Interactions of cationic bile salt derivatives with the ileal bile salt transport system

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Abstract Previous structure–activity studies of the active ileal bile salt transport system have demonstrated that a single negative charge on the side chain is essential for active transport. Furthermore, mutual inhibition studies between different pairs of bile salt substrates indicated that dihydroxy bile salts had a greater apparent affinity for the transport system than the trihydroxylated compounds and triketo bile salts had the least such affinity. In this study, a series of cationic bile salt derivatives (cholamine conjugates) were prepared with one, two, and three α-hydroxyl groups on the steroid moiety. Based on the previous observations one would expect (*1*) no active transport of any of the cholamine conjugates by the ileal transport system; (*2*) interaction of these compounds with the transport system in such a way as to inhibit the transport of bile salts, with inhibition potency of the transport of any single bile salt inversely related to the number of hydroxyl groups present on the cholamine conjugate; and (*3*) transport of triketo anionic bile salts to be most readily inhibited, trihydroxy compounds less readily inhibited, and dihydroxy bile salts least inhibited. Using everted gut sac preparations it was demonstrated that all three aforementioned expectations did occur. Furthermore, reversible inhibition of ileal absorption of taurocholate and the bile salt derivative taurodehydrocholate could be demonstrated in vivo. The dihydroxy cholamine conjugates were better inhibitors than the trihydroxy compound. Relative specificity for the bile salt system of these cationic bile salt derivatives was demonstrated in the in vivo preparation by comparing its inhibition of taurodehydrocholate absorption with their lesser capacity to inhibit glucose transport.

Supplementary key words active bile salt transport · intestinal bile salt absorption · refractory substrates

Structure–activity studies of the ileal bile salt transport system have demonstrated that a single negative charge on the substrate side chain is a critical requirement for active transport. In addition, mutual inhibition studies suggested that the apparent affinity of the transport system for the substrate increases with fewer hydroxyl groups on the steroid portion of the bile salt (1). These observations prompted the preparation and study of a series of positively charged bile salt derivatives, cholamine conjugates, with one, two, and three α-hydroxyl group substituents on the steroid moiety. Because of the positive charge it would be expected that the cholamine conjugates would be refractory to transport by the ileal transport system. However, some of these compounds might inhibit the transport of anionic bile salt substrates by binding at the site of transport. Furthermore, the inhibitory potency of the cholamine conjugates should vary inversely with the number of hydroxyl groups on the steroid ring. This paper reports the results of such studies.

METHODS

Materials

Cholic, deoxycholic, chenodeoxycholic, and lithocholic acids used in the preparation of conjugated compounds were recrystallized before use as previously described (2). [24-^{14}C]Cholic acid and [1,2-^{14}C]taurine were obtained from New England Nuclear Corp. [24-^{14}C]Lithocholic acid and [24-^{14}C]chenodeoxycholic acid were products of Mallinckrodt Chemical Works, Science Products Division. [24-^{14}C]Deoxycholic acid was obtained from the International Chemical and Nuclear Corp. Bile salts conjugated with taurine were prepared and purified as previously described (3). The cationic cholamine-conjugated bile acid derivatives were prepared and purified by the general method described previously (2).

Transport studies

In vitro studies of transport utilized the everted gut sac preparation of Wilson and Wiseman (4). All experiments were performed with small intestines from young, fasted guinea pigs of the Hartley strain. The general experimen-

Abbreviations: DMSO, dimethylsulfoxide; ser/muc, serosal/mucosal concentration ratio.

