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Characterization of the ileal Na^+/bile salt co-transporter in brush border membrane vesicles and functional expression in *Xenopus laevis* oocytes

Jonathan G. Ll. MULLINS,* R. Brian BEECHEY,* Gwyn W. GOULD,† F. Charles CAMPBELL‡ and Soraya P. SHIRAZI-BEECHEY*§

*Epithelial Function and Development Group, Department of Biochemistry, University of Wales, Aberystwyth, Dyfed SY23 3DD, Wales, †Molecular Pharmacology Group, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, and ‡Department of Surgery, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland, U.K.

The Na^+/bile salt co-transporter of the pig ileal brush border membrane has been expressed in *Xenopus laevis* oocytes. Injection of pig ileal poly $(A)^+$ RNA into oocytes resulted in the functional expression of an Na^+-gradient-stimulated taurocholate uptake within 2–5 days. The expressed Na^+-dependent taurocholate uptake exhibited saturation kinetics (apparent K_m of 48 μM), and displayed similar competitive substrate inhibition by taurodeoxycholate as the native brush border Na^+/bile salt co-transporter studied in pig ileal brush border membrane vesicles. Interestingly, injection of pig proximal and mid intestinal poly $(A)^+$ RNA into oocytes also resulted in the expression of the Na^+/bile salt co-transporter, though the Na^+-dependent transport of bile salts does not occur in brush border membrane vesicles (BBMV) isolated from pig proximal and mid intestine. This suggests that the mRNA coding for the co-transporter is present in the enterocytes lining the whole length of the small intestine, but that the function is only expressed in the brush border of the distal small intestine. The transport of D-glucose into BBMV, and the transport of methyl-α-D-glucopyranoside (a non-metabolizable hexose derivative) into oocytes were used throughout the study as methods of confirming the integrity of vesicles and oocytes.

INTRODUCTION

The enterohepatic cycling of bile salts is a major factor in the maintenance of the bile salt pool. In this cycle, conjugated bile salts secreted into the duodenum by the liver are re-absorbed by a specific transport system in the distal ileum. They are then carried back to the liver by the portal blood (Lack & Weiner, 1966). Using intestinal everted sacs, isolated cells and membrane vesicles from various species, it is now well established that the bile salts are transported by a Na^+-dependent mechanism which is localized exclusively in the distal ileum (Schiff *et al.*, 1972; Lucke *et al.*, 1978; Beesley & Faust, 1979; Wilson, 1981; Barnard & Ghishan, 1987).

Loss of the ability to absorb bile salts has a number of clinical consequences, such as secretory diarrhoea (Hofman, 1967, 1988; Meihof & Kern, 1968; Mekhijian *et al.*, 1971; Hofmann & Poley, 1972). Studies on the ontogeny of the ileal bile salt transporter have shown that in all the species studied the transporter appears post-natally, around the time of weaning (Little & Lester, 1980; Moyer *et al.*, 1989; Schwarz *et al.*, 1990). The factors that regulate the development of ileal bile salt transport have not been elucidated. The temporal relationship between the onset of weaning and the maturation of bile salt transport suggests a possible modulatory influence of diet on the ontogenic expression of this process. Such a modulatory role has been established for the intestinal Na^+/D-glucose co-transporter (Shirazi-Beechey *et al.*, 1990).

Our main objectives are to study the signals and the molecular mechanisms that are involved in regulating the expression of the intestinal bile salt transporter. To fulfil these objectives, there is a need to identify the structure of this transporter. To this end, we have functionally expressed the pig ileal bile salt transporter in *Xenopus laevis* oocytes. The expressed protein possesses functional characteristics similar to those of the native protein. These studies should allow the isolation and characterization of the cDNA encoding the intestinal Na^+-dependent bile salt co-transporter.

MATERIALS AND METHODS

Removal and storage of tissue

Suckling (14 days old) and adult pigs were anaesthetized with sodium pentabarbitone. The proximal, mid and distal regions of pig intestine were removed, and cut into segments 10 cm in length. They were flushed with ice-cold 0.9 % (w/v) NaCl (pH 7), cut open longitudinally and washed again with saline. The tissues were wrapped in aluminium foil and dropped in liquid nitrogen. They were kept at −80 °C until use. The first 1 m after the pyloric sphincter was taken as the proximal gut. The 1 m immediately prior to the ileo-caecal valve was used as the distal gut, and 1 m of small intestine between these regions was taken as the mid gut. It was shown that results obtained from material stored in this way were similar in terms of Na^+-dependent D-glucose and taurocholate transport to those obtained with fresh material.

