EFFECT OF SOME STRESSORS ON MITOSIS IN THE CORNEAL EPITHELIUM AND THE BONE MARROW ANEUPLOID CELL LEVEL IN ADRENALECTOMIZED ALBINO RATS

S. S. Timoshin, N. B. Simon, and A. P. Baranov

UDC 612.841.1.014.3:612.6.014.43

It was shown previously that exposure to stressors led to a decrease in the number of mitoses in the cornea as a result of G_2-M delay. The index of labeled nuclei and level of pathological mitoses were unchanged. It is now shown that injection of pyrogenal or contact hypothermia for 1 h to 28-30°C did not cause reactive inhibition to develop in adrenalectomized rats, but led to a significant increase in the level of pathological mitoses in the cornea from 4.3-6.3% in intact and adrenalectomized rats to 10.6-12.5% in adrenalectomized rats exposed to stress. Karyotypic analysis of the bone marrow cells under these conditions revealed a significant increase in the number of aneuploid cells (both hypo- and hyperdiploid).

KEY WORDS: stress; pathological mitoses; aneuploidy.

In previous experiments the writers showed that single and repeated exposure to stress led to a decrease in the number of mitoses in the cornea as a result of delay in the G_2-M period. The index of labeled nuclei (ILN) and level of pathological mitoses were unchanged under these circumstances [7, 8]. If adrenalectomized rats were exposed to stressors, absence of inhibition of cell division was accompanied by an increase in the number of pathological mitoses [8]. That depression of mitotic activity is determined by adrenal hormones during stress is an established fact [1, 5].

The object of the present investigation was to assess the role of adrenal hormones in the formation of pathological mitoses during exposure to extremal factors.

EXPERIMENTAL METHOD

Male albino rats weighing 140-190 g were used. Adrenalectomy was performed and postoperative care of the animals maintained in the usual way. The experiments were carried out on the 5th day after the operation. Pyrogenal was injected into the caudal vein in a dose of 2 μg/100 g body weight 6 h before sacrifice, and rats were exposed for 1 h to cold lowering their temperature to 28-30°C for 1 h by the method described previously [5] 5 h before sacrifice. To estimate the rate of mitosis and also to obtain chromosome preparations from bone marrow cells, parallel experiments were carried out with animals into which colchicine was injected in a dose of 4 μg/g body weight 2 h before sacrifice. In view of circadian changes in the response of mitosis to stress [4, 6, 8], in the experiments of group 1 the animals were sacrificed at 7 a.m. and in group 2 at 7 p.m. The state of mitoses was determined in total preparations of the cornea by the method described in [7], using Alov's suggested classification of pathological mitoses [2]. The duration of mitosis was determined in the usual way and expressed in minutes. Chromosome preparations of bone marrow cells were obtained by Ford's method [9]. At least 50 metaphase plates satisfying the criteria of selection [3] were analyzed from each animal. Aneuploid cells with fewer than 40 or more than 44 chromosomes were disregarded. A combined increase in the number of hypo- and hyperdiploid cells was considered to be true aneuploidy. The numerical results were subjected to statistical analysis by Student's method. The standard error of the duration of mitosis was determined by the method of Worthing and Geffner. Altogether 143 animals were used in the experiments.

Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 9, pp. 338-341, September, 1979. Original article submitted February 19, 1979.

 0007-4888/79/8809-1056$07.50

TABLE 1. Effect of Stressors on Mitosis and Its Duration in Adrenalectomized Rats (M ± m)

Time of sacrifice	Group of animals											
	intact			adrenalectomy			adrenalectomy + pyrogenal			adrenalectomy + hypothermia		
	number of mitoses	percent pathological mitoses	duration of mitoses	number of mitoses	percent of pathological mitoses	duration of mitoses	number of mitoses	percent of pathological mitoses	duration of mitoses	number of mitoses	percent of pathological mitoses	duration of mitoses
Morning	379±42	4,3±1,0	32±5	335±25	6,3±1,0	43±5	318±25	11,6±1,2*	55±6*	440±36	10,6±1,4*	50±7
Evening	191±17	4,9±0,7	42±5	228±16	4,6±0,8	47±5	234±25	12,5±1,8*	57±10	212±24	10,8±1,5*	81±11*

