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CHANGES IN EPIDERMAL G_2-CHALONE CONTENT AND MITOTIC ACTIVITY OF VAGINAL EPITHELIAL CELLS OF OVARIECTOMIZED RATS AFTER STIMULATION OF PROLIFERATION BY ESTRADIOL

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According to Khlopin's histogenetic classification the vaginal epithelium is one of the epidermal tissues. Accordingly the proliferation of the cells of this epithelium may evidently be regulated by epidermal chalones. For instance, their inhibitory action on DNA synthesis and mitosis of epitheliocytes has been demonstrated [4], and the presence of an endogenous epidermal G_2 chalone in this epithelium has been found immunologically [5, 7]. The vaginal epithelium is a hormone-dependent tissue. Ovariectomy leads to its hypoplasia, and administration of estrogens against this background is a powerful stimulus to proliferation, causing a marked increase in the index of labeled nuclei (ILN) and mitotic coefficient (MC) of the cells [6, 9, 10]. To judge from the few available data, the increase in mitotic activity after such stimulation is preceded by a fall in the G_2 chalone level in the epithelium [1, 8]. However, in the investigations cited G_2 chalone was determined in extracts made from the whole organ, by Mancini's immunodiffusion method, and for that reason its concentration may have depended on the completeness of extraction and on various other causes.

For the reasons mentioned above, in the investigation now described an attempt was made to characterize changes in the epidermal G_2 chalone content and to compare them with data on mitotic activity in the vaginal epithelium of ovariectomized rats at different times after stimulation of proliferation by estradiol, using a technique of quantitative immunomorphology, giving a more adequate estimate of its concentration actually in the epithelial cells.

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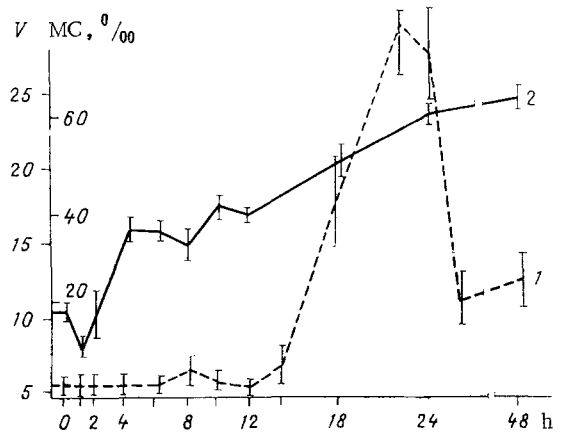

Fig. 1. Changes in level of mitotic activity (1) and epidermal G_2 chalone concentration (2) in vaginal epithelial cells of ovariectonized rats at different times after stimulation of proliferation by injection of estradiol. Abscissa, time after injection of estradiol (in h); ordinate: numbers on left represent G_2 chalone concentration (in units of change of voltage); numbers on left represent MC (in $^{o}/_{oo}$).

EXPERIMENTAL METHOD

Experiments were carried out during the winter on sexually mature female albino rats weighing 18-200 g obtained from the "Rappolovo" nursery, Academy of Medical Sciences of the USSR. The stages of the estrous cycle were determined in the animals by the vaginal films method, and ovariectomy was performed under general ether anesthesia in the stage of diestrus. The rats were given a subcutaneous injection of 17-β-estradiol (Organon, Holland) in a dose of 1 µg per rat 4 weeks after castration, inducing a high level of proliferation in the vaginal epithelium of the ovariectomized animals [3]. The rats were killed by cervical dislocation in groups of four animals at different times after injection of the hormone: 1 and 2 h, and thereafter at intervals of 2 h for 2 days, and again after 7 and 45 days. The vaginal wall was divided longitudinally into two parts. One part of the material was fixed in Bouin's fluid for counting the number of mitoses, and paraffin sections were cut to a thickness of 6 µ; the other part was used to determine the concentration of epidermal G_2 chalone in the epithelium. Histological preparations were stained with hematoxylin and eosin and the number of mitoses was counted in 1000 cells of the stratum basale of the vaginal epithelium, after which MC was calculated in promille.

