HEMATOPOIETIC ACTION OF HIGH-MOLECULAR-WEIGHT FRACTIONS OF LEUKOCYTIC PYROGEN

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UDC 612.115.38:615.832.8

By fractionation of rabbit leukocytic pyrogen (LP) on a Sephadex column and by the alcohol method a nonpyrogenic protein fraction of LP was isolated. In intact rats this fraction caused stimulation of granulocytopoiesis, an increase in the proliferative activity of the granulocytes and their absolute number in bone marrow and peripheral blood, and also an increase in the number of macrocolonies in the spleen of lethally irradiated mice on account of an increase in the number of granulocyte colonies.

KEY WORKS: leukocytes; leukocytic pyrogen; granulocytopoiesis

The writers showed previously that native leukocytic pyrogen (LP) obtained from rabbit peritoneal exudate granulocytes has not only a pyrogenic, but also a hematopoietic action. The hematopoietic effect of LP depends on the dose injected: Large doses cause temporary depression of granulocytopoiesis, a small dose stimulates it [2]. It has been suggested that LP contains factors which stimulate and inhibit hematopoiesis; later investigations have confirmed this suggestion. It has been shown that a nonpyrogenic high-molecular-weight protein fraction causes an increase in DNA synthesis by bone marrow cells, whereas the low-molecular-weight pyrogenic fraction causes a decrease in their proliferative activity on the third day after injection [1].

To develop these investigations, it was decided to study the effect of the high-molecular-weight nonpyrogenic protein fraction of rabbit LP and NDA synthesis in bone marrow cells, on the leukocyte composition of the blood, and on the morphology of the bone marrow of intact rats and on colony formation in the spleen of lethally irradiated mice.

EXPERIMENTAL METHOD

The methods of obtaining rabbit LP and of its fractionation on a Sephadex G-75 column were fully described previously [1, 2]. Fractionation of LP by ethanol was carried out with gradually increasing concentrations of ethanol at 4°C. After each precipitation the mixture was allowed to stand for 1 h to allow a residue to form, and this was then removed by centrifugation at 1000 g for 20 min. The ethanol concentration in the top layer was increased from 20 to 80%. The high-molecular-weight fraction was contained in the residue obtained by precipitation with 20 and 40% ethanol.

Pyrogenic activity was studied in rabbits. The material was injected intravenously and the rectal temperature was measured every 30 min for 3 h. Hematopoietic activity of the high-molecular-weight fraction of LP was assessed from the intensity of incorporation of labeled thymidine into DNA of the bone marrow cells, and changes in the composition of the blood leukocytes and morphology of the bone marrow of intact rats during nine days after injection. The fraction was injected intravenously as a single dose into male Wistar rats weighing 100 g (in a dose of 200 μg/100 g body weight in a volume of 1 ml). This dose was chosen because unpurified LP in this dose had an inhibitory action on hematopoiesis [3].

To study the proliferative activity of the bone marrow cells the animals were killed and the bone marrow was flushed out of the femur into medium No. 199 with the addition of EDTA-Na_2 (50 mg/100 ml medium). Thymidine-^3H was added in a dose of 2.5 μCi per sample containing 5 million cells. Bone marrow of intact rats served as the control. Five parallel tests were set up simultaneously. The mixture of bone marrow was in-

Department of Pathological Physiology, Central Institute of Hematology and Blood Transfusion, Moscow. Department of General Pathology, Institute of Experimental Medicine, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologii Meditsiny, Vol. 89, No. 2, pp. 134-137, February, 1980. Original article submitted June 12, 1979.

0007-4888/80/8902-0097$07.50

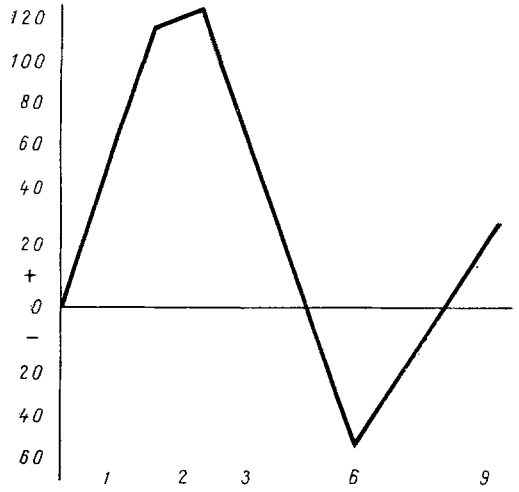

Fig. 1

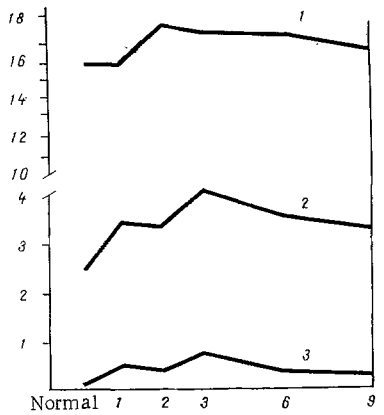

Fig. 2

Fig. 1. DNA synthesis in rat bone marrow cells after injection of nonpyrogenic fractions of leukocytic pyrogen. Abscissa, time (in days); ordinate, number of counts per minute (in percent of control).

