Diurnal rhythmicity in intestinal SGLT-1 function, $V_{\text{max}}$, and mRNA expression topography

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Received 16 June 2000; accepted in final form 31 August 2000

Diurnal rhythmicity in intestinal SGLT-1 function, $V_{\text{max}}$, and mRNA expression topography. Am J Physiol Gastrointest Liver Physiol 280: G209–G215, 2001.—Mechanisms underlying the circadian rhythmicity in intestinal sugar absorption remain unclear. To test whether this rhythmicity is caused by changes in Na+-glucose cotransporter 1 (SGLT-1) function, we measured phloridzin-inhibitable sugar fluxes as an index of SGLT-1 activity. Jejunum obtained from rats killed at 6-h intervals during a 12-h light-dark cycle (CT0 is circadian time 0 h, time of light onset) were mounted in Ussing chambers, and 3-O-methylglucose (3-OMG) fluxes were calculated before and after addition of phloridzin. 3-OMG-induced change in short-circuit current and absorptive flux were significantly greater at CT9 than at CT3. This increase was phloridzin inhibitable. Kinetic studies indicated a significant increase in SGLT-1 maximal velocity ($V_{\text{max}}$) at CT9. Food intake between CT3 and CT9 was <10% of the daily total, indicating that the increased SGLT-1 activity was anticipatory. Diurality of SGLT-1 mRNA was confirmed by Northern blotting. Expression topography analyzed by in situ hybridization revealed more intense labeling along the entire villus axis at CT9 and CT15 compared with CT3 and CT21. We conclude that diurnicity in intestinal sugar absorption is caused by periodicity in SGLT-1 $V_{\text{max}}$.

intestinal sugar absorption; Ussing chamber; sodium-glucose cotransporter; in situ hybridization; enzyme kinetics

PLASMA glucose concentration is maintained within a narrow physiological range that exhibits circadian rhythmicity (1, 21). Endocrine mechanisms involved in controlling carbohydrate homeostasis, such as plasma insulin level and glucose tolerance, exhibit striking but largely unexplained diurality in both humans (17, 32) and rodents (1, 14, 16, 21). The specific mechanisms responsible for the observed daily shifts in carbohydrate tolerance have not been identified. To provide a better understanding of the pathways regulating carbohydrate homeostasis, we have examined the expression of intestinal Na+-glucose cotransporter 1 (SGLT-1) because it is responsible for the intestinal absorption of glucose and can be an effector pathway for modulating carbohydrate disposal.

SGLT-1 expression is restricted mainly to the mature villus enterocytes of the small bowel and to the renal epithelium. In the small intestine, the energy-dependent uptake of glucose (and galactose) by apical SGLT-1 combined with its downhill flow via the basolateral GLUT-2 facilitated glucose transporter accounts for the absorption of this sugar. The expression of intestinal glucose transporters shows circadian rhythmicity and is altered in response to the carbohydrate content of the diet. Several studies comparing intestinal sugar absorption during the day versus the night have shown that in rats fed ad libitum and kept under normal physiological conditions with dark nights, sugar uptake is low in daytime and high at night, when these rodents feed (13, 15). This rhythmicity has been shown to be independent of the light cycle but cued by feeding (29, 30). This periodicity is anticipatory because it still occurs during food deprivation (15). Moreover, persistence of this functional rhythm during discontinuous parenteral feeding indicates that use of the intestine is not required (31).

Much of the earlier published work on functional rhythmicity of intestinal glucose absorption was done before the mechanisms involved in intestinal sugar absorption were elucidated and the relevant transporters were identified. Recent work has focused on the diurnal expression rhythm of GLUT-2, GLUT-5 (facilitated fructose transporter), and SGLT-1 at the mRNA and protein levels (6, 23), but physiologically important changes in the SGLT-1 function have not been studied. Although changes in 3-O-methylglucose (3-OMG) absorption have been recognized for many years, it has not been formally shown that SGLT-1 is directly responsible for the temporal changes observed, nor has it...
be determined whether the changes are caused by rhythmicity in transporter maximal velocity ($V_{max}$) or substrate affinity. To test our hypothesis that the reported diurnicity in intestinal glucose absorption is caused by changes in SGLT-1 function, we measured phloridzin-inhibitable sugar fluxes in the Ussing chamber as an index of SGLT-1 activity and performed kinetic studies. To help further understand the mechanism of SGLT-1 mRNA periodicity, we also mapped the topography of these changes by in situ hybridization.

