Regional Differences in Transport, Lipid Composition, and Fluidity of Apical Membranes of Small Intestine of Chicken

C. Garriga,* C. M. Vázquez,† V. Ruiz-Gutiérrez,‡ and J. M. Planas*,[1]

*Departament de Fisiologia-Divisió IV, Facultat de Farmàcia, Universitat de Barcelona, Spain;
†Departamento de Fisiología y Biología Animal, Facultad de Farmacia, Universidad de Sevilla, Spain;
and ‡Instituto de la Grasa y sus derivados,
CSIC, Sevilla, Spain

ABSTRACT Na^+-dependent D-glucose transport was studied in brush-border membrane vesicles from duodenum, jejunum, and ileum of 5- to 6-wk-old chickens. Regional differences were found, and both initial rates and accumulation ratio of D-glucose were higher in the proximal part of the small intestine than in the ileum. To establish the mechanism(s) underlying these differences we have studied the density of Na^+-dependent D-glucose cotransporter (SGLT1) as well as lipid composition and fluidity. Phlorizin-specific binding and Western blot analysis indicated a decrease in the amount of SGLT1 in the ileum when compared to the duodenum and jejunum. The distal part of the small intestine also showed a decrease in free cholesterol content and saturated-to-unsaturated fatty acid ratio together with an increase in lipid content and phosphatidylcholine-to-sphingomyelin ratio. These results were associated with a decrease in the diphenylhextriene fluorescence polarization found in brush-border membranes of the ileum. We can conclude that the decrease in the apical D-glucose transport found in the ileum is primarily due to a reduction in the amount of SGLT1 present in the brush-border membrane rather than the differences in the lipid composition and fluidity.

(*Key words*: brush-border membrane vesicles, sodium-glucose transporter isoform 1, phospholipid, fatty acid, cholesterol)

2002 Poultry Science 81:537–545

INTRODUCTION

The small intestine is a highly specialized organ with regional differences in structure and function. Changes in the intestinal transport of hexoses by the Na^+-dependent D-glucose cotransporter along the small intestine have been described for many species (Buddington and Diamond, 1989). In mammals, glucose uptake has been demonstrated to be higher in the apical membrane from the proximal (Freeman and Quamme, 1986) or mid-intestine (Diamond and Karasov, 1984; Thomson, 1984), depending on the animal. In chickens, we have previously shown that capacity to transport sugars decreased from the proximal to distal regions of the small intestine (Ferrer et al., 1994; Rovira et al., 1994). However the mechanism responsible for these regional differences is not yet well established. The aim of the present work was to study the D-glucose uptake using brush-border membrane vesicles (BBMV) from chicken duodenum, jejunum, and ileum and to establish the relative amount of the Na^+-dependent D-glucose cotransporter. In addition, we have simultaneously analyzed the lipid composition and fluidity of these preparations considering that many functions of biological membranes such as transport and enzyme activities are influenced by their composition and physical state (Le Grimellec et al., 1992) and also that the lipid composition and fluidity of the intestinal membranes of mammals present regional differences (Heubi and Fellows, 1985; Schwarz et al., 1985; Ibrahim and Balasubramanian, 1995). The relationships among the regional changes in sugar transport, density of Na^+-dependent D-glucose cotransporter, and membrane composition and fluidity are also discussed.

MATERIALS AND METHODS

Birds

Male white Leghorn chickens were obtained from a commercial farm[2] on the day of hatch and brought to the

Received for publication July 20, 2001.
Accepted for publication November 19, 2001.
[1]To whom correspondence should be addressed: jplanas@farmacia.far.ub.es.
[2]Gibert, Tarragona, Spain.

Abbreviation Key: BBMV = brush-border membrane vesicle; DPH = 1,6-diphenyl-1,3,5-hexatriene; FAMES = fatty acid methyl ester standards; HEPES = *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; PBS-T = PBS containing 0.05% Tween 20; PL = phospholipids; SGLT1 = sodium-glucose transporter isoform 1.

Facultat de Farmàcia. They were maintained in standardized humidity conditions with a 12L:12D photoperiod. Temperature was kept at 34 C during the first week after hatch and at 25 C thereafter. From the day of hatch, birds had free access to water and a commercial diet containing (g/kg diet): 218 crude protein, 33.5 lipid, 375 carbohydrate, 33 crude fiber, 11 lysine, 4 methionine, 10 calcium, 7 phosphorus, 5.4 sodium chloride, and 14,500 UI vitamin A/kg, 2,900 UI D_3/kg, 29 UI vitamin E/kg. The metabolizable energy content was 12.2 MJ/kg. Manipulation and experimental procedures are in accordance with the Spanish regulations for the use and handling of experimental animals.

Experiments were carried out at 5 to 6 wk of age. Birds were killed in the morning, without previous starvation, by cervical dislocation followed by exsanguination. Duodenum (pancreatic loop), jejunum (from the end of the duodenal loop to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction) were removed and immediately flushed with ice-cold saline in presence of phenyltmethanesulphonyl fluoride (0.2 m*M*), LiN_3 (0.41 μM), and benzamidine (0.1 m*M*). Segments were opened lengthwise, frozen in liquid N_2, and stored at –80 C.

BBMV Preparation

The BBMV were prepared by $MgCl_2$ precipitation, as previously described (Vázquez et al., 1997). After successive centrifugations, the final pellet containing purified BBMV was resuspended in a medium containing: 300 m*M* mannitol, 0.1 m*M* $MgSO_4$, 0.41 μM LiN_3, and 20 m*M* *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-tris(hydroxymethyl)aminomethane (Tris) (pH 7.4) to a final protein concentration of 10 to 20 mg/mL. The vesicles were frozen, stored in liquid N_2 in 150-mL aliquots, and used during a period of 15 d. During this time, the integrity and function of the vesicles were not modified (results not shown).

