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GLYCODEOXYCHOLATE TRANSPORT IN BRUSH BORDER MEMBRANE VESICLES ISOLATED FROM RAT JEJUNUM AND ILEUM

FREDERICK A. WILSON and L. LOCKIE TREANOR

Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232 (U.S.A.)

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Summary

The transport of the bile salt, glycodeoxycholate, was studied in vesicles derived from rat jejunal and ileal brush border membranes using a rapid filtration technique. The uptake was osmotically sensitive, linearly related to membrane protein and resembled D-glucose transport. In ileal, but not jejunal, vesicles glycodeoxycholate uptake showed a transient vesicle/medium ratio greater than 1 in the presence of an initial sodium gradient. The differences between glycodeoxycholate uptake in the presence and absence of a Na^+ gradient yielded a saturable transport component. Kinetic analysis revealed a K_m value similar to that described previously in everted whole intestinal segments and epithelial cells isolated from the ileum. These findings support the existence of a transport system in the brush border membrane that: (1) reflects kinetics and characteristics of bile salt transport in intact intestinal preparations, and (2) catalyzes the co-transport of Na^+ and bile salt across the ileal membrane in a manner analogous to D-glucose transport.

Introduction

The characterization of bile salt intestinal transport has been determined in vivo in man and experimental animals [1—3] and by a variety of in vitro assay systems involving whole tissue preparations [4—7] and isolated epithelial cells [8]. These studies have shown that the ileal system satisfies many of the criteria for active transport, i.e. (a) to move bile salt against an electrochemical gradient; (b) to manifest saturation kinetics when uptake rates are measured as

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

a function of increasing bile salt concentration, and (c) to be inhibited by anaerobiosis, metabolic inhibitors, and decreasing temperature and Na^+ concentration in the mucosal perfusate. In contrast, bile salt absorption across the jejunum failing to fulfill these criteria appears to be a passive process.

Recently intestinal brush border membranes have been prepared that contain an osmotically active space, presumably the membrane vesicle seen with electron microscopy [9]. D-Glucose uptake by the vesicle preparation reflected several characteristics of sugar transport in intact intestinal preparations including the accumulation of D-glucose against a concentration gradient under conditions of a Na^+ gradient (extravesicular greater than intravesicular) across the vesicle wall [9—11]. In the present report, the transport of the bile salt, glycodeoxycholate, was studied using brush border vesicles. The results obtained indicate that the ileal, but not the jejunal, brush border vesicles contain a Na^+-glycodeoxycholate co-transport system, which is capable of transient accumulation of the bile salt provided that an electrochemical potential difference for Na^+ exists across the vesicle wall.

Materials and Methods

Materials. [*carbonyl*-^{14}C]Glycodeoxycholate (California Bionuclear Corp., Sun Valley, CA) and unlabeled glycodeoxycholate (Steraloids Inc., Pawling, NY) were found to have greater than 95% purity using thin-layer chromatography [1]. [^{3}H]Dextran (New England Nuclear, Boston, MA) was used as a non-absorbable marker to measure the volume of adherent extravesicular volume. The dextran was dialyzed against distilled water before use. L-[1-^{14}C]-glucose and D-[6-^{3}H(N)]glucose were used as purchased from the supplier (New England Nuclear).

Brush border vesicle preparations. Female 180—220 g Sprague-Dawley rats fed regular chow ad libitum were killed by a blow on the head and bled. The entire small intestine was removed flushed with iced 150 mM NaCl solution, slit lengthwise to expose the mucosa and divided into ten segments of equal length numbered 1—10, proximal to distal. Segments 2—4 and 7—9 were used for study of jejunum and ileum, respectively. The brush border vesicles were prepared from mucosa scraped from these segments by a method similar to that used by Kessler et al. [12]. The scraped mucosa was diluted 20 times volume in 50 mM mannitol plus 2 mM Tris-HCl buffer, pH 7.0, and homogenized in the cold in a Sorval Omni-mixer at maximal speed for 4 min. Crystalline $CaCl_2$ was added to yield a 10 mM solution while stirring with a magnetic stirring bar in the cold for 20 min. The homogenate was centrifuged at 7700 × *g* for 15 min, and the sediment was discarded. The supernatant was spun down at 20 000 × *g* for 30 min. The pellet was homogenized with a teflon-glass homogenizer (seven strokes, 1200 rev./min) in 20 ml of a solution containing 100 mM mannitol plus 1 mM Tris/*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (Hepes) buffer, pH 7.2. $MgSO_4$ was added to yield a 0.1 mM solution. The homogenate was centrifuged at 7700 × *g* for 15 min, and the pellet was discarded. The supernatant was spun down at 20 000 × *g* for 25 min. The pellet was resuspended in 1 mM Tris/Hepes buffer, pH 7.5, plus 100 mM mannitol and 0.1 mM $MgSO_4$ with a 1 ml tuberculin syringe fitted with a 27 gauge,

