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INDUCING ACTION OF HYDROCORTISONE ON MITOTIC RHYTHM IN THE CORNEAL AND ESOPHAGEAL EPITHELIUM IN RATS

S. G. Mamontov and N. M. Suvorova

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Glucocorticoid hormones are among the leading factors responsible for adaptation of the body to changing or extremal environmental conditions. Since a persistent increase in the blood levels of adrenocortical hormones is observed in stress, elucidation of the principles governing cell multiplication under conditions of hypercorticism is an urgent problem. Various workers have found a decrease in the number of mitoses and index of [^{3}H]thymidine-labeled nuclei [15, 16] and lengthening of the periods of the mitotic cycle in animals receiving cortisone or hydrocortisone [1, 4]. As a result, glucocorticoid hormones have come to be regarded as inhibitors of cell proliferation [5]. However, this view of the effect of glucocorticoids on proliferation is opposed to their basic (adaptive) role. Inhibition of cell proliferation during prolonged exposure to a stressor, given the more rapid differentiation and death of cells taking place under conditions of hypercorticism, must inevitably lead to a reduction in the number of cells in the tissues and reduction or loss of function. In reality, this does not happen. It must be assumed that the conclusion regarding the inhibitory properties of glucocorticoids is determined by the character of the techniques used in the investigations cited above and many others. First, most studies of cell proliferation during glucocorticoid administration have been undertaken at a particular time of day. We know, however, that the sensitivity of tissues to biologically active substances, including hormones, can vary considerably depending on the time of day when the procedure is carried out [12]. With regard to glucocorticoids, it has been shown that a single injection of dexamethasone can lead to a fall in the mitotic index, can induce synchronization of cell division, or can establish a 48-h period of the mitotic rhythm instead of a 24-h period, depending on the time of injection — in the morning, afternoon, or evening [13]. Second, a transient increase in the corticosteroid level may lead to basically different results from those of prolonged hydrocortisone. If mitotic activity is studied simultaneously in several tissues of rats receiving single or repeated injections of hydrocortisone, it is found to be reduced in animals receiving a single injection of the hormone. In rats receiving injections of hydrocortisone for 1 week before sacrifice, the intensity of cell proliferation is increased. The 24-h pool of DNA-synthesizing cells also is increased [3, 8]. Structure of the mitotic rhythm during intensified cell proliferation induced by hydrocortisone has not yet been investigated. Since circadian rhythms of physiological processes at different levels play an important role in adaptation to the external environment [11], it was decided to study the structure of the mitotic rhythm in the surface epithelium of rats against the background of a prolonged rise in the glucocorticoid hormone level in animals.

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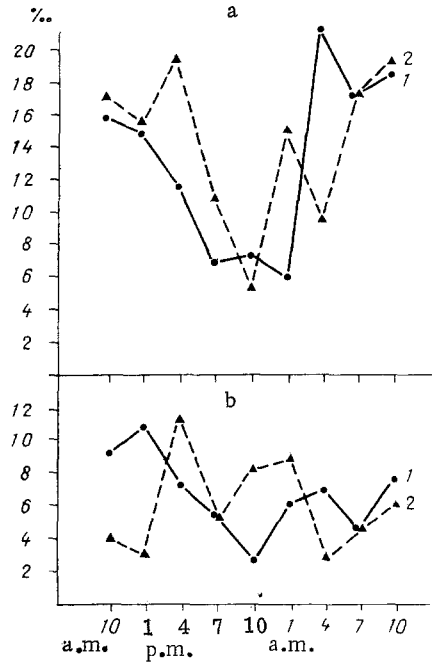

Fig. 1. Circadian rhythm of mitoses in corneal (a) and esophageal (b) epithelium of rats after repeated injections of hydrocortisone. 1) Control, 2) experiment. Abscissa, clock time; ordinate, mitotic index (in °/₀₀).

EXPERIMENTAL METHOD

Male Wistar rats were used for experiments in February. The rats were kept under natural conditions of illumination. The experimental animals were given a daily intraperitoneal injection of hydrocortisone acetate (from Gedeon Richter, Hungary) in a dose of 10 mg/100 g body weight for 7 days. The hormone was injected at 4-5 p.m., i.e., at a time of natural elevation of hormone levels in the circadian rhythm [9]. On the 7th day of the experiment, control and experimental animals were decapitated at 10 a.m., 1, 4, 7, and 10 p.m., and 1, 4, 7, and 10 a.m., four to six rats at each time. The number of mitoses in 30,000-40,000 cells for the corneal epithelium and in 6000-8000 cells for the esophageal epithelium was counted in total preparations of the cornea and histological sections of the esophagus. The mitotic index (MI) was expressed in promille. The results were subjected to statistical analysis by the Fisher–Student t test and by the Wilcoxon–Mann–Whitney nonparametric U test.