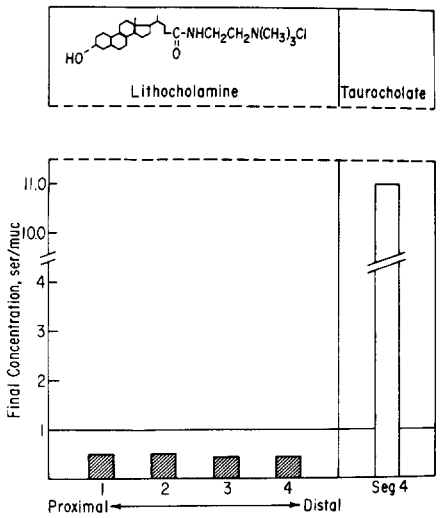

Fig. 1. Incubations of everted sacs from guinea pig small bowel with [^{14}C]lithocholamine. 9-cm gut sacs were made from the center of each quarter of intestine. Incubations with [^{14}C]taurocholate used similar sacs taken from the most distal quarter adjacent to the experimental sac. Each bar represents the average of four incubations. Initial concentrations of substrate in the serosal and mucosal compartments, 10 μg/ml. DMSO was present in 3% concentration in all incubations, including those with taurocholate. Initial volume of mucosal fluid, 10.0 ml; serosal, 1.5 ml; time, 90 min; temperature, 37°C.

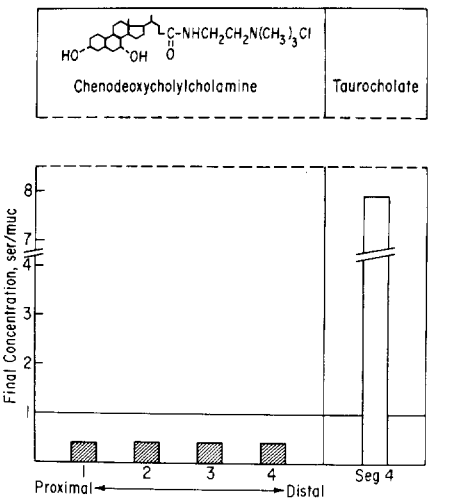

Fig. 2. Incubation of everted guinea pig intestinal sacs with chenodeoxycholylcholamine. Experimental conditions are similar to those described in Fig. 1 except that DMSO was omitted.

tal conditions were the same as those described previously (2).

Two major types of experiments were performed: (*1*) To ascertain whether the cationic bile salt derivatives were actively transported by the ileal bile salt transport system, everted gut sacs 9 cm long were prepared from the midportion of each quarter of the small bowel. The concentrations of ^{14}C-labeled bile salts and ^{14}C-labeled bile salt derivatives in the mucosal and serosal compartments at the end of the incubations were determined in a Beckman liquid scintillation counter, model LS-150, equipped with an external standard. Initially, the substrate was present in equal concentrations in the serosal and mucosal compartments. In this type of preparation, evidence for transport of solute against its concentration is the generation of a final serosal-to-mucosal concentration ratio in excess of unity. (*2*) In determining whether any of the cationic bile salt derivatives could inhibit the transport of regular substrates of the active bile salt transport system, gut sacs were prepared from the distal ileum only (segment 4). Each ileum provided four 9-cm sacs. Four animals were used for each experiment, and the sacs were staggered in the previously described manner in order to obviate differences between animals (2). This procedure permits studying the inhibitory effectiveness at three concentrations. In these experiments the substrate was present as the radioactive compound and the inhibitor contained no radioactive label. Inhibitions of active transport in such in vitro preparations can be demonstrated by depressions of final serosal-to-mucosal concentration ratios. The degree of depression of bile salt removal from the mucosal compartment is used to quantitate inhibition and permits the determination of the relative order of inhibitory potency of this series of compounds.

In these experiments one must consider the possibility of volume changes of the fluid remaining on the mucosal side. When such measurements were made in comparable incubations (5), volume changes were found to be sufficiently low to allow the assumption of constancy (10 ml) for all calculations.

Incubations containing lithocholamine required the presence of dimethylsulfoxide (DMSO) in order to enhance its solubility. Lithocholamine was dissolved in a specific amount of DMSO, which was then added to the incubation solutions to give the specified concentrations. The final concentration of DMSO was 3%. Control experiments demonstrated that DMSO when present at 2, 3, or 4% had no effect on the ability of everted gut sacs to transport bile salts. All solutions, including those containing bile salts together with the cationic derivatives, were clear.

