Sodium Ion-Coupled Uptake of Taurocholate by Intestinal Brush-Border Membrane Vesicles

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The uptake of taurocholate was studied in membrane vesicles isolated from brush borders of hamster jejunum and ileum. When an extra- to intra-vesicular gradient of Na^+ ions was present ileal vesicles took up 10 times more taurocholate than did jejunal vesicles. Accumulation of taurocholate by ileal vesicles was transient and was due to transport of this bile salt into an osmotically active intravesicular space rather than simple binding. Uptake of taurocholate was specifically dependent on Na^+ ions; NaCl and Na_2SO_4 were capable of supporting accumulation, whereas KCl , LiCl and mannitol were not. Na^+ -coupled uptake of taurocholate into ileal vesicles was inhibited by other trihydroxy bile salts, by preloading the vesicles with Na^+ and by simultaneous flow of glucose into the vesicles. Similarly, vesicular uptake of glucose was inhibited by simultaneous uptake of taurocholate. These results demonstrated that brush-border membrane vesicles prepared from ileum possess an Na^+ -coupled co-transport system for taurocholate that is similar to the active bile-salt transport system present in the intact ileum.

Previous physiological studies utilizing a variety of techniques, *in vivo* and *in vitro*, have shown that bile salts are primarily absorbed by an active-transport system that is localized in the distal portion of the small intestine, the ileum (Holt, 1964; Glassner *et al.*, 1965; Dietschy *et al.*, 1966; Schiffer *et al.*, 1972; Faust & Wu, 1966). This active-transport system is dependent on the presence of Na^+ ions in the lumen of the intestine (Holt, 1964). Replacement of Na^+ ions with other cations results in a severe decrease in active intestinal absorption of bile salts (Playoust & Isselbacher, 1964). Because of the complexity of most of the intestinal preparations used so far, the nature of the interaction between Na^+ cation and the bile-salt transport system has remained obscure.

Many of the drawbacks inherent in utilizing relatively intact tissue preparations to study the interaction between Na^+ ion and the bile-salt transport system can be avoided by using isolated plasma-membrane vesicles. These vesicles afford the opportunity to study transport properties without interference by intracellular metabolism or compartmentation and under conditions in which the composition of the medium bathing both surfaces of the membrane can be controlled. Since the development of procedures to isolate membrane vesicles from intestinal epithelial cells, this simplified system has proved to be of considerable value in studying the mechanisms involved in intestinal absorption of a

variety of organic solutes (Murer & Hopfer, 1974; Sigrist-Nelson & Hopfer, 1974; Sigrist-Nelson *et al.*, 1975).

The object of the present investigation was to utilize intestinal brush-border membrane vesicles as a system for studying intestinal absorption of bile salts. The results presented here demonstrate that ileal brush-border membranes possess an Na^+ -coupled transport system for the bile salt taurocholate and that the characteristics of this transport system are similar to those reported for the intact ileum.

Methods

Brush borders were prepared from hamster small intestine by a modification (Faust *et al.*, 1967) of the methods of Miller & Crane (1961) and Harrison & Webster (1964). Jejunal and ileal brush borders were obtained from the most proximal and most distal 10 cm of the small intestine respectively. Unless otherwise stated, all steps in the preparative procedure were carried out at 2–4°C.

An essentially pure preparation of jejunal or ileal brush borders from six to eight hamsters was suspended in approx. 20 ml of a solution containing 400 mM-mannitol, 10 mM- MgCl_2 and 20 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] adjusted to pH 7.5 with Tris. These intact brush borders were disrupted by freezing at –78°C for 20 min followed by thawing in a 37°C water bath. The disrupted brush borders were suspended with ten strokes by hand in a glass homogenizer with a

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loose-fitting Teflon pestle. The suspension was brought to 40 ml with the above medium and centrifuged at 3000g for 10 min. This centrifugation yielded a somewhat cloudy supernatant that was saved and a whitish pellet. The pellet was resuspended in mannitol medium, subjected to the freeze-thaw procedure, and centrifuged at 3000g for 10 min. This centrifugation yielded a slightly turbid supernatant and a tan pellet that, on examination by electron microscopy, appeared to consist primarily of partially disrupted brush borders and fibrous material derived from the core filaments and terminal web. The two supernatants from the 3000g centrifugations were combined, again centrifuged at 3000g for 10 min to remove any residual debris and finally centrifuged at 30000g for 60 min to obtain the brush-border membrane fraction. These membranes were suspended in a medium consisting of 400 mM-mannitol and 20 mM-Hepes/Tris buffer, pH 7.5.

