Ionic requirements for the active ileal bile salt transport system

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Abstract Taurocholate transport by everted ileal gut sacs was studied in physiological media containing graded amounts of sodium ions. Significant uphill transport of taurocholate was observed when the bulk of NaCl was replaced by osmotic equivalents of mannitol or choline chloride. Seventy-seven percent of control transport activity was observed when 36 milliequivalents per liter of Na^+ were present in the incubation medium with mannitol acting as the isosmotic replacement, and 74% of the control transport was retained when 31 milliequivalents per liter of Na^+ were present in the incubation medium with choline chloride acting as the osmotic replacement. Lowering the Na^+ concentration to 19 milliequivalents per liter (i.e., 84% replacement of Na^+) still allowed for 69% of the uphill transport observed in the control incubations. Taurodehydrocholate transport by ileal everted sacs was more sensitive to decreased Na^+ concentrations; 29% of control transport was observed at 31 milliequivalents per liter of Na^+. A kinetic analysis comparing the transport of taurocholate with taurodehydrocholate, the triketo analogue, at different concentrations of Na^+ indicated that the apparent affinity of the transport system for Na^+ is greater in the presence of taurocholate than in the presence of taurodehydrocholate. The ability of taurodehydrocholate to depress taurocholate transport is less in media of low Na^+ concentration. Finally, in vivo intestinal perfusion studies demonstrated that the depression of taurocholate absorption, following Na^+ removal, is reversible. These results are in agreement with the idea that Na^+ has a physiological role in intestinal bile salt transport, and that the affinities of the anionic bile salt and the sodium cation for the transport system appear to be cooperative in that one enhances the binding of the other.

Supplementary key words sodium ion · mannitol · choline chloride · taurocholate · taurodehydrocholate

A critical role for sodium ions has been demonstrated for the active transport of nonelectrolytes by the intestine. These observations have included transport of sugars (1) and amino acids (2). In 1964 Holt (3) demonstrated that considerable inhibition of tissue uptake of sodium cholate by open ileal intestinal slices occurred when the concentration of Na^+ in the bathing medium was reduced to 24 mM. Playoust and Isselbacher (4) showed that replacement of sodium ions by potassium ions also depressed the active transport of taurocholate.

In our structure activity studies of the ileal bile salt transport system, we ascertained that for maximal transport the bile salt substrate requires a single negative charge on the side chain. Bile salts modified to possess two negative charges on the side chain are minimally transported in in vitro (5) and in in vivo preparations (6); however, lowering the pH of the medium increases the transport of these dibasic derivatives.

These latter observations led us to speculate that the active site of the transport system contains (*1*) a positive charge for coulombic interaction with the anionic bile salts and (*2*) a negative charge, closely associated with the positively charged site, that could repel bile salt derivatives containing a double negative charge in the side chain region, thereby preventing their efficient transport. It was further suggested that this anionic site in the membrane is normally associated with Na^+; thus the orientation of the naturally occurring bile salt at the active site (with its coulombic interaction with the positive membrane charge) and the occupation of the anionic membrane site by sodium ions could occur simultaneously in a cooperative manner.

These hypotheses have led us to study the role of Na^+ in the active transport of bile salt derivatives containing uncharged side chains. However, as a prerequisite for these studies, it was necessary to investigate the effects of sodium ions on the active transport of bile salts in in vitro and in vivo preparations. The experimental results reported here demonstrate that significant uphill transport of taurocholate occurs in vitro even when 85% of the Na^+ normally present in the incubation mixture was replaced. In

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vivo preparations that allow for continual monitoring of intestinal bile salt absorption show that sodium ion deprivation causes reversible inhibition of bile salt absorption.

Comparison of the sodium ion requirement for the transport of taurocholate with that of taurodehydrocholate indicated that the shape of the steroid moeity influences the susceptibility of the transport system to decreases in the concentration of sodium ions.

MATERIALS

Cholic and dehydrocholic acids used in the preparation of conjugated bile acids were recrystallized as previously described (7). [24-^{14}C]Cholic acid and [1,2-^{14}C]taurine were purchased from New England Nuclear Corporation, Boston, Mass. Bile salts conjugated with taurine were prepared and purified as previously described (8).

