Daily Variations of Epinephrine, Norepinephrine, and β-Adrenoceptors in the Blood and Lymphoid Organs of Intact Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 128, No. 9, pp. 344-346, September, 1999
Original article submitted September 16, 1999

Daily variations of catecholamine concentrations in the blood and lymphoid organs in Wistar rats were revealed. Daily fluctuations of epinephrine and norepinephrine levels in the spleen and blood were synchronous. Circadian variations of epinephrine in the thymus, lymph nodes, and plasma were synphasic. A relationship between neurotransmitter concentrations and expression of β-adrenoceptors on thymic and splenic lymphocytes was noted.

Key Words: *epinephrine; norepinephrine; β-adrenoceptors; biorhythms; lymphocytes*

Neuroimmune relationships is a pressing problem within the framework of regulation of the immune functions. At present, several neuroendocrine pathways affecting the immune homeostasis are distinguished, one of which is the hypothalamus—*fasciculus longitudinalis*—postganglionar sympathetic fibers—lymphoid cells axis [8]. The sympathetic component of the autonomic nervous system is widely presented in lymphoid organs [4], and catecholamines produced by nerve endings regulate proliferation and differentiation of immunocompetent cells via specific membrane receptors [7,9,11]. On the other hand, lymphoid cells produce neuroactive substances, including catecholamines [2]. The role of lympho- and monokines in the realization of immunoneuroendocrine relationships has been demonstrated [6].

The functions of the immune and neuroendocrine systems are characterized by biological rhythms of activity [3,10]. However, reports about the temporal aspects of neuroimmune relationships are scanty. We investigated the circadian rhythms of epinephrine, norepinephrine (NA), and β-adrenoceptor (β-AC) levels in the blood and lymphoid organs.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 150-250 g (Rappolovo Breeding Center). The animals were kept in a vivarium with constant light-darkness regimen (light 8.00-20.00, darkness 20.00-8.00). The rats were decapitated at 7.00, 11.00, 13.00, 15.00, 19.00, 23.00, 1.00, and 3.00. Blood was stabilized with 10% EDTA, the plasma was separated by centrifugation and frozen. Lymphoid organs (thymus, spleen, and lymph nodes) were isolated immediately after decapitation, weighed, and frozen in liquid nitrogen. The levels of epinephrine and NA in the blood and lymphoid tissue were evaluated by high-performance liquid chromatography with electrochemical detection [12]. Catecholamines were extracted from lymphoid tissue homogenates and plasma by adsorption on aluminum oxide in alkaline Tris-buffer followed by desorption with 0.2 M $HClO_4$ [1]. For β-AC assay, the thymus and lymph nodes were gently crushed in a glass homogenizer on the cold, blood and splenic lymphocytes were isolated by centrifugation in Ficoll-Uropolin density gradient (d=1.076). Tritium-labeled

0007-4888/99/0009-0956$22.00

β-adrenoblocker dihydroalprenolol was used as a β-AC ligand. Radioligand assay was carried out using a previously described method [5] with minor modifications. The results were processed using the Student, Wilcoxon—Mann—Whitney, and Spearman rank correlation tests.

RESULTS

Circadian variations in the level of catecholamines in the plasma and lymphoid organs were revealed. In the plasma, a significant increase in epinephrine and NA levels was recorded at 15.00 and their minimum level at 3.00. In the thymus and mesenteric lymph nodes, the content of NA little varied over 24 h, while epinephrine level in the thymus increased at 13.00-19.00 and gradually decreased at 23.00-3.00. The highest level of epinephrine in lymph nodes was recorded at 7.00. Synchronous changes in epinephrine and NA levels were detected only in the spleen (r=0.78, p<0.05), where the minimum concentrations of both neurotransmitters were observed at 11.00 and the maximum at 19.00 for NA and at 21.00 for epinephrine (Fig. 1).

