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SHORT-TERM STUDIES OF TAUROCHOLATE UPTAKE BY ILEAL BRUSH BORDER MEMBRANE VESICLES

ANION EFFECTS

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Summary

Experiments allowing Na^+-dependent short-term uptake measurements by ileal brush border vesicles were described. Glucose uptake was compared with taurocholate uptake in the presence of NaCl, NaSCN and Na_2SO_4. In contrast to the observation made with glucose, taurocholate transport was the same for the three electrolytes, indicating electroneutral taurocholate transport.

In 1977 we reported that brush border vesicles prepared from the guinea-pig ileum were capable of Na^+-dependent uphill transport of taurocholate [1]. This indicated that the ileal bile salt transport system originally described by Lack and Weiner [2] was located in the brush border membrane. Subsequent studies on the question of the ionic requirements for taurocholate transport have been reported by this laboratory and others [3—5]. The cation requirement was found to be specific for Na^+. This laboratory found that the degree of lipid permeability to the anion had no effect on the magnitude of the taurocholate transport [3]. In a recent study on L-ascorbate transport in the guinea-pig small intestine, Siliprandi et al. [6] pointed to their similar observations of the lack of an anion effect as evidence for an electroneutral transport process. In the case of the transport of these two monovalent anions, taurocholate and L-ascorbate, a 1:1 cotransport of substrate and Na^+ by the carrier would be electroneutral. In contrast, a postulated 1:1 cotransport of the un-

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

charged molecule, D-glucose, with Na^+ would result in a net positive charge addition to the carrier. The electrogenic nature of the D-glucose transport has been demonstrated in anion studies by others in which the magnitude of the D-glucose transport was enhanced in the presence of the more lipid permeable anions that render the intravesicular space more electronegative [7].

In our previous studies cited above [3], uptake of taurocholate was measured after an incubation period of 15 s or longer following the addition of electrolyte. The methodology which required the manual starting and sampling of the incubation mixture made shorter sampling periods impractical. It was necessary to point out at that time that substrate efflux from vesicles, dissipation of the sodium gradient and other factors would be expected to complicate studies of the uptake process at these prolonged incubation times. Questions were raised concerning the conclusions derived from these 15-s studies. The inability to measure taurocholate uptake at times less than 15 s left the possibility open that NaSCN could have caused an earlier and greater overshoot peak than that evoked by NaCl or Na_2SO_4. Consequently, the data from 15-s might not have demonstrated real differences in transport with the different electrolytes.

This communication describes the patterns of vesicular uptake of taurocholate in the period from 2 to 15 s. To obtain reliable measurements for short-term incubations, a system was designed to automate the timing and termination of the incubations. In addition, uptake of taurocholate during short-term incubations of vesicles in the presence of NaCl, NaSCN and Na_2SO_4 was compared with D-[6-^{14}C]glucose uptake during parallel incubations of vesicles. The results of these experiments demonstrate that the Na^+-dependent uptake of taurocholate, unlike that for glucose, is unaffected by the nature of the Na^+ counteranion present.

Sodium [24-^{14}C]taurocholate and D-[6-^{14}C]glucose were obtained from New England Nuclear. Other materials were all of reagent grade. Leupeptin was purchased from the Protein Institute, Inc., Osaka, Japan.

Guinea-pigs of the Hartley strain (300—500-g) were used for the preparation of the brush border membranes. The procedure was the calcium method described by Schmitz et al. [8] and modified by Kessler et al. [9]. Leupeptin was added to the 50 mM mannitol/2 mM Tris-HCl (pH 7.1) homogenizing solution to make a concentration of 50 μM. The final preparation of vesicles was the same as previously reported [3]. Leupeptin is a protease inhibitor that is particularly effective against calcium-activated [10] and lysosomal proteases [11]. We have found that the addition of leupeptin prior to the homogenization step yields vesicle preparations with transport activities that are more consistent when one preparation is compared with another. All vesicles were formed from brush borders isolated from the most distal quarter of the small intestine. The experiments were performed immediately after preparation of the vesicles. Protein was determined by using the method of Lowry et al. [12].

