Twenty-Four-Hour Variations in Ornithine Decarboxylase and Acid Phosphatase in Mice (42943)

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Abstract. **Polyamines are essential for cell growth and differentiation. Ornithine decarboxylase (ODC) is the rate-limiting enzyme in polyamine biosynthesis. Acid phosphatases (AP) are lysosomal enzymes that are important in normal intracellular metabolism. Twenty-four-hour variations in these enzymes may be important in understanding the temporal responses of different tissues to various stimuli. The purpose of this study was to examine a variety of tissues for fluctuations in the levels of ODC and AP over a 24-hr period. Significant circadian variations in the amount of ODC activity were observed in all tissues examined. Activity of AP varied with time of day in the liver, kidney, and heart. The highest and lowest measurements of ODC activity were as follows: liver, 81.5 ± 7.0, 47.9 ± 4.4; colon, 11.7 ± 1.2, 3.1 ± 0.7; stomach 3.1 ± 0.4, 0.9 ± 0.1; kidney, 420.9 ± 0.9, 67.5 ± 0.8; and heart, 4.7 ± 1.0, 2.5 ± 0.2. The highest and lowest measurements of AP activity were as follows: liver 3.8 ± 0.1, 2.8 ± 0.1; kidney, 3.4 ± 0.1, 1.9 ± 0.1; and heart, 2.6 ± 0.1, 2.0 ± 0.1. These findings suggest that rhythmic fluctuations in polyamine biosynthesis and lysosomal enzymes may influence other metabolic pathways differentially throughout 24 hr.** [P.S.E.B.M. 1989, Vol 191]

Polyamines are small, highly charged cations that are important in a variety of fundamental intracellular processes (1–3). The naturally occurring polyamines, putrescine, spermidine, and spermine, are described as important modulators of nucleic acid and protein biochemistry in eukaryotic systems (4, 5). The concentrations of polyamines are elevated in rapidly dividing tissues, particularly before increases become apparent in RNA, DNA, and protein synthesis (3, 6). In addition, a depletion of intracellular polyamines causes a slowing and eventual cessation of cell growth (6, 7).

The biosynthesis of polyamines in mammals is initiated exclusively by decarboxylation of ornithine to form the diamine, putrescine. The first and rate-limiting biosynthetic step is governed by the action of the enzyme, ornithine decarboxylase (ODC; EC 4.1.1.17) (8, 9). Circadian rhythms in tissue ODC activity have been demonstrated in the liver, kidney, thymus, and small intestine of the rat and in the liver of the mouse (10–14). Disruption of rhythms in ODC activity can be caused by fasting, hypophysectomy, adrenalectomy, and pinealectomy (11, 13, 14).

Acid phosphatases (AP; EC 3.1.3.2) are lysosomal enzymes that perform various intracellular metabolic functions in many tissues, including liver and heart (15). In rats, measurements of AP activity represent an average of several chromatographically distinct active forms of this enzyme (16). Twenty-four-hour variations in AP activity have been described in the liver of rats and mice as well as in the salivary gland of mice (16–19). The timing of these rhythms undergoes circannual phase shifts even when animals are maintained under a constant photoperiod length (19). In addition, rhythms in liver AP activity phase shift with increasing age in rats (16). The factors regulating these rhythms are unknown.

Daily variations of liver ODC activity have been described in mice previously (20) but not in other organs of the mouse. Also, AP variations have not been described before in the heart or kidney. We report here

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our findings of 24-hr variations in the activity of ODC and AP in selected organs from mice.

Materials and Methods

Fifty Sprague-Dawley weanling mice (Harlan Sprague-Dawley Laboratories, Houston, TX) were randomly divided into six groups of eight to nine mice each on November 22, 1986. All groups were housed in isolation chambers under a 12-light:12-dark photoperiod (lights on 0600 hr; lights off 1800 hr) for 4 weeks. Food and water were provided *ad libitum*. On December 16, 1986, all mice were sacrificed by cervical dislocation. One group each was sacrificed at 0, 4, 8, 12, 16, and 20 hr after light onset (HALO). The liver, kidney, stomach, colon, and heart were frozen in liquid nitrogen at −80°C and stored at −20°C until assays were performed, at which time specimens were divided for separate analysis of the two enzymes.

Liver, heart, and kidney were assayed for lysosomal AP activity. Tissue AP activity in 0.25 *M* sucrose homogenate was determined according to the method of Barrett and Heath (21), using *p*-nitrophenol phosphate as substrate. Results are expressed in μmol/min/g of *p*-nitrophenol suspended tissue. ODC activity was measured by quantifying the amount of $^{14}CO_2$ released from radiolabeled L-ornithine as outlined by Russell and Snyder (22). ODC purified from *Escherichia coli* (Sigma Chemical Co., St. Louis, MO) was reconstituted in 0.9% saline (standard). Enzyme kinetics were studied by assaying the activity of homogenates of each tissue in 0.9% saline and comparing this activity with that of fixed aliquots of standard ODC. Protein content of each sample of tissue was measured according to the method of Lowry *et al.* (23). Results are expressed in pmol/hr/mg of protein in each sample.