0.5 inch needle. The suspension was centrifuged at 20 000 × *g* for 5 min, and the supernatant was taken for transport measurements. The relative purity of the vesicle preparations was monitored by sucrase activity determined using the Tris/glucose oxidase peroxidase reagent [13]. Vesicle protein was determined according to the method of Lowry et al. [14] employing crystalline bovine serum albumin (Armour Pharmaceutical Co., Chicago, IL) as a standard.

Transport measurements. The uptake of substrates by brush border vesicles was determined by a membrane filtration technique similar to that described by Hopfer et al. [9]. Incubations were performed at 25°C in 12 times 75-mm polypropylene test tubes containing, unless otherwise stated, 100 mM mannitol, 100 mM NaSCN and 1 mM Tris/Hepes buffer, pH 7.4. Incubation medium also contained ^{14}C- and ^{3}H-labeled substrates and the non-absorbable marker, dextran, at concentrations stated in the legends to figures. Brush border vesicles (0.2—1.2 mg protein) were added to complete a reaction volume of 600 μl. The incubation was terminated by the removal of a 50 μl aliquot and dilution with a 50-fold excess of cold 150 mM NaCl solution supplemented with 50 mM $MgCl_2$ and 10 mM Tris/Hepes buffer, pH 7.4. The vesicles were collected on a prewashed 0.2 μm Nucleopore filter (Pleasanton, CA) and washed with 2 ml of buffered NaCl solution. The filter was solubilized with NCS (Amersham/Searle, Arlington Heights, IL) in a scintillation vial. Scintillation fluid was added that contained: 12 g of PPO (2,5-diphenyloxazole), POPOP (1,4-bis-5-phenyl-oxazolyl-2)-benzene, Fischer Scientific Co., Fair Lawn, NJ) and 2000 ml of toluene. Radioactivity was counted using an external standardization technique to correct for quenching of the two isotopes [15]. Following correction for medium radioactivity bound to the filters in the absence of vesicle membranes, the flux rates were calculated and have the units : nmol taken up into vesicles/unit time per mg vesicle protein.

Analysis of vesicular content. Brush border vesicle membranes were incubated with an incubation medium as described above for 15 s and 15 min and filtered on Nucleopore filters. The filters were extracted with 15 ml methanol for 24 h at 25°C; the methanol extract was decanted and concentrated by evaporation under nitrogen. Separation of potential products of [^{14}C]glycodeoxycholate was carried out by thin-layer chromatography on Silica gel H (Analtech Inc., Newark, DE) using isoamyl alcohol/propionic acid/*n*-propanol/H_2O (60 : 60 : 40 : 30, by vol.) as a developing solution [1]. Controls were run in which the [^{14}C]glycodeoxycholate was added subsequent to the incubation. After separation the chromatograms were divided into sections, and the sections were counted for radioactivity.

Results

Preparations of brush border vesicles

The yield and purity of brush border vesicle membranes was routinely followed by measuring the activity of the marker enzyme, sucrase (Table I). One-third and about one-fourth of the mucosal sucrase activity was recovered in the jejunal and ileal brush border vesicles, respectively. The sucrase specific activity was 17.1 times greater in the vesicles than in the mucosal homogenate from

TABLE I

SUCRASE ACTIVITY OF BRUSH BORDER VESICLES

Specific activity is expressed as $\mu mol \cdot mg^{-1}$ protein $\cdot h^{-1}$. The purification is the ratio of the specific activities in the final brush border vesicles and the homogenate. Yield is the percentage of total sucrase activity. Values represent means ± S.E. for eight determinations.

