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Ionic Requirements for Taurocholate Transport in Rat Liver Plasma Membrane Vesicles[1]

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Abstract

As part of the enterohepatic circulation, taurocholate is taken up by hepatocytes by a Na^+-gradient-dependent, carrier-mediated process. The dependence of taurocholate uptake on the presence of a Na^+ gradient, outside greater than inside, has been studied in isolated rat liver plasma membranes. The uptake is specific for sodium, and a cotransport stoichiometry of 2 Na^+ per taurocholate taken up was found. The presence of K^+ ions inside the vesicles was also found to be essential for maximum Na^+-stimulated uptake of taurocholate, although a K^+ gradient is not required. Mg^{2+} was almost as effective as K^+ in this regard. The symport of Na^+ and taurocholate during uptake was shown to be electrogenic, so that K^+ may act as an exchange counterion preventing the accumulation of positive charge within the vesicles.

Key Words: Taurocholate transport; sodium symport; K^+ requirement; liver plasma membrane vesicles; bile acid transport.

Introduction

As part of the enterohepatic circulation, bile acids are secreted by the liver via the bile duct to the intestines and are recycled back to the liver by way of the blood. Taurocholate is a major bile acid present in rat liver (Simion *et al.*, 1984a). Its uptake by liver is a carrier-mediated process which can be stimulated by a sodium gradient [Na^+] outside greater than [Na^+] inside (Reichen and Paumgartner, 1975; Dietmaier *et al.*, 1976; Schwarz *et al.*, 1975; Anwer and Hegner, 1978). Plasma membranes isolated from rat liver retain the ability to take up taurocholate in a sodium-gradient-stimulated manner (Inoue *et al.*, 1982; Duffy *et al.*, 1983; Simion *et al.*, 1984b). This

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transport appears to be localized in basolateral plasma membranes, since isolated bile canalicular plasma membranes do not transport taurocholate directly (Simion *et al.*, 1984b). A second transport system exists intracellularly which may take up taurocholate from the cytoplasm and could be involved in secreting bile acids across the canalicular plasma membranes by exocytosis (Simion *et al.*, 1984b).

In the present study, we have investigated further the coupling of taurocholate transport to sodium ion gradients in isolated rat liver plasma membranes. In addition, the dependence of Na^+-stimulated taurocholate uptake on preincubation of the vesicles with potassium ions has been clarified.

Materials and Methods

Radioactive [^{3}H]taurocholate acid, ^{22}NaCl, and [^{14}C]thiocyanate were obtained from New England Nuclear. Plasma membranes were prepared from rat livers as described previously (Fleischer and Kervina, 1974). Taurocholate uptake by the membranes was measured using millipore filtration with initial uptake measured at 20 sec as described previously (Simion *et al.*, 1984b). Net uptake was distinguished from binding to the vesicles by preincubating the fraction with filipin (0.3 mg filipin/mg protein) for 10 min at 37°C. Filipin abolishes uptake without affecting taurocholate binding to the membrane (Simion *et al.*, 1984a, b). Filipin was a gift from Dr. Joseph Grady, the Upjohn Company. The difference in [^{3}H]taurocholate retained after filtration, with and without pretreatment with filipin, represents net uptake of taurocholate. Other conditions are as described for the individual experiments.

Results

Uptake of taurocholate by isolated rat liver plasma membranes is stimulated about 2-fold by the imposition of a sodium gradient (outside > inside). The ion gradient is specific for sodium since no enhancement of uptake is observed by substitution of 100 mM sodium chloride with 100 mM Tris-HCl, cesium chloride, choline chloride, or magnesium chloride (Fig. 1). There does not appear to be an anion specificity for uptake since replacing NaSCN with NaCl or Na-gluconate did not significantly affect the stimulated uptake rate. A potassium gradient will not substitute for the sodium gradient in stimulating taurocholate uptake (data not shown).