To allow the measurement of vesicle uptake of substrate at short time intervals, the system shown in Fig. 1 was built to automate the delivery of ice-cold stop solution (1:1 buffered electrolyte and 200 mM mannitol) into the incubation mixture at accurate pre-set times after initiation of the incubation. Mixing of the vesicles with the radioactively labeled substrate and

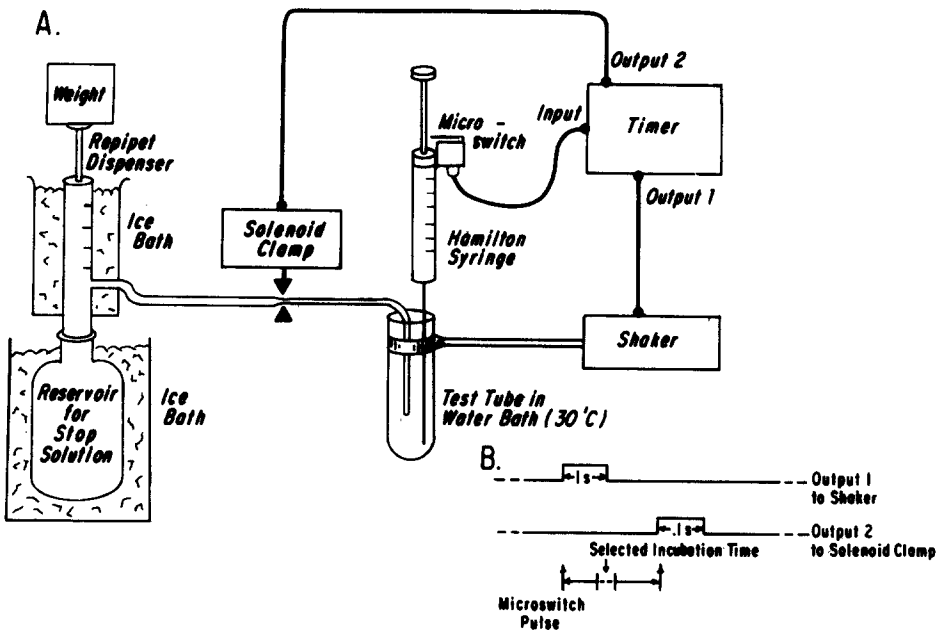

Fig. 1. Automated timing system. (A) Schematic diagram. (B) Timing diagram for control circuit.

electrolyte was also automated by this system. In this way, incubations, as short as 1 s could be performed. To compare the manual incubation technique employed in previous studies [1, 3] with the new automated system, parallel uptake experiments were performed by both methods with sodium [24-^{14}C] taurocholate with the same preparation of vesicles. As seen in Fig. 2, the values obtained for taurocholate uptake were comparable for the two systems.

Incubations were carried out at 30°C. Vesicles suspended in 200 mM mannitol with 1 mM Tris-Hepes buffer, pH 7.4, were pre-incubated for 2 min in a constant-temperature water bath at 30°C. An equal volume of temperature-equilibrated 200 mM mannitol/1 mM Tris-Hepes buffer (pH 7.4) containing sodium [24-^{14}C] taurocholate or D-[6-^{14}C] glucose was then added to yield a final concentration of 0.018 mM sodium [24-^{14}C] taurocholate or 1 mM D-[^{14}C] glucose. Following a 2-min pre-incubation, an equal volume of buffered electrolyte solution at 30°C containing either 0.018 mM sodium [24-^{14}C] taurocholate or 1 mM D-[^{14}C] glucose was added to the incubation mixture with a Hamilton syringe. A microswitch was attached to the side of the syringe so that as the delivery of the electrolyte solution was completed, the cap of the plunger activated the microswitch. This pulse to the timing circuit simultaneously began the timing for the incubation period and started the shaker for the incubation tube. A Vibrometer from Chemie-Apparatebau, Zurich, set at a medium amplitude was used for this purpose. Duration of shaking was 1 s and the frequency was 120 cycles/s. At the end of the preselected incubation time, a pulse to the solenoid clamp allowed the automated delivery of 5.5 ml of ice-cold stop solution to the incubation mixture within 0.1 s. The incubation times were set and monitored for each incubation by a Hewlett-Packard 5308A counter/timer. The incubation tube was immediately taken out of the water bath and placed in ice. The contents

