Taurocholate Transport by Brush Border Membrane Vesicles from Different Regions of Chicken Intestine[1]

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ABSTRACT Taurocholate transport was studied in brush border membrane vesicles (BBMV) isolated from chicken small (duodenum, jejunum, and ileum) and large (proximal cecum and rectum) intestines, using a rapid filtration technique. The purity of the BBMV was verified by the finding that the specific activity of sucrase (a brush border membrane enzyme marker) was severalfold greater in vesicles than corresponding values in mucosal homogenate. The functional integrity of isolated BBMV was evaluated by the uptake of D-glucose, which showed a transient increase in the presence of Na^+. A Na^+-dependence of taurocholate uptake was shown in BBMV prepared from ileum, cecum, and rectum, as taurocholate transport was transiently increased (accumulation) in the presence of a Na^+ gradient between the external medium and intravesicular medium. The magnitude of the accumulation was similar among ileum, cecum, and rectum. In contrast, BBMV prepared from duodenum and jejunum did not show any Na^+-dependent taurocholate transport, as the taurocholate uptake was not affected when a Na^+ gradient was replaced by a K^+ gradient. The use of taurochenodeoxycholate in the incubation medium inhibited Na^+-dependent taurocholate transport in the ileum, cecum, and rectum. This study is the first to show the presence of a Na^+-dependent bile salt transport in BBMV obtained from chicken ileum, proximal cecum, and rectum.

(*Key words*: ileum, cecum, rectum, bile salt, chicken)

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INTRODUCTION

Bile acids are a family of acidic sterols synthesized in the liver from cholesterol. Following synthesis and conjugation with glycine or taurine, they are secreted into bile and stored in the gallbladder. Upon the ingestion of food, the gallbladder contracts, emptying bile into the proximal small intestine, where bile acids facilitate lipid digestion and absorption. Bile acids are then absorbed from the intestine, returned to the liver via the portal circulation, and resecreted into bile.

Bile acids are largely reabsorbed by an active mechanism in the ileum and by passive mechanism in the duodenum, jejunum, cecum, and colon (Schiff *et al*., 1972; Vázquez *et al*., 1985; Molina *et al*., 1986).

The active absorption of bile acids, which was first described by Lack and Weiner (1961), has been shown in humans (Krag and Phillips, 1974) and experimental animals (Glasser *et al*., 1965; Lücke *et al*., 1978) to be restricted to the ileum, and to be mediated by an Na^+ gradient-driven transporter located at the brush border membrane of the ileocyte. The transport kinetics and specificity of this Na^+/bile acid cotransport system have been extensively studied using whole intestinal segments (Wilson and Dietschy, 1972; Heubi and Fellows, 1985), everted ileal gut sacs (Lack, 1979; Kitagawa *et al*., 1991), isolated ileocytes (Wilson and Treanor, 1975; Kitagawa *et al*., 1990), and brush border membrane vesicles (BBMV) (Kramer *et al*., 1992, 1993; Wong *et al*., 1994). However, very little is known about intestinal transport of bile acids in chickens, and the information available about the contribution of the different regions of the chicken intestine to bile acids absorption is limited, confused, and based on jejunum and ileum (Glasser *et al*., 1965; Linsay and March, 1967).

The main objective of this initial study was to show the existence of an Na^+/bile acid transporter in the ileum of chicken and to know the mechanism(s) of bile acid absorptions in the other regions of chicken intestine. We have evaluated and compared taurocholate transport across the small and large intestines of

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Abbreviation Key: BBMV = brush border membrane vesicles.

chickens, using BBMV. Experiments with the transport of Na^+-dependent D-glucose are also included to control the functional integrity of the isolated BBMV.

MATERIALS AND METHODS

Chemicals

All unlabeled reagents were obtained from Sigma Chemical.[4] D-(U-^{14}C)Glucose (specific radioactivity, 258.5 mCi/mmol) and ^{3}H-(G)-Taurocholate (specific radioactivity, 2.1 Ci/mmol) were purchased from Itisa Biomedicina, S.A.,[5] and diluted as required.

