Bile-salt inhibition of sodium ion-coupled D-glucose and L-alanine accumulation by brush-border-membrane vesicles from hamster jejunum

Robert C. BEESLEY*

Department of Physiology, Wayne State University of Medicine, Detroit, MI 48201, U.S.A.

and Robert G. FAUST

*Department of Physiology, University of North Carolina School of Medicine,
Chapel Hill, NC 27514, U.S.A.*

(Received 9 January 1980)

The effects of bile salts on Na⁺-coupled accumulation of D-glucose and L-alanine by brush-border-membrane vesicles isolated from hamster jejunum were investigated. The approximate percentage inhibition of Na⁺-coupled D-glucose accumulation produced by various bile salts at a concentration of 1 mM were: deoxycholate and chenodeoxycholate, 60%; glycine and taurine conjugates of deoxycholate and chenodeoxycholate, 40–50%; lithocholate, 45%; cholate and its glycine and taurine conjugates, <10%. Inhibition of Na⁺-coupled accumulation of D-glucose was rapid, reversible and not due to dissolution of the vesicles. Na⁺-coupled accumulation of L-alanine was also inhibited by deoxycholate. Deoxycholate but not cholate enhanced (1) the rate of Na⁺ influx, (2) the rate of influx of D-glucose and L-alanine in the absence of a Na⁺ gradient and (3) the rate of efflux of D-glucose and L-alanine from vesicles preloaded with this sugar or amino acid. Deoxycholate-stimulated efflux of D-glucose was not blocked by phlorizin, which completely prevented efflux in the absence of this bile salt. These results suggest that selected bile salts inhibit Na⁺-coupled accumulation of D-glucose and L-alanine by enhancing the rate of dissipation of the Na⁺ gradient required for substrate accumulation. In addition, bile salts may also decrease D-glucose and L-alanine accumulation by increasing the rate of efflux of these substrates across the brush-border plasma membrane.

Previous investigations utilizing relatively intact tissue preparations have demonstrated that bile salts inhibit active intestinal absorption of sugars and amino acids. This inhibition has been attributed to a variety of processes. For instance it has been suggested that bile salts inhibit absorption by directly inhibiting specific organic-solute transport systems located on the luminal or brush-border plasma membrane of the enterocyte (Frizzell & Schultz, 1970; Burke *et al.*, 1976). Alternatively, it has been suggested that inhibition of organic-solute transport may be due to an elevated intracellular Na⁺ concentration (i.e. decreased Na⁺ gradient) as a result of (1) bile salt-induced decreases in intracellular ATP concentrations (Faust & Wu, 1965) and/or (2) direct inhibition of the (Na⁺ + K⁺)-dependent ATPase located in the basal-lateral plasma membrane (Guiraldes *et al.*, 1975).

The objective of the present study was to

* To whom reprint requests should be sent.

determine whether bile salt-induced inhibition of active intestinal absorption could be explained on the basis of the interactions between bile salts and the brush-border plasma membrane. For this purpose we utilized membrane vesicles derived from brush borders of hamster jejunum. These isolated membrane vesicles, which actively accumulate sugars (Hopfer & Murer, 1974) and amino acids (Sigrist-Nelson *et al.*, 1975) in response to an imposed transmembrane Na⁺ gradient, afford the opportunity of investigating brush-border-membrane transport systems without the complications inherent in more intact tissue preparations.

The results of this investigation demonstrate that selected bile salts inhibit Na⁺-coupled vesicular accumulation and, by inference, active intestinal absorption of D-glucose and L-alanine by accelerating the rate of dissipation of the transmembrane Na⁺ gradient. In addition the bile salt-induced increase in the permeability of the brush-border plasma mem-

brane to D-glucose and L-alanine and the resultant enhancement in the rate of efflux may also contribute to the observed decrease in the amounts of accumulation of these solutes.

Methods

Jejunal brush borders and brush-border-membrane vesicles were prepared by using the freeze-thaw procedure described previously (Beesley & Faust, 1979).