Enzyme and Protein Determinations

The brush-border marker enzyme, sucrase (α-D-glucohydrolase, EC 3.2.1.48), was routinely assayed by the method of Dahlquist (1964). The basolateral membrane marker enzyme, ouabain-sensitive K^+-activated phosphatase (EC 3.6.1.3), was assayed by the method described by Colas and Maroux (1980). Protein was evaluated by a Coomassie Brilliant Blue protein assay with bovine γ-globulin as a standard (Bradford, 1976).

Assay of D-glucose Transport

The uptake of D-glucose was measured by a rapid filtration technique as previously described (Garriga et al., 1999a). Briefly, BBMV were preincubated for 30 min at 37 C in a medium containing K^+ in order to perform the uptake experiments in short-circuited conditions. Loaded BBMV had a final concentration of 200 m*M* mannitol, 50 m*M* KCl, 0.1 m*M* $MgSO_4$, 0.41 μM LiN_3, and 20 m*M* HEPES/Tris (pH 7.4). For time-course studies, vesicles were incubated between 5 s and 30 min at 37 C with a medium containing 100 m*M* mannitol, 100 m*M* NaCl, 0.1 m*M* $MgSO_4$, 0.41 μM LiN_3, 20 m*M* HEPES/Tris (pH 7.4), 45 μM valinomycin, 0.1 m*M* D-glucose, and an aliquot of D-[^{14}C]-glucose. At selected times, the uptake was quenched by adding 1 mL of an ice-cold stop solution containing 300 m*M* mannitol, 0.41 μM LiN_3, and 20 m*M* HEPES/Tris (pH 7.4). The diluted samples were rapidly filtered under negative pressure by placing 0.9 mL of the reaction mixture on a prewetted and chilled cellulose nitrate filter (0.22-μm pore filter).[3] The filter was rinsed with 5 mL ice-cold stop solution.

The filter was dissolved in a Biogreen-6 cocktail,[4] and the radioactivity retained was measured with a scintillation counter. Non-specific radioactivity binding to the filters was obtained by adding the stop solution to reaction tubes immediately after addition of the vesicles. This non-specific binding was subtracted from the total radioactivity of each sample. All experiments were performed in triplicate.

Orientation of Vesicles

Membrane orientation was studied from sucrase activity according to Del Castillo and Robinson (1982). Sucrase activity was determined in intact vesicles and in vesicles incubated for 30 min with a mixture of 3 m*M* deoxycholate and 15 m*M* EDTA.

Phlorizin Binding Measurements

Steady-state phlorizin binding was assayed at 37 C by the method described by Garriga et al. (1999a). BBMV suspensions were rapidly mixed with the incubation medium containing 100 mM mannitol, 100 m*M* NaCl or KCl, 0.1 m*M* $MgSO_4$, 20 m*M* HEPES/Tris (pH 7.4), 0.41 μM LiN_3, 50 μM phlorizin, and an aliquot of [^{3}H]phlorizin. At 5 s, the binding process was stopped by addition of 1 mL of an ice-cold stop solution containing 300 m*M* mannitol, 0.1 m*M* $MgSO_4$, 20 m*M* HEPES/Tris (pH 7.4), and 0.41 μM LiN_3. The resulting suspension was rapidly filtered under negative pressure through 0.22-μm cellulose nitrate filters and washed with 5 mL of stop solution. The radioactivity remaining in the filter was determined as described earlier. Specific [^{3}H]phlorizin binding was calculated by subtracting the non-specific binding (in the presence of K^+) from total phlorizin binding (assayed in the presence of Na^+). The density of phlorizin-binding sites was expressed as picomoles of phlorizin bound per milligram of protein with 50 μM phlorizin (B_{50}).

Western Blot Analysis of SGLT1

Measurements of sodium-glucose transporter isoform 1 (SGLT1) protein abundance in BBMV of chicken small

[3]Millipore (www.millipore.com).
[4]Sharlau, Barcelona, Spain.

intestine were performed using Western-blot analysis, as previously described (Garriga et al., 1999b). The BBMV were solubilized by boiling in the presence of SDS and 2-mercaptoethanol. Aliquots (30 mg of protein) were subjected to SDS-PAGE with an 8% linear polyacrylamide gel. After electrophoresis, the proteins were electrophoretically transferred from the unstained gel to nitrocellulose membranes in a transfer buffer containing 20 m*M* Tris, 150 m*M* glycine, and 20% methanol; transfer was for 60 min at 4 C and at constant voltage of 100 V using a Trans-Blot apparatus.[5] Non-specific binding sites were first blocked with PBS containing 0.05% Tween 20 (PBS-T) and 3% BSA.

Blots were incubated overnight at 4 C with a rabbit polyclonal antibody (kindly donated by M. Kasahara, Teikyo University, Tokyo, Japan) raised against a synthetic peptide corresponding to amino acids 564 to 575 of the deduced amino acid sequence of rabbit intestinal SGLT1 (Hediger et al., 1987) diluted to 1:5,000. In parallel experiments, nitrocellulose membranes were incubated with the same antibody that had first been adsorbed with the peptide (1 mg/mL) against which the antibody was raised (kindly provided by E. M. Wright, UCLA, Los Angeles, CA). The membranes were washed (8 × 4 min) with PBS-T. Then, anti-SGLT1 antibody was detected by ECL chemiluminiscence[6] by using a peroxidase-conjugated anti-rabbit IgG[7] as a second monoclonal antibody (1:3,000). After detection, the samples were measured by scanning densitometry.

