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BRUSH BORDER MEMBRANE VESICLES FORMED FROM HUMAN DUODENAL BIOPSIES EXHIBIT NA^+-DEPENDENT CONCENTRATIVE L-LEUCINE AND D-GLUCOSE UPTAKE

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Summary: The human duodenum actively transports L-leucine and D-glucose under Na^+ gradient conditions as demonstrated by uptake studies using brush border membrane vesicles from organ donor duodenum. Brush border membrane vesicles formed from peroral duodenal biopsies likewise demonstrate Na^+ dependent concentrative uptake of D-glucose and L-leucine. This is the first demonstration of active transport processes in human duodenum. A simple microvesiculation method to form these vesicles is described as well as its potential application to clinical medicine in studying diseases of defective intestinal transport.

Introduction: Human small intestine obtained from organ donors has been used to form brush border membrane vesicles for transport studies (1-5). Vesicles obtained from human jejunum and ileum demonstrate active transport of D-glucose and L-leucine in the presence of a Na^+ gradient (6). However, it is not known whether the human duodenum possesses monosaccharide and amino acid transporters similar to the jejunum and ileum. The purpose of the present study was two-fold: 1) to determine if the human duodenum actively transports amino acids and monosaccharides 2) to develop a technique for obtaining brush border membrane vesicles from routine endoscopic duodenal biopsies in order to assess duodenal transport functions.

Methods

Permission for these studies was obtained from the Human Rights and Research Committee of the Medical College of Wisconsin on August 13, 1984.

Brush Border Membrane Vesicles From Organ Donor Intestine

Human duodenal mucosa was obtained from six organ donors at the time of organ retrieval as previously described (1-3). The mucosa was scraped from the seromuscular layer and was stored at -70° C. Purified brush border membranes were obtained from thawed mucosal scrapings by the $CaCl_2$ precipitation method of

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Schmitz et al (7). Vesiculation was attained by a modification of the procedure of Hopfer et al (8), as previously described by us (1-3). Uptake studies were performed using 50 uM [^{14}C] D-glucose and 50 uM [^{14}C] L-leucine.

Microvesiculation method for endoscopic biopsies

Once the above transport studies had been performed, we formed brush border membrane vesicles from very small amounts of organ donor mucosa utilizing a microvesiculation method (Figure 1). We could form enough membrane vesicles to get a 3 point time course uptake curve for D-glucose and L-leucine using only 100-200 mg of mucosa. We then obtained biopsies from the descending duodenum of three patients; during fiberoptic endoscopy in two and by fluoroscopically controlled aspiration biopsy in the other. The final diagnosis of these patients was functional gastrointestinal disorder; biopsies from each patient showed no histologic abnormalities. The total biopsy weight ranged from 60 to 200 mg. Membrane vesicles were then formed from these biopsies using the same microvesiculation technique developed for small amounts of organ donor mucosa (Figure 1).

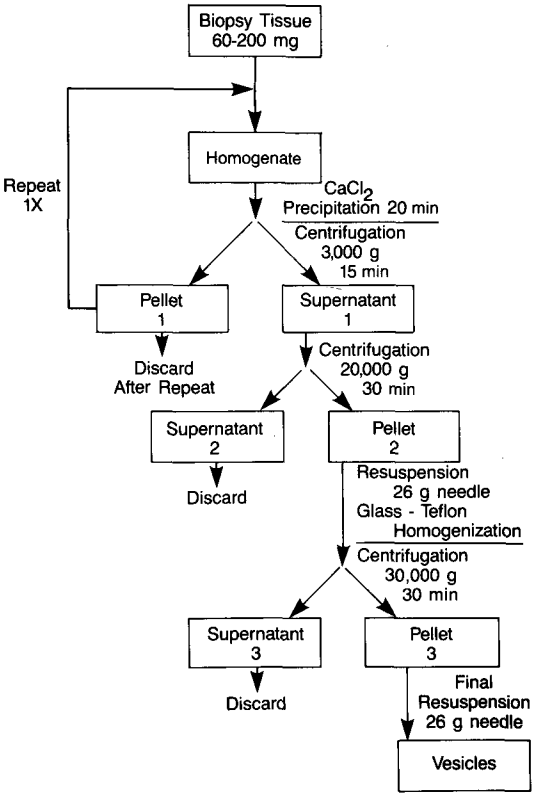

Figure 1: Microvesiculation method for the formation of human intestinal brush border membrane vesicles from peroral endoscopic or suction biopsies. Homogenization is performed in a Polytron homogenizer for 30 seconds at full speed. Homogenization buffer consists of 50 mM mannitol, 2 mM Tris-HCl, (pH 7.0); 1 mM $CaCl_2$ is used for membrane precipitation. Pellet 2 is resuspended in the desired buffer to be loaded into the vesicles.