Radioisotopes

D-[U-^{14}C]Glucose (275 Ci/mmol) and [U-^{14}C]methyl-α-D-glucopyranoside (154 Ci/mmol) were purchased from Amersham International, Amersham, Bucks, U.K., and were diluted as required. ^{3}H-[G]-Taurocholate (2.1 Ci/mmol) was purchased from New England Nuclear, and diluted as required.

Abbreviation used: BBMV, brush border membrane vesicles.

§ To whom correspondence should be addressed.

Preparation and characterization of brush border membrane vesicles (BBMV) from small intestine

The method was as described by Shirazi-Beechey *et al.* (1988). Intestinal pieces 10 cm in length were placed in 50–100 ml of buffer (100 mM-mannitol/2 mM-Hepes/Tris, pH 7.1). After the tissue was fully defrosted, the cells were removed using a Vibromixer (model E-1; Alpha Laval, Brentford, Middx., U.K.). The cells in the crude homogenate were filtered from the remaining muscle by passing through a Buchner funnel, and were collected. An aliquot of the filtered homogenate was removed for later assay of protein and marker enzyme activities. $MgCl_2$ (2.5 M) was added to a final concentration of 10 mM; the homogenate was then stirred for 20 min and centrifuged at 3000 *g* for 30 min. The supernatant was centrifuged further at 30000 *g* for 30 min. The pellet was resuspended in a buffer consisting of 100 mM-mannitol, 2 mM-Hepes/Tris, pH 7.4, and 0.1 mM-$MgSO_4$, and then manually suspended with a Dounce homogenizer. The suspension was centrifuged for a further 30 min at 30000 *g*. The final supernatant was discarded. The final pellet of BBMV was made homogeneous by passing the suspension through a 27-gauge needle 10 times. Routinely, the final suspension was made in a medium that contained 300 mM-mannitol, 0.1 mM-$MgSO_4$, 0.02% (w/v) NaN_3 and 20 mM-Hepes/Tris, pH 7.4. The temperature was maintained at 4 °C throughout the procedure. Aliquots of 40–100 μl were stored in liquid nitrogen until use.

Protein assay

Protein was assayed by its capacity to bind Coomassie Blue, according to the Bio-Rad assay technique, using bovine γ-globulin (1–100 μg of protein) as the standard. The protein values achieved by this assay were the same as those obtained after treatment of the BBMV with 0.02% (w/v) Triton X-100 prior to assay, indicating that there is complete solubilization of the membrane-bound proteins in the assay reagent.

Assay of the Na^+-dependent uptake of D-glucose into BBMV

The uptake of D-glucose was measured using a filtration stop technique as described by Shirazi *et al.* (1981) and Shirazi-Beechey *et al.* (1988, 1990). The assay was begun by the addition of 50 μl of the incubation medium to 3–5 μl of a suspension of BBMV (normally equivalent to 50–150 μg of protein). After the appropriate period, the uptake was quenched by the addition of 1 ml of ice-cold stop solution. Incubations were carried out at 37 °C. The BBMV were separated from the incubation medium by placing 0.9 ml of the quenched assay medium on a 0.22 μm-pore-size cellulose acetate/nitrate filter (Millipore) and filtered under vacuum. The filter was washed with 5 × 1 ml of stop buffer. The radioactivity retained on the filter was measured in a scintillation counter. The incubation medium contained 100 μM-D-[^{14}C]glucose (40 Ci/mol), 100 mM-NaSCN, 100 mM-mannitol, 0.1 mM-$MgSO_4$, 0.02% (w/v) NaN_3 and 20 mM-Hepes/Tris, pH 7.4. The stop solution contained 150 mM-KSCN, 0.02% (w/v) NaN_3, 0.25 mM-phlorizin and 20 mM-Hepes/Tris, pH 7.4.

