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0014-4754/84/040394-03$1.50 + 0.20/0

Sex steroids in the rat submaxillary gland during the estrus cycle

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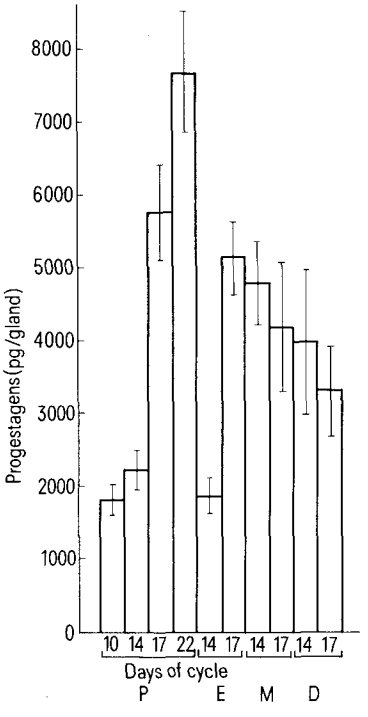
Summary. Relatively high progestagen levels were found in female rat submaxillary glands, with a maximum in the proestrus stage at 22 h and 2 minima; in proestrus between 10 and 14 h, and in estrus at 14 h. Estrogen and androgen concentrations in the gland were undetectable during most of the cycle except in the proestrus stage, when the highest level was determined at 14 h for estrogens and at 17 h for androgens.

The rat submaxillary glands are a source of polypeptide hormones[2-6], and sex steroids as well[7]. Among steroids, relatively high progestagen levels were found in the submaxillary glands of adult females and they still increased further several times during pregnancy[8]. In the present study, sex steroid fluctuations were found in the rat submaxillary gland during the estrus cycle.

Material and methods. Virgin female Wistar rats (130–180 g), maintained under controlled lighting of 12 h light and 12 h darkness, and fed pelleted food and water ad libitum, were used. Vaginal smears were taken every morning and only the rats which showed 3 consecutive 4-day cycles were chosen for the study. Submaxillary glands (5–6 per group) were excised from the rats under ether anesthesia on each day of the cycle at different hours: in proestrus (P) at 10, 14, 17 and 22 h, in estrus (E), in metestrus (M) and in diestrus (D) at 14 and 17 h. The glands were stored at −20 °C until steroid analysis was carried out by radioimmunoassay. Then the glands were homogenized with 2 ml phosphate buffer, and 200 µl of homogenate was extracted with ethyl ether for estrogen and androgen analysis, and with n-hexane for progestagen analysis. All samples were assayed in duplicate.

Estrogens were estimated according to Hotchkiss et al.[9], progestagens were measured according to Abraham et al.[10], androgens were determined according to Dufau et al.[11]. These methods were the same as described in detail previously[8]. The steroids measured are referred to as estrogens, progestagens and androgens because the antiserum used in the estradiol-17β RIA cross-reacted also with estrone (66%) and estriol (2.1%); the antibody used in the progesterone RIA cross-reacted also with pregnenolone (5%), with 20α-hydroxy-pregn-4-en-3-one (1.8%) and with 17α-hydroxy-progesterone (1.1%); the antibody used in the testosterone RIA cross-reacted also with dihydrotestosterone (20.8%), with androstenedione (7.4%) and with dehydroisoandrosterone (3%). The results of the assays are shown graphically as hormone mean values for the whole gland ± SEM.

Results and discussion. Changes in the submaxillary gland progestagen level of 4-day cycle rats are given in the figure. Progestagen concentration in the P stage was lowest at 10 and 14 h (about 2000 pg per gland), considerably increased at 17 h (up to more than 5000 pg per gland) and it reached the maximum at 22 h (above 7000 pg per gland). In the E stage at 14 h a sharp drop of progestagens (to below 2000 pg per gland) was found; this was then followed by a rapid increase in the same stage at 17 h to 5000 pg per gland. In the M and D stages a gradual decrease to about



Progestagen contents of the submaxillary gland of a female rat at different days and hours of the estrus cycle. Each bar is the mean ± SEM per gland for a minimum of 5 duplicate determinations.