Our results show that phloridzin-inhibitable periodicity in SGLT-1 activity is responsible for changes in sugar absorption. This change in activity is caused by a significant increase in SGLT-1 $V_{max}$ with no change in transporter affinity (Michaelis constant, $K_m$). These changes in transporter activity are anticipatory and are not caused by enzyme induction by the presence of an increased load of nutrients in the intestinal lumen. Northern blot analysis confirmed rhythmicity in SGLT-1 mRNA expression, and in situ hybridization showed that these changes occurred both in enterocytes in the upper villi as well as in the less mature enterocytes in the lower third of the villus. Our studies suggest that diurnal regulation of SGLT-1 occurs by a pathway distinct from the carbohydrate induction pathway (9).

MATERIALS AND METHODS

Animals. The study protocols were approved by the Harvard Medical Area Standing Committee on Animals and the Massachusetts General Hospital Subcommittee on Research Animal Care. Six-week-old female Sprague-Dawley rats (Taconic Farms, Germantown, NY) were acclimatized for at least 5 days to a 12-h photoperiod. Light onset was regarded as the beginning of the circadian time (0 h; CT0), and rats were killed at CT3, CT9, CT15, and CT21 (Fig. 1).

Chemicals. Chemicals were obtained from Sigma (St. Louis, MO). Tritiated 3-OMG ([3H]3-OMG) and 32P-labeled nucleotides were obtained from NEN (Boston, MA).

Transport and kinetic studies. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (100 mg/kg). Midline laparotomy was performed, and a 15-cm segment of proximal small intestine, beginning at 5 cm distal to the ligament of Treitz, was harvested and immediately placed in ice-cold Ringer solution. The intestine was opened along its mesenteric border, rinsed free of luminal debris, and divided into two segments. These segments were stripped of their seromuscular layers under a magnifying lens and mounted in Lucite Ussing chambers (World Precision Instruments, Sarasota, FL) with an exposed area of 0.64 cm². They were bathed on each side with 10 ml of a modified Ringer solution containing (in mM) 140 Na¹, 5.4 K¹, 1.3 Ca²⁺, 1.2 Mg²⁺, 124 Cl⁻, 2.4 HPO₄²⁻, 0.6 H₂PO₄⁻, 21 HCO₃⁻, and 5 mM HEPES. pH was adjusted to 7.4 ± 0.01. The solution was circulated using gas lift with 5% CO₂-95% O₂. Temperature was maintained at 37°C with water-jacketed reservoirs. Ten millimolar fructose was added to the serosal and mucosal chambers. Transmural potential difference (PD) was measured using calomel electrodes connected to the bathing solution with Ringer-Agar (2%) bridges. Tissues were continuously short-circuited with an automatic voltage clamp (model EVC-4000, World Precision Instruments) except during 5-s intervals every 5–10 min when the open-circuit PD was measured. Tissue resistance ($R$) was calculated from open-circuit PD and short-circuit current ($I_{sc}$) using Ohm’s law. After a 40-min period of equilibration, 3-OMG (30 mM, final chamber concentration) was added to both mucosal and serosal chambers. The maximal change in $I_{sc}$ ($ΔI_{sc}$) within 20 min of 3-OMG addition was recorded. Tissues from the same animal were paired together, and at the time of the addition of 3-OMG, 2 µCi of [3H]3-OMG was added to the serosal chamber of one tissue and the mucosal chamber of the other tissue of each pair. If baseline $R$ varied by more than 5%, between the pair, the tissues were discarded and the results were not included in the analysis. Thirty minutes later, when steady-state flux had been reached, two 15-min [3H]3-OMG fluxes were measured. Phloridzin (100 μM) was then added to both chambers, and a third 15-min [3H]3-OMG flux was measured. Response to the addition of 10⁻⁸ M vasoactive intestinal polypeptide (serosal, final chamber concentration) was used at the end of all experiments to confirm tissue viability. The radioactivity in each sample was counted using a liquid scintillation counter (1212 Rackbeta, Wallac, Gaithersburg, MD). Mucosal-to-serosal ($J_{ms}$), serosal-to-mucosal ($J_{sm}$), and net ($J_{net}$) fluxes were calculated. A mean phloridzin flux was calculated from the two fluxes measured, and this value was used in further calculations.