Measurements of ODC and AP, recorded at different times in the 24-hr period, were analyzed for significant variation by one-way analysis of variance.

Results

Significant 24-hr variations in ODC activity were found in all five tissues examined (Table I). Highest measurements of ODC activity occurred at 1100 hr (5 HALO) in liver, 0700 hr (1 HALO) in kidney and colon, and 0300 hr (21 HALO) in heart and stomach (Fig. 1). There were significant differences between highest and lowest values in each tissue. Levels of ODC activity in samples of the kidney were 10–100 times greater than in all other tissues. Measurements in liver ranged from 81.5 ± 7.0 at 1100 hr to 47.9 ± 4.4 at 2100 hr (expressed in pmol of CO_2 released/hr/g of protein). Kidney ODC activity varied from 67.5 ± 0.8 at 2300 hr to 420.9 ± 0.9 at 0700 hr, a change of over 500% during the 24-hr period. The range of change in the stomach and heart was not so great but was still significant: 2.5 ± 0.2 at 2300 hr to 4.7 ± 1.0 at 0300 hr (heart); 0.9 ± 0.1 at 2300 hr to 3.1 ± 0.4 at 0300 hr (stomach). ODC activity in the colon was measured highest at 11.7 ± 1.2 at 0700 hr and lowest at 3.1 ± 0.7 at 0300 hr, almost 300% variation during the 24-hr period.

Similarly, we found significant variations in AP activity in the liver, kidney, and heart (Table II). Higher AP activity occurred at 1500 hr (9 HALO) in the liver and heart and 0300 hr (21 HALO) in the kidney (Fig. 2). Two- to 4-fold differences between highest and lowest measurements were found in these tissues. Concentration of AP in the kidney rose in the morning from 2.0 ± 0.1 at 0700 hr to 3.4 ± 0.1 at 1100 hr (expressed as μmol of activity/min/g of tissue) and then began to decline to reach the lowest level at 0300 hr, 1.9 ± 0.1. Levels in the liver and heart rose also during daylight to peaks at 1500 hr of 3.8 ± 0.1 (liver) and 2.6 ± 0.1 (heart), then decreased during the dark.

Discussion

Circadian rhythms in enzymes other than ODC and AP have been demonstrated repeatedly. Examples include heart and skeletal muscle lactate dehydrogenase (24), liver and brain glycolytic and other enzymes (25–29), and other lysosomal enzymes such as β-acetylglucosaminadase (19). Control of these enzyme variations is complex and incompletely understood but appears to involve both alterations in substrate availability (i.e., feeding) (30, 31) and endogenous circadian rhythms (32, 33).

We have described significant circadian rhythms in the activity of both ODC and AP in several organs in mice. Circadian rhythms in ODC activity have been demonstrated previously in the liver of rats and mice

Table I. ODC activity (CO_2 pmol/hr/mg of protein, $\bar{X}$ ± SEM) in Mouse Liver, Kidney, Colon, Stomach, and Heart

Organ	Time of sample (hr)					
	0700	1100	1500	1900	2300	0300
Liver[a]	72.4 ± 5.1	81.5 ± 7.0	74.1 ± 8.8	66.9 ± 9.5	47.9 ± 4.4	54.9 ± 7.7
Kidney[a]	420.9 ± 0.9	277.4 ± 1.0	147.5 ± 0.8	112.4 ± 0.7	67.5 ± 0.8	236.1 ± 0.4
Heart[a]	3.2 ± 0.3	3.8 ± 0.7	3.8 ± 0.6	2.7 ± 0.3	2.5 ± 0.2	4.7 ± 1.0
Colon[a]	11.7 ± 1.2	3.6 ± 1.0	4.9 ± 1.1	4.3 ± 0.6	4.3 ± 1.1	3.1 ± 0.7
Stomach[a]	1.5 ± 0.3	1.2 ± 0.5	1.0 ± 0.1	1.7 ± 0.3	0.9 ± 0.1	3.1 ± 0.4

[a] $P < 0.05$.

(13), as have subsequent rhythms in cellular proliferative activity in liver after a meal (13). Ornithine, which is utilized to synthesize putrescine, is formed by the action of arginase on arginine, which is provided by the citric acid and urea cycles (5). Both of these metabolic loops have shown great 24-hr variations, and the highest measured values were coincidental with the time of feeding (28, 34).

Similar to previous findings in the rat, our results show a 2-fold daily variation in liver ODC activity. However, the timing of peak ODC activity occurred at 5 HALO in mice and at 16–20 HALO in rats (10, 13). Because we did not measure food intake, we cannot state with confidence whether the mice ate most of their food early or late during the dark period, their usual feeding time. In the laboratory, rats eat about 75% of their food in the dark phase (35, 36). We have also found that shortening the photoperiod to 6-hr light:18-hr dark does not alter the amplitude or timing of ODC rhythms in the liver (unpublished data from our laboratory). Hence, we are confident of these findings despite their variance from previous studies in rats. Our findings may represent a significant species difference in polyamine biosynthesis, or perhaps a circannual shift in timing of peak ODC activity occurred.