The uptake at zero time was measured by adding the stop solution to the BBMV before adding the incubation solution. To assay the passive uptake of glucose by the BBMV, NaSCN in the incubation medium was replaced by KSCN.

Assay of the Na^+-dependent uptake of taurocholate into BBMV

The uptake of taurocholate was measured using a filtration stop technique as described for D-glucose transport. However, the incubation medium contained 100 μM-[^{3}H]taurocholate (120 Ci/mol), 100 mM-NaCl, 100 mM-mannitol, 0.1 mM-$MgSO_4$, 0.02% (w/v) NaN_3 and 20 mM-Hepes/Tris, pH 7.4, and the stop solution contained 100 mM-mannitol, 100 mM-choline chloride, 0.02% (w/v) NaN_3, 1 mM-taurocholate and 20 mM-Hepes/Tris, pH 7.4. The uptake at zero time was measured by adding the stop solution to the BBMV before adding the incubation solution. To assay the passive uptake, and to study the cation specificity of taurocholate transport by the BBMV, NaCl in the incubation medium was replaced by the same concentration of either KCl or choline chloride.

For the kinetic studies, the taurocholate concentration was varied from 5 to 200 μM by altering the amount of unlabelled taurocholate added. For the inhibition studies, the various bile salts and amino acids were included in the uptake medium at a concentration of 1 mM.

A time period of 5 s was used to measure initial rates of transport of Na^+-dependent D-glucose and taurocholate transport. The rates were linear over this time period.

All uptake assays were routinely performed in triplicate. The variation was never greater than 10% of the mean value.

Assay of marker enzymes

The temperature of assay was 37 °C. Sucrase activity was measured using a procedure based on that of Dahlqvist (1964). Alkaline phosphatase was measured at pH 9.0 in the presence of 5 mM-$MgCl_2$. The substrate was *p*-nitrophenyl phosphate, as described by Shirazi *et al.* (1981). Succinate dehydrogenase was measured as described by Pennington (1961). α-Mannosidase was assayed according to the method of Tulsiani *et al.* (1977) Tris-resistant α-glucosidase was assayed according to the method of Peters (1976).

Isolation of pig intestinal mRNA

Total RNA from 8 g segments of adult proximal, mid or distal pig intestine was prepared by a single-step guanidium thiocyanate/phenol/chloroform extraction procedure (Chomczynski & Sacchi, 1987). Poly(A)$^+$ RNA (mRNA) was selected by oligo(dT)–cellulose chromatography (Maniatis *et al.*, 1982). Four successive extractions of the total pig intestinal RNA were performed in order to enrich with mRNA.

Total RNA and mRNA were precipitated with sodium acetate and ethanol, resuspended in water and stored at −80 °C. Total RNA and mRNA concentrations were estimated by measuring the absorbance at 260 nm (Maniatis *et al.*, 1982).

Microinjection of mRNA into *Xenopus laevis* oocytes

Xenopus laevis oocytes were microinjected according to the procedure described by Colman (1986). Ovaries were removed from adult female *X. laevis*. The ovaries were dissected, excised and placed in a modified Barth's solution [88 mM-NaCl, 1 mM-KCl, 2.4 mM-$NaHCO_3$, 15 mM-Hepes/NaOH (pH 7.6), 0.3 mM-$Ca(NO_3)_2$, 0.41 mM-$CaCl_2$, 0.82 mM-$MgSO_4$, 0.01% (v/v) gentamycin]. Oocytes were then dissected manually and the follicular layer was removed. After an overnight incubation, healthy stage V–VI oocytes were selected and injected with 50 nl of mRNA (concentration range 0.25–1.5 μg/μl). Control oocytes were injected with 50 nl of water. Oocytes were cultured for 2–5 days at 20 °C with a daily change of modified Barth's solution.

Assay of transport into oocytes

The uptake of [^{14}C]methyl-α-D-glucopyranoside and [^{3}H]taurocholate into the oocytes was measured by a dual-labelled assay. At 1 h before the uptake assay, the modified Barth's solution was replaced by a Na^+-free wash buffer (25 °C) consisting of 88 mM-choline chloride, 1 mM-KCl, 2.4 mM-$KHCO_3$, 15 mM-Hepes/Tris (pH 7.6), 0.3 mM-$Ca(NO_3)_2$, 0.41 mM-$CaCl_2$, 0.82 mM-$MgSO_4$ and 0.01% gentamycin.

