Published in final edited form as:
Surgery. 2009 July ; 146(1): 100–112. doi:10.1016/j.surg.2009.02.012.

Expression and Function of Intestinal Hexose Transporters after Small Intestinal Denervation

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Abstract

Background—The role of neural regulation in expression and function of intestinal hexose transporters is unknown.

Aim: To determine the role of intestinal innervation in gene expression and function of the membrane hexose transporters, SGLT1, GLUT2, and GLUT5 in the enterocyte.

Hypothesis: Denervation of the small intestine decreases expression of hexose transporters leading to decreased glucose absorption.

Methods—Six groups of Lewis rats were studied (n=6 each): control, 1 wk after sham laparotomy, 1 and 8 wk after syngeneic (no immune rejection) orthotopic small bowel transplantation (SBT) (SBT1, SBT8) to induce complete extrinsic denervation, and 1 and 8 wk after selective disruption of intrinsic neural continuity to jejunoileum by gut transection and reanastomosis (T/A1, T/A8). All tissue was harvested between 8AM and 10AM. In duodenum, jejunum, and ileum, mucosal mRNA levels were quantitated by real time PCR, protein by Western blotting, and transporter-mediated glucose absorption using the everted sleeve technique.

Results—Across the six groups, relative gene expression of hexose transporter mRNA and protein levels were unchanged and no difference in transporter-mediated glucose uptake was evident in any region. Glucose transporter affinity (K_m) and functional transporter levels (V_{max}) calculated for duodenum and jejunum showed no difference between the six groups.

Conclusion—Baseline regulation of hexose transporter function is not mediated tonically by intrinsic or extrinsic neural continuity to the jejunoileum.

Keywords

small bowel transplant; hexose transporters; glucose transport

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This work was presented in part at the Academic Surgical Congress Phoenix, AZ February 2007.

Introduction

The success of small bowel transplantation (SBT), although improving, has been limited (1-3). Even in the absence of immune-mediated graft rejection, intestinal transplantation is complicated by enteric dysfunction characterized by dysmotility, diarrhea, and malabsorptive syndromes which may be due, in large part, to the intestinal denervation associated with SBT (4-12). SBT necessitates complete transection of extrinsic neurons (from the central nervous system) that course through the mesentery and innervate the intestine, as well as a complete disruption of enteric myoneural continuity to the transplanted jejunoileum (intrinsic innervation) (13-15). It is well accepted that gut function is highly dependent on neural modulation of absorption and motility, two key components of normal intestinal function (6, 7,16,17). It is not surprising then that intestinal dysfunction is seen in other clinical scenarios of denervation, such as vagotomy or disorders of neural function where disruption of enteric neural input also occurs and results in intestinal dysmotility and malabsorption (18-22). Specifically, the effect that intestinal denervation has on hexose transporter expression and function is not known; such changes in innervation could be a factor in the malabsorptive syndromes associated commonly with SBT.

The current study was designed to determine the role of neural regulation in the expression and function of the primary intestinal hexose transporters of the small bowel enterocyte: sodium-glucose transporter-1 (SGLT1), glucose transporter-2 (GLUT2), and glucose transporter-5 (GLUT5), and how changes in the expression and function of these transporters may contribute to enteric dysfunction after SBT. We hypothesized that intestinal innervation plays a substantive role in regulating hexose transporter expression and function in the intestinal brush border, and therefore, denervation of the gut after SBT will result in decreased expression and ultimately function of these transporters.

Methods

Groups and Procedures

Under the policies established by our Institutional Animal Care and Use Committee in accordance with the National Institutes of Health guidelines for the humane use and care of laboratory animals, we studied 250- to 300-g male Lewis (inbred) rats (Harlan, Indianapolis, IN) between 8AM and 10AM. A set time period of the light/dark cycle was chosen, because gene expression and transport function of the hexose transporters of interest are well-known to vary diurnally (25,26). We studied two groups, each at 1 and 8 wk after operation, with one group undergoing SBT and the other subjected to transection/anastomosis (T/A) of the proximal jejunum and distal ileum. For baseline values, we also studied a group 1 wk after sham celiotomy as well as a group of non-operated (naïve) control rats.

The first group (SBT; n=12) underwent complete disruption of extrinsic innervation and intrinsic neural input to the jejunoileum via orthotopic, jejunoileal isotransplantation as described previously (23). In brief, these syngeneic Lewis rats were anesthetized using 2% inhaled isoflurane induction followed by intraperitoneal injection of 50 mg/kg sodium pentobarbital. The donor jejunoileum was resected with preservation of the portal vein and the superior mesenteric artery (SMA) with a cuff of aorta as vascular conduits. In the recipient, the jejunoileum was resected leaving a short, 3-cm portion of proximal jejunum and distal ileum for anastomosis of the transplanted jejunoileum. Revascularization of the transplanted jejunoileum in the recipient involved implantation of the donor portal vein and SMA into the recipient inferior vena cava and abdominal aorta, respectively, using 10-0 nylon suture material. Intestinal continuity was restored with end-to-end intestinal anastomoses of donor jejunum and ileum to recipient jejunum and ileum using a running 6-0 polypropylene suture. The abdomen was closed in two layers, and the animal was recovered. Animals received a

liquid diet for the first 3 postoperative days and resumed solid chow (5001 Rodent Diet, PMI Nutrition International LLC, Brentwood, MO) on postoperative day 4. The animals were given acetaminophen in their drinking water for 1 day preoperatively and 2 days postoperatively. Animals were then studied at 1 wk (SBT1; n=6) and 8 wk (SBT8; n=6).

The second group (T/A; n=12) underwent disruption of intrinsic neural continuity to their native jejunoileum via transection and reanastomosis of the proximal jejunum and terminal ileum. These animals were anesthetized as in the SBT group. Through a midventral celiotomy, the proximal jejunum was transected 3 cm from the ligament of Treitz and reanastomosed in an end-to-end fashion using a running 6-0 polypropylene suture. A similar transection and reanastomosis was then performed in the distal ileum, 10cm proximal to the ileocecal valve. The abdomen was closed in two layers, and the animals were managed similar to the SBT group in that solid food consumption was not resumed until postoperative day 4. These animals were also studied at 1 wk (T/A1: n=6) and 8 wk (T/A8: n=6).

