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Role of Vagal Innervation in Diurnal Rhythm of Intestinal Peptide Transporter 1 (PEPT1)

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Abstract

BACKGROUND: Protein is absorbed predominantly as di/tripeptides via H⁺/peptide cotransporter-1 (PEPT1). We demonstrated previously diurnal variations in expression and function of duodenal and jejunal but not ileal PEPT1; neural regulation of this pattern is unexplored.

HYPOTHESIS: Complete abdominal vagotomy abolishes diurnal variations in gene expression and transport function of PEPT1.

METHODS: 24 rats maintained in a 12-h light/dark room [6AM-6PM] underwent abdominal vagotomy; 24 other rats were controls. Four weeks later, mucosal levels of mRNA and protein were measured at 9AM, 3PM, 9PM, and 3AM (n=6 each) by quantitative real time-PCR and Western blots, respectively; transporter-mediated uptake of di-peptide (Gly-Sar) was measured by the everted-sleeve technique.

RESULTS: Diurnal variation in mRNA, as in controls, was retained post-vagotomy in duodenum and jejunum (peak at 3PM, p<0.05) but not in ileum. Diurnal variations in expression of protein and Gly-Sar uptake, however, were absent post-vagotomy (p>0.3). Similar to controls, maximal uptake was in jejunum after vagotomy (V_{max}-nmol/cm/min: jejunum vs. duodenum and ileum; 163 vs. 88 and 71 at 3AM; p<0.04); K_m remained unchanged.

CONCLUSIONS: Vagal innervation appears to mediate in part diurnal variations in protein expression and transport function of PEPT1, but not diurnal variation in mRNA expression of PEPT1.

Keywords

PEPT1; diurnal rhythm; vagotomy; rat small intestine; protein absorption

INTRODUCTION

The extrinsic nervous innervation regulates and modulates many physiologic functions of the small bowel, including both intestinal motility and absorption [1–6]. The cellular and molecular mechanisms of this neural control, however, remain largely unknown. Insights into these mechanisms will advance our understanding of the physiologic and pathophysiologic changes expected after certain forms of operative denervation, such as abdominal vagotomy and small bowel transplantation, leading potentially to improvements in clinical practice.

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Enormous interest has focused in recent years on the mechanisms regulating nutrient absorption from the lumen of the gut. In our laboratory, we characterized previously the gene expression and transport function of several mucosal nutrient-transporters in the rat small intestine, such as hexose transporters [7-10] and, most recently, the H⁺/peptide cotransporter-1 “PEPT1” [11,12]. Being the exclusive peptide transporter in the apical membrane of enterocytes, PEPT1 mediates the uptake of essentially all di/tripeptides (the major protein digestion products) in addition to certain peptide-like drugs (e.g. β -Lactam antibiotics, ACE inhibitors) from the lumen [13-15]. Similar to several other mucosal transporters, PEPT1 exhibits diurnal variations in gene expression and transport function in rodents, especially in the proximal intestine (i.e. duodenum and jejunum); this diurnal pattern occurs in temporal association with their nocturnal feeding behavior [11,16-19]. Various factors appear to regulate this diurnal rhythm, such as luminal peptide substrates, hormones, and clock genes [20-22]; however, the role of extrinsic innervation to the gut in control of PEPT1 diurnal rhythmicity, as occurs with the hexose transporters, is unexplored.

The vagus nerve represents one of the primary extrinsic innervations to the gut. Because we and others have shown that vagal innervation appears to mediate in part the diurnal variations in the expression of other mucosal proteins (e.g. hexose transporters [8,23]), our aim was to determine whether neural mechanisms mediated by the vagus nerves mediate diurnal variation in gene expression (mRNA, protein) and transport function of the peptide transporter PEPT1. We hypothesized that total abdominal vagotomy would abolish diurnal variations of gene expression and transport function of PEPT1 in the rat small intestine.

