceeds against a concentration gradient [24] and exhibits a higher transport activity in ileum than in jejunum [25, 26]. The present data show that isolated enterocytes of the guinea pig have preserved the ability to accumulate sulfate. At all sulfate concentrations jejunal cells accumulated the anion less (about 1.5-fold) than ileal cells (about 3.1-fold). This difference may reflect the higher transport capacity in the ileum [25, 26].

Despite their lower sulfate concentrations proximal cells sulfated 1-naphthol at higher rates than distal cells; this distribution of the sulfation of phenols is in good accordance with the data of others [17, 27] and may be best explained by assuming decreasing concentrations of phenol sulfotransferases from the jejunum to the ileum. Interestingly, sulfation of macromolecules (probably glycosaminoglycans) also proceeds faster in proximal cells. Besides an inhomogenous distribution of sulfotransferases involved in the metabolism of macromolecules, the present data may also be explained by assuming different amounts of acceptor molecules in proximal and distal cells.

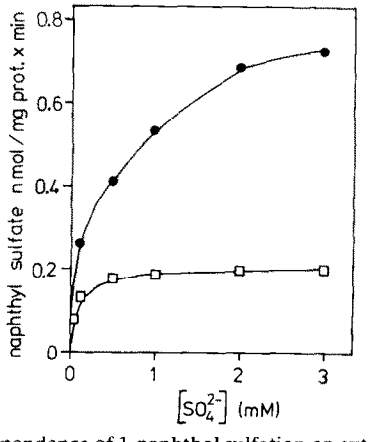

Fig. 3. Dependence of 1-naphthol sulfation on extracellular sulfate concentrations. Cells (1–2 mg protein/ml) from proximal (●) and distal (□) small intestine were preincubated for 25 min in standard medium, containing various sulfate concentrations. Then 1-[^{14}C]-naphthol (100 μM) was added and rates of 1-naphthol sulfation were determined within the following 7 min.

The rate of 1-naphthol sulfation depended upon the extracellular sulfate concentration. This indicates that guinea-pig enterocytes efficiently activate inorganic sulfate of the medium to adenosine 3'-phosphate 5'-sulfatophosphate (PAPS). With increasing extracellular sulfate concentrations 1-naphthol conjugation became saturated at about 3 mM sulfate in proximal cells, but at 1 mM sulfate in distal cells. Based on intracellular sulfate concentrations, however, half-saturation of the reaction occurs in both cell types between 0.5 and 1 mM sulfate. Thus the apparent K_m-value of the sulfate activating system is in a similar range to that in rat liver cells [12].

In view of our data suggesting saturation of 1-naphthol sulfation at about 3 mM sulfate in proximal enterocytes, the serum sulfate level of 0.85 mM in the guinea pig [28] may be below the level required for maximal sulfation of phenols *in vivo*. It therefore appears possible that the increased sulfation of tyramine and salicylamide, observed after coadministration with sulfate or cysteine, occurs largely in the upper small intestine [6, 7].

In summary, the jejunum is the part of the small intestine with the highest sulfation capacity. However, sulfation occurs at sub-optimal rates, being restricted by low intracellular sulfate levels. Thus orally administered sulfate may increase sulfation of environmental or nutritional phenols, and thereby enhance their detoxication before their entrance into the circulation.

Acknowledgements—We thank Professor Dr H. Greim for the critical discussion of the manuscript. The expert secretarial help of Ms U. Welscher is gratefully acknowledged.

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