Transport studies

In vitro studies of transport utilized the everted gut sac preparations of Wilson and Wiseman (9). The sacs were made from the ileal region of young fasted guinea pigs of the Hartley strain (7). Incubation media consisted of Krebs-Ringer bicarbonate or Krebs-Ringer phosphate solutions. Replacement of specified amounts of Na^+ by osmotic equivalents of either mannitol or choline chloride allowed for the preparation of the experimental incubation media. The pH of all incubation solutions was 7.3. All solutions were assayed for Na^+ and K^+ by flame photometry and the osmolality was checked with an Osmette A osmometer. At the end of the experiments the incubation fluids were again assayed for Na^+ and K^+, and the osmolalities were rechecked. All results are presented in terms of these final electrolytic determinations.

The concentrations of ^{14}C-labeled bile salt substrate in the mucosal and serosal compartments were determined at the end of the incubations. Aliquots, 0.2 ml, were assayed for radioactivity by means of a Beckman liquid scintillation counter, Model LS-150, equipped with an external standard (Beckman Instruments, Fullerton, Cal.). Initially the substrate was present in equal concentrations in the mucosal and serosal compartments. Active uphill transport of these substrates is evidenced by final serosal to mucosal concentration ratios in excess of unity. A quantitative assessment of the active transport is made by determining the amount of material removed from the mucosal compartment under these conditions. These measurements must consider the possibility of volume changes in the fluid remaining on the mucosal side; when such measurements were made previously (10), the changes were sufficiently low to allow for the assumption of constancy (10 ml) for all calculations.

TABLE 1. Influence of Na^+ on the in vitro transport of bile salts

Expt. No.	Substrate	Initial Concentration	Na^+	K^+	Osmotic Replacement	Substrate Transported: Final Serosal Mucosal Ratio	Substrate Transported: Transport[a] Mean ± SEM	Substrate Transported: Control
		μmole/ml	*mEq/l*	*mEq/l*			*μmoles*	%
1	Taurocholate	0.37	152	7.5		8.3	2.21 ± 0.12	100
	Taurocholate	0.37	96	7.6	mannitol	6.6	2.03 ± 0.16	92
	Taurocholate	0.37	70	8.0	mannitol	6.3	1.94 ± 0.08	88
	Taurocholate	0.37	36	7.9	mannitol	5.3	1.70 ± 0.08	77
2	Taurocholate	0.37	149	7.0		3.3	1.61 ± 0.09	100
	Taurocholate	0.37	33	7.0	mannitol	2.3	1.19 ± 0.02	74
3	Taurodehydrocholate	0.37	152	7.0		3.1	0.94 ± 0.04	100
	Taurodehydrocholate	0.37	36	6.9	mannitol	1.4	0.27 ± 0.02	29
4	Taurocholate	0.37	150	7.1		8.5	2.15 ± 0.13	100
	Taurocholate	0.37	91	7.1	choline chloride	6.8	1.99 ± 0.08	93
	Taurocholate	0.37	62	7.1	choline chloride	5.9	1.84 ± 0.10	86
	Taurocholate	0.37	31	7.1	choline chloride	4.8	1.59 ± 0.11	74
5	Taurocholate	0.37	151	6.4		6.7	1.93 ± 0.14	100
	Taurocholate	0.37	19	6.7	choline chloride	3.6	1.34 ± 0.07	69

[a] Removed from mucosal compartment. Incubation was for 60 min at 37°C except for Experiment 2, which was for 30 min. Values are the means of four ileal gut sacs. Experiments 1, 2, 3, and 4 employed Krebs-Ringer bicarbonate buffer with the Na^+ concentrations modified as shown. Experiment 5 used Krebs-Ringer phosphate buffer with Ca^{2+} omitted. Gas phase for experiments 1, 2, 3, 4 was 95% O_2, 5% CO_2; for experiment 5, 100% O_2; volume of mucosal fluid 10 ml, serosal fluid 1.5 ml.