The stoichiometry between sodium ions and taurocholate transported

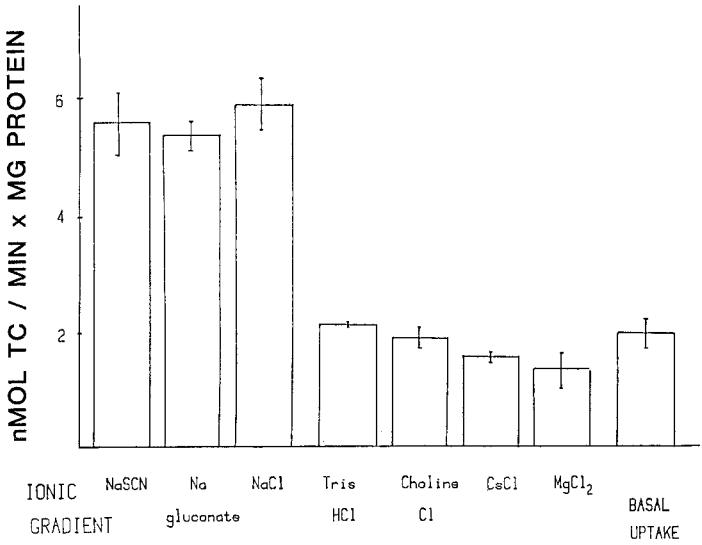

Fig. 1. Ionic specificity of the Na^+-gradient-stimulated taurocholate (TC) uptake into rat liver plasma membranes. The fraction was preincubated with 100 mM KCl, 50 mM Tris · HCl, 0.25 M sucrose, pH 7.0, for 3 min at 37°C. Uptake was then initiated by the simultaneous addition of taurocholate and salt to a final concentration of 300 μM and 100 mM, respectively. The basal taurocholate uptake rate was determined with the same taurocholate concentration (300 μM) but in the absence of an ionic gradient, after preincubation in 0.1 M Tris · HCl, 0.25 M sucrose, pH 7.0. Uptakes were measured at 20 sec as described in Materials and Methods.

during sodium-gradient-stimulated taurocholate uptake was determined directly in double labeled experiments using [^{3}H]taurocholate and [^{22}Na]-NaSCN at varying concentrations of the two substrates. The observed sodium and taurocholate uptake rates are plotted on the ordinate and abscissa, respectively, in Fig. 2. The slope of the plot, 2.0 ± 0.3 (n = 2) indicates a stoichiometry of two sodium ions taken up per taurocholate. This stoichiometry would result in an excess net accumulation of positive charge which should be counterbalanced by ionic fluxes. Membrane-permeable anions such as thiocyanate have been used as a probe for measuring ionic fluxes and estimating transmembrane potential (Papa *et al.*, 1973). We added radioactive thiocyanate with a 10 mM sodium thiocyanate gradient, just prior to the addition of [^{3}H]taurocholate. There was a net loss of [^{14}C]thiocyanate when taurocholate was taken up, suggesting that other counterion fluxes occur (Fig. 3).

The sodium-stimulated taurocholate uptake by plasma membranes requires preincubation in the presence of potassium ions. Stimulation is not observed when a 100 mM sodium gradient is imposed in the absence of