Lipid Analysis

Total lipids were extracted from the brush-border membrane by the method of Rose and Oklander (1965), using 0.02% (wt/vol) butylated hydroxytoluene as antioxidant. The lipid extract was quantified gravimetrically and kept in a stoppered vessel under nitrogen atmosphere at –30 C until the assays. Lipid and phospholipid compositions were obtained by means of the Iatroscan thin-layer chromatography/flame ionization detector technique (De Schrijver and Vermeulen, 1991). Iatroscan MK-5 was used in combination with Chromarods S, which have a precoated active silica thin layer. Chromarods S were routinely stored in 4.5 M H_2SO_4. Prior to use, the rods were washed with distilled water, dried at 110 C for 15 min, and activated by being passed through the flame of the Iatroscan detector. Each rod was spotted with a 10-mL Hamilton syringe containing 3 mL of total lipids or phospholipids (PL). To separate total lipids, rods were developed in hexane-diethyl ether-acetic acid (80:20:1, vol/vol/vol). The PL were resolved in two steps, starting with an initial development of rods in chloroform-methanol-acetic acid-water (201:84:9:6, vol/vol/vol), drying at 70 C for 10 min, and a second development in hexane-diethylether-acetic acid (80:20:1, vol/vol/vol).

Rods were scanned under the following conditions: 150 mL/min hydrogen flow, 1,750 mL/min air flow, 47 mm/s scanning speed, and 42 mm/min chart speed. A Iatrocorder TC-11 integrator was used to record and integrate the area.

Fatty Acid Analysis

Fatty acids of the total lipid extract were analyzed by gas chromatography. The samples were saponified by heating for 5 min with 5 mL of 0.2 *M* sodium methylate and heating again at 80 C for 5 min with 6% (wt/vol) H_2SO_4 in anhydrous methanol. The fatty acid methyl esters thus formed were eluted with hexane and analyzed in a Hewlett-Packard 5890 series II gas chromatograph equipped with a flame ionization detector and using an Omegawax 320 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 mm film). The initial column temperature was 200 C, which was maintained for 10 min, then programmed to increase from 200 to 230 C by 2 C/min. The injection and detector temperatures were 250 and 269 C, respectively. The flow rate of helium was 2 mL/min, the column head pressure was 250 kPa, and the detector auxiliary flow rate was 25 mL/min. Peak areas were calculated by a Hewlett-Packard 3990A recording integrator.

Individual fatty acid methyl esters were identified on isothermal runs by comparison of their retention time against those of standards. Fatty acid methyl esters were quantified by internal standarization (tricosanoic methyl ester, 23:0) by using peak-area integration.

Fluorescence Measurement

The steady-state fluorescence polarization and fluorescence anisotropy were determined as previously described (Vázquez et al., 1997), using the lipid-soluble fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). A stock solution of 2 m*M* DPH in tetrahydrofuran was prepared and stored in the dark at –30 C. BBMV equivalent to 100 mg protein was incubated at 25 C for 1 h in 2 mL of buffered saline containing 250 m*M* sucrose, 10 m*M* Tris/HCl (pH 7.4), and 2 m*M* DPH. Measurements were taken using a fluorescence spectrophotometer equipped with a polarizing filter. The excitation and emission wavelengths were 365 and 430 nm, respectively.

The steady-state fluorescence polarization (p) was calculated as follows:

$$p = (I_{vv} - I_{vh}/I_{vv} + I_{vh})$$

where I_{vv} and I_{vh} are observed intensities measured with polarizers respectively parallel to and perpendicular to the vertically oriented polarizer exciting beam. The steady-state fluorescence anisotropy (r_s) was calculated from the following ratio:

$$r_s = 2p/(3\text{-}p).$$

Corrections for light scattering were routinely made by subtracting the signal obtained from identical but unla-

[5]Bio-Rad, Hercules, CA.
[6]Amersham International, Buckinghamshire, UK.
[7]Sigma Chemical Company, St. Louis, MO.

TABLE 1. Sucrase activity of brush-border membranes from duodenum, jejunum, and ileum[1]

Intestinal region	Homogenate specific activity	n	BBMV-specific activity (nkat/mg protein)	Enrichment	Recovery[2] (%)
Duodenum	0.249 ± 0.019[c]	4	4.132 ± 0.58	14 ± 3.0	45 ± 3.5
Jejunum	1.193 ± 0.070[a]	13	11.39 ± 0.91	11 ± 0.5	42 ± 5.2
Ileum	0.365 ± 0.066[b]	5	4.164 ± 0.40	14 ± 3.6	41 ± 7.4

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

[1]Values are means ± SEM; n = number of separate isolations.

[2]Enrichment is ratio of the specific activities in final brush-border membrane vesicles preparation (BBMV) and initial homogenate. Recovery shows total activity in final brush-border preparation expressed as a percentage of activity in original homogenate.

beled samples and contributed <3% to the fluorescence of the complete system.

Chemicals

All unlabeled reagents for the transport studies were obtained from Sigma Chemical Company.[7] Measurements of the enzyme activity were carried out using purchased reagents[8] and the enhanced chemiluminiscence were from Amersham International.[6] D-[U-^{14}C]-glucose (specific activity 251 mCi/mmol) and [^{3}H]phlorizin (specific activity 46.4 Ci/mmol) were purchased.[9] The final activity of labeled substrates in the incubation medium was 0.5 to 2 mCi/mL. In the lipid composition studies, fatty acid methyl ester standards (FAMES) were obtained from Larodan Fine Chemicals.[10] The internal standard solutions were prepared by dissolving 200 mg of tricosanoid acid methyl ester in 100 mL of hexane. Calibration solutions were prepared by dissolving specific amounts of FAMES in hexane containing 2,6-ditertbutyl-*p*-cresol (butylated hydroxy-toluene) obtained from Sigma Chemical Company.[7] Other chemicals were analytical grade.[11]

Statistical Analysis

Statistical differences between intestinal regions were established by ANOVA and Snedecor's *F* test. $P < 0.05$ was taken as significant.