Fig. 2. Total leukocyte count (1) and absolute number of polymorphonuclear neutrophils (2) and of juvenile granulocytes and stab cells (3) during nine days after injection of nonpyrogenic fractions of leukocytic pyrogen. Abscissa, time (in days); ordinate, number of cells (in thousands).

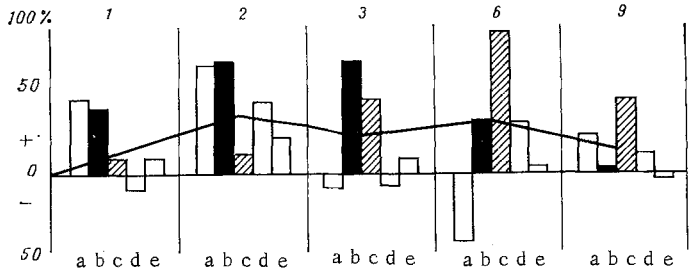

Fig. 3. Absolute numbers of individual groups of rat bone marrow cells during nine days after injection of nonpyrogenic fraction of leukocytic pyrogen: a) undifferentiated cells; b) immature granulocytes; c) mature granulocytes; d) erythroid cells; e) lymphoid cells. Abscissa, time (in days); ordinate, absolute number of cells (in percent of control).

cubated for 2 h at 37°C and then washed three times with physiological saline. The cells were destroyed by addition of 10% TCA solution to the cell residue. The acid-soluble fractions of DNA were deposited on millipore filters. The filters were placed in toluene scintillator and radioactivity counted. The number of counts per minute in bone marrow samples from intact rats was taken as 100% and deviations of activity of bone marrow of the experimental animals from this level were expressed as percentages with a plus or minus sign.

Changes in the composition of the blood leukocytes and in bone marrow morphology in the animals were studied 1, 2, 3, 6, and 9 days after injection of the fractions. Intact animals, studied at the same time as the experimental animals, served as the control. Blood was taken from the caudal vein and bone marrow from the femur. Groups of six rats were studied simultaneously and mean data calculated. Blood and bone marrow films were fixed in methanol and stained with azure II-eosin. The leukocytic formula was calculated by counting 200 cells and conversion into absolute values.

To assess the functional state of the bone marrow the following indices were studied: 1) the total number of myelokaryocytes in 1 mm^3 bone marrow; 2) the absolute number of individual forms of cells in 1 mm^3 bone

marrow, combined into the following groups: undifferentiated cells; immature granulocytes (myeloblasts; neutrophilic, eosinophilic, and basophilic promyelocytes; myelocytes and juvenile cells); mature granulocytes (stab cells, polymorphonuclear neutrophilis, eosinophils, and basophils); lymphoid and erythroid cells. Values obtained for cells of intact rats were taken as 100% and deviations from this level in the experimental groups were expressed as percentages with a plus or minus sign; 3) the mitotic index of the granulocytes – the number of mitoses per 100 cells capable of division, determined by counting 1000 granulocytes.

The effect of the high-molecular-weight fraction of LP on the number and composition of colonies in the spleen of lethally irradiated mice was studied by Till and McCulloch's method. Serial histological sections, stained with hematoxylin and eosin, were prepared for the microscopic study of the composition of the colonies.

EXPERIMENTAL RESULTS

Intravenous injection of the high-molecular-weight fractions of LP into rabbits in a dose of 15-150 μg/kg did not evoke a temperature reaction. After injection of the nonpyrogenic fraction of LP obtained on a Sephadex column, the incorporation of labeled thymidine into DNA of the rat bone marrow cells was increased after one day by 50%, and after 2 and 3 days by 112 and 115% respectively. On the 6th day the proliferative activity of the bone marrow cells fell, probably because of accumulation of mature granulocytes in the bone marrow at that time (Fig. 1).

The total leukocyte count in the peripheral blood was increased at all times of observation, reaching a maximum of 18,000 cells/mm^3 (initial level 16,000 cells/mm^3) after two days. In the course of nine days a significant increase was observed in the absolute number of granulocytes (by 50-100%) and with a shift to the left toward juvenile forms, which was maximal on the third day (Fig. 2).

