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Effect of Diabetes Mellitus and Insulin on the Regulation of the PepT 1 Symporter in Rat Jejunum

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Abstract: This investigation focused on studying the effects of insulin-dependent diabetes mellitus and insulin treatment on absorption of glycylsarcosine (Gly-Sar) across the Sprague-Dawley rat jejunum, using *in situ* perfusion in a physiologic acidic microenvironment at pH 6.0. Rats were divided into five groups: normal controls in group I, normal colchicine-treated rats in group II, normal cytochalasin-treated rats in group III, streptozotocin-induced diabetic rats in group IV, and insulin-treated diabetic rats in group V. Histologic studies of the five different groups showed morphologic changes upon induction of diabetes and treatments with colchicine and cytochalasin and several variations in post-1 month diabetic rats treated with insulin. The rate of uptake of Gly-Sar was significantly reduced in the diabetic state. The comparison of colchicine-treated and cytochalasin-treated rats to the diabetic group suggests that an intact cytoskeleton and tight junctions may play a role in jejunal dipeptide absorption. In the diabetic and insulin-treated group, the dipeptide influx rate was significantly increased compared to that of the nontreated controls. The regulation of the PepT 1 symporter was further assessed by immunostaining and Western blot analyses in the normal, diabetic, and diabetic and insulin-treated groups. Our results showed that a downregulation of PepT 1 in the diabetics seemed to be due in part to the low systemic insulin levels, and not necessarily to hyperglycemia. In addition, the results suggest a probable role of systemic insulin binding at the vascular site of the jejunal epithelium, and the role that this hormone may be playing in the regulation and probably cellular trafficking of PepT1.

Keywords: Jejunal PepT 1; diabetes type 1; insulin; PepT 1 functional expression; cytoskeleton

I. Introduction

Insulin has a wide variety of cellular effects;[1] however, little is known about its involvement in the regulation of substrate transport and metabolism in the mucosal lining of the small intestine.[2] Because insulin action necessitates its binding to its receptor, several laboratories investigated the presence of insulin receptors in the small intestinal mucosa. The results demonstrated the presence of a highly specific receptor for insulin on the vascular but not the luminal surface of the small intestine and suggested an important

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role for this hormone in the regulation of intestinal physiologic and metabolic activities.[2−4]

The PepT 1 symporter, involved in the transport of small peptides across the brush border mammalian cell membranes of the small intestine, has been cloned, and its functional characteristics have been elucidated. It is thought to play a pivotal role in the efficient absorption of dietary proteins, and a variety of peptidomimetic drugs such as β-lactam antibiotics, bestatin, angiotensin-converting enzyme inhibitors, and other therapeutically active molecules.[5,6] Furthermore, PepT 1 seems to be an important intestinal target transporter for improving the oral bioavailability of these drugs.[7−12] The expression of this symporter has been shown by immunolocalization studies to be restricted to the brush border membrane of the differentiated absorptive epithelial cells in the small intestinal villi.[13−15]

Intestinal absorption of oligopeptides has been shown to be altered in diabetes.[16−18] In rats with uncontrolled diabetes, both the length and weight of the small intestine increased.[17,19,20] This hypertrophy or hyperplasia appears to be localized mainly in the mucosa and is accompanied by substantial structural damage to the intestinal mucosa that affects intestinal absorption of oligopeptides. It seems that there is a cross talk between insulin and the regulation of PepT 1 activity that is very important in the clinical aspects of nutritional and pharmacological therapies[16,21] and that chronic insulin deficiency could lead to impairment in the oligopeptide symporter expression and/or transport activity.

In this study, to verify the possible cross talk between insulin and the PepT 1 symporter, we evaluated the effects of diabetes and insulin treatment on the functional activity of Gly-Sar uptake in the rat jejunum, and the molecular expression of PepT 1 in that tissue.

II. Experimental Section

Adult female Sprague-Dawley rats (200−250 g), *Rattus norvegicus*, were fed purina pellets and tap water *ad libidum*. All experiments were conducted with the approval of the Institutional Review Board/Animal House Committee of the American University of Beirut, and in accordance with the guidelines of the American Association for Laboratory Animal Sciences (AALAS) on Human Care and Use of Laboratory Animals.

1. Animal Treatments. Animals were divided into five different groups.

i. Normal (N) rats were fed *ad libidum* for 1 month.

ii. Normal Rats Treated with Colchicine (NCo). Rats were treated intraperitoneally (ip) with 1 mg of colchicine/kg of body weight (Sigma Chemical Co., St. Louis, MO) 24 h prior to the perfusion of the jejunum.

iii. Normal Rats Treated with Cytochalasin B (NCy). In this group, cytochalasin B (Sigma Chemical Co.) diluted in dimethyl sulfoxide (DMSO, Fluka Chemie GmbH, Sigma-Aldrich Laborchemikalien) was added to both the clearance buffer and the perfusate solution at a final concentration of 2 mM.

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iv. Diabetic Rats (D). Two groups of rats were made diabetic by a single intravenous (over the course of 30 s) injection of streptozotocin (STZ, 85 mg/kg of body weight; Sigma Chemical Co.) in saline acidified to pH 4.5 with 0.1 M citrate buffer.[22,23] The animals were treated ip with 3 cm^3 of a saline solution (NSS)/kg of body weight, twice daily (bid), as non-insulin-treated placebo controls. One group was treated for 1 month and the other for 96 h.

v. Diabetic Rats Treated with Insulin (DI). Diabetic rats were weighed weekly, and bovine insulin (0.33 unit/cm^3) was administered ip, bid, at a dose of 1 unit (in 3 cm^3 of NSS)/kg of body weight, for a period of 1 month starting day 1 post-STZ injection.