EXPERIMENTAL RESULTS

The largest number of mitoses in the corneal epithelium (Fig. 1) was found between 4 and 10 a.m., the smallest number between 7 p.m. and 1 a.m., as was found in rabbits in many other investigations [2, 7]. The amplitude of fluctuations in MI in the course of the 24-h period, expressed as the ratio between the highest and lowest values of MI, was 3.9. In rats receiving injections of hydrocortisone MI was high between 10 a.m. and 1 p.m., and high again at about 4 p.m., at a time when in the control the number of mitoses was low. Differences between MI at 4 p.m. in the control and experimental series were significant ($P = 0.007$). MI then fell until 10 p.m. and rose again at 1 a.m. ($P = 0.01$). This second peak of MI at 1 a.m. differed significantly from MI at 4 a.m. The mitotic rhythm in the corneal epithelium of rats receiving hydrocortisone, extending over the 24-h period, was thus a two-humped curve. The results suggest that the monophasic character of the mitotic rhythm in the control animals was replaced by a biphasic rhythm with a long period of 9 h (from 4 p.m. to 1 a.m.) and 15 h (from 1 a.m. to 4 p.m.). The mean daily MI in the experiments (14.1 ± 1.6 °/₀₀) was practically indistinguishable from the control (13.0 ± 1.9 °/₀₀). The amplitude of the rhythm (3.9) likewise was unchanged.

The mitotic rhythm in the esophageal epithelium of rats receiving hydrocortisone also was modified (Fig. 1b). In the control rats, the number of mitoses reached a peak at 10 a.m.–1 p.m. and fell until 10 p.m. ($P = 0.002$), after which it rose again in the morning. In rats with

hypercorticism MI was low between 10 a.m. and 1 p.m. ($P = 0.003$ compared with the controls at this period), it showed a sharp increase at 4 p.m. ($P = 0.01$) and a decrease at 7 p.m. ($P < 0.05$). Between 10 p.m. and 1 a.m., the second peak of mitoses appeared, followed by a low MI. The mean daily MI in the esophageal epithelium of the control and experimental rats was 6.6 ± 0.8 and $5.9 \pm 0.9‰$, respectively, and the amplitude of the fluctuations of MI was 4.4×4.1, i.e., practically the same.

Changes in the rhythm of mitósis observed in the corneal epithelium and in the esophageal epithelium of rats after prolonged administration of hydrocortisone compared with the control are thus similar in principle. The rhythm becomes biphasic in character but the amplitude and mean daily values of MI remain unchanged.

Changes in the character of the rhythm in response to hydrocortisone may be due to several factors. It is now considered [10] that tissues are a set of cell populations with different proliferative potential and making unequal contributions to the over-all parameters of proliferative activity. They may perhaps exhibit unequal competence as regards factors inducing synchronized initiation of DNA synthesis in the cells or of a mitotic rhythm. Unequal sensitivity of cells to glucocorticoids, due to different numbers of receptors for these hormones, has been demonstrated for cells of lymphoma L-1210 [14]. A similar situation cannot be ruled out for normal tissues. Considering that under conditions of hypercorticism the proliferative pool is increased [8], it can be postulated that heterogeneity of the cell populations constituting the proliferative fraction increases even more. The appearance of two peaks of mitoses during the 24-h period can be explained by complex interaction between cell populations differing in their sensitivity to regulatory factors, and adrenocortical hormones. The possibility likewise cannot be ruled out that during a prolonged rise in the blood hormone levels, a parallel process takes place — shortening of the mitotic cycle in a certain proportion of cells. The possibility of a considerable decrease in the duration of mitosis after injection of hydrocortisone has been established for certain tissues [3, 6].

It was shown previously [7] that adrenalectomy flattens out the mitotic rhythm in the tissues by reducing the amplitude of fluctuations in mitotic activity during the 24-h period, and it reduces the intensity of cell proliferation. Comparison of these data with results of the present investigation shows that glucocorticoid hormones are an important factor determining the level and rhythm of cell divisions.

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