EFFECT OF VARIOUS DOSES OF RETINOL ACETATE ON RNA AND TOTAL PROTEIN CONTENT IN HAMSTER OVARIES AND ENDOMETRIUM

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KEY WORDS: retinol acetate; total protein; endometrium; ovary.

In the present state of knowledge vitamin A can be regarded as an important regulator of cellular metabolism and of processes of cell division and differentiation, with its main effect on organs of epithelial nature [6, 9, 10, 12]. Both an excess and a deficiency of retinol in the body lead, in particular, to disturbance of RNA and protein synthesis and also to their metabolic conversions, as has been conclusively demonstrated by studies of several organs [7, 8]. However, data in the literature on the dynamics of changes of this kind in organs of the female reproductive system are extremely limited. Nevertheless the level of proliferative and protein-synthesizing processes in these organs is very high because of their great functional importance [3, 5, 11]. This explains the urgency of the study of the basic principles governing the effect of vitamin A on nucleic acid and protein metabolism in the female reproductive organs.

EXPERIMENTAL METHOD

A quantitative histochemical analysis was made of the RNA and total protein (TP) content in the ovaries and uterus of animals receiving 50,000 and 80,000 I.U. of vitamin A. Altogether 110 female CBA × C57BL mice aged 3-4 months were used in the experiment. A 3.44% solution of retinol acetate (RA) in soy oil was used as the vitamin A preparation. RA was administered daily for 10 days in a dose of 5000 and 8000 I.U. by means of a gastric tube. Animals receiving soy oil by the same method served as the control. Before the beginning and at the end of the experiment the time course of the estrous cycle (EC) was determined by the vaginal smear method. The animals were decapitated. The organs were fixed in Carnoy's fluid. Paraffin sections 6 μ thick were stained with gallocyanin and chrome alum by Einarson's method to determine RNA and with Amido black 10B to demonstrate TP. The optical density of the nuclei and cytoplasm of the cells in the structures chosen for study was measured on a "Reichert" (Austria) cytospectrophotometer by a single-wave, multiple-point method [1]. The RNA and TP content was expressed in conventional units [2].

EXPERIMENTAL RESULTS

The study of the RNA and TP content in the ovaries and uterus of animals receiving different doses of RA showed a number of general patterns.

The RNA level in the cytoplasm of cells of the follicular epithelium (FE) of the intact mice, oocytes (O), cells of the theca interna (TI), corpora atretica (CA), and interstitial tissue (IT) reached peak values in diestrus (D). For O it was 25.14 ± 1.49, for FE 82.24 ± 1.44, for TI 54.93 ± 1.49, for CA 58.02 ± 3.56, and for IT 54.57 ± 1.86. The cyclic pattern of time course of the RNA content was observed most significantly ($P < 0.001$) in FE, less significantly ($P < 0.05$) for O. Values of the parameters for TI, CA, and IT were not significant ($P > 0.05$). Minimal values of the RNA level in the cytoplasm of the structures mentioned above were recorded in estrus (E). They were most significant in FE (67.94 ± 2.42; $P < 0.001$) and in O (19.06 ± 1.44; $P < 0.001$).

The cytoplasmic RNA content reached a maximum in the corpora lutea (CL) in E (64.33 ± 2.44) and a minimum in D (59.17 ± 2.4). However, the results were not significant ($P > 0.05$).

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The maximal RNA level in the nucleus and cytoplasm of cells of the integmentary epithelium (IE) and glandular epithelium (GE) was observed in E ($P < 0.001$). The content of nuclear RNA in IE and GE was 54.6 ± 1.2 and 41.2 ± 2.8 respectively. The RNA level in the cytoplasm of the above-mentioned structures reached 93.5 ± 2.3 and 65.0 ± 2.6. Minimal values for the nuclear RNA content (IE 35.1 ± 1.4, GE 26.3 ± 1.3), and of the cytoplasmic RNA content (IE 41.9 ± 0.8, GE 27.6 ± 2.3) in the endometrium were observed during D.

The time course of the TP content in the cytoplasm of ovarian structures of intact animals during EC differed somewhat in character from changes in the RNA level. Meanwhile cyclic fluctuations in the content of the above substances in the endometrium coincided.

The highest TP content (81.8 ± 2.37; low level of significance, $P < 0.05$) was observed during proestrus (P). In O the corresponding increase in the TP level during P (82.47 ± 2.49) was not significant ($P > 0.05$).

Cyclic fluctuations in the TP content in TI, CA, and IT coincided with the similar changes in the RNA level. Maximal values of TP in the structures listed above were recorded in D (66.77 ± 2.53, 67.21 ± 2.39, and 66.41 ± 2.64), minimal values in E (63.66 ± 63.17, 65.09 ± 3.19, and 64.38 ± 3.27). However, none of the results obtained were significant ($P > 0.05$).

The highest TP level in CL was recorded during E (74.41 ± 4.48; $P < 0.05$), the lowest during D (66.27 ± 2.53; $P > 0.05$).

