BBA Report

BBA 71083

Daily rhythmic change in the transport of histidine by everted sacs of rat small intestine

SHŪ FURUYA* and YASUMI YUGARI**
Institute for Protein Research, Osaka University, Osaka (Japan)
(Received May 24th, 1971)

SUMMARY

Fluctuations in the intestinal transport of L- and D-histidine were measured in rats on three feeding schedules under conventional lighting conditions, with a dark night. In rats fed *ad libitum*, the transport of L-histidine through the everted intestine showed a daily rhythmic change, being high at 4 p.m. and low in the early morning. In rats adapted to daytime feeding, the transport of L-histidine was highest at 6 a.m. and low at night. In starved rats, the rhythmicity was maintained for at least one day of fasting. Transport of D-histidine showed no daily fluctuation.

Recently, findings on the daily rhythmic changes in higher animals have accumulated. For example, the habit of food intake[1–3], the movement of isolated intestine[4], amino acid level of blood[5], concentration of amino acids in various organs[6–8], and the enzyme activities metabolizing amino acids in the liver, such as tyrosine aminotransferase (EC 2.6.1.5)[2,7–12], and tryptophan oxygenase (EC 1.13.1.12)[8,9,13] are all rhythmic phenomena.

Active transport of amino acids through the intestine comes after the digestion of food and is followed by the metabolism of amino acids in the body. Therefore, the active transport of amino acids is expected to change rhythmically concomitant with the rhythm of food intake or of amino acid metabolism.

In this paper, we report a daily rhythmic change in the active transport of L-histidine by everted sacs of the rat small intestine, and a phase shift of the rhythm caused by manipulation of the feeding time.

Male Wistar strain rats, weighing 140–180 g at the beginning of the experiments, were used. Ambient temperature was 25–27°. Conventional lighting conditions were used,

*Present address: National Institute of Animal Industry, Chiba, Japan.
**Present address: Central Research Laboratories, Ajinomoto Co., Inc. Kawasaki, Kanagawa, Japan.

with a dark night. Rats were fed on laboratory chow and were allowed free access to water at all times. In Expt. 1, rats were freely accessible to food at any time. In Expt. 2, rats were fed from 9 a.m. to 3 p.m. In Expt. 3, rats were preliminarily fed from 6 p.m. to 6 a.m. every day for one week and then starved. Rats were sacrificed every 3 h in Expts. 1 and 2, and in Expt. 3 at 7 a.m. and 7 p.m.

Transport activity of histidine was measured using everted sacs of intestine by the method of Wilson and Wiseman[14]. Everted jejunum, each segment about 8 cm long, was filled with 0.8 ml of Krebs–Ringer bicarbonate buffer (pH 7.4)[15], containing 4 mM L- or D-histidine and 0.2% glucose, saturated with a mixture of 95% O_2 and 5% CO_2. The filled sac was placed in a 25-ml erlenmeyer flask containing 10 ml of the same solution, and the flask was shaken at 80 oscillations/min for 1 h at 37° in an atmosphere of 95% O_2 and 5% CO_2. Transport activity was expressed as the ratio of the histidine concentration in the serosal (inner) fluid to that in the mucosal (outer) fluid at the end of incubation. Histidine concentration was determined colorimetrically by a modification of the method of MacPherson[16] adapted for an AutoAnalyser (Technicon) system (Y. Yugari, see footnote★).

In Expt. 1, L-histidine transport activity in rats fed *ad libitum* under conventional lighting was high during the night and low in the early morning (Fig. 1, ●—●). A slight rise in the ratio was observed at 10 a.m.

The rhythmic change in histidine transport was observed only in the case of the L-isomer. The concentration ratio of D-histidine was 0.72 ± 0.01 (S.E.) at 1 p.m., and 0.71 ± 0.02 (S.E.) at 7 p.m.

Recently, it was realized that the daily light–dark cycle was significant in controlling daily rhythmic changes. Rats are accustomed to eat during the night as nocturnal animals, and, therefore, the light–dark cycle and the fast–eating cycle are

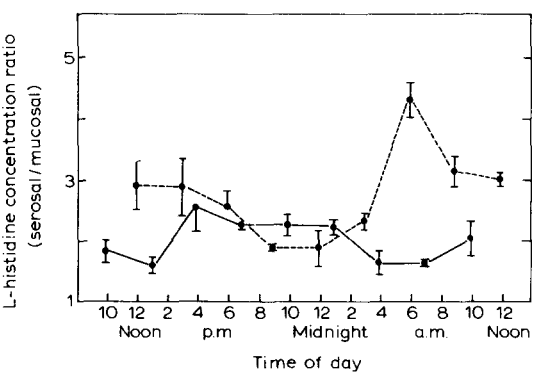

Fig. 1. Daily rhythmic changes in active transport of L-histidine in rat small intestine. ●—●, rats fed *ad libitum*; ●- - -●, rats adapted for 3 weeks to feeding from 9 a.m. to 3 p.m. The transport activity of L-histidine by everted sacs is expressed as the ratio of the L-histidine concentration in serosal fluid to that in mucosal fluid. Each point represents the mean value for three rats with the standard error.

