APPEARANCE OF SIGNS OF STRESS IN MICE AFTER TRANSPLANTATION OF LYMPHOCYTES FROM SYNGENEIC ANIMALS AFTER HYPOKINESIA

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UDC 616.45-001/3+616-003.93

KEY WORDS: stress; regeneration, lymphocytes.

Besides its well-known symptom-complex, stress also causes disturbance of normal and reparative growth of several internal organs [3, 7-10, 12]. Preliminary exposure of animals to stress has been shown to lead to more intensive proliferation in the liver during its subsequent regeneration [11, 13].

Since lymphocytes of animals undergoing operations have the property of stimulating proliferation in the organs of intact syngeneic recipients [1] it was considered interesting to study whether the basic features of stress and the increased readiness for subsequent regeneration are transmitted by lymphoid cells, and the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 300 male (CBA × C56BL/6)F_1 mice weighing 18-20 g. Stress was induced by keeping the future donors for 17 h in cages restricting their movements considerably [4-6]. Splenocytes, prepared by the method described previously, were transplanted intravenously into syngeneic recipients in a dose of $5 \cdot 10^7$ cells in 1 cm^3 of medium 199 per mouse [1]. The recipients were intact mice and animals anesthetized with ether and undergoing resection of the central lobe of the liver (about 30%). Intact and partially hepatectomized mice, serving as recipients for splenocytes of intact donors, and also hepatectomized and intact animals not exposed to any other kind of treatment, served as the controls.

The recipients, like the donors, were killed by cervical dislocation 2, 3, and 7 days after transplantation of splenocytes. The organs were weighed on torsion scales and fixed in Carnoy's fluid. Paraffin sections of the liver were stained with hematoxylin and eosin. The mitotic index (MI) of the hepatocytes was calculated in promille after counting 6000-10,000 liver cells from each animal.

EXPERIMENTAL RESULTS

Restriction of motor activity of the animals for 17 h caused a decrease in body weight and the weight of the thymus, spleen, and liver by 17.6, 12.2, 35.3, and 10.5% respectively. The weight of the adrenal after 17 h of hypokinesia did not differ significantly from the control values, due to the too short period of observation.

Splenocytes from animals exposed to hypokinesia (SAH) differed significantly in their properties from splenocytes of normal animals. SAH 17 h after restriction of motor activity were able to induce some signs of stress in syngeneic recipients. For instance, injection of SAH into intact recipients caused more marked and prolonged hypertrophy of the adrenals than injection of splenocytes from normal animals. The weight of the adrenal was significantly increased by 1.5 times after 2 days under the influence of SAH, and after 3 days it was 2.2 times greater than in recipients of normal splenocytes.

Atrophy of the thymus, caused by injection of lymphoid cells, also was more marked in recipients of SAH (by 33%) than in recipients of normal splencytes (by 17.7%). Seven days

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 98, No. 8, pp. 146-148, August, 1984. Original article submitted October 3, 1983.

 0007-4888/84/9808-1022$08.50

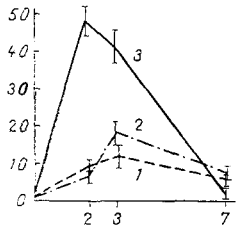

Fig. 1. Dynamics of changes in MI of hepatocytes in regenerating liver (1) after injection of splenocytes of intact donors (2), and donors exposed to hypokinesia (3). Abscissa, time (in days); ordinate, MI (in ‰).

after transplantation of lymphoid cells from animals exposed to hypokinesia, unlike after injection of splenocytes from normal donors, a threefold enlargement of the spleen was observed, a characteristic feature of the latter poststress period [2].

Unlike splenocytes from normal animals, SAH led to a decrease in MI in the liver of intact mice 48 h after transplantation compared with the intact control (0.25 ± 0.02 ‰ in the experimental recipients, 0.58 ± 0.17‰ in the control recipients, and 0.71 ± 0.13‰ in intact mice). Under conditions of preliminary stress induced in the recipients by reaction of one lobe of the liver, differences also were found in the properties of the splenocytes from mice exposed to hypokinesia and normal mice, although they were less marked.

In hepatectomized recipients SAH induced greater delay of growth of the animal than the operation alone or the operation combined with transplantation of lymphoid cells from the spleen of normal mice (by 17 and 14% respectively). Moreover, in hepatectomized recipients, splenocytes of donors exposed to hypokinesia caused more prolonged involution of the thymus. After 7 days, when substantial normalization of the weight of the thymus was observed in partially hepatectomized mice and hepatectonized animals which received normal splenocytes, these signs were virtually completely absent in recipients of SAH.