Between 8 and 12 oocytes were then incubated at 30 °C in 500 μl of uptake medium containing, unless otherwise stated, 88 mM-NaCl, 1 mM-KCl, 2.4 mM-$NaHCO_3$, 15 mM-Hepes/NaOH (pH 7.6), 0.3 mM-$Ca(NO_3)_2$, 0.41 mM-$CaCl_2$, 0.82 mM-$MgSO_4$, 10 μCi of [^{14}C]methyl-α-D-glucopyranoside and 30 μCi of [^{3}H]taurocholate. The concentration of methyl-α-D-glucopyranoside and taurocholate was routinely adjusted to 100 μM. For the kinetic studies, however, the concentration of taurocholate ranged from 5 to 200 μM. After the indicated time interval, the uptake was stopped by the addition of 5 ml of ice-cold stop solution. The stop solution was of similar composition to the Na^+-free wash buffer, except that 5 mM unlabelled methyl-α-D-glucopyranoside and 1 mM unlabelled taurocholate were added, in order to reduce non-specific binding of the radiolabelled solutes.

Oocytes were washed a further four times in 5 ml of cold stop solution. After individual oocytes were dissolved in 20 μl of formic acid, 4 ml of scintillation fluid was added to each oocyte and the radioactivity associated with each oocyte was counted in a liquid scintillation counter.

Radioactivity counting channels

In the dual-labelling experiments, the counting channels were set so that only the counts falling in the lowest 10% of the ^{3}H energy spectrum (0–0.002 MeV) were taken to represent [^{3}H]taurocholate uptake, and only the highest 40% of the ^{14}C energy spectrum (0.09–0.15 MeV) was taken to represent the [^{14}C]-methyl-α-D-glucopyranoside uptake. This meant that the interference in either channel from the other isotope was negligible. The advantage of dual-labelled uptake experiments in this context was that a value for the uptake of two different substrates could be obtained for each individual oocyte.

RESULTS AND DISCUSSION

Characteristics of BBMV

BBMV prepared from proximal, mid and distal pig intestines, irrespective of the age of the animal, were enriched (12–17-fold for sucrase and 10–12-fold for alkaline phosphatase) compared with the initial homogenate. These enrichments are similar to the values reported in BBMV isolated from intestines of various species (Shirazi *et al.*, 1981; Shirazi-Beechey *et al.*, 1988, 1990). The activity of marker enzymes of cellular organelles, namely succinate dehydrogenase, α-mannosidase and α-glucosidase, were not enriched in the final BBMV.

Transport in BBMV

Na^+-dependent D-glucose transport. In the presence of Na^+, significant transport of D-glucose was observed in BBMV isolated from the proximal, mid and distal intestines of suckling and adult pigs. This transport showed the characteristics of concentrative accumulation (Shirazi-Beechey *et al.*, 1988) of D-glucose. There was negligible glucose transport into intestinal BBMV, either in the presence of K^+ or in the absence of any cations. This indicates that the transport of D-glucose across the brush border membrane is energized by a Na^+ gradient (Crane, 1962).

The initial rates of Na^+-dependent D-glucose transport in BBMV prepared from various regions of the small intestine of adult and suckling pigs are shown in Table 1. Initial rates of transport were higher in BBMV prepared from adult pig proximal intestine and mid intestine than in those prepared from the distal region. Rates in intestinal BBMV prepared from 14-day-old suckling piglets were lower than in the adult animals, but displayed a similar regional pattern. These findings are consistent with those of other workers, who have shown that the rate of Na^+-dependent glucose transport decreases aborally (Bluet *et al.*, 1986).

The uptake at equilibrium (30 min) of D-glucose into the vesicles was very similar in BBMV prepared from adult intestine (60.5 pmol/mg of protein) to that from the suckling animal (52.5 pmol/mg of protein), confirming that the observed age-related differences in rates of transport were not due to differences in the size of the vesicles.