In the kinetic studies, aliquots of 3-OMG were added to both mucosal and serosal chambers every 5 min, resulting in final concentrations of 2, 4, 8, 16, 32, and 64 mM. Maximal $J_{ms}$ ($J_{ms,max}$) at each dosage was recorded. A double reciprocal graph of data, with 1/[3-OMG] as the x-axis and 1/$ΔI_{sc,max}$ as the y-axis, was drawn. $K_m$ and $ΔI_{sc,max}$, which reflects transporter $V_{max}$, were determined using the x- and y-intercepts. Although Ussing chamber measurements reflect transepithelial glucose transport kinetics, SGLT-1 activity is critical for this transport, as confirmed by the observed reduction after the addition of phloridzin, and hence the values are referred to as SGLT-1 $V_{max}$ and $K_m$.

Food intake. The food intake of 12 rats, kept in pairs, was recorded over 3 days, and the mean food consumption per rat was calculated for each 24-h cycle and the 6-h period between CT3 and CT9. Percentage of total daily intake eaten between CT3 and CT9 was calculated.

Northern blotting analysis. After rats were killed by CO₂ inhalation, the first 5 cm of proximal small intestine, beginning 2 cm distal to pylorus, were removed. The first 2 cm were frozen in isopentane and kept in a −80°C freezer for use in the in situ hybridization studies. The distal 3 cm were flushed with cold phosphate-buffered saline and everted, and the mucosa was scraped. Mucosa was rapidly frozen in liquid nitrogen, and RNA was later extracted as previously described (5). Equal amounts (20 µg) of RNA were electrophoresed on 1% formaldehyde-agarose gels. RNA integrity and quantity were confirmed by ethidium bromide staining. RNA was transferred to Hybond-N filters (Amersham) and hybridized to a [32P]-labeled rat SGLT-1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA as previously described (20). Blots were washed twice in 2× SSC, 0.1% SDS (1× SSC is 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) for

CT0
↓

Light period
↓

CT12
Dark period
↓

CT24

Fig. 1. The light-dark cycle of the rats. Arrows indicate the circadian times (CT, in hours) at which experiments were carried out.
Table 1. Diurnal variation in rat jejunal 3-OMG transport

<table>
<thead>
<tr>
<th>Time</th>
<th>Baseline $I_{ac}$, $\mu$A/cm$^2$</th>
<th>Baseline $R$, $\Omega$ cm$^2$</th>
<th>$\Delta$amo, $\mu$A/cm$^2$</th>
<th>3-OMG Fluxes, $\mu$mol·cm$^{-2}$·h$^{-1}$</th>
<th>Post-Phloridzin 3-OMG Fluxes, $\mu$mol·cm$^{-2}$·h$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$J_{m-s}$</td>
<td>$J_{s-m}$</td>
</tr>
<tr>
<td>CT3</td>
<td>72.0 ± 4.9</td>
<td>82.4 ± 3.6</td>
<td>84.4 ± 7.2</td>
<td>2.83 ± 0.16</td>
<td>1.37 ± 0.30</td>
</tr>
<tr>
<td>CT9</td>
<td>78.1 ± 7.8†</td>
<td>66.8 ± 2.7†</td>
<td>139.8 ± 11.0†</td>
<td>4.38 ± 0.24‡</td>
<td>1.52 ± 0.19</td>
</tr>
<tr>
<td>CT15</td>
<td>58.4 ± 4.2</td>
<td>80.7 ± 4.5</td>
<td>119.0 ± 11.9</td>
<td>2.89 ± 0.34</td>
<td>0.96 ± 0.15</td>
</tr>
<tr>
<td>CT21</td>
<td>47.2 ± 4.8</td>
<td>79.9 ± 4.2</td>
<td>116.7 ± 8.5†</td>
<td>3.01 ± 0.26</td>
<td>0.87 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 10$–12 for short-circuit current ($I_{ac}$), tissue resistance ($R$), and change in $I_{ac}$ ($\Delta$amo); 5–6 for 3-O-methylglucose (3-OMG) fluxes; and 4–5 for post-phloridzin fluxes, $J_{m-s}$, mucosal-to-serosal flux; $J_{s-m}$, serosal-to-mucosal flux; $J_{net}$, net flux; CT, circadian time (in hours). $*P < 0.05$ compared with CT21; †$P < 0.05$ compared with CT3; ‡$P < 0.05$ compared with CT3, CT15, and CT21 by ANOVA and post hoc Tukey test.

