Circadian Variations in the Metabolic Reaction of Human Blood Lymphocytes to Hormonal Stimuli in Health and Immunodeficiency

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Circadian variations in the sensitivity of blood lymphocyte succinate dehydrogenase to thymalin and hydrocortisone are shown. The maximal sensitivity to thymalin is observed in the morning, and to hydrocortisone in the evening. The range of fluctuations in this parameter is wider in patients with immunodeficiency than in donors. Moreover, the direction of the lymphocyte metabolic reaction to the studied agents was found to be disturbed in the patients. The findings may be useful for designing chronotherapeutic schemes with the use of hormonal immunomodulating agents.

Key Words: *circadian variations; succinate dehydrogenase; lymphocytes; hormones; immunodeficiency states*

The functioning of the immune system is known to be regulated by endocrine factors, the leading role in this process being played by thymic hormones and glucocorticoids [6-8]. It should be noted that the results of studies of the immunotropic effects of the above hormones are rather contradictory. Both thymosine and glucocorticoid hormones have been found to act both as immunostimulating and immunosuppressive agents, depending on their concentration in the body, the status of the immune system, the experimental conditions, and other factors [1,2,5]. The type of the immunomodulating effect of hormones may be related to the status of the lymphocyte receptor system and the direction of hormone-induced metabolic shifts in the cell. Hence, it is impossible to understand the mechanisms of endocrine immunoregulation without investigating the specific features of the lymphocyte reaction to hormones under various conditions of hormonal exposure. In this work we studied changes in the activity of blood lymphocyte succinate dehydrogenase (SDH) after incubation of lymphocytes with thymalin and hydrocortisone at various times of the day in donors and patients with T-cell immunodeficiency.

MATERIALS AND METHODS

Thirty women aged 22 to 40 (20 healthy and 10 with inflammatory gynecologic diseases) were examined. Blood was collected from the ulnar vein twice a day, at 10 o'clock in the morning and at 8 o'clock in the evening. The T-lymphocyte subpopulation was estimated by the E-rosette formation test [3]. The total count of T cells (total E-rosette-forming cells, E-RFC), the content of T helpers (early E-RFC), T suppressors (recovered E-RFC), and poorly differentiated T cells (complex E-RFC), and the T helper/T suppressor ratio were assessed. Lymphocyte sensitivity to immunomodulators was assessed by changes in the activity of cytochemically detected SDH in lymphocytes after

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TABLE 1. Content of E–RFC per 100 Mononuclear Cells in Venous Blood of Donors and Patients in the Morning and Evening ($M \pm m$)

Time of investigation, h	Total T-cell count	Cell counts			T helpers/T suppressors
		T helpers	T suppressors	poorly differentiated T cells	
		Donors			
10	46±2.71	33±2.29	26±1.69	41±1.37	1.34±0.12
20	42±1.96	34±2.29	26±2.39	37±2.55	1.51±0.15
		Patients			
10	35±3.99	26±2.88*	23±3.21	30±2.22*	1.17±0.16
20	34±4.12*	24±2.09*	20±2.42	28±3.78*	1.19±0.10

Note. Asterisk shows reliable differences ($p<0.05$) *vs.* the relevant group of donors.

TABLE 2. Circadian Variations of Lymphocyte Sensitivity to Thymalin and Hydrocortisone in Donors and Patients with Immunodeficiency

Agent	Time of investigation, h	Examinees whose lymphocytes reacted with a change of SDH, %	
		donors	patients
Thymalin	10	40	71
	20	30	14
Hydrocortisone	10	55	29
	20	61	80

Note. The total number of examinees is 100%.

incubation of blood with thymalin and hydrocortisone [2,4]. The enzyme activity was assessed by the mean number of formazan granules per lymphocyte. The results were statistically processed using Wilcoxon-Mann-Whitney's test.

RESULTS

T-cell immunodeficiency manifesting by a reduction of the relative content of total, early, and complex E-RFC was detected in all the patients examined. The changes in the composition of the T-cell subpopulation were more pronounced in the evening hours (Table 1).

A study of lymphocyte sensitivity to thymalin revealed that in the morning the lymphocytes of the majority of examinees reacted to incubation with the agent by changes in SDH activity (Table 2), circadian fluctuations in this parameter being greater in the patients.

In both donors and patients blood lymphocytes reacted to thymalin by either a reduction or an increase of SDH activity (Table 3). The direction of this reaction in donors depended on the baseline activity of the enzyme. When the mean content of SDH was no more than 10.6 granules per lymphocyte, thymalin increased it, whereas at a mean level of at least 11.5 granules the agent reduced it. Such a relationship was not observed in the patients. This may be indicative of a disordered metabolic reaction of lymphocytes to factors of endocrine regulation in immunodeficiency.

A study of lymphocyte sensitivity to hydrocortisone demonstrated that this parameter was also liable to change in the course of 24 h, the difference between the morning and evening values being greater in the patients (Table 2). In the morning SDH activity was reduced under the influence of hydrocortisone both in normal subjects and in patients. Conversely, in the evening the lymphocytes of the majority of donors reacted to hydro-

TABLE 3. Circadian Variations in the Direction of Lymphocyte Reaction to Thymalin and Hydrocortisone in Donors and Patients

Agent	Time of investigation, h	Change of SDH activity	Examinees showing a definite direction of SDH reaction, %	
			donors	patients
Thymalin	Increase	10	25	40
		20	43	–
	Decrease	10	75	60
		20	57	100
Hydrocortisone	Increase	10	–	–
		20	27	75
	Decrease	10	100	100
		20	73	25

Note. The total number of examinees sensitive to the agent is 100%.

cortisone by a reduction of SDH, whereas in the majority of patients the response to this agent was an increase of enzyme activity (Table 3).

Hence, similarly as with thymalin, the lymphocytes of patients with immunodeficiency demonstrated an altered metabolic reaction to glucocorticoids in comparison with the lymphocytes of normal subjects. It is possible that a disturbed pattern of cellular reaction to endocrine stimuli is one of the pathogenetic mechanisms underlying the development of an immunopathological process.

The results demonstrate circadian variations in metabolic reactions of lymphocytes to regulatory influences and their individual nature both in donors and in patients with disturbed function of the immune system. The wider range of circadian fluctuations of this parameter in the patients may be indicative of increased synchronization of biorhythms of their immune system functioning and be a sign of stress and lowered compensatory potential.

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