Purification of the brush-border membranes was demonstrated by the more than 3-fold increase, as compared with isolated intact brush borders, in the specific activity of the membrane marker enzymes (Eichholz, 1967) sucrase (EC 3.2.1.26) and maltase (EC 3.2.1.20). Electron photomicrographs of the brush-border membrane fraction showed that it was homogeneous and composed primarily of membrane-bound vesicles.

Unless otherwise stated in the text, the following procedures were used to measure uptake of taurocholate by the brush-border membrane vesicles. Uptake was initiated by addition of 1 vol. of vesicles (approx. 2 mg of protein/ml) to 3 vol. of incubation medium to give the following final concentrations: 150 mM-NaCl, 100 mM-mannitol, 20 mM-Hepes/Tris buffer, pH 7.5, and 50 μ M-[14 C]taurocholate (2.6 μ Ci/ml). Incubation was carried out at 25°C in an oscillating water bath. At various times a sample (0.1 ml) of the incubation mixture was removed and uptake was terminated by diluting the sample 20-fold in ice-cold medium identical in composition with the incubation medium, but without the labelled bile salt. The diluted suspension was filtered on 0.45 μ m Millipore filters that retained more than 95% of the vesicles. The filters were washed twice with 2 ml of ice-cold medium, dissolved and counted in Aquasol (New England Nuclear Corp., Boston, MA, U.S.A.) in a liquid-scintillation spectrophotometer. For each experiment the amount of bile salt retained by the filters themselves was determined and subtracted from that retained when vesicles were present. Results are expressed as the amount of bile salt taken up per mg of vesicle protein. Protein was determined by the method of Lowry *et al.* (1951).

Sodium [24 - 14 C]taurocholate (sp. radioactivity 52 mCi/mmol) was purchased from New England Nuclear, and D-[U- 14 C]glucose (sp. radioactivity 284 mCi/mmol) was from Amersham/Searle (Arling-

ton Heights, IL, U.S.A.). Sodium glycocholate and sodium cholate were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All chemicals used were reagent grade or 'ultrapure' grade and were made up in triple-distilled water.

Results

The time course for uptake of taurocholate by brush-border membrane vesicles isolated from two different regions of the small intestine is shown in Fig. 1. Vesicles isolated from ileum rapidly took up taurocholate. Taurocholate uptake was transient reaching a maximum accumulation of approx. 2 nmol/mg of protein in 1–3 min and then decreasing to approx. 0.5 nmol/mg of protein over the next 30–60 min. In contrast, vesicles isolated from jejunal mucosa did not exhibit rapid uptake of taurocholate. In fact, the maximum amount of taurocholate taken up by jejunal vesicles was only about one tenth of that observed with ileal vesicles.

To distinguish between binding of taurocholate to the vesicle membrane and transport of this bile salt into the intravesicular space, uptake of taurocholate was measured as a function of the osmolarity of the incubation medium. Increasing or decreasing the osmolarity of the incubation medium and the resultant change in the volume of the intravesicular space should have a significant effect on transport, while causing little or no change in binding. As shown in Fig. 2, uptake of taurocholate by ileal vesicles was

Fig. 1. Uptake of taurocholate by brush-border membrane vesicles from ileum and jejunum

Uptake was initiated at zero time by addition of vesicles to a medium containing (final concn.) 100 mM-mannitol, 150 mM-NaCl, 0.05 mM-taurocholate and 20 mM-Hepes/Tris buffer, pH 7.5. Values are expressed as the mean $\pm$ S.D. for four experiments. $\bullet$, Ileum; $\blacksquare$, jejunum.

strongly dependent on the osmolarity of the incubation medium. The amount of taurocholate bound to the membranes, as estimated by extrapolation to infinite osmolarity, represented less than 7% of that taken up by vesicles at the highest osmolarity tested.