RESULTS

Purity of the BBMV Preparation

Table 1 shows the specific activities of sucrase in homogenates of duodenum, jejunum, and ileum. The specific activity of sucrase in the jejunum was significantly higher than that obtained in the mucosa from duodenum and ileum. The concentration of sucrase activity in the final brush-border membrane pellet from different regions was uniformly 11- to 14-fold greater than in the starting homogenate, and the recoveries of sucrase were from 41 to 45%. There was no significant difference in enrichment and recovery between segments. Brush-border membrane fractions presented low activity of ouabain-sensitive K^+-activated phosphatase. The activity of this enzyme was reduced during the isolation procedure in any segment studied (results not shown), indicating little basolateral contamination. The membrane orientation studies indicate that 92 ± 2% (duodenum), 93 ± 5% (jejunum), and 93 ± 3% (ileum) of the vesicle population were outside-out oriented.

Transport of D-Glucose Across BBMV

Figure 1 shows the time course of D-glucose transport into the BBMV along the three regions of the chicken small intestine. In the presence of a 100 m*M* extravesicular-to-intravesicular Na^+ gradient, D-glucose uptake exhibited a typical transient increase in the intravesicular concentration of sugar in three segments, at 5 s of incubation. The overshoot disappeared when the Na^+ gradient was replaced by a K^+ gradient. Uptake of D-glucose at equilibrium (30 min) was identical in the presence or absence of Na^+ gradient and was not changed in any of the segments. The mean value of the vesicular volume was 0.56 ± 0.09 μL/mg of protein for the duodenum, 0.59 ± 0.08 μL/mg of protein for the jejunum, and 0.61 ± 0.07 μL/mg of protein for the ileum. The accumulation ratios were 3.43 in the duodenum, 3.21 in the jejunum, and 2.16 in the ileum.

Specific Phlorizin Binding

Figure 2 shows that the density of phlorizin-binding sites was significantly lower in the ileum (34.5 ± 3.7 pmol of phlorizin bound/mg protein) when compared to the duodenum and jejunum (50.1 ± 2.7 and 51.2 ± 1.9 pmol phlorizin/mg protein, respectively). No differences were found between duodenum and jejunum.

Immunoblots

Figure 3A shows a typical Western blot with rabbit polyclonal antibody raised against the 564-575 amino acid sequence of rabbit SGLT1. The antibody recognized a single band of 75 kDa, which was blocked by preab-

[8]Boehringer, Manheim, Germany.
[9]New England Nuclear Research Products, Dreieich, Germany.
[10]Malmö, Sweden.
[11]Merck, Darmstadt, Germany.

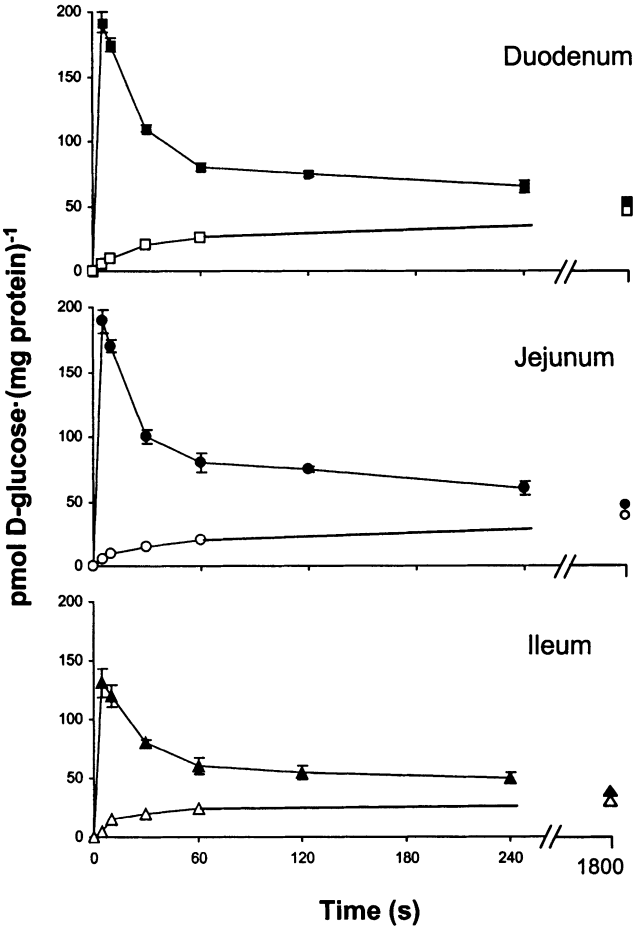

FIGURE 1. Time course of D-glucose uptake in the brush-border membrane vesicle of duodenum (D), jejunum (J) and ileum (I) from chicken. Vesicles were incubated (5, 10, and 30 s and 1, 2, and 30 min) with 0.1 m*M* D-glucose, in the presence of a Na^+ gradient (filled symbols) or in absence of a Na^+ gradient (empty symbols). The initial rates (expressed as pmol D-glucose/(mg of protein per s) were 38.4 ± 0.9, 37.9 ± 0.9, and 26.3 ± 0.7, for D, J, and I, respectively). Results are expressed as means ± SEM of three to four separate membrane preparations per segment.