Na^+-dependent taurocholate transport. Na^+-dependent transport of taurocholate was found in only the distal intestine of adult pigs (initial rate 43.0±1.6 pmol/s per mg of protein). As can be seen from Fig. 1, there was an enhanced uptake of taurocholate into ileal membrane vesicles in the presence of an inwardly directed Na^+ gradient, with accumulation at concentrations (17-fold) above equilibrium values, the 'overshoot' phenomenon. In the presence of an inward K^+ gradient uptake was negligible, and no overshoot was observed. Na^+-dependent transport of taurocholate was absent from all intestinal regions of suckling animals. This observation is in accord with previous findings that the Na^+-dependent bile salt transporter is restricted to the distal intestine, and absent before weaning (Little & Lester, 1980; Moyer *et al.*, 1989). These regional and developmental characteristics contrast with those seen for Na^+-dependent D-glucose transport.

The Na^+-dependent taurocholate co-transporter found in pig ileal BBMV was temperature-dependent; rates were enhanced 5-fold at 37 °C compared with 25 °C. Experiments were carried out to determine the substrate-dependence of taurocholate transport in BBMV isolated from the distal gut of adult pigs. The uptake

Table 1. Initial rates of Na^+-dependent D-glucose transport in BBMV prepared from various regions of suckling and adult pig intestine

BBMV were prepared from the proximal, mid and distal intestines of suckling (14-day-old) and adult pigs ($n = 4$ for both ages). The uptake experiments were performed as described in the Materials and methods section. Data represent the means±S.D. for three assays.

	Rate of transport (pmol/s per mg of protein)		
Animals	Proximal	Mid	Distal
Suckling	56±5	75±7	34±4
Adult	241±6	318±8	134±5

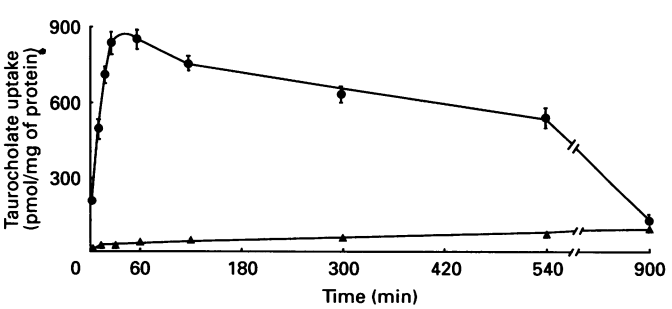

Fig. 1. Time course of taurocholate uptake by BBMV prepared from the distal intestine of adult pigs

BBMV were prepared as described in the Materials and methods section. The transport of taurocholate into the BBMV was measured in the presence of NaCl (●) or KCl (▲). The assay conditions were as described in the Materials and methods section. Each point represents the mean±S.D. for three assays.

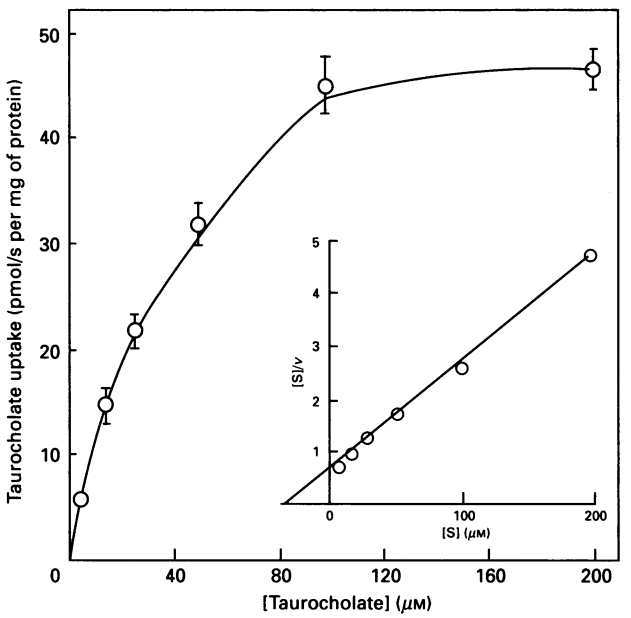

Fig. 2. Kinetics of Na^+-dependent taurocholate uptake in pig distal intestinal BBMV