2. Animal Preparation. i. Surgical Procedures. All rats were weighed and then anesthesized by ip injection of 50 mg of pentobarbital sodium salt/kg of body weight. Each animal was tracheotomized and laid dorsally on a 37 °C heating pad throughout the experiment. The abdominal and groin regions were dissected, and a 10 cm segment of the upper jejunum, situated 15 cm from the pylorus, was exposed, keeping the mesenteries intact. A 3 mm horizontal inlet slit in the anti-mesenteric side was made in the cranial part of the jejunal segment. Another 3 mm outlet slit was similarly made 10 cm caudal to the inlet. A 1 mm inner diameter L-shaped glass catheter was introduced into each of the outlet and inlet slits ligated to the intestine to fit the jejunal lumen. A 3 mm polyethylene tubing connected to an infusion pump was then attached to the jejunal inlet and the other end drained into the collecting vessel. The rate of perfusion through the intestinal segment was kept at 0.8 mL/min throughout the experiment. The intestine was covered with cotton imbibed with Tris buffer to prevent intestinal dryness.

ii. Perfusion of the Upper Jejunum. There were two major steps in each perfusion experiment. First, 10 mL of oxygenated (aerated with 95% O_2 and 5% CO_2; Air-liquid, Beirut, Lebanon) Tris buffer was infused to clear the 10 cm jejunal lumen from debris. Second, 50 mL of an oxygenated Tris buffer solution containing ≈0.02 μCi/mL [*glycyl*-2-^{3}H]-glycylsarcosine (American Radiolabeled Chemical Inc., St. Louis, MO) was mixed with 0.5 mM unlabeled glycylsarcosine (Sigma Chemical Co.). The solutions were perfused through the intestinal segment, and ≈1.6−4.0 mL samples were collected from the outlet at 2, 4, 6, 8, 10, 15, 20, 25, and 30 min with a hypodermic needle inserted into the polyethylene tubing in the outlet. Perfusate was assayed for [*glycyl*-2-^{3}H]glycylsarcosine in 0.5 mL aliquots added to 4 mL of Ecolume scintillation cocktail (ICN Biochemicals, Irive, CA) in a liquid scintillation spectrometer (Packard Liquid Scintillation Counter, Tri-Carb 2750 TR/LL).

iii. Preparation of Histology Sections. Tissues were prepared and sectioned as follows. Jejunal tissues were removed from normal and treated rat groups at different times. They were fixed in formaldehyde for 48 h and then processed for routine light microscopy according to standard methods. The tissue blocks were cut into 5 μm sections. Sections from each group were stained with hematoxylin and eosin (H&E stain) or with periodic acid schiff (PAS stain) and viewed under the light microscope. The different groups showed the basic pattern and arrangements of layers in the intestinal wall, and these are mucosa, the submucosa, the musculosa, and the serosa.

iv. Western Blotting. The rats were sedated with halothane (MTC Pharmaceuticals, Cambridge, ON) and sacrificed by decapitation. The jejunum was cut transversely, and the lumen was washed with phosphate-buffered saline (PBS, 1 M, pH 7.4, 1 × 2 min). The luminal mucosa was collected by scraping the luminal side with a glass slide. The samples were rapidly frozen in liquid nitrogen and stored at −80 °C until they were used. On the day of the experiment, the samples were transferred on ice, weighed, and diluted in homogenization buffer (1 g of tissue in 10 mL of homogenization buffer). The homogenization buffer has the following composition: 125 mM Trizma base (pH 6.8) (Sigma-Aldrich, Oakville, ON), 0.1 M phenylmethanesulfonyl fluoride (PMSF) (Sigma-Aldrich), 0.5 mg/mL aprotinin (Sigma-Aldrich), 0.5 mg/mL leupeptin (Sigma-Aldrich), and 0.7 mg/mL pepstatin (Sigma-Aldrich). The samples were homogenized with a Polytron and after that were diluted by adding the same quantity of Laemmli 2× buffer [125 mM Tris buffer (pH 6.8) (Sigma-Aldrich), 10% sodium dodecyl sulfate (SDS) (Sigma-Aldrich), 20% glycerol (J. T. Baker, Phillisburg, NJ), 0.1% bromophenol blue (J. T. Baker), and 10% β-mercaptoethanol (Sigma-Aldrich)]. After that, this mixture was centrifuged at 10 000 rpm for 3 min. The supernatant containing the proteins was kept, and the pellet was discarded. The samples were heated at 100 °C for 5 min and were sonicated. The protein samples diluted in sample buffer 2× [17% glycerol, 9% β-mercaptoethanol, 10% SDS, 1 M Tris buffer (pH 6.7), and 1−2 mg of bromophenol blue] were loaded at a density of 50 μg/lane, then separated via 7.5% polyacrylamide gel electrophoresis, and finally transferred to nitrocellulose membrane Hybond ECL (Amersham Biosciences, Baie-d'Urfé, PQ). The membranes were blocked with 5% nonfat skim dry milk (Nestlé Canada Inc., North York, ON) and 0.05% Tween 20 (Biorad-Life Science Research Division, Mississauga, ON) in PBS (1×, pH 7.4) for 2 h at room temperature and probed with rat peptide-1 antisera (1:1000; provided by D. E. Smith) in 5% nonfat dry milk and 0.05% Tween 20 in PBS overnight at 4 °C on a shaker. Then the membranes were washed (3 × 10 min) with 0.05% Tween 20 in PBS and were incubated with anti-rabbit IgG horseradish peroxidase-linked $(ab^1)_2$ fragment [from donkey, 1:5000 (Amersham Biosciences)] for 1 h at room temperature on a shaker. After the gels had been washed with 0.05% Tween 20 in PBS (5 × 10 min) and with PBS only (1 × 10 min), bands were visualized

