The Diurnal Rhythm of the Intestinal Transporters SGLT1 and PEPT1 Is Regulated by the Feeding Conditions in Rats

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ABSTRACT The intestinal Na\(^+\)/glucose cotransporter 1 (SGLT1) and H\(^+\)/peptide cotransporter 1 (PEPT1) play important roles in the absorption of carbohydrate and protein. Although they exhibit a diurnal rhythm in their expression and function, the factors responsible for this are unclear. In the present study, we examined the effects of various feeding conditions on the diurnal rhythm of intestinal SGLT1 and PEPT1. Rats were divided into 1 of 4 groups: group 1 was fed, group 2 was food deprived for 1–4 d, group 3 was food deprived for 4 d and then refed for 1 or 2 d, and group 4 was fed during the daytime (0900–1500 h) for 10 d. In fed rats, the SGLT1 protein level was significantly higher at 2000 h than at 0800 h. However, in rats deprived of food for 2–4 d, protein levels did not differ between 0800 and 2000 h. In contrast, the SGLT1 messenger RNA (mRNA) level was significantly higher at 2000 h than at 0800 h in rats deprived of food for 4 d. Refeeding for 2 d after 4 d of food deprivation returned the diurnal variation in SGLT1 and PEPT1 protein expressions to normal. Consuming food during the daytime only shifted the peaks of SGLT1 and PEPT1 mRNAs and protein expressions from the dark phase to the light phase. These findings suggest that food intake, rather than the light cycle, greatly affects the diurnal rhythm of SGLT1 and PEPT1 expressions.


KEY WORDS: \(\bullet\) circadian rhythm \(\bullet\) Na\(^+\)/glucose cotransporter \(\bullet\) H\(^+\)/peptide cotransporter \(\bullet\) food deprivation \(\bullet\) refeeding

The small intestinal epithelial cells have a major role in the digestion and the absorption of dietary carbohydrates and proteins, which are mainly absorbed as glucose and as di- and tripeptides, respectively. The transport of glucose and small peptides through the brush border membranes of intestinal epithelial cells is mediated by Na\(^+\)/glucose cotransporter 1 (SGLT1)\(^1\) (1) and H\(^+\)/peptide cotransporter 1 (PEPT1)\(^2\), respectively, and therefore these 2 transporters play important nutritional and physiological roles in the small intestine.

The functional activities of these 2 transporters are flexible to be able to adapt to various nutritional and pathophysiological conditions, such as dietary conditions, development, and diseased states (3,4). In addition, the transport activities and the expression of SGLT1 (5–9) and PEPT1 (10,11) showed a diurnal rhythm. For example, in rats that eat ad libitum, glucose uptake is low in the daytime and high at night (5,6). In accordance with the functional change of glucose transport, SGLT1 messenger RNA (mRNA) and protein levels were also increased near the onset of the dark period (7–9). These diurnal rhythms are consistent with a nocturnal dietary load, because rats show a nocturnal feeding behavior.

Recently, Tavakkolizadeh et al. (9) proposed that there are 2 distinct and separate pathways regulating SGLT1 expression and function in the intestinal epithelial cells. One pathway utilizes gut luminal signals to induce the diurnal variation, and the other is a daily anticipatory mechanism preparing the intestine for an expected increase in nutrients before exposure to the luminal contents. These 2 factors may be involved in the regulation of other nutrient transporters and may be greatly affected by feeding conditions. Thus, in the present study, we assessed how food intake can modulate the diurnal rhythm of intestinal SGLT1 and PEPT1 expressions under various feeding conditions, i.e., food deprivation, refeeding, and scheduled daytime feeding. We already demonstrated that the diurnal rhythm of intestinal PEPT1 protein expression is disrupted by food deprivation (11).

MATERIALS AND METHODS

Animals. Animal studies were performed in accordance with the Guidelines for Animal Experiments of Kyoto University. Male Wistar rats, weighing 160 g, were obtained from Japan SLC. Rats were housed in stainless-steel cages in a temperature-controlled room at 22 ± 0.5°C, with a 12-h light/dark cycle (0800–2000 h).