METHODS

Handling of animals, surgical procedures, and conduct of experiments were performed only after approval from the Mayo Clinic Institutional Animal Care and Use Committee in accordance with the NIH Guidelines for the Humane Use and Care of Laboratory Animals. Male Lewis rats weighing 200-250 g (Harlan, Indianapolis, IN) were acclimatized to a 12-h photoperiod room (lights on only from 6AM to 6PM) with free access to water and standard rat chow (5001 Rodent Diet, PMI Nutrition International LLC, Brentwood, MO). Twenty four rats underwent subdiaphragmatic, total abdominal vagotomy (see below, Abdominal Vagotomy); another 24 rats served as normal, unoperated controls. Daily weights of each rat and the chow consumed separately per light and dark hours were tabulated for 4 wk postoperatively. To determine the presence or absence of diurnal rhythmicity in the expression and function of PEPT1, 6 rats at each of four time points (9AM, 3PM, 9PM, 3AM) were killed, and the levels of mRNA, protein, and transport activity for PEPT1 were measured in duodenum, jejunum, and ileum.

Abdominal Vagotomy

Rats were anesthetized initially using 2% inhaled isoflurane followed by intraperitoneal injection of 50 mg/kg sodium pentobarbital. A midline incision (3-5 cm) was performed, and the gastroesophageal junction (GEJ) was identified using blunt and sharp dissection as necessary. Using $\times 2$ to $\times 3$ optical magnification, both anterior and posterior vagus nerves were identified, lifted off the esophagus, ligated with 6-0 silk sutures, and 1-cm sections excised. Homeostasis was achieved as needed with electric cauterization or by topical pressure. The abdominal wall was closed in two layers using a running 5-0 polyglactin suture. Postoperatively, all rats were maintained in a 12-h light/dark facility (1 rat per cage) and allowed free (yet monitored) access to chow and water containing acetaminophen for the first 48 h postoperatively.

Tissue Harvest

At the time of tissue harvest, rats were anesthetized with inhaled isoflurane and intraperitoneal pentobarbital. After celiotomy, successful vagotomy was confirmed by visual observation of the distended stomach [8]. The duodenum was then cannulated just distal to the pylorus, and the small intestine was flushed with cold (4°C) Ringer's solution. The small intestine was excised and placed immediately in cold (4°C), oxygenated (95% O₂/5% CO₂) Ringer's solution. The proximal duodenum was used for the experiments of transport function using everted sleeves (see below, Uptake Function), and the distal duodenum was used for measurements of mRNA and protein. Similarly, mid-jejunum and mid-ileum were studied. The mucosa was scraped bluntly using a glass slide into cold, phosphate-buffered saline (PBS). Samples for mRNA analysis were placed in RNA stabilization buffer (RNALater, Qiagen, Valencia, CA) and stored immediately at -80°C. The samples for protein analysis were collected separately, placed in cold RIPA buffer containing Halt protease inhibitors (Pierce, Rockford, IL) and PMSF (phenylmethane sulfonyl fluoride solution; Sigma Aldrich, St. Louis, MO), and stored at -80°C. For histomorphometry, 0.5-cm portions of each anatomic segment were pinned on a support and fixed in 10% buffered formalin.

mRNA Measurement

Real-time, reverse transcription, polymerase chain reaction (RT-PCR) was used to quantitate mRNA levels of PEPT1 as described previously [11]. Mucosal samples stored in RNA stabilization buffer were thawed on ice and homogenized; RNA was isolated using the RNeasy Midi kit (Qiagen). RNA was then reverse transcribed into cDNA using the Super Script III kit (Invitrogen, Carlsbad, CA); cDNA levels of PEPT1 and the stably expressed housekeeping gene glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were then determined using RT-PCR in a 7500 Thermocycler using Taqman® chemistries with primers and fluorescently-labeled probes in assay mixes (Applied Biosystems, San Francisco, CA). Standard curves from serial dilutions of known copy numbers were used to calculate copy numbers of cDNA for each sample. All samples were run as duplicates with 2 µl of cDNA added to 23 µl of master mix. PCR was carried out at 50 °C for 2 min, then 95°C for 10 min followed by 40 cycles of 15 s at 95°C and 1 min at 60°C during which fluorescence measurements were made. Transporter copy numbers were normalized to copy numbers of GAPDH from each sample. Within each major group (control and vagotomy), all samples underwent RT-PCR simultaneously using the same reverse transcription kit to minimize the possibility of error and variability within the same group [24,25]. Moreover, to enable direct comparisons between individual groups (i.e. anatomic segments at each time point) across the two major groups (control vs. vagotomy), we ran a further set of analyses of RT-PCR ensuring quantification of copy numbers using cDNA from simultaneous reverse transcription for all compared individual groups.