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with ECL Lumi-Light plus Western Blotting Substrate, and the bound antibody was detected on X-ray film. To confirm equal loading of proteins, the blots were also immunoprobed with a monoclonal antibody against β-actin (Abcam Limited, 1:20000 dilution).

v. Immunofluorescence Microscopy. Two proximal jejunal specimens (1 cm) were removed for each rat that was sedated and sacrificed by decapitation. The samples were embedded in tissue-Tek (OCT) compound (CANEMCO Supplies, St-Laurent, PQ) and promptly frozen as described in Western Blotting. The frozen sections, 3 μm thick, were cut with a cryostat, mounted on glass slides, fixed for 20 min with 4% paraformaldehyde (Cedarlane Laboratories Ltd., Hornby, ON), and washed with PBS (1 M, pH 7.4, 3 $\times$ 5 min) at room temperature. They were permeabilized with a solution containing 10% NGS (normal goat serum, from Sigma-Aldrich) and 1% BSA (bovine serum albumin, from Sigma-Aldrich) in PBS and 0.1% Triton X-100 (Sigma-Aldrich) for 30 min. The sections were then washed with PBS (3 $\times$ 5 min) and incubated overnight at 4 °C with primary rat peptide-1 antisera (1:1000 dilution) in PBS and 0.1% Triton X-100 containing 2% NGS and 0.2% BSA. After the sections had been washed with PBS (3 $\times$ 5 min), they were incubated with RNase from bovine pancreas (1 mg/mL) for 20 min, washed with PBS (3 $\times$ 5 min), and then incubated for 1 h at room temperature with secondary goat anti-rabbit IgG-Alexa Fluor 488 (2 μg/mL, from Molecular Probes, Eugene, OR) in PBS and Triton X-100 containing 2% NGS and 0.2% BSA. They were then washed with PBS (3 $\times$ 5 min), incubated with 150 nM propidium iodide (Sigma-Aldrich), diluted in PBS for 20 min, and washed again with PBS (3 $\times$ 5 min). The sections were mounted in FluoroGuard antifade reagent (Biorad-Life Science Research Division) and were visualized and photographed with a Nikon Eclipse E 1000 microscope (PerkinElmer Life Sciences, Markham, ON) equipped with a Nikon Cooplix 950 camera (Nikon Canada Inc., Mississauga, ON).

vi. Statistical Analysis. Statistical analysis and comparisons were made using the analysis of variance for multiple factors (ANOVA) utilizing the Benferroni penalty method. Values were expressed as arithmetic means $\pm$ the standard error of the mean, and P values of ≤ 0.05 indicate significant differences.

III. Results

1. Histological Sections. i. Jejunum of Normal Rats. Normal intestinal tissue morphology was depicted (Figure 1A), including the following. The mucosa presented villi formed of simple columnar epithelium consisting of goblet, surface absorptive, and enteroendocrine cells. Few nuclei of lymphocytes were present within the epithelium. The surface absorptive cells possessed well-organized densely packed microvilli forming the striated border. Their tips were covered with a thick coat of glycocalyx that was demonstrated well with the PAS stain (Figure 1A, arrow 2) and rich in disaccharides and dipeptidases. The goblet cells were well-stained and calyx-like (Figure 1A, arrow 1) and produce mucinogen which when released into the intestinal lumen becomes hydrated, forming mucus that protects the intestinal lining. The basement membrane was present underlying the epithelium. The core of the villus was composed of loose connective tissue, vessels lying centrally, smooth muscle fibers, and other cells of the connective tissue. This portion of the mucosa is named the lamina propria. The lamina propria terminated at the muscularis mucosa that was composed of a band of smooth muscle fibers, a few layers thick. Located within the mucosa were many simple tubular intestinal glands and few lymphatic nodules.