In vivo studies

Studies of the ability of these cationic bile salts to inhibit in vivo intestinal absorption of the test substrates, taurodehydrocholate and taurocholate, utilized the guinea pig preparation and perfusion apparatus described by Heaton and Lack (6). The animals were maintained under light ether anesthesia. In each experiment the cystic duct was tied off and the common bile duct cannulated for bile collections. Ileal segments were perfused at a constant rate in the following order.

(*1*) A solution containing a specified concentration of ^{14}C-labeled sodium taurocholate or ^{14}C-labeled sodium taurodehydrocholate was perfused for 50 min.

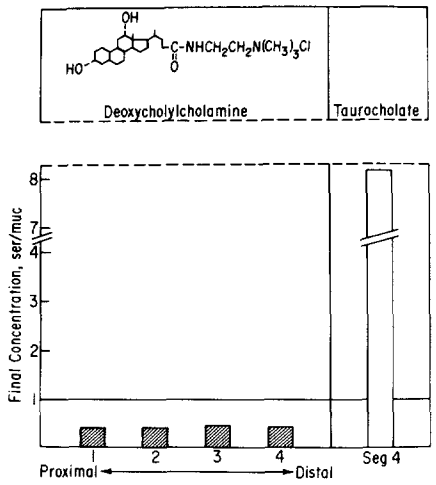

Fig. 3. Incubations of everted guinea pig gut sacs with deoxycholylcholamine. Experimental conditions are similar to those of Fig. 2.

(*2*) A solution containing the same concentration of either ^{14}C-labeled sodium taurodehydrocholate or taurocholate plus the cholamine derivative being investigated was perfused for 50 min.

(*3*) The control solution used in (*1*) was then perfused for 50 min.

All solutions were in isotonic saline with 0.01 M sodium phosphate buffer, pH 7.0. Intestinal absorption of perfused substrate was quantitated by measuring the rate of its appearance in bile collected via the common bile duct cannula.

Liver perfusion experiments

To demonstrate that the inhibition of taurodehydrocholate observed in the in vivo perfusion experiments took place at the intestinal site rather than in the liver, experiments were performed in which the liver was perfused directly. A mesenteric vein was cannulated with fine polyethylene tubing that was then connected to a multispeed, slow-infusion pump (Harvard Apparatus Co., Millis, Mass.). Solutions of sodium taurodehydrocholate, with and without the inhibitor deoxycholylcholamine, made up in isotonic saline buffered at pH 7.3 with 0.01 M sodium phosphate buffer were infused into the vein at a rate of 0.20 ml/min. The concentration of taurodehydrocholate was selected such that the liver was presented with approximately the same amount as the average value that was found to be excreted by the livers in the ileal perfusion experiments. The concentration of the inhibitor was in the same ratio to the substrate concentrations as in the ileal perfusions.

Glucose absorption experiments

To study the effect of one of these compounds on in vivo glucose transport, it was necessary to use a different technique for measuring absorption. A solution of glucose was made up in Krebs-Ringer phosphate buffer and perfused through the ileum in the usual way. The effluent was collected in 10-min aliquots, and its glucose concentration was measured by the enzymic Glucostat (Worthington Biochemical Corp.) method. Any decrease in concentration was considered to represent absorption. After 50 min the perfusion fluid was changed so that it now contained a bile salt derivative as well as the same concentration of glucose. After a further 50 min, glucose alone was perfused again.

RESULTS

Figs. 1–3 depict results of a series of experiments designed to determine whether the cholamine derivatives can be actively transported by everted guinea pig gut sacs. Each of the bars represents the average of four individual gut sac incubations. In no instance was the final serosal-to-mucosal concentration ratio in excess of 1.0. Because active transport was not observed with these compounds, it was necessary to demonstrate that these particular ileum preparations were capable of active bile salt transport. Simultaneous gut sac incubations were performed with ^{14}C-labeled sodium taurocholate. These control sacs were taken from ileal sections (segment 4) immediately adjacent to the experimental sacs. In all instances, active transport of taurocholate was observed. Not shown are comparable experiments with the trihydroxy derivative, cholylcholamine. It has previously been demonstrated (2) that this compound, like the others shown here, cannot be transported by the ileum.

