CHANGES IN MITOTIC ACTIVITY AND DESTRUCTION OF LYMPHOCYTES IN THE RAT THUMUS AFTER HYDROCORTISONE ADMINISTRATION AT DIFFERENT TIMES OF DAY

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A similar type of diurnal rhythm of the number of mitoses was found in the lymphocytes of the thymus of 30-day-old rats 4 h after injection of hydrocortisone and in control animals. The number of dying lymphocytes in the control rats did not change during the 24-h period. The number of destroyed cells rose sharply 4 h after injection of hydrocortisone, but the degree of destruction of the lymphocytes was greater during the night and early morning than during the afternoon and evening.

KEY WORDS: diurnal rhythm of mitosis; thymus lymphocytes; hydrocortisone; destruction of lymphocytes.

Certain unfavorable factors which increase the secretion of glucocorticoid hormones by cells of the adrenal cortex are known to reduce the number of mitotically dividing cells in various tissues and, in particular, in the organs of the lymphoid system. At the same time it has been shown that if the intensity of the factor is high, mortality of the lymphocytes is increased by a degree which also depends on the age and state of the organism. Naturally, to assess the complex dynamics of proliferative and destructive processes in lymphoid tissue it is essential to have quantitative data characterizing these processes. It has been shown in a few published papers that there is a diurnal rhythm of mitotic activity in the organs of the lymphoid system. Quantitative changes in cell destruction in the course of the 24-h period has been investigated to a much smaller degree. There is an almost complete absence of data on the possible unequal sensitivity of lymphocytes to the action of glucocorticoid hormones in different sections of the 24-h period. Yet with regard to other tissues it has been conclusively shown that various inhibitors and stimulators of cell multiplication differ in their effects on mitotic activity when used at different times of day, i.e., in different phases of the diurnal rhythm of mitosis or the rhythm of the other phases of the mitotic cell cycle [4].

The object of this investigation was to study the diurnal rhythm of mitotic activity and of processes of thymocyte destruction in albino rats under normal conditions and after injection of hydrocortisone.

EXPERIMENTAL METHOD

Experiments were carried out on 67 albino rats aged 30 days. Hydrocortisone was injected intraperitoneally into the experimental rats as a single dose of 5 mg/100 g body weight 4 h before sacrifice, at 10 a.m., 2, 6, and 10 p.m., and 2 and 6 a.m. Control rats received an injection of the corresponding volume of physiological saline at the same time of day. The thymus was fixed in Carnoy's fluid and sections (5 μ) were stained with Carazzi's hematoxylin.

The subcapsular zone of the cortex of the thymus was investigated by examining 10,000-15,000 cells in each case. The mitotic index (MI) was determined in promille and the indices of pycnotic nuclei were calculated in percent, using cells with clearly defined pycnotic nuclei and also large, darkly stained granules arising as a result of death of the thymocytes, and not less than 0.3-0.5 of the diameter of the nuclei of normal lymphocytes in diameter.

The experiments were carried out in June. All quantitative data were subjected to statistical analysis by the Fisher–Student method.

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TABLE 1. Diurnal Changes in MI in Cortical Cells of the Rat Thymus under Normal Conditions and after Injection of Hydrocortisone at Various Times of Day and Night

Time	Control		After injection of hydrocortisone		Normal response
	MI, ‰	P	MI, ‰	P	
2 p.m.	4,7	0,01	6,2	0,04	0,03
6 p.m.	7,2	0,39	8,0	0,50	0,56
10 p.m.	6,0	0,01	7,0	0,001	0,06
2 a.m.	3,9	0,17	4,4	0,38	0,17
6 a.m.	4,6	0,63	5,0	0,44	0,34
10 a.m.	4,3		4,5		0,34
Mean MI for 24-h period	5,1		5,9		0,39

TABLE 2. Diurnal Changes in Destructive Processes in Cortex of Thymus of Rats Aged 30 Days under Normal Conditions and after Injection of Hydrocortisone at Different Times of Day and Night

Time	Percentage of pycnotic nuclei	
	control	after injection of hydrocortisone
2 p.m.	0,5	24
6 p.m.	0,4	26
10 p.m.	0,3	20
2 a.m.	0,4	45
6 a.m.	0,5	50
10 a.m.	0,5	30
Mean value for 24-h period	0,4	32

EXPERIMENTAL RESULTS

The data given in Table 1 show that a diurnal rhythm of MI was present in the control rats of this age, and can be represented by a monomodal curve reaching a maximum of the number of mitoses at 6 p.m. and a minimum at 2 a.m. ($P = 0.002$).

These results do not agree with those obtained by other workers, who found a maximal number of mitoses in the cortex of the thymus of adult rats [2, 9, 10, 12, 13] and adult mice [1, 8, 11] in the morning and a minimal number in the evening. These differences in the results could be explained by age changes in the character of the rhythm of mitoses. It has been shown [3, 5] that in the early stages of postnatal ontogeny there is a regular reorganization of the character of the rhythm of mitosis in several tissues, leading to the establishment of the definitive rhythm. Evidence of the instability of the mitotic rhythm in the thymus of young rats (aged 28 days) is given by observations [6, 7] showing that they are most numerous at 1-2 p.m. and least numerous at 3 p.m., although the number of mitoses is also considerable between 5 and 10 p.m.

After injection of hydrocortisone 4 h before sacrifice of the animals (Table 1) no change was found in the character of the mitotic rhythm or in the mean-daily mitotic activity of the thymocytes. These findings agree with those of an investigation [6] which showed that when the same doses of hydrocortisone were given to rats aged 28 days, a significant decrease in MI was found only 12-16 h after injection. However, if much larger doses of cortisone were given, a sharp decrease in the number of mitoses in cells of the thymus was found 4 h after its injection [1].

The results giving the characteristics of destructive processes are given in Table 2.

They show that in the control animals the number of dying lymphocytes remained virtually unchanged

during the 24-h period. The mean index of pycnotic nuclei for the 24-h period was 0.4%. This result is close to that of an investigation [12] which showed that the number of pycnotic cells in the thymus of mice aged 24 weeks is 0.8-0.9%. On the other hand, it has also been shown [11] that in the subcapsular zone of the mouse thymus there are differences in the number of pycnotic lymphocytes at different times of the 24-h period, with a maximum (1.1%) at 6 p.m. and a minimum (0.56%) at 6 a.m.

The results of the present experiments showed that 4 h after injection of hydrocortisone the percentage of dying cells rose sharply (Table 2). Despite considerable individual differences in the number of these cells, the mortiality of the cells varied at different times. In the late morning, afternoon, and evening (10 a.m.-10 p.m.) the mortality of the cells was lower than at night and in the early morning (2 and 6 a.m. ($P = 0.004$). These results support the view that the sensitivity of the thymocytes to the destructive action of hydrocortisone differs at different times of the day and night.

The smallest numbers of dying lymphocytes were found to occur at about the same time as the highest values of MI, and vice versa. This suggests that cells in the premitotic phase of the cycle are most sensitive to the harmful action of hydrocortisone.

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