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EFFECT OF HYPOKINESIA ON NUCLEIC ACID AND PROTEIN METABOLISM IN CELLS OF RAT LYMPHOID ORGANS

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KEY WORDS: hypokinesia; nucleic acids; proteins; lymphoid organs.

One response of the body to hypokinesia is a reduction in weight of the lymphoid organs and in the number of lymphoid cells they contain [6]. During prolonged limitation of movement, biochemical changes also may take place in the cells of lymphoid organs [3, 4]. Lymphocytes are known to play an important role in the maintenance of homeostasis during prolonged exposure to stress under extremal conditions [1]. They have the functions of synthesis, storage, and transport of nucleoproteins, which can be assimilated by cells of other tissues.

The object of this investigation was to study the effect of hypokinesia on thymocytes and splenic lymphocytes with particular reference to nucleic acid and protein metabolism, for it is these parameters which mainly define the trephocyte function and humoral activity of lymphoid cells.

EXPERIMENTAL METHOD

Adult (about 80 days old) male Wistar rats, weighing 200-250 g at the beginning of the experiment, were used. Animals of the experimental and control groups were kept in the same room on a standard diet, but the experimental animals were kept in special restraint cages, greatly restricting their mobility. During the experiment, which lasted 22 days, the state of the animals was assessed periodically on the basis of their behavior, body weight, and blood picture. After decapitation of the animals their organs (thymus and spleen) were weighed, washed in cold physiological saline, and homogenized in a glass homogenizer with Teflon pestle. Cell suspensions were obtained by pressing the homogenates through Kapron tissue, and the stroma was removed. The cells were washed and suspended in medium No. 199. The thymocytes thus obtained were used for analysis immediately, and lymphoid cells were isolated from the spleen cell suspension by centrifugation in medium containing Ficoll and Verografin [10]. The number of lymphocytes was counted by the standard method in a Goryaev's chamber. The intensity of synthesis of DNA, RNA, and protein was determined from the rates of incorporation into their molecules of the corresponding

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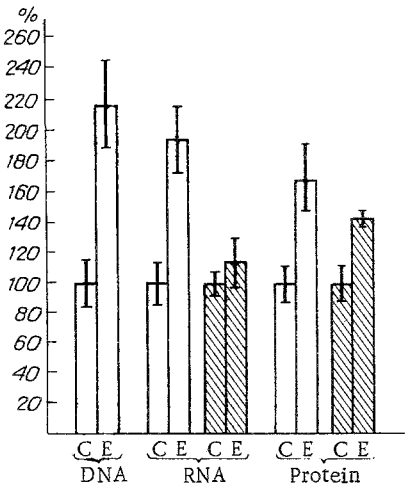

Fig. 1. Indices of nucleic acid and protein metabolism in rat thymocytes. Unshaded columns — intensity of biosynthesis; shaded columns — content of substances specified. C) Control; E) experiment (animals after hypokinesia for 22 days).

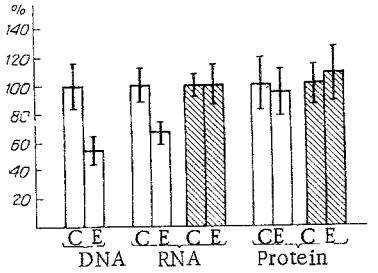

Fig. 2. Indices of nucleic acid and protein metabolism in splenic lymphocytes. Legend as to Fig. 1.

radioactive precursors: 5-methylthymidine-^{3}H (specific activity 11.8 mCi/mmole), 5-uridine-^{14}C (specific activity 370 mCi/mmole), and leucine-^{14}C (specific activity 270 mCi/mmole).

Lymphocytes were incubated in medium containing labeled precursors of nucleic acids and samples were prepared for analysis as described by Forsdyke [13]. To determine the intensity of protein biosynthesis, 10^{6} lymphocytes were incubated for 30 min at 37°C in 1 ml medium No. 199, containing 2 μCi leucine-^{14}C. The cells were washed to remove radioactivity and lipids and nucleic acids were isolated from them. The protein preparations thus obtained were dried at 105°C and hydrolyzed in 80% formic acid. The radioactivity of the samples was measured on an automatic liquid scintillation counter, using a toluene–PPO–POPOP system as scintillation fluid. The intensity of biosynthesis of nucleic acids and protein was judged from the number of radioactive fissions per microgram substrate (specific radioactivity). Protein was determined by Lowry's method, total nucleic acids after Spirin [7], DNA after Burton [12], and RNA as the difference between (DNA + RNA) and DNA.

The numerical data given in this paper are arithmetic mean values obtained by analysis of material from 10 animals. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

After hypokinesia for 22 days the body weight of the experimental animals was 48 g less than that of the controls and a marked decrease in the weight of their lymphoid organs also was observed. The weight of the thymus of the experimental rats, for instance, was 65% of the weight of the thymus of the control rats, compared with 57% for the spleen, in agreement with data in the literature [6]. It will be clear from Fig. 1 that during hypo-

kinesia the intensity of DNA and RNA synthesis in thymocytes left in the gland after loss of much of its cell mass was approximately twice as high as in the control ($P < 0.001$). A tendency was noted for the RNA content in the cells to increase. The protein content in the thymocytes was increased by 44% ($P < 0.01$) and the intensity of protein biosynthesis was increased by 69% ($P < 0.002$). Consequently, accumulation of nucleic acids and proteins in the cells of the thymus was intensified, and this was possibly connected with activation of the trephocyte function.