Table 1. Average Blood Glucose Levels of Various Groups[a]

group	blood glucose level (mg/dL)
normal rats	113 $\pm$ 3[a]
rats treated with STZ for 96 h	458 $\pm$ 23[b]
rats treated with STZ for 30 days	576 $\pm$ 23[c]
diabetic rats treated with insulin for 30 days (1 IU/kg of body weight, bid)	408 $\pm$ 31[d]

[a] $N = 8$ rats. ANOVA test: $p < 0.0001$ for a vs b, a vs c, and a vs d, $p = 0.0027$ for b vs c, $p = 0.2162$ for b vs d, and $p = 0.0007$ for c vs d.

ii. Jejunum of Diabetic Rats. As shown in Figure 1B, many histological alterations of the 1 month STZ-diabetic rat jejunum existed as compared to a normal rat jejunum (Figure 1A). The glycocalyx was interrupted, leaving openings and channels between enterocytes particularly at the tips of villi (Figure 1B, arrow 1). The epithelial lining was elevated with pale or lightly colored enterocytes (Figure 1B, arrow 2). Goblet cells were deeply stained with irregular shapes. In addition, the lamina propria was edematous with irregular spacing between the epithelium, the basement membrane, and the rest of the lamina propria (Figure 1B, arrow 3). Moreover, the blood vessels were dilated, and there was an increase in the number of inflammatory cells (Figure 1B, arrow 4). As for the musculosa layer, it was noticed that muscle fibers showed hypertrophy in some locations (Figure 1C, arrow 1). These alterations were also observed in 96 h STZ-diabetic rats.

iii. Jejunum of Diabetic Rats Treated with Insulin. After STZ-diabetic rats had been treated with insulin for 30 days, an average blood glucose level of 408 $\pm$ 31 mg/dL (Table 1) was recorded. Histological sections of the jejunum of the diabetic group treated with insulin showed several variations compared to the jejunum of the normal and the non-insulin-treated diabetic rats with average glucose levels of 113 $\pm$ 3 and 576 $\pm$ 23 mg/dL, respectively (Table 1 and Figure 1D). The glycocalyx in the insulin-treated group was less interrupted as compared to that of the nontreated diabetics (Figure 1B, arrow 1) but showed thickening of the PAS stain (Figure 1D, arrow 1). Goblet cells and enterocytes looked normal. Moreover, the mucosa and lamina propria were also close to normal, wherein fewer inflammatory cells were noticed. In addition, minimal edema of the submucosa and little muscle fiber hypertrophy were noticed, which was similar to the case with diabetic jejunum (Figure 1C, arrow 1).