Experimental design. Rats were allowed free access to water and to a standard laboratory diet for 7 d and then were randomly distributed into 1 of 4 groups: group 1 was fed, group 2 was food deprived for 1–4 d, group 3 was food deprived for 4 d and then refed for 1 or 2 d, and group 4 was fed only during the daytime (0900–1500 h) for 10 d.

\(^1\) Supported in part by the 21st Century COE Program “Knowledge Information Infrastructure for Genome Science” and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.
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E-mail: inui@kuhp.kyoto-u.ac.jp.
\(^3\) Abbreviations used: GAPDH, glyceraldehydes-3-phosphate dehydrogenase; mRNA, messenger RNA; PEPT1, H\(^+\)/peptide cotransporter 1; SCN, suprachiasmatic nucleus; SGLT1, Na\(^+\)/glucose cotransporter 1.
After each treatment, rats were killed at specified times and a 10-cm length of duodenum was removed. Then, the intestinal mucosa was scraped and rapidly frozen in liquid nitrogen for later preparation of brush border membranes and total RNA.

**Western and Northern blot analyses.** Preparation of brush border membranes and Western blot analysis were performed as described previously (10–14). Total RNA was isolated by TRIzol reagents (1 mL/100 mg tissue) (Invitrogen Japan KK) according to the manufacturer’s directions. Northern blot analysis was carried out as described (10,11,14).

**Data analyses.** Data are expressed as means ± SEM and were analyzed with StatView (version 5.0, SAS Institute). Body and intestinal weight data were subjected to two-way ANOVA (duration of food deprivation and time of day) (Fig. 1). When significant effects were found (P < 0.05), group differences were analyzed further by the post-hoc Fisher’s protected least squares difference multiple comparison test. SGLT1 protein and mRNA expression levels were compared in fed and 4-d food-deprived rats by unpaired t tests. SGLT1 protein and mRNA expression levels between 0800 and 2000 h of food deprived or refed rats were also compared by unpaired t tests.

**RESULTS**

**Body and intestinal weights.** For body and intestinal weights, there was no interaction between the duration of food deprivation and time of day (Table 1). Both were decreased by the duration of food deprivation (Table 1, P < 0.05); weights in 1- and 2-d refed rats were greater than those in 4-d food-deprived rats (P < 0.05).

**Expression of intestinal SGLT1 protein and mRNA levels in 4-d food-deprived rats.** Protein and mRNA levels of SGLT1 were decreased in 4-d food-deprived rats at both 0800 and 2000 h, compared with fed rats (Fig. 1, P < 0.05). Expression levels for villin (cytoskeletal protein) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were also decreased in 4-d food-deprived rats at both 0800 h (ratio to fed, villin: 1.00 ± 0.06 vs. 0.49 ± 0.058, GAPDH: 1.00 ± 0.02 vs. 0.69 ± 0.01, n = 6, P < 0.05) and 2000 h (ratio to fed, villin: 1.00 ± 0.03 vs. 0.21 ± 0.019, GAPDH: 1.00 ± 0.128 vs. 0.73 ± 0.07, n = 6, P < 0.05).

**Effect of food deprivation on the diurnal rhythm of intestinal SGLT1 protein and mRNA levels.** In the fed and 1-d food-deprived rats, the level of SGLT1 protein was higher at 2000 h than at 0800 h (Fig. 2, P < 0.05), whereas that of villin was not different (ratio to 0800 h, fed: 1.00 ± 0.12 vs. 0.97 ± 0.05; 1-d food-deprived: 1.00 ± 0.10 vs. 0.87 ± 0.12, n = 6). However, after food deprivation for 2–4 d, SGLT1 protein expression did not differ between 0800 and 2000 h (Fig. 2). In contrast to protein expression, the SGLT1 mRNA level in 4-d food-deprived rats was higher at 2000 h than at 0800 h (ratio to 0800 h, 1.00 ± 0.059 vs. 2.21 ± 0.24, n = 6, P < 0.05), although that of GAPDH was not (ratio to 0800 h, 1.00 ± 0.13 vs. 0.95 ± 0.14, n = 6).