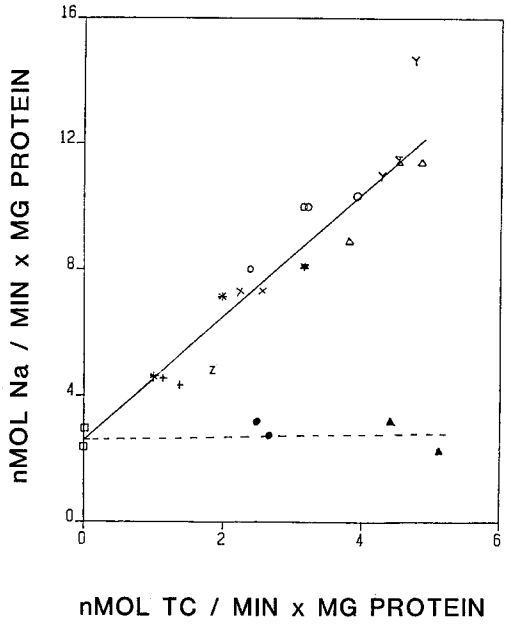

Fig. 2. Stoichiometry of sodium ion to taurocholate taken up during sodium-gradient-stimulated taurocholate uptake by plasma membranes. Plasma membranes were preincubated with 0.1 M KCl, 0.05 M Tris · HCl, 0.25 M sucrose, pH 7.0, for 3 min before the [^{22}Na]-NaSCN and [^{3}H]taurocholate were added simultaneously at varying concentrations. The uptake rate of ^{22}Na is plotted against that for [^{3}H]taurocholate. The slope of the graph provides the stoichiometry. Data from two plasma membrane preparations is shown: 9 mM NaSCN, 0 μM TC (□); 9 mM NaSCN, 90 μM TC (+); 9 mM NaSCN, 160 μM TC (*); 9 mM NaSCN, 230 μM TC (X); 9 mM NaSCN, 300 μM TC (○); 9 mM NaSCN, 415 μM TC (Y); 9 mM NaSCN, 530 μM TC (△); 18 mM NaSCN, 230 μM TC (⧖); 18 mM NaSCN, 300 μM TC (✱); 23 mM NaSCN, 180 μM TC (Z). The basal taurocholate uptake rate was measured after preincubating the plasma membranes in 0.1 M Tris · HCl, 0.25 M sucrose, pH 7.0, at 37°C for 3 min; the final concentrations of sodium thiocyanate and taurocholate were 9 mM NaSCN, 300 μM TC (●); and 9 mM NaSCN, 660 μM TC (▲).

potassium (Simion *et al.*, 1984b). The uptake rate is enhanced by preincubating the plasma membranes for 3 min with potassium chloride before uptake is initiated by the addition of taurocholate and sodium ions (Fig. 4A). Preincubation at potassium chloride concentrations beyond 150 mM causes little additional enhancement of the uptake rate (data not shown). A longer

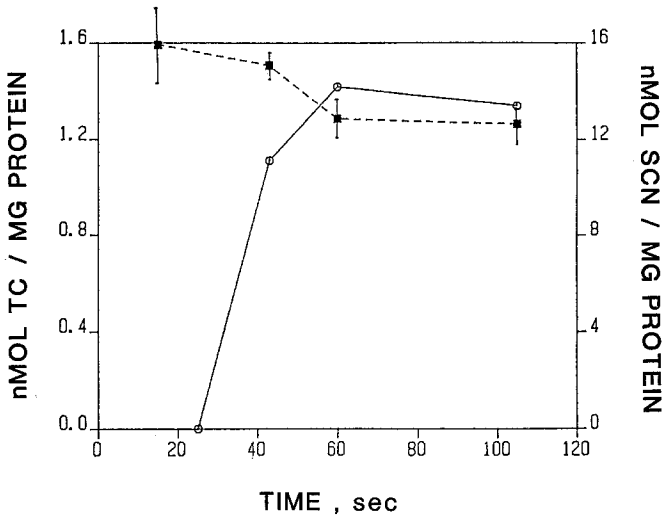

Fig. 3. Electrogenicity of sodium-gradient-stimulated taurocholate uptake by plasma membranes. After preincubation for 3 min at 37°C in 0.1 M KCl, 0.05 mM Tris · HCl, and 0.25 M sucrose, pH 7.0, sodium [^{14}C]thiocyanate was added to give a 10 mM gradient (time 0). An aliquot of the reaction medium was removed within 15 sec to measure thiocyanate binding to the membrane before [^{3}H]taurocholate was added at 25 sec to give a final concentration of 300 μM. Taurocholate uptake (O) and thiocyanate binding (■) by the plasma membranes were determined by millipore filtration. When taurocholate is not added, thiocyanate binding to the membranes remains constant.

preincubation time is required with lower potassium chloride concentration (15 mM: Fig. 4B), but the same maximal stimulation is obtained. At higher potassium chloride concentrations (100 mM), the time lag is reduced. These results suggest that for Na^+-stimulated uptake to occur, potassium ions must first diffuse into the intravesicular space. When the potassium salt is added together with the taurocholate and sodium gradient to start the uptake process, sodium stimulation of taurocholate uptake is not observed (Fig. 4B). When the barrier to the potassium is reduced by preincubating with valinomycin (10 μg valinomycin/mg protein), the time lag is decreased (Fig. 4B). A potassium gradient inside > outside is not required for Na^+-gradient-stimulated taurocholate uptake, since stimulation is observed when the K^+ concentration outside is increased by adding K^+ together with the pulse of Na^+ and taurocholate (data not shown). Preincubation of the plasma membranes for 3 min with 100 mM magnesium chloride instead of potassium chloride also supports sodium-gradient-stimulated uptake of taurocholate, although magnesium is somewhat less effective than potassium in facilitating