The absolute number of myelokaryocytes was increased in the bone marrow of these animals, maximally on the second day (by 30%), and it remained high until the end of the observations. During the first six days the number of immature granulocytes increased by 30-60% ($P < 0.05$), maximally after two days, and the number of mature granulocytes increased by 10-75% over the period of nine days, the increase being most significant on the 6th day (Fig. 3).

Stimulation of granulocytopoiesis also was observed under the influence of the nonpyrogenic protein fraction of rabbit LP obtained by alcoholic fractionations. The fraction obtained by precipitation with 40% ethanol possessed stronger hematopoietic activity. The absolute leukocyte count in the peripheral blood was increased after the first day and until the end of observation by 50-100%, maximally on the 6th day – to 18,500 cells/mm^3 (initially 9,500 cells/mm^3), mainly on account of an increase in the absolute number of granulocytes. The number of polymorphs showed the greatest increase (by 166%) on the 6th day ($P < 0.05$). After the second day the leukoocytic formula for the peripheral blood showed a shift to the left; the absolute number of juvenile cells and stab cells was significantly increased (threefold) after 6 days.

The index of mitoses of the granulocytes was increased at all times, and significantly (by 84%) after two days. The total number of myelokaryocytes showed no significant change, despite the fact that the absolute number of immature granulocytes was significantly increased at all times of observation, and maximally (by 138%) after nine days. Starting from the 6th day the number of mature granulocytes decreased, but not significantly, possibly on account of their increased release into the peripheral blood stream.

After injection of this nonpyrogenic fraction of LP into lethally irradiated mice, an increase in the number of macrocolonies (by 54%) was observed on account of an increase in the number of colonies of granulocytes. Similar changes in hematopoiesis also were observed under the influence of the fraction obtained by precipitation with 20% ethanol, but they were less marked.

These investigations thus showed that rabbit leukocytes, activated by aseptic inflammation, secrete a group of substances including factors stimulating hematopoiesis. The question of whether these factors are identical with leukocytic pyrogen, as Kampschmidt et al., [1, 4-6] suggest, requires further study.

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ANALYSIS OF FOOD-MOTIVATED EXCITATION AT THE THALAMIC NEURONAL LEVEL IN RABBITS

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UDC 612.833.3-06: 612.826.4

Activity of 34 neurons in the ventral posteromedial thalamic nucleus – a relay in the central pathway for taste – was investigated in fed rabbits before and after elicitation of a food response from them to stimulation of the lateral hypothalamus. The appearance of volley activity in 26.4% of neurons in response to lateral hypothalamic stimulation is particularly interesting, for it evidently may reflect recruiting of neurons of this thalamic nucleus into food-motivated excitation.

KEY WORDS: rabbit thalamus; motivated excitation.

Food-motivated excitation, arising in pacemaker points of the hypothalamic structure of the brain has been shown [6-8, 10, 11] to spread to neurons at both subcortical and cortical levels of the brain, to create a widely branched system that acts as the structural and functional basis for goal-directed behavior. The neuronal correlates of the participation of structures such as the hypothalamus and neocortex in this case are not so much changes in general activity of the neurons as changes in the configuration of their discharge activity [1, 2, 4, 5, 9].

It was therefore decided to investigate unit activity in another brain structure which could become involved in food-motivated excitation, namely the ventral posteromedial nucleus of the thalamus. Food-motivated excitation in the rabbit was created by electrical stimulation of the lateral hypothalamus, giving rise to an additional demand for food in satiated rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 27 male rabbits, weighing 3-3.5 kg. As a preliminary measure, before unit activity was recorded, the fed rabbits were tested for the presence of a food response to stimulation of the lateral hypothalamus, causing an additional demand for food. The lateral hypothalamus was stimulated electrically through a bipolar nichrome wire electrode with factory insulation, inserted by the "wandering electrode" method, so that the region of the lateral hypothalamus could be accurately identified with coordinates taken from Sawyer's atlas (P 1.5, I 1.5, H 12-15 mm) and a marked food response could be obtained in the satiated rabbit. The parameters of the stimulating current were: frequency 50 Hz, pulse duration, 3 msec, voltage from 2 to 5 V.

Activity of neurons of the thalamic nucleus which coordinates P 4, I 3, H 10-12 mm was recorded extracellularly by glass microelectrodes (unanesthetized rabbit, stereotaxic fixation), amplified on the MZ-4 apparatus (Nihon Kohden), and recorded on magnetic tape.

Statistical analysis of unit activity recorded 200 sec before and 200 sec after stimulation of the lateral hypothalamus was carried out with respect to the mean frequency (in spikes/sec) and the degree of regularity of discharge activity, determined as the coefficient of variation of the mean frequency or the mean value of interspike intervals, by means of the NTA-1024 analyzer and Iskra-122 calculator.

The location of the macro- and microelectrode was determined in laminar brain sections processed by the photo-express method.

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 0007-4888/80/8902-0100$07.50