AUTORADIOGRAPHIC INVESTIGATION OF PROTEIN SYNTHESIS IN MYOCARDIAL MUSCLE AND INTERSTITIAL CELLS DURING FORMATION OF COMPENSATORY HYPERFUNCTION OF THE HEART

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KEY WORDS: interstitial component; heart; emergency stage of compensatory hyperfunction.

In response to an increased functional load the heart develops compensatory hyperfunction, which may lead to hypertrophy. In the initial stage of hyperfunction, the mechanisms of urgent adaptation of the heart come into play. This stage is generally called the emergency stage [2, 3]. In the emergency stage protein has been shown to accumulate in the myocardium [4, 10-13, 15]. In the few investigations by autoradiography which have been undertaken an increase in incorporation of labeled precursors of protein synthesis into cardiomyocytes has been found [7-9, 14]. The intensity of labeling of the interstitial component of the myocardium was not, however, analyzed by the workers cited above. Nevertheless, the present writers showed previously that interstitial cells participate directly in the formation of hypertrophy of the heart [5, 6].

The object of this investigation was to study protein synthesis in myocardial muscle and interstitial cells in intact animals and during the formation of compensatory hyperfunction of the heart.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male August rats weighing 130-170 g. Coarctation of the abdominal aorta was used as a model with which to study compensatory hyperfunction of the heart. The operation was performed under pentobarbital anesthesia (0.05 mg/g body weight) by the method described in [7]. The diameter of the probe was 0.71 mm.

For autoradiography the animals were divided into two equal groups, in each of which five intact rats were used as the control. The animals of group 1 were given an intraperitoneal injection of a solution of [5-^{3}H]uridine in a concentration of 7 μCi/g body weight 3 h before sacrifice. The rats of group 2 were given an intraperitoneal injection of [2-^{3}H]-leucine in a concentration of 6 μCi/g body weight 1 h before sacrifice. The material was collected 1, 5, and 10 days after coarctation of the aorta. These times of the experiment correspond to the emergency stage of compensatory hyperfunction of the heart [2, 3]. Five specimens of tissue measuring 1 mm^3 were taken from the wall of the left ventricle. The specimens were fixed by Karnovsky's method [1], postfixed with OsO_4, and embedded in epoxide resins. Three sections 1 μ thick also were cut from each block for autoradiography. The sections were mounted on slides and covered with type M nuclear emulsion. The autoradiographs were exposed for 16 weeks, then developed, stained with an aqueous solution of toluidine blue, and mounted in synthetic resin.

The autoradiographs were analyzed by counting grains of silver above the various myocardial cells. The material was studied under the microscope with a magnification of 900.

EXPERIMENTAL RESULTS

Data on incorporation of labeled leucine into the various components of the myocardium are given in Figs. 1 and 2.

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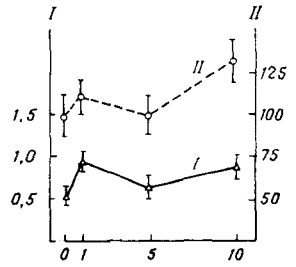

Fig. 1

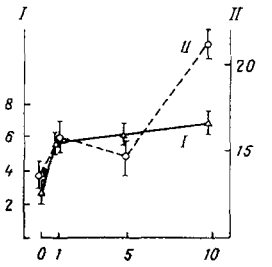

Fig. 2

Fig. 1. Changes in intensity of labeling of rat cardiomyocytes during formation of compensatory hyperfunction of the heart. I) Uridine (Ag/visual field); II) leucine (Ag/9600 μ^2). Here and in Fig. 2, abscissa: time after operation (in days).

Fig. 2. Changes in intensity of labeling of interstitial component of rat myocardium during formation of compensatory hyperfunction of the heart. I) Uridine (Ag/cell); II) leucine (Ag/9600 μ^2).

On analysis of the results the biphasic character of incorporation of labeled leucine into the cardiomyocytes was apparent (Fig. 1). The first peak of labeling was observed 24 h after coarctation of the aorta. At that time the number of silver grains per area of one field of vision (9600 μ^2) was 110.23 ± 7.31 ($P < 0.04$), which corresponded to an increase in incorporation of labeled leucine by 19% compared with the control. Later the intensity of labeling fell, virtually to the control level (91.93 ± 4.78), and this was followed by an increase in incorporation of leucine to 131.21 ± 0.35 ($P < 0.001$), i.e., by 42%.