RESULTS

Diurnal variation in baseline $I_{ac}$ and $R$. Baseline $I_{ac}$ were higher during the light cycle (CT3 and CT9) than during darkness, reaching a significant difference when CT3 was compared with CT21 (Table 1). Tissue resistance was significantly lower at CT9, when SGLT-1 activity was at its peak, compared with CT3, when SGLT-1 was least active.

Diurnal variation in 3-OMG-induced $\Delta$amo and fluxes. At CT9, before the onset of darkness and the start of feeding, 3-OMG-induced $\Delta$amo was significantly greater than at CT3. $J_{m-s}$ was significantly higher at CT9 compared with all other time points. 3-OMG $J_{net}$, however, was significantly higher at CT9 only compared with CT3, consistent with the significant increase in 3-OMG-induced $\Delta$amo. There were no significant differences in $J_{s-m}$ among the four time points (Table 1).

Effect of phloridzin on 3-OMG fluxes. Phloridzin, a specific inhibitor of SGLT-1 (7), abolished the increase in $J_{m-s}$ and $J_{net}$ observed at CT9. Fluxes in the presence of phloridzin were not significantly different among the four time points (Fig. 2).

Diurnal variation in $V_{max}$. Kinetic studies demonstrated that the increase in 3-OMG flux was caused by an increase in SGLT-1 $V_{max}$ and not $K_m$. At CT9, when the 3-OMG flux was at its maximum, $V_{max}$ had increased by a factor of 3. There was no significant

10 min at room temperature, once in 1× SSC, 0.1% SDS for 15 min at 65°C, and twice in 0.1% SDS for 20 min at 65°C and exposed on Kodak XAR-5 film at −80°C. Densitometry was performed using NIH software. SGLT-1 hybridization signals were standardized with GAPDH signals before comparisons. Four rats were studied per time point.

In situ hybridization. Digoxigenin-labeled sense and antisense probes for SGLT-1 were synthesized from linearized expression plasmids containing 2.5 kb of the SGLT-1 sequence and alkali-hydrated to an average length of 500 bp. In situ hybridization was performed on cryosections (15 μm) of fresh frozen tissue from the proximal small intestine (1–2 cm from the pylorus) as we have previously described (2). The hybridization buffer consisted of 50% formamide, 5× SSC, 2% blocking reagent (Roche, Indianapolis, IN), 0.02% SDS, and 0.1% N-lauroylsarcosine. The probe concentration was ∼100 ng/ml. Sections were immersed in slide mailers in hybridization solution and hybridized at 68°C for 66 h. This step inactivates the endogenous alkaline phosphatases. Sections were washed three times in 2× SSC and for 2 × 30 min in 0.2× SSC at 68°C. The hybridized digoxigenin-labeled probes were visualized with alkaline phosphatase-conjugated anti-digoxigenin Fab fragments (1:5,000, Roche) and BCIP/NBT substrate (Kirkegard and Perry Laboratories). Sections were then rinsed in (mM) 100 Tris, 150 NaCl, 25 EDTA, pH 9.5, and coverslipped with glycerol gelatin medium (Sigma).

Pictures were taken with a digital spot camera attached to a Nikon E600 microscope and imported into Adobe Photoshop (Adobe Systems, Seattle, WA). The exposure settings and sharpness enhancement were identical for each picture.

Statistical analysis. Data are presented as means ± SE. Probability of $P < 0.05$ was taken as significant. $P$ values were estimated using ANOVA with post hoc Tukey intergroup comparison. Computations were performed using a commercially available statistical package (Statistica, version 4.3, StatSoft, Tulsa, OK).

Effect of phloridzin on diurmiocity of 3-O-methylglucose (3-OMG) fluxes in proximal small intestine. Pre- and postphloridzin net sugar fluxes ($J_{net}$) for the 4 time points studied are shown. The white boxes represent prephloridzin, and the gray boxes represent postphloridzin 3-OMG fluxes. At CT9, there is a significant increase in $J_{net}$ observed at CT9. Fluxes in the presence of phloridzin were not significantly different among the four time points (Fig. 2).
Table 2. Diurnal variation in transepithelial glucose transport kinetics

