EXPERIMENTAL BIOLOGY

Effect of Epiphysectomy on the Circadian Dynamics of Antiradical Activity of Chalone-Antichalone System of Albino Rat Liver

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Antiradical activity of the chalone-antichalone system in the liver of albino rats is characterized by circadian rhythms. The production of tissue regulators of proliferation is synchronized with the 24-h light/darkness cycle. Active phase of the rhythm of antiradical activity falls on night hours. Epiphysectomy caused disappearance of the circadian rhythm of activity of the liver chalone-antichalone system in albino rat.

Key Words: *epiphysectomy; antiradical activity; chalones*

One of the key problems of experimental chronobiology is to clear out the mechanisms regulating circadian rhythms of mitotic activity of tissues. Studies of proliferation regulation revealed tissue specific inhibitors of DNA synthesis and mitosis, chalones. Chalone antagonists stimulating proliferation were detected (antichalones, or growth factors). Chalones and antichalones are components of the same dynamic chalone-antichalone system (CAS) [6]. The proportion of their concentrations determines the level of proliferative activity and maintains cell homeostasis in the tissue.

The formation of proliferation biorhythms can be realized through rhythmic production of tissue regulators of cell division [1,5]. The production of chalones of all interphase periods (G_1, S, and G_2) is characterized by circadian rhythms.

Rhythmic activity of the proliferative system is regulated at different levels [1,2]. It seems that the effects of whole-body regulators of proliferation should be realized through the tissue regulator system [9], which can be seen from organ and tissue specificity of cell multiplication rhythms.

The presence of circadian biorhythm (CR) of chalone production suggests the involvement of the epiphysis in its formation; it is assumed to play the leading role in the regulation of the circadian and seasonal biorhythms of body functions [3,4].

We studied the role of the epiphysis in the formation of tissue proliferation regulators production CR.

MATERIALS AND METHODS

Experiments were carried out on 96 outbred male albino rats (170-200 g). The animals were adapted to light:darkness 12:12 regimen (day from 6.00 to 18:00) for 20 days before the experiment and had free access to water and food during the experiment. After adaptation period, the rats were divided into 2 groups: control (n=48) and experimental (n=48). Epiphysectomy (resection of a fragment of the bone with the underlying epiphysis) was carried out in experimental animals. All painful manipulations

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were carried out in accordance with regulations on work with experimental animals. The animals were decapitated under ether narcosis on day 40 after epiphysectomy every 3 h from 18.00 till 15.00.

Biorhythms of tissue regulators of proliferation were studied on albino rat liver, characterized by homogeneity of tissue composition. The livers were removed, frozen in liquid nitrogen, and stored in a freezer. Complex extracts of liver chalone and antichalone were prepared by cleansing the liver lobes from the connective tissue capsule and homogenizing the tissue. Water-soluble components were removed from the liver with distilled water and subjected to centrifugation and fractionation with cold (4°C) ethanol. The fraction obtained by precipitation between 70 and 87% saturation was used.

The CAS has emerged as a complex of antioxidant defense from free radicals excess; its components are characterized by potent antiradical activity [8]. This characteristic underlies the definition of biological activities of hepatic chalone and antichalone preparations. Antiradical activity of hepatic chalone and antichalone preparations was evaluated by chemiluminescence quenching in a system generating free radicals and expressed in arbitrary units per mg protein. It is known that hepatic chalone reduces, while antichalone increases the antiradical activity of a system generating free radicals [7]. The mixture of hepatic chalone and antichalone was prepared and their antiradical activity was evaluated at Department of Medical Biology and Genetics (headed by Professor A. N. Pashkov) of N. N. Burdenko State Medical Academy, Voronezh.

The results were statistically processed using the Fisher—Student method. The CR and ultradian rhythm (UR) of the liver CAS antiradical activity were detected and their periods were evaluated by two methods in parallel: spectral analysis of antiradical activities and the least squares method.

RESULTS

Periodical changes in the liver CAS antiradical activity were detected in control rats; these changes were characterized by a monophasic rhythm throughout 24 h (Fig. 1, *a*). The active phase was observed during the night hours. The liver CAS antiradical activity was maximum at 21.00 and significantly ($p<0.05$) surpassed the minimum values at 14.00. The mean liver CAS activity during the dark phase (479±13 arb. units/mg protein) was higher ($p<0.05$) than that during the light phase (232±32 arb. units/mg protein). The data of spectral analysis and analysis by the method of least squares indicate rhythmic organization of hepatic CAS antiradical activity with a period approximating 24 h in intact animals. The results of analysis by the method of least squares showed the presence of a UR (with a period of about 7 h).

