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Research Article

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Ontogenesis of Taurocholate Transport by Rat Ileal Brush Border Membrane Vesicles

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Abstract

Developmental aspects of taurocholate transport into ileal brush border membrane vesicles were studied in 2-wk-old (suckling), 3-wk-old (weanling), and 6-wk-old (adolescent) rats. Taurocholate uptake (picomoles per milligram protein) into brush border membrane vesicles prepared from 2-wk-old rats was similar under Na^+ and K^+ gradient conditions (outside greater than inside). By contrast, uptake in 3- and 6-wk-old rats was significantly enhanced at 20 s, and at 1, 2, and 5 min of incubation in the presence of a Na^+ gradient when compared with a K^+ gradient incubation ($P < 0.05$). Under isotope exchange conditions, a plot of active uptake velocity versus taurocholate concentration (0.10–1.0 mM) in 2-wk-old rat membrane vesicles was linear and approached the horizontal axis, suggesting the absence of active transport. However, similar plots in 3- and 6-wk-old rats described a rectangular hyperbola, indicating a Na^+-dependent, saturable cotransport system. Woolf-Augustinsson-Hofstee plots of the uptake velocity versus concentration data from 3- and 6-wk-old rat brush border membrane vesicles yielded V_{max} values that were not significantly different, 844 and 884 pmol uptake/mg protein per 120 s, respectively. The respective K_m values were 0.59 and 0.66 mM taurocholate. The induction of an electrochemical diffusion potential by incubating K^+-loaded vesicles with valinomycin did not significantly enhance taurocholate uptake in 2-, 3-, or 6-wk-old rat vesicle preparations.

These data indicate that taurocholate transport into rat ileal brush border membrane vesicles is mediated by an electroneutral, sodium-coupled, cotransport system that is incompletely developed in the 2-wk-old suckling rat but fully developed by the time of weaning at 3 wk of age.

Introduction

The enterohepatic circulation of bile salts is important for the maintenance of a bile salt pool of sufficient size and concentration to permit absorption of dietary lipid. Experimental observations in immature laboratory animals and in human infants suggest that active transport of bile acids in the ileum is not fully operational, and that the bile salt pool size is diminished during the fetal and neonatal periods (1–5). By contrast, in mature animals, bile salt reabsorption is an active process, the specific features of which have recently been thoroughly elucidated by techniques allowing the study of substrate transfer into brush border membrane vesicles (6–12). These studies have shown that bile salt transport is mediated by a Na^+-dependent cotransport system that is similar to that described for glucose, amino acids, and inorganic phosphate (13). The active transport mechanism for bile salts is located on the ileal brush border membrane; transport across the duodenal and jejunal mucosa appears to occur by passive diffusion. In this report, we present the first description of the postnatal development of taurocholate transport into ileal brush border membrane vesicles.

Methods

Preparation of brush border membrane vesicles. Brush border membrane vesicles were prepared from 14-d-old, 21-d-old, and 6-wk-old Sprague-Dawley rats (Sasco Laboratories, Omaha, NE). The animals were killed by cervical dislocation. The distal one-third of the small intestine was removed, everted, and scraped with a glass slide. All steps in this preparation were conducted at ~4°C. Using a Waring blender at maximal speed, the mucosal scrapings were homogenized for 3 min in 60 ml of 300 mM mannitol, 5 mM EGTA, and 12 mM Tris HCl (pH 7.1). 240 ml of ice-cold, distilled water was added. The homogenate was treated with 3 ml of 1 M $MgCl_2$ and centrifuged in a rotor (model J2-21; Beckman Instruments, Inc., Fullerton, CA) at 5,000 rpm for 15 min. The supernatant was then centrifuged at 15,000 rpm for 30 min. The resulting pellet was resuspended in 60 ml of 60 mM mannitol, 5 mM EGTA, and 12 mM Tris HCl (pH 7.1), and homogenized in a Potter-Elvehjem tube for 10 strokes at the highest speed. The homogenate was treated with 0.6 ml of 1 M $MgCl_2$ and centrifuged at 5,000 rpm for 15 min. The supernatant was spun at 15,000 rpm for 30 min. The pellet was resuspended in 30 ml of 250 mM mannitol and 20 mM Hepes Tris (pH 7.4) and centrifuged at 20,000 rpm for 30 min. Using a tuberculin syringe with a 25 gauge needle, the pellet was resuspended in the desired volume of preincubation solution, the composition of which is described in each figure legend. The protein concentration was measured by the method of Lowry et al. (14), using bovine serum albumin as a standard.