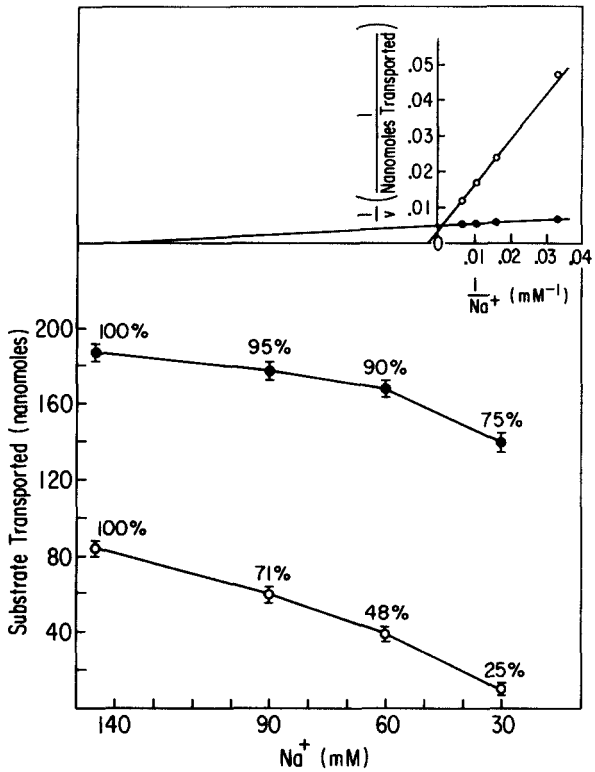

Fig. 1. Comparison of the in vitro transport of taurocholate with taurodehydrocholate in media of different Na^+ concentrations. Solutions were Krebs-Ringer bicarbonate with different amounts of Na^+. Mannitol was used as the osmotic replacement for NaCl. ●, Transport of taurocholate (each point is the mean ± SEM of 8 gut sacs). ○, Transport of taurodehydrocholate (each point is the mean ± SEM of 16 gut sacs). Concentration of substrate, 28 nmoles/ml.

The apparatus used to perfuse intestinal segments in situ was essentially that described previously (11). It was modified to allow for the independent perfusion of any one of four solutions, with no significant lapse in time when one perfusion solution was replaced by another. Absorption of [^{14}C]taurocholate from the perfused segment of intestine was quantitated by measuring the rate of its appearance in the bile that was recovered by means of a cannula in the common bile duct. With this preparation it is possible to compare the in vivo intestinal absorption of taurocholate from media of high Na^+ concentration with that from Na^+-depleted solutions. The Na^+-containing perfusion solution, isotonic Na_2SO_4 (buffered with 0.02 M sodium phosphate), was that described by Csaky and Zollicoffer (12) as suitable for in vivo perfusion. The Na^+-free solutions consisted of isotonic mannitol with 0.02 M Tris buffer. All solutions were buffered at pH 7.0.

TABLE 2. Inhibition of in vitro transport of taurocholate by taurodehydrocholate at regular and low Na^+ concentrations

Na^+	Initial Concentration of Taurocholate	Initial Concentration of Taurodehydrocholate (Inhibitor)	Taurocholate[a] Transported	Inhibition
mEq/l	*μmoles/ml*	*μmoles/ml*	*μmoles ± SEM*	%
150	0.37	0	2.46 ± 0.04	
150	0.37	18.0	1.43 ± 0.06	42 ± 2.1
46	0.37	0	2.06 ± 0.05	
46	0.37	18.0	1.56 ± 0.04	24 ± 2.8

[a] Removed from the mucosal compartment. Each value is the mean ± SEM of 11 gut sacs. Krebs-Ringer bicarbonate buffer solutions were used and modified with mannitol as the isosmotic replacement for NaCl. Incubation was for 60 min at 37°C. Gas phase, 95% O_2, 5% CO_2. Volume of mucosal fluid 10 ml, serosal fluid 1.5 ml.

