Circadian Rhythm of the Mitotic Index in the Rumen Epithelium of Sheep

Tohoku Journal of Agricultural Research
Vol. 33, Nos. 3–4, March 1983
Printed in Japan

Circadian Rhythm of the Mitotic Index in the Rumen Epithelium of Sheep

Shyuichi OHWADA and Hideo TAMATE

Laboratory of Animal Morphology, Faculty of Agriculture, Tohoku University, Sendai 980, Japan

(Received, January 31, 1983)

Summary

Mitotic indices in sheep rumen epithelium were examined at 3:00, 7:00, 11:00, 15:00, 19:00 and 23:00, and the circadian rhythm of the index were determined. When sheep were fed once daily at 18:00 with orchard grass hay or high concentrate type meal, the mitotic index showed the lowest value at 19:00, 1 hr after the meal, and there were little differences between the mitotic index at another times. When sheep were fed twice daily at 6:00 and 18:00, the lowest mitotic index appeared at 7:00 and the peak was 23:00, 5 hrs after the evening meal. The circadian rhythm of the mitotic index in twice daily fed sheep was less clear than that in the sheep fed once daily. The mitotic index and its circadian rhythm are probably related to the frequency and the time of food intake.

Sakata and Tamate (1) have reported that there was a circadian rhythm of the mitotic index in sheep rumen epithelium and stated that the rate of epithelial cell division may be affected by the food intake. There was, however, a clear difference in the circadian rhythm in their two experiments in which sheep were fed at 7:00 or 11:00. Thus, we re-examined the circadian rhythm of the mitotic index in sheep rumen epithelium.

Materials and Methods

Three male Corriedale sheep, 35–40 kg body weight, were used for the study. They were fed with 1 kg of orchard grass hay in Exp. 1 (n=8), and with 200 g of orchard grass hay and 800 g of concentrate in Exp. 2 (n=6), once daily at 18:00. In Exp. 3 (n=8), they were fed twice daily at 6:00 and 18:00 with 100 g of orchard grass hay and 400 g of concentrate with an automatic feeder.

Rumen papillae were biopsied from *atrium ruminis* with a pair of Chevarier-Jackson specimen forceps through the rumen fistula at 3:00, 7:00, 11:00, 15:00, 19:00 and 23:00. Biopsied samples were fixed in phosphate-buffered glutaraldehyde, dehydrated with ethanol and embedded in Paraplast (Lancer, U.S.). Four-micron thick sections were stained with periodic acid-Schiff-hematoxylin.

The number of mitotic figures were counted in the basal cells of the epithelium and the mitotic indices were calculated. The student's t-test was used in statistical analyses.

Results

The results of the experiments showed the presence of a circadian rhythm of the mitotic index in sheep rumen epithelium (Fig. 1). The indices showed wide fluctuations ranging from 0.14 to 1.32%, from 0.28 to 0.95%, and from 0.25 to 1.45% in Exp. 1, 2 and 3, respectively.

In Exp. 1, sheep were fed with orchard grass hay at 18:00. The mitotic index showed the lowest value at 19:00, then increased and reached the peak between