Morphologic purity of the brush border membrane vesicle preparation. Membranes were fixed for 90 min in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). The suspensions were centrifuged at 50,000 *g* for 1 h to form pellets. The pellets were then fixed in 1.5% osmium tetroxide, dehydrated in graded ethanols, and stained en bloc with uranyl acetate. After embedding in Epon 812, thin sections were cut and stained with uranyl acetate and lead citrate. Sections were examined at 9,000–2,200× in a 300-electron microscope (Philips Electronic Instruments, Inc., Mahwah, NJ).

Functional purity of the brush border membrane vesicle preparation. Lactase, sucrase, and Na^+-K^+-ATPase activities were measured by previously described methods (15, 16). Leucine aminopeptidase was measured by a Boehringer kit (no. 124869; Boehringer Mannheim Biochemicals, Indianapolis, IN).

Transport measurements. Uptake of radiolabeled taurocholate was

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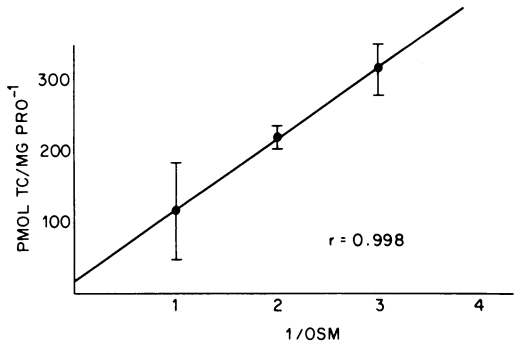

Figure 1. Effect of osmolarity on taurocholate uptake in ileal vesicles from 6-wk-old rats. Membranes were preloaded with 280 mM mannitol, and 20 mM Hepes Tris buffer (pH 7.4). Incubation (37°C) was initiated by the addition of 0.1 mM taurocholate, 50 mM NaCl, 20 mM Hepes Tris buffer (pH 7.4), and mannitol in sufficient concentration to give the indicated osmolarity, i.e. 1,000 mosM, 500 mosM, or 333 mosM. The incubation time was 30 min (equilibrium). Each point represents the mean±SE for six determinations. TC, taurocholate; pro, protein.

measured by the rapid filtration technique (17). All incubations were done at 37°C and were initiated by addition of 50 μl vesicle suspension to 150 μl incubation solution. The composition of the incubation medium is noted in the legend of each figure. At each desired incubation time interval a 30-μl aliquot was removed and diluted in 1 ml of ice-cold "stop solution" which consisted of 100 mM mannitol, 20 mM Hepes Tris (pH 7.4), 20 mM $MgSO_4$, 100 mM choline chloride, and 0.1 mM taurocholate. The cold, diluted reaction mixture was immediately pipetted onto a prewetted filter (cellulose nitrate, 0.45 μm pore size; Sartorius Filters, Inc., Hayward, CA) and kept under suction. The filter was rinsed with 5 ml of ice-cold stop solution and then dissolved in Bray's solution. Radioactivity was counted in a scintillation counter (model LS 4000; Beckman Instruments, Inc.). Results are expressed as picomoles of taurocholate uptake per milligram of vesicle protein. Experiments were performed in duplicate on different days using freshly prepared vesicles. Statistical significance was analyzed by the unpaired *t* test.

Materials. [^{3}H]Taurocholic acid (6.6 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Taurocholic acid was purchased from Calbiochem-Behring Corp. (San Diego, CA). All other chemicals were reagent grade.