Table 1 presents data that assess the ability of these compounds to inhibit the active transport of taurocholate, taurodehydrocholate, and taurochenodeoxycholate. It can be seen that lithocholylcholamine (one hydroxyl group) is the most effective inhibitor of taurocholate transport. Approximately 50% inhibition of transport was observed at a concentration of 0.20 μmole/ml. In contrast, cholylcholamine was the least potent inhibitor; 1.52 μmoles/ml, the highest concentration tested, inhibited transport by 35%. The two dihydroxy compounds, chenodeoxycholylcholamine and deoxycholylcholamine, were equal in their potency and were better inhibitors than the trihydroxy compound but less active than the monohydroxylated derivative. 50% inhibition values obtained by interpolation of these data are 0.34 μmole/ml for chenodeoxycholylcholamine and 0.36 μmole/ml for deoxycholylcholamine.

When deoxycholylcholamine was tested for its ability to inhibit taurodehydrocholylcholamine, the 50% inhibition value was determined to be 0.17 μmole/ml, approximately twice the potency observed when taurocholate was used as the test substrate. Deoxycholylcholamine was least effective in inhibiting transport of the dihydroxy bile salt tauroche-

TABLE 1. In vitro inhibition of bile salt transport

Expt. No.	Initial Concentration (μmoles/ml) Substrate	Inhibitor	Substrate Transported: Final Serosal/Mucosal Ratio	μmoles Transported[a]	% of Control
				mean ± SEM	
1	Taurocholate	Lithocholamine			
	0.37	0	6.7	1.92 ± 0.12	100
	0.37	0.050	3.8	1.23 ± 0.19 a	64
	0.37	0.100	3.2	1.16 ± 0.12 b	60
	0.37	0.200	2.4	0.91 ± 0.08 b	48
2	Taurocholate	Deoxycholylcholamine			
	0.37	0	17.0	2.71 ± 0.09	100
	0.37	0.20	9.5	2.21 ± 0.30 NS	82
	0.37	0.39	3.6	1.27 ± 0.19 a	47
	0.37	0.78	1.3	0.45 ± 0.11 b	17
3	Taurocholate	Chenodeoxycholylcholamine			
	0.37	0	7.8	2.14 ± 0.03	100
	0.37	0.20	4.8	1.53 ± 0.16 b	71
	0.37	0.39	2.6	0.90 ± 0.31 a	42
	0.37	0.78	1.0	0.22 ± 0.04 b	10
4	Taurocholate	Cholylcholamine			
	0.37	0	8.3	2.14 ± 0.09	100
	0.37	0.38	6.3	1.88 ± 0.09 b	88
	0.37	0.76	5.6	1.80 ± 0.14 a	84
	0.37	1.52	3.6	1.39 ± 0.14 b	65
5	Taurodehydrocholate	Deoxycholylcholamine			
	0.37	0	4.8	1.22 ± 0.13	100
	0.37	0.047	3.8	0.92 ± 0.04 NS	76
	0.37	0.095	3.1	0.79 ± 0.06 a	65
	0.37	0.190	2.4	0.58 ± 0.04 b	48
6	Taurochenodeoxycholate	Deoxycholylcholamine			
	0.37	0	3.5	1.82 ± 0.13	100
	0.37	0.20	2.3	1.57 ± 0.05 NS	86
	0.37	0.39	1.4	1.21 ± 0.05 b	66
	0.37	0.78	1.1	0.79 ± 0.05 b	43
7	Taurochenodeoxycholate	Taurodehyrocholycholamine			
	0.37	0	3.8	1.86 ± 0.08	100
	0.37	0.37	3.3	1.76 ± 0.09 NS	95
	0.37	0.74	3.6	1.86 ± 0.12 NS	100
	0.37	1.48	3.7	1.79 ± 0.15 NS	96
8	Taurodehydrocholate	Chenodeoxycholylcholamine			
	0.37	0	17.0	2.71 ± 0.09	100
	0.37	0.10	9.5	2.21 ± 0.30 NS	82
	0.37	0.20	3.6	1.27 ± 0.19 a	47
	0.37	0.39	1.3	0.45 ± 0.11 b	17