3000 pg per gland in the D stage at 17 h was observed. In all groups of rats there was a lack of estrogens and androgens, except in some groups investigated in the P stage. Estrogens concentration started in the P stage at 50 pg per gland at 10 h, reached a maximum at 14 h (about 60 pg per gland), and rapidly decreased during the following hours down to an undetectable level in the E stage at 14 h. The androgens level, undetectable in the P stage at 10 h, increased suddenly at 14 h (up to 50 pg per gland), reached a peak at 17 h (above 60 pg per gland) and afterwards fell sharply, to zero at 22 h.

From the present results it is evident that the progestagen level in the rat submaxillary gland homogenates during the estrus cycle was not constant but underwent fluctuations depending on the stage of the cycle and hour of the day. Progestagen content in this gland in the P stage was minimal in the morning, increased in the afternoon, and reached a maximum at night. Similar differences in progestagen concentration were found in proestrus in ovarian tissue[12-14] and also in ovarian venous plasma and peripheral blood[15,16]. In all these investigations preovulatory progestagens began to increase in the afternoon of the P stage and reached a maximum several hours later. The 2nd minimum of progestagen level in the submaxillary gland was found in the E stage at 14 h, while in the ovaries it was observed in the same stage just at 9 h[12]. The increase of submaxillary gland progestagen level in the afternoon in the E stage and the subsequent slow decrease of these hormones in the M and D stages correlate with progestagen concentration in peripheral blood plasma during those stages, but not with their concentration in ovarian vein blood[15]. Estrogen and androgen were undetectable in the rat submaxillary gland during most stages of the estrus cycle except the P stage when trace quantities were found with a maximum for estrogens at 14 h and for androgens at 17 h. Since plasma estrogen and androgen concentrations are low during the E and M stages, increase in the D stage and reach a maximum in the morning (estrogens) or in the afternoon (androgens) of proestrus[17-19], the content of these steroids in the submaxillary gland appears to correlate with this pattern.

In conclusion, steroid concentration in the female rat submaxillary glands in the consecutive stages of the estrus cycle is well correlated with the steroid level in the peripheral blood. This similarity strongly suggests the external origin of submaxillary gland hormones. On the other hand, the possibility cannot be excluded that there is some synthesis of progestagens in the submaxillary gland itself, although the rate is rather low[7,20].

1 The author is indebted to Prof. Dr S. Stokłosowa for her critical remarks during preparation of the manuscript and to the World Health Organization Special Programme of Research, Development and Research Training in Human Reproduction for support.

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0014-4754/84/040396-02$1.50 + 0.20/0

Pineal indols and testosterone affect exploratory activity of male rats

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Summary. The testosterone level has an inverse relation to activity in the open-field test. This is more important in red light than in white light. Pineal indols do not disturb this action. Some of these results are consistent with the assumption that androgens play a role on the exploratory activity of adult subjects.

The pineal gland may play some role in the general motor and exploratory behavior of male rats following pinealectomy. Some investigators reports changes in the wheel-running score[1-3] or exploratory activity as studied in the open-filed test[4]. Treatment with pineal extract or with melatonin results in modification of exploratory activity[1,4,5]. The possible mechanism of action underlying these behavioral effects are not clear at this time. It is accepted that the pineal gland produces antigonadotropic agents[6,7] and that the gonadal hormones play an important part in exploratory activity. Female rats are more active and defecate less than males. But females, which have been exposed to androgens in early neonatal life, are comparable with males[8,9]. The purpose of this study was to investigate the possible role of melatonin and 5-methoxytryptophol on exploratory behavior in the open-field test and its relationship to testosterone action.

Methods. Subjects and treatment. 108 adult male Wistar rats were divided into 9 groups of 12 animals each. The animals were kept on a standard light-dark schedule (12:12). All the rats were castrated and 20 days afterwards each group received daily s.c. injections of testosterone propionate (TP), 10 µg, 50 µg or 500 µg, dissolved in peanut oil, for 10 days. At each level of testosterone a first group received 1 mg of 5-methoxytryptophol s.c., a 2nd group 1 mg of melatonin and a 3rd group the vehicle (absolute alcohol