Unless otherwise stated in the text, the following procedures were used to measure Na⁺-coupled vesicular accumulation of D-glucose and L-alanine. To see more clearly the stimulatory effect of Na⁺ on sugar and amino acid accumulation above the equilibrium value the vesicles were equilibrated with these substrates before addition of NaCl. Equilibration was accompanied by incubating brush-border-membrane vesicles (approx. 1 mg of protein/ml) for 20 min at 23°C in 0.5 ml of a medium consisting of 400 mM-mannitol, 20 μ M-D-[¹⁴C]glucose (5.68 μ Ci/ml), 20 μ M-D-[³H]mannose (15 μ Ci/ml) and 20 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] adjusted to pH 7.5 with Tris. When amino-acid transport was studied, glucose was replaced by 15 μ M-L-[¹⁴C]alanine (2.46 μ Ci/ml). Uptake was subsequently initiated by addition of 0.5 ml of a solution identical in composition with the equilibration medium, but containing 0–1.5 mM-bile salt and 200 mM-NaCl, which replaced the 400 mM-mannitol.

To measure vesicular uptake of D-glucose and L-alanine in the absence of an Na⁺ gradient, vesicles were pre-equilibrated with sodium by incubation for 30 min at 23°C in a medium containing 100 mM-NaCl, 200 mM-mannitol and 20 mM-Hepes/Tris buffer, pH 7.5. Uptake was subsequently initiated by addition of D-[¹⁴C]glucose or L-[¹⁴C]alanine to give final concentrations of 45 and 35 μ M respectively.

When measuring uptake of Na⁺ the incubation medium consisted of 200 mM-mannitol, 100 mM-²²NaCl (60 μ Ci/ml) and 20 mM-Hepes/Tris buffer, pH 7.5. Cholate or deoxycholate, when present, were at a concentration of 1 mM.

For efflux studies vesicles were pre-loaded with sugar or amino acid by incubation for 30 s in a medium containing 100 mM-mannitol, 150 mM-NaCl, 20 μ M-D-[¹⁴C]glucose or 15 μ M-L-[¹⁴C]alanine and 20 mM-Hepes/Tris buffer, pH 7.5. Efflux was initiated by addition of 0.1 ml of the pre-loaded vesicles to 2 ml of a medium containing 400 mM-mannitol and 20 mM-Hepes/Tris buffer, pH 7.5. Bile salts, when present in these studies, were at a concentration of 1 mM.

The amount of hexose, amino acid or Na⁺ present within the vesicles was determined by using a modification (Beesley & Faust, 1979) of the rapid

Millipore filtration technique of Hopfer *et al.* (1973). When Na⁺ gradient-dependent uptake of sugar and amino acid was being measured, the stopping and wash solutions were identical with the incubation medium, but lacked the labelled substrate. To measure Na⁺ uptake and substrate uptake in the absence of an Na⁺ gradient, the stopping and wash solution used was 0.3 M-KCl. The results are expressed as the amount of substrate taken up per mg of vesicle protein. Protein was determined by the method of Lowry *et al.* (1951).

D-[U-¹⁴C]Glucose (sp. radioactivity 284 Ci/mol), D-[1-³H]mannose (sp. radioactivity 750 Ci/mol), L-[U-¹⁴C]alanine (sp. radioactivity 164 Ci/mol) and ²²NaCl (carrier-free) were purchased from New England Nuclear (Boston, MA, U.S.A.). Sodium salts of the bile acids were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All chemicals used were reagent or 'ultrapure' grade and were made up in deionized water.

Results

Bile salt inhibition of Na⁺-coupled D-glucose accumulation

The effect of deoxycholate on vesicular uptake of D-glucose, which is actively transported by the small intestine, and D-mannose, which is not actively transported, is shown in Fig. 1. In the absence of bile salt, addition of NaCl to vesicles equilibrated with these sugars resulted in rapid uptake of additional

Fig. 1. Effect of deoxycholate on D-glucose and D-mannose uptake by brush-border-membrane vesicles. Vesicles were pre-equilibrated with D-glucose and D-mannose. At zero time (indicated by the arrow) uptake was initiated by addition of either NaCl alone (○ and □) or NaCl plus 1 mM-deoxycholate (● and ■) and the time course of vesicular uptake of D-glucose (○ and ●) and D-mannose (□ and ■) was determined. Values are expressed as means $\pm$ S.D. for four experiments.