As controls for anesthesia and celiotomy, we used rats 1 wk after a sham laparotomy (sham: n=6). After identical anesthesia and celiotomy, the entire jejunoileum was manipulated manually for 5 min. Postoperatively, the animals were managed in the same way as the experimental groups. Naïve, unoperated rats served as baseline controls (control: n=6). All rats (controls and experimental) were housed in a strictly controlled, 12-h light-dark cycle animal facility and were fed standard rat chow. Feeding patterns were monitored closely by measuring the weight of food consumed at the beginning and the end of each light-dark cycle. Animal weights were recorded at the initial procedure and at harvest. A group of naïve rats were used to assess normal feeding patterns and weight gain in rats over 8 wk.

Tissue Harvest

Because of the known diurnal variation in expression and function of hexose transporters in the rat small intestinal mucosa, all tissue was harvested within one hour of 9AM (24-26). We chose this time point, not to evaluate the effects of denervation on diurnal variation in gene and protein expression, but rather to evaluate the effects of denervation on baseline expression. After anesthetization, the duodenum was cannulated at the pylorus, and the entire small intestine was flushed with chilled (4°C) mammalian Ringer's solution (in mM: 128 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 20 $NaHCO_3$; pH 7.3-7.4; 290 mOsm) to remove luminal content and decrease activity of intraluminal proteases. The small intestine was then excised rapidly using sharp dissection and placed immediately in chilled (4°C), oxygenated (95% O_2/5% CO_2) mammalian Ringer's Solution. The mucosa was scraped bluntly from portions of each intestinal segment using a glass slide into cold, phosphate-buffered saline (PBS). Half of the mucosal scrapings were placed into RNA stabilization buffer (RNALater, Qiagen, Valencia, CA), snap frozen in liquid nitrogen, and stored at -80°C. The remaining mucosal scrapings were placed in cold PBS containing protease inhibitors (Complete, Roche Applied Sciences, Indianapolis, IN) before being snap-frozen in liquid nitrogen and stored at -80°C.

mRNA Measurements

Real-time reverse transcription polymerase chain reaction (RT-PCR) was used to quantitate mRNA levels (27,28). The mucosal samples stored in RNA stabilization buffer were thawed on ice and then homogenized by repeated passing of the solution through a 20-gauge needle. RNA was isolated using the RNeasy Midi kit (Qiagen, Valencia, CA). Samples not to be used immediately were stored at -80°C.

DNase digestion (Invitrogen DNase, amplification grade) was performed on the isolated RNA to remove contaminating DNA. Then, 1.7 μg of the RNA was reverse transcribed into cDNA

using the Super Script III kit (Invitrogen, Carlsbad, CA) using random hexamer primers. The resultant cDNA was stored at -20°C until real-time RT-PCR was used to quantitate mRNA levels for SGLT1, GLUT2, GLUT5, and the stably expressed housekeeping gene, glyceraldehyde-6-phosphate dehydrogenase (GAPDH) against which levels were quantitated and normalized. RT-PCR was performed using Taqman® chemistries with primers and fluorescently-labeled probes in assay mixes from Applied Biosystems (Applied Biosystems, San Francisco, CA). The standard procedure from Applied Biosystems of 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min was used. Standard curves from serial dilutions of known copy numbers were used to calculate the number of cDNA copies for each sample. All copy numbers of protein transporters were normalized to copy numbers of GAPDH, allowing estimates of expression per enterocyte. All RT-PCR runs were performed in triplicate.

Protein Measurements (Western blot)

Samples stored in PBS containing protease inhibitors were thawed on ice and placed in RIPA lysis buffer also containing protease inhibitors to prevent protein degradation. These samples were then homogenized using a Kontes Pellet Pestle (Fischer Scientific, Pittsburg, PA). The protein-containing supernatant was then separated by centrifugation at 5000 × g for 15min. Protein concentrations were measured by the bicinchoninic acid method (Pierce, Rockfor, IL). Then, 200ug of protein was separated on a 10% SDS-PAGE gel (Bio-Rad, Hercules, CA); the proteins on the gel were transferred electrically to a PVDF membrane (Millipore, Bedford, MA). The membranes were blocked using 5% milk in tris-buffered saline with TWEEN (TBS-T). To quantitate protein and GAPDH on the same Western Blot, the membranes were cut between GAPDH and the specific proteins of interest: SGLT1 and GLUT2. The cut gels were then incubated overnight at 4°C with primary antibody (SGLT1 [1:3000] and GLUT2 [1:500] antibody from Chemicon International, Temecula, CA; GAPDH [1:500] antibody from US Biological, Swampscott, MA). After incubation with the primary antibody, the membranes were rinsed three times with TBS-T and incubated with a secondary antibody (1:10000) in TBS-T containing 5% milk. Horseradish peroxidase-conjugated, goat anti-rabbit IgG was used for SGLT1 and GLUT2, (Sigma, St. Louis, MO and Upstate, Lake Placid, NY, resp), and horseradish peroxidase-conjugated, goat anti-mouse IgG was used for GAPDH (Sigma, St. Louis, MO). After rinsing, the protein bands were visualized with a colorimetric reaction using Opti-4CN (Bio-Rad, Hercules, CA) substrate. Amplified Opti-4CN substrate kit (Bio-Rad) was used to enhance the SGLT1 and GLUT2 bands. The membranes were scanned, and the Scion Range program (Scion Corp, MA) was used for semi-quantitative measurements of protein levels based on band densitometry. All protein measurements were normalized to levels of GAPDH. Western blots for GLUT5 were unable to be performed because of lack of a reliable antibody to GLUT5 despite multiple attempts to purchase and develop our own antibody.