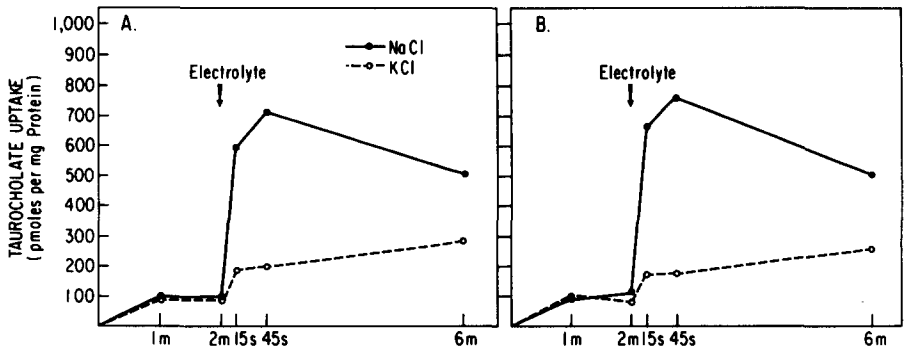

Fig. 2. Parallel incubations of brush border membrane vesicles with sodium [24-^{14}C] taurocholate by two methods. (A) Manual method, in which each data point represents a sample that is manually removed from the incubation mixture, added to stop solution, filtered and washed. (B) Automated new method for short-time incubations in which each data point represents a separate incubation that is stopped, filtered and washed. In both methods the electrolyte solution is 100 mM NaCl, 1 mM Hepes-Tris (pH 7.4), incubation temperature 30° C.

were then filtered, washed and processed for scintillation counting, as previously reported [1].

Electrolyte solutions: NaCl, KCl, NaSCN and KSCN were 100 mM in 1 mM Tris-Hepes buffer (pH 7.4). Osmolarities as determined by the freezing-point method were 200 mosM. Na_2SO_4 and K_2SO_4 prepared at 67 mM in the same buffer assayed as 162 mosM. Na^+ activity for Na_2SO_4 was 90 mM.

All points presented in the figures are the average of three to five individual incubations. Data are presented on the basis of pmol substrate transported per mg vesicular protein. The protein concentrations ranged from 1.25 mg/kg to 2.25 mg/ml. The values for Na^+-dependent cotransport of taurocholate and D-glucose were determined by subtracting the uptake in the presence of the potasssium salts of the anion from the uptake in the presence of the sodium salts.

In previous studies published by our laboratory, the Na^+-dependent taurocholate uptake was determined by the subtraction of the uptake in the vesicles derived from the proximal quarter of the small intestine from the uptake in the vesicles derived from the distal quarter. However, to standardize the experimental parameters in the comparison of D-glucose transport with taurocholate transport, it was necessary to utilize the potassium values as controls in order to correct for passive fluxes and binding since D-glucose is transported in all regions of the guinea-pig small intestine. Previous studies have shown that the use of either non-transporting (jejunal) tissue or the substitution of K^+ for Na^+ in ileal tissue is equally valid as controls for taurocholate [3].

Fig. 3 presents the uptake curves for the Na^+-dependent transported sodium [24-^{14}C] taurocholate with the three electrolytes used in the anion studies. A deviation in linearity was seen in all three curves between 2 and 4 s. The data for the three electrolytes were obtained on different days with different vesicle preparations. Thus, these curves are not intended for comparison with each other, but for examination of the nature of the cotransport process during these short incubation periods. The shortest incubation time in which the magnitude of the taurocholate transport was sufficient to ensure an accurate measurement of substrate uptake was 3 s.