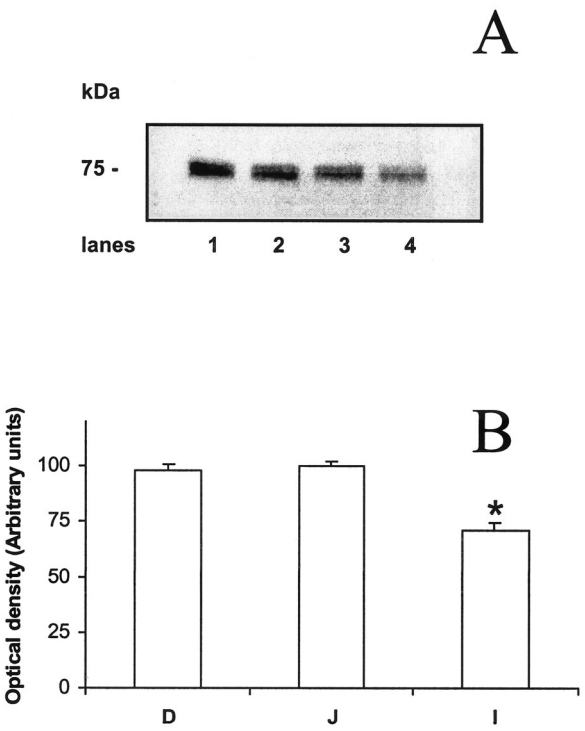

FIGURE 3. (Panel A) Western blot analysis of sodium-glucose transporter isoform 1 (SGLT1) in the brush-border membrane vesicle (BBMV) of rabbit small intestine (Lane 1) and in BBMV from duodenum (D; Lane 2), jejunum (J; Lane 3) and ileum (I; Lane 4) of chicken. Samples (30 mg of protein/lane) were blotted using a rabbit polyclonal antibody raised against synthetic peptide corresponding to amino acids 564 to 575 of the deduced amino acid sequence of rabbit intestinal SGLT1. (Panel B) Relative abundance measured by optical densitometry. Results show the means ± SEM of three different experiments. Molecular mass standard is shown on the left. *Significant differences (Student's *t*-test; $P < 0.05$).

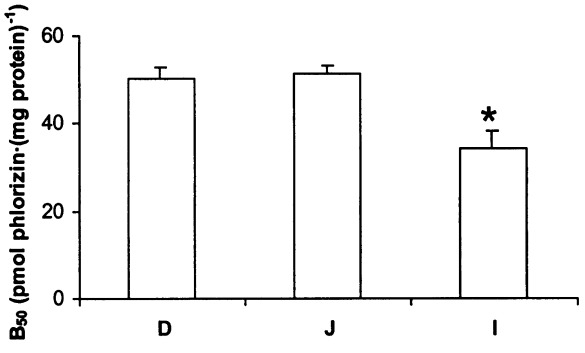

FIGURE 2. Specific phlorizin binding to the brush-border membrane vesicle of duodenum (D), jejunum (J), and ileum (I) from chicken. Values are means ± SEM of five separate experiments, expressed as picomoles of phlorizin bound per milligram of protein at a phlorizin concentration of 50 μM (B_{50}). The B_{50} values (in pmol/mg of protein) were: 50.1 ± 2.7 (D), 51.2 ± 1.9 (J), and 34.5 ± 3.7 (I). *Significant differences (Student's *t*-test; $P < 0.05$).

sorbing it with the antigenic peptide. Figure 3B shows the densitometric analysis of three separate assays that indicated the abundance of SGLT1 in ileum was 30% less when compared with duodenum and jejunum, without differences between these proximal segments.

Correlation Between Maximal Transport Rates, Specific Phlorizin Binding, and Densitometric Analysis of SGLT1 Immunoblots

There was a positive correlation between the initial rates and both the specific phlorizin binding and the optical density determined by Western blot analysis. These correlations are defined by the equations y = 0.73x + 1.17 (r = 0.9954) and y = 0.75x + 1.03 (r = 0.9967), respectively. The relative values of the three parameters in duodenum, jejunum, and ileum are represented in the histogram of Figure 4. Identical patterns were found among the three parameters along the small intestine. In all cases, there are significant decreases in the ileum in comparison with the proximal segments.

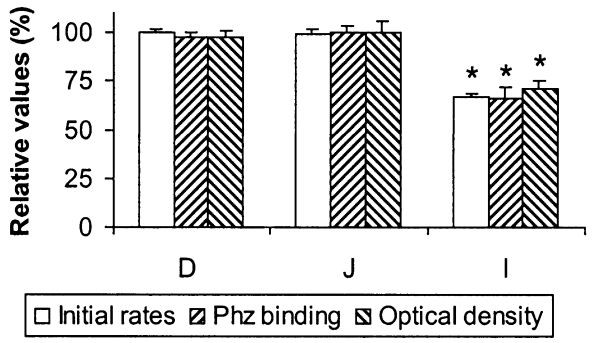

FIGURE 4. Relative values of initial rates of D-glucose, specific binding using 50 μM phlorizin (Phz; B_{50}), and optical density determined by Western blot analysis. Results show the means ± SEM of three different experiments. *Significant differences (Student's t-test; $P < 0.05$.

Lipid Composition of BBMV

Brush-border membrane lipid composition of all segments studied is shown in Table 2. There was a significant regional increase in the lipid/protein ratio, with no differences between duodenum and jejunum, and a high ratio in ileum. The contents of PL, free fatty acids, triglycerides, and cholesterol ester were not modified. Higher values in the molar ratios of free cholesterol/PL were found in the duodenum and jejunum when compared to the ileum, as a result of an increase in free cholesterol levels of these segments. No differences were observed between the duodenum and jejunum.

The PL composition is shown in Table 3. The major PL in all studied segments were phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine. Phosphatidylinositol and sphingomyelin were found at lesser concentrations. Among the individual PL, duodenum and jejunum had lower phosphatidylcholine levels, which resulted in lower phosphatidylcholine/sphingomyelin ratio of these segments when compared to the ileum. No differences were noted for the other phospholipids or between duodenum and jejunum.

The fatty acid composition in brush-border membranes of different segments is given in Table 4. The major fatty acids in all these segments were palmitic (16:0), stearic (18:0), oleic (18:1, n-9), and linoleic (18:2, n-6). Arachidonic acid was present although at low concentration. A decrease in the level of saturated acids (mainly stearic acid) together with an increase in the level of unsaturated acids (mainly oleic acid) was found in the distal segments. These changes resulted in a decrease in the ratio saturated/unsatutared fatty acids in ileal lipids.