TABLE 2. Effect of Stressors on Level of Aneuploidy in Bone Marrow Cells of Adrenalectomized Rats

Time of sacrifice	Group of animals											
	intact			adrenalectomy			adrenalectomy + pyrogenal			adrenalectomy + hypothermia		
	aneuploid cells, %											
	hypodiploid	hyperdiploid	total	hypodiploid	hyperdiploid	total	hypodiploid	hyperdiploid	total	hypodiploid	hyperdiploid	total
Morning	5,8	0,4	6,2	8,3	0,5	8,8	11,1	1,2	12,3*	12,0	1,5	13,5*
Evening	8,3	0,8	9,1	11,3	0,5	11,8	10,0	0,8	10,8	12,0	1,3	14,6*

Note: In Tables 1 and 2 asterisk denotes significant ($P < 0.05$) difference compared with control.

EXPERIMENTAL RESULTS

During determination of mitotic activity in the cornea of intact rats a clearly defined rhythm of cell division was observed: the number of mitoses at 7 a.m. was twice that found at 7 p.m. (Table 1). There was no significant change in the duration of mitosis.

Adrenalectomy caused no significant changes in the number of dividing cells or in the duration of mitosis. The amplitude of the circadian variations in the number of mitoses was smaller than in intact animals, although the differences between the number of mitoses in the morning and evening of adrenalectomized rats remained statistically significant. Cooling and injection of pyrogenal into the adrenalectomized rats caused no change in the number of dividing cells but led to a significant increase in the duration of mitosis in the morning group after injection of pyrogenal and in the evening group after cooling. The most striking feature of these experiments was an increase in the level of atypical mitoses following exposure to stressors. The number of pathological mitoses in the cornea of the intact rats was 4.3 and 4.9% in the morning and evening respectively (the differences are not significant). The principal forms of pathology of mitosis were various types of c-mitosis: colchicine-like metaphases, scattering and deletion of chromosomes, asymmetrical telophases, and pseudoanaphases. Adrenalectomy caused no significant changes in the numbers of atypical mitoses: the small increase in their number in the morning was not statistically significant ($P > 0.1$). Exposure of the adrenalectomized rats to stressors led to a significant increase in the number of pathological mitoses. They exceeded 10% in all the experimental groups. No circadian changes were found in the level of atypical mitoses after exposure to extremal factors. The spectrum of pathological mitoses in the experimental animals did not differ significantly from that observed in the control group.

Karyotypic analysis (Table 2) revealed no significant differences between the number of aneuploid cells in the intact rats in the morning and evening ($P > 0.1$). No significant changes likewise were found in the number of aneuploid cells after adrenalectomy. Exposure to stressors led to a significant increase in the number of aneuploid cells, both hypo- and hyperdiploid, in the bone marrow of the adrenalectomized rats in the morning. In the evening, however, a significant increase took place only in response to cooling; injection of pyrogenal caused no significant changes.

The detection of aneuploidy by the colchicine method as early as 6 h after exposure to stress is evidence that, besides damage to mitosis, repair processes are also disturbed in adrenalectomized rats during stress, or the system controlling entry of damaged and aneuploid cells into mitosis is impaired. The protective effect of a delay in cell division during exposure to radiation has been demonstrated. The present experiments indirectly confirm that inhibition of cell division prevents the appearance of pathological mitoses following exposure to nonspecific extremal factors. The cause of disturbance of cell division in the present experiments could be a direct effect of the stressors on mitosis. For a variety of reasons the writers conclude that the pathology of mitosis was due to a series of metabolic disturbances arising in response to the action of stressors on adrenalectomized rats.

The results of these experiments indicate that the uniform distribution of genetic information among the cells is disturbed and, as a result, subpopulations with an unbalanced number of chromosomes appear. This may be an important link in the chain of pathological processes developing during the action of extremal factors on animals with depressed adrenal function.

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