Uptake Studies

Uptake studies were carried out by the rapid filtration technique of Hopfer et al (8) as previously described (1-3). Briefly, the vesicles were suspended to a final protein concentration of 5-10 mg/ml. Membrane protein was measured by the method of Lowry et al (9). Incubation media contained the following in a 250-ul final volume: 10 mM Tris-HEPES (pH 7.5), 150 mM NaCl, and 50 uM of either radiolabeled D-glucose or L-leucine. Studies were initiated by the addition of vesicles containing 250-750 ug of membrane protein into a tenfold dilution of incubation medium containing 50 uM of radiolabeled substrate and were conducted at room temperature. At specified time intervals, 50-ul aliquots of the reaction mixture were transferred to a cold (0°C) stop solution (composition equal to the incubation media and containing [^{3}H]-L-glucose to correct for incomplete washing of the [^{14}C] substrates) and filtered on wetted 0.45 um membrane filters. The filters were washed with 4 ml of ice-cold rinse solution of the same composition as the stop solution but containing no [^{3}H]-L-glucose and were processed for dual isotope counting as previously described (1). There was negligible nonspecific binding of any of the labeled substrates to either the membranes or the nitrocellulose filters. Results are expressed as picomoles of uptake per milligram of membrane protein (1). Using different membrane preparations, qualitatively identical results are obtained but because of variations in the equilibrium uptake values, results of representative experiments are shown.

Measurement of sucrase enrichment from the mucosal homogenate to the purified membrane vesicle pellets was done by the method of Dahlqvist et al (10). D-[U-^{14}C] glucose, L-[U-^{14}C] leucine and L-[^{3}H] glucose were obtained from New England Nuclear, Boston, MA. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Results

Sucrase enrichment

Approximate enrichment of sucrase activity from crude mucosal homogenate to final brush border membrane vesicle pellets were as follows: duodenum-20 fold, jejunum-19 fold (both by conventional method); duodenum-16 fold (by microvesiculation method). These enrichments demonstrate excellent purifications of brush border membranes for vesicle studies.

Organ donor membrane vesicles using the standard method: D-glucose and L-leucine uptake

Figures 2 and 3 demonstrate the time course uptakes of representative experiments under sodium gradient conditions for D-glucose and L-leucine respectively into organ donor intestine membrane vesicles. Duodenal brush border membrane vesicles actively transport D-glucose and L-leucine under these conditions. Although the membrane purifications are equivalent, the ratios of duodenal peak uptakes to the final (60 minute) uptakes are somewhat smaller than those in the jejunum. These results, however, demonstrate that there is indeed Na^{+}-dependent secondary active transport of D-glucose and L-leucine by the human duodenum similar to the human jejunum and ileum (6).

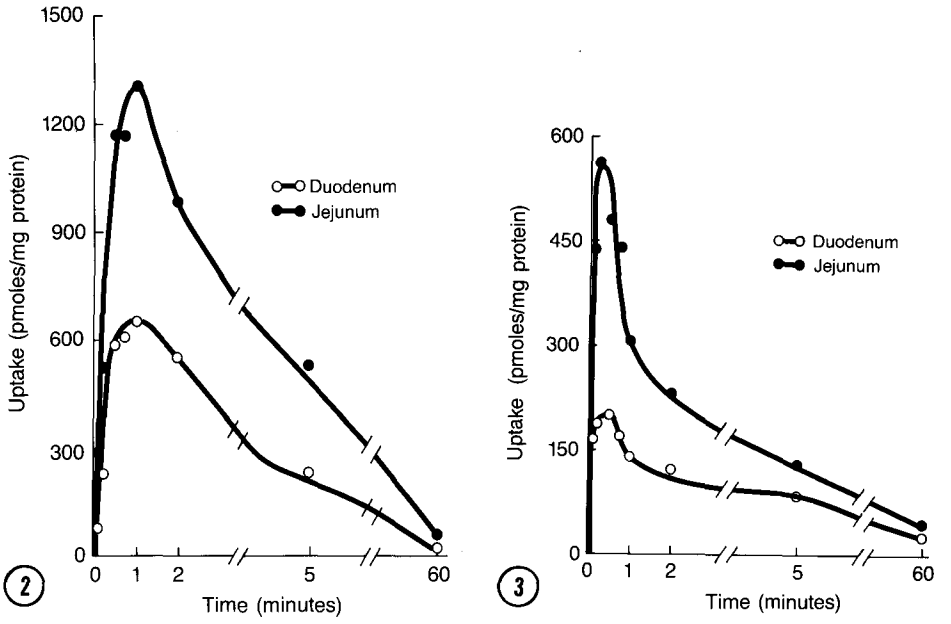

Figure 2: Uptake of [^{14}C] D-glucose into brush border membrane vesicles obtained from organ donor intestine. Vesicles were loaded with 150 mM KCl, 10 mM Tris-HEPES buffer (pH 7.5). Extravesicular incubation medium consisted of 150 mM NaCl, 10 mM Tris-HEPES (pH 7.5) and 50 uM [^{14}C] D-glucose.

Figure 3: Uptake of [^{14}C] L-leucine into brush border membrane vesicles obtained from organ donor intestine. Vesicles were loaded with 150 mM KCl, 10 mM Tris-HEPES buffer (pH 7.5). Extravesicular incubation medium consisted of 150 mM NaCl, 10 mM Tris-HEPES (pH 7.5) and 50 uM [^{14}C] L-leucine.