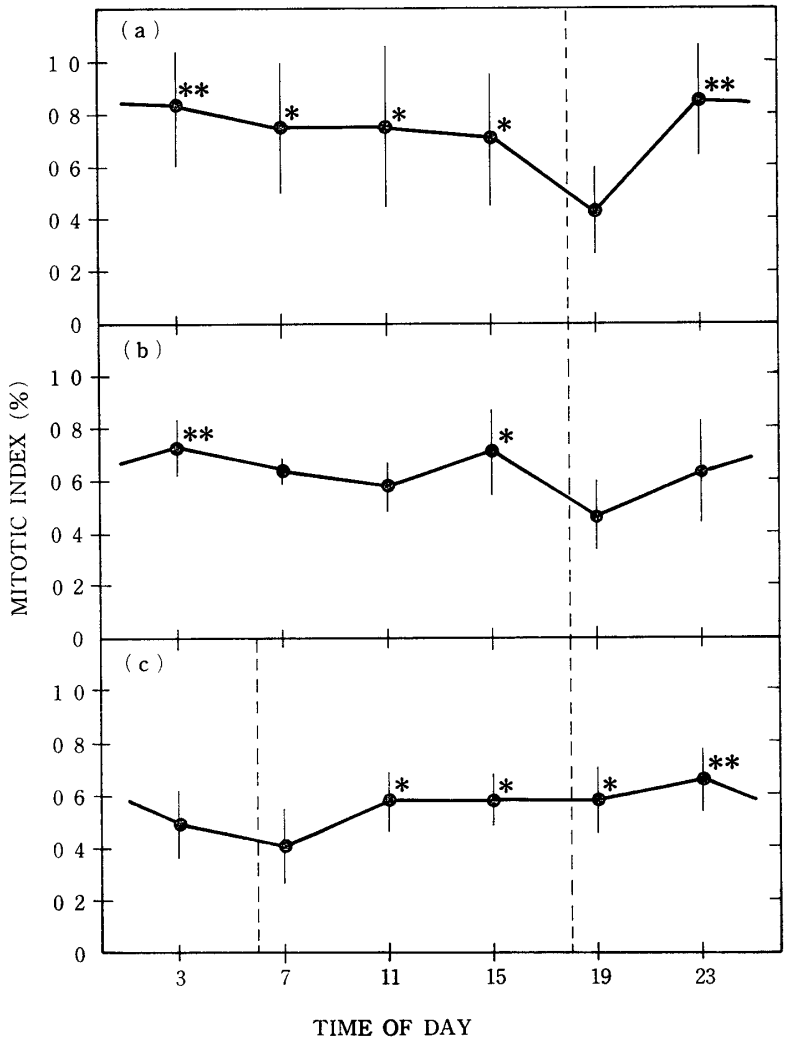

FIG. 1. Circadian rhythm of mitotic index in sheep rumen epithelium. Each point represents mean ± standard error of the mean. Borken line shows time of meal. Significantly (*: $p<0.05$, **: $p<0.01$) different from nadiar value at 19:00 (a, b) and at 7:00 (c).

23:00 and 3:00 (Fig. 1a). There were no differences between the mitotic indices at 3:00, 7:00, 11:00, 15:00 and 23:00.

In Exp. 2, in which sheep were fed with high concentrate type meal at 18:00, the circadian rhythm of the mitotic index showed a similar pattern with that in Exp. 1 (Fig. 1b), though the circadian variation was less clear than that in Exp. 1.

When sheep were fed twice daily at 6:00 and 18:00 (Exp. 3), the mitotic index did not show a two cycle pattern in a day (Fig. 1c). Nadiar value appeared at 7:00, 1 hr after the morning meal and peak value at 23:00, 5 hrs after the evening meal (Fig. 1c). The mean value and the variations of the mitotic index at every time in the circadian rhythm were reduced compared with that of once daily fed sheep (Exp. 1 and 2).

Discussion

In laboratory animals, the epithelia of the alimentary tract show circadian rhythms of cell proliferating activity expressed by the mitotic and labeling index as well as the mitotic rate (2–9). Sheep ruminal epithelium also showed the circadian rhythm of the mitotic index (1). But the pattern of the rhythms were quite different between the two experiments in which sheep were fed once daily at 7:00 or 11:00.

The circadian rhythms of the mitotic index obtained in the experiments of the once daily fed sheep (Exp. 1 and 2) in this study were similar to the pattern when sheep were fed at 7:00 in their report (1). In these rhythms, the mitotic indices showed the lowest values only 1 hr after the meal and maintained relatively high values during a day.

These results suggest that the food intake may supress the mitotic index or that some products of rumen fermentation may inhibit the cell proliferating activity in rumen epithelium. Treatment with Na-butyrate inhibits DNA synthesis of ruminal epithelial cells *in vitro* (10, 11). Sakata and Tamate (12, 13) reported that the volatile fatty acids (VFA) stimulated the mitotic activity in sheep rumen epithelium. In these reports, however, the rise of the mitotic index after the VFA injection into rumen was observed 24 hrs after the administration. Therefore, their conclusion is not directly usable for the circadian rhythm of the mitotic index in rumen epithelium.