Membrane Fluidity

The steady-state fluorescence polarization (p) and fluorescence anisotropy (r_s) data for DPH-labeled brush-border membrane preparations from all segments studied are given in Table 5. In the duodenum and the jejunum, both variables were higher than those found in the ileum, indicating that the membranes from proximal small intestine were less fluid. No differences were observed between the duodenum and the jejunum.

DISCUSSION

The aim of this study was to establish the regional differences in the activity of SGLT1 in the small intestine of chickens and to analyze which mechanisms are responsible for these changes. For this purpose, we have measured Na^+-dependent D-glucose uptake, estimated the number of SGLT1 transporters by the specific binding of phloridzin and Western blot analysis, and studied the membrane fluidity/lipid composition in BBMV isolated from chicken duodenum, jejunum, and ileum. Our data indicate that Na^+-dependent D-glucose transport is decreased in the ileum when compared to the duodenum and the jejunum. The time course and total uptakes of Na^+-D-glucose cotransport were studied using short-circuited BBMV to avoid possible effects of regional changes in the permeability of electrolytes. The three intestinal segments studied showed a transient overshoot of D-glucose. The initial rates and accumulation ratios in the ileum were around 30% lower than those in the duodenum and the jejunum (Figure 1). These regional differences in the activity of SGLT1 cannot be attributed to variations in vesicle preparations, as the purification and size of BBMV, as measured from the enrichment of sucrase and Na^+-dependent D-glucose uptake at equilibrium, respectively, were similar in all three intestinal regions (Table 1).

Similar results have been observed in the distal small intestine from chicken (Ferrer et al., 1994; Rovira et al., 1994), rats (Freeman and Quamme, 1986), monkey (Ibrahim and Balasubramanian, 1995), mouse (Diamond and Karasov, 1984), and rabbit (Thomson, 1984). How-

TABLE 2. Brush-border membrane lipid composition in duodenum, jejunum, and ileum of chicken[1]

Lipid (% wt/wt)	Duodenum	Jejunum	Ileum
Lipid/protein (w/w)	0.64 ± 0.07[b]	0.65 ± 0.03[b]	1.8 ± 0.4[a]
Total phospholipid (PL)	74.2 ± 2.3	64.2 ± 4.7	69.0 ± 3.0
Free cholesterol (FC)	27.3 ± 1.5[a]	28.0 ± 1.1[a]	20.0 ± 1.2[b]
Free fatty acids	6.7 ± 1.0	6.4 ± 1.8	6.8 ± 0.5
Cholesterol ester	4.7 ± 0.2	3.4 ± 0.3	5.5 ± 1.0
Triglycerides	3.9 ± 0.7	3.4 ± 0.8	2.5 ± 0.6
FC/PL	0.38 ± 0.01[a]	0.44 ± 0.03[a]	0.28 ± 0.02[b]

[a,b]Values in each row with no common superscript differ significantly ($P < 0.05$).

[1]Values represent means ± SEM from at least five separate membrane preparations.

TABLE 3. Brush-border membrane phospholipid composition in duodenum, jejunum, and ileum of chicken[1]

Phospholipid[2] (% wt/wt)	Duodenum	Jejunum	Ileum
PC	29.0 ± 0.9[b]	26.9 ± 3.4[b]	35.8 ± 4.5[a]
PS	29.3 ± 2.9	33.7 ± 2.4	29.0 ± 6.7
PE	29.3 ± 2.6	31.1 ± 1.8	29.2 ± 4.3
PI	8.0 ± 0.6	7.5 ± 1.4	6.7 ± 2.3
SM	3.1 ± 0.7	4.4 ± 0.7	3.0 ± 0.6
PC/SM	5.8 ± 0.1[b]	5.9 ± 0.7[b]	9.4 ± 1.2[a]

[a,b]Values in each row with no common superscript differ significantly ($P < 0.05$).

[1]Values represent means ± SEM from at least five separate membrane preparations.

[2]PC = phosphatidylcholine; PS = phosphatidylserine; PE = phosphatidylethanolamine; PI = phosphatidylinositol; SM = sphingomyelin.

ever, in ducklings, the maximal capacity to transport sugars is found in the ileum (Thomas et al., 1996).

To investigate whether the regional changes in the sugar transport were correlated with changes in the density of SGLT1 protein along the chicken small intestine, the specific binding of phlorizin and Western blot analysis were performed. When phlorizin was used as a competitive inhibitor of D-glucose uptake in the intestinal brush-border membrane (Garriga et al., 1999a), the specific phlorizin binding in the ileum was lower than those in the duodenum and the jejunum (around 30%), with no significant differences between these two proximal segments. When the specific phlorizin binding (B_{50}) was plotted against the initial rate, a highly linear correlation was found, indicating that the observed changes in the Na^+-dependent D-glucose transport along the small intestine are directly correlated with modifications in the amounts of SGLT1 transporter. With adult chickens, Garriga et al. (1999a) described differences in the specific phlorizin binding between jejunum, ileum, and rectum that fully account for the changes in the sugar transport rates observed in chicken adapted to diets containing varying amounts of Na^+.

Studies on the density of SGLT1 protein by Western blot analysis showed a single band of 75-kDa immunoreactive protein in all intestinal regions analyzed. Results of blot densitometry showed regional changes in the abundance of SGLT1 protein, with a decrease in the brush-border membranes from the ileum when compared to the duodenum and the jejunum, with no differences between these proximal segments (Figure 3). All these results show a clear correlation between SGLT1 protein abundance and the SGLT1-mediated glucose transport along the chicken small intestine.

In previous studies (Garriga et al., 1999a,b), we reported no differences in the specific phlorizin binding and the amounts of SGLT1 protein determined by Western blot analysis between jejunal and ileal BBMV. In these studies, we used 12-wk-old chickens adapted to a diet that consisted of a mixture of wheat and barley (1:1) with a carbohydrate content of 626 g/kg diet. The composition of this diet is significantly different from the commercial diet supplied to the 5-wk-old chickens used in the present study. The commercial diet contained half of the amount of carbohydrate, 375 g/kg. This marked difference might explain an enhanced expression of hexose transporters in the intestine of animals fed with a diet containing higher amounts of sugars (Diamond and Karasov, 1984).

