Circadian rhythms of intestinal sucrase and glucose transport: cued by time of feeding

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STEWART, NANCY R., AND JEFFREY S. FIERSTEIN. Circadian rhythms of intestinal sucrase and glucose transport: cued by time of feeding. Am. J. Physiol. 230(3): 731-735. 1976. In the rat, under constant illumination, the activities of the digestive enzyme sucrase and the absorptive transport system for glucose follow circadian rhythms on ad lib. and controlled feeding regimens. In response to controlled feeding, (1400-1800 h or 0200-0600 h, EST), both rhythms shift with time and the general level of activities are enhanced. Sucrase activity peaks before feeding and transport activity peaks during feeding. Feeding is a synchronizer for these digestive-enzymes activities; the maximum activity of a function may occur prior to as well as subsequent to the daily onset of the synchronizer. The rhythms of these functions results from previous days' feeding patterns.

digestion; absorption; enzyme activities; feeding schedules

THE CAPACITY OF THE MUCOSAL CELLS of rat intestine to absorb L-histidine and to hydrolyze maltose and naphthylamide (LNAase) in vitro and to absorb L-histidine and glucose in vivo has been reported to exhibit circadian rhythmicity (4, 6, 17). With normal light-dark cycle and ad lib. feeding, the highest activities for all 3 parameters measured in vitro were observed at night, as might be expected of a nocturnal animal that feeds mainly during the dark hours (8, 19). The time of day for maximal activities was shifted by changes in the feeding pattern, indicating that feeding is a synchronizer, that is, an environmental factor determining the temporal placement of these rhythms (7).

This paper reports changes in the activity patterns and the daily average levels of activity of the digestive enzyme sucrase and the absorptive transport system for glucose in response to an artificial feeding schedule. Special interest was paid to the temporal relationship of daily peak activity periods and the daily time of feeding, that is, the time of day of the synchronizer.

An abstract of this work has been published (18).

METHODS

In this study we were interested in observing two aspects of these intestinal functions: the existence of circadian rhythms and the effect of feeding schedule on these functions. We therefore attempted to provide an environment and daily routine that was as constant and uniform as possible for each of the five groups of animals that comprised the two ad libitum feeding experiments and three controlled feeding experiments. Two groups of rats were fed from 1400 to 1800 h and one group was fed from 0200 to 0600 h, 12 h out of phase. No change in the general vivarium environment or in daily routine was reported during the duration of the study. Constant lighting was employed to remove a conflicting environmental cue, that is, the light-dark cycle.

Experimental conditions. Male Sprague-Dawley rats, 100-125 g, were obtained from Perfection Breeders, Inc. The animals were housed individually in a locked, constant-environment suite (animal room with a preparation room) with free access to water. They were fed commercial rat chow (Purina laboratory chow no. 5001). All handling was done during the half-hour period prior to feeding or from 1330 to 1400 h, when food was available ad libitum. In controlled feeding experiments, food was removed from the cages during the 5-min period after the end of feeding. The animals were otherwise left undisturbed.

Upon arrival the rats were allowed a 3-day acclimatization period during which the light cycle used by the breeder was continued, i.e., light from 0600 to 2000, and feeding was ad libitum. This was followed by a 6-day experimental period during which the rats were either continued on the ad libitum feeding regimen (the ad lib group) or restricted in food access to a single 4-h period, 1400-1800 h or 0200-0600 h (the controlled groups). Constant lighting was used during the experimental period. Beginning on the morning of the 7th day, groups of three to five rats were sacrificed by cervical dislocation at specific times of day during the ensuing 24 h.

Procedures. The middle three-fourths of the small intestine was removed, rinsed with buffer, and everted. Three-centimeter portions from the proximal, middle, and distal areas were taken for transport studies. The mucosa was scraped from the remainder of the intestine for sucrase and protein assays.

3-O methylglucose or 3,5-methylid glucoside transport was assessed by a modification of the tissue uptake procedure of Crane and Mandelstam (2). The uptake of the sugar was determined in triplicate in each animal. Six tissue rings, approximately 3-5 mm in width, were cut from each intestinal segment. Two rings from each area were distributed to each of three flasks containing 5 ml of medium. The rings were incubated at 37°C and shaken at 180 oscillations/min. The incubation media was Krebs-Hanseleit bicarbonate buffer, pH 7, equili-
brated with 95% O₂-5% CO₂, containing cold sugar and tracer amounts of that sugar plus n-[1-³H(N)]mannitol. In experiments with 3-O-methylglucose, the sugar was present in a 5 mM concentration and incubation time was 30 min; when β-methyl-d-glucoside was used, the concentration was 10 mM and incubation time was 15 min.