D-glucose but not D-mannose into the vesicles. Accumulation of D-glucose was transient, reaching a maximum in 30–90s, and then decreasing to the original equilibrium value over the next 30min. Although some Na⁺-stimulated transient accumulation of D-glucose was also observed when the incubation mixture contained deoxycholate, this bile salt did decrease both the rate of uptake and the maximum accumulation. Thus, in the presence of 1 mM-deoxycholate the amount of D-glucose accumulated by the vesicles in 30s was only 33% of that observed in the absence of this bile salt. It should be noted that deoxycholate did not significantly alter the final equilibrium concentrations of D-glucose and D-mannose retained by the vesicles.

The concentration dependence of deoxycholate inhibition of D-glucose accumulation is shown in Fig. 2. Increasing the concentration of deoxycholate in the incubation medium resulted in a progressive decrease in the amount of D-glucose accumulated by the vesicles. For instance, 0.25 mM-deoxycholate inhibited D-glucose accumulation by 28%, whereas 1.5 mM-deoxycholate resulted in almost 90% inhibition.

Deoxycholate inhibition of vesicular D-glucose accumulation was both rapid and reversible (Figs. 3a and 3b). Thus, the magnitude of inhibition of accumulation obtained when deoxycholate was added to the vesicles simultaneously with the initiation of uptake was identical with that observed when vesicles were preincubated with deoxycholate

(Fig. 3a). Furthermore, vesicles preincubated in a relatively high concentration of deoxycholate and subsequently diluted to obtain a lower deoxycholate concentration accumulated D-glucose to essentially the same extent as vesicles exposed only to the lower concentration of the bile salt (Fig. 3b).

Deoxycholate was not the only bile salt that inhibited vesicular accumulation of D-glucose. A comparison of the effects of a variety of bile salts (1 mM) on D-glucose accumulation is shown in Table 1. Unconjugated dihydroxy bile salts, deoxycholate and chenodeoxycholate, were the most potent inhibitors, giving approx. 60% inhibition of accumulation. The glycine and taurine conjugates of dihydroxy bile salts were slightly less effective, causing 40–50% inhibition of accumulation, where-

Fig. 2. Concentration dependence of deoxycholate inhibition of Na⁺-coupled D-glucose
Uptake was initiated by addition of NaCl and sufficient deoxycholate to give the indicated final concentration. The amount of D-glucose accumulated in 30s is shown and expressed as the mean $\pm$ s.d. for three experiments.

Fig. 3. Rapidity (a) and reversibility (b) of deoxycholate inhibition of Na⁺-coupled D-glucose accumulation by brush-border-membrane vesicles
In both (a) and (b), vesicles were preincubated for 10min at room temperature in a medium that contained D-glucose and the indicated initial concentration of deoxycholate. At the end of the preincubation period the vesicle suspension was diluted with a medium containing sufficient deoxycholate to obtain the indicated final concentration. In (a), uptake was initiated by addition of NaCl immediately after dilution. In (b), the incubation mixture was held at room temperature for an additional 5min before initiation of uptake. In all experiments the concentrations of vesicles, NaCl and D-glucose in the final incubation mixture were the same. The amount of D-glucose accumulated in 30s is shown and expressed as the mean $\pm$ s.d. for four experiments. Note that preincubation with deoxycholate does not increase its inhibitory effect (a) and that deoxycholate inhibition is reversible (cf. a and b).

Table 1. *Comparison of the effects of various bile salts on Na⁺-coupled D-glucose accumulation*

Uptake was initiated by addition of NaCl. The concentration of bile salt, when present, was 1 mM. The amount of D-glucose taken up in 30 s is shown and expressed as the mean $\pm$ S.D. for four experiments.