Cellulose nitrate filters for transport studies (0.22 μm pore size, 25 mm diameter) were from Millipore Ibérica.[6] The scintillation liquid (Ready protein) was from Beckman.[7] Protein was determined using the Bio-Rad[8] kit, and kit for determination of the activity of the marker enzyme sucrase was from Boehringer Mannheim.[9]

Animals

Hubbard chickens were provided through the courtesy of a commercial farm[10] on the day of hatch and brought to the Facultad de Farmacia. They were maintained in standardized humidity conditions with a 12:12 light-dark cycle. Temperature was kept at 34 C during the 1st wk after hatch and at 25 C thereafter. The birds had free access to water and a commercial diet[11] containing 18% protein, 4.5% lipid, and 7.5% cellulose. At age of 4 to 5 wk, chickens were anaesthetized with urethane without previous starvation, and segments of duodenum (pancreatic loop), jejunum (yolk sac region), ileum, proximal cecum, and rectum were removed. Once the adherent mesenteric tissue was trimmed off, the intestinal segments were opened lengthwise and washed with ice-cold saline (NaCl, 0.9%). The mucosa was scraped off with a glass slide, wrapped in aluminum foil, frozen in liquid nitrogen, and kept at –80 C until use.

BBMV Preparation

Brush border membrane vesicles were isolated from mucosa scrapings by a $MgCl_2$ precipitation method (Vázquez *et al*., unpublished data). Briefly, the mucosal scrapings were homogenized for 30 s in 100 mmol/L mannitol, 2 mmol/L HEPES/Tris, pH 7.1, using a Waring blender at maximal speed, and filtered through a buchner funnel. The homogenate was treated with 10 mmol/L $MgCl_2$, stirred for 20 min, and centrifuged at 3,000 *g* for 30 min. The supernatant was centrifuged at 30,000 *g* for 35 to 40 min. The resultant pellet was resuspended in 100 mmol/L mannitol, 2 mol/L HEPES/Tris, pH 7.4 and 0.1 mmol/L $MgSO_4$. This suspension was homogenized with a glass-teflon homogenizer, brought to 35 to 40 mL volume with the same buffer, and centrifuged at 30,000 *g* for 35 to 40 min. The final pellet containing the purified BBMV was suspended in a medium containing 300 mmol/L mannitol, 0.1 mmol/L $MgSO_4$, and 20 mmol/L HEPES/Tris, pH 7.5. The final suspension was homogenized by passing the suspension through 25-gauge and 28-gauge needles. The vesicles were frozen and stored in liquid nitrogen in aliquots. All the steps were carried out at 4 C. The integrity of the vesicles was determined by measuring Na^+-dependent D-glucose uptake after 5 s of incubation.

Enzyme and Protein Determinations

The BBMV preparations were routinely assayed for marker enzyme activities. The brush border marker enzyme, sucrase, was assayed by the method of Dahlquist (1964), and the basolateral membrane marker enzyme, ouabain-sensitive K^+-activated phosphatase activity, was measured according to Colas and Maroux (1980) using *p*-nitrophenyl phosphate as a substrate. Protein was evaluated by a Coomassie Brilliant Blue protein assay using a bovine gamma-globulin as a standard (Bradford, 1976).

Assay of D-Glucose Transport

The uptake of D-glucose was measured at 25 C by a rapid filtration technique. The BBMV were loaded in a medium containing 300 mmol/L mannitol, 0.1 mmol/L $MgSO_4$, and 20 mmol/L HEPES/Tris, pH 7.4. Reactions were started by mixing 5 to 10 mL of vesicle suspension (equivalent to 75 to 125 mg of protein) to 100 mL of the incubation medium containing 100 mmol/L mannitol, 0.1 mmol/L $MgSO_4$, 20 mmol/L HEPES/Tris, pH 7.5, 0.1 mmol/L ^{14}C-D-glucose, and either 100 mmol/L NaSCN or 100 mmol/L KSCN. At selected times, the uptake was stopped by the addition of 1 mL of an ice-cold stop solution. The BBMV were separated from the incubation medium by placing 0.9 mL of the reaction mixture on presoaked cellulose nitrate filters (0.22 μm pore size Millipore filter), and rinsed with 5 mL ice-cold stop solution. The stop solution containing 150 mmol/L KSCN, 0.25 mmol/L phlorizin, and 20 mmol/L HEPES/Tris, pH 7.5.

The radioactivity retained on the filter was measured using a scintillation counter. The uptake at time zero was measured by adding the stop solution after adding the incubation medium to vesicles, and subtracted from the total radioactivity of each sample. The Na^+-dependent D-glucose transport was determined as the difference in the presence of Na^+ and the absence of Na^+ (presence of K^+). A time period of 5 s was used to measure initial rates of transport of Na^+-dependent D-glucose.

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[5]Itisa, Biomedicina, S.A., 28049 Fuencarral, Madrid, Spain.
[6]Millipore Ibérica, S.A., 08008 Barcelona, Spain.
[7]Beckman, S.A., 28034 Madrid, Spain.
[8]Bio-Rad, S.A., E-28100 Alcobendas, Madrid, Spain.
[9]Boehringer Mannheim, S.A., 08006 Barcelona, Spain.
[10]Copoll, 41200 Alcalá de Guadaira, Sevilla, Spain.
[11]Sanders, S.A., 08006 Barcelona, Spain.

