Circadian Rhythm of Ornithine Decarboxylase Activity in Small Intestine of Fasted Rats (43449)

KAZUMA FUJIMOTO,* D. NEIL GRANGER,* LEONARD R. JOHNSON,† V. HUGH PRICE,* TOSHIIE SAKATA,‡ AND PATRICK TSO*,[1]

Department of Physiology, Louisiana State University Medical Center, Shreveport, Louisiana 71130; Department of Physiology,† University of Tennessee Medical Center, Memphis, Tennessee 38163; and Department of Internal Medicine I,‡ Oita Medical College, Oita 879-55, Japan*

Abstract. **The aim of this study was to determine whether the circadian changes in ornithine decarboxylase (ODC) activity of different segments of the small intestine were governed by factors other than food intake. First, the effects of fasting on mucosal ODC activity were examined. The results indicate that mucosal ODC activity in 24 hr and 48 hr fasted rats decreased significantly compared with *ad libitum*-fed rats. Second, the circadian rhythm of mucosal ODC activity was characterized by measuring mucosal ODC activity in fasted rats at four time points (09:00, 15:00, 21:00, and 03:00 hr; light period: 06:00–18:00 hr). The results from this study indicate that there is a detectable baseline ODC activity in different segments of fasting intestine. In duodenum, mucosal ODC activity was highest at 15:00 hr (light period), a time at which the rat was normally not eating. In jejunum and ileum, mucosal ODC activity increased between 21:00 and 03:00 hr (dark period). The observation that small intestine exhibits a distinct circadian rhythm of ODC activity in fasted rats suggests that not only food but also intrinsic factors can modulate physiologic oscillations in mucosal ODC activity.** [P.S.E.B.M. 1992, Vol 200]

Ornithine decarboxylase (ODC; EC 4.1.1.17) is a key rate-limiting enzyme in the formation of polyamines. In rats and other animals, the activity of ornithine decarboxylase undergoes circadian rhythm in brain (1), kidney (2), lymphoid tissues (3, 4), bone marrow (4), adrenal gland (5, 6), and testis (7). The circadian rhythm in ODC activity exhibited in these organs seems to be mediated by humoral factors, such as pituitary hormones (2–4, 6, 8).

ODC activity in the liver and gastrointestinal tract of rats exhibits a circadian rhythm (9, 10) that is significantly influenced by feeding (9, 10). The feeding-induced increase in ODC activity in intestinal mucosa appears to be mediated by luminal nutrients (10–15) and humoral factors (14, 15). The height and width of villi (16, 17), the number of cells undergoing mitosis, and DNA synthesis in crypt epithelia (18, 19) also exhibit circadian rhythms. Several functions of the small intestine also exhibit circadian variations, e.g., absorption of amino acids and glucose (16, 20) and disaccharidase activity (20–23).

Although food intake plays an important role in regulating the rhythm of the gastrointestinal tract, the contribution of other factors in modulating intestinal biorhythms remains undefined. For example, Saito *et al.* (21, 22) demonstrated that the disaccharidase activity in intestinal mucosa undergoes a circadian rhythm in fasted rats (21) and in isolated rat jejunal segments (22), indicating that the rhythmic changes in disaccharidase activity cannot be attributed entirely to food intake. The aim of this study was to determine whether mucosal ODC activity in different segments of small bowel exhibits a circadian rhythm and whether this biorhythm is influenced by factors other than food intake.

Materials and Methods

Animals. Male Sprague-Dawley rats (280–330 g) were used in this study. They were housed in wire-

[1] To whom requests for reprints should be addressed at Department of Physiology, LSU Medical Center, 1501 Kings Highway, Shreveport, LA 71130.

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bottomed cages placed in a room illuminated from 06:00 hr to 18:00 hr (12:12-hr light:dark cycle) and maintained at 21 ± 1°C. Rats were fed *ad libitum* on the light:dark schedule for 2 weeks before they were divided into the various experimental groups.

