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OVARIAN MORPHOLOGY DURING PRECOCIOUS SEXUAL MATURATION OF THE RAT INDUCED BY ELECTROCHEMICAL STIMULATION OF THE BRAIN

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The present study concerns some morphological and cytochemical aspects of follicular growth in the ovary of the immature rat subjected to electrochemical stimulation of the hypothalamus or to electrochemical stimulation and combined treatment with estradiol benzoate.

Three groups of animals were investigated. One group of 25 animals was electrochemically stimulated in the hypothalamus on day 23. A second group of 25 animals received electrochemical stimulation of the hypothalamus in combination with administration of estradiol benzoate (0.125 µg) on day 23. Finally a third group of 25 animals was used as controls.

Groups of 5 animals were then sacrificed on day 23 (one hour after the electrochemical stimulation), day 24, 25, 26 and 27. Uteri and ovaries were rapidly dissected, blotted and weighed. One of the ovaries was prepared for light microscopical examination whereas the other one was kept in the deep freezer for subsequent steroid analysis.

A differential count of the follicles was carried out according to the method described by PEDERSEN (1969). Only medium and large follicles were included. The total number of medium and large follicles was then calculated per mg ovarian weight in the three groups of animals. A decreasing number of medium and large follicles per mg ovarian weight was found during the period of observation in the stimulated groups as well as in the control group. This finding may be due to an increase in the interstitial tissue in all three groups. An analysis of variance revealed that the diffe-

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rence between the various age groups significantly exceeded the variations between the stimulated groups and the control group ($P < 0.001$). No statistically significant variation was found between the two stimulated groups or between each of the stimulated groups and the control group.

Since the statistical analysis of the total number of medium and large follicles per mg ovarian weight did not show any significant difference between the two stimulated groups and the control group it was felt that an assessment of the total number of large follicles in one ovary might yield some further information.

The number of large follicles per ovary (type 5 *a*, *b*, 6 and 7, classification of PEDERSEN and PETERS 1968) was calculated in the three groups of animals.

The mean number of large follicles observed in the ovaries of the electrochemically stimulated animals treated with 0.125 µg estradiol benzoate significantly exceeded the mean number of large follicles observed in the control group ($P < 0.001$). Furthermore the difference between the group of electrochemically stimulated animals treated with 0.125 µg estradiol benzoate and the group of animals treated only with electrochemical stimulation significantly exceeded the difference between the age groups ($P < 0.001$). It can therefore be concluded that the treatment with estradiol benzoate was followed by a statistically significant increase in the number of large follicles when compared to the other experimental group and the controls.

No statistically significant difference was found between the follicular population in the group of electrochemically stimulated animals that did not receive estradiol benzoate and the control group. Nevertheless there was clear evidence for a more advanced sexual maturation in the group of animals treated with electrochemical stimulation (e. g. uterine weight, age at vaginal opening etc.) Ovaries obtained on day 27 from five animals treated with electrochemical stimulation and from 5 control animals of the same age were therefore subjected to a cytochemical assessment of DNA in the granulosa cells of the large follicles. The follicles were grouped in three categories. In the first category only follicles with a diameter about 175 to 200 µ were included. In the second category follicles with a diameter between 250 and 350 µ were included. In the third category follicles with a diameter of 400 µ or more were included. These three categories of follicles roughly correspond to type 5, 6 and 7 of the classification of PEDERSEN and PETERS (1968). The granulosa cells obtained from the follicles were smeared on glass slides and subjected to a semi-quantitative microfluorometric method as described by RUCH (1966). The distribution of granulosa cell nuclei in G_1-phase (the pre-synthetic interval of the cell cycle) and S-phase (the synthesizing phase) was analysed and compared with a standard cell population of rat thymocytes representing the diploid value of DNA.

The mean values obtained from the cell nuclei in G_1-phase and S-phase were calculated and expressed in arbitrary units. In all types of follicles investigated the mean value of DNA per granulosa cell nucleus was exceeding the diploid control value. It is therefore suggested that all follicles investigated were at the stage of proliferation. The increase in DNA per cell nucleus in the granulosa cells of the control ovaries was statistically highly significant and related to the increased size of the follicles ($P < 0.001$).

A statistically significant difference was also found between the group of electrochemically stimulated animals and the control group ($P < 0.01$). The data obtained indicate that the proliferative rate in the large follicles obtained from the electro-

chemically stimulated animals was significantly decreased. Although some cell nuclei were found in the S-phase, the majority of the cells was in the G_1-phase corresponding to the standard diploid DNA value of the thymocytes.

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RÉSUMÉ

MORPHOLOGIE DE L'OVAIRE DE RATTE AU COURS DE LA MATURATION SEXUELLE PRÉCOCE INDUITE PAR STIMULATION ÉLECTROCHIMIQUE DE L'HYPOTHALAMUS

Des rattes de 23 jours subissent une stimulation électrochimique de l'hypothalamus suivie ou non d'une injection de benzoate d'estradiol. A 27 jours le nombre de grands follicules par ovaire (types 5, 6, 7, classification de PEDERSEN et PETERS, 1968) est significativement plus élevé chez les rattes stimulées et traitées à l'estradiol que chez les rattes témoins. Bien que la stimulation électrochimique seule provoque une accélération de la maturation sexuelle (poids de l'utérus, ouverture vaginale), le nombre de grands follicules dans l'ovaire n'est pas différent de celui observé chez les témoins.

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