EFFECT OF STRESSORS ON THE MITOTIC REGIME AND SOME EVENTS OF THE CELL CYCLE IN THE CORNEAL EPITHELIUM OF ADRENALECTOMIZED RATS

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Previous investigations showed that the action of stressors causes inhibition, due to adrenal hormones, of mitotic activity in the corneal epithelium. The level of pathological mitoses (PM) and the index of labeled nuclei (ILN) were unchanged under these circumstances [3, 6, 7]. Injection of pyrogenal or contact hypothermia of the adrenalectomized rats caused an increase in the level of PM [7-9].

The object of the present investigation was to detect changes in the cell cycle determining the increase in the number of PM in the cornea or accompanying it, during the action of stressors on adrenalectomized rats.

EXPERIMENTAL METHOD

Experiments were carried out on 118 male rats weighing 140-190 g on the 5th day after adrenalectomy. Contact hypothermia of the rats for 1 h to 28-30°C and intravenous injection of pyrogenal in a dose of 2.5 μg/100 g were carried out by the method described previously [3, 9]. Autoradiography, determination of ILN and the label intensity (LI), counting of mitoses, and determination of the PM level were carried out by the methods described in [3, 6]. To estimate the duration of mitosis, parallel experiments were carried out on animals into which colchicine was injected in a dose of 4 μg/g 2 h before sacrifice. The mitotic index (MI) was expressed as the mean number of mitoses in 100 fields of vision.

The DNA content was determined cytophotometrically with control for the height of the sections. The height of the paraffin sections were determined on the ORIM microscope by the method in [2]. The optical density of the nuclei was measured on a laboratory cytophotometer designed in the Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR. The results of the cytophotometric measurements were analyzed as described in [5]. The main aim of the cytophotometric analysis was to detect the level of tetraploid nuclei, characterizing the duration of the G_2-M period. Nuclei located in the zone of distribution of metaphase figures on the histogram (95-110 conventional units) were regarded as tetraploid.

The investigation was carried out 6 and 24 h after the end of exposure to stress. Material was obtained toward 6 or 7 p.m. Together with investigations on adrenalectomized animals, a cytophotometric analysis of the DNA content also was made on animals with intact adrenals, exposed to stress.

The numerical results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Analysis of the mitotic regime 6 h after exposure to stress confirmed previous observations of elevation of the PM level in the cornea of adrenalectomized rats after exposure to stressors [8, 9]. The PM level was raised in the cornea of intact and adrenalectomized animals receiving pyrogenal or exposed to hypothermia (Table 1).

Just as in the previous experiments, the main form of PM in the intact and adrenalectomized rats consisted of mitoses with signs of disturbance of the mitotic apparatus, colchicine-like metaphases, scattering and deletions of chromosomes in metakinesis, asymmetric mitoses, and pseudoanaphases. The increase in

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TABLE 1. Effect of Stressors on Mitotic Regime, ILN, and LI in Corneal Epithelium of Adrenalectomized Albino Rats (M ± m)

Experimental conditions	MI	PM, %	ILN	LI
Intact animals (control)	161,7±19,6	4,6±0,4	9,2±0,4	32,3
Adrenalectomy (control)	140,5±17,4	3,9±0,3	7,9±0,3	26,5
6 h after exposure				
adrenalectomy + pyrogenal	198,0±22,1	15,4±1,0*	10,3±1,0	29,8
adrenalectomy + hypothermia	214,7±25,5‡	13,9±1,8*	6,7±0,9†	21,9
24 h after exposure				
adrenalectomy + pyrogenal	251,8±23,5*	16,8±1,5*	4,7±0,3*	12,5
adrenalectomy + hypothermia	263,3±26,9*	9,8±1,6*	12,5±1,2*	37,8

*Changes significant relative to "intact" and "adrenalectomized" control.

†Changes significant relative to "intact" control only.

‡Changes significant relative to "adrenalectomized" control only.

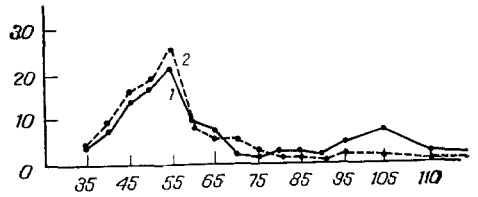

Fig. 1

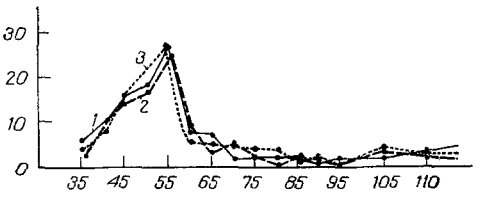

Fig. 2

Fig. 1. Distribution of DNA content in sections through corneal nuclei of intact rats (1) and rats exposed to stress (2). Abscissa, DNA content (in relative units); ordinate, number of nuclei (in %).