Fraction	Specific activity	Purification	Yield
I. Jejunum			
Homogenate of intestinal scrapping	65.6 ± 6.2	1.0	100
Brush border vesicles	1114.5 ± 91.0	17.1	33
II. Ileum			
Homogenate of intestinal scrapping	9.8 ± 1.5	1.0	100
Brush border vesicles	167.8 ± 18.5	17.1	26

jejunum and ileum. The specific activity of sucrase is similar to that in other preparations of vesicles from intestinal brush borders [10].

Uptake of radiolabeled D- and L-glucose

In order to validate the transport assay and provide studies for comparison with bile salt transport, initial experiments were performed with D- and L-glucose. Fig. 1 shows the typical features of glucose uptake by jejunal and ileal brush border vesicles. First, in the presence of a NaSCN gradient across the membrane (outside greater than inside), 1 mM D-glucose showed an overshoot phenomenon, i.e. the amount of D-glucose present in the vesicle transiently exceeds the equilibrium value. The equilibrium value was reached after 6 min and was identical for D- and L-glucose under all experimental conditions. No overshoot for D-glucose was seen after the Na^+ gradient was dissipated across

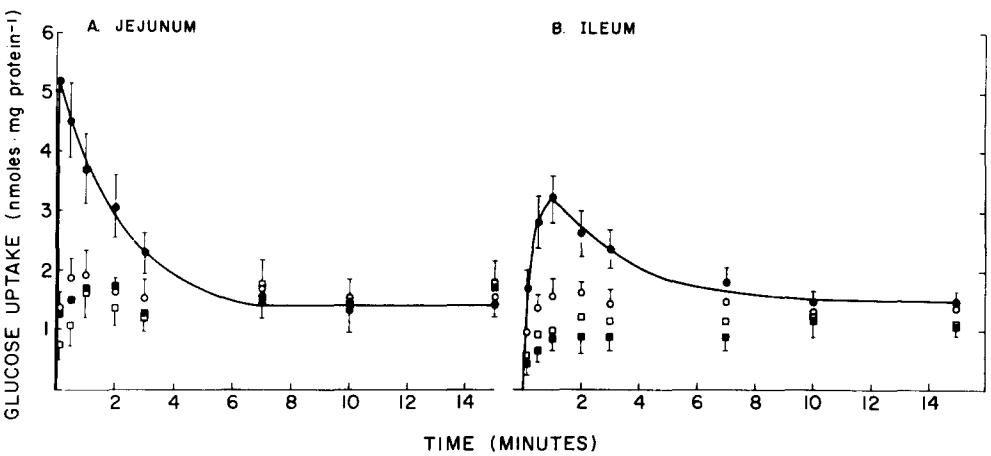

Fig. 1. Time course of 1 mM D- and L-glucose uptake by brush border membrane vesicles isolated from jejunum (A) and ileum (B). The uptake was initiated either by the addition of vesicles to the complete incubation medium (100 mM NaSCN gradient at time zero; ●, D-glucose; ■, L-glucose) or by the addition of D-[^{3}H]glucose and L-[^{14}C]glucose after the membrane had been preincubated for 15 min with the complete medium (abolition of the NaSCN gradient; ○, D-glucose; □, L-glucose). Means ± S.E. for 18–24 determinations at each time period are shown by the vertical bars.

the vesicle wall. Second, maximal D-glucose uptake was higher in jejunal (Fig. 1A) than ileal vesicles (Fig. 1B). Ratios obtained for maximal D-glucose uptake during a Na^+ gradient and glucose uptake during Na^+ equilibrium were 3.5 and 2.3 in the jejunum and ileum, respectively. Third, the time of maximal D-glucose uptake occurred at 15 and 60 s in the jejunal and ileal vesicles, respectively. Thus, the time of maximal D-glucose uptake was directly related to the absolute ratio of maximum/equilibrium uptake. Finally, L-glucose failed to show the overshoot phenomenon in the presence or absence of a Na^+ gradient. Thus, these data reflect previously described characteristics of uptake for 1 mM D- and L-glucose across the wall of brush border vesicles from jejunum and ileum [9,10].