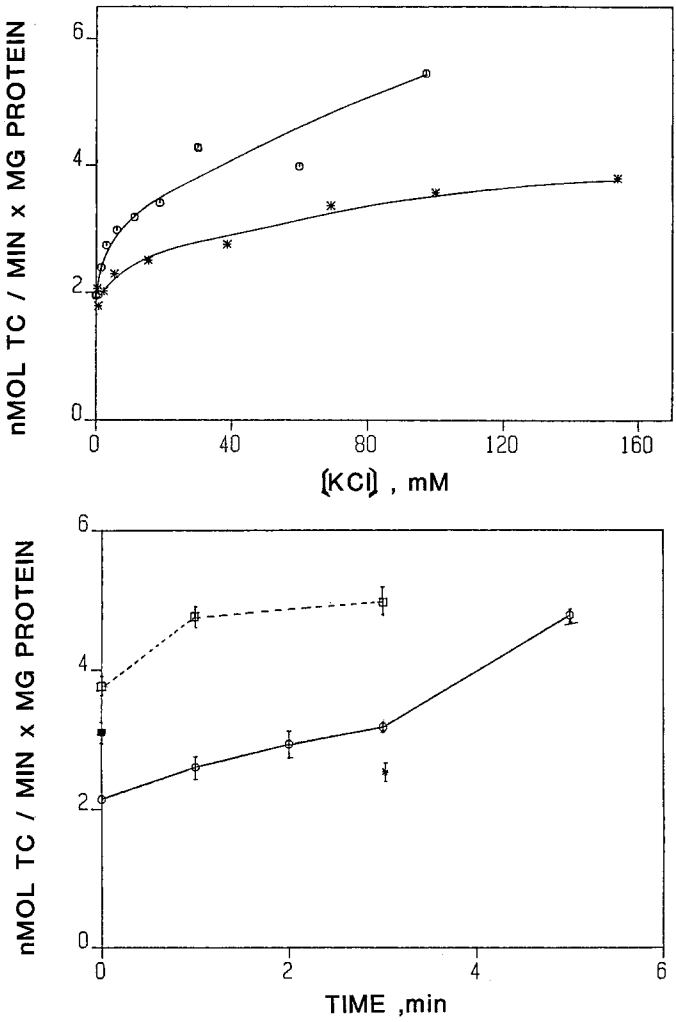

Fig. 4. Effect of preincubation with varying concentrations of potassium chloride on the sodium-gradient-stimulated uptake of taurocholate by plasma membrane vesicles. (A) Dependence of the taurocholate uptake on the concentration of potassium chloride in the preincubation medium. Plasma membranes were preincubated in 0.05 M Tris · HCl, 0.25 M sucrose, pH 7.0, and varying concentrations of KCl for 3 min at 37°C, before the uptake reaction was started by the addition of taurocholate and NaSCN to give final concentrations of 300 μM and 100 mM, respectively. Data for two different preparations are shown (○, *). (B) The time dependence for the effect of preincubation with potassium ions on Na^+-stimulated taurocholate uptake. The period for which the plasma membranes were preincubated in 15 (○) or 100 (□) mM KCl, in 0.05 M Tris · HCl, 0.25 M sucrose, pH 7.0, was varied before the uptake reaction was initiated by the addition of taurocholate and NaSCN to give final concentrations of 300 μM and 100 mM, respectively. For the zero time points, the potassium chloride was added with the sodium thiocyanate and taurocholate. The initial rate of taurocholate uptake was determined as previously described. When 15 mM KCl is added to plasma membranes preincubated 3 min with 10 μg valinomycin/mg protein, partial restoration of stimulation is detected at the zero time point (■). The basal uptake rate was determined after preincubation for 3 min in 0.1 M Tris · HCl, 0.25 M sucrose, pH 7.0 (*).

