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EFFECT OF CHRONIC PYROGENAL STRESS ON THE MITOTIC REGIME AND NUMBER OF DNA-SYNTHESIZING CELLS IN THE CORNEAL AND LINGUAL EPITHELIUM OF ALBINO RATS

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The effect of prolonged stress on the mitotic regime and number of DNA-synthesizing cells in the corneal and lingual epithelium was studied in response to pyrogenal. Injection of pyrogenal for 5 days caused a decrease of 41% in the number of mitoses in the corneal and lingual epithelium. The decrease in the number of dividing cells did not correlate with changes in the rate of mitosis. The number of pathological mitoses in the corneal epithelium of intact rats remained unchanged during stress. The index of labeled nuclei in the corneal and lingual epithelium of the control rats was 12.6 and 10.8 respectively, which did not differ significantly from their values in the experimental animals (12.2 and 12.2). KEY WORDS: mitotic activity; stress; pyrogenal; DNA synthesis.

In previous investigations the writers found a decrease in the mitotic activity of the corneal and lingual epithelium following single [6] and repeated [7] injections of pyrogenal. The inhibition of cell division was found to be mediated through adrenal hormones [8], increased secretion of which is observed under the influence of pyrogenal [2, 3].

The object of the present investigation was to use pyrogenal stress as a model with which to study the effect of prolonged stress on the number of DNA-synthesizing cells and the mitotic regime of the corneal and lingual epithelium. The study of this problem is also of applied interest, for pyrogenal is used, in particular, for the treatment of dermatological diseases, an important link in the pathogenetic mechanism of which is stimulation of mitotic activity and disturbance of DNA synthesis [12].

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 140-190 g. Pyrogenal was injected into the caudal vein in a dose of 5 μg/100 g body weight between 11 a.m. and 12 noon daily for five days. Intact animals served as the control. The animals were decapitated six h after the last injection, and 1 h before sacrifice the rats were given an intraperitoneal injection of thymidine-^{3}H in a dose of 0.6 μCi/g body weight. Since it was impossible to obtain satisfactory autoradiographs of the corneal epithelium after administration of this

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TABLE 1. Effect of Five Injections of Pyrogenal on Some Indices of Adrenal Function (M ± m)

Group of animals	Wt. of adrenals, mg	Cholesterol content, μg % /g tissue	Blood 11-HS concentration μg percent	
			total 11-HS	protein-bound 11-HS
Control	20,4±0,30	0,46±0,053	30,3±4,3	24,7±9,7
Experimental	37,46±0,17	0,33±0,016	30,9±3,1	23,1±4,8
P	<0,01	<0,05	>0,5	>0,5

TABLE 2. Effect of Chronic Pyrogenal Stress on Some Indices of Proliferation in the Corneal and Lingual Epithelium (M ± m)

Group of animals	Cornea			Tongue			
	mean No. of mitoses	% of pathological mitoses	ILN, %	mean No. of mitoses after colchicine given*	mean No. of mitoses	ILN, %	mean No. of mitoses after colchicine given*
Control	154,6±16,2	6,0	12,6±0,8	594,8±86,1	84,1±11,8	10,8±1,2	271,2±20,3
Experimental	92,2±11,6	6,2	12,2±0,6	323,7±50,4	50,4±5,3	12,2±1,1	176,2±21,0
P	<0,01	>0,5	>0,5	<0,02	<0,02	>0,5	<0,005

*As in Russian original – Consultants Bureau.

dose of thymidine, the eye enucleated immediately after sacrifice was also incubated in medium 199 with thymidine-^{3}H (1 μCi/ml) at 37°C for 1 h. The corneas were washed in medium 199 and fixed in Carnoy's fluid. The second eye and the tongue were fixed immediately after sacrifice. Autoradiographs were prepared in the usual way from the incubated cornea and tongue. Cells synthesizing DNA were counted in the stratum basale and stratum spinosum of the corneal epithelium and on the lateral surface of the tongue. The index of labeled nuclei (ILN) was calculated after examination of 2000-3000 nuclei and expressed in percent. The mitotic regime in the corneal epithelium was determined in total preparations. Mitotic activity was judged from the number of mitoses in 100 fields of vision. Pathological mitoses were counted in accordance with Alov's classification [1], after which the number of pathological mitoses was expressed as a percentage of the total number of dividing cells. To exclude any change in the rate of mitosis during stress, in parallel experiments the animals were given an injection of colchicine, in a dose of 2 μg/g, 3 h before sacrifice. The number of blocked mitoses in the cornea and tongue was counted in 100 fields of vision. The adrenals of the rats were weighed on analytical scales, the cholesterol content in the adrenals was determined by Lifschitz's method [11], and the blood 11-hydroxysteroid level was determined by the method of Pankov and Usvaitova [4]. Altogether 36 rats were used in the experiments. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The data in Table 1 (an increase in weight of the adrenals, a decrease in their cholesterol content) points to the development of a stressor reaction to the five injections of pyrogenal. The absence of any increase in the blood 11-HS level can be explained by the increased consumption and intensified metabolism of glucocorticoids in the tissues under the influence of pyrogens [13].

Just as in the previous experiments, after injection of pyrogenal daily for five days the number of dividing cells in the corneal and lingual epithelium decreased by 41 and 37% respectively (Table 2). The number of pathological mitoses in the cornea of the intact rats was 6%. The main form of pathology was C-mitosis and its varieties.

Chronic stress caused no change in the level of pathological mitoses.

In the experiments with colchicine statistically significant differences remained between the control and experiment. It can accordingly be concluded that the decrease in the number of mitoses found as a result of chronic stress in fact reflects a decrease in mitotic activity and is not the result of a decrease in the rate of mitosis itself. Counting the number of DNA-synthesizing cells in the corneal and lingual epithelium revealed no significant difference between the results in the control and experimental groups. The index of labeled nuclei in the corneal and lingual epithelium on the intact animals was 12.6 and 10.8 respectively, and in rats receiving pyrogenal 12.2 and 12.2 respectively ($P > 0.5$). These results are in agreement with those of Yoshida's experiments [14], which demonstrated no change in ILN in the cornea of rabbits exposed to electric shock

for five days. The stability of ILN during prolonged stress, accompanied by inhibition of mitotic activity, suggests a number of possible explanations of the nature of the inhibition of cell division during stress: 1) diurnal variations in the response of the mitotic regime to stress [5, 9]; 2) prolonged holdup of the cells in G_2, G_2 growth [10]; 3) polyploidization of the epithelial nuclei under the influence of chronic stress.

These hypotheses require experimental verification.

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