ODC Activity in Rat Small Intestine. *Experimental design.* Two series of studies were performed. In the first series (Series A), we studied the effects of fasting on ODC activity in intestinal mucosa. Four different groups of rats were used in this study: (i) normally fed; (ii) fasted 12 hr before ODC assay; (iii) fasted 24 hr before ODC assay; and (iv) fasted 48 hr before ODC assay. Water was available *ad libitum* to all groups throughout the experiment. Six rats were studied in each group. In the second series (Series B), we characterized the circadian rhythm of mucosal ODC activity in fasted rats. To achieve this goal, we measured mucosal ODC activity at four different times of the day: 09:00 hr (09:00–09:30 hr), 15:00 hr (15:00–15:30 hr), 21:00 hr (21:00–21:30 hr), and 03:00 hr (03:00–3:30 hr). Rats were fasted exactly at either 24 hr or 48 hr before the time of sacrifice. There were 12 rats each in the eight groups of fasted animals.

Collection of intestinal mucosa for determination ODC activity. The animals were anesthetized and then euthanized. The small intestine was excised and divided into four segments of equal length. The most proximal 5 cm of the first segment (duodenum) was used as well as the distal 5 cm of each of the four bowel segments (labeled as I-1, I-2, I-3, and I-4, from proximal to distal). Mucosa from each segment was obtained by scraping with a glass slide over an ice-cold glass plate.

ODC assay. ODC activity was assayed by a radiometric technique in which the amount of $^{14}CO_2$ liberated from *levo*-[1-^{14}C]ornithine (52.3 mCi/mmol; New England Nuclear, Boston, MA) was measured (13). Mucosal scrapings were collected as described above and placed in 2 ml of 0.1 *M* Tris hydrochloride buffer (pH 7.4) containing 1 m*M* EDTA, 50 μM pyridoxal 5′-phosphate, and 5 m*M* dithiothreitol. The tissues were homogenized twice with a Polytron tissue homogenizer for 15 sec and centrifuged at 30,000*g* for 30 min. Protein content was determined by Lowry *et al.* (24). A 200-μl aliquot of the supernatant was incubated in stoppered vials in the presence of 3.5 nmol of *levo*-[1-^{14}C]ornithine for 15 min at 37°C. The $^{14}CO_2$ liberated by the decarboxylation of ornithine was trapped on a piece of filter paper impregnated with 20 μl of 2 *N* NaOH, which was suspended above the reaction mixture. The reaction was terminated by the addition of 0.3 ml of 10% trichloroacetic acid. Radioactivity of the $^{14}CO_2$ trapped in the filter paper was measured in an aqueous, miscible scintillant (Poly-Flour; Packard Instrument Co., Downers Grove, IL). The samples were counted for 10 min in a liquid scintillation spectrometer (LKB model 1209 Rackbeta). Results are expressed as picomoles CO_2 produced per milligram protein per hour (pmol CO_2/mg protein/hr).

Statistics. Results are expressed as mean ± SE. Data of Series A were evaluated by two-way analysis of variance, in which orthogonal decomposition for linear comparison was carried out (25). Data of Series B were evaluated by one-way analysis of variance, and multiple comparisons were carried out with the method of least significant difference (25). Differences were considered significant if the probability of the difference occurring by chance was less than five in 100 ($P < 0.05$).

Normal Feeding Patterns of Rats. We also studied the normal feeding pattern of 30 rats. Each rat was adapted to a 30 × 25 × 25-cm testing chamber equipped with a pellet-sensing eatometer (Astec, Fukuoka, Japan) for 1 week. Rats were allowed free access to standard pellet rat chow (mean weight: 48.4 ± 0.6 mg; range: 44–55 mg). The number of pellets consumed was automatically recorded with a computer. This system has been described in detail elsewhere (26). After the rats were adapted to the chamber, the feeding patterns of 30 rats were recorded for 2 days. A meal was defined as the consumption of >10 pellets for a period greater than 10 min (26, 27).

Results

ODC Activity in Rat Small Intestine. Figure 1 shows the effect of feeding and fasting on ODC activity in the mucosa of various small intestinal segments. The rats were fasted for either 12, 24, or 48 hr. ODC activities of all five segments were not significantly different between the 24-hr and the 48-hr fasted rats ($F[1,60] < 1$, NS). The mucosal ODC activity was low in all intestinal segments of 24-hr and 48-hr fasted rats except I-4, in which significant ODC activity was observed even in the rats fasted for 48 hr. In contrast, mucosal ODC in rats fed *ad libitum* was significantly