Modifications in the membrane lipid composition and, consequently, in membrane microviscosity could affect the function of many membrane-bound proteins, such as Na^+-dependent D-glucose cotransporter (Brasitus et al., 1979; Carrière and Le Grimellec, 1980; Fernández et al., 1984). The highest changes in membrane lipid composition were observed in apical membranes from the ileum. Distal brush-border membranes were shown to have

TABLE 4. Brush-border membrane fatty acid composition in duodenum, jejunum, and ileum of chicken[1]

Fatty acid (% wt/wt)	Duodenum	Jejunum	Ileum
14:0	1.2 ± 0.3	1.9 ± 0.2	1.6 ± 0.3
16:0	20.9 ± 0.7	23.1 ± 1.2	21.2 ± 0.7
16:1(n-7)	2.2 ± 1.4	2.0 ± 0.4	2.2 ± 0.2
18:0	26.6 ± 0.7[a]	21.3 ± 0.7[b]	18.3 ± 0.2[c]
18:1(n-9)	13.1 ± 0.2[c]	16.0 ± 0.4[b]	18.2 ± 0.7[a]
18:2(n-6)	24.6 ± 1.7	22.7 ± 2.1	23.7 ± 0.9
20:4(n-6)	7.3 ± 0.9	6.7 ± 0.4	8.5 ± 0.5
Saturated	48.7 ± 1.7[a]	46.7 ± 0.3[a]	41.3 ± 0.7[b]
Monounsaturated	16.7 ± 0.5[b]	19.7 ± 1.3[a,b]	21.9 ± 0.6[a]
Polyunsatured	51.3 ± 1.7[b]	53.5 ± 0.4[b]	58.9 ± 0.6[a]
Saturated/unsaturated	0.96 ± 0.07[a]	0.88 ± 0.02[a]	0.70 ± 0.02[b]

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

[1]Values represent means ± SEM from at least four separate membrane preparations.

TABLE 5. The steady-state fluorescence polarization (pDPH) and steady-state fluorescence anisotropy (r_s) in duodenum, jejunum, and ileum of chicken[1]

	Duodenum	Jejunum	Ileum
P_{DPH}	0.356 ± 0.002[a]	0.362 ± 0.005[a]	0.342 ± 0.004[b]
r_s	0.260 ± 0.001[a]	0.264 ± 0.004[a]	0.244 ± 0.003[b]

[a-b]Values in each row with no common superscript differ significantly ($P < 0.05$).

[1]Values represent means ± SEM from at least eight separate membrane preparations.

higher molar ratios of lipid-to-protein, phosphatidylcholine-sphingomyelin, PL-free cholesterol, and unsaturated-saturated than brush-border membranes from the duodenum and the jejunum (Tables 3 to 5). These results are consistent with studies of DPH fluorescence polarization found in the ileum, which indicated a decreased membrane microviscosity in the apical membranes isolated from the ileum when compared to those from the duodenum and the jejunum. These results are consistent with the study of Ibrahim and Balasubramanian (1995) in monkey small intestine and Dudeja et al. (1989) in human small intestine, which showed that the distal portion of the small intestine was more fluid mainly due to regional changes in cholesterol, phosphatidylcholine and unsaturated fatty acid content. However, studies in rat (Heubi and Fellows, 1985) and rabbit (Schwarz et al., 1984) showed that brush-border membranes isolated from ileum are less fluid than those from duodenum and jejunum, mainly due to increased content of cholesterol and a greater degree of saturation in the fatty acid composition (Schwarz et al., 1985). These different results might be explained by differences in the animal species and by the effects of different diets.

The highest membrane fluidity observed in the ileum from chicken intestine might indicate that the SGLT1 turnover number in the ileum is higher than in the duodenum and the jejunum. However, when the turnover number of SGLT1 protein was calculated as the relationship between the initial rates of 0.1 m*M* D-glucose transport and the number of specific phlorizin binding sites measured at a phlorizin concentration of 50 μM (Garriga et al., 1999a), no significant differences were found between the three intestinal regions studied indicating that the higher fluidity of brush-border membranes isolated from the chicken ileum did not increase the turnover number of the SGLT1 cotransporter in this segment. However, variations in fluidity recorded along the small intestine might correspond to changes in localized domains of the brush-border membrane and might have little effect on the fluid environment of the Na^+-D-glucose cotransporter (Le Grimellec et al., 1992).

Although there are previous studies demonstrating a positive correlation between membrane fluidity and Na^+-dependent D-glucose transport in rat (Brasitus et al., 1979; Heubi and Fellows, 1985; Freeman and Quamme, 1986) or rabbit (Schwarz et al., 1984; Thomson, 1984), there is a lack of agreement in the literature and other authors demonstrated an inverse relationship between fluidity and D-glucose transport in intestinal or kidney brush-border membranes (Carrière and Le Grimellec, 1980; Fernández et al., 1984).

In conclusion, we found regional differences in the apical Na^+-dependent D-glucose uptake in BBMV that are due to variations in the expression of Na^+-dependent D-glucose transporter, because there was a decrease in the abundance of the cotransporter in the distal part of the small intestine. In addition, changes in the lipid composition and an increase in the fluidity of ileal BBMV were found without direct evidence of correlation between these modifications and Na^+-glucose transport. The regional changes of the abundance of the cotransporter indicated the presence of molecular mechanisms that regulated the expression of Na^+-D-glucose cotransporter. Further studies using specific cDNA to the Na^+-D-glucose cotransporter may provide a better understanding of this regulation.