Fig. 3 shows the dependency of vesicular taurocholate uptake on the concentration of NaCl in the

Fig. 2. Effect of medium osmolarity on uptake of taurocholate by ileal vesicles

Vesicles were incubated in a medium containing the following: 50mM-NaCl, 10mM-Hepes/Tris buffer, pH 7.5, 0.025mM-taurocholate and sufficient mannitol to give the indicated osmolarity. The incubation time was 2min.

Fig. 3. Effect of NaCl concentration on the uptake of taurocholate by ileal vesicles

Vesicles were incubated in mannitol medium containing 0.05mM-taurocholate and the indicated concentration of NaCl, which replaced mannitol iso-osmotically. The amount of taurocholate taken up in 1 min is shown and expressed as the mean $\pm$ s.d. for four experiments.

incubation mixture. Increasing the NaCl concentration from 12.5 to 100mM resulted in an approx. 4-fold increase in the amount of taurocholate taken up by ileal vesicles in 1 min. Raising the NaCl concentration further, to 150mM, caused only a small additional stimulation.

As shown in Table 1 uptake of taurocholate by ileal vesicles was specifically dependent on a transmembrane gradient of Na⁺ ions. Uptake was initiated by addition of vesicles, which had been prepared in an Na⁺-free medium, to a solution containing this ion, thus establishing a transmembrane Na⁺ gradient. Extra- to intra-vesicular gradients of NaCl and Na₂SO₄ produced a dramatic increase in taurocholate accumulation when compared with that obtained in mannitol medium alone. When the Na⁺ gradient was eliminated by preloading the vesicles with NaCl, so that the intra- and extra-vesicular concentrations of Na⁺ were the same, taurocholate accumulation was decreased to the value observed in the absence of this cation. Imposition of transmembrane gradients of KCl or LiCl did not result in significant stimulation of accumulation.

As shown in Table 2, uptake of taurocholate by ileal vesicles was inhibited when a second trihydroxy bile salt was present. Increasing concentrations of glycocholate or cholate caused a progressive decrease in the amount of taurocholate accumulated. For instance, as little as 0.05mM-glycocholate caused a 32% inhibition of taurocholate accumulation, whereas 0.1mM-cholate resulted in almost 60% inhibition.

As shown in Table 3, vesicular accumulation of taurocholate was also inhibited by D-glucose, a hexose that is accumulated by jejunal and ileal vesicles (Table 3; Murer & Hopfer, 1974; Hopfer *et al.*, 1976). The inhibitory effect of glucose on vesicular taurocholate accumulation was essentially blocked by phlorrhizin, a potent competitive inhibitor of Na⁺-dependent D-glucose transport (Alvarado & Crane,

Table 1. Effectiveness of various ions in stimulating taurocholate accumulation by ileal vesicles

The amount of taurocholate accumulated in 1 min is shown and expressed as the mean $\pm$ s.d. for four experiments.

Addition	Concn. (mM)	Taurocholate accumulation (nmol/mg of protein)
Mannitol	300	0.06 $\pm$ 0.04
NaCl	150	2.84 $\pm$ 0.23
Na ₂ SO ₄	75	2.52 $\pm$ 0.25
KCl	150	0.22 $\pm$ 0.11
LiCl	150	0.18 $\pm$ 0.09
NaCl*	150	0.06 $\pm$ 0.05

* Vesicles were preloaded with NaCl by incubation in mannitol medium containing 150mM-NaCl for 30min at 25°C.

Table 2. *Inhibition of vesicular taurocholate accumulation by glycocholate and cholate*

The amount of taurocholate accumulated in 1 min is shown and expressed as the mean $\pm$ s.d. for four experiments.