Bile salt	D-Glucose accumulation (pmol/mg of protein)	Inhibition (%)
None	612 $\pm$ 15	0
Cholate	568 $\pm$ 19	7
Glycocholate	608 $\pm$ 12	1
Taurocholate	556 $\pm$ 18	9
Deoxycholate	236 $\pm$ 18	61
Glycodeoxycholate	328 $\pm$ 10	46
Taurodeoxycholate	360 $\pm$ 22	41
Chenodeoxycholate	258 $\pm$ 22	57
Glycochenodeoxycholate	318 $\pm$ 30	48
Taurochenodeoxycholate	328 $\pm$ 19	46
Lithocholate	337 $\pm$ 20	45

as lithocholate, a monohydroxy bile salt, caused 45% inhibition. Trihydroxy bile salts, cholate and its glycine and taurine conjugates, were not very effective inhibitors and decreased D-glucose accumulation by less than 10%.

Deoxycholate inhibition of Na⁺-coupled L-alanine accumulation

As shown in Fig. 4 addition of NaCl to brush-border-membrane vesicles equilibrated with L-alanine resulted in a rapid transient accumulation of this amino acid. Deoxycholate inhibited both the rate of uptake and the maximum accumulation of L-alanine. In the presence of 1 mM-deoxycholate, the amount of L-alanine accumulated within the vesicles 45 s after initiation of uptake was only 44% of that in the absence of this bile salt.

Effect of cholate and deoxycholate on D-glucose and L-alanine uptake in the absence of an Na⁺ gradient

In the absence of an Na⁺ gradient, uptake of D-glucose and L-alanine results from movement of these solutes down their concentration gradients rather than accumulation as occurs in the presence of an Na⁺ gradient. The effects of deoxycholate and cholate on uptake of D-glucose and L-alanine by vesicles previously equilibrated with NaCl are shown in Table 2. A potent inhibitor of Na⁺-coupled accumulation of D-glucose and L-alanine, deoxycholate stimulated movement of these solutes down their concentration gradients into the vesicles. In contrast cholate, an ineffective inhibitor of Na⁺-coupled accumulation of D-glucose, had little effect on uptake of D-glucose and L-alanine in the absence of an Na⁺ gradient.

Effect of deoxycholate on efflux of D-glucose and L-alanine

If, as suggested by the results presented above (Table 2), deoxycholate increases the permeability of the brush-border membrane to D-glucose and L-

Fig. 4. *Effect of deoxycholate on Na⁺-coupled L-alanine accumulation by brush-border-membrane vesicles* Vesicles were pre-equilibrated with L-alanine. At zero time (indicated by the arrow) uptake was initiated by addition of either NaCl alone (O) or NaCl plus sufficient deoxycholate to give a final concentration of 1 mM (●). Values are expressed as means $\pm$ S.D. for four experiments.

Table 2. *Effect of cholate and deoxycholate on uptake of D-glucose and L-alanine in the absence of a sodium ion gradient*

Vesicles were equilibrated with NaCl. Uptake was initiated by addition of vesicles to a medium containing D-glucose or L-alanine and the indicated bile salt (1 mM).

Substrate	Addition	Substrate uptake (pmol/mg of protein)	
		10 s	30 min
D-Glucose	None	24.9	55.8
D-Glucose	Deoxycholate	38.0	53.3
D-Glucose	Cholate	25.3	56.4
L-Alanine	None	24.3	42.3
L-Alanine	Deoxycholate	33.6	44.7
L-Alanine	Cholate	22.4	40.4

Table 3. *Effect of deoxycholate on efflux of D-glucose and L-alanine from vesicles*

Vesicles were pre-loaded with substrate (D-glucose or L-alanine). Efflux was initiated by addition of 1 vol. of vesicles to 20 vol. of substrate-free medium containing the indicated additions. When present the bile salt concentration was 1 mM and the phlorizin concentration was 0.1 mM. The amount of substrate retained by the vesicles 0, 15 or 30 s after initiation of efflux is shown and expressed as the mean $\pm$ S.D. for four experiments.