Uptake of radiolabeled glycodeoxycholate

The first series of experiments were performed to validate the assay system for bile salt. As shown in Fig. 2, the uptake rate for glycodeoxycholate in the ileal brush border vesicles increased in a linear fashion with respect to increasing vesicle protein. The determination may be described by a linear regression which has essentially a zero intercept. Thus, dividing vesicle uptake by vesicle protein is an appropriate method of normalizing determinations. Further, it had to be resolved whether the uptake of glycodeoxycholate by the brush border vesicles represents adsorption to the membrane surface, incorporation into the membrane or transport into an intravesicular space. If the latter process is involved, the uptake of glycodeoxycholate should be dependent on the intravesicular volume. Intravesicular volume was decreased by increasing the medium osmolarity with sorbitol, a relatively impermeable solute. As shown in Fig. 3, the amount of 0.2 mM glycodeoxycholate taken up by the vesicles was inversely proportional to medium osmolarity from 166 to 2000 mM, thus directly related to intravesicular space. However, uptake was estimated by extrapolation to infinite medium osmolarity and suggested that

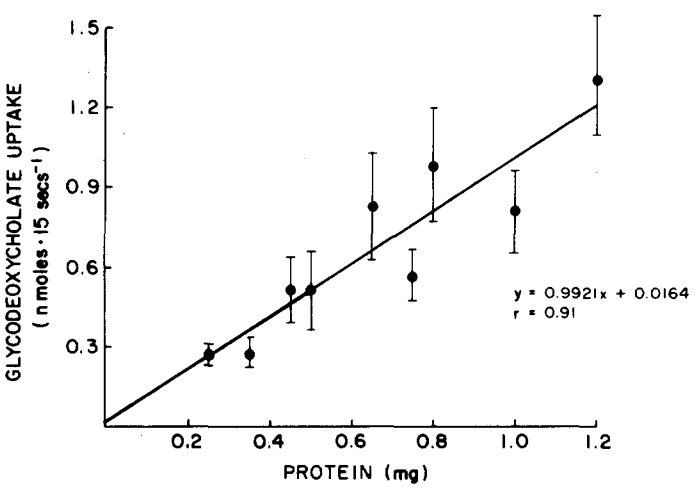

Fig. 2. Effect of membrane protein on 0.2 mM glycodeoxycholate uptake by ileal brush border membrane vesicles. The uptake was determined in the presence of increasing amounts of membrane protein and Na^+ equilibrium after 15 min of incubation. Values are means ± S.E. for 16 determinations. S.E. for the slope of the least-squares regression line is 0.12.

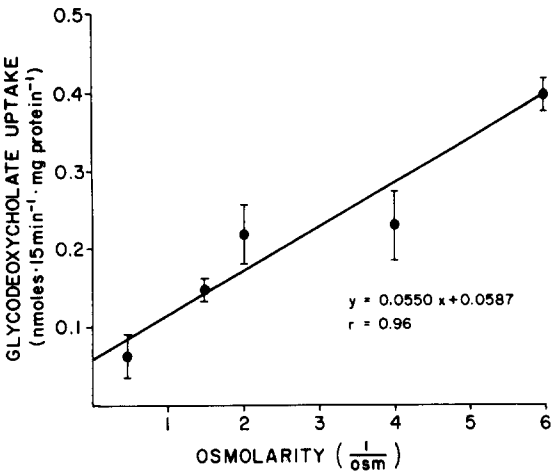

Fig. 3. Effect of medium osmolarity on 0.2 mM glycodeoxycholate uptake by ileal brush border membrane vesicles. The uptake was determined in the presence of increasing amounts of sorbital to give the indicated osmolarity of the incubation solution plotted in units of osM^{-1}. Mean values ± S.E. are for 22–24 determinations obtained at Na^+ equilibrium following 15 min of incubation. S.E. for the slope of the least squares regression line is 0.05.

some glycodeoxycholate interacted with the membrane. Thus, at physiologic tonicity (300 mM), the amount of glycodeoxycholate not found in the intravesicular space may account for 24% of the total uptake of the bile salt by the brush border vesicles. It should also be noted that analysis of the vesicular content after 15 s and 15 min of incubation revealed that more than 95% of the radiolabel found in the vesicle extract had an R_F value identical to that of glycodeoxycholate.