TABLE 1. Specific activities of marker enzyme and initial rates of Na^+-dependent D-glucose transport in brush border membrane vesicles (BBMV) prepared from different regions of the chicken intestine[1]

Region	Sucrase						K^+-activated phosphatase						Initial rates	
	BBMV		E		R		BBMV		E		R			
D	0.23	0.06[a]	16.4	3.7	45.6	5.4	1.82	0.7	2.1	0.8	4.7	1.0	58	8[a]
J	0.52	0.09[b]	21.1	3.1	42.7	4.9	0.82	0.28	1.7	0.4	4.5	1.0	125	21[c]
I	0.19	0.05[a]	13.6	1.7	39.2	2.3	2.83	0.55	1.5	0.6	1.8	0.7	228	23[b]
C	0.08	0.02[b]	17.5	2.7	33.3	5.7	1.83	0.68	2.1	0.5	4.9	1.5	65	5[a]
R	0.05	0.02[b]	12.9	3.0	32.8	7.6	1.40	0.16	2.6	0.4	5.6	1.2	30	6[a]

[a–c]Values in each column with no common superscript differ significantly ($P < 0.05$).

[1]Values are means SEM for at least four different membrane preparations per region. Specific activities are in micromoles of glucose formed per milligram of protein per minute (sucrase) and micromoles of *p*-nitrophenol formed per milligram of protein per 30 min (ouabain-sensitive K^+-activated phosphatase). Initial rates are in picomoles per milligram of protein per second. E = enrichment, ratio of the specific activity of the BBMV to that of the homogenate; R = recovery, total activity in the BBMV as a percentage of the total activity in the homogenate; D = duodenum; J = jejunum; I = ileum; C = cecum; R = rectum.

Assay of Taurocholate Transport

The uptake of taurocholate was measured using the filtration technique as described for D-glucose transport, at 25 C. The incubation medium containing 0.63 mmol/L (^3H) taurocholate, 100 mmol/L mannitol, 0.1 mmol/L $MgSO_4$, 20 mmol/L HEPES/Tris, pH 7.5, and either 100 mmol/L NaCl or 100 mmol/L KCl. The stop solution contained 100 mmol/L mannitol, 100 mmol/L KCl, 1 mmol/L taurocholate, and 20 mmol/L HEPES/Tris, pH 7.5. For the inhibition studies, taurochenodeoxycholate was included in the uptake medium with a concentration of 100 mmol/L.

Statistical Analysis

All results were subjected to one-way ANOVA, and represent means SEM from several experiments, each at least in triplicate. Comparison between different experimental groups was evaluated by the two-tailed Student's *t* test, and were considered statistically different at $P < 0.05$. In the case of cecum and rectum, measurements of at least three to five pieces of each were pooled for analysis.

RESULTS AND DISCUSSION

Purity of BBMV Preparations and Vesicle Integrity

The purity of the BBMV preparations was determined by evaluating the membrane marker enzyme, sucrase, for the brush border membranes, and ouabain-sensitive K^+-activated phosphatase for the basolateral membranes in the mucosal homogenate and in the purified microvillus membranes. Table 1 shows enzyme marker specific activities, enrichment, and recoveries for both enzymes in all regions studied. There was a significant increase in the specific activity of sucrase in BBMV prepared from jejunum when compared with the other regions of the intestine. The BBMV prepared from cecum and rectum showed the lowest levels of specific activity of sucrase. Sucrase was enriched 13- to 21-fold compared with total mucosal homogenate, and recoveries were high (33 to 46%) in all regions of the intestine. These values are similar to those reported in BBMV isolated from rat (Raul *et al*., 1988; Takase and Toshinao, 1990) and chicken (Moreno *et al*., 1995) intestines. The BBMV were not enriched in K^+-activated phosphatase relative to initial homogenate in any fractions, and recoveries were low. Ileum showed the highest levels in the specific activity of this enzyme.

The integrity of the BBMV preparations was evaluated by examining the Na^+-dependent D-glucose transport. In agreement with reported findings (Vázquez *et al*., unpublished data), D-glucose uptake into BBMV was markedly stimulated by an inwardly directed Na^+ gradient. The initial rates of Na^+-dependent D-glucose uptake by BBMV prepared from different regions of the intestine are shown in Table 1. The BBMV prepared from jejunum and ileum exhibited a significant increase in the initial rate whereas the rectum showed the lowest values when compared with other regions. These findings are not consistent with those of other workers, who found a decrease in hexose transport from proximal to distal regions (Ferrer *et al*., 1994; Ibrahim and Balasubramanian, 1995). These studies establish the presence of Na^+ coupled D-glucose transport in all studied segments, and, therefore, that these BBMV remained functionally intact for bile salt transport studies.