Inhibitor	Concn. (mM)	Taurocholate accumulation (nmol/mg of protein)	Percentage of control
None	—	2.05 $\pm$ 0.12	100
Glycocholate	0.05	1.40 $\pm$ 0.20	68
Glycocholate	0.10	0.99 $\pm$ 0.12	48
Glycocholate	0.25	0.53 $\pm$ 0.07	26
Glycocholate	0.50	0.36 $\pm$ 0.05	18
Glycocholate	1.00	0.05 $\pm$ 0.04	2
Cholate	0.10	0.85 $\pm$ 0.10	41
Cholate	1.00	0.06 $\pm$ 0.04	3

1962). In the absence of glucose, phlorrhizin had little or no effect on the uptake of taurocholate.

The effect of taurocholate on accumulation of D-glucose by ileal and jejunal vesicles is also shown in Table 3. Taurocholate (1 mM) caused a 49% inhibition of D-glucose accumulation by ileal vesicles. In contrast, taurocholate did not significantly decrease D-glucose uptake by vesicles isolated from jejunum.

Discussion

Several laboratories have utilized membranes isolated from intestinal epithelial cells to investigate the mechanisms responsible for intestinal absorption of bile salts. Wilson & Treanor (1977) isolated brush-border membranes from rat intestine and examined uptake of several bile salts by this preparation. Although the membranes did take up bile salts, the characteristics of uptake by the brush-border membranes were quite different from those for intestinal absorption. For instance, although the kinetic parameters of bile-salt absorption by the intact jejunum and ileum differ considerably (Glassner *et al.*, 1965; Schiffer *et al.*, 1972), those of the isolated jejunal and

ileal membranes were essentially the same. Furthermore, uptake of taurocholate by the isolated membranes was not inhibited by structurally similar bile salts, boiling or treatment with trypsin and was similar in sonicated brush borders and phospholipid liposomes. Taken together these results suggested that uptake of bile salts by the brush-border membranes prepared by Wilson & Treanor (1977) represented partitioning of these amphiphilic substances between the aqueous medium and the membrane lipids rather than ligand-specific binding to receptors or transport of the bile salts.

Recently, Lack *et al.* (1977) published a short report comparing the uptake of taurocholate by proximal (jejunal) and distal (ileal) brush-border membrane vesicles isolated from guinea pig. They showed that, when NaCl was added to vesicle suspensions, ileal vesicles took up more taurocholate than did jejunal vesicles. Whether stimulation of taurocholate uptake after addition of NaCl was specifically dependent on Na⁺ and/or Cl⁻ or whether other cations and anions would be equally effective was not determined. Furthermore, no attempt was made to ascertain whether the uptake represented binding or transport.

Critical to the interpretation of results obtained in studies of the uptake of substrates by vesicle preparations is the distinction between binding and transport. In the present study the relative contributions of binding and transport to the observed vesicular uptake of taurocholate was assessed by measuring taurocholate uptake as a function of the osmolarity of the incubation medium. The results demonstrated that binding could account for less than 10% of the uptake and that uptake of taurocholate by ileal vesicles was due primarily to transport of this bile salt into the osmotically active space within the vesicles.

Uptake of taurocholate by ileal vesicles was dependent on an extravesicular to intravesicular gradient of Na⁺ ions. This finding suggests that this bile salt may be transported via an Na⁺ ion and

Table 3. *Mutual inhibition of vesicular accumulation of taurocholate and glucose*

Uptake was initiated by addition of vesicle to mannitol medium containing substrate (0.05 mM-taurocholate or 0.02 mM-glucose), inhibitor and 150 mM-NaCl. Incubation time was 1 min when the substrate was taurocholate and 30 s when it was glucose.