Glucose Absorption

We measured transporter-mediated glucose absorption using a modified everted sleeve technique as described by Karasov and Diamond (29). The targeted segment of intestine was everted over a steel rod with pre-formed grooves at 1-cm intervals so that the mucosal surface was exposed externally. The segment was secured with two 5-0 silk ties, and the redundant edges were excised leaving a 1-cm everted segment. These intestinal sleeves were kept in chilled (4°C) mammalian Ringers bubbled with 95% O_2/5% CO_2 until ready for absorption experiments. Prior to measurements of absorption, the tissues were transferred to a 38°C bath, preincubated in 8 ml of mammalian Ringers bubbled with 95% O_2/5% CO_2 for 5min, and then placed in 8-ml of 38°C mammalian Ringers with iso-osmotic replacement of NaCl using either 1, 20, or 50mM d-glucose. The solution was stirred at 1,200 rpm to mix the “unstirred layer.” Radiolabelled glucose probes (0.5 to 1 μCi of ^{14}C-D-glucose and 1 to 2 μCi of ^{3}H-L-glucose) were included in the test solution to measure the different modes (active, passive) of glucose

absorption (see below). After incubation in the glucose solution for 1 min, the tissues were removed, rinsed quickly in 30 ml of chilled mammalian Ringers stirred at 1,200 rpm, and placed in glass scintillation vials. One-half ml of tissue solubilizer (Perkin-Elmer, Boston, MA) was added with the segments kept in a 50°C water bath for 3 h. After complete solubilization, 10 ml of scintillation counting cocktail (Opti-Flour, Perkin-Elmer, Shelton, CT) was added, and probe counts were determined using techniques of dual-marker liquid scintillation counting with a standard quench curve.

Extensive preliminary validation studies using the everted sleeve technique for transporter-mediated glucose absorption were carried out previously in our laboratory using jejunal segments of 300- to 350-g Sprague-Dawley rats (Harlan, Indianapolis, IN) incubated for varying time periods (0.5, 1, 2, 4, or 8min) at six different glucose concentrations (1, 5, 10, 15, 20, and 50 mM) to determine the kinetics of the transporter system and select optimal incubation times and concentrations. Michaelis-Menten plots were made, and K_m and V_{max} values were calculated using Lineweaver Burke plots and were compared to published values for validation (30,31). Additionally, phlorizin, a known inhibitor of the primary apical glucose transporter, SGLT1, was used at 0.05 mM, 0.1 mM, and 0.2 mM concentrations to antagonize transporter-mediated glucose absorption to further validate the technique (32-34).

Analysis of Data

mRNA and protein levels—To determine relative changes in gene expression of mRNA and protein levels, the measurements of SGLT1, GLUT2, and GLUT5 were normalized to levels of GAPDH, the housekeeping gene. For mRNA, all samples to be compared were subjected to the same reverse transcription run, in duplicate (values meaned), and run simultaneously during the real time RT-PCR. Similarly, the relative expressions of protein for SGLT1 and GLUT2 were compared on the same Western Blot to prevent potential errors in loading; all samples were also run in duplicate (values meaned). Values of mRNA, protein, and glucose uptake were meaned across rats in each group and a grand mean calculated per group.

Glucose absorption—To calculate transporter-mediated glucose uptake, total glucose uptake needed to be corrected for any adherent glucose in the unstirred (non-absorbed) layer and for passive, non-carrier mediated absorption. ^{3}H-L-glucose is not absorbed by transporter-mediated uptake and was thus used to correct for this adherent glucose and passive uptake. Carrier-mediated uptake was measured in duplicate for each segment and calculated as $nmol \cdot cm^{-1} \cdot min^{-1}$. Uptake was plotted as Lineweaver-Burke plots to allow calculation of V_{max} and K_m using classic Michaelis-Menten kinetics.

Statistical Analysis

Statistical analysis was performed using JMP software. Kruskal-Wallace was used to make comparisons across study group with Wilcoxon rank sums used for direct comparisons of groups. P values were corrected according to the Bonferoni method, and a corrected p value of ≤ 0.05 was considered significant. All data are reported as the median ± interquartile range or mean ±SE unless otherwise specified; n values are number of rats.

Results

All surviving animals tolerated the experimental procedures. No differences in chow consumption were appreciated over this 8-wk period either (data not shown).

Transporter Expression

Relative expression of SGLT1 mRNA among the six groups was not different in duodenum (0.02-0.03, p=0.2), jejunum (0.02-0.04, p=0.6), nor ileum (0.01-0.02, p=0.2) (Figure 1). Relative expression of mRNA was less in ileum versus duodenum and jejunum. Similarly, protein expression as measured by semiquantitative Western blotting techniques did not differ among the six groups in duodenum (0.85-1.14, p=0.1), jejunum (0.44-1.00, p=0.1), or ileum (1.11-1.57, p=0.4) (Figure 2).

Relative expression of GLUT2 mRNA was also unchanged in duodenum (0.02-0.3, p=0.7), jejunum (0.02-0.03, p=0.6), and ileum (0.001-0.004, p=0.6) (Figure 3). Relative expression of mRNA was less in ileum versus duodenum and jejunum. Protein expression for GLUT2 demonstrated no difference in the jejunum (0.9 - 1.5, p>0.02) nor in the ileum (0.94-1.75, p=0.02). A difference was observed, however, across the duodenum (0.75-1.82, p=0.0004). GLUT2 protein expression was greater in the experimental groups (T/A1, TA/8, SBT1, and SBT8) compared to the sham and controls ($p<0.04$, Figure 4).

Relative expression of GLUT5 mRNA also remained unchanged in duodenum (0.001-0.004, p=0.3), jejunum (0.01-0.02, p=0.1), and ileum (0.001-0.002, p=0.1) (Figure 5), and relative expression of mRNA was much less (by a factor of 10) in ileum versus duodenum and jejunum.