[a] Removed from mucosal compartment. Incubation was for 90 min at 37°C. Values are the means from four gut sacs. NS, not significantly different from control; a, significantly different from control, $P < 0.05$; b, significantly different from control, $P < 0.01$; paired t test. Controls are assigned a value of 100%.

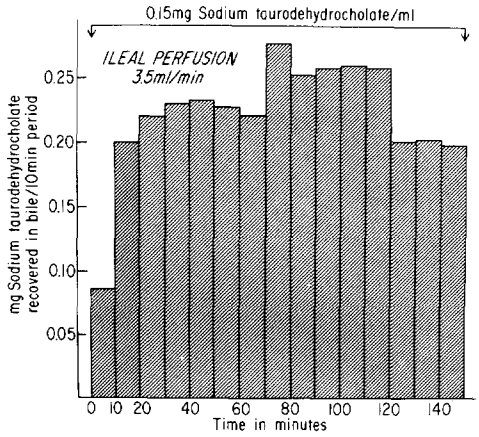

Fig. 4. Recovery of [^{14}C]taurodehydrocholate in bile during ileal perfusion with sodium taurodehydrocholate. Each bar represents total recovery for a 10-min period. Initiation and termination of ileal perfusion are indicated by the arrows.

nodeoxycholate. In this case, 0.65 μmole/ml would be necessary to inhibit transport by 50%.

The ability of these compounds to inhibit the in vivo absorption of bile salts from the ileum was studied with sodium taurocholate and sodium taurodehydrocholate as the test substrates. As shown previously (6), taurocholate, when perfused through the ileum, is absorbed at a fairly constant rate. This was also found to be true for sodium taurodehydrocholate (**Fig. 4**).

Fig. 5 depicts a typical in vivo inhibition experiment. Throughout the entire experiment, 0.15 mg/ml taurodehydrocholate was perfused through the ileum of the guinea pig. Between 50 and 100 min, deoxycholylcholamine, 0.15 mg/ml, was perfused together with taurodehydrocholate. It can be seen that inhibition of taurodehydrocholate absorption took place. This inhibition was reversed during the last 50 min, when no inhibitor was present. Repeating the above experiment with the concentration of inhibitor increased to 0.30 mg/ml showed that taurodehydrocholate

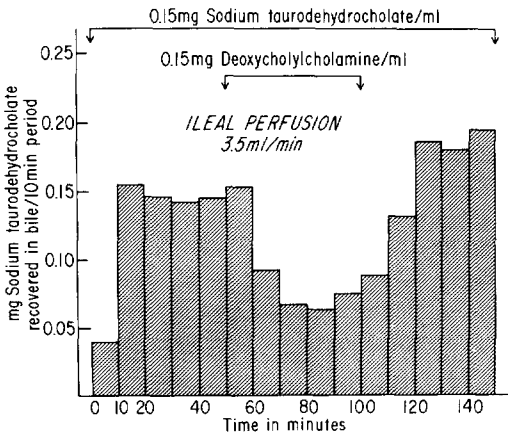

Fig. 5. Effect of deoxycholylcholamine on ileal absorption of taurodehydrocholate.