Epiphysectomy led to disappearance of the CR of liver CAS antiradical activity. Fluctuations in the antiradical activity throughout 24 h exhibited no relation to the light/darkness hours (Fig. 1, *b*). The common monotonous fluctuations of antiradical activity had two statistically negligible peaks at 24.00 and 9.00. The mean antiradical activity of the liver CAS was statistically the same during the light (589±61 arb. units/mg protein) and dark phases (525±75 arb. units/mg protein). The results of spectral analysis and analysis by the method of least squares showed the absence of CR and a clear-cut UR with a period of about 9.5 h.

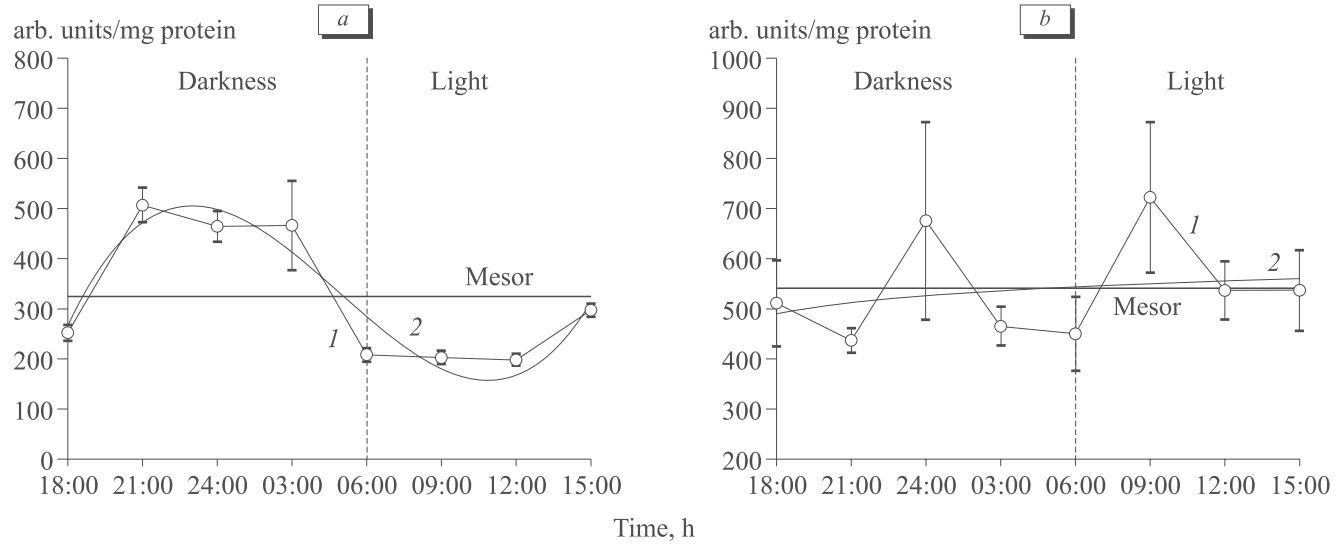

Fig. 1. Time course of rat liver CAS antiradical activity. *a*) control; *b*) experiment. *1*) antiradical activity; *2*) smoothed curve.

Hence, removal of the epiphysis lead to dissociation of the circadian constituent of the liver CAS activity and illumination conditions and to complete disappearance of the CR of antiradical activity on day 40 after epiphysectomy. These data indicate that the modulating effect of the epiphysis on the formation of proliferation CR is realized through CAS. On the other hand, emergence of the proliferation UR seems to be due to the cyclic pattern of metabolic processes in tissues. This is confirmed by the fact that the dynamics of antiradical activity of the liver CAS in epiphysectomied animals is characterized by UR alone.

As proliferation stimulators and inhibitors are present in tissues simultaneously, the final result of their effects is determined by their proportion. Since the hepatic chalone inhibits LPO and the antiradical component of the free-radical system, while antichalone stimulates antiradical activity [7], it seems that during the night hours, in the active phase of antiradical activity of the liver CAS, the chalone/antichalone proportion is shifted towards the latter. Passive phase of the antiradical activity CR observed during the daytime is caused by reduced concentration of antichalone and predominance of the chalone component. This assumption is in line with published data on circadian dynamics of chalone content in tissues, but is in need of experimental verification.

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