Glucose Uptake

Transporter-mediated glucose uptake demonstrated saturation kinetics over the three substrate concentrations for all six groups in each intestinal segments (Figure 6A). Baseline kinetic values were determined using the naive controls. The K_m in duodenum was less than in jejunum (4.5±0.3 vs 6.0±0.3 mM; p=0.002); V_{max} was 3.6±0.2 nmol/cm/min in duodenum and 4.6±0.3 nmol/cm/min in jejunum (p=0.002). Total measured transporter-mediated uptake was also greatest in jejunum and duodenum compared to ileum in the controls at saturation levels of substrate ($p \leq 0.03$)(Figure 6A). Phlorizin (0.2mM), an SGLT1 inhibitor, resulted in near-complete inhibition of transporter-mediated glucose uptake in duodenum and jejunum (jejunal data shown, Figure 6B) and complete inhibition of transporter-mediated glucose uptake in the ileum compared to transport in the absence of phlorizin (data not shown, $p<0.0001$). The sham group exhibited comparable findings to the controls, although there was no difference in K_m measured between duodenum and jejunum (4.4±0.3 mM vs 4.0±0.5 mM, p=0.06).

In the T/A1 and T/A8 groups, no change in total transporter-mediated glucose uptake was evident in duodenum, jejunum, or ileum compared to the controls and sham groups (Figure 7). Furthermore, calculated K_m and V_{max} values for duodenum and jejunum were also unchanged compared to controls. K_m did not differ in duodenum and jejunum (T/A1: 4.0±1 vs 5.3±0.5 mM, p=0.1; T/A8: 3.5±0.4 vs 4.6±0.5 mM, p=0.2).

In both SBT1 and SBT8, total transporter-mediated glucose uptake also was unchanged compared to controls (Figure 7). Calculated K_m and V_{max} values were similar as well. V_{max} was also similar in transplanted jejunum compared to native duodenum (SBT1: 5.6±0.7 vs 5.0 ±0.4 nmol/cm/min, p=0.06; SBT8: 4.6±0.6 vs 3.6±0.4 nmol/cm/min, p=0.15). Furthermore, transplanted jejunal K_m was not different from native duodenal K_m (SBT1: 5±1 vs 6.2±1 mM; p=0.12 and SBT8: 4.6±1 vs 4.8±1 mM, p=0.15).

Discussion

The exact regulatory mechanisms controlling and modulating hexose transporter expression and function have not been elucidated comprehensively. Because expression and function of the hexose transporters vary diurnally (27,28), we hypothesized that expression and function is mediated predominantly by neural mechanisms and that complete extrinsic intestinal

denervation, such as that inherent to SBT (whereby the extrinsic neurons to the gut are transected completely and continuity of the intrinsic, enteric myoneural innervation is disrupted) would cause a decrease in intestinal hexose transporter expression and a subsequent decrease in hexose transport. To further implicate a specific neural pathway (extrinsic versus intrinsic [intramural] neural continuity), we included a group of animals with complete but selective disruption of intrinsic, intestinal myoneural continuity to the jejunoileum from the duodenum proximally and the large intestine distally via transection and reanastomosis of the proximal and distal small intestine; we carefully, however, maintained the extrinsic neural input. We elected to study hexose transporter expression and function at 9AM because this represents baseline expression and function and circumvents confounding factors such as luminal substrates.

There were no differences in relative expression of mRNA for all three of the intestinal hexose transporters (SGLT1, GLUT2, and GLUT5) compared to the housekeeping gene, GAPDH, in native duodenum or in the jejunoileum after intestinal transection/reanastomosis or SBT. These findings were consistent regarding protein expression of SGLT1 and GLUT2 as well. While a minor difference was observed for GLUT2 protein expression only in the duodenum, the fold change was so small that this likely bears no clinical importance; the mild increase was noted in both experimental groups (SBT and T/A) and did not occur in the sham operated group, and thus may represent an effect of intestinal transection. Our study cannot shed further insight into this small change in mRNA expression. Additionally, the findings with SGLT1 correlated with functional uptake studies of glucose using the everted sleeve technique, where transporter-mediated glucose uptake remained unchanged across all six experimental groups for each intestinal segment. Furthermore, kinetic analyses of receptor affinity (K_m) and number of functional transporters (V_{max}) also did not demonstrate any changes after denervation (intrinsic or extrinsic) of the small intestine. GLUT5 transports primarily fructose, and our functional studies did not assess GLUT5 activity.

Our study also analyzed GLUT5 levels of mRNA. GLUT5 transports fructose which is one of the primary hexoses ingested by rats. Any implications about GLUT5 function are limited, because we have no good means of measuring GLUT5 protein, and we did not assess fructose uptake because this study was designed to address glucose uptake. We can, however, say that GLUT5 mRNA, similar to GLUT2, is expressed to very low levels under baseline conditions in the ileum, most likely because virtually all fructose is absorbed in the proximal gut. This finding of a marked decrease in GLUT5 and GLUT2 mRNA in the ileum differs from the relative expression of mRNA for other mucosal transporters such as SGLT1; in our study, although mRNA for SGLT1 is somewhat less than in the duodenum and jejunum, the relative decrease is much smaller than for GLUT2 or GLUT5. In addition, mRNA for the peptide transporter PEPT1 is expressed in similar relative copy numbers to its expression in the duodenum and jejunum (35), possibly because of more protein reaching the distal small intestine from enterocyte shedding or other processes requiring uptake of protein.

These findings suggest that extrinsic and intrinsic intestinal innervations do not appear to play a major role in regulating baseline gene expression or function of hexose transporters in the jejunoileum. The malabsorptive syndromes and diarrhea seen after SBT and intestinal resections do not appear to be secondary to abnormalities in hexose transporters, either in their expression (mRNA, protein) or their function (glucose uptake). Abnormalities in enteric function may relate solely to changes in motility affecting luminal substrate concentrations or abnormalities in water and electrolyte absorption and secretion secondary to extrinsic denervation, or they may be related to other unidentified mechanisms not related to intestinal denervation (3,5,7,36-39).

