The investigations thus showed that injection of SP leads to an increase in its hypothalamic concentration, and this may perhaps be responsible for increased resistance to emotional stress.

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CHARACTER OF CHANGES IN CYTOSTATIC AND CYTOTOXIC FUNCTIONS OF MOUSE SPLENOCYTES AFTER IMMOBILIZATION STRESS

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KEY WORDS: stress; cytostatic activity; normal killer cells.

Intensive stress has been shown [1-4] to lead to a substantial decrease in activity of the system of natural cell-mediated resistance of the organism in the early period after stress. In view of recent evidence of the widespread biological significance of normal, or natural, killer cells (antitumor resistance, elimination of cells contaminated with viruses and bacteria, participation in the control of proliferation and differentiation of cells of the hematopoietic system, etc.), a very varied spectrum of undesirable after-effects for the body can be postulated as a result of this poststress modulation of the natural killer system.

Meanwhile exposure to stress can modify functions of other populations of immunocompetent cells. Investigations demonstrating the essential role of cytostatic interaction (by which is meant interaction between effector cells and target cells, leading not to death of the latter, but to restriction of their proliferation), both during tumor growth and during metastasization [8], and also, possibly, during regeneration, have recently been published. For instance, the cytostatic action of activated macrophages on tumor cells has been demonstrated [14], and evidence has been obtained of antigen-specific cytostasis of suppressor cells, generated by immunization of C57BL/6 mice by allogeneic P-815 cells [6]. It has been suggested that normal killer cells can also exert a cytostatic action on various target cells [5]. The place and role of the system of cell-mediated cytostatic activity (CA) in the normal organism, during tumor growth, and also in noncancer diseases (for example, in stress states), have not yet been explained.

It was accordingly decided to study the effect of stress action on the intensity of the cytostatic function of splenocytes and to compare it with the dynamics of normal killer cells

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TABLE 1. Effect of Immobilization Stress on CA of Splenocytes of CBA Mice at Different Times after Stress (M ± m)

Time after exposure to stress, days	ICA of splenocytes relative to P-816 target cells, %	P (relative to control)
Control	58±3,4	—
1	30±2,8	<0,05
2	48±4,1	—
3	54±3,9	—
5	66±3,6	—
8	75±3,7	<0,05
14	78±4,1	<0,05
21	60±4,8	—

Legend. Averaged results of three series of experiments; at least 12 animals taken at each point.

(NKC) activity at different times of the poststress period.

EXPERIMENTAL METHOD

Male CBA mice weighing 16-18 g were used. Immobilization stress was inflicted for 6 h on animals lying supine and with their limbs fixed. The mice were decapitated 1-21 h after cessation of the stress reaction and the isolated splenocytes [3] were used to determine NKC activity relative to VAC-1 target cells labeled with radioactive chromium [3], and also to determine their CA. The cytostatic action of the splenocytes was tested by the method in [7], modified by the writers, based on recording the decrease in RNA synthesis in the target cells. Cells of a mastocytoma P-815, transplanted in DBA/2 mice, and insensitive to the action of NKC in the test with ^{51}Cr, were used as target cells. Splenocytes used as effector cells were treated beforehand with actinomycin D, which blocks DNA-dependent RNA synthesis, in a dose of 1 μg/ml per 10^6 cells in 1 ml medium to prevent incorporation of [^{3}H]uridine into the effector cells. As was shown previously [9, 12], treatment of this kind did not affect NKC activity. Target cells, in a concentration of 2×10^4 per 100 μl, were incubated with effector cells (100 μl) in the ratio of 1:20 in complete culture medium (medium RPMI 1640 + 10% fetal calf serum + 1 mM glutamine + 100 U monomycin), in wells of 96-well plates. After 4 h, 50 μl of [^{3}H]uridine (specific radioactivity 1 mCi) was added to the cell mixture in each well and the plates were incubated for 4 h at 37°C. The cells were then deposited on filters by means of a 12-channel harvester. Radioactivity was determined with a scintillation counter. The index of cytostatic activity (ICA) was calculated by the equation:

$$ICA = \frac{\text{Quantity of Impulse (experiment} - \text{control efficiency)}}{\text{Quantity of Impulse in control mice}} \cdot 100.$$

The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Data on the effect of immobilization stress for 6 h on CA of the mouse splenocytes at different times of the poststress period are given in Table 1. During the first 24 h after stress CA was found to decrease by half. Depression of cytostatic activity was temporary, and by the 3rd day values of CA were restored to the control level. Starting with the 3rd day after stress and, in particular, by the 8th-14th days, CA was 20-40% higher than in the control. In some experiments the level of activity of cells with cytostatic function remained high even 3 weeks after exposure to stress.