The spleen, like the thymus, lost much of its cell mass, but the intensity of DNA and RNA biosynthesis in its residual cells was lower than in the control ($P < 0.001$), although the nucleic acid content was unchanged. It will be clear from Fig. 2 that the decrease in the intensity of RNA synthesis took place simultaneously and approximately to the same degree as that of DNA. The change in RNA metabolism caused by hypokinesia evidently affected dividing cells and different types of RNA were involved.

Meanwhile, the rate of incorporation of leucine-^{14}C into proteins and the protein content in the splenic lymphocytes were virtually indistinguishable from normal (Fig. 2). The fact that hypokinesia had no effect on protein metabolism although RNA biosynthesis was reduced could be evidence of complex mechanisms of reorganization of metabolism in the spleen cells, taking place at different levels of cellular integration. For example, the possibility cannot be ruled out that RNA-dependent inhibition of protein synthesis is balanced by activation of translation of certain classes of enzyme proteins which participate in the realization of the trephocyte function of the lymphocytes. Evidence in support of this possibility is given, in particular, by data on activation of acid DNase in the spleen of animals under the influence of space flight [3], during which they were exposed to restricted motor activity. DNA of the lymphocytes can be reutilized by newly synthesizing cells only in the form of fragments [15], and since DNase is responsible for DNA degradation, activation of this enzyme ought to stimulate the trephocyte function of the lymphocytes. It can be tentatively suggested that during prolonged hypokinesia, biosynthesis of DNA hydolases in the splenic lymphocytes is increased, despite the fact that total protein synthesis remains unchanged.

The results are evidence that during hypokinesia similar morphological changes in the thymus and spleen are accompanied by different biochemical changes in their lymphoid cells. Destruction of the lymphoid organs during hypokinesia is regarded as a manifestation of a stress reaction resulting from the raised blood steroid hormone concentration [5]. In the present experiments loss of their cell mass by the thymus and spleen was evidently associated with the action of corticosterone, the level of which in the blood and adrenals is raised in hypokinesia of this duration [2]. Only T lymphocytes are known to be sensitive to corticosteroid hormones [8, 9]. These account for 100% of cells in the thymus. The spleen contains 35% of T lymphocytes and 55% of B lymphocytes [14, 15]. It may be postulated that loss of the lymphoid mass in the spleen takes place on account of T cells, and mainly B lymphocytes are left, in which nucleic acid biosynthesis is inhibited. Unlike the spleen, the thymus showed hyperfunction during 22-day hypokinesia. This was evidently due to the fact that its increased destruction is chronic, and activation of nucleic acid and protein biosynthesis is reparative and aimed at compensating (albeit partially) destruction of the organ. Participation of splenic lymphocytes in the formation of adaptive reactions during hypokinesia is probably limited to the early stages of development of the process, whereas the role of the thymus in it is longer and more important. The possibility cannot be ruled out that activation of the trophic function of the thymus is a compensatory reaction, aimed at correcting the deficiency of structural materials when tissue catabolism is intensified during hypokinesia. The fact that hyperfunction of the thymus develops against the background of a raised blood level of a hormone with an action directed toward the thymus deserves special attention. In a physiological situation of such complexity as chronic hypokinesia, not only the cell composition of the organ may be changed, but also the biochemical state of its cells, including their sensitivity to hormones. The results suggest that lymphoid cells of the thymus and spleen play different roles in mechanisms of adaptation of the body to prolonged hypokinesia.

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CHANGES IN HEMODYNAMICS AND OXYGEN SUPPLY DURING MASSIVE INJECTION OF HOMOLOGOUS BLOOD THROUGH AN ARTERIOVENOUS SHUNT

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Injection of an excess of homologous blood during major operations on the heart, lungs, and great vessels causes an increase in the number of posttransfusion complications. These complications, affecting the recipient, exhibit considerable polymorphism and have been called the "massive transfusion syndrome" or "homologous blood syndrome" [1-8].

One of the main manifestations of this syndrome is a disturbance of the general hemodynamics and of the oxygen supply to the recipient at different levels. It was therefore decided to study these disturbances experimentally in order to elucidate certain aspects of its pathogenesis. An arteriovenous shunt [8] was used as experimental model of the extracorporeal circulation in this investigation.

EXPERIMENTAL METHOD

Experiments were carried out on 42 dogs weighing 9-20 kg (nine recipients and 33 blood donors). The animals were first heparinized (3 mg/kg) and anesthetized with pentobarbital (30 mg/kg). Blood was obtained from the donors on the day of the experiment by bleeding from the femoral artery into a vessel containing heparin (50 mg/liter). Before the blood was taken the blood of the donors and of the donor and recipient was cross-matched. The total volume of donors' blood to be exchanged was introduced into a vessel connected to the femoral artery and vein. Normovolemic injection of freshly prepared homologous blood (taken from at least three donors) was then carried out into the experimental dogs by means of an arteriovenous shunt. Exchange of blood took place on account of the arteriovenous pressure gradient at a rate of 90-110 ml/min. The volume of donors' blood injected in all the experiments was 110-120 ml/kg body weight of the recipient. The duration of perfusion was 30 min.

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