EFFECT OF NOCICEPTIVE STIMULATION ON MITOTIC ACTIVITY OF EPITHELIAL CELLS IN SKIN SURROUNDING A WOUND AT DIFFERENT AGE PERIODS

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Wounds 1 cm long, down to the cutaneous muscle, were inflicted on rats aged 11 days, 2 months, and 2.5 months in the subscapular region. Nociceptive stimulation was applied to the animals by means of an electric current 48 h after the operation. Mitotic activity was determined in cells of the stratum basale of the skin epithelium adjacent to the wound. Nociceptive stimulation was found to reduce the mitotic index of the cutaneous epithelial cells of the animals of all age groups studied by a considerable degree (by several times). An increase in the mitotic index was observed from the early to the later stages of postnatal ontogeny.
KEY WORDS: nociceptive stimulation; incised wound; mitotic index.

Nociceptive stimulation, which frequently accompanies wound healing, is known to be a factor that inhibits cell proliferation in various epithelial tissues [4-7, 11]. It has also been shown that nociceptive stimulation depresses mitotic activity in the cutaneous epithelium, not from the very beginning of postnatal life but only after a certain stage has been reached. However, the age aspects of the dynamics of mitotic activity in the epidermis of the skin during nociceptive stimulation has been inadequately studied. Only a few investigations have been published [9, 12, 13] in which the authors set out mainly to determine the effect of nociceptive stimulation on mitotic activity of the cutaneous epithelium in the early stages of postnatal development in animals of different species.

It was decided to continue these investigations with the aim of studying the effect of brief nociceptive stimulation on mitotic activity of the epithelium of the skin adjacent to a wound defect, at different age periods. Before the age of 10 days, short-term nociceptive stimulation is known not to cause any changes in the dynamics of cell division of the injured cutaneous epithelium [9].

EXPERIMENTAL METHOD

Experiments were carried out on 36 noninbred male albino rats of three age groups: 11 days (group 1), 2 months (group 2), and 2.5 months (group 3). Twelve animals were used at each time (six control and six experimental).

Full-thickness (down to the cutaneous muscle) incised wounds 1 cm long were inflicted with a safety razor blade in the region below the right scapula. The operations were performed under ether anesthesia. Allowing for the diurnal rhythm of mitotic activity [3, 8], the experiments were carried out from 8 to 9 a.m., when mitotic activity is maximal. The wounded animals of each age group were divided into two equal and experimental control groups. The experimental animals, in a special cage, were stimulated with an electric current of 30 V for 5 min, 48 h after wounding, when the number of mitoses in the skin adjacent to the wound would have reached a maximum [10]. The control animals were kept under corresponding conditions except that nociceptive stimulation was not applied.

Pieces of tissue measuring 1 cm^2 in area, including the wound and adjacent part of the skin, were excised from the animals of all three age groups 30 min after infliction of nociceptive stimulation (during this time the

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cells subjected to nociceptive stimulation would have completed their division and dividing cells which have not yet reacted to it would have started to appear). The biopsy material was fixed in Zenker's fluid and embedded in paraffin wax; sections were cut perpendicularly to the wound, 5 μ in thickness, and stained with Carrazzi's hematoxylin.

The number of dividing cells was counted in 5000 cells of the stratum germinativum of the epidermis. The counting began 2-3 fields of vision away from the wound edge. The mitotic index (MI) was expressed in promille. The results of all the series of experiments were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The following results were obtained. In rats aged 11 days the level of mitotic activity in the skin adjacent to the wound defect was $4.2 \pm 0.2‰$, whereas after nociceptive stimulation it was significantly reduced, to $1.3 \pm 0.09‰$ ($P < 0.001$). In the animals aged 2 months MI in the control was $7.3 \pm 0.45‰$, whereas after nociceptive stimulation it was $3.26 \pm 0.3‰$ ($P < 0.001$). In the rats aged 2.5 months, nociceptive stimulation led to a decrease in MI from $8.9 \pm 0.4‰$ in the control to $3.0 \pm 0.5‰$ in the experiment ($P < 0.001$).

Following nociceptive stimulation sharp inhibition of cell division was thus observed, in agreement with the observations of other workers who studied the effect of nociceptive stimulation under rather different conditions [2, 14]. Nociceptive stimulation reduced MI in the animals of group 1 by two thirds, in the animals of group 2 by more than half, and in the animals of group 3 by almost two thirds, i.e., the character of the reaction of the cutaneous epithelium to nociceptive stimulation was the same in the animals of all three age groups.

