EFFECT OF HORMONAL STIMULATION ON THE ACTION OF X-RAY IRRADIATION ON DNA SYNTHESIS IN UTERINE EPITHELIAL CELLS

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The action of x-ray irradiation on DNA synthesis in the uterine epithelial cells of ovariectomized mice depending on the level of hormonal stimulation was studied by autoradiography using thymidine-H^3. After local irradiation in a dose of 400 R the decrease in the index of labeled nuclei varied with the dose of dihydrostilbestrol administered before irradiation and on the phase of the mitotic cycle of most cells of the uterine epithelium.

Autoradiographic investigation on animals in vivo with thymidine-H^3 have shown that the number of labeled cells decreases in several organs during irradiation [1, 3, 5, 7, 14].

The uterine epithelium of ovariectomized mice receiving injections of estrogens is a convenient model with which to study these processes. Work has been carried out in which the temporal parameters of the mitotic cell cycle and changes in the number of cells in the stage of DNA synthesis have been determined by autoradiography in the uterine epithelium [2, 4, 7, 9-11].

Few investigations into the action of irradiation on the epithelium of the reproductive organs have been published [8, 12].

The object of this investigation was to study the action of x-ray irradiation on the initiation of DNA synthesis by the epithelial cells of the mouse uterus depending on the level of hormonal stimulation.

EXPERIMENTAL METHOD

Experiments were carried out on 224 female (CBA × C57B1/6J) F_1 hybrid mice weighing 18-20 g. All the animals were ovariectomized 14 days before the experiment began. An oily solution of dihydrostilbestrol was used in the experiments. To study changes in the number of cells in the stage of DNA synthesis the animals received an injection of Soviet thymidine-H^3 with a specific activity of 1.4 Ci/mmole. Local irradiation of the experimental animals was carried out on the RUM-7 apparatus (filter 3.57 mm Al, half-thickness layer 2 mm). The focal distance from the anode to the uterine cornua was 10.5 cm. The mice received a single dose of irradiation (400 R, dose rate 63 r/min).

In the experiments of series I the ovariectomized mice received a subcutaneous injection of dihydrostilbestrol in a dose of 1 μg. The animals were divided into three groups. In group 1, in which the index of labeled nuclei (ILN) was determined at various times of injection of the hormone, the mice were sacrificed 6, 12, 16, 18, 24, 26, and 30 h after the injection of dihydrostilbestrol. Eight mice were killed at each time. Thymidine-H^3 was injected intraperitoneally in a dose of 0.7 μCi/g 1 h before sacrifice. Ovariectomized animals not receiving the hormone acted as the control.

In the second group of mice the action of irradiation on the uterine epithelium was studied in ovariectomized mice stimulated with estrogen. The animals were injected with thymidine-H^3 1 h before sacrifice.

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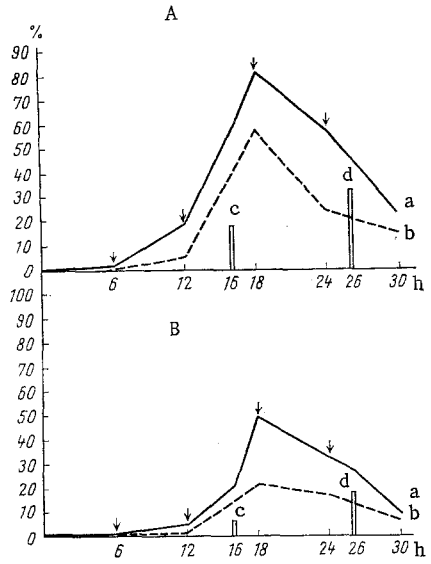

Fig. 1. Changes in ILN in uterine epithelial cells after local irradiation of mice in a dose of 400 R at various times after injection of dihydrostilbestrol in doses of 1 μg (A) and 0.05 μg (B): a) group 1; b) group 2; c and d) group 3 (killed 10 and 20 h, respectively after irradiation). Arrows indicate time of irradiation. Abscissa, time after injection of hormone (in h); ordinate, change in ILN (in %).

Eight mice were irradiated at each time, 6, 12, 18, and 24 h after the injection of dihydrostilbestrol. All the animals were killed 6 h after irradiation.

The mice of group 3 were irradiated 6 h after injection of the hormone and sacrificed 10 and 20 h after irradiation.

In series II the scheme of the experiment was the same as in series I except that dihydrostilbestrol was given in a dose of 0.05 μg.

