CIRCADIAN RHYTHMS IN CONNECTIVE TISSUE

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The study of circadian rhythms in rat connective tissue showed that its cells (especially histiocytes) are characterized by circadian rhythms of their metabolic processes. This is manifested as a distinct diurnal dynamic of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activity. During adaptation to high-altitude hypoxia SDH and LDH activity was appreciably higher than in the control but the dynamics of changes in the activity of these enzymes during the 24-h period was similar in its general features to that in the control group. It is postulated that circadian metabolic rhythms in connective tissues are relatively stable: They show no sign of breaking down during adaptation to high-altitude conditions.

KEY WORDS: rat connective tissue; oxido-reductases; diurnal rhythm; adaptation to hypoxia.

There is now extensive literature on circadian rhythms in the cells and tissues of animals under physiologically normal and abnormal conditions, including during exposure to extremal factors such as hypoxia [1, 10]. However, data on changes in enzyme activity in the course of the 24-h period are still very limited [7] and they virtually do not exist for connective-tissue cells.

The object of this investigations was to study circadian rhythms of some enzymes in rat connective tissue under normal conditions and during adaptation to hypoxia.

EXPERIMENTAL METHOD

Noninbred male albino rats were used. The cellular composition of the subcutaneous connective tissue and adaptivity of various oxido-reductases (succinate dehydrogenase – SDH, lactate dehydrogenase – LDH, malate dehydrogenase – MDH, monoamine oxidase – MAO) and hydrolases were investigated.

Material was obtained from two groups of animals; those of group 1 (20 rats) were kept at an altitude of 770 m and those of group 2 (15 rats) were kept for 3 days in the Tyan'-Shan' Mountains at an altitude of 3200 m. To study the diurnal rhythm, three animals of each group were decapitated at each time at intervals of 6 h. Pieces of skin from the back were excised and fixed for 24 h in 12% formalin. Films were prepared from the subcutaneous connective tissue and stained with Weigert's iron-hematoxylin in Lillie's modification [4]. The number of the principal cell forms in the stained films was counted in areas between blood vessels and the results expressed as percentages for: fibroblasts, histiocytes, mast cells, and leukocytes. Histochemical analysis was carried out on fresh unfixed films of connective tissue. SDH activity was determined by Nachlas's method [8], activity of LDH, MDH, and NAD-diaphorase by Burstone's method [2], activity of MAO and acid and alkaline phosphatases by Pearse's method [5], ATPase as described by Padykula and Herman [9], and nonspecific esterase by the method of Bascy and Vadasz [6], and the results estimated visually according to a five-point system.

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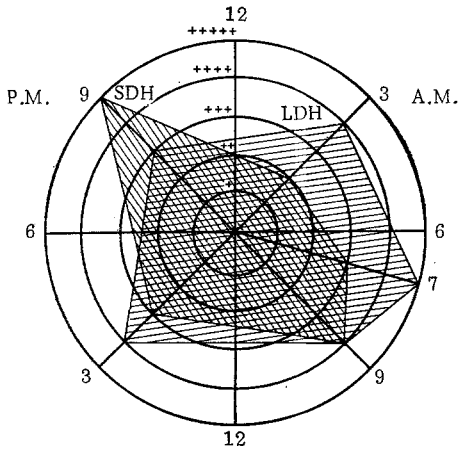

Fig. 1

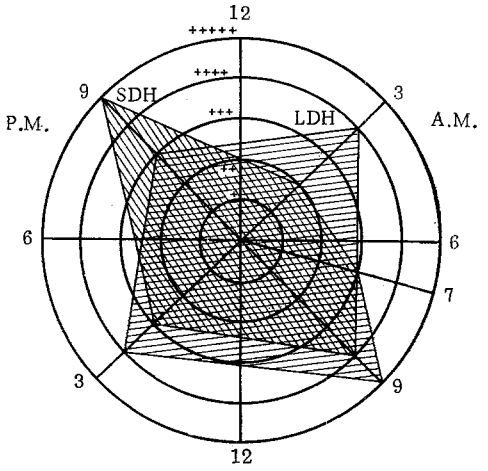

Fig. 2

Fig. 1. Circular graph of changes in diurnal activity of SDH and LDH in histiocytes (group 1). Here and in Fig. 2, time of day and night shown on outside ring. Plus signs show estimated enzyme activity on five-point system.