3-O-methylglucose was obtained from Calbiochem and β-methyl-d-glucoside from Sigma. 3-O-methyl-[¹⁴C]d-glucose and β-[¹³C(N)]mannitol were obtained from New England Nuclear Corporation and β-methyl-[¹⁴C(U)]d-glucoside was obtained from Calatomic.

The mucosal scrapings were homogenized in 0.9% NaCl for 3 min in a Waring blender. Aliquots were taken for the immediate assay, or were frozen for later assay, of sucrase by the Dahlqvist method (3) as modified by Lloyd and Whelan (11) and for protein by the method of Lowry et al. (12) with bovine serum albumin used as a standard.

Data calculations and evaluation. Daily food intake was calculated as the weight of food pellets placed in the cage minus the weight of pellets removed from the cage 24 or 4 h later plus those pieces retrieved from below the cage. Scattered powdered chow or that mixed with urine was not collected.

The results of the transport studies are reported as the tissue-to-medium (T/M) ratio (mM sugar, tissue/mM sugar, medium) corrected for the mannitol space. A tissue water value of 80% of the tissue wet weight was used in these calculations. The mannitol space was calculated as the tissue ³H counts per minute/³H counts per minute per milliliter of medium. This space was determined for each transport sample and averaged 36% of the tissue volume. The correction for this volume was done by calculating the ¹⁴C counts per minute that would be in the volume of medium equal to the corresponding mannitol space and subtracting this value from the total ¹⁴C counts per minute found in the tissue prior to the calculations of the millimolar concentration of sugar in the tissue.

The specific activity of sucrase was calculated as micromoles sucrose hydrolyzed per minute per milligram of protein. These assays were done in duplicate or quadruplicate.

The data from the groups on each feeding regimen were pooled. Data are presented as mean ± SD or SE. A two-tailed t test was used to determine the levels of significance. The rhythmicity of the sucrase activity was similar in both ad lib groups and the 1400-1800-h controlled fed groups, but the overall magnitudes differed about 30%, necessitating normalization to the first time point. The variability in the 3-O-methylglucose transport activity between the two replicates was not sufficiently great to necessitate normalization of the data. Nonnormalized specific activities were used for the calculation of the average daily activities. The two 0500-h values were averaged and the mean was used.

RESULTS

Condition of animals. Change in body weight per day, body weight, and food intake of all groups of animals are shown in Fig. 1. In the ad lib group, food intake increased approximately 1 g/day and the body weights increased approximately 7 g/day throughout the 6 days of the experimental period. Similar food consumption and weight gain were observed on the 7th day for the remaining animals as well.

The body weight and daily weight gains were similar in the ad lib and the controlled rats prior to the experimental period. The controlled groups lost weight for the first 2 days of the experimental period. The loss in weight stopped on the 3rd day. From then to the end of the experimental period these rats increased their food intake by 0.75 g/day and had a weight gain of approximately 1.5 g/day. This increase in body weight continued through the 8th day as shown by the data from the remaining animals. In other experiments, animals have been maintained on the 1400-1800-h controlled feeding regimen for 6 days and have gained almost the same amount of weight. The animals have been successful in maintaining their body weight.

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FIG. 1. Daily body weights (upper graph), change in body weight/day (middle graph), and daily food intake (lower graph) of rats fed ad libitum or restricted to 4-h feedings at 0200-0600 h (rest 2-6) or 1400-1800 h (rest 14-18). Each value represents mean ± SD, n = 50, from day 3 through day 7; on day 8, n = 12 in ad lib group and 20 in both restricted groups.
CIRCADIAN RHYTHMS OF INTESTINAL TRANSPORT

The majority of the eating in the ad lib group appeared to be in the early morning hours, although some eating occurred throughout the 24-h period. Around-the-clock eating is also seen in rats fed ad libitum under conventional light-dark cycles (8, 19). When it was dark from 2000 to 0800 h, the majority of the eating occurred throughout the 12-h dark period and maximal activity occurred during the 6-h period between 2130 and 0330 h (8). Comparison of our observations with this work suggests that the exposure to constant light may have caused a slight shift in the peak eating time, away from the evening hours.