Na^+ gradient-dependent uptake of glycodeoxycholate

The uptake of 0.2 mM glycodeoxycholate by jejunal and ileal brush border vesicles was determined with respect to the length of incubation. As shown in Fig. 4A, the rates of glycodeoxycholate uptake by jejunal vesicles were similar in the presence and absence of a Na^+ gradient between the external medium and intravesicular medium. Steady-state levels for the bile salt uptake were achieved within 3 min. In contrast, the presence of a Na^+ gradient across the ileal vesicle wall stimulated a transient overshoot in the uptake of glycodeoxycholate (Fig. 4B). At the peak of the overshoot (15 s), the accumulation of glycodeoxycholate was approximately twice the equilibrium value. After the peak of stimulated uptake, the amount of bile salt rapidly decreased to equilibrium values, indicating efflux from the vesicles. The final level of uptake for glycodeoxycholate in the presence and absence of the Na^+ gradient in jejunal and ileal vesicles was similar suggesting that equilibrium was established. These results indicate that the imposition of a large extravesicular to intravesicular electrochemical potential difference for Na^+ provided the driving force to effect the transient movement of glycodeoxycholate into the ileal brush border vesicle against its concentration gradient. Despite such a potential difference, no uphill or active transport was seen in jejunal vesicles for glycodeoxycholate. Thus, these events occurring at the level of the brush border mem-

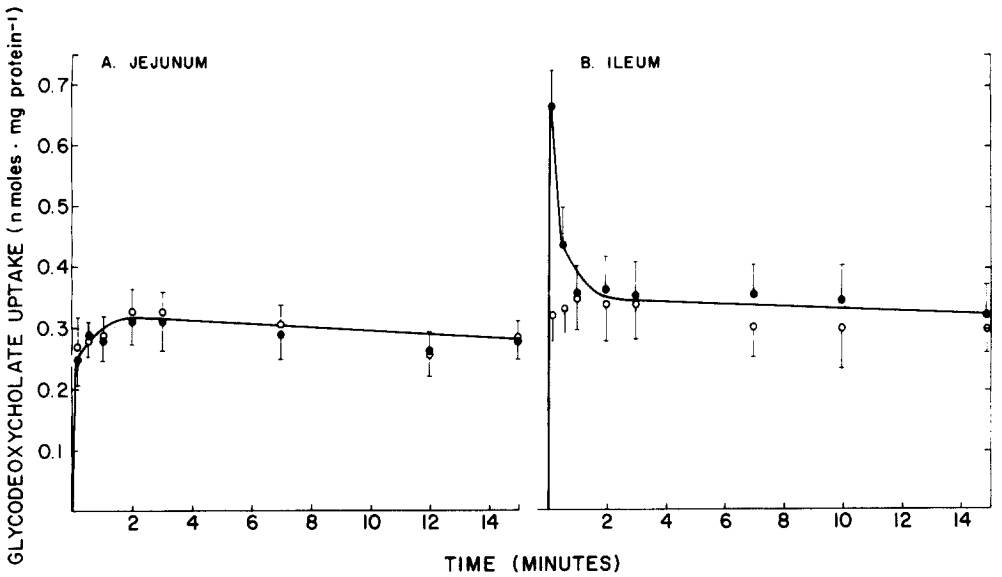

Fig. 4. Time course of 0.2 mM glycodeoxycholate uptake by brush border membrane vesicles isolated from jejunum (A) and ileum (B). The uptake was initiated either by the addition of vesicles to the complete incubation medium (100 mM NaSCN gradient at time zero; •, glycodeoxycholate) or by the addition of [^{14}C]glycodeoxycholate and [^{3}H]dextran after the membrane had been preincubated for 15 min with the complete medium (abolition of the NaSCN gradient; ○, glycodeoxycholate). Mean values ± S.E. for 22—25 determinations at each period are shown by the vertical bars.

brane appear to reflect differences in bile acid transport that were previously described in vivo [1] and in vitro using whole intestinal tissue [7] and isolated epithelial cells [8].