Protein Measurement

Levels of total cellular protein for PEPT1 were measured semi-quantitatively using our well-characterized technique with Western blots [11]. Tissue samples stored in RIPA buffer containing Halt protease inhibitors and PMSF were thawed on ice; the presence of protease inhibitors was used in attempt to minimize protein degradation. Samples were homogenized using a Kontes Pellet Pestle (Fisher Scientific, Pittsburg, PA). The protein-containing supernatant was separated by centrifugation at 5000 × g for 15 min. Protein concentrations were measured by the bicinchoninic acid method (Pierce); 200 µg of protein was resolved on a 10% SDS-PAGE gel (Bio-Rad, Hercules, CA) and transferred electrically to a PVDF membrane (Millipore, Bedford, MA). Membranes were blocked using 5% milk in Tris-buffered saline with Tween (TBS-T). GAPDH was used as a stably expressed “housekeeping” protein. Membranes were incubated overnight at 4°C with primary antibody for PEPT1 (Santa

Cruz Biotechnology, Santa Cruz, CA), and GAPDH antibody (US Biological, Swampscott, MA). After incubation with primary antibody, membranes were rinsed 3 times with TBS-T and incubated with secondary antibody in TBS-T containing 5% milk using horseradish peroxidase-conjugated, goat anti-rabbit IgG for PEPT1 and anti-mouse IgG for GAPDH (Sigma). Protein bands visualized with a colorimetric reaction using Opti-4CN Substrate kits (Bio-Rad) for GAPDH and amplified Opti-4CN for PEPT1 were scanned, and Scion Image (Scion Corp, Frederick, MA) was used for semiquantitative measurements based on band densitometry. Protein measurements were normalized to GAPDH as a technique designed to estimate amount of protein per enterocyte.

Uptake Function

We measured transporter-mediated uptake of the di-peptide Glycyl-Sarcosine (Gly-Sar), a non-hydrolysable substrate for PEPT1 [12,21], using a modified everted sleeve technique as we described previously [9]. Intestinal segments (1 cm) were everted over a pre-grooved steel rod and secured with silk ties, thereby exposing the mucosal surface externally. The intestinal “sleeves” were kept in chilled (4°C) Ringer's solution bubbled with 95% O₂/5% CO₂. The sleeves were transferred into 8 ml of warmed (38°C), Gly-Sar-free incubation medium (in mM: 129 NaCl, 5.1 KCL, 1.4 CaCl₂, 1.3 NaH₂PO₄, and 1.3 Na₂HPO₄; pH 6.0) [11,19] for 5 min bubbled with 95% O₂/5% CO₂ and stirred at 1,200 rpm. Then, the sleeves were placed in 8 ml of 38°C incubation medium containing 0.02, 1, 5, 20, or 40 mM Gly-Sar maintaining iso-osmotic conditions with replacement with appropriate amounts of NaCl. One μ Ci of ¹⁴C-Gly-Sar was included in the test solution to measure total uptake of Gly-Sar, from which the transporter-mediated uptake by PEPT1 was calculated (see below). After 1-min incubation, sleeves were removed, rinsed in 30 ml of ice-cold (Gly-Sar-free) incubation medium stirred at 1,200 rpm for 20 s, placed in glass scintillation vials containing 1 ml of tissue solubilizer (Perkin-Elmer, Boston, MA), and kept in a 50°C water bath for 3 h. After complete solubilization, 15 ml of scintillation counting cocktail (Opti-Flour, Perkin-Elmer, Waltham, MA) was added, and DPMs of ¹⁴C were determined using liquid scintillation.

Transporter vs. non-transporter mediated uptake—The method of estimating transporter vs. non-transporter mediated uptake of Gly-Sar was described previously [9]. To calculate transporter-mediated uptake, total uptake needed to be corrected for passive diffusion and mucosal adherence (non-transporter mediated “uptake”). Non-transporter-mediated uptake at lesser concentrations is best estimated from observed uptake at markedly greater concentrations [11,26]. As the substrate concentration increases, non-transporter mediated passive uptake increases linearly before and after the transporter is saturated; thus, the linear increase in total uptake after the transporter is saturated is attributed “only” to non-transporter-mediated “uptake”, i.e. passive diffusion and mucosal adherence. We used 20- and 40-mM concentrations of Gly-Sar (at which a linear increase in total uptake was observed) to estimate non-transporter-mediated “uptake” at the lesser concentrations (0.02, 1, 5 mM). Subtraction of the estimated, non-transporter-mediated uptake from observed total uptake allowed estimation of the transporter-mediated uptake.