**Effect of refeeding on diurnal rhythm of intestinal transporters.** SGLT1 protein levels did not differ between 0800 and 2000 h after 1-d refeeding (ratio to 0800 h, 1.00 ± 0.08 vs. 1.01 ± 0.48, n = 6). But, after 2-d refeeding, a diurnal variation of SGLT1 protein levels was observed between 0800 and 2000 h (1.00 ± 0.07 vs. 1.20 ± 0.06, n = 6, P < 0.05). Similarly, PEPT1 protein levels were not different between 0800 and 2000 h after 1-d refeeding (1.00 ± 0.09 vs. 0.99 ± 0.15, n = 6), but were different after 2-d refeeding (1.00 ± 0.15 vs. 1.36 ± 0.06, n = 6, P < 0.05).

![Table 1](https://academic.oup.com/jn/article-abstract/134/9/2211/4688755/Downloaded-from)”

**TABLE 1**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Body weight</th>
<th>Small intestinal weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0800 h</td>
<td>2000 h</td>
</tr>
<tr>
<td></td>
<td>g</td>
<td>mg/10 cm intestinal segment</td>
</tr>
<tr>
<td>Fed</td>
<td>225.0 ± 3.4a</td>
<td>218.2 ± 5.2a</td>
</tr>
<tr>
<td>Food deprived, d</td>
<td>196.7 ± 1.8c</td>
<td>198.8 ± 3.2c</td>
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<tr>
<td></td>
<td>179.2 ± 2.4d</td>
<td>179.2 ± 1.8d</td>
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<tr>
<td></td>
<td>166.5 ± 2.1e</td>
<td>169.7 ± 3.2e</td>
</tr>
<tr>
<td></td>
<td>157.5 ± 3.5f</td>
<td>152.2 ± 3.9f</td>
</tr>
<tr>
<td>Refed, d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>193.3 ± 1.8c</td>
<td>198.3 ± 3.4c</td>
</tr>
<tr>
<td></td>
<td>204.2 ± 1.8b</td>
<td>207.2 ± 1.3b</td>
</tr>
</tbody>
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* Values are means ± SEM, n = 6. Values in a column without a common letter differ, P < 0.05.
Effect of daytime feeding on diurnal rhythm of SGLT1 and PEPT1.

After the rats consumed food ad libitum (normal feeding: N) or were fed from 0900 to 1500 h (daytime feeding: D) for 10 d, SGLT1 and PEPT1 expression levels were determined at different times during a 24-h period (0400, 0800, 1200, 1600, 2000, 2400 h) on d 11. The peak of SGLT1 protein expression in rats fed during the daytime shifted to 0400 h from 2000 h in rats that consumed food ad libitum (Fig. 3B). The peak of PEPT1 protein expression in rats fed from 0900 to 1500 h was also changed to 1200 h (Fig. 3C).

DISCUSSION

The diurnal rhythm of glucose transport was thought to persist during starvation (6), but the present study demonstrated that the diurnal rhythm of apical SGLT1 protein expression is abolished in rats deprived of food for 4 d. Similarly, the diurnal rhythm of PEPT1 protein expression was also abolished by 4 d of food deprivation (11). Thus, food intake is an important factor for setting the diurnal rhythm of intestinal nutritional transporters. Interestingly, the diurnal rhythm of the SGLT1 mRNA level was maintained in the 4-d food-deprived rats, suggesting that posttranslational regulation may be involved in the disruption of the diurnal rhythm of apical SGLT1 protein expression. Kipp et al. (15) recently demonstrated that most of the cellular SGLT1 is present in an intracellular pool and that this pool may be involved in regulating the abundance of SGLT1 at the apical cell surface. Because we measured only SGLT1 protein expression at brush border membranes, a change in the cellular distribution of SGLT1 protein between the cytoplasmic pool and apical membranes may be responsible for the effect of food deprivation.

