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VARIATIONS IN THE PROGESTERONE CONTENT OF THE RAT ADRENAL GLAND DURING THE OESTROUS CYCLE

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SUMMARY

The progesterone content of the adrenal gland of the rat was found to increase significantly in the evening of pro-oestrus, similar to the ovarian progesterone of the same rats, and to decrease in early oestrus. During these phases of the oestrous cycle there was no significant difference between the amounts of progesterone contained in one pair of ovaries and one pair of adrenals. In late oestrus, however, ovarian progesterone increased again significantly whereas adrenal progesterone remained low and reached a minimum in early met-oestrus. Although there is little doubt that the main physiological function of adrenal progesterone is that of a precursor steroid, the present findings emphasize the necessity of taking its variations into account when attempts are made to explain the events of the oestrous cycle.

INTRODUCTION

The adrenal gland of the rat contains progesterone in quantities which are comparable to those in the ovary [1, 2]. This adrenal progesterone is not only used as a precursor in the synthesis of the main adrenal secretion products but is also secreted into the blood stream [3–5]. In anaesthetized rats undergoing abdominal surgery, the rate at which progesterone was secreted by the adrenal gland was of the same order as its ovarian secretion rate [2].

For an explanation of the events of the oestrous cycle it is important to know whether adrenal progesterone production shows cyclic variations. In the present experiments the progesterone content of the adrenal glands was measured during different phases of the oestrous cycle and compared with the amount of progesterone present in the ovaries of the same animal.

METHODS

Experiments were carried out on virgin Wistar rats (120–200 g) which were kept under controlled lighting conditions with 12 h red light (14:00–02:00 h) and 12 h white light. This time schedule was chosen in order to bring the beginning of the period of late pro-oestrus into a convenient hour of the working day. Daily vaginal smears were taken to assess the phase of the cycle. Only rats which exhibited at least three regular cycles of 4 day length were used. They were killed by rapid decapitation either 8 h after onset of white light (midday) or 3 h after onset of red light (evening). The adrenal glands and the ovaries were dissected out and put into a deep freeze (−21°C) for 1–10 days. The tissues were homogenized in an all glass homogenizer using ethylacetate to extract the steroids. Purification of the extracts, separation of individual steroids by paper chromatography in the E_2B and E_1 system [6] and the quantitative estimation of progesterone and pregnenolone by gas–liquid chromatography was carried out as described previously [1, 7].

RESULTS

In Fig. 1 it can be seen that the amount of progesterone contained in one pair of adrenal glands is of the same order as that in one pair of ovaries. During the oestrous cycle the adrenal progesterone content was lowest during oestrus and early met-oestrus and high during di-oestrus. In the evening of the day of pro-oestrus there occurred a significant rise ($P < 0.05$) in adrenal progesterone, similar to the increase in ovarian progesterone during this phase of the cycle. A significant decrease ($P < 0.05$) in adrenal progesterone was seen during early oestrus, similar to the fall in ovarian progesterone. During these phases of the cycle the amounts of progesterone contained in the adrenal glands and in the ovaries were not significantly different. However, in the evening of the day of oestrus the adrenal progesterone remained low whereas the ovarian progesterone increased again. It was now 80% higher than the adrenal progesterone ($P < 0.01$). On

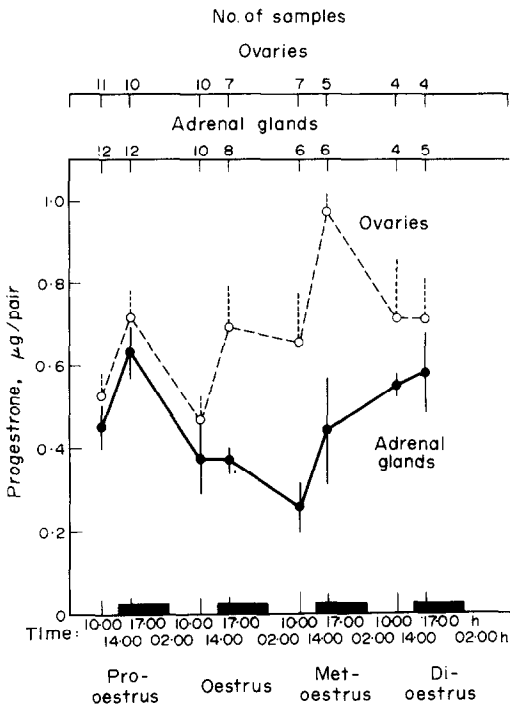

Fig. 1. Progesterone contents of the adrenal glands and ovaries of rats during the oestrous cycle. Rats kept under controlled lighting conditions: 12 h white light (02:00–14:00 h), 12 h red light (14:00–02:00 h, dark bars). Progesterone content expressed as µg/pair glands, mean values ± standard error of the mean.

the day of met-oestrus adrenal progesterone was even lower than during oestrus, whereas the ovarian progesterone remained high and increased further in the evening of this day ($P < 0{\cdot}02$). During this phase there was also a rise in adrenal progesterone, however the individual variations were large. Whereas the adrenal glands of two rats contained 0·86 and 0·84 µg progesterone per pair, in one rat there was only 0·14 µg present. The adrenal progesterone content in early di-oestrus was double that in early met-oestrus ($P < 0{\cdot}01$). The ovarian progesterone was lower than in late met-oestrus and the difference between the progesterone content of ovaries and adrenal glands was no longer significant.