Villous Height

The formalin-fixed tissues from both groups (control and vagotomy) were embedded in paraffin, sectioned parallel to the villous axis, and stained with hematoxylin and eosin. Maximum villous height was measured from above the crypt to the tip of the villous at 10x magnification using an optical reticule with a micrometer. Measurements from each specimen were made on at least 6 slides with at least three measurements per slide.

Statistical Analysis

All levels of mRNA and protein were expressed as the ratio of PEPT1 to the housekeeping gene (GAPDH) in an attempt to estimate gene expression per enterocyte. Transporter-mediated uptake of Gly-Sar was measured in nmol/cm/min with Lineweaver-Burke plots used to calculate $V_{\max}$ and K_m . Data are reported as median $\pm$ interquartile range (IQR). Kruskal-Wallis analysis was used to compare non-parametric data across multiple groups; Wilcoxon rank sums were used for direct comparisons between individual groups. P-values were corrected according to the Bonferroni method, only corrected p values of < 0.05 were considered significant, and n values are number of rats.

RESULTS

Food Intake/ Weight Gain

Rats in both groups (control and vagotomy) displayed a nocturnal-based feeding pattern through the entire 4-wk period (until tissue harvest); greater than 70% of chow intake occurred between 6PM and 6AM ($p<0.001$). During the first postoperative week (Days 1-6), vagotomized rats consumed lesser amounts of food (as total-daily intake) compared to controls ($p\leq 0.003$; Figure 1); however, by the end of the first week (Day 7), there was no difference between both groups either in total daily-intake or in night/day ratio of food consumption per rat ($p>0.1$). During the 4-wk interval, the rats in the control group gained a median weight of 103 g (IQR: 97-110 g), while vagotomized rats gained a median weight of 95 g (IQR: 70-109 g) ($p>0.2$).

mRNA Expression

Diurnal patterns of expression—Similar to the pattern exhibited by controls, mRNA levels of PEPT1 varied diurnally in the duodenum and jejunum of vagotomized rats (peak at 3PM, trough at 3AM; $p<0.05$; Figure 2A-B), whereas ileal mRNA levels had no diurnal variations either in controls or in the vagotomy group ($p=0.2$). The median relative fold-changes (peak over minimum levels) in the duodenum and jejunum of control rats were 5- and 4-fold, respectively. Similarly, median fold-changes in vagotomized rats were 4- and 3-fold in the duodenum and jejunum, respectively.

Segmental anatomic expression—In controls, there were no differences between the anatomic segments at any time point in the relative expression levels of mRNA per enterocyte ($p>0.2$); after vagotomy, however, this lack of difference between anatomic segments was retained only at 3PM (when mRNA levels peak), but at other time points (9AM, 9PM, 3AM), transcription of mRNA (per enterocyte) was greater aborally in the small bowel of vagotomized rats (ileum>jejunum>duodenum; $p<0.01$). Furthermore, when site-specific segments from both groups were compared individually (control vs. vagotomy for a given segment at each time point), vagotomized rats had greater mRNA levels than controls at 9PM in duodenum and 3PM in jejunum ($p<0.02$; Figure 3A-B); there were no differences in ileal mRNA levels between any of the groups at all time points ($p>0.1$; Figure 3C).

Protein Expression

Diurnal patterns of expression—Levels of total cellular protein for PEPT1 showed a slight, albeit statistically significant, increase during the dark phase (9PM and 3AM) in the jejunum and ileum of control rats (<1.5 -fold changes across time points; $p<0.05$; Figure 4A). After vagotomy, however, these diurnal variations in total cellular protein seen in controls were abolished in all anatomic segments of vagotomized rats ($p>0.2$; Figure 4B).

Segmental anatomic expression—In both groups (control and vagotomy), there were no measurable differences between the anatomic segments in total cellular protein for PEPT1 ($p>0.3$). Moreover, when comparing each anatomic segment between groups (control vs. vagotomy at each time point); no differences were seen between any site-specific segments in levels of total protein (for PEPT1) per enterocyte ($p>0.1$).