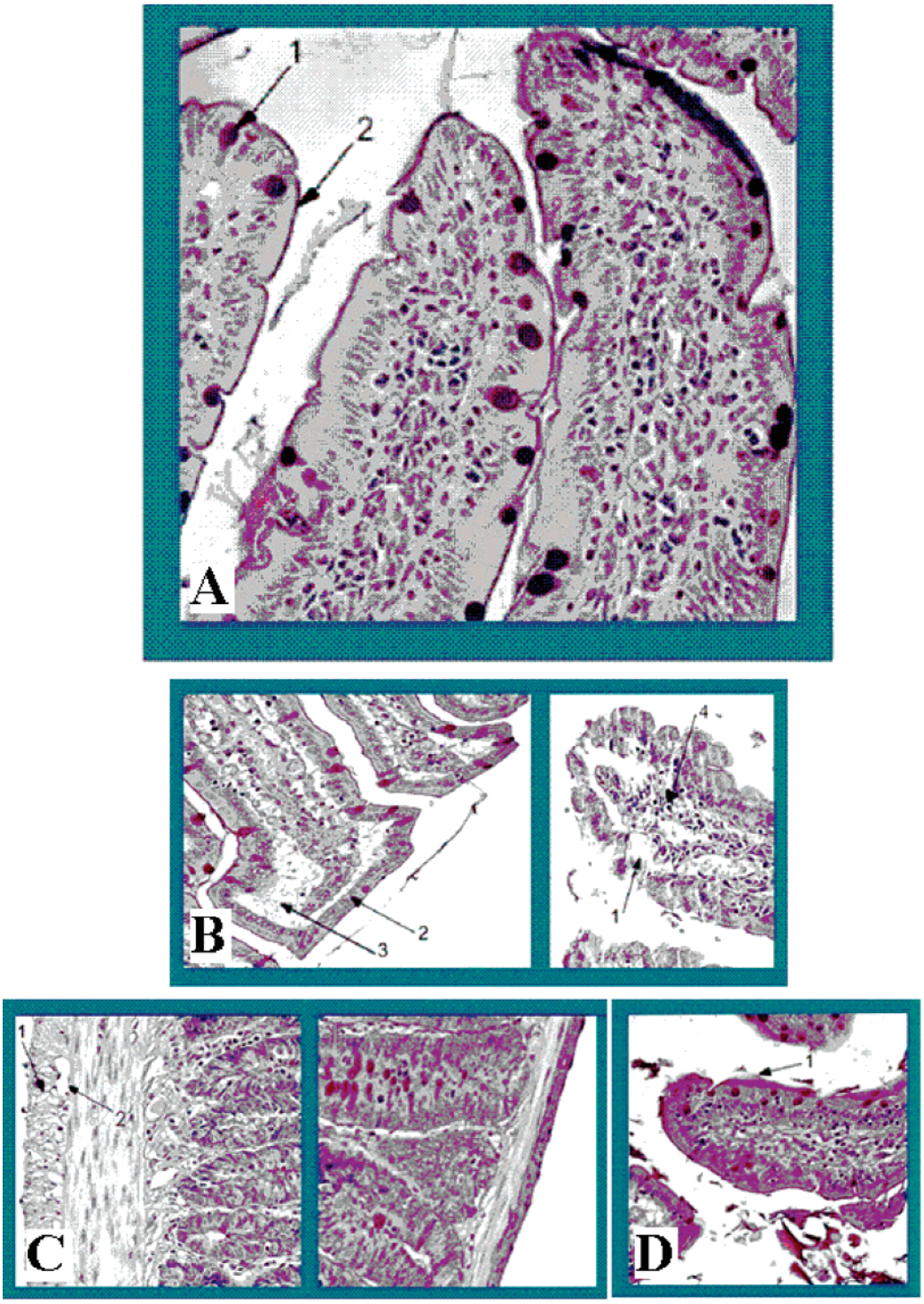

Figure 1. Photomicrographs showing the histology of rat jejunum (400×, PAS). (A) Normal rat jejunum: (1) goblet cells and (2) glycocalyx. (B) Diabetic rat jejunum: (1) interrupted glycocalyx, (2) elevated epithelial lining with lightly colored enterocytes, (3) edematous lamina propria, and (4) increase in the number of inflammatory cells. (C) Diabetic rat jejunum: (1) hypertrophy of muscle fibers and (2) dilation of vessels. (D) Diabetic rat jejunum treated with insulin: (1) normal glycocalyx.

iv. Jejunum of Normal Rats Treated with Colchicine. Figure 2A shows the effect of colchicine treatment on the jejunum of normal rats, indicating an increased level of infiltration of the epithelium by white blood cells in addition to irregularities in the alignment of the cells of the villi. There was also a widening in the space between the epithelium and the basement membrane. In some places, mainly the apical sides of the villi, the space is wider, indicating possible sloughing of the epithelium from the lamina propria or widening of the lamina rara. Vasodilation in the villi was

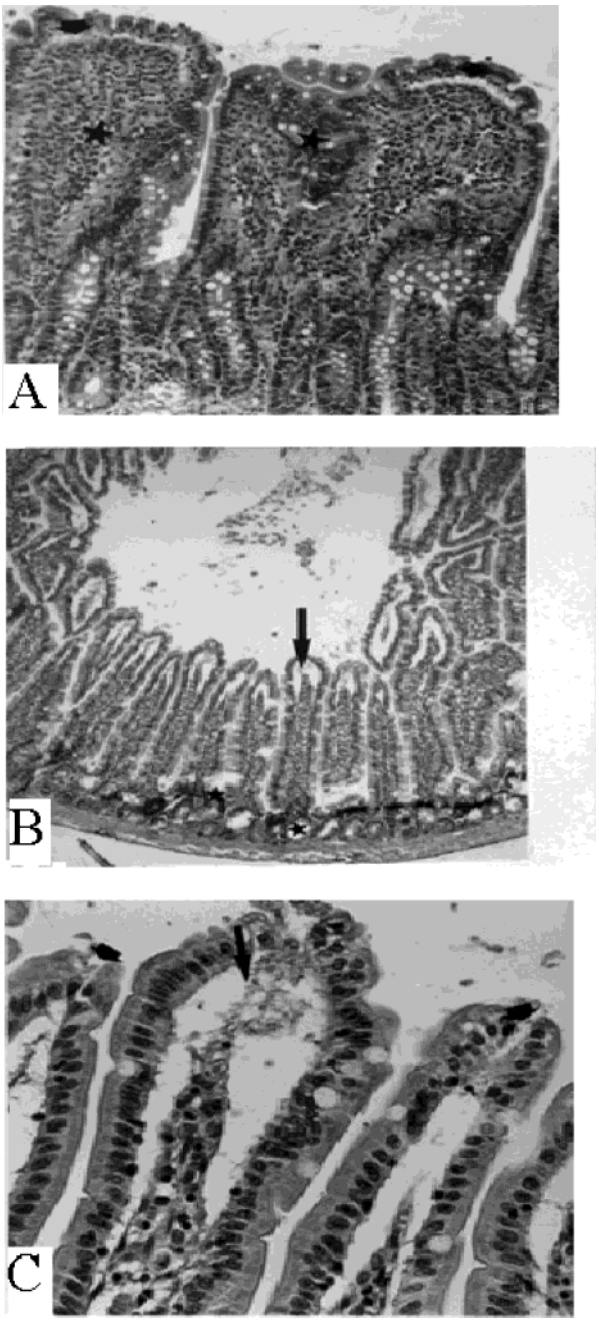

Figure 2. Photomicrographs showing the histology of rat jejunum (hematoxylin and eosin). [A (100×)] Colchicine-treated rat jejunum. Morphological alterations were noticed: more mucosal lesions with heavy infiltration of the lamina propria by leukocytes (star), shortening and broadening of the villi, widening of the subepithelial space, and apparent disintegration of the basement membrane (arrow). [B (45×) and C (250×)] Cytochalasin B-treated rat jejunum. The jejunal histology exhibited severe alterations that included the villi and the crypts (star), a great widening of the subepithelial space (arrow), and the apparent disruption of the basement membrane. In addition, channel-like pores or canals (arrowhead) were noted linking directly the lumen to the lamina propria inside the villus.

observed with possible oozing of blood cells and fluid, thus leading to a spacing between the basement membrane and epithelium or the basement membrane and the lamina propria. In addition, the vessels between the mucosa and submucosa or even musculosa were also dilated (Figure 2A).

v. Jejunum of Normal Rats Treated with Cytochalasin B. Panels B and C of Figure 2 show alterations caused by cytochalasin B on the normal rat jejunum. These included broadening and leaflet formation of villi, few tunnel-like spacings between the lumen and the core of the villus, further detachment of the basement membrane, and formation of obvious intercellular spaces. Comparing the effects of colchicine and cytochalasin B on the structure of the intestine, we can conclude that the effects of cytochalasin B were more pronounced than normal and the morphological changes were more prominent, reaching even the crypts (Figure 2B,C).

