Effects of Adrenal Glands on Bone Marrow Hemopoietic Microenvironment

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Effects of bilateral adrenalectomy on the hemopoiesis-inducing microenvironment and bone marrow hemopoietic cells were studied. It was shown that adrenal hormones regulate secretory activity of the hemopoiesis-inducing microenvironment, in particular its nonadherent fraction. Adrenalectomy did not change the content of hemopoietic islets, number of erythro- and granulocytopoietic precursors, and blood indexes. The data suggest that structural and functional properties of the hemopoiesis-inducing microenvironment determined by the great variety and high plasticity of organ and intersystem regulatory interactions for a long time compensate the effects of hypocorticism on the requirements for mature blood cells.

Key Words: *adrenalectomy; hemopoietins; hemopoietic islets; hemopoietic precursors; blood system*

A large body of data obtained in *in vivo* and *in vitro* experiments demonstrates both the stimulatory and suppressive effects of the pituitary-adrenal and sympathoadrenal systems on hemopoiesis [7,10,11,12]. These ambiguous results can be explained by diversity of adrenalectomy-induced changes in metabolic processes [10,11] or by effects of regulatory hormones on hemopoietic precursors mediated by the cells of the hemopoietic microenvironment (T cells and macrophages) [19].

In this connection, the effects of adrenalectomy on the hemopoiesis-inducing microenvironment (HIM) and its interrelation with hemopoiesis in mice are of particular interest.

MATERIALS AND METHODS

Experiments were performed on 56 outbred albino male and female rats obtained from the collection of the Laboratory of Experimental Biomedical Modeling (Institute of Pharmacology, Tomsk Research Center).

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Four weeks before examinations, 2-month-old animals were subjected to bilateral adrenalectomy or sham-operated. Five days postoperation, the mortality rate in mice receiving 5% glucose in physiological saline was 9-12%, which agreed with published data [17]. Blood indexes were measured in 3 experimental series (Table 1).

Spontaneous secretion of erythropoietic (EPA) and colony-stimulating (CSA) activities of myelokaryocytes was determined by culturing erythroid (CFU-E), granulocytic (CFU-G), and granulocyte-macrophage (CFU-GM) colony-forming units (CFU) in the semisolid culture medium [5]. Nonadherent or adherent (over 1 h) nuclears in various concentrations ($1\text{-}6\times10^6$ cells/ml) were incubated in complete culture medium consisting of 90% RPMI-1640 medium (Sigma), 10% fetal bovine serum (ICN Pharmaceuticals), 10 mM HEPES (Sigma), 40 mg/liter gentamicin, 280 mg/liter L-glutamine (Sigma), and 2.5×10^{-5} M 2-mercaptoethanol (Sigma) for 24 h. EPA and CSA (per 10^6 cells/ml and per femur) were calculated taking into account the cellularity of the nonadherent and adherent fractions. The results were expressed in arbitrary units by counting colonies per 10^5 cultured myelokaryocytes.

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TABLE 1. Blood Indexes in Adrenalectomized and Sham-Operated (100%) Mice ($X \pm m$)

Blood indexes	Females		Males			
	series I		series II		series III	
	sham-operation	adrenal-ectomy	sham-operation	adrenal-ectomy	sham-operation	adrenal-ectomy
Bone marrow TNK	100±4	82±5*	100±7	120±7*	100±5	81±5
Neutrophilic granulocytes						
immature	100±8	84±18	100±17	94±12	100±22	92±12
mature	100±8	74±5*	100±7	148±7*	100±9	88±9
Lymphoid cells	100±11	86±8	100±13	125±7	100±5	76±6*
Erythroid cells	100±13	81±17	100±20	87±16	100±31	83±30
TNL	100±14	188±13*	100±3	129±8*	100±6	97±10
Segmented neutrophils	100±14	202±26*	100±18	71±15	100±13	63±6*
Lymphocytes	100±12	188±15*	100±10	154±11*	100±10	118±13
Reticulocytes			100±14	60±5*		
Thymus TNK	100±7	100±12	—	—	100±6	102±12

Note. TNK and TNL, total number of karyocytes and leukocytes, respectively. Here and in Table 3: *$p<0.05$ compared with sham-operated animals.