Transporter-Mediated uptake of Gly-Sar

Diurnal pattern of uptake—Uptake of Gly-Sar in both control and vagotomy groups demonstrated saturation kinetics in all three anatomic segments consistent with transporter-mediated uptake. In control rats, uptake of Gly-Sar varied diurnally in duodenum and jejunum with values of Gly-Sar uptake (nmol/cm/min) being greater at 3PM and 9PM compared to 9AM for all concentrations ($p<0.05$; Figure 5A-B); in the ileum, no significant diurnal variation was noted in Gly-Sar uptake ($p>0.5$; Figure 5C).

In contrast, in vagotomized rats, all diurnal variations in transport function of PEPT1 (di-peptide uptake) were abolished completely; when measured at 4 wk post-vagotomy, no diurnal variation in Gly-Sar uptake was noted in duodenum, jejunum, or ileum ($p>0.1$; Figure 6A-C).

Uptake kinetics—The calculated $V_{\max}$ (in nmol/cm/min, a function of the number of apical transporters participating actively in uptake) varied diurnally in the proximal intestine of control rats (3PM vs. 9AM: 104 vs. 62 in duodenum, and 185 vs. 101 in jejunum, $p<0.03$; Figure 6A), while $V_{\max}$ remained unchanged in the ileum. In contrast, after vagotomy, $V_{\max}$ remained unchanged across time points at all anatomic segments (3PM vs. 9AM: 83 vs. 73 in duodenum, and 138 vs. 137 in jejunum, $p>0.2$; Figure 7B). K_m did not differ amongst the segments at all time points for both groups ($p>0.1$, Figure 8A-B). When comparing $V_{\max}$ values between controls and vagotomized rats at each time point in each anatomic segment, no differences were present in duodenal and ileal segments ($p>0.2$); however, in the jejunum, vagotomized rats had a lesser $V_{\max}$ value compared to controls at 3PM (when uptake peaks in controls), but greater values than controls at 9AM and 3AM ($p<0.5$).

Villous Height

In both groups as expected, median villous height was greater in duodenum and jejunum compared to ileum (0.47 and 0.48 vs. 0.29 mm, respectively in controls; 0.51 and 0.49 vs. 0.32 mm, respectively in vagotomy; $p<0.0001$). There were no differences in villous heights between controls and vagotomized rats for each site-specific segment ($p>0.05$).

DISCUSSION

Diurnal rhythmicity of absorptive function in the small intestine in rodents is a fascinating but poorly understood phenomenon. Diurnal variations in expression and function of mucosal transporters serve, presumably, to match the expected amounts of nutrients being delivered “diurnally” to the gut lumen; the mechanisms entraining these “anticipatory” rhythms are not known [16,27]. Recent studies from our laboratory and others suggested that extrinsic nervous innervation, primarily the vagal innervation, modulates in part this diurnal expression of mucosal hexose transporters [8,23,28,29]; however, and to the best of our knowledge, no groups have investigated the role of vagal innervation in control of gene expression, transport function, and diurnal rhythm of the mucosal transporter “PEPT1”, whose physiologic and clinical importance has been well recognized recently.

In this study, we delineated the impact of total abdominal vagotomy on baseline levels and diurnal variations in gene expression and absorptive function of PEPT1 throughout the rat small intestine. In order to assess the effect of vagal ablation on PEPT1 expression and function,

we needed to show that the amount of food intake, pattern of food intake (light vs. dark cycle), and body weight were unchanged after vagotomy at the time of harvest. In fact, there was an initial post-operative lag in food intake and weight gain in vagotomized rats in the first week post-op. Our previous studies addressing hexose transporters showed a similar initial lag in vagotomized rats compared to sham laparotomy and normal controls [7,8], most likely related to the vagal ablation; however, at the time of harvest four weeks later, there were no differences between controls and vagotomized rats in terms of feeding pattern (nocturnal feeding), amounts of food consumed daily, or overall weight gain, consistent with our past work. This sustained rhythmicity of nocturnal feeding after vagotomy correlated with the persistence of a diurnal variation in mRNA transcription in the duodenum and jejunum of vagotomized rats (as in controls); however, despite rhythmicity in feeding and **mRNA expression**, the diurnal variations of protein **expression** and transport function that occurred in controls were abolished when measured 4 wk after vagotomy. These data suggest that neither the feeding pattern of rats nor the diurnal rhythm of **mRNA expression** are modulated by vagal innervation; however, diurnal variations of protein expression and transport function appear to be mediated in part by the vagal input to and/or from the small bowel.