<table>
<thead>
<tr>
<th>Time</th>
<th>$K_m$, mM</th>
<th>$\Delta I_{sc,max}$, $\mu A/cm^2$</th>
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<tbody>
<tr>
<td>CT3</td>
<td>27.4 ± 5.7</td>
<td>81.6 ± 11.9</td>
</tr>
<tr>
<td>CT9</td>
<td>45.2 ± 10.6</td>
<td>258.9 ± 35.1*</td>
</tr>
<tr>
<td>CT15</td>
<td>28.0 ± 6.2</td>
<td>172.7 ± 35.0</td>
</tr>
<tr>
<td>CT21</td>
<td>18.6 ± 5.8</td>
<td>97.8 ± 20.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6–8 tissues at each time point. Kinetic studies were performed in the Ussing chamber. Transporter affinity (Michaelis constant, $K_m$) and transporter maximal velocity ($\Delta I_{sc,max}$, $V_{max}$) were calculated as described in MATERIALS AND METHODS. There were no significant changes in $K_m$ among the 4 time points, but $\Delta I_{sc,max}(V_{max})$ was significantly higher at CT9 compared with CT3. *P < 0.05 by ANOVA and post hoc Tukey test.

Food intake. The mean daily food intake of each rat was 17.1 ± 0.8 g. Between CT3 and CT9, when maximal increase in 3-OMG flux occurred, rats consumed only 6.6 ± 1.9% (2.4 ± 0.7 g) of their total daily intake. Diurnicity in rat food intake has been documented previously (12).

SGLT-1 mRNA analysis. To confirm that the SGLT-1 expression rhythm was present in the rats used in the in situ hybridization studies, RNA extracted from adjacent distal mucosa was analyzed by Northern blotting. As shown in Fig. 4, and in accord with our previous study (23), SGLT-1 mRNA levels were increased at CT9 and CT15, compared with the level at CT3. The increased mRNA levels in mucosal scrapings at CT9 and CT15 could have been because of increased transcription within a given subset of villus enterocytes or an increased fraction of villus cells transcribing SGLT-1. To distinguish between these possibilities, the villus distribution of SGLT-1 mRNA was examined using in situ hybridization.

Hybridization with antisense probe resulted in labeling of the enterocyte layer in intestinal villi, whereas hybridization with sense probe produced no labeling (Fig. 5A). In Fig. 5, representative results are shown for the enterocyte labeling observed in small intestinal sections collected at CT3, CT9, CT15, and CT21. Labeling commences in cells as they exit the crypt, in agreement with the observation of others (4, 20). The labeling intensity was increased in villi collected at CT9 and CT15 compared with those collected at the other times. A higher-magnification view of labeled villi cut approximately in the vertical plane is shown in Fig. 5B. The single layer of enterocytes visible in these villi allows direct comparison among the four time points. The enterocytes on CT9 and CT15 tissues are

Fig. 3. Circadian rhythmicity of maximal transepithelial glucose transport change in short-circuit current ($\Delta I_{sc,max}$; reflecting maximal transporter velocity ($V_{max}$)). Kinetic studies were performed in the Ussing chamber at the stated time points, and SGLT-1 $\Delta I_{sc,max}$ ($V_{max}$) and transporter affinity (Michaelis constant, $K_m$) were calculated as described in MATERIALS AND METHODS. At CT9, when the maximal 3-OMG flux was observed, SGLT-1 $\Delta I_{sc,max}$ ($V_{max}$) was significantly higher than at CT3, when the 3-OMG flux was at its lowest (*P < 0.05 $\Delta I_{sc,max}$ at CT9 vs. CT3, ANOVA and post hoc Tukey test; n = 6–8 tissues).

Fig. 4. Temporal pattern of SGLT-1 mRNA expression in proximal small intestinal mucosa. A: Northern blot analysis of RNA extracted from the jejunal mucosa of rats killed at the indicated times was performed as described in MATERIALS AND METHODS. Message levels were quantified with scanning densitometry and indexed to the intensity obtained for CT3. The upper hybridization band (arrow) was used for comparisons, but both bands exhibit parallel changes in intensity ($P < 0.05$ compared with CT3, ANOVA and post hoc Tukey test; n = 4 tissues).