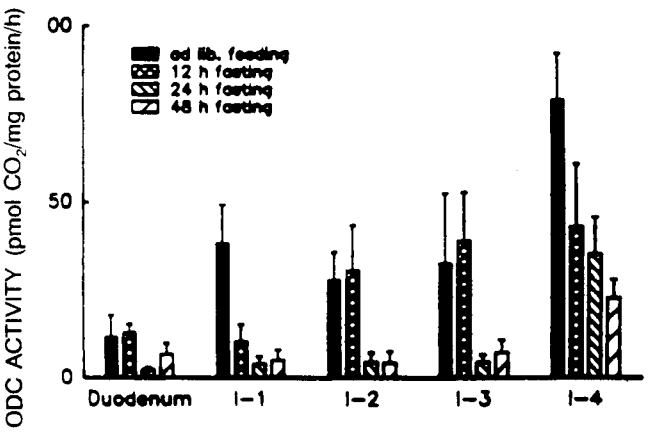

Figure 1. Ornithine decarboxylase activity in intestinal mucosa from rats fed *ad libitum* or fasted at 09:00 hr. I-1 and I-2 correspond to the jejunum and I-3 and I-4 correspond to the ileum. Six rats in each group were studied and the results are expressed as mean ± SE.

higher than in comparable segments from the fasted rats (24 hr and 48 hr combined, $F[1,60] = 28.5$, $P < 0.01$). Mucosal ODC activity in rats fasted for only 12 hr did not decrease significantly, except in I-1, compared with rats fed *ad libitum*. However, it was significantly higher when compared with the 24-hr or 48-hr fasted rats in all segments (24 hr and 48 hr combined, $F[1,60] = 6.0$, $P < 0.05$).

Figure 2 shows the circadian rhythm of ODC activity in the duodenum of fasted rats. For 24-hr fasted rats, maximal ODC activity was observed at 15:00 hr, i.e., 3 hr before the dark period. The ODC activity at 15:00 hr was significantly higher than at the other three time points and this difference was statistically significant ($P < 0.01$ for each). The ODC activities at 09:00, 21:00, and 03:00 hr were not significantly different. For 48-hr fasted rats, the ODC activity also peaked at 15:00 hr ($P < 0.01$, for each comparison to ODC activity at other three time points). No significant differences were observed between the 24-hr and 48-hr fasted rats.

In contrast to the duodenum, the ODC activity of the jejunum (I-1) did not increase at 15:00 hr for 24-hr and 48-hr fasted rats (Fig. 3). In these segments, ODC activity of 24-hr and 48-hr fasted rats peaked in the dark period (21:00 hr and 03:00 hr), with ODC activity at these time points significantly higher than at times in the light period ($P < 0.01$ for each comparison). It should be emphasized that this circadian rhythm of ODC activity was observed despite the absence of nutrients in the intestinal lumen.

Figure 4 shows the circadian rhythm in ileum (I-3). Similar to the jejunum, ODC activity in the ileum of 24-hr and 48-hr fasted rats increased markedly in

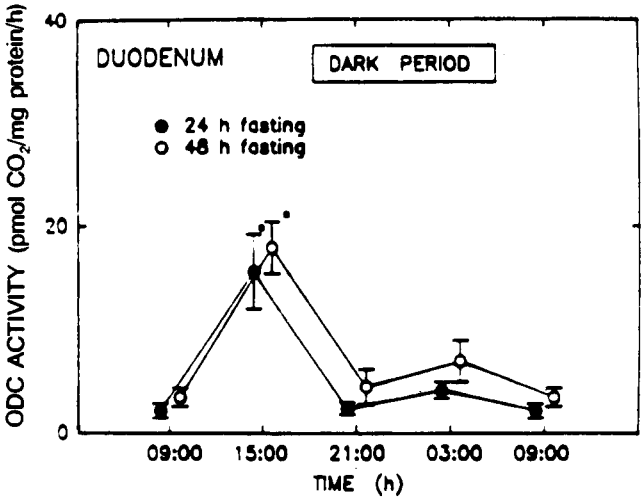

Figure 2. Circadian variations of ornithine decarboxylase activity in duodenal mucosa of fasted rats. The dark period is 18:00–06:00 hr. Two groups of rats were used: fasted for 24 hr and fasted for 48 hr. In each group of rats, we studied four time points: 09:00 hr, 15:00 hr, 21:00 hr, and 03:00 hr. $^{*}P < 0.01$, compared with the values at the other three time points. Twelve rats were used for each time point. Values are expressed as mean ± SE.