The weight of the thymus in the animals of this group at all times of observation was the same (2, 3, and 7 days after the operation and transplantation of splenocytes it was 25.0 ± 2.5, 21.0 ± 2.4, and 24.2 ± 1.5 mg respectively), whereas in animals of the other groups 7 days after transplantation it was increased by 50 and 100% (33.9 ± 2.7 and 42.5 ± 3.9 mg).

Determination of mitotic activity of the hepatocytes in mice undergoing resection of the liver followed by transplantation of splenocytes of normal mice and of donors exposed to hypokinesia showed that lymphoid cells from stressed animals, unlike those from normal animals, increased MI of the hepatocytes and also, evidently, accelerated completion of regeneration of the liver, for in the late stages of observation this parameter in these animals quickly returned to normal (Fig. 1). The final answer to the question of the nature of the changes observed in the number of dividing hepatocytes will be given by experiments to determine the duration of the mitotic cycle. On the basis of phase analysis of cell division it can be postulated that under these experimental conditions there is both an increase in the number of cells starting to divide and also some lengthening of all the phases of mitosis.

The results on the whole are evidence that transplantation of SAH induces changes in body weight and in the weight of the thymus, adrenals, and spleen in the recipients that are similar to those observed in stress. SAH, like stress itself [11, 13], also increased the number of mitoses in the liver during its subsequent regeneration.

Consequently, not only signs of stress, but also the particular features of the response of stressed animals appeared in recipients of SAH. By contrast with splenocytes from hepatectomized animals, lymphoid cells from animals exposed to hypokinesia inhibit hepatocyte proliferation in intact recipients, evidence that the phenomenon of transmission of regeneration information by lymphocytes is determined by changes in their properties that arise not only as a result of stress. The question of which population of lymphoid cells induced signs of stress in recipients requires further analysis.

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CHANGES IN CONTRACTILE FUNCTION OF THE HEART IN EMOTIONAL-PAINFUL STRESS

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UDC 613.863-07:612.12-008.3-07

KEY WORDS: emotional-painful stress, dynamics, contractile function of the heart.

Previous investigations have shown that severe emotional-painful stress (EPS) is accompanied by phasic changes in the metabolism and structure of the heart, and also in the blood eosinophil count, which reflects the response of the pituitary-adrenal system [2, 3, 6, 7]. It was found that the greatest disturbances of the oxidative and phosphorylating functions of the cardiac mitochondria and injuries to the structure of the cardiomyocytes of focal contractural and necrotic type are observed after the end of EPS — at a time when the eosinopenia induced by stress is suddenly replaced by eosinophilia [3-5, 9]. The question of how the dynamics of changes in the contractile function of the heart (CHF) correlates with the development of disturbances of cardiac metabolism and structure in EPS has not yet been answered.

The aim of this investigation was to study CHF of the heart in animals at times after EPS corresponding to the temporal parameters of responses of the pituitary-adrenal system, and also to the phases of development and regression of stress-induced disturbances of metabolism and structure of heart muscle [3, 5, 8].

EXPERIMENTAL METHOD

Experiments were carried out on 111 male Wistar albino rats weighing 200-300 g, divided into four groups. The control group (experiments of series I) consisted of 30 animals. EPS in the form of an anxiety neurosis was produced in the course of 6 h in 81 rats by the method described in [10]. The animals were anesthetized 2 h (series II, 24 rats), 45 h (series III, 34 rats), and 96 h (series IV, 23 rats) after the end of the procedure with pentobarbital sodium in a dose of 8 mg/100 g body weight, artificially ventilated, after which the chest was opened and the pressure in the left ventricle measured by means of a VI 6-6 TN electromanometer and N-105 oscilloscope [1] or RM-6000 polygraph (Nihon Kohden, Japan). CFH was estimated on the basis of the following parameters: the developed pressure P_d, the rate of contraction V_c and relaxation V_r of the ventricle, the index of intensity of functioning of structures (IFS) — the product of the heart rate (HR) and developed pressure, divided by the weight of the left ventricle. CHF was analyzed under conditions of relative rest and maximal load on the heart, due to compression of the aorta for 30 sec. Calculation of CSH and statistical analysis of its values under isometric conditions were done after 5 and 25 sec.

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 0007-4888/84/9808-1024$08.50