Kinetics of the Na^+ gradient-dependent uptake of glycodexycholate

Vesicles isolated from the ileum were used to characterize the kinetics of the active transport of glycodeoxycholate. For kinetic analysis, the ileal uptake, J, is given by the Eqn. 1.

$$J = \frac{(V)(C_m)}{K_m + C_m} + (P)(C_m) \quad (1)$$

where, J is the vesicle uptake in nmol · min^{-1} · mg^{-1} protein; V is the maximal vesicle uptake in nmol · min^{-1} · mg^{-1} protein; C_m is the bile salt concentration in mmol · l^{-1} in the incubation medium; K_m is the Michaelis constant in mmol · l^{-1}; P is the permeability coefficient in nmol · min^{-1} · mg^{-1} protein · mmol · l^{-1}. The first term gives the magnitude of the active flux and describes a rectangular hyperbola; the second term gives the magnitude of passive flux and describes a straight line. The rate of passive flux must be subtracted from the experimental uptake, J, in order to determine the rate of active uptake. The required permeability coefficient (P) of glycodeoxycholate for the ileal vesicles was obtained in two ways. First, with increasing C_m (3—4 times K_m) the first term approximates a constant, and J continues to increase in a linear manner with the slope equal to P. Second, it was assumed that Na^+-independent uptake of bile salt in ileal vesicles is passive and that the slope of the linear uptake

corresponds to P. The passive permeability coefficients obtained by these two methods were averaged, and the average value was subtracted from the measured uptake to yield the active component of glycodeoxycholate in ileal vesicles [8].

The effect of increasing concentrations of glycodeoxycholate on the rate of bile salt uptake in the absence and presence of a 100 mM Na^+ gradient is shown in Fig. 5. In the absence of a sodium gradient the rate of glycodeoxycholate uptake increased linearly with increasing bile salt concentration throughout a range from 0.1 to 1.0 mM. In the presence of a Na^+ gradient, the rate of vesicle uptake described a hyperbolic component with respect to the concentration of glycodeoxycholate in the incubation medium. However, a passive component of uptake by ileal vesicles in the presence of a Na^+ gradient was quantitatively important. The slope of the linear portion of the curve for ileal uptake in the presence of the Na^+ gradient approached that for uptake in the absence of a Na^+ gradient. The regression curve for glycodeoxycholate (J against C_m) that describes the asymptotic linear component in the presence of the Na^+ gradient, $Y = 1.7800\ X + 0.7017$ (S.E. of the slope is 0.21) approached the one for passive permeability in the absence of the Na^+ gradient, $Y = 1.7242\ X + 0.0088$ (S.E. of the slope is 0.18). When the average of the slopes was subtracted from experimental values, the kinetic curve for active transport (dashed line) was obtained. The value of V (0.72 nmol · mg^{-1}) and K_m