Diurnal variations of **mRNA expression** in the proximal intestine of control and vagotomized rats had very similar patterns; mRNA levels peaked in anticipatory fashion 3 h before the dark cycle when most of the feeding occurred and declined to minimal levels 3 h before the light cycle when feeding had decreased. Preservation of this same diurnal pattern of **mRNA expression** after vagotomy (i.e. matched peak and trough levels with those of controls) reinforces the concept that these anticipatory rhythms of **mRNA expression** are not controlled or modulated by vagal innervation but rather entrained by other regulatory mechanisms, related probably to the role of peripheral clock genes in the gastrointestinal tract, which in turn, may be subject to control or modulation by other luminal, hormonal, and/or neural factors [30-32].

Although an overall diurnal rhythmicity of mRNA levels was retained after vagotomy, several changes were noted in the absolute levels of **mRNA for** PEPT1 in the duodenum and jejunum in the two groups. Vagotomized rats had greater levels of total cellular mRNA of PEPT1 in duodenum (at 9PM) and jejunum (at 3PM) compared to the innervated control of rats; there were no differences between ileal segments from both groups. These differences might be related to loss of vagal input (vagal modulation) on cellular **mRNA expression** at the times when **expression** of mRNA for PEPT1 is maximal (late light phase, early dark phase); no differences were noted at other times, raising the possibility that vagal innervation modulates signals controlling times of increased **mRNA expression (transcription and/or stability)**. Indeed, in the vagally innervated control rats, the total cellular mRNA levels were similar across all three segments (duodenum, jejunum, and ileum), while after vagotomy, greater mRNA levels occurred in the more distal small intestine (ileum > jejunum > duodenum) at several time points in the diurnal rhythmicity, again suggesting a vagal modulation.

From a functional standpoint (peptide absorption), the changes in total cellular protein of PEPT1 and peptide uptake were equally interesting. Ultimately, protein absorption requires a functional peptide transporter in the apical membrane. Vagotomy led to a loss of the diurnal variation in total cellular levels of PEPT1 (as measured by the semiquantitative Western blots) despite the ongoing diurnal variations in mRNA, suggesting a potential vagal modulation of protein **expression** as has been postulated for hexose transporters [8,23,29]. But, measurement of total cellular protein by Western blots may not differentiate between functional and non-functional PEPT1 transporters or between intracellular stores of PEPT1 and apical, membrane-bound PEPT1 where the transport occurs. Therefore, it was necessary to evaluate actual transport function of PEPT1 to determine functional capacity for protein absorption. Consistent with the loss of diurnal expression of PEPT1 protein after vagotomy, diurnal variations in transport of Gly-Sar by PEPT1 were also absent after vagotomy, no longer peaking at 3PM

and 9PM as in control rats. When the kinetics of uptake were evaluated, there was a loss of the diurnal variation in $V_{\max}$, a measure of the number of “functional” PEPT1 transporters. In addition, the calculated K_m , a measure of transporter affinity for the substrate, remained unchanged, reinforcing the concept that the loss of the diurnal pattern of uptake after vagotomy is not a result of changes in type of transporter or protein conformation, but rather a result of a loss of diurnal variations in the number of transporters expressed in the apical membrane of enterocytes after vagotomy.

A possible interpretation of these overall findings in PEPT1 expression (i.e. loss of rhythmicity of both protein expression and transport function after vagotomy) is that the mechanisms entraining these rhythms might have been impacted directly by loss of vagal input; thus, vagal input may modulate some aspect(s) of posttranscriptional and/or posttranslational processing; intracellular PEPT1 transporter proteins could be targeted for immediate breakdown and/or not recruited to the apical membrane to participate in peptide uptake. As stated above, vagal modulation of protein translation for hexose transport has also been noted both by us [8] and by others [23,29]. In addition, there is good experimental evidence for recruitment of the hexose transporter GLUT2 to the apical membrane of the enterocyte by translocation from intracellular pools of transporter in response to greater concentrations of luminal substrate [33-36]; furthermore, some evidence suggests that PEPT1 may also be regulated in part by similar mechanisms of apical translocation [22,37,38]. Our study suggests strongly that vagal innervation modulates some aspect of the cellular regulation of PEPT1; however, under our experimental design, we cannot determine further the actual mechanism(s).