2. Immunohistochemistry. The results of immunolocalization of PepT 1 in different groups (treated and untreated) are shown in Figure 3. The results indicate strong staining of PepT 1 at the brush border jejunal membrane with weak fluorescence at the crypt cell site and no staining at the top villus site. Furthermore, less staining of PepT 1 labeling is observed in STZ-treated rat jejunum (Figure 3B) than in normal rat jejunum (Figure 3A), whereas in diabetic rats treated with insulin for 1 month (Figure 3C), there was upregulation of PepT 1 fluorescence. In addition, Figure 3D shows the normal rat jejunum blocked with a synthetic peptide, indicating a competition experiment showing specificity of the PepT 1 localization.

3. Western Blotting. Figure 4 shows the effect of STZ treatment of rats on the expression of PepT 1 in the rat jejunum. As shown in Figure 4, the level of PepT 1 expression has been drastically reduced in STZ-treated rats. However, in diabetic rats treated with insulin for 1 month, the expression of PepT 1 was upregulated to a near-normal state.

4. Glycylsarcosine Uptake across Intact Rat Jejunum at pH 6 for the Five Different Groups. To verify if insulin treatment prevents abnormal uptake across intact rat jejunum, in all groups, the rat jejunum was perfused with a Tris buffer solution at pH 6.0 (maximum influx environmental pH[24]) containing 0.5 mM nonlabeled Gly-Sar and ≈0.02 μCi/mL labeled [*glycyl*-2-^{3}H]Gly-Sar. By measuring the rate of uptake of the dipeptide in $\times 10^{-9}$ moles versus time in minutes, we noticed an exponential increase (Figure 5A) in the number of moles absorbed followed by a steady state linear influx after 8 min. The slope of this steady state uptake (Figure 5B) was indicative of the influx rate of the dipeptide across the jejunal brush border lumen. The values of the influx rate are reported in Table 2, which clearly showed differences among the various experimental groups. It is worth noting the major reduction in the rate of Gly-Sar uptake in the diabetic versus the normal group; however, treatment of

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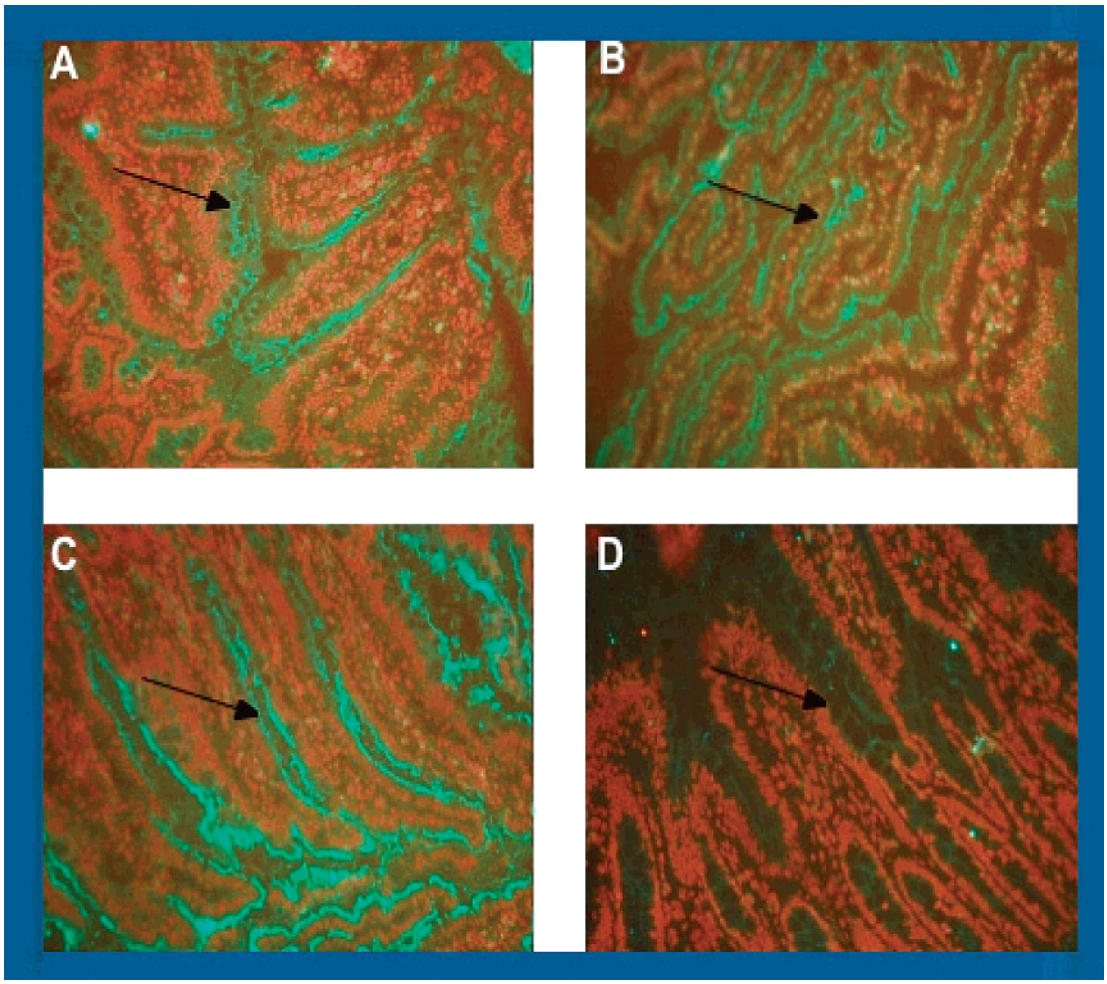

