Intestinal Absorption of Unconjugated Dihydroxy Bile Acids: Non-Mediation by the Carrier System Involved in Long Chain Fatty Acid Absorption

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Experiments were performed using isolated mucosal cells from the rat jejunum or using the perfused jejunum in the anesthetized rat to test whether lipophilic unconjugated dihydroxy bile acids are absorbed from the proximal small intestine via the same carrier mechanism involved in the uptake of long chain fatty acids. With isolated jejunal mucosal cells, the cellular uptake rate of deoxycholic acid or chenodeoxycholic acid increased linearly with time, showed no evidence of saturation, and was not decreased by the presence of a monospecific antibody to the membrane fatty acid binding protein. In contrast, olate uptake was saturable, was inhibited by the same antibody, but was not affected by the presence of chenodeoxycholic acid or deoxycholic acid. Bile acid uptake by isolated enterocytes occurred at one-eighth the rate of fatty acid uptake if expressed in relation to total solute concentration; if expressed in relation to monomeric concentration, initial bile acid uptake was four orders of magnitude slower than fatty acid uptake. In the isolated perfused jejunal segment, chenodeoxycholic acid and deoxycholic acid uptake was not influenced by the presence of the antibody to membrane fatty acid binding protein, whereas absorption of oleate was inhibited by more than 70%. These experiments indicate that absorption of unconjugated lipophilic dihydroxy bile acids in the rodent jejunum does not involve the carrier mediated uptake mechanism involved in the absorption of long chain fatty acids—the mechanism is likely to be passive nonionic diffusion.


The absorption of endogenous unconjugated bile acids from the distal small intestine is a normal physiologic event in humans (1), and absorption of unconjugated bile acids from anywhere in the small intestine may occur in pathological conditions causing increased bile acid deconjugation (2). Efficient absorption of exogenous unconjugated bile acids is desirable when they are administered for therapeutic purposes, such as cholesterol gallstone dissolution (3) or to correct inborn errors of bile acid biosynthesis, such as cerebrotendinous xanthomatosis (4).

Previous studies have shown that lipophilic dihydroxy bile acids, such as chenodeoxycholic acid (CDC) or deoxycholic acid (DC), are rapidly absorbed by the perfused jejenum, but that cholic acid, an unconjugated trihydroxy bile acid, is absorbed more slowly (5-9). Because the absorption rate of unconjugated bile acids is inversely proportional to their hydrophilicity, as assessed by octanol/water partition coefficients (10), it has generally been assumed that bile acids are absorbed passively by partitioning into the lipid domains followed by subsequent “flipflop” across the lipid bilayer. The rates at which unconjugated bile acids permeate artificial bilayers have recently been reported and have been shown to be inversely proportional to the hydrophilicity of the molecule (11).

Whether passive absorption is the sole mechanism of absorption of lipophilic unconjugated bile acids has not been examined experimentally. Recent studies on the mechanism of long chain fatty acid uptake by isolated intestinal cells have suggested that fatty acid absorption is, at least in part, a carrier mediated process. Two lines of evidence have supported this hypothesis. First, long chain fatty acid uptake has been shown to be saturable; second, fatty acid uptake can be inhibited by a monospecific antibody prepared against a membrane fatty acid binding protein (MFABP) (12,13). This antibody also has been shown to inhibit olate uptake by isolated hepatocytes (14,15) and cardiomyocytes (16).

The chemical structure of unconjugated dihydroxy bile acids can be considered to be analogous to hydroxy fatty acids such as ricinoleic acid in that an acidic group is present at the end of a hydrophobic body with one or more hydroxyl groups. We hypothesized that the intestinal absorption of lipophilic unconjugated bile acids might also use the putative carrier system involved in long chain fatty acid absorption. If so, the absorption of such unconjugated bile acids should be saturable, should inhibit that of fatty acids, and should be inhibited by the monospecific antibody to MFABP. Two experimental approaches were used to test this hypothesis: The first measured the rate of uptake of solutes by isolated rat jejunal enterocytes; and the second measured the rate of uptake of solutes by a jejunal segment perfused in single pass fashion in the anesthetized rat. For both, the uptake rate of the unconjugated bile acids CDC and DC was compared with that of olate. The effect of increasing bile acid concentration on uptake of olate was also examined using isolated enterocytes. Finally, the effect of added monospecific antibody to MFABP on the uptake of either bile acids or olate was defined using both systems.

MATERIALS AND METHODS

Materials. [24,14C]Chenodeoxycholic acid (CDC) and [24,14C]deoxycholic acid (DC) were prepared using a modification of the method of Tserng et al. (17). The final products, with a specific activity of 50 μCi/μmol, were purified by thin-layer chromatography (TLC). [9,10-3H]-Oleic acid, L-[2,3-3H]-alanine, [1,2,14C]- and [1,2,3H]-polyethylene glycol 4000 ([14C]PEG, [3H]PEG) and Aquasol

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were from New England Nuclear, Dreieich, West Germany. Collagenase (type I) was from Worthington, Freehold, NJ. Nonradioactive CDC was generously provided by Diamalt AG, Raubling, West Germany; and DC was purchased from Aldrich Chemical Co., Milwaukee, WI. Oleic acid, bovine serum albumin (fraction V, essentially fatty acid free), D-glucose, L-glutamine, L-alanine, insulin (bovine), PEG 4000, and HEPES were from Sigma Chemie GmbH, Deisenhofen, West Germany.

All reagents were of analytical grade; doubly distilled, deionized water was used in all experiments. Glassware was acid washed.

Wistar rats were obtained from the Zentralinstitut für Versuchstiere, Hannover, West Germany, and were fed a standard chow diet (Altromin 1314).