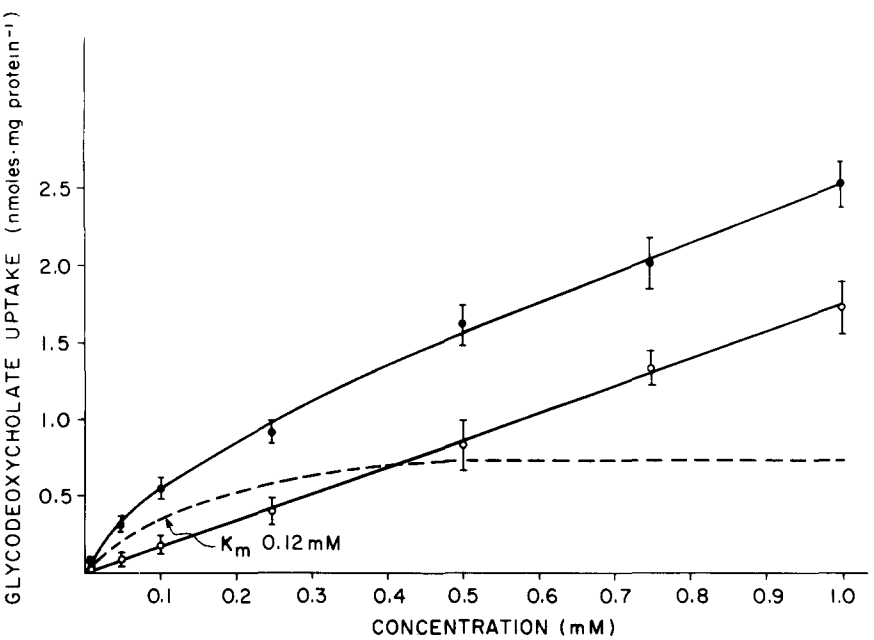

Fig. 5. Relationship between glycodeoxycholate concentration and the Na^+ gradient-dependent and -independent rates of uptake of the bile salt. Incubations were for 15 s (in the presence of 100 mM NaSCN gradient; •, glycodeoxycholate) and 15 min (absence of NaSCN gradient; ○, glycodeoxycholate). ------, the Na^+ gradient-dependent component for glycodeoxycholate uptake calculated by subtracting a passive permeability coefficient from experimentally determined rates of ileal vesicle uptake as described in the text. Values are means ± S.E. for 22—26 determinations.

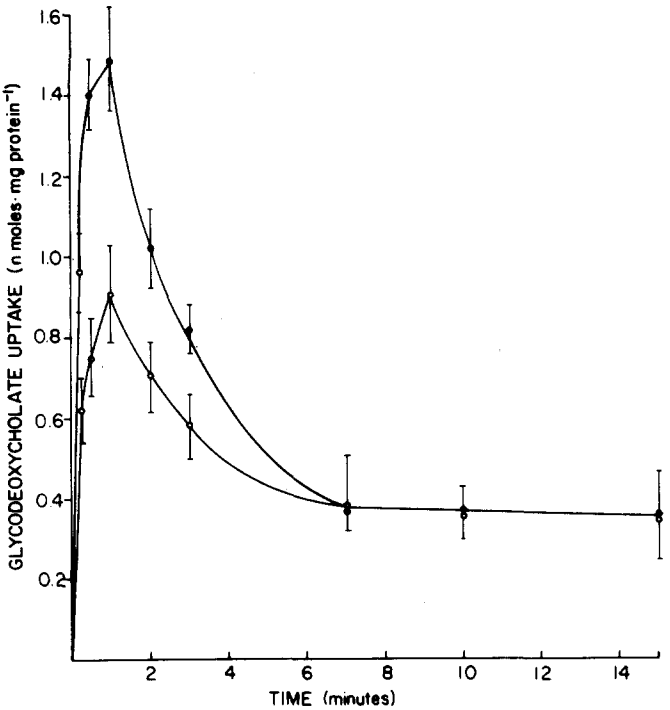

Fig. 6. Influence of K^+ diffusion potential on the Na^+ gradient-stimulated uptake of glycodeoxycholate by brush border membrane vesicles isolated from ileum. The membrane vesicles were preloaded with 100 mM KCl, 100 mM mannitol and 1 mM Tris/Hepes. The uptake was initiated by the addition of vesicles to a medium containing 100 mM NaCl, 100 mM mannitol, 5 mM KCl, 1 mM Tris/Hepes, 0.2 mM [^{14}C]glycodeoxycholate, [^{3}H]dextran and 2% ethanol in the presence (●———●) and absence (○———○) of valinomycin (8 $\mu g \cdot mg^{-1}$ protein). Mean values ± S.E. for six determinations at each period are shown by the vertical bars.

(0.125 mM) were derived from the curve for active transport based on the Lineweaver-Burk plot.

Influence of K^+ diffusion potential on uptake of glycodeoxycholate

The membrane potential of the brush border vesicles can be manipulated by imposing diffusion potentials across the membrane [11]. Since diffusion potentials depend on the relative mobility of cations and anions, the ionophore, valinomycin, was used to increase the permeability of the vesicle membrane to K^+. Vesicles were preloaded with KCl and the Na^+ gradient-dependent uptake of 0.2 mM glycodeoxycholate was examined in the absence and in the presence of valinomycin. As shown in Fig. 6, a transient overshoot in the uptake of glycodeoxycholate occurred under conditions of a Na^+ gradient (medium greater than vesicle) and a K^+ gradient (vesicle greater than medium). The addition of valinomycin further enhanced the overshoot in the glycodeoxycholate uptake to four times that at equilibrium. The equilibrated uptake values of the control and the valinomycin-containing membranes were identical.