In twice daily fed sheep (Exp. 3), the mitotic index showed a low value 1 hr after the morning meal, but the value did not decrease 1 hr after the evening meal. The first experiment of twice daily fed sheep was performed 4 weeks after a change from a once daily feeding in which the sheep were fed at 18:00 and in which the mitotic index showed the lowest value at 19:00. The nadiar value shifted from evening to morning when the feeding was changed as above. It is expected that in twice daily feedings, sheep reflected a light and dark cycle in nature,

because they are dirnal animals and they graze mainly after dawn and before twilight (14).

In sheep fed twice daily, the variation of the mitotic index in the circadian rhythm reduced and the rhythm appeared less clear than that in sheep fed once daily. This may relate to the fact that the frequent feeding reduces the dirnal variation in the concentration of ruminal metabolites including VFA (15, 16).

Proliferating activity of ruminal epithelium in sheep is stimulated by insulin infusion (17) and is influenced by infusion of catecholamines (18). Proliferation activity of intestinal epithelium is controlled by many factors including the neural and endocrine systems, as reviewed by Tutton (19). Sympathectomy causes the circadian rhythm of the mitotic index in intestinal crypts of laboratory animals to vanish (20, 21). In ruminal epithelium, too, the cell proliferating activity may be controlled by the endocrine and neural systems which are related to food intake and rumen fermentation products.

Acknowledgement

We thank Dr. T. Senshu for his suggestions and kind permission to use the automatic feeder. This study was partly supported by Grant-in-Aid for Scientific Research from the Ministry of Education of Japan (No. 56560274).

References

1) Sakata, T. and Tamate, H., *Res. Vet. Sci.*, **24**, 1 (1978)
2) Burns, E.R., Scheving, L.E., Fawcett, D.F., Gibbs, W.M. and Galatzan, R.E., *Anat. Rec.*, **184**, 265 (1976)
3) Hamilton, E., *Cell Tissue Kinet.*, **12**, 91 (1979)
4) Izquierdo, J.N. and Gibbs, S.J., *Exp. Cell Res.*, **71**, 402 (1972)
5) Neal, J.V. and Potten, C.S., *Cell Tissue Kinet.*, **14**, 581 (1981)
6) Scheving, L.E., Burns, E.R. and Pauly, J.E., *Am. J. Anat.*, **135**, 311 (1972)
7) Sigdestad, C.P., Bauman, J. and Lesher, S.W., *Exp. Cell Res.*, **58**, 159 (1969)
8) Tutton, P.J.M., *Virchows Arch. B. Zellpath.*, **13**, 68 (1973)
9) Wolfsberg, M.F., *Exp. Cell Res.*, **35**, 119 (1964)
10) Galfi, P., Veresegyhazy, T., Neogrady, S. and Kutas, F., *Zbl. Vet. Med. A*, **28**, 259 (1981)
11) Galfi, P., Neogrady, S. and Kutas, F., *Biol. Cell*, **42**, 103 (1981)
12) Sakata, T. and Tamate, H., *J. Dairy Sci.*, **61**, 1109 (1978)
13) Sakata, T. and Tamate, H., *J. Dairy Sci.*, **62**, 49 (1979)
14) Dulphy, J.P., Remond, B. and Theriez, M., "*Digestive Physiology and Metabolism in Ruminants*", ed. by Y. Ruckenbush and P. Thivend, MTP Press Ltd., Lancaster, England, pp. 103 (1980)
15) Bath, I.H. and Rook, J.A.F., *J. Agric. Sci.*, **61**, 341 (1963)
16) Jensen, K. and Wolstrup, J., *Acta Vet. Scand.*, **18**, 108 (1977)
17) Sakata, T., Hikosaka, K., Shiomura, Y. and Tamate, H., *Brit. J. Nutr.*, **44**, 325 (1980)
18) Ohwada, S. and Tamate, H., *Tohoku J. Agr. Res.*, **33**, 142 (1982)
19) Tutton, P.J.M., *Med. Biol.*, **55**, 201 (1977)
20) Tutton, P.J.M., *Virchows Arch. B Cell Path.*, **19**, 151 (1975)
21) Klein, R.M. and Torres, Jr. J., *Cell Tissue Res.*, **195**, 239 (1978)