ACKNOWLEDGMENTS

The rabbit polyclonal antibody raised against the synthetic peptide corresponding to amino acids 564 to 575 of the rabbit intestinal SGLT1 sequence and the antigenic peptide were kindly provided by M. Kasahara (Teikyo University, Tokyo, Japan) and E. M. Wright (UCLA University, Los Angeles, CA), respectively. We are indebted to M. García-Rocha (Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona, Barcelona, Spain) for valuable technical advice in Western blot experiments. C. Garriga was a recipient of a Formació d'Investigadors grant from Generalitat de Catalunya. This work was supported by grants PB96/1255 from the Ministerio de Educación y Cultura and 1999-SGR-00271 from the Generalitat de Catalunya (Spain).

REFERENCES

Buddingtton, R. K., and J. M. Diamond. 1989. Ontogenetic development of intestinal nutrient transporters. Annu. Rev. Physiol. 51:601–619.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Brasitus, T. A., D. Schachter, and D. Mamouneas. 1979. Functional interaction of lipids and proteins in rat intestinal microvillus membranes. Biochemistry 18:4136–4144.

Carrière, B., and C. Le Grimellec. 1980. Effects of benzyl alcohol on enzyme activities in kidney brush-border membranes. Biochim. Biophys. Acta 600:406–420.

Colas, B., and S. Maroux. 1980. Simultaneous isolation of brush-border and basolateral membrane from rabbit enterocytes. Biochim. Biophys. Acta 600:406–420.

Dahlquist, A. 1964. Method for assay of intestinal disaccharidases. Anal. Biochem. 7:18–25.

Del Castillo, J. R., and W. L. Robinson. 1982. The simultaneously preparation of basolateral and brush-border membrane vesicles from guinea pig intestinal epithelium and the determination of the orientation of the basolateral vesicles. Biochim. Biophys. Acta 688:45–46.

De Schrijver, R., and D. Vermeulen. 1991. Separation and quantification of phospholipids in animal tissues by Iatroscan TLC/FID. Lipids 26:74–76.

Diamond, J. M., and W. H. Karasov. 1984. Effect of dietary carbohydrate on monosaccharide uptake by mouse small intestine in vitro. J. Physiol. (London) 349:419–440.

Dudeja, P. K., J. M. Harig, R. Ramaswamy, and T. A. Brasitus. 1989. Protein-lipid interactions in human small intestinal brush-border membranes. Am. J. Physiol. 257:G809–G817.

Fernández, Y. J., R. A. M. Boigegrain, C. D. Cambon-Gros, and S. E. Mitjavila. 1984. Sensitivity of Na^+-coupled D-glucose uptake, $Mg2^+$-ATPase and sucrase to perturbations of the fluidity of brush-border membrane vesicles induced in n-aliphatic alcohols. Biochim. Biophys. Acta 770:171–177.

Ferrer, R., M. A. Gil, M. Moretó, M. Oliveras, and J. M. Planas. 1994. Hexose transport across the apical and basolateral membrane of enterocytes from different regions of the chicken intestine. Pflüg. Archiv. 426:83–88.

Freeman, H. J., and G. A. Quamme. 1986. Age-related changes in sodium-dependent glucose transport in rat small intestine. Am. J. Physiol. 14:G208–G217.

Garriga, C., M. Moretó, and J. M. Panas. 1999a. Hexose transport in the apical and basolateral membranes of enterocytes in chickens adapted to high and low NaCl intakes J. Physiol. (London) 514:189–199.

Garriga, C., N. Rovira, M. Moretó, and J. M. Planas. 1999b. Expression of Na^+-D-glucose cotransporter in brush-border membrane of the chicken intestine. Am. J. Physiol. 276:R627–R631.

Hediger, M. A., M. J. Coady, T. S. Ikeda, and E. M. Wright. 1987. Expression cloning and cDNA sequencing of the Na^+/glucose co-transporter. Nature 330:379–381.

Heubi, J. E., and J. L. Fellows. 1985. Postnatal development of intestinal bile salt transport. Relationship to membrane physico-chemical changes. J. Lipid Res. 26:797–805.

Ibrahim, S. A., and K. A. Balasubramanian. 1995. Lipid composition and membrane fluidity of monkey small intestinal brush-border membrane: Regional differences. Indian. J. Biochem. Biophysics 32:290–294.

Le Grimellec, C. L., G. Friedlander, E. H. ElYandouzi, P. Zlatkine, and M. C. Giocondi, 1992. Membrane fluidity and transport properties in ephitelia. Kidney Int. 42:825–836.

Rose, H. G., and M. Oklander. 1965. Improved procedure for the extraction of lipids from human erythrocytes. J. Lipid Res. 6:428–431.

Rovira, N., M. E. Soriano, and J. M. Planas. 1994. Ontogenetic and regional changes in kinetic constants of α-methyl-D-glucoside transport in chicken small intestine. Biochem. Soc. T. 22:262S.

Schwarz, S. M., S. Ling, B. Hostetler, J. P. Draper, and J. B. Watkins. 1984. Lipid composition and membrane fluidity in the small intestine of the developing rabbit. Gastroenterology 86:1544–1551.

Schwarz, S. M., B. Hostetler, S. Ling, M. Mone, and J. B. Watkins. 1985. Intestinal membrane lipid composition and fluidity during development in the rat. Am. J. Physiol. 248:G200–G207.

Thomas, V., B. Pichon, G. Crouzoulon, and H. Barre. 1996. Effect of chronic cold exposure on Na^+-dependent D-glucose transport along small intestine in ducklings. Am. J. Physiol. 271:R1429–R1438.

Thomson, A. B. R. 1984. Effect of region of intestine and unstirred layers on uptake of sugars into rabbit intestine. Q. J. Exp. Physiol. 69:497–505.

Vázquez, C. M., N. Rovira, V. Ruiz-Gutierrez, and J. M. Planas. 1997. Developmental changes in glucose transport, lipid composition, and fluidity of jejunal BBM. Am. J. Physiol. 273:R1086–R1093.