Discussion

The data presented in this study describe the uptake of the bile salt, glycodeoxycholate, into isolated intestinal brush border membrane vesicles. Certain observations indicate that the measured glycodeoxycholate uptake represents transport into an intravesicular space. (a) The equilibrium uptake of the bile salt decreased with increasing medium osmolarity as predicted for osmotically active vesicles. (b) Glycodeoxycholate exhibited overshooting uptake in ileal vesicles under conditions of a Na^+ gradient. (c) The bile salt was taken up in ileal vesicles to the same extent as D-glucose under similar experimental conditions. However, the presence of uptake following extrapolation to infinite medium osmolarity, i.e. zero intravesicular space, suggests that glycodeoxycholate also interacts with the membrane. Whether this occurs by adsorption onto or partitioning into the brush border membrane is unknown. The latter possibility is likely because (a) uptake was seen despite correction for the extravesicular fluid volume with a relatively impermeable solute, dextran, and (b) previous studies indicated significant partitioning of bile salts into non-vesiculated brush border membranes [16].

The present results show that the imposition of an extravesicular to intravesicular Na^+ gradient brings about the transient accumulation of the glycodeoxycholate in ileal, but not jejunal, brush border membrane vesicles that reaches a level two-fold higher than that attained at equilibrium. The rate of uptake of glycodeoxycholate into the ileal vesicles may be considered the sum of contributions from a Na^+ electrochemical gradient-dependent transport system and a Na^+ gradient-independent process. When the latter component is subtracted from uptake values for glycodeoxycholate in the presence of a Na^+ gradient a saturable concentration curve is derived for the Na^+ gradient-dependent system. The glycodeoxycholate concentration at one-half maximal uptake (K_m) by ileal vesicles is similar to that described for conjugated dihydroxy bile salt in whole intestinal tissue following correction for unstirred water layer effects [7] and for epithelial cells isolated from the rat ileum [8].

The role of the electrochemical membrane potential in glycodeoxycholate transport was studied by determining the uptake of the bile salt by brush border membrane vesicles under conditions in which the membrane potential was mediated by valinomycin. When valinomycin was added to K^+-loaded brush border membranes, efflux of K^+ was induced, and an electrochemical potential, inside negative, was generated. The development of this membrane potential facilitated greater influx of Na^+ [17]. Thus, the uptake of glycodeoxycholate transported with Na^+ was stimulated further, and a greater overshoot was seen. This observation suggests that one of the determinants of glycodeoxycholate transport across the ileal brush border membrane is membrane potential in addition to the Na^+ and glycodeoxycholate gradients.

Earlier reports on the influence of Na^+ on the uptake of bile salt in intestinal segments implicated a role for Na^+ in the transport of bile salt [4,7]. The increased movement of the bile salt may be a specific effect of the Na^+ electrochemical gradient on the mediated transport of bile salt across the ileal brush border membrane in a manner analogous to the mechanism for intestinal transport of D-glucose [18]. In this scheme the movement of sodium from the

lumen into the cell down its concentration gradient provides the energy to drive glucose against its concentration gradient. The low intracellular sodium concentration necessary for continued operation of the system is maintained by the active extrusion of this cation across the basolateral cell membrane with energy derived from adenosine triphosphate (ATP) hydrolysis by ouabain-sensitive ($Na^+ + K^+$)-ATPase. This is consistent with ouabain-inhibition of bile salt transport into hepatic cells [19] and epithelial cells isolated from the ileum [8]. The vesicle uptake of glucose and glycodeoxycholate is not dependent upon ATP since the uptake by these membranes deals only with that portion of the transport process which takes place at the luminal cell surface across which the Na^+ gradient is artificially imposed. The simplicity of the vesicle preparation also suggests that differences in jejunal and ileal bile salt transport are explained by a brush border membrane component peculiar to the ileum that couples Na^+ and bile salt movement across the cell wall.

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