In order to assess the impact of vagotomy on mucosal histomorphometry, we measured villous height in three anatomic segments in controls and vagotomized rats. Changes in villous height (and thus the number of enterocytes/cm) could affect the results of di-peptide uptake (per 1-cm segment), although our measurements of expression of mRNA and protein would not be affected, because these values were measured relative to the stably-expressed housekeeper gene GAPDH and thus reflect indirectly the levels of mRNA and protein per enterocyte. Indeed, our measurements showed that there were no differences between the two groups in the villous height (for each corresponding segment), suggesting that vagotomy did not appear to cause a change in number of enterocytes. We cannot exclude the possibility of a change in enterocyte function, however, at least by this method of histomorphometry.

Our study, however, has several limitations that must be acknowledged that could affect the interpretation of our results. Vagotomy did lead to an obvious gastric distention at the 4-wk time postoperatively. This gastric distention, although not affecting the diurnal pattern of food intake or weight gain of the animals, may have altered the timing of delivery of nutrients to the small intestine via a change in gastric emptying, a known effect of vagotomy. Similarly, vagotomy may alter transit through the small intestine. Different patterns of food delivery to the proximal and distal small intestine could affect the diurnal rhythms of protein expression and transport function via non-vagal mechanisms entraining these diurnal rhythms. Interestingly, the diurnal “anticipatory” patterns of gene expression were not affected by vagotomy, although the absolute levels were increased after vagotomy. Another potential limitation of our study is that we had no control group undergoing “sham laparotomy” to control for the anesthesia and celiotomy; however, previous studies from our laboratory addressing hexose transporters [8] and PEPT1 [12] found no effects of sham laparotomy on gene expression, transport function, or on feeding patterns of rats.

CONCLUSION

While vagal innervation to the small bowel does not appear to regulate or modulate the diurnal rhythms of **mRNA expression** for PEPT1, vagotomy does appear to mediate, in part, the

diurnal variations of protein **expression** and transport function. This vagal control may have important implications in gut function after vagotomy, small bowel transplantation, bowel resection, or even in patients with short gut.

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FIGURE 1.

Pattern of food intake of normal control rats (NC) and vagotomized rats (Vag) for the first postoperative week; thereafter, food intake was virtually identical (data not shown).

FIGURE 2.

Relative expression levels of mRNA in A) control and B) vagotomized rats for PEPT1 in the three anatomic segments at four time-points (each major group was studied separately using two different sets of analysis of RT-PCR). Levels of mRNA **varied diurnally and** peaked at 3PM in both groups. In controls, there was no difference between the intestinal segments in relative expression of mRNA (per enterocyte) at each time point; however, relative expression of mRNA varied between intestinal segments of vagotomized rats (peak in ileum).

FIGURE 3.

Variations in levels of mRNA between both groups (control and vagotomy) in A) duodenum, B) jejunum, and C) ileum at four time-points during the day (individual groups from both major groups were compared directly using additional sets of analysis of RT-PCR). mRNA expression was greater in vagotomized rats (compared to controls) in duodenum (at 9PM) and jejunum (at 3PM).

FIGURE 4.

Variations in levels of protein in A) controls and B) vagotomized rats. Small but significant ($p<0.05$) diurnal changes were noted in protein levels in the jejunum and ileum of controls, but not in vagotomized rats.

FIGURE 5.

In control rats, transporter-mediated uptake of Gly-Sar at three concentrations in A) duodenum, B) jejunum, and C) ileum at four time-points. Gly-Sar uptake varied diurnally in duodenum and jejunum but not in ileum.

FIGURE 6.

In vagotomized rats, transporter-mediated uptake of Gly-Sar at three concentrations in A) duodenum, B) jejunum, and C) ileum at four time points. Gly-Sar uptake did not vary diurnally in any intestinal segment.

FIGURE 7.

Values for $V_{\max}$ in each intestinal segment at four time points in A) control rats and B) vagotomized rats. In control rats, $V_{\max}$ varied diurnally in duodenum and jejunum but not in ileum. There was no variation of $V_{\max}$ in rats after vagotomy.

FIGURE 8.

Values of K_m in each intestinal segment at four time points in A) control rats and B) vagotomized rats. K_m remained constant in both groups in each segment.