All the experimental animals were killed by decapitation. The epithelium of the uterine cavity was used as the test object.

For autoradiography sections 5 μ in thickness were covered with type M radiosensitive emulsion. The sections were exposed in a refrigerator at 4°C for 2 weeks. After development with amidol developer the sections were stained with Carazzi's hematoxylin. The number of labeled nuclei was counted in at least 3000 cells of the uterine epithelium in the resulting autoradiographs.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1A (a) that 6 h after a single injection of dihydrostilbestrol the value of ILN was low. It rose gradually to reach 59.8% 16 h after injection of the hormone. The maximal number of synthesizing cells (81.5%) was observed 18 h after the injection of dihydrostilbestrol. The increase in number of cells synthesizing DNA then fell somewhat and 30 h after injection of the hormone it was only 23.2%. These results agree with those obtained by Perrotta [10] and Roslyakova [4]. The results of the experiments in which animals were irradiated at various times after the injection of dihydrostilbestrol are given in Fig. 1A (b). The curve of ILN in the experimental mice was considerably lower at all times of the investigation than that for the animals of group 1. This decrease was irregular, and it depended on the phase of stimulation of DNA synthesis induced by the hormone given to the animals before irradiation. The greatest decrease in ILN was observed when the mice were irradiated 6 h after administration of dihydrostilbestrol, i.e., before the beginning of the increase in ILN, and were sacrificed 12 h after injection of the hormone, and also when the animals were irradiated 18 h after injection of the dihydrostilbestrol, i.e., during the maximum of DNA synthesis, and were sacrificed 24 h after injection of the hormone. In the first case ILN was 28.9% of the control value compared with 41.3% in the second. If the animals were irradiated 12 and 24 h after the injection of dihydrostilbestrol and sacrificed 6 h after irradiation, the values of ILN were 70.4 and 65.9% of the control, respectively. The control consisted of unirradiated mice sacrificed at the same times after injection of the hormone as the irradiated animals. Irradiation of mice 6 h before the maximal increase in ILN induced by hormonal stimulation, i.e., 12 h after the injection of dihydrostilbestrol, thus did not prevent this rise; all that happened was that the maximum was 30% lower than in the unirradiated animals.

It is interesting to note that in mice irradiated 6 h after injection of dihydrostilbestrol, i.e., before the mass initiation of DNA synthesis in the cells, and were sacrificed 10 h after irradiation, i.e., 16 h after injection of the hormone, as the results for the experiments of group 3 (Fig. 1A,c) show, hormonal stimulation of DNA synthesis was considerably inhibited. If the mice of the same group were sacrificed 20 h after irradiation, i.e., 26 h after injection of dihydrostilbestrol (Fig. 1A, d), ILN was considerably higher, namely 68.2% of the control. These results evidently indicate that the response of the cells to irradiation is more likely to be manifested as the delay of onset of DNA synthesis than of its continuous inhibition.

The results of the experiments of series II are given in Fig. 1B. They show that the increase in DNA synthesis in the uterine epithelium of the ovariectomized mice after a single injection of 0.05 μg dihydrostilbestrol was also observed after injection of 1 μg of the hormone, in this case after 18 h. However ILN

in all groups of this series was nearly 50% lower than in the animals of series I. After irradiation of the experimental animals the ILN curve, just as in series I of the experiment, was much lower than the curve for the control animals. By contrast to the experiments of series I, with a lower dose of the hormone a sharp decrease in ILN was also observed during the period of its maximum, i.e., if the animals were killed 18 h after injection of dihydrostilbestrol.

The results of these experiments confirm those of Perrotta [12], who showed that stimulation of DNA synthesis in the uterine epithelium of ovariectomized mice is inhibited more sharply if the animals are irradiated 6 h after injection of the hormone, i.e., before an appreciable increase in the number of cells synthesizing DNA.

It was also found that irradiation of mice 12 h after injection of the hormone (1 μg) at a time when ILN was slightly increased, but the overwhelming majority of cells later contributing to the sharp rise in the number of DNA-synthesizing cells had not yet entered the S period, no longer prevented the increase in ILN. Meanwhile, in the presence of the weak stimulant effect (dose of the hormone 0.05 μg), irradiation in the same period considerably retarded the entry of the cells into the period of DNA synthesis. The inhibitory action of the same dose of irradiation thus depended in these experiments on the dose of hormone given before irradiation and also on the phase of the mitotic cycle in which most cells of the uterine epithelium were found.

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