BBMV were prepared from pig distal ileum. The incubation medium contained 100 mM-NaCl, 100 mM-mannitol, 0.1 mM-$MgSO_4$, 0.02% (w/v) NaN_3 and 20 mM-Hepes/Tris, pH 7.4, in addition to [^{3}H]taurocholate (120 Ci/mol) at 5, 15, 30, 50, 100 and 200 μM. For the determination of passive uptake of taurocholate into the BBMV, NaCl was replaced by KCl. The initial rates were measured with 5 s incubations and were calculated by subtracting the uptake in the presence of a K^+ gradient from that with a Na^+ gradient. The data are presented as a Michaelis–Menten curve and (inset) as a Hanes–Woolf plot ([S]/v against [S]). Each point represents the mean ± S.D. for four determinations.

Table 2. Effect of bile salts and free amino acids on Na^+-dependent taurocholate uptake

BBMV were prepared from adult pig distal intestine as described in the Materials and methods section. The transport of taurocholate into BBMV was measured at 37 °C, in the presence of 100 μM-[^{3}H]-taurocholate (120 Ci/mol), 100 mM-NaCl, 100 mM-mannitol, 0.1 mM-$MgSO_4$, 0.02% (w/v) NaN_3 and 20 mM-Hepes/Tris, pH 7.4. The bile salts and the amino acids glycine and taurine were each added to the incubation medium at a concentration of 1 mM.

Competitor	Inhibition (%)
Cholate	56
Glycocholate	56
Glycodeoxycholate	89
Glycochenodeoxycholate	92.5
Taurodeoxycholate	90
Taurochenodeoxycholate	93
Glycine	0
Taurine	0

displayed saturation kinetics. A Hanes–Woolf plot of the results obtained is shown in Fig. 2. Plots fitted by linear regression gave a $V_{max.}$ value of 46 ± 1.2 pmol/s per mg of protein, and a K_m of 40 ± 3 μM.

The ability of various bile salts and their constituent amino acids to modulate Na^+-dependent taurocholate transport into adult pig ileal BBMV was investigated. The transport was inhibited by all of these naturally occurring bile salts, but the amino acids displayed no inhibition (Table 2). At inhibitor concentrations of 1 mM, the inhibition was greater with the dihydroxy bile salts (90%), than with the trihydroxy bile salts (56%). It has been shown by us and other workers that the inhibition of Na^+-dependent taurocholate transport by the bile salts is of a competitive nature (Schiff *et al.*, 1972; Dietschy, 1976).

Since bile salts have detergent-like properties which could have a deleterious effect on the integrity of the membrane, it was important to show that the observed inhibition of Na^+-dependent taurocholate transport was not due to detergent action of the bile salts on the brush border membrane. The initial rates of Na^+-dependent glucose transport into ileal BBMV were measured in the presence of 1 mM concentrations of the bile salts. The rates of D-glucose transport were the same in the presence and absence of these salts, indicating that no solubilization of the membrane had occurred.

Table 3. Functional expression of Na^+-dependent D-glucose transporter in *Xenopus* oocytes

mRNA was isolated from adult proximal, mid and distal pig intestine. A total of 72 oocytes were injected with 50 nl of mRNA (50 ng), 24 with mRNA from the proximal region, 24 with mRNA from the mid region and 24 with mRNA from the distal region; 20 oocytes were injected with 50 nl of water. Subsequently, oocytes were incubated for 4 days at 20 °C. Thereafter, the Na^+-dependent uptake of [^{14}C]methyl-α-D-glucopyranoside by the oocytes was measured as described in the Materials and methods section. Phlorizin (1 mM) was used as the inhibitor of the transport system.

Oocytes injected with:	Uptake of methyl-α-D-glucopyranoside in oocytes (pmol/h per oocyte)	
	No inhibitor	With inhibitor
Water	2.5 ± 0.4	2.3 ± 0.3
Proximal gut mRNA	30.2 ± 2.1	7.5 ± 0.7
Mid gut mRNA	38.0 ± 3.5	9.7 ± 0.8
Distal gut mRNA	19.5 ± 1.4	4.9 ± 0.5

Table 4. Functional expression of Na^+-dependent taurocholate transport in *Xenopus* oocytes

mRNA was isolated from the proximal, mid and distal portions of the adult pig small intestine. The procedure for oocyte injection was similar to that described in the legend to Table 3. The Na^+-dependent uptake of [^{3}H]taurocholate by the oocytes was measured as described previously. Taurodeoxycholate (1 mM) was used as the inhibitor.