Upon sacrifice, smaller fat deposits were seen in the subcutaneous area, peritoneal cavity, and thymic mediastinal region in the controlled groups. Gross macroscopic examination of the intestinal tracts indicated an increase in the food storage capacity of the stomach in the controlled groups, but no difference in the general appearance or length of the small intestine between the groups. Carcasses of several animals from the 1400–1800-h controlled groups were examined further and all organs were found to be within normal size limits. Food material was found in the stomach and small intestine of the controlled rats at all times except 1–2 h before feeding and at all times in the ad lib animals. There were no significant differences in the weight of the mucosal scrapings per intestine between the two groups. The weight of the scrapings was 1.104 ± 116 mg (mean ± SD, n = 50) in the ad lib group and 1.044 ± 229 mg (mean ± SD, n = 50) in the 1400–1800-h controlled group.

Intestinal function. The protein content of the mucosal homogenates was uniform. No circadian rhythmicity of protein content could be detected and there was no significant difference between the two feeding regimens.

In other work we have studied animals at several times of day on the 6th, 8th, and 23rd days of controlled feeding. There was no significant difference between the activity values determined on these days and those on the 7th day. The rhythms appear to be established by the 6th day and are stable for at least 23 days.

The combined results of the experiments are shown in Fig. 2 (sucrase) and Fig. 3 (transport). In the ad lib group, both intestinal functions were higher during the early morning and lower during the early evening. Maximum sucrase activity was detected at 0700 h and minimum activity at 2100 h. Maximum transport was observed at 0900 h and minimum transport at 2100 h. The exposure of the animals to constant light presumably resulted in this drift of the peak activities away from the evening hours as previously observed in ad libitum feeding studies employing conventional light-dark cycles (5, 14). Thus, both the major eating period and the time of peak activities of these intestinal functions appear to be slightly shifted in the same direction, away from evening hours.

In the controlled group, fed between 1400 and 1800 h, the activity patterns were quite different from those in the ad lib group. Sucrase activity was higher during the late morning–early afternoon period, peaking at 1300 h, and was lower during the evening–early morning period, with a minimum at 0500 h. Transport activity was higher in the late afternoon–early evening period, peaking at 1700 h, and was lower during the morning, with a minimum at 0700 h.

In both groups the sucrase activity peaked prior to transport activity. However, the effect was accentuated in the controlled group. Moreover, in the controlled group, the sucrase rhythm actually anticipated the feeding time of day since maximal activity was observed at 1300 h, whereas feeding began at 1400 h.

When the feeding period was changed to 0200–0600 h, 12 h out of phase with the original feeding period, the rhythms shifted by 12 h. The rhythms are superimposable when presented in respect to the time of day of feeding and are thus independent of other environmental cues or factors related to nominal time.

Restriction of feeding also resulted in an enhanced average daily activity level for both functions. Sucrase specific activity rose from 0.064 ± 0.003 (mean ± SE) in the ad lib group to 0.076 ± 0.004 (mean ± SE) in the 1400–1800-h controlled group, P < 0.05. Transport T/M values increased from 2.53 ± 0.20 (mean ± SE) in the ad lib group to 3.55 ± 0.27 (mean ± SE) in the 1400–1800-h controlled group, P < 0.01. This rise in general activity is distinct from any rise due to modified diets in rate (1,
used the rhythms are directly and quite precisely related to the time of day of feeding and independent of other potential factors. It is also most noteworthy that the daily rise in sucrase activity anticipates the daily feeding period. These two observations stress the fact that, although the time of day of feeding synchronizes the rhythms of various digestive-absorbptive functions, the rhythms observed on any one day are a function of the feeding schedule of previous days rather than a direct response to the stimulus of food.

The cause of the general increase in activities in the controlled fed animals is unclear. Although they did not determine average daily levels, others (9, 10, 13) have reported increases in transport activity when rats were restricted in food intake.

The observations of the existence of circadian rhythmicity in activities of enzymes and transport systems and the nonuniform response of these rhythms to feeding bring into focus questions about feeding experiments in general. In experiments purporting to show dietary-induced changes in the level of enzymes and/or transport systems, it must first be shown that the changes are not merely the result of a shift in the rhythm induced by the feeding procedures used, whether they be forced feeding, changes in the time of feeding, pair feeding, or feeding a less acceptable diet that may be consumed by animals or humans in a self-induced altered feeding pattern. Obviously, the choice of sampling time should be made on the basis of a point in the diurnal rhythm and not on the basis of a time of day.

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REFERENCES