RESULTS

Table 1 gives the results of four experiments in which the transport of taurocholate and taurodehydrocholate by everted ileal sacs incubated in media of different Na^+ ion concentrations was measured. Experiment 1 demonstrates that at 36 mM Na^+, 77% of the transport is retained. When the incubation time was reduced from 60 to 30 min, Experiment 2, the same results were obtained. In these studies mannitol was used to maintain isosmolality. Experiment 4 shows that replacement of mannitol with choline chloride yields the same results, i.e., 74% of control activity is retained at 31 mM Na^+. In contrast, taurodehydrocholate transport was inhibited to a greater extent under such circumstances, 29% of control activity being retained at 36 mM Na^+; this is shown in Experiment 3. These experiments employed Krebs-Ringer bicarbonate solutions and were limited in the amount of Na^+ reduction by the requirement for the bicarbonate buffer. When Krebs-Ringer phosphate buffer was used (Experiment 5), a final concentration of 19 mM Na^+ was achieved. Under these conditions uphill translocation of taurocholate was observed at 69% of control values.

Fig. 1 shows dose response data comparing the transport of taurocholate with taurodehydrocholate by gut sacs in media of 145, 90, 60, and 30 mM Na^+. A double reciprocal plot of transport vs. sodium ion concentration demonstrates that the triketo compound, taurodehydrocholate, is much more sensitive to the presence of this ion than is the transport of its (trihydroxy) natural analogue.

Table 2 compares the ability of taurodehydrocholate (18 mM) to inhibit the transport of taurocholate in the presence of high and low Na^+ concentrations. In normal Krebs-Ringer bicarbonate media, 42%

inhibition of taurocholate transport is observed. At concentrations of 46 mM Na^+ the transport of taurocholate is inhibited by 24%. These two values are significantly different ($P < 0.001$).

Figure 2A depicts a single in vivo perfusion experiment. Ileal taurocholate absorption is significantly reduced in the absence of Na^+ and this effect is reversible. Figure 2B summarizes five such perfusion experiments. Perfusions of the proximal small bowel, which does not have the active transport system, resulted in insignificant absorption that was not changed when isotonic mannitol replaced isotonic sodium sulfate (**Fig. 3**).

DISCUSSION

Previous workers who have studied Na^+ requirements of the ileal bile salt transport system suggested that active transport of bile salts was highly dependent on the presence of Na^+ ions. Holt (3), studying tissue uptake of unconjugated cholic acid by open intestinal rings made from rat ileum, reported a marked depression when Na^+ was replaced by either Li^+ or K^+. They also found that tissue accumulation was somewhat greater when choline chloride was used as the isosmotic replacement. Playoust and Isselbacher (4) incubated everted ileal (hamster) sacs in Na^+-depressed media with Li^+ and K^+ serving as cationic replacements. Tissue uptake, as well as accumulation in the serosal space, was depressed more than 60% when the Na^+ concentration was reduced to 60 mM. In these experiments the initial concentration of taurocholate in the serosal compartment was zero.

The work described in this paper was designed to assess uphill transport of taurocholate and taurodehydrocholate in sodium ion-depleted incubation solutions. Consequently the initial concentrations of substrate in both the mucosal and the serosal compartments were equal, and the transport was assessed by measuring the removal of substrate from the mucosal compartment. Furthermore, in these studies, the isosmotic replacement of NaCl was either mannitol or choline chloride. This avoided the complications that might arise from the use of K^+ or other cations such as Li^+, Rb^+, and Cs^+, which have been shown capable of penetrating the mucosal cells and eliciting biological effects in addition to acting as Na^+ replacements (2).

The data presented in Table 1 demonstrate a remarkable residual capacity of guinea pig everted gut sacs to transport taurocholate against its own concentration gradient when the sodium ion concentration is depressed. The concentration of taurocholate that was chosen, 0.37 μmoles/ml, insures against complications that might arise from self-association or micelle formation. Lowering the sub-