Results

Purity of the brush border membrane vesicle preparation. Electron photomicrographs of the brush border membrane vesicles showed closed vesicles and no contamination with other subcellular organelles. Leucine aminopeptidase, sucrase, and lactase activities were enriched 8–14-fold when the final vesicle preparation was compared with starting mucosal homogenate. There was no enrichment of Na^+-K^+-ATPase, a marker for basolateral membranes.

Binding. Brush border membrane vesicles are osmotically sensitive compartments (17). Therefore, binding to the vesicle surface can be differentiated from transport into the osmotically sensitive intravesicular space by performing incubations in solutions of increasing osmolarity. At infinite osmolarity the intravesicular volume is zero and uptake should represent binding only. Fig. 1 illustrates taurocholate uptake at 30 min (equilibrium) vs. osmolarity^{-1} in brush border membrane vesicles prepared from 6-wk-old rats. Mannitol was the osmotically active solute. Extrapolation to infinite osmolarity revealed that <10% of taurocholate uptake resulted from binding. A similar linear relationship was determined for uptake in vesicles prepared from 2-wk-old rats; that is, <10% binding (data not shown). This result is similar to previously reported values of 7% binding by hamster brush border membranes (11) and 16% binding in rat brush border membranes (diluted in a stop solution containing unlabeled taurocholate [10]). Calculations in this report do not consider the minimal influence of binding on the total taurocholate uptake.

Sodium dependence. It is well-established that taurocholate transport by the ileum is Na^+-dependent in mature laboratory animals (6–8, 10–12). Fig. 2, *A–C*, shows taurocholate uptake as a function of time and cationic gradient in 2-, 3-, and 6-wk-old rat ileal brush border membrane vesicles, respectively. The vesicles were prepared in Na^+- and K^+-free buffer solutions and then incubated in a Na^+- or K^+-containing buffer (pH 7.4, 37°C), thus creating a cationic gradient directed from the extravesicular to the intravesicular space. In all preparations there was a rapid initial uptake during the first 2 min of incubation and then a slower accumulation until equilibrium was reached at 30 min. In 2-wk-old rat vesicles (Fig. 2 *A*) the magnitude of taurocholate uptake in the presence of a Na^+ or