Fig. 5. Representative results are shown for the enterocyte labeling obtained in small intestinal sections collected at CT3, CT9, CT15, and CT21. Labeling commences in cells as they exit the crypt, in agreement with the observation of others (4, 20). The labeling intensity was increased in villi collected at CT9 and CT15 compared with those collected at the other times. A higher-magnification view of labeled villi cut approximately in the vertical plane is shown in Fig. 5B. The single layer of enterocytes visible in these villi allows direct comparison among the four time points. The enterocytes on CT9 and CT15 tissues are
more strongly labeled than enterocytes on CT3 and CT21 tissues, suggesting a higher expression of SGLT-1 message. This apparent qualitative difference was observed in three independent experiments, although no attempt was made to quantify it. It should be noted that no SGLT-1 signal was observed in crypts in any sections examined (not shown). Thus our results indicate that the increased expression of SGLT-1 at CT9 and CT15 is caused by increased transcription in all the cells normally expressing SGLT-1.

**DISCUSSION**

Our results show periodicity in intestinal sugar uptake and SGLT-1 function, with the rise to maximal activity occurring just before darkness and the nocturnal increase in feeding. We used phloridzin-inhibitable 3-OMG flux as an index of SGLT-1 activity. 3-OMG is a glucose analog that is transported by SGLT-1 but not metabolized by the enterocyte (19), thus allowing the study of transport function in the absence of confounding alterations in enterocyte metabolism. The abolition of the diurnal increase in sugar uptake by phloridzin provides strong evidence that this glucose cotransporter is responsible for the observed rhythmicity in intestinal sugar absorption. The data show, for the first time, that the changes in SGLT-1 mRNA and protein previously reported (6, 23) are reflected in the physiologically important transporter function. Our kinetic studies showed that the significant increase in $V_{\text{max}}$ could account for the observed increase in sugar flux. Although the flux $K_m$ did show modest temporal changes, we do not believe that cotransporter affinity contributes to the periodicity in sugar fluxes for two reasons. First, the changes did not achieve statistical significance. Second, the cotransporter affinity tended to decrease when the fluxes were highest. Previous reports of diurnicity in SGLT-1 transporter protein, as observed on Western blots, further support this finding (23). Other groups have also found periodicity in other intestinal enzymatic functions, such as sucrase activ-
ity, to be caused by changes in $V_{\text{max}}$, with no changes in $K_{\text{m}}$ (8, 18).

Diet and the presence of food in the intestinal lumen affect intestinal sugar absorptive capacity. However, the animals in our study consumed <10% of their daily total during the interval when the maximum change in SGLT-1 activity was observed (from CT3 to CT9). Thus the observed changes in 3-OMG flux and SGLT-1 $V_{\text{max}}$ are not brought about by the presence of food in the intestinal lumen per se but occur in anticipation of the bulk of the feeding that occurs early in the dark phase. Presence of anticipatory rhythmicity in intestinal digestive and absorptive functions has been reported before and is supported by the observation that the rhythms persist during starvation (15, 25). The timing of these changes is consistent with our previous work (23), which showed maximal SGLT-1 protein at the CT9 time point (denoted as 1600 h in that study). In addition, other groups have shown the onset of maximal glucose uptake to coincide with the beginning of the feeding time (15, 30).

Our studies have confirmed diurnicity in SGLT-1 mRNA expression. The experiments showed the diurnal peak in mRNA expression and function to coincide, but the design was not sufficiently refined to document any lag time that may exist between the message and the function peaks. We looked at SGLT-1 mRNA localization using in situ hybridization, where studies revealed more intense labeling in villi collected in the afternoon and evening (CT9 and CT15) than in those collected in the morning. It should be noted that the presence of or an increase in the mRNA levels does not necessarily indicate the concurrent presence of SGLT-1 protein or activity in the enterocyte but is an indicator of the cell’s commitment to express the transporter. This hybridization pattern demonstrates that mature enterocytes, in the upper third of the intestinal villus, are able to alter the amount of mRNA expressed by the cell on a daily basis. This observation is contrary to what has been previously hypothesized. Dietary factors have long been known to regulate intestinal digestive function in rodents (8) and humans (24). More recently, Ferraris et al. (9–11) and others (22) have confirmed that SGLT-1 transcription, translation, and activity are modified by diet. These investigators proposed a model in which dietary-induced changes take 3–5 days to occur, because only enterocytes exiting the crypt are able to alter SGLT-1 expression and replace the old cells with the previous SGLT-1 expression level along the villus. Other groups have reported acute increases in SGLT-1 activity induced by luminal glucose, although changes in SGLT-1 mRNA expression were not studied (28). However, because sampling in these studies was limited to a single segment of the day, diurnal changes were not detected. Our studies, on the other hand, clearly demonstrate rapid changes in SGLT-1 mRNA expression and function in mature enterocytes. It is likely that there are two distinct and separate pathways regulating SGLT-1 expression and function in intestinal epithelial cells. One pathway utilizes gut luminal signals to induce changes, whereas the other is a daily anticipatory mechanism preparing the intestine for an expected increase in nutrients before exposure to the luminal contents. Two sets of data support this hypothesis. First, isolated loops of rat jejunum show the same circadian rhythm in disaccharidase activity as unligated jejunum, but the overall enzyme activities were not inducible by a carbohydrate-rich diet as was found in the unligated jejunum (27). Second, the circadian changes in intestinal enzyme activities were shown to be independent of the changes induced by dietary protein or carbohydrate (26). Intermediary signals involved in these two distinct pathways are not yet identified. Our earlier work (23) implicates hepatocyte nuclear factor-1 (HNF-1) in regulating diurnal periodicity of SGLT-1 transcription. Whether this factor is involved in dietary regulation of SGLT-1 expression and what signals lie upstream from this nuclear transcription factor remain to be determined.

