IMMUNOCORRECTIVE EFFECT OF TUFTSIN AFTER EXPOSURE TO IONIZING RADIATION

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The basis for this investigation was provided by data on the action of the tetrapeptide tuftsin (Thr-Lys-Pro-Arg), a fragment of the Ig molecule [11], on parameters of natural and acquired immunity. Tuftsin has been shown to stimulate phagocytosis, the bactericidal and antitumor activity of macrophages and polymorphonuclear leukocytes of the blood [6, 9], and immunogenesis and antibody production [8]. The present writers also found [1, 2, 4] a central activating action of tuftsin on emotional behavior (especially if depressed), and an indirect action on monoaminergic (MA) processes in the brain [3].

In view of the high immunostimulating activity of tuftsin and its effect on the MA systems of the limbic brain and hypothalamus, modulating adaptive processes and, in particular, those of immune homeostasis [5], it was considered necessary to continue the study of the action of tuftsin in various pathological processes characterized by depression of the protective properties of the organism.

This paper describes a study of the efficacy of tuftsin in immunodepression caused by exposure to ionizing radiation.

EXPERIMENTAL METHOD

Experiments were carried out on male mice of the B line, weighing 18-21 g, reared at the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR. The animals were irradiated in a dose of 450 cGy on an IGUR apparatus (four cesium sources, uniformity of irradiation dosage ±2%). The degree of immunodepression was assessed from changes in the humoral immune response in the spleen of the irradiated animals. Sheep's red blood cells (SRBC), injected intraperitoneally in a dose of $3 \cdot 10^8$ cells 2 days after irradiation, were used as the test antigen. On the 5th day after immunization cell suspensions were prepared from the spleen by expression into medium 199 in a homogenizer with imperfectly ground pestle. The number of cells in the suspension was counted in a Goryaev's chamber and their viability determined by the trypan blue test. The intensity of the immune response was determined as the number of cells secreting hemolysin of the IgM class in the spleen, using the method of local hemolysis without agar [3].

Tuftsin* was injected intraperitoneally in a dose of 20 mg/kg, the same as that used by other workers in experiments on CBA mice [4]. To assess the protective action of tuftsin it was injected for 5 days before irradiation, and to study its immunocorrective properties it was given for 5 days starting from the day of irradiation. In parallel control experiments, physiological saline was injected into the mice instead of tuftsin.

EXPERIMENTAL RESULTS

The data in Table 1 show the degree of radiation depression of the immune response in the irradiated mice. The number of antibody-forming cells (AFC) in the spleen of animals of

*The tuftsin was synthesized by V. N. Kalizevich and A. Z. Ardemasova at the Chemical Research Institute, A. A. Zhdanov Leningrad University.

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TABLE 1. Effect of Injection of Tuftsin before and after Irradiation on Immune Response in Animals' Spleen

Series of experiments	Number of experiments	Weight of spleen, mg	Number of nucleated cells, $\times 10^6$	Number of antibody-forming cells	
				per 10^6 nucleated cells	per whole spleen
Intact animals (control)	18	122±8	75,0±5,3	252±38	18870±1408
Irradiation	20	44±6 †	9,7±1,0 †	32±3 †	308±44 †
Tuftsin before irradiation	20	48±7 †	11,2±0,9 †	38±4 †	421±48 †
Tuftsin after irradiation	20	56±9 •	24,5±2,1 †	211±36*	5045±726 †

*$P < 0.01$.
†$P < 0.001$.

a given experimental group was significantly less than in the control. Meanwhile a marked reduction in weight of the organ and in the total number of nucleated cells was observed.

Injection of tuftsin into the animals before irradiation did not affect the development of immunodepression caused by irradiation. As Table 1 shows, mean values for the intensity of the immune response in the spleen of these mice were practically identical with those recorded in the group of animals exposed to irradiation without preliminary injection of tuftsin. It can accordingly be concluded that tuftsin has no protective action on the immune system against the effects of ionizing radiation on the body.