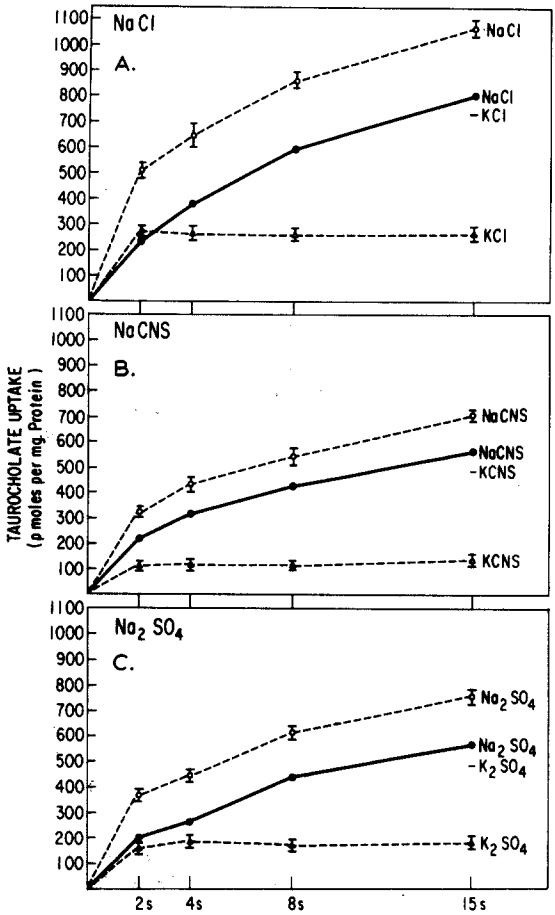

Fig. 3. Uptake curves for sodium [24-^{14}C] taurocholate in the presence of the following buffered electrolyte solutions: (A) 100 mM NaCl and 100 mM KCl, (B) 100 mM NaSCN and 100 mM KSCN, (C) 67 mM Na_2SO_4 and 67 mM K_2SO_4. The automated method (Fig. 1) was used. Each data point of the dashed lines represents the mean ±S.E. of three to five individual incubations.

The data from uptake studies with 3-s incubations for D-[6-^{14}C] glucose and sodium [24-^{14}C] taurocholate in the presence of the Na^+ and K^+ salts of SCN^-, Cl^-, and SO_4^{2-} are shown in Table I. The transport of D-glucose and of taurocholate differ in the influence of anion permeability on the magnitude of transport. Na^+-dependent D-glucose uptake by these vesicles was dependent on the nature of the anionic counterion. When SO_4^{2-} was replaced by Cl^-, uptake was doubled. Replacing the Cl^- by SCN^- doubled it once again. In contrast, the Na^+-dependent uptake of taurocholate was not altered when SO_4^{2-} was replaced by Cl^- or SCN^-. Conditions for the experiment were such that the amount of glucose and taurocholate uptake during the incubation of vesicles in the media containing SO_4^{2-} were comparable.

By minimizing the complicating factors of substrate efflux and an altered Na^+ gradient, these short incubation studies allow the elucidation of parameters more specific to the influx of the substrate. Thus, a stronger hypothesis can be developed on the molecular events involved in the absorption of bile salts in the intestine.

TABLE I

GLUCOSE AND TAUROCHOLATE UPTAKE BY ILEAL BRUSH BORDER VESICLES DURING 3-s INCUBATIONS

Uptake values represent pmol per mg protein. Each value represents the average ± S.E.M. of four determinations.

Anion in incubation medium	D-Glucose uptake			Taurocholate uptake		
	Cation in incubation medium		Sodium-dependent uptake (Na^+—K^+)	Cation in incubation medium		Sodium-dependent uptake (Na^+—K^+)
	Na^+	K^+		Na^+	K^+	
SO_4^{2-}	1137 ± 25	797 ± 6	340	449 ± 16	188 ± 3	261
Cl^-	1431 ± 50	796 ± 33	635	437 ± 10	187 ± 10	250
SCN^-	2186 ± 84	867 ± 33	1319	436 ± 9	145 ± 5	291

In our present studies on the rate of taurocholate transport over the time period of 2 to 15 s, deviation from the initial uptake rate was seen as early as 2 to 4 s. In our selection of a 3-s incubation time for the anion studies, our limitations concerning the apparent lack of linearity were similar to those of Siliprandi et al. [6] in their study of L-ascorbate transport.

The patterns observed for the transport of D-glucose and taurocholate in the presence of NaSCN, NaCl and Na_2SO_4 indicate that these two transport systems differ significantly in their electrogenic nature. In agreement with the observations of others, the Na^+-dependent D-glucose uptake by intestinal brush border membrane vesicles was affected by the lipid permeability of the anions present. Although the stoichiometry of Na^+ and taurocholate binding on the postulated carrier during transport is not known at present, our observation that taurocholate transport was not enhanced when Na_2SO_4 was replaced by either NaCl or NaSCN suggests that uphill cotransport of taurine-conjugated bile salts may involve Na^+ and the bile salt anion occupying the carrier in a 1:1 ratio. To this extent, the mechanism of transport of such bile salts could be expected to be different from that of D-glucose but similar to that postulated for L-ascorbate [6] and phosphate under the appropriate pH conditions [13].

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