The maximal TP level in IE (177.4 ± 5.2) and in GE (156.0 ± 3.9) during the EC was observed in E, whereas in D it reached a minimum (67.9 ± 0.9 in IE, 61.9 ± 2.2 in GE). These results were significant ($P < 0.001$).

In animals receiving 50,000 I.U. of RA the same cyclic pattern of fluctuations in the RNA and TP content could be observed as in the control. As regards the levels of these substances, comparison of these parameters during the stages of EC in the control and experimental animals revealed an increase in the RNA and TP content in all structures of the ovaries and uterus studied in mice receiving 50,000 I.U. of RA, but they differed in their level of significance.

The most significant increase in the level of cytoplasmic RNA was observed in FE (86.67 ± 1.42; $P < 0.05$) and in TI (61.0 ± 2.37; $P < 0.05$). Relative to the control it amounted to 5.39 and 8.5% respectively.

The peak level of nuclear RNA in IE (69.2 ± 2.3) and in GE (56.7 ± 1.1) exceeded the corresponding values in the control by 26 and 38%. The RNA content in the cytoplasm of these structures increased by 46% (134.0 ± 6.2) and by 33% (86.7 ± 3.3). The results are highly significant ($P < 0.001$).

The TP level was increased most significantly in the cells of FE (88.52 ± 1.41; $P < 0.05$), which was 8.18% above the corresponding value in the control. The TP content in the cytoplasm of cells of IE (257.0 ± 4.1) and GE (201.4 ± 3.5) was increased by 45 and 29% respectively.

The EC ceased in animals receiving 80,000 I.U. of RA. This dose of the compound caused a decrease in the RNA and TP content in all ovarian structures of the mice compared with the control. Similar changes also were recorded in the endometrium.

The decrease in the cytoplasmic RNA level was most significant ($P < 0.001$) in O — by 67.1% (6.28 ± 1.31) and in FE — by 45.7% (36.91 ± 1.52). In the remaining ovarian structures the decrease in the RNA content in the cytoplasm of their cells was not significant ($P > 0.05$). The RNA level in the cells of IE (24.9 ± 0.43) and GE (17.4 ± 0.67) was significantly ($P < 0.001$) reduced by 54.3% and 57.8%. The RNA content in the cytoplasm of these structures of the endometrium was reduced by 71% (26.7 ± 2.1 and 19.4 ± 0.4 respectively).

The TP level showed a significant ($P < 0.001$) decrease in O — by 21.4% (60.19 ± 2.47), and in FE — by 10.9% (67.04 ± 2.42). In the other ovarian structures the decrease in the TP content was not significant ($P > 0.05$).

The TP level in the cytoplasm of IE and GE cells was reduced by 75.4% (43.6 ± 1.6 in IE) and by 76.4% (37.8 ± 2.1 in GE).

Consequently, the cyclic pattern of fluctuations in the RNA and TP content, reflecting activity of protein synthesis, is more characteristic of the epithelial structures of the rodent ovary and uterus and less characteristic of oocytes and of lutein and theca-glandular structures. Injection of RA in a dose of 50,000 I.U. caused an increase in the RNA and TP

content in all structures studied. The most significant quantitative changes in these substances were observed in FE and TI of the ovaries, and also in IE and GE of the uterus. The stimulating effect of 50,000 I.U. of RA on the epithelial and theca-glandular structures of the ovaries and endometrium was exhibited most strongly in those phases of EC that are characterized by the highest level of their functional activity.

Vitamin A in a dose of 80,000 I.U. inhibits protein synthesis in all structures of the ovaries and uterus described above. FE and O in the ovaries are more susceptible to the action of toxic doses of retinol whereas the theca-glandular structures exhibit greater resistance.

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EFFECT OF HIGH DOSES OF RETINOL ACETATE ON 3-β-OL-STEROID DEHYDROGENASE AND ALKALINE PHOSPHATASE ACTIVITY IN MOUSE OVARIES

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Sex steroid production is the most important parameter of ovarian function. Processes of steroid production involve several ovarian structures, and no strict specialization in the production of any one hormone evidently exists among them. This is shown by the presence of a full complement of enzymes catalyzing the metabolic conversions of hormones of both estrogen and progesterone series in all the histophysiological components of the ovary that take part in steroid production [4, 9]. The data cited above indicate that the ovary is a system of hormonally active structures connected in a functional hierarchy and maintaining steroid homeostasis at an adequate level. The principal role among ovarian endocrine formations is probably played by follicles, which are not only independent steroid-producing structures, but also the sources of formation of the corpora lutea (CL) and, to some extent also, of the interstitial gland (IG) of the ovaries.

It is natural to suggest that factors with epitheliotropic action, which affect secretory processes and are characteristic of glandular structures, will take part in the realization of ovarian endocrine function. They include vitamin A. Data in the literature on the effect of retinol on the steroidogenic activity of ovarian structures are very incomplete [7, 8, 10].

The object of this investigation was to study the basic principles governing the effects of different doses of vitamin A on synthesis and secretion of female steroid hormones.

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