Oocytes injected with:	Cation in incubation medium	Uptake of taurocholate into oocytes (pmol/h per oocyte)	
		No inhibitor	With inhibitor
Water	Na^+	2.6 ± 0.9	2.7 ± 0.7
Proximal gut mRNA	Na^+	9.1 ± 1.9	4.1 ± 0.4
Mid gut mRNA	Na^+	10.6 ± 1.9	4.6 ± 0.4
Distal gut mRNA	Na^+	10.2 ± 1.1	4.1 ± 0.3
Distal gut mRNA	K^+	4.4 ± 1.7	4.2 ± 1.0
Distal gut mRNA	Choline	4.1 ± 0.9	–

Functional expression of Na^+-dependent D-glucose and bile salt transport in the plasma membrane of *Xenopus* oocytes

Na^+-dependent D-glucose transport. Injection of 50 ng of mRNA isolated from proximal, mid and distal regions of the pig intestine resulted in 12-, 15- and 8-fold increases respectively in

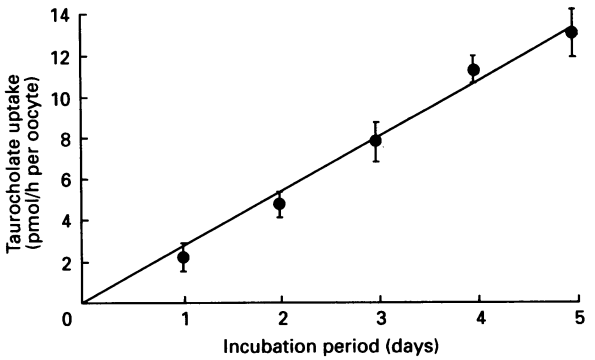

Fig. 3. Relationship between incubation period and the expression of Na^+-dependent taurocholate transport in oocytes

mRNA isolated from pig distal intestine was injected into oocytes as described in the legend to Fig. 5. Subsequently, oocytes were incubated for 1–5 days at 20 °C. After each day, 10 oocytes were assayed for the uptake of [^{3}H]taurocholate, as described in the Materials and methods section.

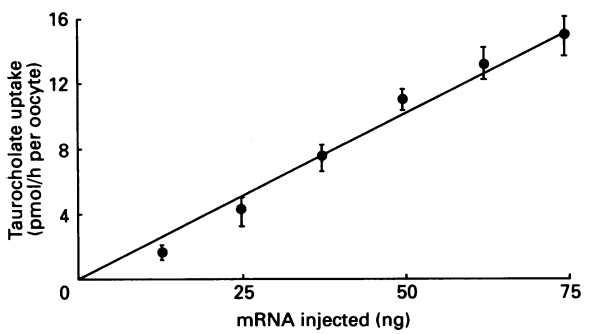

Fig. 4. Relationship between the amount of ileal mRNA injected into oocytes and the expression of Na^+-dependent taurocholate transport

mRNA was isolated from pig distal intestine. Seven sets of eight *Xenopus laevis* oocytes were injected with different concentrations of mRNA to give 0, 12.5, 25, 37.5, 50, 62.5 and 75 ng of ileal mRNA in a 50 nl volume of water. Subsequently, oocytes were incubated and assayed as described previously.

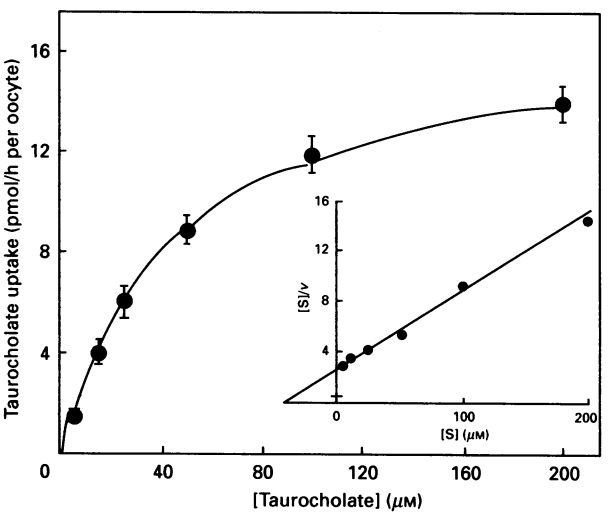

Fig. 5. Kinetics of the expressed Na^+-dependent taurocholate transporter in *Xenopus laevis* oocytes