Fig. 2. Distribution of DNA content in sections through corneal nuclei of intact (1) and adrenalectomized (2) rats and of adrenalectomized rats exposed to stress (3). Legend as in Fig. 1.

the number of PM during hypothermia was not accompanied by changes in the spectrum of the aberrations, whereas during pyrogenal stress there was a twofold increase in the number of pseudoanaphases and an increase in the number of bridges from 0.2% of all PM in intact rats to 1.6% in adrenalectomized animals receiving pyrogenal. No significant changes took place in the total number of mitoses in adrenalectomized rats exposed to stress. Under these circumstances the number of anaphases was increased by 3.2 times compared with the control. Anaphase delay in mitosis was rare [1]. Its appearance is characteristic of processes accompanied by inhibition of protein synthesis [1, 11]. Injection of pyrogenal into adrenalectomized rats caused no change in ILN or LI at this time of investigation. Hypothermia of the rats led to a decrease in ILN and IM (Table 1). The changes were significant relative to the control.

Cytophotometric determination of the DNA content was carried out only on the pyrogenal model. In this group of experiments changes arising in animals with intact adrenals and exposed to stress were compared with the response of adrenalectomized animals to stress. The investigations were carried out 6 h after injection of pyrogenal.

After injection of pyrogenal into rats with intact adrenals an increase in the number of tetraploid nuclei in the corneal epithelium took place from 3.2% ($\sigma = 1.3$) in the control rats to 10.9% ($\sigma = 1.9$) in rats exposed to stress (Fig. 1). No change in the number of nuclei of other classes of ploidy could be observed. This agrees with the results of determination of MI under these conditions [4], indicating a decrease in the number of mitoses. The increase in the number of tetraploid nuclei was probably the result of delay of the cells in the G_2-M period, characteristic of the action of stressor situations [10]. Injection of pyrogenal into adrenalectomized rats caused no changes in the number of tetraploid nuclei 6 h after the injection (Fig. 2).

The number of PM in adrenalectomized rats receiving pyrogenal still remained high 24 h after the injection (Table 1). The PM level at this time of the investigation in the cornea of animals exposed to hypo-

thermia showed a tendency to return to normal, although it still remained significantly higher than in the control (Table 1). The total number of mitoses in the cornea of the cooled rats and rats receiving pyrogenal was increased, but the results of the experiments with colchicine indicated that this phenomenon differed in its nature. The increase in the number of dividing cells after injection of pyrogenal was connected with lengthening of the time of mitosis, whereas the increase in mitotic activity during hypothermia was true in character and took place on account of an increase in the number of cells taking part in mitosis. Autoradiographic analysis revealed a considerable decrease in ILN and LI in the cornea of rats receiving pyrogenal. The decrease in ILN and LI taking place after 6 h in rats exposed to hypothermia was replaced 24 h after the end of hypothermia by a significant increase in both indices.

The experimental results indicate that the increase in the number of PM in the cornea of rats with reduced powers of adaptation is not an isolated process in the cell cycle. Hypothermia or administration of pyrogenal to adrenalectomized rats caused disturbances in the S period, whereas in animals with normal powers of adaptation lengthening of the G_2-M period took place under the influence of stressors. This did not happen in adrenalectomized rats during stress.

The character of relations of cause and effect between the elevation of the PM level and changes in the cell cycle of the corneal epithelium of the adrenalectomized rats under the influence of stressors requires analysis on its own account.

LITERATURE CITED

1. I. A. Alov and L. S. Strochkova, Byull. Éksp. Biol. Med., No. 5, 605 (1978).
2. P. A. Gorelikov, Tsitologiya, No. 11, 1341 (1975).
3. N. B. Simon, S. S. Timoshin, and N. B. Murzina, Byull. Éksp. Biol. Med., No. 7, 87 (1979).
4. S. S. Timoshin, Vestn. Dermatol., No. 1, 38 (1971).
5. S. S. Timoshin, Byull. Éksp. Biol. Med., No. 2, 217 (1980).
6. S. S. Timoshin and N. B. Murzina, Byull. Éksp. Biol. Med., No. 8, 248 (1978).
7. S. S. Timoshin, N. B. Simon, and A. P. Baranov, in: Scientific and Technical Progress and Circumpolar Medicine. Fourth Conference [in Russian], Vol. 2, Novosibirsk (1978), p. 146.
8. S. S. Timoshin et al., in: Mechanisms of Injury, Resistance, Adaptation, and Compensation [in Russian], Vol. 1, Tashkent (1976), p. 342.
9. S. S. Timoshin, N. B. Simon, and A. P. Baranov, Byull. Éksp. Biol. Med., No. 9, 338 (1979).
10. L. V. Uryvaeva and V. M. Faktor, Tsitologiya, No. 9, 1100 (1974).
11. S. Pathnak and M. McGill, J. Cell Biol., 63, 258 (1974).