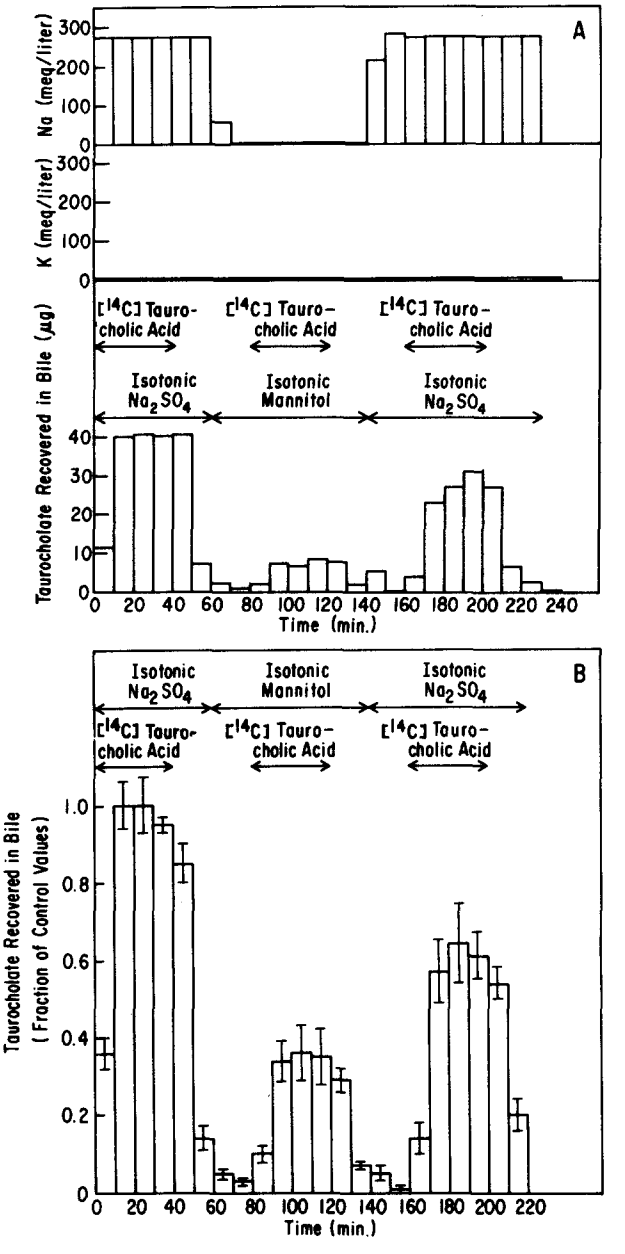

Fig. 2A. Recovery of [^{14}C]taurocholate in a guinea pig's bile during its ileal perfusion. The perfusion rate was 4 ml/min. Arrows indicate the types of perfusing solutions. When [^{14}C]-taurocholate was present in the perfusing solutions its concentration was 20 μg/ml. Each bar represents the recovery of taurocholate during a 10 min period. Also shown are the compositions of the perfusing solutions determined on aliquots of all fluid samples as they emerged from the perfused segment. **B.** Biliary recoveries of [^{14}C]taurocholate from five guinea pigs during ileal perfusion. Results are normalized and presented as fractions of the control values (averages of the third and fourth perfusion periods). The experimental conditions are the same as those described for Fig. 2A. The data from the experiment of Fig. 2A are included. Variance shown is ±SEM.

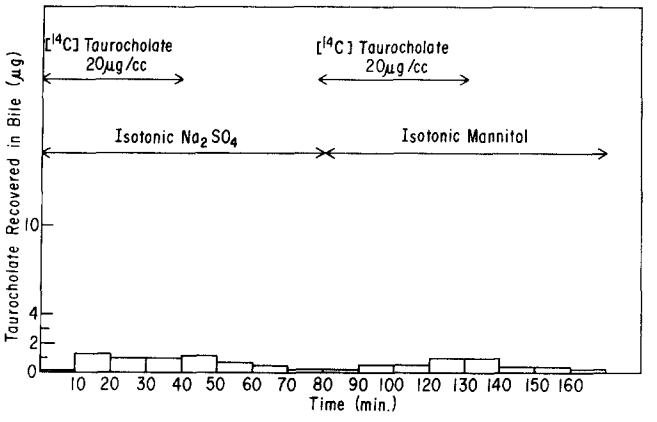

Fig. 3. Recovery of [^{14}C]taurocholate following perfusion of the proximal small bowel of a guinea pig with [^{14}C]taurocholate in solutions of isotonic Na_2SO_4 and isotonic mannitol. Perfusion rate was 4.0 ml/min; [^{14}C]taurocholate concentration, 20 µg/ml.

strate activity to 0.028 µmoles/ml did not alter the results (Fig. 1).