Cloning of CFU-E, CFU-G, and CFU-GM was performed by culturing nonadherent bone marrow nuclears (3×10^5 viable cells/ml) in methyl cellulose for 3 and 6 days [5]. Human recombinant erythropoietin (0.5 U/ml, Sigma) and mouse recombinant granulocyte colony-stimulating factor (1 ng/ml, ICN) were used as specific growth stimulators.

Hemopoietic islets were isolated using 0.05% collagenase (Sigma) [14]. Erythroid, erythrogranulocytic, and granulocytic hemopoietic islets were identified by the morphology of cells associated with the central element and stained with azure II-eosin.

The results were analyzed by Student's t test. The relationship between secretory activity of bone marrow nuclears and their concentration in the culture was expressed graphically basing on the linear dependence of this parameter in the specified concentration range [5].

RESULTS

Some authors believe that physiological effects of the adrenal glands on hemopoiesis are mediated through vascular tone and metabolic processes [11]. On the other hand, HIM is assumed to be the main factor con-

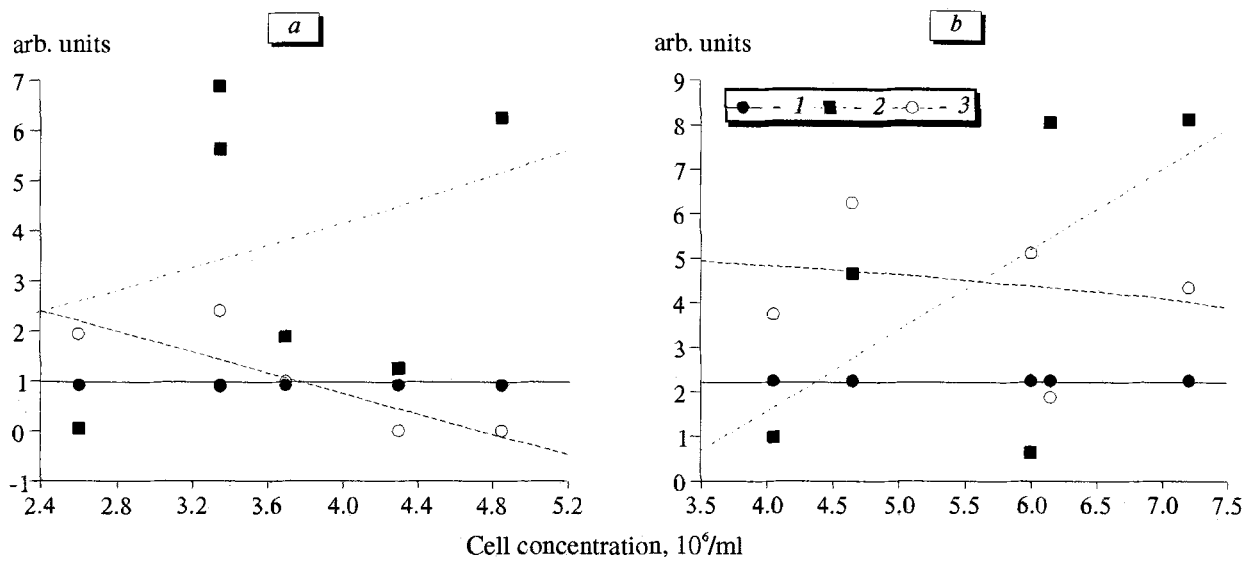

Fig. 1. Erythropoietic activity as a function of concentrations of nonadherent (*a*) and adherent (*b*) bone marrow cells in culture. Here and in Fig. 2: initial culture medium (without supernatant, *1*) and supernatants of myelokaryocytes obtained from sham-operated (*2*) and adrenalectomized mice (*3*).

trolling functions of the hemopoietic tissue [2,7, 9,18] and mediating the regulatory influences of neuroendocrine organs and systems [15].

Adrenalectomy did not affect the content of adherent and nonadherent elements in the bone marrow but modulated their secretory activity. The production of EPA by individual cells and the total nonadherent fraction decreased 2-3-fold, while the total CSA of the adherent fraction was reduced primarily due to low secretion of fibroblast-stimulating cytokines (Table 2). These processes are probably responsible for the considerable decrease in the number of fibroblastic CFU (CFU-F) in myelokaryocyte culture from adrenalectomized mice (Table 3).