Duodenal biopsy membrane vesicles using the microvesiculation method: D-glucose and L-leucine uptake

Table 1 shows the uptake of D-glucose and L-leucine into duodenal vesicles prepared from small amounts (200 mg) of organ donor mucosa using the microvesiculation method. The vesicles were loaded with 150 mM choline chloride (instead of KCl) to demonstrate the effects of an inward Na^+ versus a K^+ gradient. All other transport conditions are similar to Figures 2 and 3. The data in Table 1 demonstrate concentrative uptake at 0.5 minutes well above equilibrium (at 60 minutes) with a Na^+ gradient (but not with a K^+ gradient) for D-glucose and L-leucine. Uptakes of D-glucose and L-leucine under Na^+ gradient conditions were actually higher than those obtained using the standard vesiculation method, demonstrating that the microvesiculation technique is suitable for very small amounts of mucosa.

Table 2 shows the time course uptakes for D-glucose and L-leucine, respectively, under sodium gradient conditions into brush border membrane vesicles formed from duodenal biopsies.

Table 1

Na^+ DEPENDENT D-GLUCOSE AND L-LEUCINE INTO HUMAN ORGAN DONOR MUCOSA BRUSH BORDER MEMBRANE VESICLES (MICROVESICULATION TECHNIQUE)

	(4 Different Preparations) Uptake (picomoles/milligram protein)		
Time (minutes)	0.1	0.5	60
D-glucose			
Na^+	345 ± 32	693 ± 66	128 ± 15
K^+	23 ± 1	36 ± 3	102 ± 8
L-leucine			
Na^+	300 ± 38	445 ± 67	183 ± 15
K^+	90 ± 11	181 ± 18	172 ± 12

All three biopsy preparations demonstrated concentrative uptake of D-glucose and L-leucine. The small amount of material allowed only 3 time points to be studied for each substrate, thus no figure is given. The intervals at 0.1, 0.5 and 60 min, were chosen to demonstrate concentrative uptake based on the results shown for organ donor vesicles as in Figs. 2 and 3. Vesicle loading and "incubation" buffers were similar to those in Figures 2 and 3. Concentrative uptake of D-glucose and L-leucine into the membrane vesicles well above the final equilibrium point at 60 minutes is again demonstrated indicating Na^+ activated carrier mediated uptake. In fact, these uptakes using the microvesiculation procedure on biopsies are of equal if not greater magnitude to those obtained with the conventional method using organ donor mucosa (Figures 2,3 and Table 1). These studies show that functional duodenal membrane vesicles can be prepared from clinical biopsy material.

Table 2

Na^+ DEPENDENT D-GLUCOSE AND L-LEUCINE TRANSPORT INTO HUMAN DUODENAL BIOPSY BRUSH BORDER MEMBRANE VESICLES (MICROVESICULATION TECHNIQUE)

	(3 Different Preparations) Uptake (picomoles/milligram protein)		
Time (minutes)	0.1	0.5	60
D-glucose	895 ± 290	1565 ± 300	244 ± 58
L-leucine	440 ± 60	617 ± 36	280 ± 53

Discussion

This study demonstrates that the human duodenum is capable of secondary active transport of neutral amino acids and D-glucose under sodium gradient conditions. This is the first direct demonstration of active transport processes in the human duodenum. The magnitude of these processes at the membrane level is somewhat less in the duodenum than in the jejunum. This data is potentially relevant in the treatment of patients with short bowel syndrome where the duodenum is intact and may serve as an organ for adaptive absorption of nutrients and sodium. The present study also demonstrates that vesicles formed from biopsy material are comparable or superior to organ donor intestinal vesicles prepared from much greater amounts of mucosal tissue.

Endoscopic duodenal biopsies have been shown to be histologically equivalent to proximal jejunal biopsies in their diagnostic accuracy for diseases such as celiac sprue (11). We have demonstrated in this study that these biopsies are qualitatively the functional equivalent of jejunal biopsies. (This has been attempted in one other instance using only jejunal biopsies, but no active transport was demonstrated and the very low uptakes were likely a result of vesicle swelling since the vesicles were loaded with mannitol) (12). These findings have important ramifications for the study of small intestinal dysfunction in conditions such as sprue (13), chronic alcoholism (14) and diabetes (15) where there may be abnormal glucose and amino acid uptake related to altered transporter density or function. This technique may also be applied to the study of patients with inborn defects of amino acid transport such cystinuria (16), Hartnup's disease (17) and dicarboxylic aminoaciduria (18). Recently, the human duodenum has been shown to secrete bicarbonate in vivo thereby protecting the mucosa from damage due to hydrochloric acid. This capacity to secrete HCO_3 appears to be decreased in patients with duodenal ulcer disease (19). Thus, vesicles formed from duodenal ulcer patients may allow the characterization of defects in their HCO_3 transport system. Finally, biopsy-derived brush border membrane vesicles will be useful in assessing functional aspects of small intestinal transplantation in animals (20) and man. In conclusion, we demonstrate active transport processes for glucose and neutral amino acids in human duodenum. Using this information, we present a simple method to assess proximal intestinal transport function in patients using material easily obtained from routine clinical procedures.

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