Preparation of enterocytes. Jejunal mucosal cells of male Wistar rats (200–250 g body weight) that were fasted overnight were prepared by a vascular collagenase perfusion technique (12). The final combined mucosal cell preparation was filtered through nylon gauze (70 μm pore size), centrifuged at 70 × g for 2 min, and washed twice using the incubation medium which contained 125 mM NaCl, 2.6 mM KCl, 5.7 mM NaH2PO4, 1.2 mM KH2PO4, 10 mM HEPES buffer and 5.5 mM glucose (pH 7.4). Thereafter the cells were diluted to 2 × 10⁶ cells/ml in incubation medium (20°C). To maintain the physiological transcellular ion gradients of Na⁺ and K⁺, it was essential to use the cells within 2 hr after preparation and to prevent their exposure to temperatures below 20°C, as such is known to reduce Na⁺/K⁺-ATPase levels markedly (18). Viability and purity of the mucosal cell preparations were assessed by phase contrast microscopy and the ability to exclude trypan blue dye (16). Additional criteria of viability were based on the intracellular K⁺-concentration determined by atomic absorption spectroscopy as well as the release of lactate dehydrogenase (LDH) (19,20).

The isolated enterocytes retained both morphological and functional integrity. Phase contrast microscopy of the isolated mucosal cell preparations demonstrated that 92 ± 4% (M ± SD) of the cells revealed the typical features of intestinal epithelium with oval or elongated cell bodies, basal nuclei, and prominent brush borders at the apical pole. Of these cells, 93 ± 5% excluded trypan blue; the intracellular K⁺-concentration remained >85 mM; and loss of cellular LDH was less than 15% during 3 hr after preparation of the cells. As another criterion of their functional integrity, the uptake kinetics of L-[2,3-³H]alanine, which is known to be actively transported, were examined as described (12). With increasing L-[³H]alanine concentrations, the cellular influx showed saturation kinetics with a Vₘₙₐₓ of 17.0 nmol × min⁻¹ per 10⁶ mucosal cells and a Kₘ of 2.3 mM.

Cellular uptake studies. Isolated jejunal mucosal cells (125 μl, 2 × 10⁶ cells/ml) were incubated with 368 μM CDC or 368 μM DC or with 173 μM [³H]oleate bound to albumin in molar ratios of 1:1 and 2:1, prepared as previously described (12). The final incubation volume was 1 ml, the temperature 37°C. After certain incubation periods, 200 μl sample aliquots were pipetted into 3 ml of ice-cold incubation medium or in experiments with [³H]oleate into 3 ml ice-cold 0.5% albumin in incubation medium (stop/chase-solution) (16). After vacuum filtration, the cell associated radioactivity was measured as described previously (12). Nonspecific association of radioactivity to filters and cells was determined in each experiment by adding the cold stop solution before the addition of corresponding aliquots of cells and radioactive solutes. This blank always constituted less than 2% of the incubated radioactivity and was subtracted from all determinations. All incubations were performed in triplicate and all observations were confirmed with at least three separate cell preparations. The fraction of [³H]oleate incorporated into cellular lipids and the amount oxidized to CO2 during the course of uptake was determined as previously described (21).

Antibody inhibition studies. To determine the effect of the antibody to the MFABP on cellular influx of CDC, DC and oleate, 2 ml of the cell suspension (2 × 10⁶ cells/ml) were incubated for 30 min at room temperature in gently rotating polypropylene tubes with 100 μg of the IgG-fraction of the antiserum to the MFABP isolated from rat liver or of the preimmune serum as controls (16). After centrifugation and washing the cells three times in medium, the viability of the cells remained >90% as determined by trypan blue exclusion. The cells were then diluted to 2 × 10⁶ cells/ml and solute uptake was examined as described above.

Absorption of solutes by in vivo single pass perfused jejunal segments. Male Wistar rats (200–250 g body weight) were fasted overnight and anesthetized with pentobarbital. Jejunal segments of 25 cm length were cannulated beginning at the ligament of Treitz. The perfusate contained 85 mM NaH₂PO₄/45 mM NaH₂PO₄ (pH 7.4). PEG 4000 containing [¹⁴C]PEG or [³H]PEG was added to a concentration of 5 g/l (5 μCi) and used as a nonabsorbable marker of the perfusate. After flushing the intestinal lumen with 20 ml of this medium, perfusion was started at a flow rate of 2 ml/min. The abdominal cavity was closed and the body temperature of the anesthetized rat, as well as of the perfusion medium, were maintained at 37°C. After an equilibrium period of 20 min, the absorption rate of 500 μl bolus injections with increasing quantities (37.5–375 nmol) of [¹⁴C]CDC or [¹⁴C]DC, as well as of 0.1–5 μmol [³H]oleate in the presence of equimolar concentrations of Na taurocholate was examined. The effusate was collected over a period of 10 min, at which time no further radioactivity appeared in the effluent. For all perfusion studies it was shown that the recovery of PEG was between 98–100%. The fraction of solute absorbed was calculated from the difference of the total amount of radioactivity infused in relation to the amount of radioactivity that was recovered. Absorption rates (nmol solute/min/25 cm jejunal segment) were analyzed as a function of the logarithmic mean of the solute concentration in the perfused segment, using the assumption that the concentration of fatty acid at the beginning of the test segment was equal to that of the infused bolus (22). Clearance of solutes (ml/min) was defined as the rate of solute absorption divided by the logarithmic mean concentration of the solutes in the perfused segment (22). The clearance calculated in this manner is certainly a very crude measure of the permeation ratios, but does permit uptake rates of different ligands to be compared.

For determination of the effect of the anti-MFABP on the overall absorption process of bile acids and fatty acids, the gut lumen was perfused in a recirculating