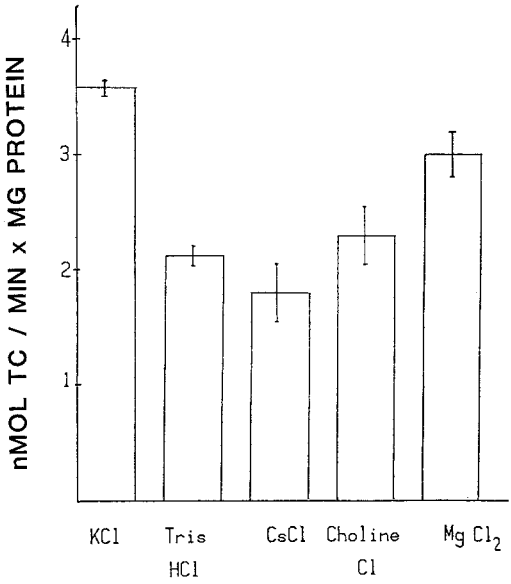

Fig. 5. Ability of different "internalized" cations to support sodium-gradient-stimulated uptake of taurocholate into plasma membrane preparations. After preincubation for 3 min at 37°C in 0.05 M Tris · HCl, 0.25 M sucrose, pH 7.0, supplemented with 0.1 M potassium chloride, cesium chloride, Tris chloride, choline chloride, or magnesium chloride, taurocholate uptake was initiated by the addition of taurocholate and sodium thiocyanate to final concentrations of 300 μM and 100 mM, respectively.

stimulation (Fig. 5). MgCl at 5 mM, a more physiological concentration, shows some stimulation (data not shown) but is not nearly as effective as physiological concentrations of K^+ (40 mM) which show about half maximal stimulation (Fig. 3). Choline, cesium, and Tris cations cannot substitute for potassium in supporting the stimulation of taurocholate uptake (Fig. 5).

Discussion

The ionic dependence of taurocholate uptake into rat liver plasma membrane vesicles has been investigated. Taurocholate uptake can be stimulated by a sodium gradient ($[Na^+]_{outside} > [Na^+]_{inside}$) after preincubation of the vesicles with potassium. The sodium-gradient-stimulated taurocholate uptake mechanism in plasma membranes resembles that observed in hepatocytes in several respects, suggesting that we are investigating the same

transport mechanism. (1) In the absence of a sodium gradient the apparent maximal uptake velocity of taurocholate is significantly reduced without there being a significant change in the observed Michaelis constant (Scharschmidt and Stephens, 1981; Simion *et al.*, 1984b). (2) Phalloidin, which competitively inhibits bile acid uptake by hepatocytes (Petzinger and Frimmer, 1980), also inhibits sodium-gradient-stimulated taurocholate uptake by plasma membrane vesicles (Simion *et al.*, 1984b). (3) In the present study, we find that two sodium cations are transported for each taurocholate anion taken up during sodium-gradient-stimulated taurocholate uptake. Anwer and Hegner (1978) also reported a sodium-to-taurocholate stoichiometry of 2:1 for sodium-stimulated taurocholate uptake in hepatocytes.

Taurocholate uptake is stimulated specifically by a Na^+ gradient. No other anion or cation that was tested would substitute for the sodium ions. However, we found that the presence of potassium ions is required for sodium ion-gradient-stimulated taurocholate uptake. Our present observations suggest that a K^+ gradient is not required, but that potassium has to permeate into the membrane vesicles for Na^+-stimulated uptake. The requirement for potassium is not absolute, since magnesium will substitute for potassium in fulfilling this role. However, at physiological concentrations, potassium is much more effective than magnesium and is probably the cation involved. Potassium may be extruded as an exchange counterion to the sodium/taurocholate cotransport, preventing the accumulation of positive charge inside the vesicles. That magnesium can partially substitute for potassium explains why Inoue *et al.* (1982) observed sodium-stimulated taurocholate uptake in the absence of added potassium but in the presence of 10 mM Mg^{2+}. In this and other studies of sodium-coupled taurocholate uptake into vesicle preparations, the vesicles were routinely preincubated with either magnesium or potassium, so that any requirement for these cations in facilitating sodium-stimulated taurocholate uptake would not have been discerned. Additional examples of systems in which potassium plays a role in facilitating sodium cotransport are the uptake of glutamate into rabbit renal brush border vesicles (Schneider and Sacktor, 1980) and brain synaptosomes (Kanner and Sharon, 1978).

Acknowledgments

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