Acid phosphatase activity in the liver showed a significant daily fluctuation with a peak activity measured at 1500 hr (9 HALO). These findings are consistent with circadian rhythms that have been shown in young rats (4 months old) and in mice held under a 12-hr light:12-hr dark photoperiod during the winter (19). The paucity of information on the role of acid phosphatase in cellular rhythms precludes speculation on its controlling factors; however, acid phosphatase may play a role in rhythms of intracellular energy metabolism

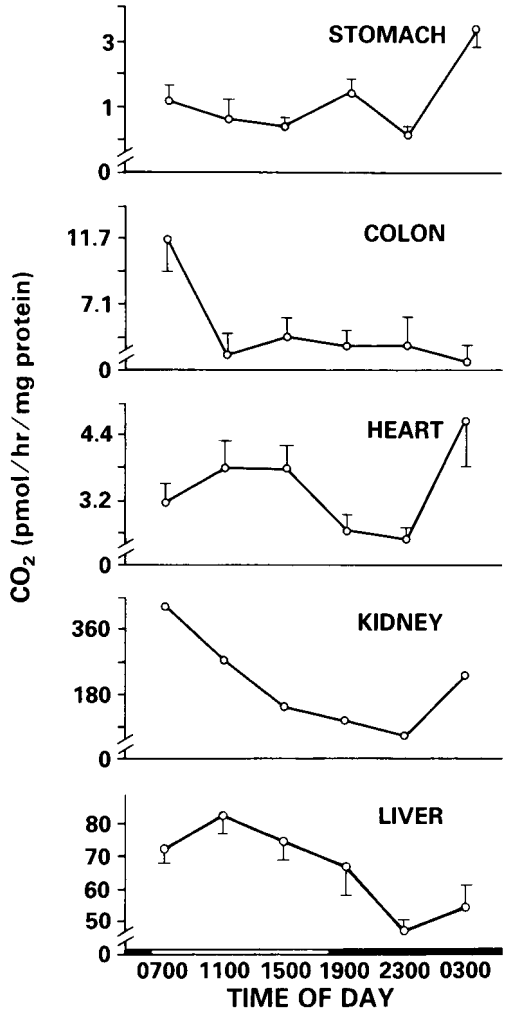

Figure 1. ODC activity (±SE) of stomach, colon, heart, kidney, and liver at six sampling times during a 24-hr period. Time expressed in hours (Central Standard Time).

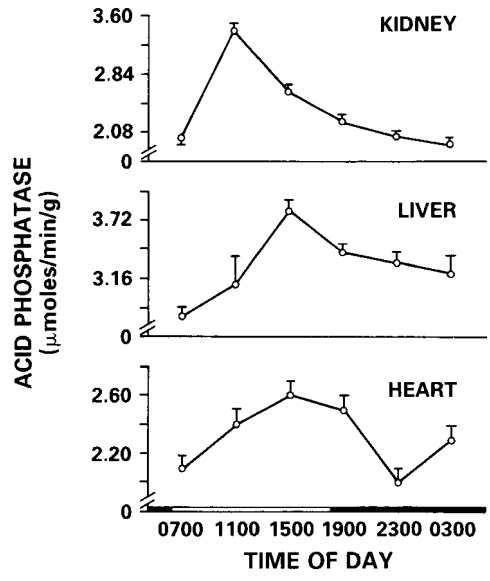

Figure 2. AP activity (±SE) of kidney, liver, and heart at six sampling times during a 24-hr period. Time expressed in hours (Central Standard Time).

Table II. AP activity (μmol/min/g tissue, $\bar{X}$ ± SEM) in Mouse Liver, Heart, and Kidney

Organ	Time of sample (hr)					
	0700	1100	1500	1900	2300	0300
Liver[a]	2.8 ± 0.1	3.1 ± 0.3	3.8 ± 0.1	3.4 ± 0.1	3.3 ± 0.1	3.2 ± 0.2
Heart[a]	2.1 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.0 ± 0.1	2.3 ± 0.1
Kidney[a]	2.0 ± 0.1	3.4 ± 0.1	2.6 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	1.9 ± 0.1

[a] $P < 0.05$.

(15, 18). We also documented significant daily variation in the activity of ODC and AP in the heart and kidney in mice. The role of these rhythms in the function of the heart and kidney remains to be discovered.

Circadian variations of DNA synthesis and of the mitotic index have been demonstrated throughout the entire murine gastrointestinal tract (37). Daily rhythms of ODC have been examined by others in the rat small intestine (12). Peak ODC levels occurred during the dark period (16–20 HALO). In contrast, in our study mouse colon and stomach showed highest ODC activity late in the dark (21 HALO) or just at the transition between darkness and light, at 0–21 HALO when fed *ad libitum*. It is interesting that the epithelia of these two tissues show a trophic response to gastrin administration.

We have demonstrated circadian rhythms in the ODC activity of the liver, heart, kidney, colon, and stomach of freely fed mice held under a 12-hr light:12-hr dark photoperiod. We also demonstrated significant daily circadian rhythms in the activity of AP in mouse liver, heart, and kidney. These findings implicate polyamine biosynthesis and lysosomal enzymes as potentially important mediators of chronobiologic events in rodents.

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