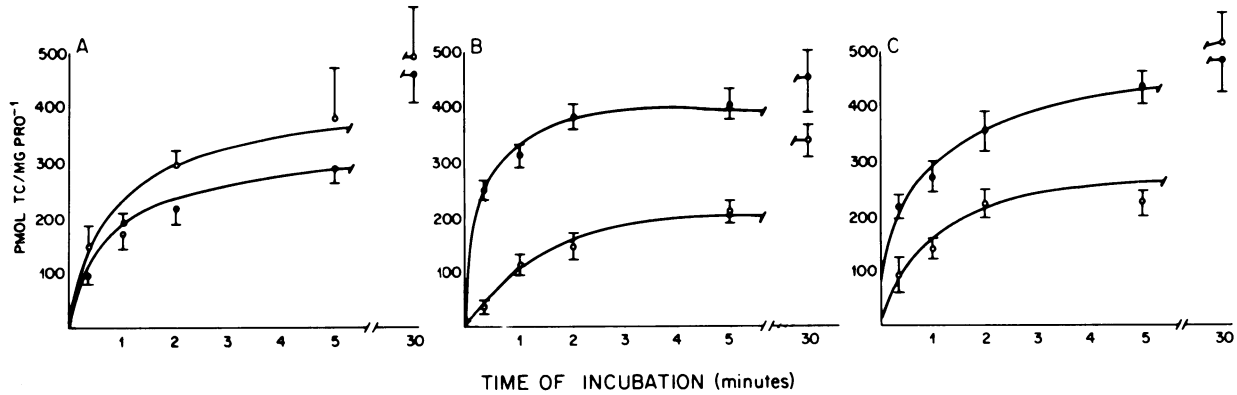

Figure 2. Uptake of taurocholate as a function of time and Na^+ (●) or K^+ (○) gradient in 2-wk (*A*), 3-wk (*B*), and 6-wk (*C*) vesicle preparations. In each instance, vesicles were preloaded with 280 mM mannitol, 20 mM Hepes Tris (pH 7.4), and then incubated at 37°C in the following: (●), 100 mM NaCl, 20 mM Hepes Tris (pH 7.4), 100 mM mannitol, 1.0 mM [^{3}H]taurocholate; or (○), 100 mM KCl, 20 mM Hepes Tris (pH 7.4), 100 mM mannitol, and 0.1 mM [^{3}H]taurocholate. Each point represents the mean±SE from eight or more determinations. Pro, protein; TC, taurocholate.

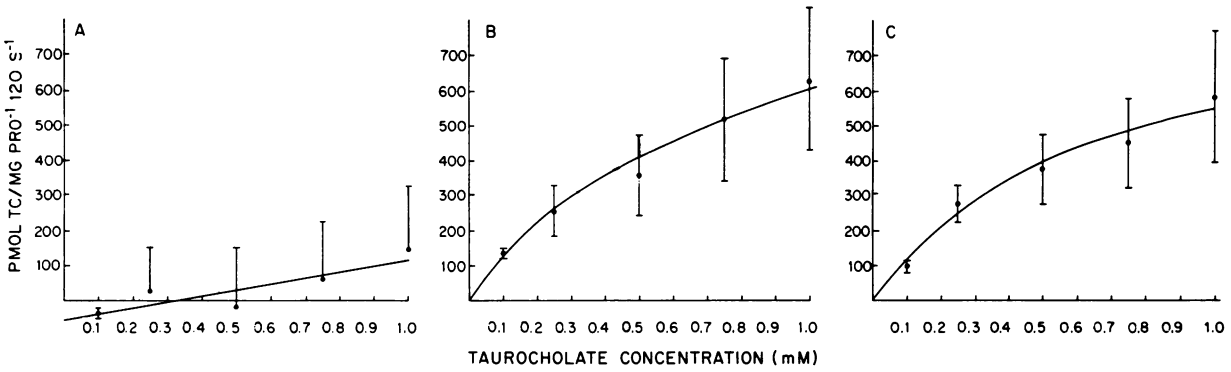

Figure 3. Kinetics of taurocholate transport in 2-wk (*A*), 3-wk (*B*), and 6-wk (*C*) vesicle preparations. Ileal membrane vesicles were preloaded with 100 mM mannitol, 100 mM choline chloride or Na^+ chloride, 20 mM Hepes Tris (pH 7.4), and unlabeled taurocholate at the indicated concentrations. Incubations (37°C, 120 s) were initiated in an identical solution excepting the presence of tracer amounts of [^{3}H]taurocholate and 6 μg gramicidin/mg protein. Each data point represents the arithmetic difference±the standard error of the difference between tracer uptake in the presence of Na^+-preequilibrated and choline-preequilibrated conditions (n = 8). TC, taurocholate; pro, protein.

K^+ gradient condition was not significantly different over the 30 min of incubation ($P < 0.05$). In the 3- and 6-wk-old rat ileal vesicles, however, a Na^+ outside greater than Na^+ inside gradient stimulated an initial taurocholate uptake that was significantly greater ($P < 0.05$) than uptake with K^+ outside greater than K^+ inside. These differences occurred at 20 s and at 1, 2, and 5 min of incubation. Equilibrium values at 30 min were similar.