In adrenalectomized animals, hemopoietic activity of HIM elements (primarily EPA production by nonadherent bone marrow cells) was inversely related to their concentration in culture (by contrast to that in sham-operated mice, Figs. 1 and 2). Nonadherent cells in concentrations above 3.6×10^6 nuclears/ml produced cytokines suppressing the growth of erythroid colonies.

The deficiency of hormones of the pituitary-adrenal and sympathoadrenal systems strengthened the inverse (for cytokines stimulating CFU-G and CFU-GM) and direct (for substances activating CFU-F growth) concentration dependences of CSA secretion by nonadherent cells (Fig. 2). At the same time, the slope of curves representing CSA secretion by bone marrow adherent cells practically did not change.

Thus, adrenal hormones in intact mice execute fine regulation of the secretory activity of HIM, particularly of its nonadherent fraction. However, low production of hemopoietins under conditions of balanced hemopoiesis [3] attests to an important role of cell-cell contacts in proliferation and differentiation of hemopoietic cells in the absence of factors disturbing hemopoiesis [15]. This primarily concerns hemopoietic islets, the major structural and functional elements of the hemopoietic tissue and the sites of intense proliferation and differentiation of stem cells from committed to mature forms [13].

Our experiments showed that structural and functional organization of the bone marrow in intact mice practically did not depend on regulatory influences of hormones secreted by the adrenal medulla and cortex. The number of hemopoietic islets and their main types determining the intensity of hemopoiesis in adrenalectomized mice were similar to that in sham-operated animals (Table 3).

The influence of glucocorticoids and catecholamines on cloning of hemopoietic precursors is extensively studied [4,7]. However, we revealed no adrenalectomy-related changes in hemopoiesis, in particular in the number of bone marrow committed precursors

TABLE 2. Secretory Activity of Bone Marrow Cells (arb. units) in Adrenalectomized and Sham-Operated Mice ($X\pm m$)

Activity	Sham-operated mice				Adrenalectomized mice			
	nonadherent myelokaryocytes		adherent myelokaryocytes		nonadherent myelokaryocytes		adherent myelokaryocytes	
	10^6/ml	total activity	10^6/ml	total activity	10^6/ml	total activity	10^6/ml	total activity
EPA	0.98±0.23	10.98±2.32	0.76±0.21	7.32±1.54	0.33±0.08*	4.05±0.99*	0.81±0.22	4.81±1.16
CSA_g	1.1±0.21	11.59±2.67	1.14±0.27	12.48±2.31	0.81±0.2	10.32±2.0	1.03±0.24	7.82±1.99
CSA_f	2.39±0.53	27.95±5.0	1.6±0.22	17.0±3.22	1.97±0.34	30.66±4.29	1.5±0.31	8.96±0.99*
CSA_t	3.49±0.64	37.2±6.64	3.04±0.27	31.26±2.56	2.78±0.48	38.45±5.98	2.45±0.36	15.53±2.77*

Note. CSA_g was determined by the growth of CFU-G and CFU-GM; CSA_t was determined by the growth of CFU-F in the culture of intact myelokaryocytes; CSA_f, the total colony-stimulating activity. *Differences between groups are significant at $p<0.05$.