A novel mechanism may lie in the results observed for the K_m values in the controls. Based on the traditional concept of intestinal hexose transport, SGLT1 is the only available transporter at the apical membrane for glucose transport and the remainder of glucose absorption occurs via solvent drag (40-43). In the controls, however, the calculated K_m was significantly greater in the jejunum than in the duodenum, suggesting that there is either a conformational change in SGLT1 expression in the duodenum versus the jejunum possibly by post-translational processing or there is more than one transporter involved in apical glucose absorption. Recent studies have suggested that GLUT2, a facilitated glucose transporter, is trafficked to the apical membrane via signaling through SGLT1 and accounts for the dramatic rapid increase in glucose absorption beyond saturation levels (44-47). This difference in K_m (duodenum compared to jejunum) was not seen in the other five groups, which may represent an unremarkable finding, or it may represent an effect on the enterocytes of intestinal denervation and/or postoperative inflammation (because this finding was not observed in the shams either). In this proposed mechanism, expression of mRNA or protein for the hexose transporter is unchanged, but the ratio of functional transporters (SGLT1, GLUT2, or others) that are present at the apical membrane is altered, which suggests a post-translational mechanism. Clearly, the data presented here are insufficient to solidify this model and further investigations into apical, transporter-mediated glucose uptake and regulation of hexose transporter expression *and* function are indicated.

Conclusion

Intestinal denervation after syngeneic SBT does not affect expression of intestinal hexose transporters or function. The glucose transporter, SGLT1, may have different conformations dependent on its anatomic location along the intestine, or apical transporter-mediated glucose absorption may be mediated by more than one glucose transporter whereby post-translational regulation of hexose transporter function may serve as a neurally mediated pathway for intestinal adaptation.

Acknowledgements

The authors are grateful to Sigurd Lenzen and Christopher Corpe for providing the entire cDNA sequences for GLUT2 and GLUT5, resp., and to Deborah Frank for her assistance in the preparation of the manuscript.

This work was supported in part by a grant from the National Institutes of Health (DK39337 - Dr. Michael G. Sarr, Principle Investigator).

Abbreviations Used in Text

GAPDH, glyceraldehyde-6-phosphate dehydrogenase
GLUT2, glucose transporter-2
GLUT5, glucose tranporter-5
PBS, phosphate-buffered saline
RT-PCR, reverse transcription polymerase chain reaction
SBT, small bowel transplantation
SBT1, 1 wk after small bowel transplantation
SBT8, 8 wk after small bowel transplantation
SGLT1, sodium-glucose transporter-1
SMA, superior mesenteric artery
T/A1, 1 wk after transection and reanastomosis
T/A8, 8 wk after transection and reanastomosis
TBS-T, Tris-buffered saline with toluene

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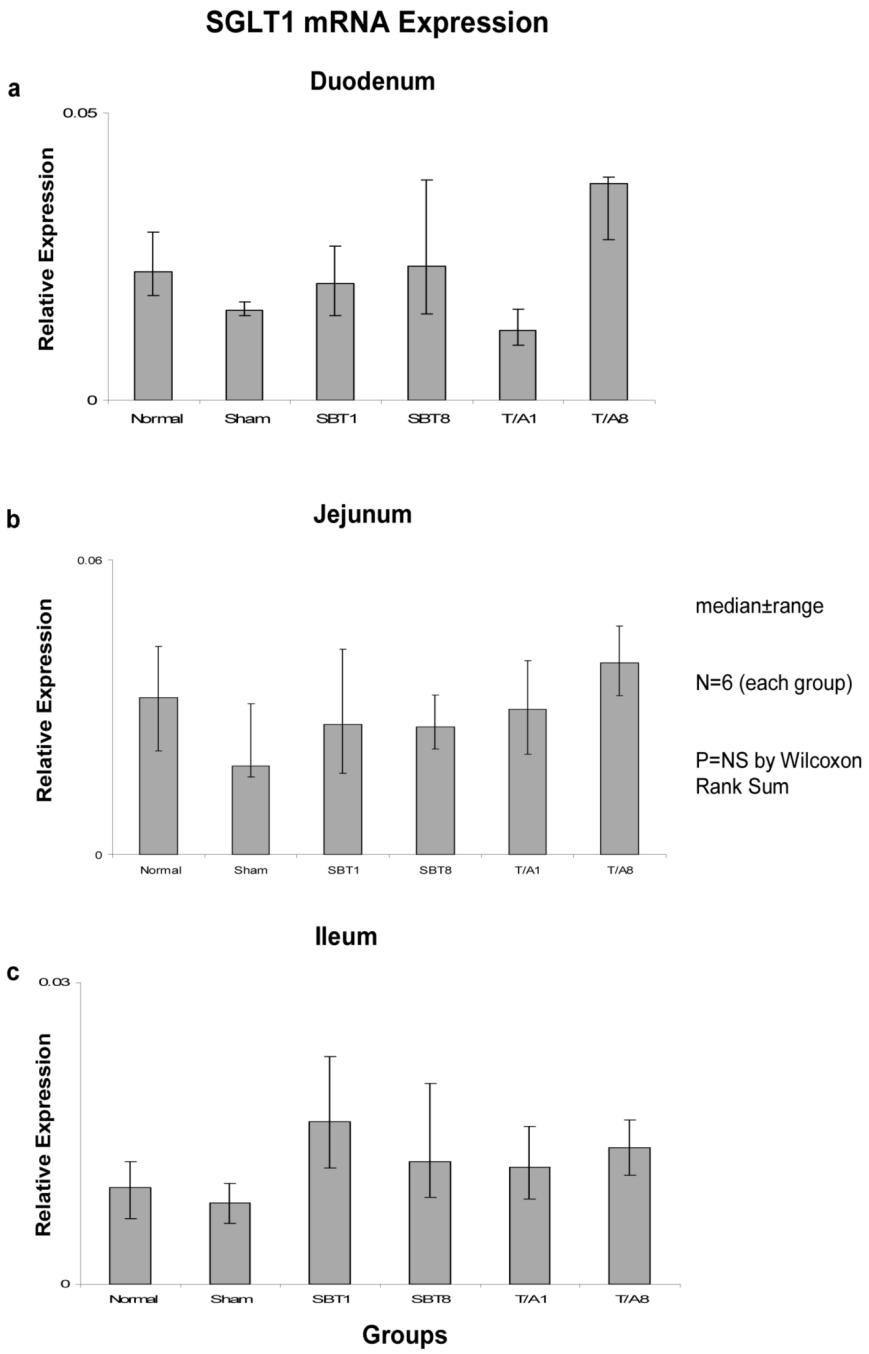

Figure 1.