Saturability of uptake. The uptake of radiolabeled taurocholate was studied under isotope exchange conditions. The vesicles were preloaded with Na^+ or choline and unlabeled taurocholate in such quantities that, during incubation, intravesicular and extravesicular concentrations of each were equal. Gramicidin, an ionophorous antibiotic that increases cation permeability, was included in the incubation solution to diminish any residual Na^+ gradient. There was a tracer quantity of extravesicular, labeled taurocholate. Fig. 3, *A–C,* shows these results from ileal vesicle preparations in 2-, 3-, and 6-wk-old rats, respectively. The curves depicted in Fig. 3 represent the arithmetic differences, ±the standard error of the differences between tracer uptake in the presence of Na^+-preequilibrated and choline-preequilibrated conditions. Unlabeled taurocholate concentrations ranged from 0.1 to 1.0 mM. The curves should represent the carrier-mediated component of taurocholate transport. Data from 2-wk-old rats was linear and closely approximated the horizontal axis. By contrast, the plots for 3- and 6-wk-old rats described a rectangular hyperbola. Using the Woolf-Augustinsson-Hofstee equation, K_m and V_{max} values were calculated for 3- and 6-wk-old rats. These values were K_m = 0.59 mM and V_{max} = 844 pmol/120 s per mg protein for the 3-wk-old rat brush border membrane preparation, and K_m = 0.66 mM and V_{max} = 884 pmol/120 s per mg protein for the 6-wk-old rat preparation. The K_m values are approximately one-half the highest taurocholate concentration used in the tracer exchange experiments (1.0 mM). Initial experiments included data points at 1.5 and 2.0 mM taurocholate. Uptake rates at these taurocholate concentrations markedly disrupted the otherwise linear or hyperbolic nature of the curve between 0.1 and 1.0 mM taurocholate. Specifically, uptake rates were diminished by as much as 50% at the higher bile salt concentrations, possibly indicating a bile-salt–mediated disruption of membrane integrity.

Membrane potential. Valinomycin is an ionophore that mediates the movement of K^+ down its concentration gradient (18). Results of experiments in which a K^+ diffusion potential was imposed across the vesicle membrane are shown in Fig. 4. Ileal brush border membrane vesicles from each age group were preloaded with K^+ (K^+ inside greater than K^+ outside) and then incubated in the presence of a Na^+ gradient (Na^+ outside greater than Na^+ inside) with or without valinomycin. In the presence of valinomycin, a rapid efflux of intravesicular K^+ generated an electrochemical potential with the intravesicular space relatively negative. The imposition of a membrane potential did not significantly alter taurocholate uptake in 2-, 3-, or 6-wk-old rat vesicle preparations. A single additional experiment was performed to control for the presence of intravesicular K^+. In this situation, taurocholate uptake was determined in the presence of valinomycin, an Na^+ gradient (Na^+ outside greater than Na^+ inside), and either a K^+ inside greater than K^+ outside (K^+ preloaded), or no K^+ gradient. Again, imposition of a membrane potential did not significantly influence taurocholate uptake after 20 s or after 1 min of incubation (n = 5).

Discussion

The bile salt pool size in newborn infants is decreased when compared with surface-area–matched healthy adults (4). The

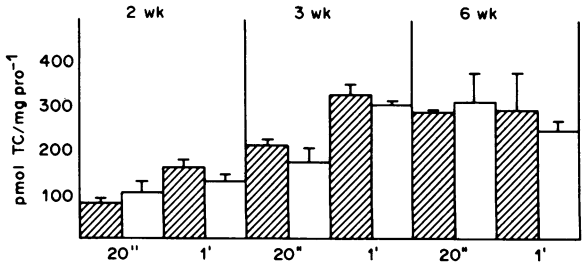

Figure 4. Effect of a valinomycin-induced membrane potential on Na^+-dependent taurocholate uptake. Brush border membrane vesicles were preloaded with 100 mM KCl, 100 mM mannitol, and 20 mM Hepes Tris (pH 7.4) at room temperature for 1 h. Incubations were done at 37°C in 100 mM NaCl, 100 mM mannitol, and 20 mM Hepes Tris (pH 7.4), with (▨) or without (□) valinomycin, 10 μg/mg protein. Each point represents mean±SE for six or more determinations. Pro, protein; TC, taurocholate.

factors involved in the maintenance of a sufficient bile salt pool include the rate of bile salt synthesis, concentration of bile salt by the gallbladder, and efficient reabsorption of bile salt conjugates in the intestine. The latter component of bile salt homeostasis has been examined by several investigators. These studies have been performed on immature laboratory animals and on human infants using such techniques as villus incubation, in vivo perfusion, and everted gut sacs, and they have demonstrated that the active reabsorption mechanism in the ileum is not present in the mammalian neonatal period (1–3, 5). In an effort to define more exactly the membrane site for postnatal development of taurocholate transport in the rat ileum, we have used brush border membrane vesicles prepared from the ileal mucosa of 2-, 3-, and 6-wk-old rats.