Comparative data on the character of changes in cytotoxic activity and CA of the mouse splenocytes after stress are given in Fig. 1. The similarity of the time course of activity of these two immunologic parameters will be noted. The poststress fall of CA was observed to

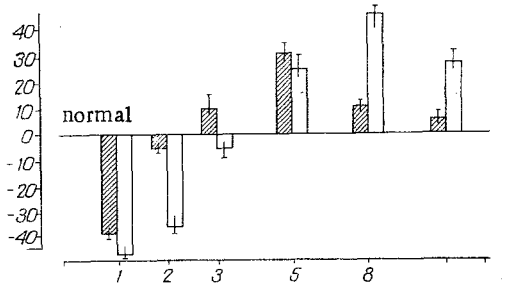

Fig. 1. Changes in cytotoxic and cytostatic activity of mouse splenocytes after stress. Abscissa, time after stress (in days); ordinate, changes in cytotoxic and cytostatic activity of splenocytes relative to control (in %). Unshaded columns—cytostatic activity of NKC; shaded columns — CA.

be abolished about 24 h earlier than depression of NKC activity. Activation of the cytostatic system of the cells after depression also preceded recovery of function of the natural cytotoxicity system a little. Otherwise their time course was virtually identical.

On the basis of these data a few suggestions may be put forward regarding relations between NKC and cells performing the function of cytostasis. NKC in the spleen, bone marrow, and also other organs and tissues can simultaneously perform not only a cytolytic, but also (and, possibly, mainly) a cytostatic function, and through this mechanism they may exercise control over proliferative processes, in the hematopoietic system in particular. By using the cytostatic mechanism they can limit the development of atypical cells, when their elimination in a more effective way (by cytolysis) is impossible. In that case we are dealing with the same cell population. At the same time, we have no direct evidence that these cells do not belong to different populations.

What is the possible biological role of these changes in activity of cells which possess cytostatic and cytolytic properties during exposure of the organism to stimuli of a stressor nature? On the one hand, depression of activity of this cell population may lead to rapid realization of the proliferative activity of a wide pool of precursor cells, including cells of the lymphoid series, resulting in an increase in weight of the spleen and in the number of nucleated cells in it. The end result of these events may be an increase in resistance of the body to certain unfavorable external environmental factors. On the other hand, depression of the cytostatic function of these splenocytes after a stress reaction may be the mechanism of stress-induced potentiation of tumor growth and of the development of autoimmune processes, in which the initiating effect of stress has been accepted by a number of workers and widely discussed in the immunologic literature [10, 11, 13, 15].

During exposure to stress not only was depression of NKC activity thus discovered, but depressed activity of the system of cellular cytostatic activity also was demonstrated; the latter evidently plays an important role in the limitation of tumor growth and the development of diseases with an autoimmune component, when the body is exposed to extremal environmental factors.

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EFFECT OF LITHIUM COMPOUNDS ON CARDIAC ARRHYTHMIAS INDUCED BY STROPHANTHIN IN CONSCIOUS RATS*

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KEY WORDS: strophanthin; cardiac arrhythmias; lithium chloride; lithium hydroxybutyrate.

An important role in the cardiotoxic action of cardiac glucosides is played by the neurogenic component. In particular, one state of strophanthin-induced arrhythmias is connected with hyperactivity of the sympathetic nervous system [8, 9, 11, 13, 14]. The writers showed previously that lithium compounds depress arrhythmias whose genesis is attributable to hyperactivity of the sympathetic nervous system [3, 4]. In the investigations cited, anesthetized cats were used.

Since general anesthesia may affect the control of the cardiac rhythm, in the investigation described below the effects of lithium salts were studied on a model of strophanthin-induced cardiac arrhythmias in unanesthetized animals.

EXPERIMENTAL METHOD

Experiments were carried out on 100 unanesthetized male Wistar rats weighing 140-160 g. The action of strophanthin and of lithium preparations was evaluated on the basis of the ECG which was recorded in standard leads II and III and in a chest lead. The active electrode for the chest lead was located at the level of the cardiac impulse. The ECG was recorded during periods when the animals were resting quietly lying on their back. Toxic doses of strophanthin K, namely 1.4-1.6 ml of a 0.05% solution per 100 g body weight, were used and caused death of the animals in 100% of cases. Lithium chloride and hydroxybutyrate were used in the form of 10% solutions. All drugs were injected into the jugular vein: strophanthin in the course of 2 min, lithium salts in the course of 5-10 min.

EXPERIMENTAL RESULTS

Three stages of changes in cardiac activity could be distinguished in the time course of strophanthin poisoning in most (24 of 30) animals (Fig. 1). In the first stage, which can be called the stage of primary disturbances of the cardiac rhythm, the heart rate slows, and conductivity is disturbed in the form of a high level atrioventricular block, with single or grouped idioventicular contractions (Fig. 1b). The longest duration of this stage was 3 min. The second stage was characterized by disappearance of the above-mentioned disturbances (Fig. 1b). This stage lasted from 1 to 3 min. The third stage, the stage of secondary disturbances of the cardiac rhythm, was characterized by complex changes of rhythm in the form of well-marked ectopic ventricular automatism against the background of A-V dissociation, changing into ventricular tachycardia (Fig. 1d, e). This stage occurred 5-7 min after injection of stro-

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