MORPHOLOGICAL AND PHYSIOLOGICAL FEATURES OF MAST CELLS OF SUBCUTANEOUS CONNECTIVE TISSUE IN RATS WITH ARTERIAL HYPERTENSION

G. A. Gavrisheva and A. S. Loktionov UDC 616.12-008.331.1-07:616.592-008.953.6-07

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Biologically active substances contained in mast cell granules and liberated as a result of their degranulation can induce considerable changes in the microcirculation [1, 5, 10]. However, very little is known of the state and role of the mast cells in essential hypertension, accompanied by marked disturbances of the microcirculation.

The aim of this investigation was to study the functional activity of mast cells from subcutaneous connective tissue of rats with experimental arterial hypertension.

EXPERIMENTAL METHOD

Mast cells of subcutaneous connective tissue of rats were studied in four series of experiments: series I) animals with adrenal-regenerative arterial hypertension [14] 4, 8, and 12 weeks after the operation; II) animals undergoing mock operations according to the same model [14] at the same times after the operation; III) Okamoto rats aged 1-1.5 and 6-10 months, with genetically determined spontaneous hypertension; IV) intact animals (control). The arterial pressure (BP) was measured in the caudal artery by a photoelectric method. Under pentobarbital anesthesia (5 mg/kg, subcutaneously) pieces of subcutaneous connective tissue were taken from the region of the spine and film preparations were made and stained with toluidine blue [6]. The total number of mast cells was determined in 50 fields of vision (magnification 400), and subsequently expressed per 10 fields of vision, and the relative percentages of degranulated and undegranulated forms of cells were counted, reflecting the physiological activity of the mast cell population. The second function test consisted of quantitative determination of serotonin in the cytoplasm of undegranulated forms. Serotonin was demonstrated by Falck's method [9] and estimated quantitatively by cytofluorometry [4, 8]. The intensity of fluoresence (after subtraction of the background) was measured in the cytoplasm of 50 randomly chosen mast cells in each preparation. The serotonin concentration was expressed in conventional fluorescence units, based on the intensity of fluorescence of a standard source. The mean intensity of fluorescence of the cells was determined from the results of the measurements for each preparation and histograms of distribution of cells by intensity of fluorescence were constructed for ranges of 0-10, 10-20, 20-30, 30-40, and over 40 conventional units. The results were subjected to statistical analysis by Student's test. Total distributions were compared by the chi-square test [7].

EXPERIMENTAL RESULTS

A parallel increase in the absolute number of mast cells was observed throughout the period of investigation in rats with adrenal regenerative arterial hypertension and in animals undergoing the mock operation. This process was accompanied by changes in the ratio between the numbers of degranulated and undegranulated forms of cells. The number of degranulated mast cells was increased 4 weeks after the operation in animals of both series (Table 1). By the 12th week the ratio between degranulated and undegranulated forms had returned to normal in rats undergoing the mock operation, whereas in the animals of series I there was a significant increase in the level of degranulation. The question arises whether the observed increase in degranulation of the mast cells was connected with the rise of BP. This question was partly answered by determination of the relative percentages of degranulated and undegranulated forms in rats with spontaneous hypertension (series III). In these animals the total number of mast cells of the subcutaneous connective tissue was the same as

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TABLE 1. Characteristics of Mast Cells of Subcutaneous Connective Tissue of Rats with Arterial Hypertension

Group of animals	Time of investigation after operation, weeks	Number of animals	Absolute number of mast cells (in 10 fields of vision)	Relative percentage of forms of mast cells		Seretonin concentration in cytoplasm of mast cells, conventional units	BP, mm Hg
				undergranulated	degranulated		
Series IV (control)	—	18	71,8±6,6	52,4±2,3	47,6±4,0	24,0±1,3	100,0±10,0
Rats with adrenal regenerative hypertension (series I)	4	20	77,0±5,6	35,4±3,8*	64,6±3,7*	18,2±0,9**	171,0±4,6**
	8	11	86,1±6,2	32,9±3,9*	67,1±3,3*	17,2±1,0*	157,0±4,4**
	12	14	100,0±7,4	42,4±2,2**	57,6±2,2*	20,2±1,0**	159,0±3,7**
Rats undergoing mock operation (series II)	4	14	66,0±6,5	27,6±4,0*	72,4±2,2*	18,5±1,2**	146,0±10,0**
	8	16	82,0±4,2	45,1±3,4	54,9±3,6	19,0±3,6	127,0±10,9
Rats with spontaneous hypertension (series III):	12	14	95,0±9,0	53,4±4,0	46,6±3,7	25,9±1,4	129,0±3,5
Aged 1-1.5 months	—	10	76,8±9,5	41,6±4,9**	58,4±4,7**	22,0±1,3	161,0±3,8**
Aged 6-10 months	—	10	75,9±4,5	26,8±3,1*	73,2±3,5*	19,1±2,6	201,0±13,2**

Legend. *P < 0.01, **P < 0.05 compared with control.