Lucke et al. (10), using brush border membrane vesicles from the adult rat ileum, have demonstrated the presence of a saturable Na^+-taurocholate cotransport system. A similar taurocholate transport mechanism has been characterized by Wilson and co-workers, who used membrane vesicles prepared from the renal cortex of adult rats (9). Our studies in 3- and 6-wk-old rats confirm the Na^+-dependent nature of taurocholate transport; however, under the conditions of our experiments there was no "overshoot" phenomenon. An overshoot represents a transient intravesicular accumulation of substrate, e.g., taurocholate, in excess of the uptake value at equilibrium. Previous studies of bile acid transport in adult guinea pigs (8), hamsters (11), and rats (10) have indicated that there is a slight overshoot that is maximal at about 1 min of incubation and is ~1.4 times the equilibrium uptake value. We have demonstrated that ileal brush border vesicle permeability to ^{22}Na decreases with the rat's increasing age (unpublished observations). Therefore, it is possible that 3- and 6-wk-old ileal membrane vesicles have sufficient permeability for Na^+ to dissipate the Na^+ gradient before the time when the bile acid carrier displays an overshoot for taurocholate in the adult rat. (As a control measure, our ileal brush border membrane vesicles were incubated in the presence of 0.1 mM glucose and 100 mM Na^+ gradients. The abrupt and dramatic overshoot that is characteristic of Na-glucose cotransport was readily apparent.) The 2-wk-old (suckling) rat ileal brush border membrane does not appear to transport taurocholate by a Na^+-dependent mechanism since there were no significant differences in uptake in the presence of a Na^+ or K^+ gradient.

A direct effect of Na^+ on the transport system for taurocholate and the saturable nature of the transport system is indicated by the tracer exchange experiments in 3- and 6-wk-old rats. The respective V_{max} values of 844 and 884 pmol/120 s per mg protein indicate that the active transport capabilities of the ileal brush borders are similar. The K_m values are also similar, suggesting that the Na-taurocholate cotransport system does not substantially change between 3 and 6 wk of age in the rat. In 2-wk-old rat membrane preparations there are no significant differences in tracer exchanges across membranes that are Na^+ or choline equilibrated; consequently, taurocholate transport during the suckling period does not appear to be an active, carrier-mediated process.

Humoral or dietary factors responsible for the appearance of the Na^+-coupled cotransport system are unclear. Little and Lester (3) could accelerate the appearance of active bile salt transport by treating pregnant rats or their suckling pups with dexamethasone. Related observations were made by Watkins et al. (19), when they studied bile salt pool size and synthesis rates in premature infants whose mothers had received dexamethasone before delivery. Thus, it appears that glucocorticoids, already known to mediate several intestinal changes at the time of weaning in the rat (20), may play an integral role in the maturation of bile salt transport.

The importance of a membrane potential on taurocholate transport is somewhat controversial. Some investigators suggest an electroneutral process (7, 12), while others emphasize the electrogenic nature of bile acid transport (9, 10). We created an "inside" diffusion potential using valinomycin, but no stimulation of taurocholate uptake occurred either when uptake was compared with a control incubation without valinomycin or a control incubation without intravesicular K^+. Our results, therefore, support the concept of an electroneutral transport process in all three age groups.

In summary, taurocholate transport into rat ileal brush border membrane vesicles is mediated by an electroneutral, Na^+-coupled cotransport mechanism that is not fully operational in suckling rats. The active transport mechanism appears in the ileal brush border membrane between 2 and 3 wk of age, and the capacity for active taurocholate transport in weanling and adolescent rats is similar.

Acknowledgments

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