In addition Table 1 and Fig. 1 indicate that the relative impairment of transport due to decreased sodium ion concentration is greater with taurodehydrocholate than with taurocholate. One possible explanation for this observation might be the more rapid depletion of the substrate pool from the mucosal compartment in the experiments using taurocholate. Presumably, the rate of transport of a given substrate from the mucosal to the serosal compartment would depend, in part, on the concentration of the substrate in this mucosal pool. Thus, under conditions where substrate is rapidly transported (high sodium ion concentration), the concentration of substrate in the mucosal compartment at any time after the onset of transport would be lower than under conditions that impair substrate transport (low sodium ion concentrations). Therefore, initial differences between taurocholate transport at high and low sodium ion concentrations might be partially obscured after 60 min incubation if the more rapid depletion of the mucosal taurocholate concentration in the presence of high sodium offset the decreased rate of transport of taurocholate due to low sodium ion. Taurodehydrocholate transport, on the other hand, would be affected less by this mechanism, since taurodehydrocholate is removed from the mucosal compartment much more slowly than taurocholate, even at high sodium ion concentrations.

Against this interpretation of these experiments are the data given in Experiment 2, Table 1, which demonstrate that inhibition of taurocholate transport after 30 min is equivalent to that measured after 60 min. In addition the final concentration of taurocholate present in the high sodium ion incubation after 30 min is 0.21 µmoles/ml, a value comparable to that for taurodehydrocholate remaining after 60 minutes, 0.28 µmoles/ml (see Experiment 3). However, lowering the Na^+ concentrations resulted in 71% inhibition of taurodehydrocholate compared to 26% for taurocholate. Therefore the differences of transport in Na^+-depleted media are more consistent with a greater susceptibility of taurodehydrocholate to sodium ion concentration.

Substitution of the three alpha hydroxyl groups of taurocholate with the three keto groupings in taurodehydrocholate results in significant distortion of the steroid moiety (13). This alteration could be expected to result in less efficient attachment of the dehydrocholate conjugates for the ileal bile salt transport system. In vitro and in vivo (7, 11) inhibition studies have shown that the triketo bile salts are the weakest inhibitors of the transport of natural bile salts, and are in themselves most readily inhibited. Such observations are in agreement with the concept that modification of the shape of the steroid lowers its affinity for the transport system.

Additional evidence for this idea was obtained in experiments employing the cholamine conjugates of bile acids (14). These compounds are modified natural conjugated bile salts with the anionic site replaced by a positively charged quaternary amine. These cationic derivatives are not actively transported but may inhibit the transport of anionic bile salts. Taurodehydrocholate was the compound most readily inhibited in in vitro and in vivo preparations. The cholamine conjugate of dehydrocholic acid was without effect as an inhibitor.

The data in Fig. 1 include a kinetic analysis comparing the transport of taurocholate and taurodehydrocholate at various sodium ion concentrations. The intercepts on the abscissa indicate that these data are in accord with the idea that the affinity of the transport system for Na^+ is less in the presence of taurodehydrocholate (with its distorted steroid) as compared with taurocholate.

Table 2 shows that taurodehydrocholate is less effective as an inhibitor of taurocholate transport when the Na^+ concentration is lowered. This would be expected if the sodium in turn affected the affinity of the bile salt for the transport system, but was more effective in this regard for taurocholate than for taurodehydrocholate.

The in vivo studies indicate that Na^+ has a physiological role in intestinal bile salt absorption, and demonstrate that the inhibition observed at low sodium ion concentration is reversible.

In their totality, the results presented would suggest a cooperative role between bile salts and sodium

ions, each enhancing the initial binding of the other to the transport system. The real but relatively low quantitative requirement for sodium ions when taurocholate is transported, when compared with taurodehydrocholate, could be related to its better stereochemical fit in the system.

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