Figure 3. Immunohistochemistry of PepT 1 in different rat groups: (A) normal rat jejunum, (B) diabetic rat jejunum, (C) insulin-treated diabetic rat jejunum, and (D) normal rat jejunum blocked with a synthetic peptide.

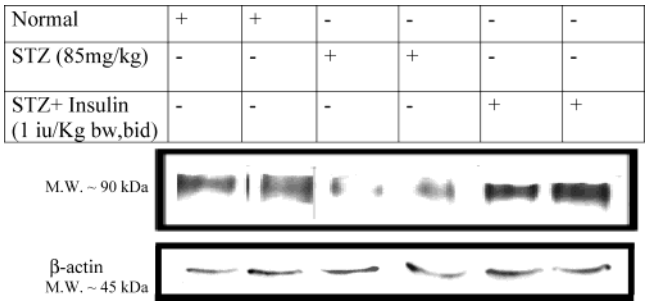

Figure 4. Western blotting of PepT 1 in different rat groups with β-actin as a protein loading marker. The first two bands are for normal control rats, the second two for diabetic rats, and the last two for diabetic insulin-treated rats.

diabetic rats with insulin resulted in a substantial compensatory influx profile (Table 2).

Table 2. Results of the Influx Rate of Gly-Sar across the Rat Jejunum of the Five Different Groups at pH 6[a]

group	influx rate at pH 6.0 (μM cm^{-2} min^{-1})
normal rats	15.9 ± 0.1[a]
normal rats treated with colchicine	8.7 ± 0.1[b]
normal rats treated with cytochalasin	12.7 ± 0.2[c]
diabetic rats (96 h)	7.7 ± 0.1[d]
diabetic rats (1 month)	6.6 ± 0.1[e]
diabetic rats treated with insulin	13.0 ± 0.2[f]

[a] $N = 8$ rats. ANOVA test: $p \leq 0.0002$ for a vs b, a vs c, a vs d, a vs e, a vs f, and e vs f.

IV. Discussion

The intestinal jejunal epithelium plays a prominent role in nutrient and drug absorption via an oligopeptide symporter (PepT 1) involving an H^+ transmembrane electrochemical gradient.[25,26] A stagnant unstirred diffusion layer with a pH between 5.5 and 6.0[10] mainly controls the jejunal microenvironment. This pH is believed to be regulated by the mucin-

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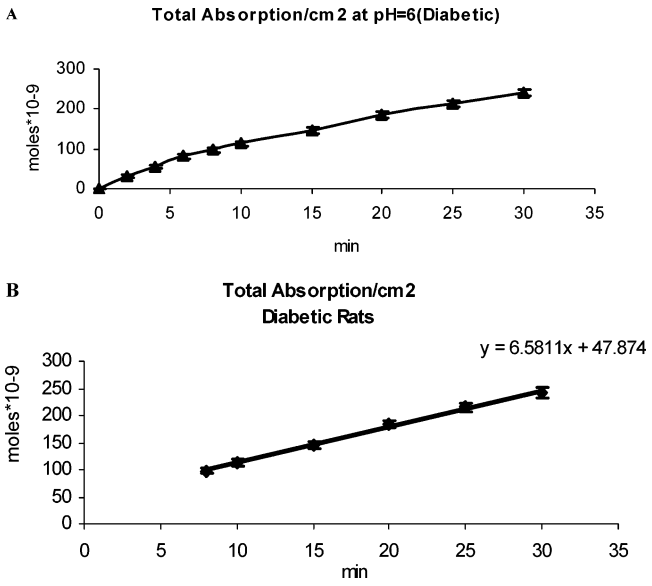

Figure 5. (A) Representative plot for the uptake of Gly-Sar across the 10 cm jejunal segment with time (1 month diabetic group at pH 6.0). (B) Best linear curve for the data obtained from the steady state profile in panel A. The slope of the line is indicative of a constant influx rate across the lumen of the jejunum.

secreting goblet cells that also secrete trefoil peptides,[27] as well as by the Na^+-H^+ exchanger.[28] Our study using a stable, hydrolysis-resistant dipeptide substrate,[29] namely, Gly-Sar (0.5 mM), which was perfused for 30 min at a rate of 0.8 mL/min, showed a constant influx rate profile across the jejunal epithelium after perfusion initiation for approximately 10 min (Figure 5). Hence, the aim is to determine the transport profile of the H^+/peptide symporter across normal, diabetic, and insulin-treated and diabetic rat jejunum at pH 6.0 (maximal influx rate in normal rats[24]) of the perfusion solution, thus not varying the pH of the unstirred stagnant layer at the apical side of the enterocytes.