Expression of SGLT1 mRNA in (**a**) duodenum, (**b**) jejunum, and (**c**) ileum; the animal groups are as outlined in the text. mRNA is shown as "relative expression" whereby the copy numbers of the transporter transcripts of interest are normalized to the copy numbers of the housekeeping gene GAPDH in attempt to allow an estimate of total cellular expression per enterocyte. NC = naïve control, sham-sham celiotomy; SBT1 and SBT8 = small bowel iso-transplantation at 1 wk and 8 wk postoperatively, resp; and T/A1 and T/A8 = jejunal and ileal transection/ reanastomosis at 1 wk and 8 wk postoperatively, resp..

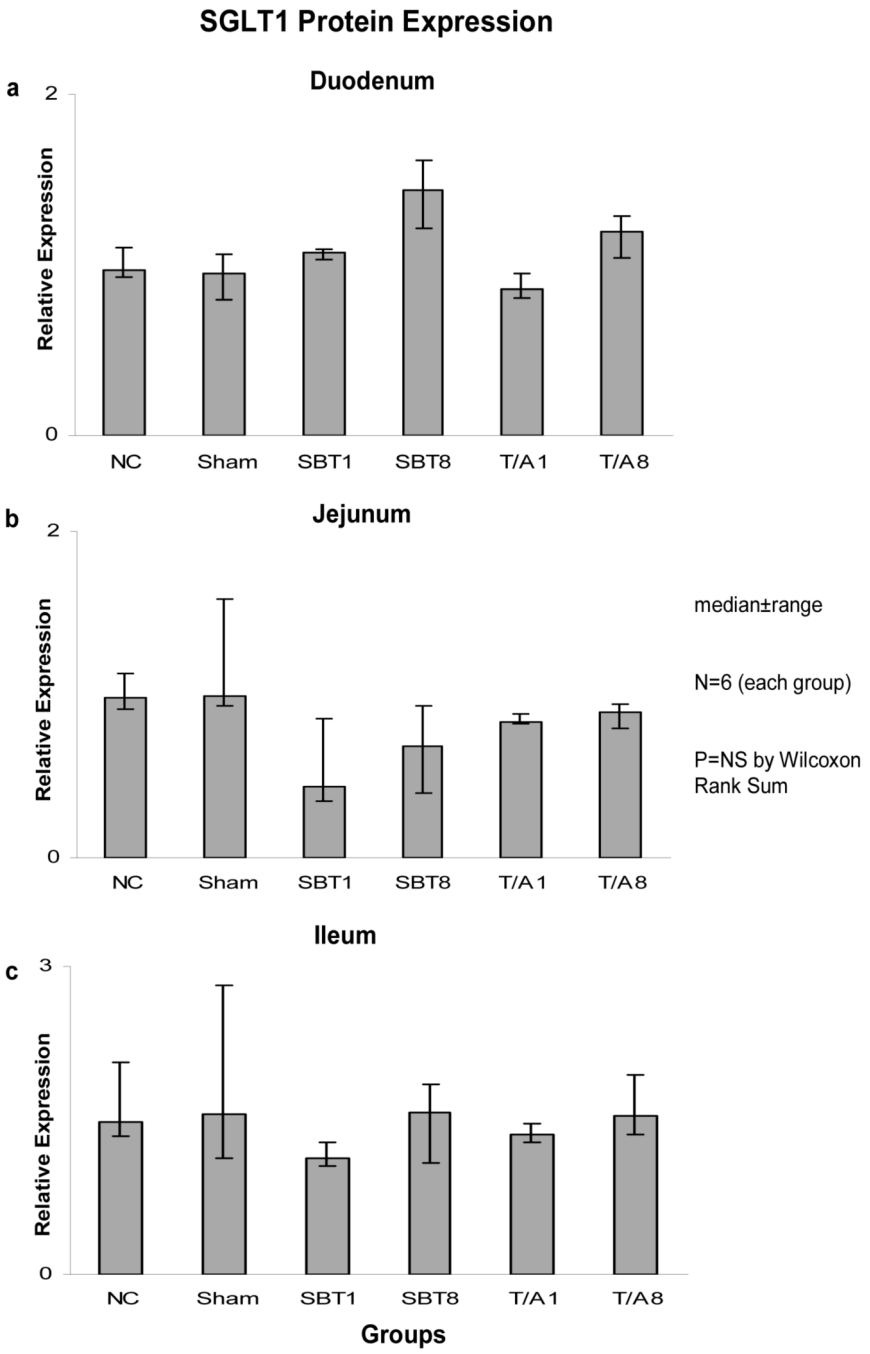

Figure 2.

Expression of SGLT1 protein in (**a**) duodenum, (**b**) jejunum, and (**c**) ileum. SGLT1 protein is shown as "relative expression" whereby the semi-quantitative density measurements from the Western blots are normalized to the density measurements of the housekeeper protein GAPDH.