Interesting results were obtained in the series of experiments in which tuftsin was given after irradiation. In this case tuftsin had a distinct stimulating effect on immunogenesis. The population of antibody-producing cells in the spleen of animals of this experimental group was many times greater than that in mice exposed to irradiation only. When tuftsin was given, recovery of the immune response was so great that when the number of AFC per 10^6 nucleated cells was counted, it was almost identical with that in immunized intact animals.

The action of tuftsin thus observed can evidently be partly explained by its activating effect on the pool of precursors of antibody producers. This is confirmed by experiments which showed absence of correlation between the degree of increase in the total number of nucleated cells and the number of AFC, the increase in the latter being the greater. For instance, the number of AFC in the spleen was increased by more than 16 times compared with their number in mice irradiated without subsequent injection of tuftsin. Meanwhile the increase in the number of nucleated cells was smaller. Correlation between the immunocorrective action of tuftsin on irradiated animals and its known activating effect on cells of the monocytic—macrophagal series and, in particular, on the immunogenic activity of macrophages [10], likewise cannot be ruled out. This hypothesis is all the more probable because there is evidence of an inhibitory action of irradiation on cells of the mononuclear phagocytic system [10].

Considering the common regulatory mechanisms shared by nervous and immunological processes, the similarity in ligand—receptor interaction on membranes of nerve cells and immunocompetent cells, and also the modulating role of the limbic—hypothalamic complex on the intensity of the immune response, it can be postulated that the central effects of tuftsin make a definite contribution to activation of the immune response.

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ACTION OF RENIN ON EFFECTS OF ELECTRICAL STIMULATION OF THE VENTROMEDIAL HYPOTHALAMUS

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The renin—angiotensin system plays an important role in the regulation of vascular tone [1, 7], for renin is formed in the kidneys when the circulation in them is reduced. Renin, as an enzyme, has been shown to activate angiotensinogen — a protein found in the blood — and to convert it into angiotensin I, from which is subsequently formed angiotensin II, which has high pressor activity [3, 8].

Recent investigations have shown that the brain has its own renin—angiotensin system, which contains the same components as the renin—angiotensin system of plasma [4-6, 10, 11] and which evidently participates in the central regulation of arterial pressure (BP), water and electrolyte homeostasis, and other functions of the body. Consequently, many workers have directed their efforts toward the study of different effects of the biologically active substances in the renin—angiotensin system of the plasma, when applied centrally [12-14].

The object of this investigation was to study the action of renin, injected into the lateral cerebral ventricles, on some autonomic parameters and on the effects of electrical stimulation of negative emotiogenic centers of the hypothalamus.

EXPERIMENTAL METHOD

Experiments were carried out on 16 male Chinchilla rabbits weighing from 1.5 to 2.5 kg. The animals were immobilized in a frame and bipolar nichrome electrodes were implanted in the ventromedial nuclei of the hypothalamus, with an interpolar distance of 0.3-0.5 mm. Electrical stimulation of the hypothalamus was carried out with square pulses 1 msec in duration, frequency 50 Hz, and strength 100-300 μA. Renin was injected through a cannula into the right lateral cerebral ventricle in doses of 10, 20, and 30 μg/kg in a volume of 20 μl physiological saline. Partially purified renin from the dog's kidney (batch No. 246, provided by Dr. E. Hass, Cleveland, Ohio), was used in the experiments.

Respiration and BP in the femoral artery were recorded in all the experimental animals by means of piezoelectric and strain-gauge transducers connected to a Mingograf-34 (Siemens-Elema, Sweden). The ECG also was recorded in standard lead II. In some experiments the EEG was recorded in several cortical regions: sensomotor, parietal, and visual, on an eight-channel electroencephalograph of the EEG-80 type (Medicor, Hungary).

At the beginning of each experiment background values of BP, ECG, respiration, and EEG were recorded. Changes in these parameters were then studied in response to stimulation of the ventromedial nuclei of the hypothalamus. Renin was then injected into the lateral ventricle and the time course of changes in the above parameters was analyzed for 2 h. Against

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