Substrate	Additions	Time (s)	Substrate retained	
			(pmol/mg of protein)	(%)
D-Glucose	None	0	382 $\pm$ 19	100
D-Glucose	None	30	122 $\pm$ 8	32
D-Glucose	Deoxycholate	30	55 $\pm$ 4	14
D-Glucose	Phlorizin	30	375 $\pm$ 16	98
D-Glucose	Phlorizin + deoxycholate	30	202 $\pm$ 10	53
L-Alanine	None	0	110 $\pm$ 8	100
L-Alanine	None	15	80 $\pm$ 3	73
L-Alanine	Deoxycholate	15	41 $\pm$ 4	37

Fig. 5. *Effect of cholate and deoxycholate on the uptake of Na⁺ by brush-border-membrane vesicles*

Uptake was initiated by addition of vesicles to media containing ²²NaCl without bile salt (Δ), with 1 mM-cholate (▼) or with 1 mM-deoxycholate (▲). Values are means for two determinations or means $\pm$ S.D. for three determinations and are expressed as percentage Na⁺ uptake. The Na⁺ uptake after 30 min in the absence of bile salts was taken as 100%.

released. Deoxycholate enhanced efflux so that approx. 85% of the D-glucose was released in 30 s. Similar bile salt stimulation of efflux was observed with vesicles pre-loaded with L-alanine. Phlorizin, a potent inhibitor of active intestinal D-glucose transport, essentially abolished D-glucose efflux. In the presence of deoxycholate, however, phlorizin caused only a partial decrease in D-glucose efflux.

Effect of cholate and deoxycholate on Na⁺ uptake

As shown in Fig. 5 incubation of vesicles in a medium containing cholate did not significantly alter the rate of uptake of Na⁺. In contrast inclusion of deoxycholate in the incubation medium resulted in a marked increase in the rate of Na⁺ uptake. Thus 45 s after initiation of uptake the amount of Na⁺ present in vesicles incubated with deoxycholate was approx. 1.8 times greater than that present in vesicles incubated without this bile salt. It is noteworthy that the final equilibrium Na⁺ content of the vesicles was essentially the same regardless of whether bile salts were present or not.

Discussion

One of the concerns when utilizing amphipathic compounds such as bile salts to study their effects on membrane transport phenomena is that any observed inhibition of transport might simply reflect dissolution of the membrane. In the present study bile-salt inhibition of Na⁺-coupled accumulation of D-glucose and L-alanine by brush-border-membrane vesicles cannot be accounted for by solubilization or disruption of the vesicle membrane. As was shown in the Results section, deoxycholate, a potent inhibitor of Na⁺-coupled D-glucose and L-alanine accumulation, did not significantly alter the final equilibrium amounts of D-glucose, L-alanine and Na⁺ retained by the vesicles. Calculations based on these equili-

alanine then this bile salt might be expected to enhance efflux of these solutes from the vesicles. To determine if this speculation was correct, the effect of bile salts on the efflux of substrates from brush-border-membrane vesicles was studied (Table 3).

Vesicles pre-loaded with D-glucose or L-alanine were diluted into sugar- and amino-acid-free medium with or without deoxycholate and re-incubated. Re-incubation resulted in rapid efflux of D-glucose from vesicles preloaded with this sugar. After only 30 s 68% of the vesicular D-glucose had been

brium values indicate that, in the absence of deoxycholate, the intravesicular volume was $1.21 \pm 0.04 \mu\text{l}/\text{mg}$ of protein (mean $\pm$ s.d.; $n = 7$), whereas that in the presence of 1 mM-deoxycholate was $1.19 \pm 0.07 \mu\text{l}/\text{mg}$ of protein (mean $\pm$ s.d., $n = 7$).

Our findings argue strongly against the notion that direct bile-salt inhibition of D-glucose and L-alanine transport systems are responsible for the decrease in Na⁺-coupled transport of these organic solutes. If that were the case, then inhibitory bile salts such as deoxycholate would not only inhibit Na⁺-gradient-dependent accumulation of D-glucose and L-alanine, but would also decrease the rate of passive influx and efflux of these organic solutes. Our results show that deoxycholate actually enhances the passive inward and outward flux of D-glucose and L-alanine.