Analysis of correlations showed a synchronous time course of epinephrine concentration in the thymus, mesenteric lymph nodes, and plasma (r=0.67, p<0.05). Circadian changes in NA level in lymphoid organs and blood did not correlate.

The mean daily levels of both neurotransmitters were the highest in the spleen and the lowest in the plasma (Fig. 2).

β-AC assay in lymphocytes at 11.00 showed that cells of different lymphoid organs express different number of β-AC: 15,620 in the spleen, 7640 in lymph nodes, and 3500 in the thymus, while their dissociation constants (K_d) were 12.4, 11.4, and 12.5 nM, respectively. At 15.00, the number of β-AC in the

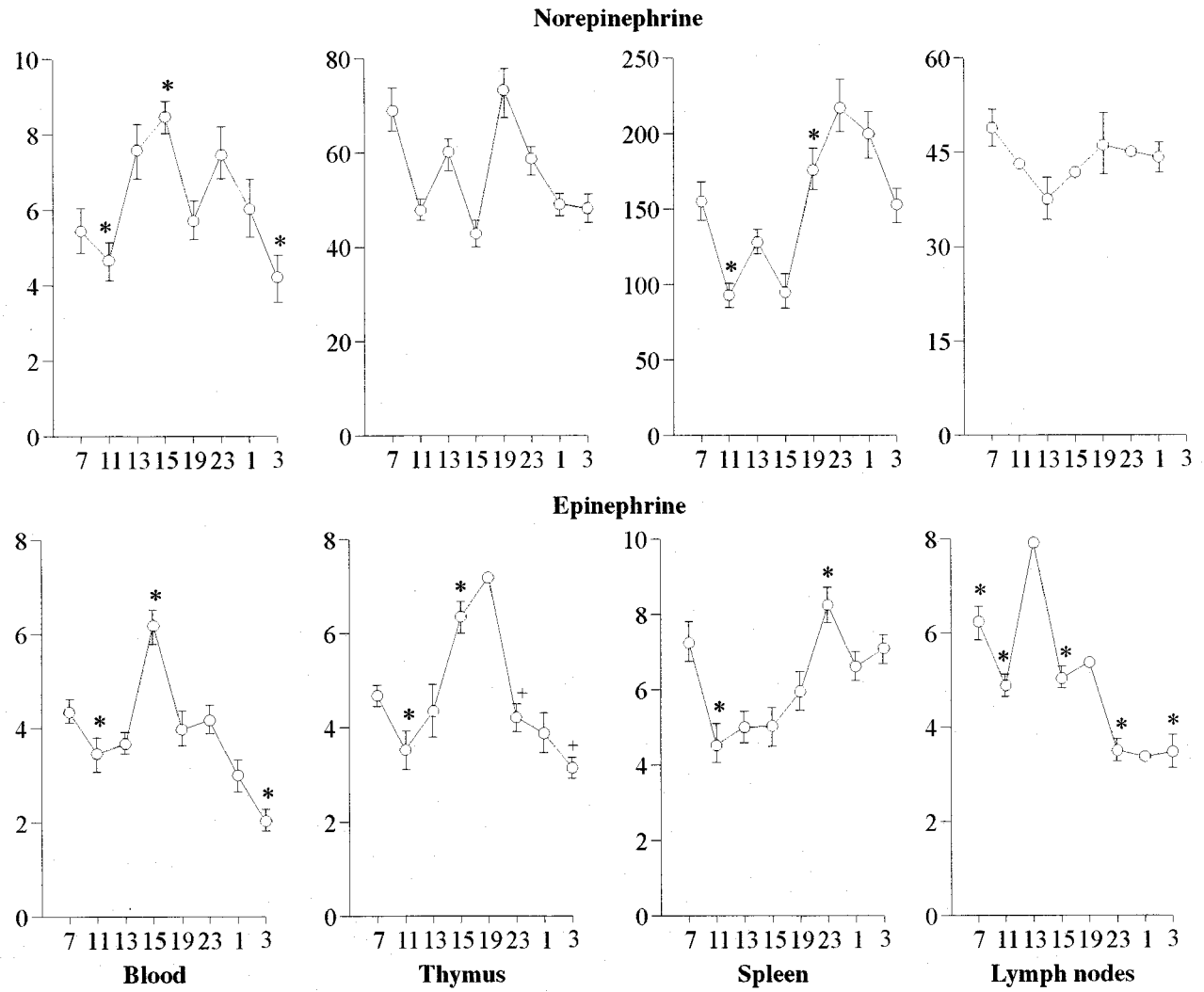

Fig. 1. Circadian rhythms course of epinephrine and norepinephrine concentrations in the blood and lymphoid organs of Wistar rats. Abscissa: time, *,+Differences are significant (p<0.05). Here and in Fig. 2: Ordinate: neurohormone content in the plasma (ng/ml) and lymphoid organs (ng/g).

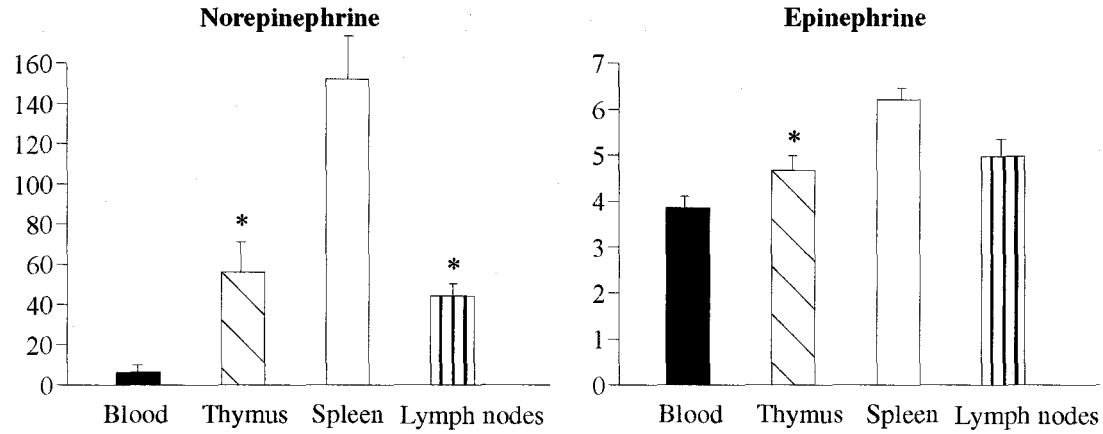

Fig. 2. Mean 24-h content of epinephrine and norepinephrine in the plasma (ng/ml) and lymphoid organs (ng/g) of Wistar rats. *$p<0.05$ *vs.* the spleen.

thymus increased to 6740 receptors/cell, and simultaneously high-affinity receptors appeared (K_d=0.73 nM, up to 1900 receptors/cell).

A relationship between the concentration of neurotransmitters and the number of β-AC on thymocytes was revealed: the rise in epinephrine concentration in the thymus (at 15.00) was paralleled by an increase in the number of β-AC on thymocytes. Hence, potential response of immunocompetent cells to a regulatory stimulus from the sympathetic nervous system is determined by the concentration of the corresponding neurotransmitter in this lymphoid organ at a certain time of the day.

These findings are in line with our previous data on circadian rhythms of the immune response in mice [3]: minimum formation of antibody-producing cells in the spleen was observed after immunization in the evening and at night. Assuming that mice and rats have the same biorhythms of physical activity and similar circadian rhythms of catecholamine concentrations in lymphoid organs and blood, we can see a negative correlation between the level of immune response and epinephrine and NA concentrations in the spleen at the moment of immunization. This agrees with the data on increased immune response and interleukin production in mice after chemical sympathectomy [9].

Thus, chronobiological parameters of the immune and neuroendocrine systems are closely related, which is important for the regulation of the immune status and immune reactions.

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