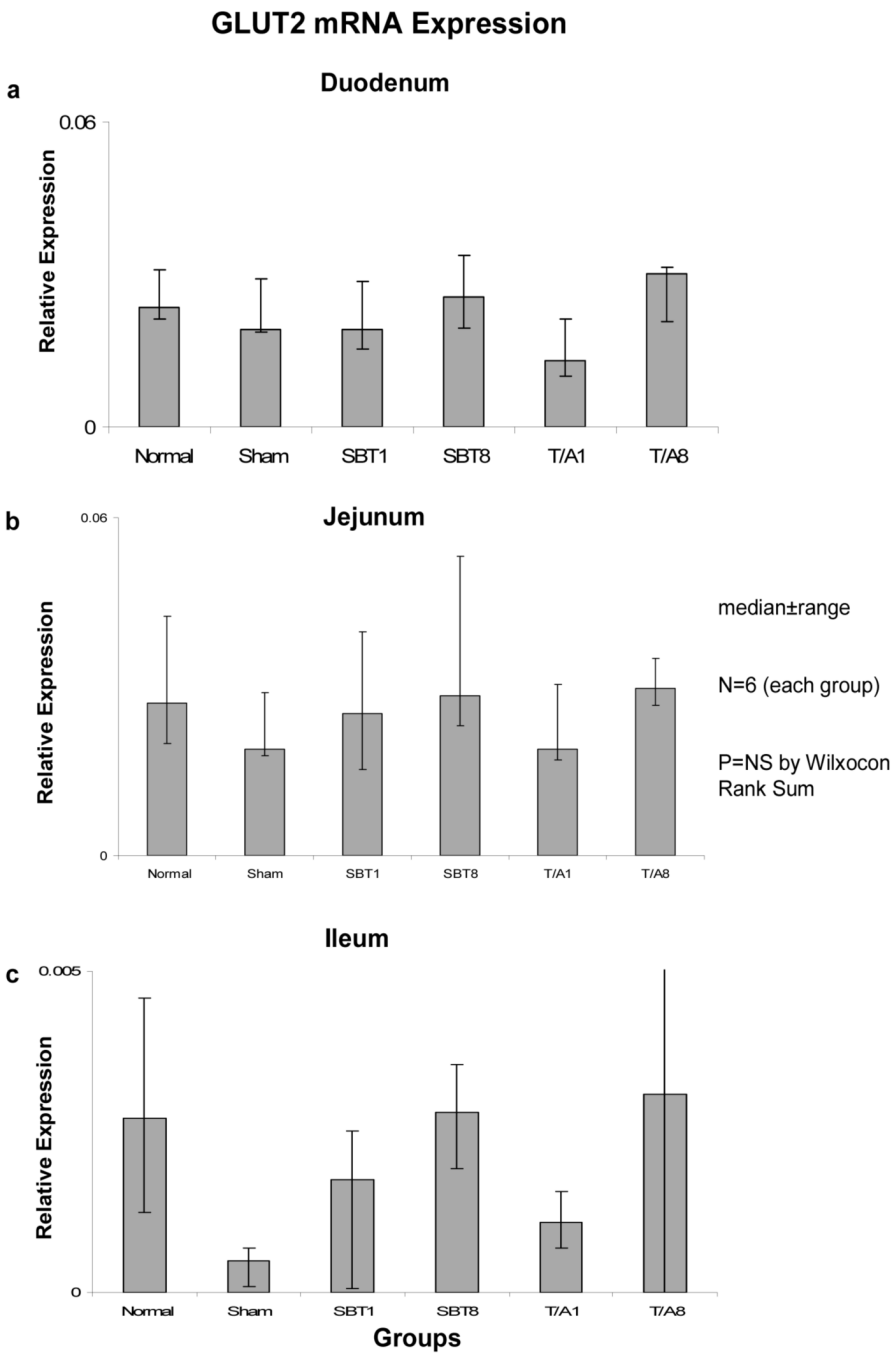

Figure 3.
Expression of GLUT2 mRNA in (**a**) duodenum, (**b**) jejunum, and (**c**) ileum. Relative expression of mRNA is as described in the legend of Figure 1.

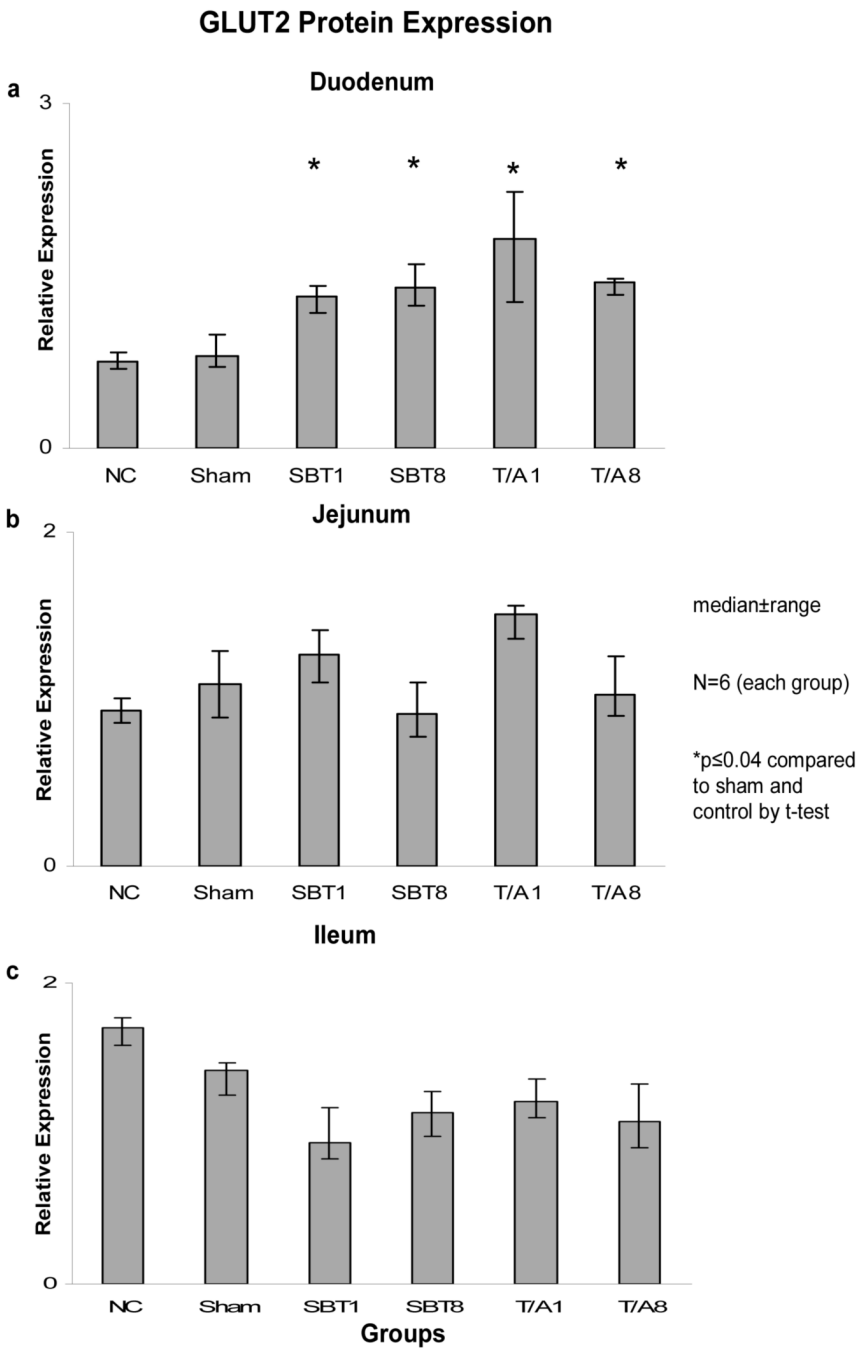

Figure 4.