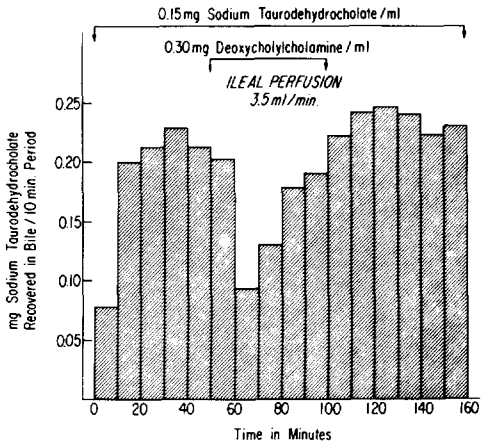

Fig. 6. Effect of deoxycholylcholamine at a concentration of 0.3 mg/ml on ileal absorption of taurodehydrocholate.

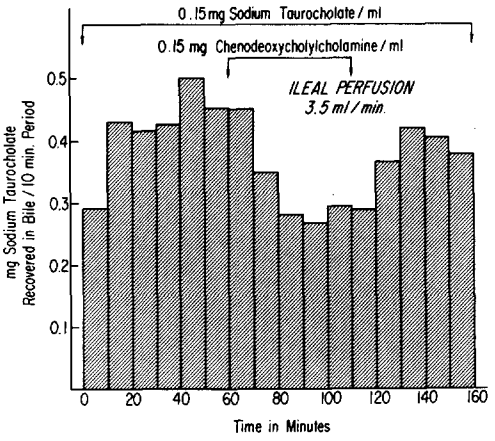

Fig. 7. Effect of chenodeoxycholylcholamine on ileal absorption of taurocholate.

absorption was initially depressed. However, as demonstrated in **Fig. 6**, the initial inhibition was followed by a gradual increase in absorption to approximately 120% of the predeoxycholylcholamine levels. This apparent alteration of permeability precluded the testing of cationic compounds at concentrations in excess of 0.15 mg/ml. **Fig. 7** shows that under such conditions taurocholate absorption is inhibited by chenodeoxycholylcholamine.

Table 2 gives the results of 12 in vivo experiments. Percentage inhibition was calculated from the average of the recovery in the two preincubation periods and the highest postinhibition period. This control value was then compared with the mean recovery in the last three periods of inhibition. Comparison of experiments 1, 2, and 3 with those numbered 7, 8, and 9 shows that inhibition of taurocholate absorption is less pronounced than that of tauro-

TABLE 2. In vivo inhibition of taurocholate and taurodehydrocholate absorption in guinea pigs

Expt. No.	Substrate	Concentration	Inhibitor	Concentration	Substrate Transported[a]		% Inhibition	Average
					Control	Experimental		
		μmoles/ml		*μmoles/ml*	*μmoles/10 min*			
1	Taurocholate	0.28	Chenodeoxycholylcholamine	0.29	0.79	0.50	37	
2	Taurocholate	0.28	Chenodeoxycholylcholamine	0.29	0.88	0.54	39	
3	Taurocholate	0.28	Chenodeoxycholylcholamine	0.29	1.51	0.79	47	41
4	Taurodehydrocholate	0.28	Cholylcholamine	0.28	0.43	0.36	16	
5	Taurodehydrocholate	0.28	Cholylcholamine	0.28	0.54	0.34	37	
6	Taurodehydrocholate	0.28	Cholylcholamine	0.28	0.61	0.53	13	22
7	Taurodehydrocholate	0.28	Chenodeoxycholylcholamine	0.29	0.34	0.085	75	
8	Taurodehydrocholate	0.28	Chenodeoxycholylcholamine	0.29	0.33	0.13	61	
9	Taurodehydrocholate	0.28	Chenodeoxycholylcholamine	0.29	0.47	0.17	64	67
10	Taurodehydrocholate	0.28	Deoxycholylcholamine	0.29	0.72	0.23	68	
11	Taurodehydrocholate	0.28	Deoxycholylcholamine	0.29	0.27	0.10	62	
12	Taurodehydrocholate	0.28	Deoxycholylcholamine	0.29	0.30	0.13	57	62

[a] Methods of selecting the 10-min collection periods for these calculations are described in the text. Dry weights of the intestinal sections, determined at the end of the experiments, ranged between 307 and 568 mg. Therefore, comparisons of transport during control periods between any two separate experiments are not valid. However, the nature of the perfusion apparatus and procedure ensured that the amount of mucosal surface in any single experiment was the same for the control and experimental periods. Control, minus inhibitor; experimental, plus inhibitor.