Changes in the epidermal G_2 chalone content in the vaginal epithelium were assessed by a quantitative immunofluorescence method. Pieces of tissue were frozen in isopentane, cooled with liquid nitrogen, cryostat sections were cut to a thickness of 6 µ, and the sections were treated with monospecific immune serum against epidermal G_2 chalone by the indirect Coons' method [5]. Fluctuations in the intensity of fluorescence characterizing the G_2 chalone content in the tissue were determined with the LYUMAN I-3 luminescence microscope, equipped with a photometric system (LOMO, Leningrad), consisting of FMEL-1A microfluorometric attachment and FEU-39A photoelectronic multiplier, UBPV-1 direct current source, and U5-9 amplifier. The voltage of the force of current was 1500 V and the input resistance on the amplifier 10^6 Ω. The intensity of fluorescence was measured in units of change of voltage induced by fluctuations in strength of the current as the result of a change in the photic flux recorded by the photoelectronic multiplier. For a more accurate measurement of fluctuations of voltage, a V7-26 voltmeter, connected in parallel to the voltmeter of the matching unit of the U5-9 amplifier was used. Disks of uranium glass ZhS-19, 0.03 and 1.5 mm thick, were used as standards of intensity of fluorescence. The field diaphragm of the microscope was stopped down almost to the limit to exclude "discoloration" of neighboring regions of the epithelium during photometry, and its position remained unchanged during the investigation. Background fluorescence of tissues located close to the epithelium was very small and equal in all preparations, and it was therefore considered that fluctuations in the intensity of fluorescence in the epithelium depended only on its content of the test antigen. At all times sections of material obtained from four animals were investigated. Altogether 20 measurements were made

on each of several sections from each animal. The numerical results were subjected to statistical analysis. Significance of differences was assessed by the Fisher–Student t test.

EXPERIMENTAL RESULTS

Data on changes in the level of mitotic activity and the content of epidermal G_2 chalone in the vaginal epithelial cells of ovariectomized rats during stimulation of proliferation by estradiol are given in Fig. 1. It will be clear from Fig. 1 that 1 h after injection of estradiol the G_2 chalone content fell in the epithelium, but later its concentration increased gradually up to a maximum after 48 h. A peak of mitotic activity occurred at 22-24 h. Mitotic activity returned practically to its initial level and the concentration of G_2 chalone also fell almost to normal after 7 days. After 45 days neither the mitotic activity nor the concentration of inhibitor differed from the initial values characteristic of ovariectomized animals.

The fall in concentration of the inhibitor 1 h after stimulation may indicate that the cells have already started their preparations for mitotic division and have freed themselves from endogenous G_2 chalone. However, after 2 h the concentration of the inhibitor returned to its initial level, and after 4 h it was much higher. Later, until 8 h, at a time when the cells had not yet started to divide, the G_2 chalone concentration in the tissue remained at practically the same level. A further rise in its concentration took place against the background of massive entry of the cells into the G_2 phase of the cell cycle and mitosis, reaching a maximum by 48 h, i.e., toward the time when mitotic activity was considerably reduced. Immediately after a small decline, the G_2 chalone concentration in the epithelium thus began to rise. Despite this, the endogenous G_2 chalone was unable to influence entry of the cells into mitotic division. This fact confirms observations showing that exogenous administration of epidermal chalones after hormone injection cannot change the proliferative response of the tissue [4].

The present investigation confirmed in principle previous data on changes in the G_2 chalone concentration in tissue extracts at different times after hormonal stimulation, studied by the quantitative immunodiffusion method [1, 8]. However, we found fluctuations in its concentration at rather different times. The main cause of the observed differences was evidently the use of different methods to assess the content of epidermal G_2 chalone in the epithelium. In the investigations cited the G_2 chalone concentration was determined in extracts prepared from the whole organ, and for that reason its concentration could be dependent on the degree of extraction. Moreover, during investigations by this method, the increase in thickness of the epithelium after mitotic divisions was not completely taken into account, for it has only a very slight effect of the total thickness of the vaginal wall and on the content of total protein in the extract, relative to which the authors cited estimated the G_2 chalone concentration. The increase in the G_2 chalone concentration observed in the extract during and after the period of mitotic division could be due to thickening of the epithelial layer, and not reflect changes in the concentration of the inhibitor on the epithelial cell. Accordingly, we considered it more appropriate to use quantitative immunomorphology to determine the concentration of the test antigen, for it enables the antigen to be assayed directly in the cytoplasm of the epithelial cells.

It can be postulated on the basis of these results that after a very small decrease in the G_2 chalone concentration, synthesis of the chalone begins, with the aim of suppressing the sharply rising level of proliferation. Synthesis of the inhibitor returns to its initial state during extinction of the proliferative response to hormonal stimulation. The endogenous G_2 chalone level in the vaginal epithelium thus changes depending on the degree of proliferative activity of the cells. Fluctuation found in the concentration of the inhibitor support the hypothesis of the existence of negative feedback in the regulation of tissue homeostasis.

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