Increased rates of passive flux of Na⁺, D-glucose and L-alanine across the brush-border membrane could result in inhibition of vesicular accumulation, and presumably active intestinal absorption, by two distinct mechanisms. The first, and perhaps the most important, mechanism would be the effect of increasing the passive influx of Na⁺. An increase in the rate of influx of Na⁺ would accelerate the dissipation of the Na⁺ gradient and thus produce the observed inhibition of Na⁺-coupled accumulation of D-glucose and L-alanine. The second mechanism would involve the increase in the permeability of the brush-border membrane to D-glucose and L-alanine. The deoxycholate-induced increase in this permeability would increase the rate of efflux of D-glucose and L-alanine, thereby decreasing net influx and inhibiting Na⁺-coupled accumulation of these organic solutes.

The precise mechanism by which deoxycholate increases the permeability of brush-border membranes to Na⁺, D-glucose and L-alanine has not been demonstrated by the present investigation. However, our studies regarding efflux of D-glucose help to clarify the problem. In the absence of deoxycholate, efflux of D-glucose was primarily mediated via a specific sugar transport system. Thus when the D-glucose translocation system was inhibited by phlorizin, efflux of D-glucose was essentially abolished. In the presence of deoxycholate, however, efflux was only partially blocked by phlorizin. The latter finding suggests that, in the presence of deoxycholate, efflux of D-glucose can occur via a pathway that is distinct from the hexose transport system. Similarly deoxycholate-induced increases in Na⁺ and L-alanine permeability may also be mediated via routes that are different from the specific transport systems for these solutes.

A variety of dihydroxy as well as monohydroxy bile salts inhibited Na⁺-coupled vesicular accumulation of D-glucose. The order of inhibitory potency of different bile salts can be roughly correlated with their tendency to partition into the brush-border membrane. This tendency can be estimated from the passive permeability coefficients of the bile salts (Schiff *et al.*, 1972; Wilson & Dietschy, 1972) and their ability to bind non-specifically to brush-border membranes (Wilson & Treanor, 1977). Thus, in general, dihydroxy bile salts were more inhibitory than trihydroxy bile salts and the unconjugated form of a given bile salt was more inhibitory than the glycine or taurine conjugate. There were, however, some exceptions to this correlation. Lithocholate was less inhibitory than deoxycholate and cholate caused essentially the same inhibition as taurocholate and glycocholate. These exceptions suggest that other properties of the bile salts, such as their effectiveness in perturbing lipid-lipid and lipid-protein interactions, may also contribute to the observed increase in membrane permeability and the resultant inhibition of Na⁺-coupled accumulation of D-glucose and L-alanine.

This investigation was supported by National Institute of Health grants AM 07998, 25343 and 21063. We thank Dr. W. B. Im and Dr. K. Y. Ling for invaluable assistance in measuring Na⁺ fluxes and for many helpful discussions.

References

- Beesley, R. C. & Faust, R. G. (1979) *Biochem. J.* **178**, 299–303
- Burke, V., Gracey, M., Thomas, J. & Malajczuk, A. (1976) *Aust. J. Exp. Biol. Med. Sci.* **54**, 391–402
- Faust, R. G. & Wu, S.-M. L. (1965) *J. Cell Comp. Physiol.* **65**, 435–448
- Frizzell, R. A. & Schultz, S.G. (1970) *Biochim. Biophys. Acta* **211**, 589–592
- Guiraldes, E., Lamabadusuriya, S. P., Oyesiku, J. E. J., Whitfield, A. E. & Harries, J. T. (1975) *Biochim. Biophys. Acta* **389**, 495–505
- Hopfer, U. & Murer, H. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 484–488
- Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248**, 25–32
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Schiff, E. R., Small, N. C. & Dietschy, J. M. (1972) *J. Clin. Invest.* **51**, 1351–1362
- Sigrist-Nelson, K., Murer, H. & Hopfer, U. (1975) *J. Biol. Chem.* **250**, 5674–5688
- Wilson, F. A. & Dietschy, J. (1972) *J. Clin. Invest.* **51**, 3015–3025
- Wilson, F. A. & Treanor, L. L. (1977) *J. Membr. Biol.* **33**, 213–230