Fig. 2. Circular diagram of changes in diurnal SDH and LDH activity in histiocytes during adaptation to hypoxia (group 2).

EXPERIMENTAL RESULTS AND DISCUSSION

Determination of the relative percentages of the principal cell types in the intact rats of group 1 gave results similar to those in the literature [3]: fibroblasts 56.3%, histiocytes 40.5%, leukocytes 2.6%, mast cells 0.6%.

Histochemical analysis revealed substantial differences between the main types of cells as regards activity of the enzymes tested. The relative percentages of cells with high activity of certain oxido-reductases varied: For instance, cells with high NAD-diaphorase activity accounted for only 25% of the total number of cells, whereas the number of cells with high LDH and MDH activity was about equal, namely about 33%. Determination of diurnal changes in the cellular composition and activity of the enzymes studied yielded nonhomogeneous results. No definite changes could be found in the relative percentages of fibroblasts, leukocytes, and mast cells. However, investigation of the activity of oxido-reductases such as SDH and LDH in cells of the histiocytic series did reveal diurnal fluctuations. It follows from Fig. 1 that SDH activity rose steadily after 3 a.m. to reach a maximum during the evening at 9 p.m. The small decrease in the afternoon (3 p.m.) was not significant. LDH activity was highest in the morning (from 7 to 9 a.m.), after which it fell gradually until 9 p.m. It is interesting to note that LDH activity was higher than SDH activity during most of the 24-h period, and it was only in the evening that a narrow "peak" of SDH activity above the LDH level occurred. Changes in the diurnal activity of these enzymes in histiocytes were accompanied by changes in the number of cells with high activity of the enzymes specified: During the afternoon their total content was 15% lower than during the evening and night. As regards the fibroblasts, mast cells, and leukocytes, the diurnal rhythm of their metabolism was less marked than in histiocytes.

The changes in activity during the 24-h period can be regarded as a manifestation of the circadian rhythm of metabolism in connective-tissue cells. Should that be the case, they evidently ought to be preserved substantially unchanged in cells cultured in vitro. A maximum of LDH activity was found during the morning. Since rats lead a predominantly nocturnal mode of life and their activity is maximal at night, the possibility cannot be ruled out that the increase in LDH activity during the second half of the night and in the morning is an expression of a compensatory response of the connective tissue to an increased content of products of anaerobic glycolysis at this period of the 24 h. The relatively low SDH activity in the connective-tissue cells in this period can also be regarded as a manifestation of this compensatory response, aimed at reducing the oxygen deficiency for the parenchymatous cells by reducing the oxygen demand of the actual connective tissue. It is difficult at present to decide which of these alternatives is more likely to be correct.

Investigation of the connective tissue of animals during adaptation to high-altitude conditions (after a

stay of 3 days at an altitude of 3200 m) gave results similar with those obtained in the control group. Changes in the cellular composition of the connective tissue also were very slight. Activity of SDH, LDH, ATPase, and alkaline phosphatase in the connective-tissue cells (chiefly the histiocytes) was appreciably higher at this time than in the control but the dynamics of changes in the activity of these enzymes during the 24-h period repeated in its general features that in the control animals (Fig. 2). Just as in the control, LDH activity reached a maximum in the morning (by 9 a.m.) and during the greater part of the day it was higher than the SDH activity; only toward 9 p.m. did it fall below the SDH activity.

Circadian rhythms of connective-tissue metabolism are thus relatively stable: They show no sign of breaking down during adaptation to high-altitude conditions.

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