Considering the results of this investigation and also data published previously, indicating that endogenous opioid peptides cause vomiting and changes in various autonomic parameters in animals (BP, PR, RR, etc.) [2, 4, 7, 10], it can be postulated that endogenous opioid peptides (and β-endorphin, in particular) are directly involved in the genesis of the vestibulo-autonomic disorders in motion sickness.

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ACCELERATION OF VERTICAL MIGRATION OF CORNEAL EPITHELIAL CELLS IN ALBINO RATS DURING CHRONIC IMMOBILIZATION STRESS

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In previous investigations the writers showed that repeated exposure to stress (sublethal hyperthermia, hypoxia, and fixation in the supine position, five times) causes activation of cell division and DNA and RNA synthesis in the corneal epithelium of albino rats [1].

In this investigation the effect of chronic immobilization stress on the kinetics of corneal epithelial cells from the basal layer into higher layers was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 49 male albino rats weighing 160-190 g. The animals were exposed for 1 h daily to fixation in the supine position for 5 days. After the final exposure to stress the animals were given an intraperitoneal injection of ^{3}H-thymidine (0.6 μCi/g body weight). Because the cornea has no blood supply, an additional application of 5 μCi of ^{3}H-thymidine was made to its surface.

The animals were killed and the cornea removed for investigation from the animals of group 1 one hour after the end of the final fixation and injection of ^{3}H-thymidine, from the animals of group 2 after 24 h, and of group 3 after 72 h. For each experimental group there was a corresponding control group of intact animals.

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TABLE 1. Effect of Chronic Immobilization Stress on Number of Cells in Corneal Epithelium and on DNA Synthesis at Different Times after Injection of ^{3}H-Thymidine (M±m)

Time after injection of ^{3}H-thymidine	Group of animals	No. of cells in field of vision	DNA synthesis	
			ILN %	IL
1	Control	60,00±1,29	3,39±0,17	32,90±0,92
	Experiment	56,20±2,83	4,91±0,39*	46,65±1,21*
24	Control	58,50±0,77	7,50±0,53	17,15±0,91
	Experiment	49,90±2,51*	10,30±1,04*	23,46±0,77*
72	Control	57,50±0,65	7,90±0,36	16,11±0,91
	Experiment	58,30±1,17	10,50±0,50*	21,45±0,92*

Legend. *P < 0.05.

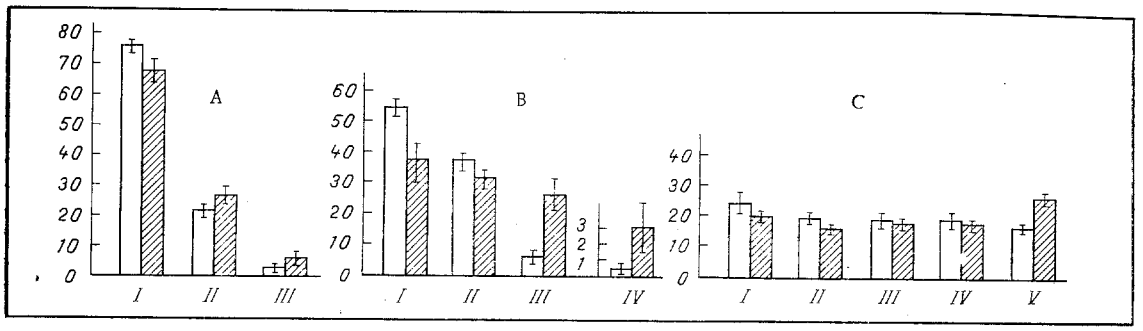

Fig. 1. Distribution (in %) of labeled nuclei among layers of corneal epithelium of albino rats during chronic immobilization stress 1 h (A), 24 h (B), and 72 h (C) after injection of ^{3}H-thymidine. Abscissa, layers of corneal epithelium; ordinate, number of labeled nuclei in layer as a percentage of total number of labeled nuclei in cornea. Unshaded columns – control, shaded – experiment.