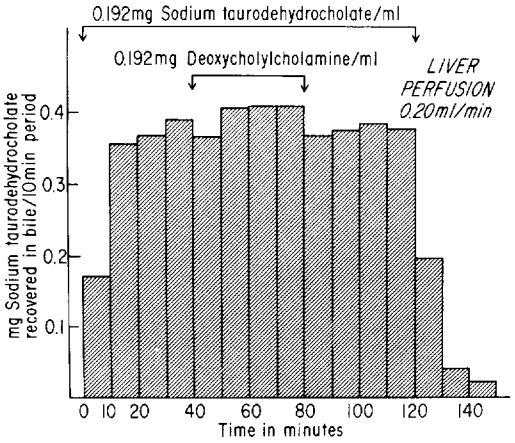

Fig. 8. Absence of the effect of deoxycholylcholamine on hepatic uptake and biliary excretion of taurodehydrocholate. Arrows indicate the order of perfusions into a mesenteric vein. In this experiment, 98% of the injected taurodehydrocholate was recovered in the bile. In another, comparable experiment, 102% of dehydrocholate was recovered.

dehydrocholate. In addition, one sees that the trihydroxy compound is a less effective inhibitor than either of the two dihydroxylated compounds tested, chenodeoxycholylcholamine and deoxycholylcholamine.

The results of a liver perfusion experiment are shown in **Fig. 8.** In this experiment, 98% of the infused taurodehydrocholate was recovered. It can be seen that the recovery rates of taurodehydrocholate were not affected when an equal concentration of deoxycholylcholamine was infused simultaneously.

Table 3 compares the ability of chenodeoxycholylcholamine to inhibit the in vivo absorption of taurodehydrocholate with its effects on glucose absorption. The concentration of inhibitor was selected so that its inhibitory potency against taurodehydrocholate absorption approximated the 50% inhibition value (actually 53%). Under these conditions, glucose absorption was inhibited in three of the four experiments; the average for the four was 10% inhibition. Differences between these two groups of experiments were significant ($P < 0.005$).

DISCUSSION

These investigations were designed to test whether the previously described structure–activity observations in the ileal bile salt transport system would permit preparation of a series of compounds with specific properties. We attempted to design a series of bile salt derivatives none of which would be transported themselves but of which some could react in a predictable manner with the ileal transport system and behave as refractory substrates. Two reasons could justify such an exercise. Not only would the previous characterizations of the transport system be further documented, but the systematic preparation of refractory substrates could provide experimental prototypes for the ultimate preparation of more potent and specific inhibitors of the ileal bile salt transport system.

Previous studies of structural requirements of the substrate for active transport by the ileal transport system included the effects of the charge on the side chain as well as the effects of hydroxylation of the steroid nucleus. It was

TABLE 3. In vivo inhibition of intestinal absorption in guinea pigs: comparison of inhibitions by chenodeoxycholylcholamine of glucose and taurodehydrocholate absorption

Expt. No.	Substrate	Concentration	Inhibitor	Concentration	Substrate Transported[a]		% Activity	Average
					Control	Experimental		
		μmoles/ml		*μmoles/ml*	*μmoles/10 min*			
1	Taurodehydrocholic	0.29	Chenodeoxycholylcholamine	0.16	0.76	0.38	50	
2	Taurodehydrocholic	0.29	Chenodeoxycholylcholamine	0.16	0.42	0.16	38	
3	Taurodehydrocholic	0.29	Chenodeoxycholylcholamine	0.16	0.25	0.14	56	
4	Taurodehydrocholic	0.29	Chenodeoxycholylcholamine	0.16	0.50	0.22	44	47[b]
5	Glucose	0.67	Chenodeoxycholylcholamine	0.16	5.38	3.89	72	
6	Glucose	0.67	Chenodeoxycholylcholamine	0.16	6.11	5.22	85	
7	Glucose	0.67	Chenodeoxycholylcholamine	0.16	1.83	1.61	88	
8	Glucose	0.67	Chenodeoxycholylcholamine	0.16	1.06	1.22	115	90[b]

[a] Control, minus inhibitor; experimental, plus inhibitor.