Taurocholate Uptake into BBMV

Figure 1 shows the time course of taurocholate transport into BBMV from different regions of the chicken intestine. In the presence of a 100 mmol/L Na^+ gradient across the membrane vesicle, there was a transient increase in the intravesicular concentration of taurocholate for ileum, cecum, and rectum, with a maximum after 30 min of incubation (overshoot phenomenon) in these regions of the intestines. The magnitude of the overshoot was similar among ileum, cecum, and rectum (1.49 0.2-fold for ileum, 1.59 0.15-fold for cecum, and 1.42 0.16-fold for rectum). The imposition of a K^+ gradient resulted in no overshoot uptake for these regions (Figure

TABLE 2. Effect of taurochenodeoxycholate (100 m*M*) on Na^+-dependent taurocholate uptake in brush border membrane vesicles (BBMV) prepared from chicken intestine[1]

Uptake	Taurocholate		Taurocholate plus taurochenodeoxycholate	
	(pmol/mg protein/min)			
Ileum	2.7	0.7	0.14	0.02
Cecum	3.6	0.9	0.21	0.6
Rectum	1.4	0.1	0.07	0.02

[1]Values represent means SEM for at least four different membrane preparations per region.

1). However, BBMV prepared from duodenum and jejunum did not show any Na^+-dependence of taurocholate uptake and no overshoot phenomenon, because taurocholate transport was not affected when KCl was replaced by NaCl (Figure 1).

Therefore, a Na^+-dependent taurocholate transport is present in the ileum, cecum, and rectum of chickens, but not in duodenum and jejunum. This Na^+ requirement for bile salt transport has been well studied in humans (Krag and Phillips, 1974) and some animal species (Glasser *et al*., 1965; Lücke *et al*., 1978), and its presence has been restricted to the ileum. This ileal transport is mediated by an Na^+-gradient-driven transporter, which has been recently identified and characterized from a number of mammalian species, including hamster (Wong *et al*., 1994), rat (Shneider *et al*., 1995), and human (Wong *et al*., 1995).

Interestingly, the equilibrium uptake values of taurocholate were similar among vesicles prepared from jejunum, cecum, and rectum. However, BBMV prepared from duodenum showed the lowest values and ileal vesicles the highest values in the equilibrium uptake of taurocholate. The same results were observed by Kramer *et al*. (1993) when comparing BBMV prepared from rabbit jejunum and ileum. However, the equilibrium uptake of taurocholate was lower in ileum than in jejunum of rat BBMV (Lücke *et al*., 1978).

It is well known that taurocholate (Schiff *et al*., 1972) is inhibited by other bile salts. In studies of transport