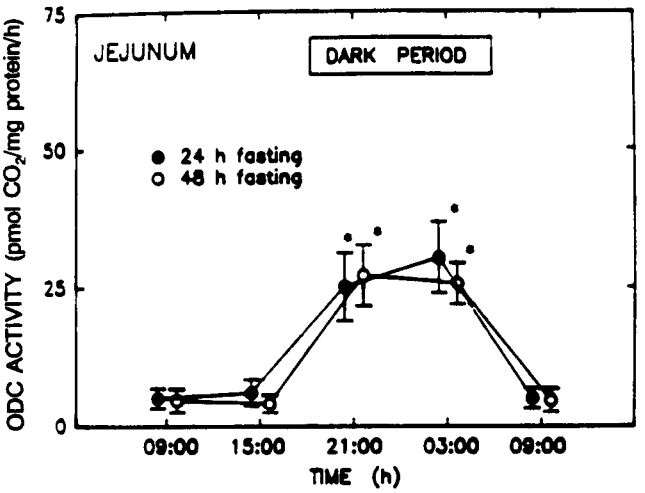

Figure 3. Circadian variations or ornithine decarboxylase activity in mucosa of jejunum (I-1 segment) of fasted rats. The dark period is 18:00–06:00 hr. Two groups of rats were used: fasted for 24 hr and fasted for 48 hr. In each group of rats, we studied four time points: 09:00 hr, 15:00 hr, 21:00 hr, and 03:00 hr. $^{*}P < 0.01$, compared with the values in light period. Twelve rats were used for each time point and the results are expressed as mean ± SE.

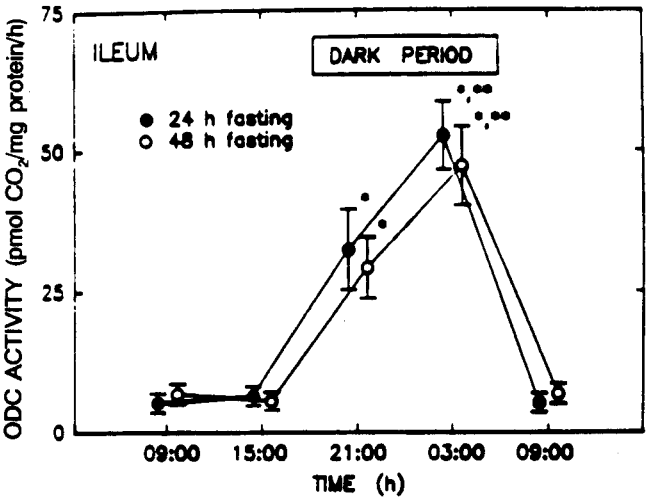

Figure 4. Circadian variations of ornithine decarboxylase activity in mucosa of ileum (I-3 segment) of fasted rats. The dark period is 18:00–06:00 hr. Two groups of rats were used: fasted for 24 hr and fasted for 48 hr. In each group of rats, we studied four time points: 09:00 hr, 15:00 hr, 21:00 hr, and 03:00 hr. $^{*}P < 0.01$, compared with the values in light period. $^{**}P < 0.01$, compared with the values at 21:00 hr. Twelve rats were used for each time point and the results are expressed as mean ± SE.

the dark period ($P < 0.01$ for each comparison to ODC activity at light period), and peaked at 03:00 hr. Mucosal ODC activity in ileum at 03:00 hr was significantly higher than at 21:00 hr in both 24-hr and 48-hr fasted rats ($P < 0.01$ for each comparison).

Feeding Patterns of Rats. A typical feeding pattern of a rat fed *ad libitum* for 2 consecutive days is depicted in Figure 5. Rats normally do not eat during 06:00–15:00 hr in the light period. During that period, only three rats of 30 rats tested ate one meal. Feeding

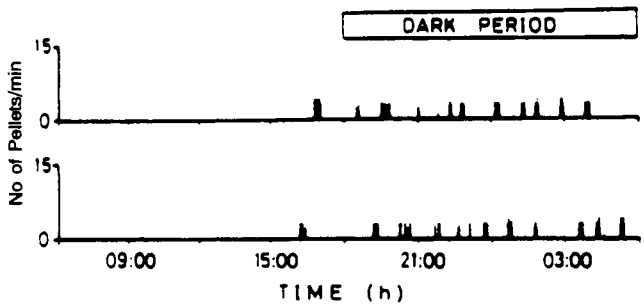

Figure 5. Typical feeding pattern of a rat fed *ad libitum* during 2 consecutive days. The dark period is 18:00–06:00 hr. The vertical bar represents the number of pellets consumed per min (mean weight of standard pellet, 48.4 ± 0.6 mg).

with a single meal was observed in the demonstrated feeding pattern during 15:00–18:00 hr before the dark period, when most of 30 tested rats started to eat one or two meals. The rats normally consumed most of their meals during the dark period, and approximately 10 meals were observed in each rat during the night period.