[b] Data were analyzed by statistical analysis of the logarithms of these percentage values. These numbers are significantly different. $t = 4.68$, $P < 0.005$ by Student's t test.

observed that cholylcholamine with a cationic side chain is not transported by everted ileal gut sacs (2). In other studies, bile salt derivatives containing two negative charges on the side chain were transported minimally, suggesting that a single negative charge on the side chain is necessary for active transport. This hypothesis was supported by in vivo and in vitro transport studies in media of different pH levels (7, 8). The requirement for a single negative charge applies only to the side chain region, because the introduction of an additional anionic charge at position 3 of the steroid does not preclude transport (9).

There is no requirement for a specific hydroxyl substituent on the steroid for transport (1, 2). However, in vivo and in vitro mutual inhibition studies have indicated that the apparent affinity of the bile salt for the transport system is greater with fewer hydroxyl groups. These studies showed that the effectiveness of one bile salt in inhibiting the transport of another correlated with the number of hydroxyl groups (2, 6). Dihydroxylated bile salts were more potent inhibitors of the transport of the trihydroxylated bile salts. Also germane to the present work was the finding that the triketo compounds, taurodehydrocholate and glycodehydrocholate, were both the poorest inhibitors and the compounds whose transport was most easily inhibited.

With these observations in mind it was considered reasonable to test a series of cationic bile salts having the same conjugated cholanic acid structure but with varying numbers of α-hydroxyl groups. This series of compounds could be expected to behave with respect to the ileal transport system in the following ways: (*1*) None of these compounds should actually be transported by the ileal gut sac preparations because the requisite anionic charge would be absent; (*2*) the potency of such compounds in inhibiting the transport of anionic bile salts should vary approximately inversely with the number of hydroxyl groups present on the inhibitor. In addition, one would expect the triketo cholamine compound to show the least inhibitory potential.

The results of the present studies demonstrate that none of the cholamine conjugates tested were actually transported by everted gut sacs. The data also show that inhibition of the transport of taurocholate by these substances is greater when the number of hydroxyl groups is decreased. The triketo cationic derivative has no capacity for inhibition.

One should also expect that any substance interacting with the transport system would be most effective in inhibiting the transport of taurodehydrocholate and least effective against taurodeoxycholate. This was also found to be the case.

These results also indicate that coulombic interaction (i.e., ion-pair formation) between substrates and inhibitors is not significant in explaining these results because such interactions, were they to occur, would result in equivalent inhibitions for all pairs of substances tested. Furthermore, experiments in which the aqueous–chloroform partition of [^{14}C]taurocholate was studied in the presence of excess chenodeoxycholylcholamine (under conditions of concentration and ionic strength approximating those described) suggest that a maximum of 2.0% of the bile salt could exist as an ion-pair complex with the cationic derivative.

The in vivo perfusion studies are consistent with the results obtained from the everted gut sac experiments. Thus, taurocholate is not inhibited as well as taurodehydrocholate. Furthermore, the cholamine compounds with two hydroxyl groups are better inhibitors than the trihydroxy compound cholylcholamine.

The present studies indicate that the cholamine conjugates of bile acids behave in a manner predicted from and consistent with the previous observations of the structure–activity determinants operative in the ileal bile salt transport system. The hepatic perfusion studies confirm that the action of these conjugates is on the intestinal mucosa. Relative specificity of the effect of the cholamine conjugates for the bile acid transport system is suggested by the mutual inhibition studies and by their minimal and inconsistent effect on intestinal glucose absorption.

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