mRNA was isolated from pig distal intestine as described in the Materials and methods section. *Xenopus laevis* oocytes were microinjected with 50 nl (50 ng) of mRNA or with 50 nl of water. Subsequently oocytes were incubated for 4 days at 20 °C with a daily change of modified Barth's solution. Thereafter, the uptake of taurocholate into the oocytes was measured as described in the Materials and methods section. The concentration of taurocholate was varied over the range 5–200 μM. Eight ileal mRNA-injected oocytes and eight water-injected oocytes were incubated at each of the following substrate concentrations: 5, 15, 25, 50, 100 and 200 μM. For kinetic analysis, the uptake values obtained for the water-injected oocytes at the different concentrations were subtracted from those for the mRNA-injected ones.

the rates of transport of methyl-α-D-glucopyranoside over the background levels found in water-injected oocytes. This mRNA-induced uptake was Na^+-dependent and was inhibited by phlorizin, a competitive inhibitor (Alvarado & Crane, 1962) of hexose transport (Table 3). The uptake was not observed when Na^+ was replaced by K^+ or choline ions. This indicates that the microinjection of mRNA isolated from all regions of pig intestine can bring about the expression of the intestinal Na^+-dependent glucose transport protein in *Xenopus* oocytes (Table 3). We have used the expression of Na^+-dependent methyl-α-D-glucopyranoside (a non-metabolizable analogue of D-glucose) transport in oocytes (Hediger *et al.*, 1987; Ikeda *et al.*, 1989) as a method of confirming the functional integrity of the oocytes.

Na^+-dependent taurocholate transport. Na^+-dependent taurocholate transport was also expressed in the same oocytes injected with mRNA isolated from proximal, mid and distal regions of adult pig intestine. The rate of transport was increased 4–5-fold over the background levels found in water-injected oocytes, irrespective of the source of mRNA (Table 4). After correction for non-specific taurocholate transport (shown in the water-injected oocytes), the expressed taurocholate uptake was five times higher in the Na^+ incubation medium than in incubation media containing either K^+ or choline ions. The expressed transport was inhibited by 80% by 1 mM-taurodeoxycholate (Table 4). This level of inhibition is similar to that observed with the native ileal brush border membrane transporter (Table 2). The rate of expressed transport increased linearly with the time of incubation with mRNA (Fig. 3) and with the amount of mRNA injected (Fig. 4).

Na^+-dependent taurocholate transport in the oocytes injected with ileal mRNA exhibited saturation kinetics. Kinetic analysis, using a Hanes–Woolf plot, gave a $V_{max.}$ value of 16 pmol/h per oocyte and a K_m of 48 μM (Fig. 5). This K_m value compares well with that of 40 μM found for native Na^+-dependent taurocholate transporter in BBMV.

The above data indicate that the taurocholate transporter expressed in the plasma membrane of the oocytes has similar functional and kinetic characteristics to the native bile salt transporter. Interestingly, the microinjection of pig proximal and mid intestine mRNA, as with ileal mRNA, caused the expression of the Na^+-dependent taurocholate transport protein in *Xenopus* oocytes (Table 4), although the transport activity is seen only in BBMV prepared from the distal intestine (Fig. 1). This observation suggests that the mRNA coding for the Na^+-dependent bile salt co-transporter is present in the enterocytes throughout the small intestine, but that the transport function is only observed in the brush border membrane of the distal region.

Whether the transport protein in the upper gut is totally absent, or is resident in an inactive form, remains to be clarified. There may be factors that repress the expression of the active protein in the upper intestine. Our results suggest that the regulation of the expression of the transport protein may occur at either a translational or a post-translational level.

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