Intestinal sucrase, lactase, and maltase activities also have circadian periodicity phased to the feeding pattern. Similar to glucose absorption, the rhythm of these enzymes is anticipatory (29). Moreover, this rhythm is independent of the use of the intestine (31). These observations have led investigators to propose the existence of a neuroendocrine mechanism to regulate genes involved in intestinal carbohydrate digestion and absorption (25, 31). Of equal importance, a significant body of work has shown that the insulin level in rats undergoes circadian variation with maximal plasma concentrations late in the afternoon, just before the peak period of food intake (1, 3). Moreover, the nighttime exaggeration of glucose and insulin responses during an oral glucose tolerance test in rats demonstrates that insulin sensitivity itself undergoes a circadian variation (16). Persistence of the periodicity in plasma insulin levels in fasted animals (1), or those given a constant glucose infusion (3), suggests that the serum glucose level is not driving this rhythm. These results suggest the presence of an entrainable neuroendocrine mechanism that not only regulates intestinal sugar absorption but also prepares the whole organism, in part by adjusting the plasma insulin levels, for the expected food intake. The finding that truncal vagotomy abolishes rhythmicity in food intake, insulin secretion, and glucose tolerance in normal rats indicates that the vagus nerve plays an important role in these carbohydrate regulatory mechanisms (21). Because intermittent parenteral nutrition maintains diurnicity in intestinal disaccharidases and glucose absorption, we propose that the hypothesized regulatory neuroendocrine axis has an endocrine/nutritional afferent limb that regulates the efferent output of the vagus nerves. The specific molecular events underlying these two limbs remain to be elucidated.

At CT9, when SGLT-1 was most active, tissue resistance was significantly lower compared with CT3, when SGLT-1 was least active. Although this is an interesting observation, its basis and significance are presently unclear. During the light cycle, the baseline $I_{sc}$ was higher than during the dark cycle. This sug-
suggests the presence of a diurnal change in baseline electrolyte fluxes, including Cl\(^-\), Na\(^+\), and/or HCO\(_3\)-. Although we have not yet examined the possible mechanisms underlying these changes, the phenomenon may be important and warrants further study.

In summary, we have shown that the periodicity in intestinal sugar absorption is caused by changes in SGLT-1 induction and function. These functional changes are anticipatory and brought about by changes in the SGLT-1 \(V_{\text{max}}\). Rhythmicity in SGLT-1 mRNA expression was confirmed and shown to be caused by increased mRNA expression by all enterocytes that normally express SGLT-1 rather than a specific subset of villus enterocytes. We propose the presence of an anticipatory pathway regulating intestinal SGLT-1 expression and function. This pathway, unlike others previously described, results in acute and rapid changes in the SGLT-1 induction in mature, as well as immature, enterocytes along the villus axis. Further work on mechanisms involved in this anticipatory control of intestinal absorption and systemic metabolism of sugars could reveal new therapeutic avenues in dealing with intestinal malabsorption syndromes as well as diabetes mellitus.

The authors gratefully acknowledge the assistance of Lihua Zhang in performing the Northern blot analysis. This study was supported by National Institutes of Health Grants DK-47326 (S. W. Ashley), HD-31215 (L. L. Levitsky), and DK-54399 (D. B. Rhoads) and by March of Dimes Grant 1-FT99–221 (D. B. Rhoads).

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