Discussion

In this study, we demonstrated that mucosal ODC activity in duodenum, jejunum, and ileum in 24-hr and 48-hr fasted animals was significantly lower than that in rats fed *ad libitum*. This observation confirms previous reports that ODC activity decreased in fasted rats and was markedly stimulated (>10-fold) in refeeding rats (10–14). In another study, it was demonstrated that ODC activity of small intestine in rats feeding *ad libitum* exhibited a circadian rhythm that was highest in the dark period. However, the peak in mucosal ODC activity could be shifted to the light period when rats were fed in the light period (10). These studies indicate that feeding is an extremely potent stimulant for mucosal ODC activity. Our results also indicate that the presence of food in the intestinal lumen may mask an intrinsic circadian rhythm of mucosal ODC activity that is manifested even in fasted animals.

Saito *et al.* (21) and Stevenson and Fierstein (20) have demonstrated that intestinal disaccharidase activity increases just prior to feeding in rats that have been trained to feed at a scheduled time. Based on this observation, Saito *et al.* (21) concluded that the rise of disaccharidase activity reflects an involvement of the nervous system and that the anticipation of food intake acts as a trigger for stimulation of mucosal disaccharidase activity.

Rats fed *ad libitum* seldom eat before 15:00 hr in the light period. Rats started to eat at 15:00–18:00 hr just before the dark period. Mucosal ODC activity in fasted rats increased markedly in the duodenum at 15:00 hr, which is before the rats started to eat. Thus, it is unlikely that the increase in duodenal mucosal ODC activity is regulated by the presence of food in the lumen. It is likely that the anticipation of food triggers the increase in duodenal mucosal ODC activity.

Mucosal ODC activity in jejunum and ileum of fasted rats increased between 21:00 and 03:00 hr, which is the dark period, when rats normally eat. Thus, it appears that the circadian rhythm of jejunal and ileal mucosal ODC activity does not require nutrients in the lumen for expression. However, it is likely that the presence of food during the dark period may greatly enhance the nocturnal increase in jejunal and ileal ODC activity. At present, the physiologic significance of the increased mucosal ODC activity during the dark period in fasted rats remains unclear. There are, however, several advantages to the circadian rhythm of jejunal and ileal mucosal ODC activity. First, the concentration of luminal ornithine, the primary substrate for ODC, is highest during feeding. Second, during feeding, the intestine is exposed to noxious agents in the food that may damage the intestinal mucosa. The damaged mucosa would have to be repaired and this would probably involve proliferation of intestinal epithelial cells. Since polyamines have been demonstrated to play an important role in intestinal growth and ODC is the first rate-limiting enzyme in the production of polyamines (13, 15), it would be physiologically advantageous for ODC activity to be highest during the period of feeding (dark period).

Although the identity of the modulators of mucosal ODC activity remains unknown, pituitary hormones exert a significant influence on ODC activity in many organs (2–6, 8, 28). We did not study the effects of pituitary hormone administration and/or hypophysectomy on ODC activity in the present studies, because these interventions change the nocturnal feeding behavior of rats (29–31). Other humoral factors, such as insulin and epidermal growth factor, may also play a role in the regulation of intestinal ODC activity (11, 15, 32, 33). Mucosal ODC activity increased at a different time in duodenum (15:00 hr) as compared with the remainder of the small intestine (21:00–03:00 hr). This observation suggests that either local factors mediate the biorhythm, or that the duodenum exhibits a different sensitivity to the specific circulating factors.

We have demonstrated in this study that circadian rhythm of ODC activity exists in rat small intestine and that this does not require nutrients in the lumen for expression. Furthermore, the results of this study underscore the need to perform studies of intestinal mucosal ODC activity under comparable physiologic conditions, i.e., in fed versus fasting, in duodenum versus jejunum and ileum, and at discrete time points.

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