FIGURE 2 Effect of food deprivation on the diurnal rhythm of rat intestinal SGLT1 protein expression. A: Western blot analyses were carried by using intestinal brush border membranes isolated at 0800 h and 2000 h from fed (F) and 1–4 d food-deprived (D1–D4) rats. Representative data are shown. B: SGLT1 protein abundances were expressed relative to the value at 0800 h. Values are means ± SEM, n = 6. *Different from 0800, P < 0.05.

FIGURE 3 Effect of daytime feeding on diurnal rhythm of intestinal SGLT1 (A, B) and PEPT1 (A, C) protein expressions. A: Rats were allowed normal feeding (ad libitum) (N) or daytime feeding from 0900 to 1500 h (D) for 10 d, and intestinal brush border membranes were prepared on d 11. Western blot analyses were carried out with these samples. Representative data are shown. B and C: SGLT1 and PEPT1 protein abundances were expressed as relative to the value at 0400 h. Values are means ± SEM, n = 5.
The importance of food intake was also confirmed by refeding and daytime feeding experiments. In these situations, the diurnal rhythm of SGLT1 and PEPT1 was synchronized with feeding conditions. The changes in SGLT1 expression in rats fed from 0900 to 1500 h were consistent with the glucose transport activity reported by Stevenson et al. (16). These findings suggest that food intake, rather than the light cycle, is responsible for setting the diurnal rhythm of intestinal SGLT1 and PEPT1. This is in contrast to the hypothesis that the circadian pacemaker in the suprachiasmatic nucleus (SCN) of the mammalian hypothalamus is primarily adjusted by light-induced phase shifts (17). Recently, Damiola et al. (18) examined the effect of daytime feeding on the peripheral clock genes (Per1, Per2, Per3, and Cry1) and demonstrated that daytime feeding completely reversed the phase of the oscillator in peripheral cells but had little effect on the central oscillator in the SCN. This finding suggests that the food-induced phase resetting of peripheral clocks is not controlled solely by the SCN pacemaker. Although it remains to be determined whether the peripheral clock genes are involved in the expression of SGLT1 and PEPT1, they may directly or indirectly affect the rhythm of intestinal epithelial cells.

Although refeeding and daytime feeding modulated the diurnal rhythm of intestinal SGLT1 and PEPT1 expressions, the mechanisms involved may differ. In the refed rats, transcriptional regulation may not be involved, because the rhythm of SGLT1 and PEPT1 (11) mRNA expressions was retained during food deprivation. On the other hand, daytime feeding caused the peak of SGLT1 and PEPT1 mRNA expressions to shift from the dark phase to the light phase, suggesting that transcriptional regulation contributes to this effect. The changes in abundance of protein and mRNA of both transporters were not correlated, perhaps due to the differences in transcription rate, translation rate, and stability of mRNA and protein. All these findings suggest that food intake can modulate the diurnal rhythm of SGLT1 and PEPT1 expressions by multiple pathways. It was reported that luminal glucose concentration in the proximal small intestine varied with the time of day, namely, high in the nighttime and low in the daytime (19). This variation was similar to the diurnal rhythm of SGLT1 expression in our studies, suggesting that luminal concentration of glucose induced by food intake may affect the setting of the diurnal rhythm of SGLT1.

In conclusion, we demonstrated that the diurnal rhythms in the protein expression of SGLT1 and of PEPT1 in the intestine were altered by food-intake conditions in food-deprived and refed rats; posttranslational regulation may be responsible for the change in the diurnal rhythm of SGLT1 and PEPT1 expressions, whereas in rats fed during the daytime only, transcriptional regulation seems to be involved in the phase shift of both transporters. Overall, the findings suggest that food intake, rather than the light cycle, is responsible for setting the diurnal rhythm of intestinal SGLT1 and PEPT1 expressions.

LITERATURE CITED