Expression of GLUT2 protein in (**a**) duodenum, (**b**) jejunum, and (**c**) ileum. Relative expression of protein is as described in the legend of Figure 2.

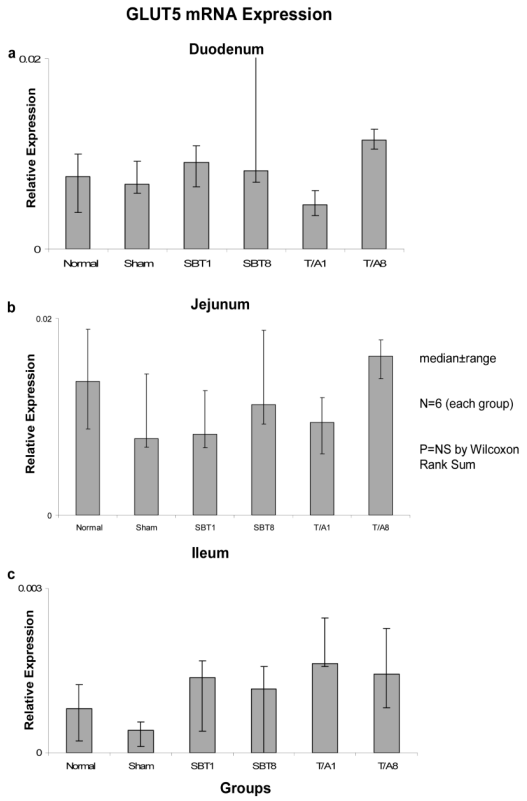

Figure 5.
Expression of GLUT5 mRNA in (**a**) duodenum, (**b**) jejunum, and (**c**) ileum. Relative expression of mRNA is as described in the legend of Figure 1.

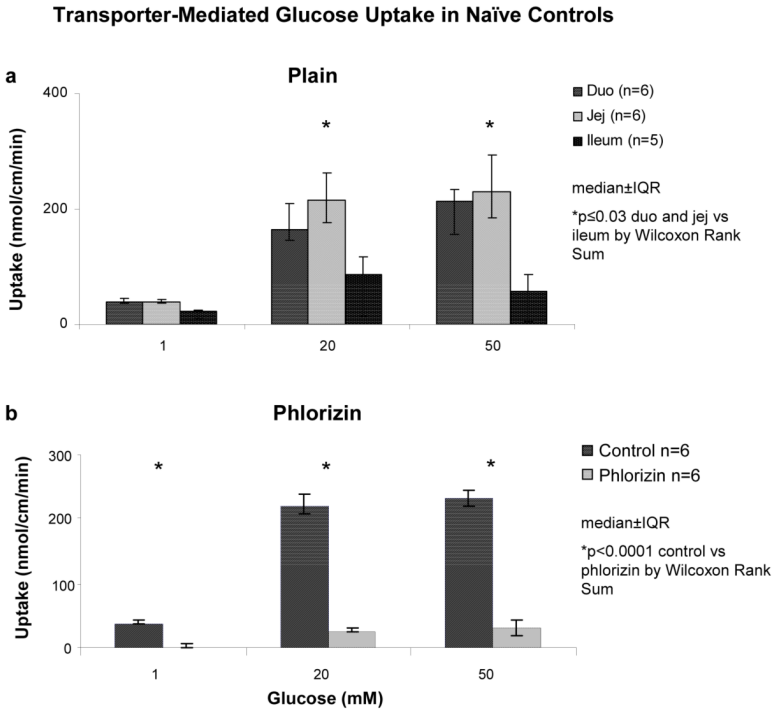

Figure 6.

Transporter-mediated glucose uptake in controls at (**a**) 1, 20, and 50 mM concentrations of d-glucose in all three segments in “plain” uptake solution; (**b**) effect in jejunum of 0.2 mM phlorizin (SGLT1 inhibitor) in uptake solution.

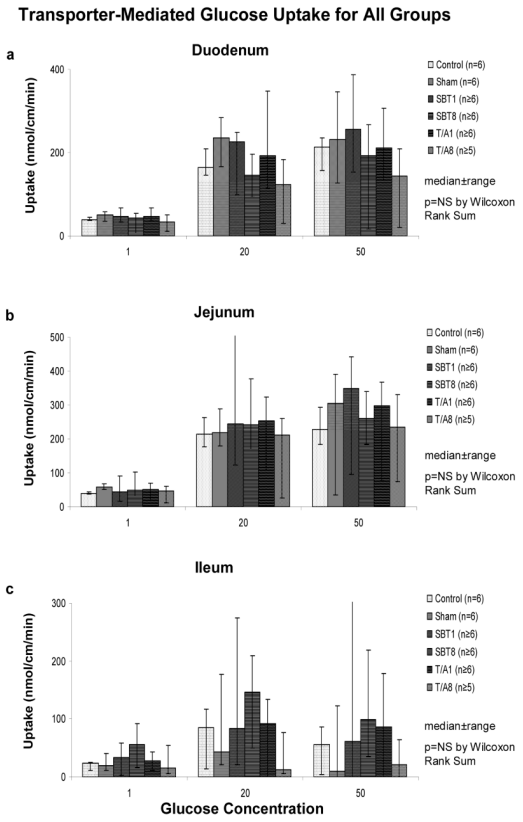

Figure 7.
Transporter-mediated glucose uptake in all groups in (**a**) duodenum, (**b**) jejunum, and (**c**) ileum.