With age, MI of the cutaneous epithelial cells changes and a general tendency was observed for it to rise both in the control and in the experimental series, but the character of the changes differed. As already stated, in the control groups MI for animals aged 11 days was $4.2 \pm 0.2‰$, for animals aged 2 months $7.3 \pm 0.45‰$, and for animals aged 2.5 months $8.9 \pm 0.4‰$. The differences between all three of these values are statistically significant. In the experimental groups, however, MI for the animals of these three age groups was 1.3 ± 0.09, 3.26 ± 0.3, and $3.0 \pm 0.5‰$, respectively. The difference between the value of MI in groups 1 and 2 and in groups 1 and 3 is significant.

Consequently, according to these results the mitotic activity of cells of the injured cutaneous epithelium is higher in the period of sexual maturation than at the age when these cells first begin to respond to nociceptive stimulation.

The results are evidence that during development the ability of cells of the cutaneous epithelium to divide varies. This phenomenon may probably be connected with the increased action of sex hormones. We know, for example, that estrogens stimulate cell division in the organs directly connected with them (mammary gland, epithelium of the uterus, pituitary gland, etc.) [1]. It is not yet clear whether sex hormones affect the intensity of cell division in other tissues and, in particular, under the conditions of nociceptive stimulation.

Analysis of these results suggests that sex hormones act on the mitotic activity of cell of the cutaneous epithelium during regeneration, stimulating this process both under normal conditions and during nociceptive stimulation. They evidently participate also in the mechanism of formation of the nociceptive response itself. Further experiments must either confirm or disprove these hypotheses.

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QUANTITATIVE HISTOENZYMIC ANALYSIS OF THE ADENOHYPOPHYSIS AND ADRENAL CORTEX IN THE EARLY STAGES OF INVOLUTION

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To assess the changes in the neuroendocrine system during involution a method of quantitative histoenzymic analysis was used. Activity of NAD- and NADP-oxidoreductases, acid and alkaline phosphatases, glucose-6-phosphate dehydrogenase, and 3β-OH-steroid and 11β-OH-steroid dehydrogenases. Differences were found in the structural and metabolic basis for physiological adjustments of the neuroendocrine system during the course of the estrous cycle in the early stages of involution. An initial reduction in transport from cells to vessels was demonstrated in the aging animals, although functional activity of the intracellular organoids was preserved.
KEY WORDS: cytospectrophotometry; histoenzymic analysis; adenohypophysis; adrenal cortex; age involution.

Modern views on age changes in the neuroendocrine system (NES) are based principally on the results of physiological and biochemical investigations [1, 4, 7]. Only a few morphofunctional studies using quantitative cytochemical analysis have been undertaken [6, 8]. In previous investigations the writers showed that disturbances of hormonal homeostasis appear in rats at the age of 12 ± 2 months, in the form of lengthening of the estrous cycle on account of diestrus and desynchronization of the cyclic function of the ovaries, adrenal cortex, and thyroid gland [3].

In this investigation an attempt was made to analyze the morphofunctional state of the adenohypophysis and adrenal cortex in the early stages of involution in order to determine objective structural-functional criteria that could bring about the age disintegration of the NES discovered previously.

EXPERIMENTAL METHOD

Experiments were carried out on 48 female albino rats aged 12 ± 2 months (24 animals) and 4-5 months (24 animals) using 6 rats for each phase of the cycle. The activity of the following enzymes was determined in cryostat sections (10 μ thick) through the pituitary and adrenal glands: NAD- and NADP-oxidoreductases (NADO, NADPO) and acid (AcP) and alkaline (AlP)phosphatases; in addition, activity of glucose-6-phosphate dehydrogenase (G6PD) and 3β-OH-steroid and 11β-OH-steroid dehydrogenases (3β-OH-SD, 11β-OH-SD) was determined in the adrenal. The intensity of the histoenzymic reactions was assessed quantitatively with the MUF-5 microspectrophotometer by scanning in visible monochromatic light. The numerical results were analyzed by special program on the EC-1020 computer [5]. Besides estimation of the mean value and dispersion, mathematical analysis of the results of cytospectrophotometry included comparison of histograms, giving the minimal percentage of cells in which enzyme activity changed during the transition from one phase of the estrous cycle to another [2].

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