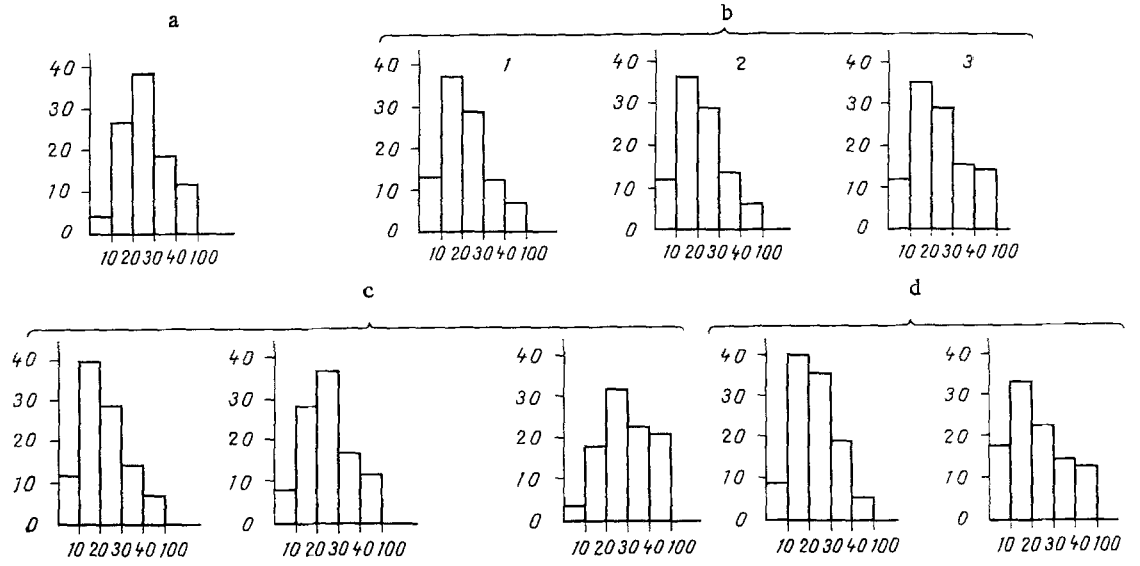

Fig. 1. Distribution of mast cells of subcutaneous connective tissue by intensity of serotonin fluorescence. Abscissa, intensity of fluorescence (in conventional units); ordinate, number of mast cells with different degrees of fluorescence (in % of total number of cells); a) intact rats (control); b) adrenal regenerative hypertension; c) mock operation; d) spontaneous hypertension. 1, 4) 4 weeks after operation; 2, 5) 8 weeks; 3, 6) 12 weeks; 7)rats aged 1-1.5 months; 8) rats aged 6-10 months.

in the control in both age groups. Meanwhile the percentage of degranulated forms in these animals was significantly increased, especially in rats aged 6-10 months. It was shown previously in intact noninbred rats aged 1.5-10 months that the total number of mast cells in the subcutaneous connective tissue and the percentage of degranulated forms were independent of the animals' age [3]; the increase in degranulation of the mast cells in Okamoto rats was therefore evidently connected with the duration of hypertension. It could accordingly be postulated that the increase in the number of degranulated forms in rats with adrenal regenerative hypertension was due to elevation of BP. Factors contributing to increased degranulation of the mast cells in arterial hypertension may be specific neurotransmitters of nerve endings [12, 13] and other humoral and hormona factors [11].

Degranulation is the final stage in the life cycle of mature mast cells [2]. Accumulation of serotonin in their cytoplasm, which takes place parallel with accumulation of other biologically active substances (histamine, heparin, and so on) [15], can be used as a relative

parameter of maturity of the undegranulated mast cells. To estimate the state of the undegranulated cells at different stages of maturation more completely, a quantitative analysis was made of the serotonin concentration in mast cell granules. A reciprocal relationship was found between the serotonin content and the intensity of degranulation in animals of all groups (Table 1). This kind of relationship is perfectly understandable, for in the course of a decrease in the percentage of undegranulated forms the proportion of immature cells with a relatively low serotonin content among them could increase. To test this hypothesis, the distribution of mast cells was analyzed according to their serotonin content (Fig. 1). When this distribution was compared in the animals of series I and II it was shown that in the early stages after the operation (4 weeks) essentially the same shift took place in the histogram to the left on account of an increase in the relative number of cells with a low serotonin content in both series. In both cases the difference of the histogram for the control, when compared by the chi-square test, was significant ($P < 0.05$). At the 8th week the distribution in animals undergoing the mock operation no longer differed significantly from the control, and at the 12th week a significant increase was observed in the percentage of cells with a high serotonin content. In rats with adrenal regenerative hypertension the distribution of cells by serotonin content was virtually identical at all times after the operation. Investigations on rats with spontaneous hypertension showed that at the age of 1-1.5 months undegranulated forms with an average intensity of fluorescence predominated and there was a relatively small number of cells with both high and low serotonin content (Fig. 1). In animals aged 6-10 months a tendency was observed toward equalization of the histogram due to an increase in the number of cells with both high and low serotonin content.

The mast cell system is thus in a state of functional stress in animals with arterial hypertension of varied genesis and this is reflected in intensification of degranulation and increased production of immature forms.

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