Material for investigation was taken at 2-3 p.m. The corneas were fixed in a mixture of ethanol and acetic acid (3 : 1) for 1 h. Autoradiographs were prepared and the index of labeled nuclei (ILN) and intensity of thymidine labeling (IL) were determined by methods described previously [3, 4]. ILN was expressed as a percentage of the total number of cells. Sections passing through the center of the cornea were chosen for investigation. In each cornea the distribution of labeled cells among the layers of the cornea was studied, with distinction of five layers: basal (I), spinous (II), intermediate (III), inner squamous (IV), and surface (V) [2]. The number of labeled cells in the layer was expressed as a percentage of the total number of labeled cells. The ratio between pairs of daughter cells, dividing in the horizontal plane (both cells remained in the basal layer) was determined 24 h after injection of the isotope. When daughter pairs of cells were counted the following criteria were used for guidance: No other cells were present between the daughter cells, and there were no labeled cells in their immediate vicinity. The difference between the number of grains of silver above the nuclei being compared did not exceed five [5]. The mean number of epithelial cells per field of vision was determined by counting cells in 50 fields of vision in each preparation (ocular 12 × 1.25; objective 100).

EXPERIMENTAL RESULTS

Just as in the previous investigations [1], 1 h after injection of ^{3}H-thymidine at the end of exposure to stress stimulation of DNA synthesis was observed. Values of ILN and IL in the cornea of the experimental animals were increased by 1.4 times (Table 1).

Analysis of the distribution of labeled cells among the layers of epithelium showed that 76% of labeled nuclei in the cornea of the control animals were in the basal layer, 22% in the spinous layer, and only single labeled nuclei were found in the intermediate layer at the periphery of the cornea. A reduction in the number of DNA-synthesizing nuclei in the basal layer was observed at this time in the cornea of the rats exposed to stress, but an increase in their number was found in the spinous and intermediate layers (from 2.0 to 5.4, P < 0.05; Fig. 1).

A twofold increase in ILN was found in the cornea of the experimental and control animals 24 h after injection of ^{3}H-thymidine and the end of the final fixation, while at the same time IL was reduced in the control by 1.8 times and in the experiment by 1.9 times, i.e., also almost by half. This indicates that virtually all cells which were in the S phase and had incorporated ^{3}H-thymidine after the end of exposure to stress underwent mitotic division. At this stage of the investigation there was a significant decrease in the average number of cells per field of vision (from 58.5±0.8 in the control to 49.0±2.5 in the experiment, $P < 0.01$). Differences in the distribution of DNA-synthesizing nuclei among the layers of the cornea in the control and experimental animals became more marked than in the previous group of experiments. A significant decrease in the number of DNA-synthesizing nuclei in the basal layer in the experimental animals (55% in the control, 38% in the experiment, $P < 0.001$) was accompanied by a more than fourfold increase in the number of DNA-synthesizing nuclei in the intermediate layer (6.6% in the control, 29.9% in the experiment; $P < 0.02$). In the inner squamous layer IV the number of DNA-synthesizing nuclei increased tenfold (0.3% in the control, 3.2% in the experiment, $P < 0.002$).

Values of ILN and IL, characterizing DNA synthesis in the corneas of the control and experimental animals, 72 h after the end of exposure to stress and injection of ^{3}H-thymidine showed no significant change compared with their values in the previous series of experiments. On analysis of the distribution of DNA-synthesizing nuclei among the layers of the cornea a significant difference in the number of labeled cells was observed only in surface layer V (17.0% in the control, 26.6% in the experiment; $P < 0.001$).

Chronic exposure to stress thus increased the velocity of vertical migration of the cells from the basal layer toward the outer layers of the cornea.

There is no general agreement as yet regarding the mechanism of vertical migration of cells. Besides the view that this process is due to forces generated directly or indirectly by the dividing cells [6, 7], it has also been suggested that vertical migration of cells is independent of mitotic activity [8]. An essential role in the future fate of the daughter cells is played by orientation of the mitotic apparatus. A vertical (relative to the basal layer) arrangement of the mitotic apparatus leads to "vertical mitosis." According to the view described in [5], "vertical mitosis" characterizes to some degree the state of the processes of differentiation. In that case what we are discussing is not the formation of morphologically different cells immediately after mitosis, but the ultimate fate of the cells. The ratio between the numbers of horizontally and vertically arranged pairs of daughter cells in the corneas of intact animals 24 h after the end of exposure to stress and injection of ^{3}H-thymidine was 2.0±0.3, whereas in animals exposed to stress this ratio was reduced to 0.5±0.07 ($P < 0.001$), i.e., a marked shift toward intensification of differential processes was observed. An essential role in this shift evidently belongs to increased secretion of glucocorticoids which, as is generally accepted, stimulate the specific function of cells and differential processes and inhibit processes of cell division. Acceleration of vertical migration, observed in the present experiments, together with diminution of the cell population, stimulates proliferative processes.

Acceleration of vertical migration of cells plays a definite role in restoring the integrity of the tissue barrier, and it is also an important mechanism for eliminating from the cell population, those cells which have substained injury during exposure to stress.

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