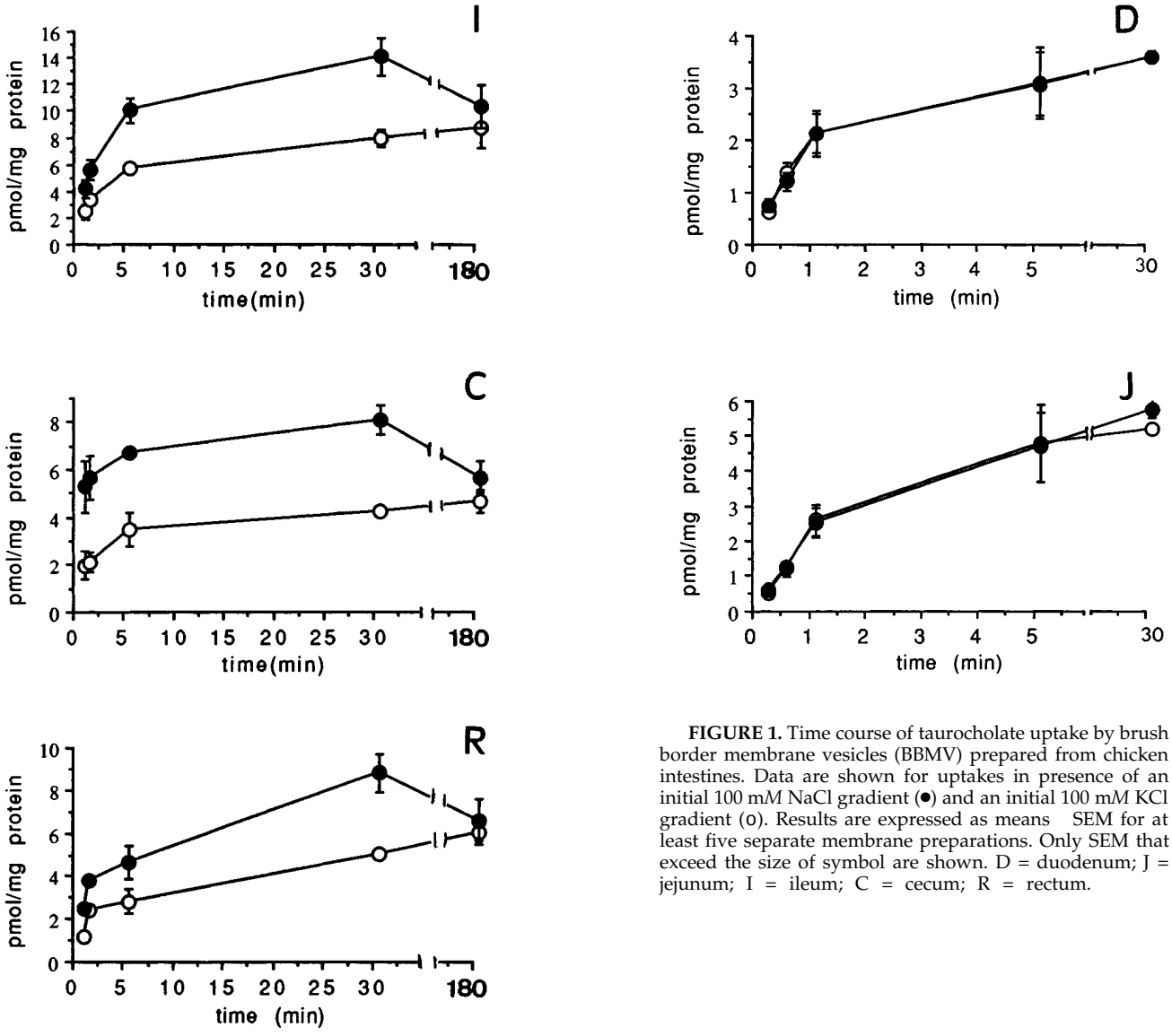

FIGURE 1. Time course of taurocholate uptake by brush border membrane vesicles (BBMV) prepared from chicken intestines. Data are shown for uptakes in presence of an initial 100 m*M* NaCl gradient (●) and an initial 100 m*M* KCl gradient (○). Results are expressed as means SEM for at least five separate membrane preparations. Only SEM that exceed the size of symbol are shown. D = duodenum; J = jejunum; I = ileum; C = cecum; R = rectum.

specificity, dihydroxy bile acids, such as taurochenodeoxycholic acid and taurodeoxycholic acid, were more effective competitors of taurocholate uptake than trihydroxy bile acids or taurocholic acid. These studies, in agreement with previous reports (Kramer *et al*., 1992; Wong *et al*., 1994), revealed that 100 mmol/L of taurochenodeoxycholate was an effective inhibitor of taurocholate in BBMV of ileum, cecum, and rectum. Table 2 shows the inhibition of taurocholate transport by taurochenodeoxycholate, which was similar among ileum, cecum, and rectum (95.2 3.7, 94 8.5, and 95 2.5% for ileum, cecum, and rectum, respectively). We have previously shown, in rat ileum, that the inhibition of Na^+-dependent taurocholate transport by bile salts is of a competitive nature (Molina *et al*., 1986).

Taken together, these results are in accord with previous findings showing that the Na^+-dependent bile salt transporter was restricted to the distal small intestine (Lücke *et al*., 1978; Kramer *et al*., 1993), whereas this Na^+/bile salt cotransport system was absent from duodenum and jejunum (Wilson and Treanor, 1975; Lack *et al*., 1977). This is the first study to show the presence of a Na^+-dependent bile salt transport in BBMV prepared from the ileum, cecum, and rectum of chickens, and its specific inhibition by other bile salts. *In vivo* (Lindsay and March, 1967) and *in vitro* (Glasser *et al*., 1965) studies using everted gut sacs have found differences in the relative absorptive capacity of different parts of the chicken small intestine, and showed that an active transport of taurocholate might occur only in the distal half of the small intestine of chickens; however, these authors did not study the absorptive capacity of bile salts in the cecum and rectum of chickens. Kinetic and molecular studies of chicken Na^+-dependent bile salt transport are currently under investigation in our laboratory.

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