As regards the interstitial component of the myocardium, at the beginning of the emergency stage no significant changes could be found in it; nevertheless, a certain trend similar to that in the muscles could perhaps be detected. A significant increase in incorporation of the label into protein in the interstitial cells could not be found until the 10th day of the experiment. During this period labeling of the interstitial cells increased by 60% compared with the control (Fig. 2).

The time course of RNA labeling during the formation of compensatory hyperfunction of the heart differed for the muscle cells and interstitial cells of the myocardium.

The curve of uridine incorporation into the muscle cells resemble in its shape that for labeled leucine, but the intensity of labeling of RNA 24 h after coarctation of the aorta was was 76% higher than the control.

Activity of labeled RNA in the interstitial cells rose steadily from 2.74 ± 0.090 grains of silver per cell in the control to 6.57 ± 0.130 ($P < 0.001$) at the end of the emergency stage of compensatory hyperfunction. The increase in incorporation of labeled precursors of RNA synthesis by 105% compared with the control, taking place 24 h after coarctation of the aorta, and the subsequent increase in this value to 139% point to reorganization of the metabolism of the interstitial cells.

This investigation thus revealed an increase in the incorporation of labeled precursors of protein synthesis into the muscle and interstitial cells of the myocardium at all times of the emergency stage. The increase in the level of functional activity of the interstitial cells was probably due to their participation in the formation of adaptive reactions and the need to strengthen the connective-tissue skeleton of the heart at a time when activity of all structures of the myocardium is intensified.

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EFFECT OF ENDOGENOUS GLUCOCORTICOIDS ON MORPHOLOGY OF THE MOUSE THYMUS AND SPLEEN

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Dependence of T cell reactivity to transplantation H-Y antigens on the physiological level of endogenous glucocorticoids (corticosterone) in inbred mouse lines was demonstrated previously [6, 7]. A decrease in the level of endogenous glucocorticoids in adrenalectomized mice is accompanied by widening of the cortex of the thymus and the appearance of an immune response to H-Y antigens [4]. The thymus affects the abundance of the T-dependent zones in the white pulp of the spleen [9] and controls differentiation of stem cells in the spleen toward myelopoiesis or erythropoiesis [5]. The cortex of the thymus contains cortisone-sensitive precursors of T lymphocytes, whereas the medulla of the thymus contains cortisone-resistant precursors of T lymphocytes. Precursors of T lymphocytes migrate from the thymus into the spleen, where they are converted into mature immunocompetent cells [10].

This paper describes a study of the possible dependence of the ratio between the cortex and medulla of the thymus and the white and red pulp of the spleen on the endogenous corticosterone level in inbred lines of mice of haplotypes $H\text{-}2^k$ and $H\text{-}2^b$.

EXPERIMENTAL METHOD

Female CBA, C57BL/6, and AKR mice aged 3-4 months, which differed in their reactivity to H-Y antigens of skin grafts [7, 11], were used. Mice were obtained from the Inbred Animals Nursery, Academy of Medical Sciences of the USSR (Stolbovaya). The plasma corticosterone level was determined by the method in [8]. Blood was taken from the retro-orbital plexus with a Pasteur pipet at the same time of day and all mice were kept under identical conditions. The thymic and splenic indices (weight of organ in g/body weight in g) × 100, was determined for the animals. Morphometric studies of histological sections of the thymus and spleen, stained with hematoxylin and eosin or azure-eosin, were carried out by means of an ocular grid [1]. The number of points on the ocular grid which coincided with the corresponding morphological structures of the thymus (cortex and medulla) and spleen (white and red pulp) was counted in sections. In sections of each organ 40 measurements were made, and the distribution of 1000 points of the ocular grid determined. In the experiments of series I intact mice were used, adrenalectomized mice in series II. The adrenalectomized mice were given NaCl solution. The thymus and spleen were obtained on the 5th and 10th days after bilateral adrenalectomy. In one experiment hydrocortisone was injected subcutaneously into intact CBA and C57BL/6 mice in a dose of 0.025 mg (1.25 mg/kg body weight) daily for 10 days, after which the thymic and splenic indices were determined; control animals were given phys-

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