Our data on the histological studies of the diabetic rat jejunum compared to the normal control revealed major morphological changes such as an interrupted glycocalyx and opening of channels between enterocytes. In addition, irregular spacing between the epithelium, basement membrane, and lamina propria with dilatation of blood vessels and an increase in the number of inflammatory cells and muscle fiber hypertrophy were also observed. All of this agrees with what was reported in the literature that in a diabetic state there is an increase in the absorbing surface area per unit length of the intestinal jejunum. An increase in gastric and intestinal permeability was noticed before the development of clinical diabetes.[30] Hypertrophy or hyperplasia appears to be mainly localized at the mucosa but not in the underlying tissues.[17,20] In the diabetic rat jejunum from rats treated with insulin for a period of 1 month, histological sections revealed several variations compared to the normal and the non-insulin-treated diabetic rats; however, with a modest return close to the normal, fewer inflammatory cells were noticed. In the rats treated with insulin for 96 h, similar histological changes such as the post-1 month diabetes were observed in our study.

Even though the uptake of Gly-Sar across the rat jejunum differed among the rat models, thus to say normal, 1 month diabetic, 1 month diabetic and treated with insulin, and 96 h diabetic rats, our results showed that there was no reversal in plasma glucose levels back to the normal state. This triggers the question of whether assessment of blood glucose levels is enough to monitor the role of insulin in cellular metabolism and growth in the diabetic state. To further explain the role of diabetes in the jejunal influx rate of Gly-Sar, at a physiologic pH of 6.0, in the normal state, we observe a maximal influx rate of 15.9 ± 0.1 μM cm^{-2} min^{-1}, whereas in a diabetic state, the maximal influx rate at pH 6.0 is 6.6 ± 0.1 μM cm^{-2} min^{-1}, with major statistical differences between the two subgroups.

The different influx rates of the dipeptide across the 10 cm rat jejunum at pH 6.0 raise the question of the role of diabetes in altering Gly-Sar absorption. Two assumptions can therefore be made. First, diabetes seems to regulate dipeptide absorption probably by affecting the cytoskeleton of the enterocytes[23] and the translocation of PepT 1 to the cell surface.[22] The cytoskeleton of euokaryotic cells consists of three highly abundant major protein families: microfilaments, microtubules, and intermediate filaments as well as a growing number of associated proteins.[31] The emerging exciting biological aspects of these proteins are their involvement in cell signaling and vesicle trafficking.[31] Our histological results agree with the literature concerning the changes in the cytoskeleton in the diabetic state, as well as in colchicine-treated rats.[24] Our results with influx rates for the colchicine- and cytochalasin-treated groups strongly support the idea that an intact cytoskeleton is the regulatory factor in dipeptide absorption. Second, the decrease in the influx rate of the dipeptide in the diabetic state may be in part due to a downregulation of the symporter and/or to an affinity change of the symporter for the H^+ as a result of the diabetic state.

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Previous studies showed upregulation of the PepT 1 in rats with diabetes that was uncontrolled for 96 h.[22] However, our results showed less staining of PepT 1 where the transporters are localized at the brush border 96 h as well as 1 month after diabetes had been induced. Hence, these findings support the idea that with time, diabetes decreased the number of PepT 1 transporters found on the apical side. In the diabetic group treated with insulin, the influx rate at pH 6.0 was significantly increased compared to that for the nontreated group while approaching a value close to the normal state. This indicated that insulin treatment for 1 month could be regulating the trafficking of the PepT 1 transporter back to the luminal surface even in the presence of mild hypertrophy or hypertrophy in the treated state. These results suggest the presence of a highly specific receptor for insulin on the vascular[1,2,18,32−34] but not the luminal surfaces of the jejunum and that insulin may contribute to the regulation of PepT 1 symporter density and/or functioning. It has been reported in the literature that insulin does not stimulate the uptake of glucose and amino acids by the adult intestine but stimulates DNA synthesis, thus accelerating the recruitment of PepT 1 at the brush border membrane.[2,3,14,33−36]

Furthermore, our results showed that in diabetic animals not treated with insulin, the PepT 1 symporter was downregulated at the brush border, while in the diabetic rats treated with insulin, the apparent level of the symporter was higher than that of normal animals. This further supports our findings that in the diabetic state, insulin treatment is essential for the upregulation of the PepT 1 level to compensate for an efficient absorptive profile for the H^+-dependent symporter. Although in insulin-treated diabetic rats the plasma glucose levels were still above normal physiologic levels (Table 1), the influx rate of Gly-Sar was close to normal (Table 2). Therefore, it can be inferred that the downregulation of PepT 1 in this diabetic state was the consequence of low systemic insulin levels and not due necessarily to hyperglycemia. In addition, these results further suggest that H^+ activates the jejunal PepT 1 probably without influencing the substrate affinity.[29]

The findings of this study propose that insulin treatment enhances Gly-Sar absorption by increasing the abundance of PepT 1 at the luminal site. Questions may be raised about the physiological relevance of these results. In IDDM (insulin-dependent diabetes mellitus), individuals who are not promptly and effectively treated with insulin may show downregulation of PepT 1 in their gastrointestinal tract. Our results clearly indicate that immediate insulin treatment will effectively compensate for the downregulation of PepT 1 at the brush border luminal site of the jejunum and probably at other localized sites of the gastrointestinal tract.

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