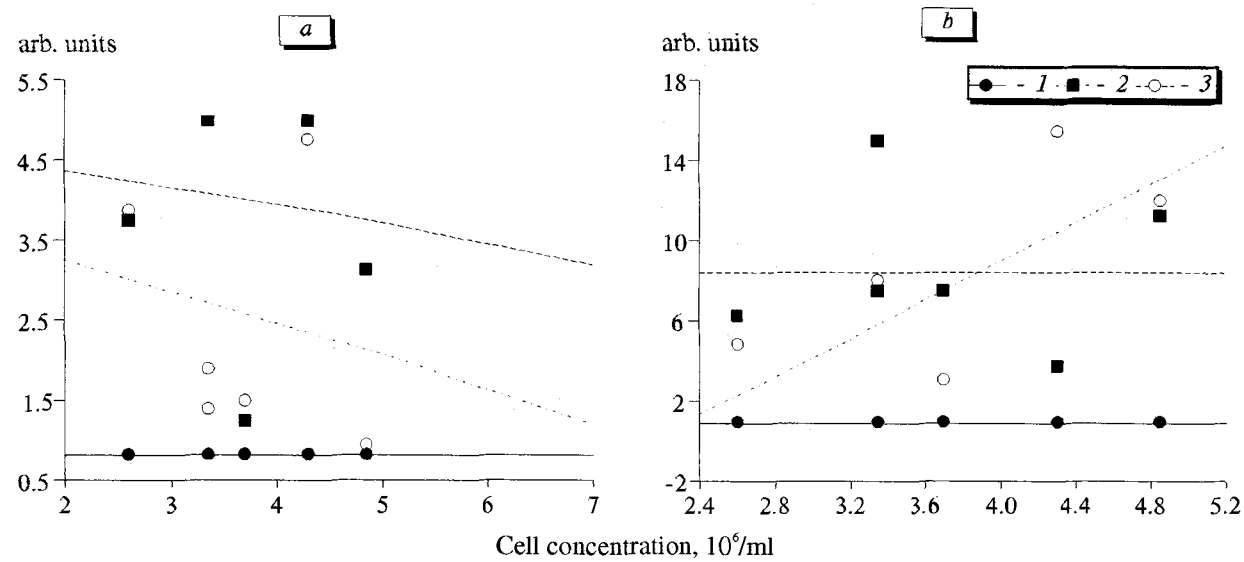

Fig. 2. Secretion of colony-stimulating activity for CFU-G, CFU-GM (*a*), and CFU-F (*b*) as a function of the concentration of nonadherent myelokaryocytes in culture.

of myelopoiesis (Table 3) and mature cells. Our findings agree with previous data [14,15] and cast some doubt on the efficiency of direct regulation of proliferation and differentiation of hemopoietic cells by adrenal hormones under conditions of balanced hemopoiesis.

We observed no clearly defined changes in blood indexes in male and female mice 1 month after bilateral adrenalectomy. The total content of bone marrow karyocytes in adrenalectomized mice increased or decreased compared with sham-operated animals, while the cellularity of the thymus remained at the control level (Table 1).

The variability of myelograms in males was due to the corresponding changes in the absolute number of neutrophilic granulocytes, while in females it was due to a 1.3-fold decrease ($p<0.01$) in the content of bone marrow lymphoid cells in the femur (Table 1). At the same time, the number of immature neutrophilic granulocytes remained practically unchanged.

It is believed that adrenalectomy reduces the total cellularity of the bone marrow [1] and the number of splenic CFU due to cell migration [6]. In our experiments, the effects of hypocorticism on cell redistribution associated with the recruitment of bone marrow mature neutrophils to the circulation were observed only in one experiments (Table 1).

Hormones of the adrenal glands play a role in the regulation of erythron. Bilateral adrenalectomy induces hypoplasia of the bone marrow erythroid stem [8] and anemia [16]. In our experiments, adrenalectomy caused reticulocytopenia not associated with considerable changes in the intensity of bone marrow erythropoiesis (Table 1).

White blood cell count in male mice (Table 1) increased 1.3-1.9-fold due to a 2-fold increase in the number of segmented neutrophils and/or 54-88% increase in lymphocytes, while in female animals, adrenalectomy caused no significant changes in the total content of leukocytes. At the same time, neutropenia, eosinopenia, and monocytopenia were found in the hemogram.

Thus, there is possibility for participation of adrenal hormones in the regulation of hemopoiesis mediated by cells of the hemopoietic microenvironment (T cells, macrophages, and stromal mechanocytes) carrying receptors for catecholamines and glucocorticoids. At the same time, structural and functional properties of HIM determined by great variety and plasticity of organ and intersystem regulatory interactions compensate the effects of hypocorticism on the requirements for mature blood cells in the absence of extreme factors.

TABLE 3. Concentration of Hemopoietic Islets and Hemopoietic Precursors in the Bone Marrow of Adrenalectomized and Sham-Operated (100%) Mice ($X \pm m$)

Indexes	Sham-operated mice	Adrenalectomized mice
Total number of hemopoietic islets	100±4	96.9±11
granulocytic	100±8	95.8±19
erythroid	100±10	113.3±22
erythrogranulocytic	100±9	82.4±8
CFU-E	100±37	120.8±28
CFU-G	100±30	163±37
CFU-F	100±26	30±10*

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