★Samples containing L- or D-histidine and 0.1% sulfanilic acid in 0.1 M HCl were mixed at flow rates of 0.03 ml/min and 2.50 ml/min, respectively. After the air was introduced at 2.00 ml/min, the reaction proceeded in the following sequence: mixing – 5% $NaNO_2$, 0.23 ml/min – mixing – 15% $Na_3PO_4 \cdot 12H_2O$, 0.60 ml/min – mixing – water, 2.00 ml/min. Color developed was measured with a Klett No. 54 filter and recorded. Details will be reported separately.

synchronized. In order to discriminate between the effects of these two cycles on the daily fluctuation of L-histidine transport activity through the intestine, rats were fed during the daytime from 9 a.m. to 3 p.m. every day for 3 weeks, being kept under conventional lighting with a dark night. Rats adapted soon to the daytime feeding schedule, and their body weight gained similarly to those on an *ad libitum* feeding schedule. As shown in Fig.1 (●- - -●), the transport activity showed a peak at 6 a.m., 3 h ahead of scheduled feeding time, and a trough from 9 p.m. to midnight. The phase of daily rhythmic change in transport activity shifted about half a day by feeding during the daytime with respect to that of rats fed *ad libitum*.

These results suggest that the daily rhythmic change in the intestinal transport of L-histidine is a consequence of the rhythm in food intake, as in the case of hepatic tyrosine aminotransferase activity[8,12], and not of the light–dark cycle.

In Expt. 3 (Fig.2), rats were fed from 6 p.m. to 6 a.m. for one week and then starved. During the fed control period, the concentration ratio of L-histidine was high at 7 p.m. and low at 7 a.m. On the first day of starvation, L-histidine transport activity was high at 7 p.m., exactly the same as in the control period, and low at 7 a.m. the next morning. On the second day, the concentration ratio was again slightly raised at 7 p.m. Although cyclic food intake is a prerequisite for the daily rhythmic change in the active transport of L-histidine, the rise in the concentration ratio may not be a direct consequence of the fed–fasted cycle, but of some mechanism which is driven by feeding on the time when rats expect to be fed, in other words, by food as a "reward". This will explain the fact that a peak in transport activity preceded feeding by 3 h, as shown in Expt. 2. Similar phenomena have been observed in hepatic tyrosine aminotransferase activity[8,12,17] and concentration of cycloleucine in the liver[6].

The daily rhythmic change in intestinal transport of L-histidine was shown to coincide with that of food intake and hepatic metabolism of amino acids. The mechanism needs to be elucidated further.

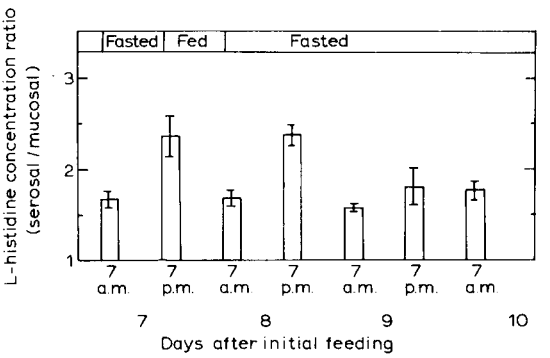

Fig. 2. Daily rhythmic change in active transport of L-histidine in rat small intestine during a 3-day fasting period. Rats were fed on laboratory chow from 6 p.m. to 6 a.m. ("fed") and starved from 6 a.m. to 6 p.m. ("fasted") every day for a week. Then rats were starved from 6 a.m. on the eighth day for 3 days. Active transport of L-histidine was measured at 7 a.m. and at 7 p.m. The transport activity is expressed as the ratio of the L-histidine concentration in serosal fluid to that in mucosal fluid. Each point represents the mean value for three rats with the standard error.

The authors are grateful to Prof. M. Suda, Institute for Protein Research, Osaka University, for his encouragement of this work.

REFERENCES

1 P.S. Siegel and H.L. Stackey, *J. Comp. Physiol. Psychol.*, 40 (1947) 365.
2 M.J. Zigmond, W.J. Shoemaker, F. Larin and R.J. Wurtman, *J. Nutr.*, 98 (1969) 71.
3 T. Kimura, T. Maji and K. Ashida, *J. Nutr.*, 100 (1970) 691.
4 E. Bünning, *Naturwiss.*, 45 (1958) 68.
5 R.D. Feigin, H.G. Dangerfield and W.R. Beisel, *Nature*, 221 (1969) 94.
6 E.F. Baril and V.R. Potter, *J. Nutr.*, 95 (1968) 228.
7 R.J. Wurtman, W.J. Shoemaker and F. Larin, *Proc. Natl. Acad. Sci. U.S.*, 59 (1968) 800.
8 R.W. Fuller, *Proc. Soc. Exp. Biol. Med.*, 133 (1970) 620.
9 V.R. Potter, R.A. Gebert and H.C. Pitot, *Adv. Enzyme Regul.*, 4 (1966) 247.
10 V.R. Potter, M. Watanabe, J.E. Becker and H.C. Pitot, *Adv. Enzyme Regul.*, 5 (1967) 303.
11 R.J. Wurtman and J. Axelrod, *Proc. Natl. Acad. Sci. U.S.*, 57 (1967) 1954.
12 R.W. Fuller and H.D. Snoddy, *Science*, 159 (1968) 738.
13 M.I. Rapoport, R.D. Feigin, J. Bruton and W.R. Beisel, *Science*, 153 (1966) 1642.
14 T.H. Wilson and G. Wiseman, *J. Physiol.*, 123 (1954) 116.
15 H.A. Krebs and K. Henseleit, *Hoppe-Seylers Z. Physiol. Chem.*, 210 (1932) 33.
16 H.T. MacPherson, *Biochem. J.*, 40 (1946) 470.
17 M. Watanabe, V.R. Potter and H.C. Pitot, *J. Nutr.*, 95 (1968) 207.