Source of vesicles	Substrate	Inhibitor (mM)	Substrate accumulation (nmol/mg of protein)
Ileum	Taurocholate	None	2.40 $\pm$ 0.25
Ileum	Taurocholate	Glucose (1.0)	1.49 $\pm$ 0.21
Ileum	Taurocholate	Phlorrhizin (0.1)	2.35 $\pm$ 0.17
Ileum	Taurocholate	Glucose (1.0) + phlorrhizin (0.1)	2.31 $\pm$ 0.18
Ileum	Glucose	None	0.24 $\pm$ 0.02
Ileum	Glucose	Phlorrhizin (0.1)	0.01 $\pm$ 0.01
Ileum	Glucose	Taurocholate (1.0)	0.13 $\pm$ 0.01
Jejunum	Glucose	None	0.60 $\pm$ 0.07
Jejunum	Glucose	Taurocholate (1.0)	0.56 $\pm$ 0.04

organic solute co-transport system similar to that described in the sodium-gradient hypothesis for active intestinal absorption of sugars (Crane, 1962) and amino acids (Schultz & Curran, 1970). According to this hypothesis an extracellular to intracellular gradient of Na^+ ions provides the energy for active transport of organic solutes. Movement of Na^+ ion down its electrochemical gradient from the lumen across the brush-border plasma membrane into the cell would be coupled with the translocation of organic solute in the same direction, resulting in intracellular accumulation of the solute.

Support for the notion of co-transport of Na^+ and bile salts can be derived from the results demonstrating mutual inhibition between glucose and taurocholate for uptake into ileal vesicles. This inhibition was not due to competition for a common carrier or transport site. Thus phlorrhizin, which inhibits uptake of glucose by competing for the sugar-transport site (Alvarado, 1967), did not inhibit taurocholate uptake by ileal vesicles. Furthermore, uptake of glucose by jejunal vesicles, which possess a transport system for this sugar (Murer & Hopfer, 1974), was not inhibited by taurocholate. The finding that taurocholate inhibition of glucose uptake was restricted to ileal vesicles and the fact that phlorrhizin, a sugar-transport inhibitor, blocked the effects of glucose on taurocholate uptake, indicate that mutual inhibition between glucose and taurocholate was dependent on the simultaneous transport of these organic solutes into the vesicles.

The inhibitory interaction between the flows of glucose and taurocholate was probably due to competition for the Na^+ gradient that is required for rapid uptake of these organic solutes (present paper and Murer & Hopfer, 1974). Previous investigations (Murer & Hopfer, 1974) have demonstrated that uptake of glucose by brush-border membrane vesicles is mediated via a co-transport system that transports both glucose and Na^+ from the extravesicular medium to the intravesicular space. In addition, it has been shown (Murer *et al.*, 1975) that co-transport of one organic solute with Na^+ into the vesicles results in accelerated dissipation of the transmembrane electrochemical gradient for Na^+ and thereby inhibits Na^+ -coupled uptake of other organic solutes. Thus the inhibitory effect of glucose on taurocholate uptake into ileal vesicles can be explained on the basis of accelerated dissipation of the Na^+ gradient. The fact that the flow of taurocholate into the vesicles inhibited Na^+ -coupled uptake of glucose suggests a similar mechanism of inhibition and indicates that transport of this bile salt, like that of glucose, occurs via an Na^+ -coupled co-transport system.

Active intestinal absorption of bile salts is due to the operation of a specific active-transport system, which is restricted to the ileum (Holt, 1964; Glassner *et al.*, 1965; Dietschy *et al.*, 1966; Schiffer *et al.*,

1972; Faust & Wu, 1966). This system was apparently preserved in isolated ileal vesicles. Thus these vesicles, in contrast with jejunal vesicles, rapidly transported taurocholate across the vesicle membrane, resulting in transient intravesicular accumulation or 'overshoot' of this bile salt. Furthermore, similarities between the taurocholate transport characteristics of the ileal vesicles and those of the intact intestine (i.e. specific dependence on Na^+ ions and inhibition by other structurally related actively transported bile salts) indicate that the Na^+ -coupled taurocholate-transport system, which is present in ileal vesicles, is involved in active intestinal absorption of bile salts.

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