The mean weight of the adrenal glands was 56·6 ± 2·4 mg/pair, that of the ovaries 55·7 ± 1·9 mg/pair. There were no significant changes in the mean weights of either tissue during the different phases of the cycle.

The pregnenolone content of the same adrenal glands increased during pro-oestrus from 0·26 ± 0·04 µg/pair (midday) to 0·55 ± 0·12 µg/pair (evening) ($P < 0{\cdot}05$). In the morning of the day of oestrus it had fallen to 0·23 ± 0·05 µg/pair ($P < 0{\cdot}05$) and did not change significantly in the evening of this day (0·31 ± 0·1 µg/pair).

The pregnenolone in the ovaries was not measured because of the presence of 3β-hydroxy-5α-pregnan-20-one in ovarian tissue [9]. This steroid could not be quantitatively separated from pregnenolone in the E_1-chromatogram and both steroids had the same retention time on the 3·8% SE-30 column of the gas chromatograph. 3β-Hydroxy-5α-pregnan-20-one has so far not been detected in rat adrenal tissue.

DISCUSSION

The experiments have shown that the progesterone content of the adrenal glands varies throughout the oestrous cycle. Similar to the ovarian progesterone, the adrenal progesterone increased significantly in late pro-oestrus and was low in early oestrus. These are phases of the cycle during which large changes in the function of endocrine organs occur within very short time intervals. Thus it is possible that the high progesterone concentrations observed three hours after onset of red light in the evening of pro-oestrus are not the highest values reached in this phase of the cycle. Equally, the adrenal and the ovarian progesterone production might not reach their maxima at exactly the same time. In the evening of the day of oestrus there was a second rise in ovarian progesterone content whereas the adrenal progesterone remained low. Further rises in ovarian progesterone occurred during met-oestrus leading to a peak in the evening of this day. Adrenal progesterone, on the other hand, fell during oestrus and reached a minimum in the morning of met-oestrus. It rose again during di-oestrus.

The rise in ovarian progesterone production in the evening of the day of pro-oestrus is now generally believed to be the consequence of the LH release in the afternoon of this day. The second rise in the evening of the day of oestrus might be attributed to the presence of new corpora lutea which reach a maximum in size (and progesterone production) during late met-oestrus. This assumption is supported by the observations that isolated, superfused rat ovaries which were removed during met-oestrus produced two to four times more progesterone than ovaries removed during oestrus or pro-oestrus [9].

Changes in ovarian progesterone contents are reflected by changes in the ovarian secretion rates of progesterone [2, 7, 10]. Whether the adrenal progesterone content is also an index for the rates at which progesterone is secreted by the adrenal gland is still unknown. Whereas stress had little effect on the ovarian progesterone content [2] the adrenal progesterone content was increased by stressful stimuli, as long as

the corticosterone secretion did not reach maximal rates. For example, rats exposed to cold or to exsanguination under ether anaesthesia showed a parallel increase in adrenal corticosterone (B) and progesterone (Prog) contents [1, 2] and the ratio B/Prog was about 3·5:1 similar to that found in unstressed rats. In rats which had undergone prolonged abdominal surgery the adrenal progesterone contents were lower than in unstressed rats whereas their adrenal corticosterone content reached maximal values [2]. The ratio B/Prog was 17:1.

It is possible that variations in the secretion of ACTH during the oestrous cycle could account for the observed adrenal progesterone changes. A systematic study of the peripheral plasma concentrations of ACTH and corticosterone is required before this explanation can be accepted.

It is also tempting to suggest that the LH released in the afternoon of pro-oestrus increases adrenal progesterone. However, equine LH was ineffective [13] and only large doses of human, but not ovine LH increased the progesterone concentrations in peripheral plasma of ovariectomized, dexamethasone treated rats [12]. Prolactin is the second pituitary hormone which shows a peak in its plasma concentration in the afternoon of pro-oestrus. An increase of more than 10-fold was reported to occur between 10:00 and 15:00 h when values in the order of 3 m.u./ml were observed [14]. In ovariectomized, dexamethasone treated rats the i.v. injection of as much as 10 units prolactin/100 g body weight caused a rise in the plasma progesterone concentrations to values which were 70% higher than those in ovariectomized, not dexamethasone treated rats [12]. It remains to be seen whether the injection of more physiological quantities of prolactin have the same effect. In all probability, the simultaneous increase in the release of several pituitary hormones will be responsible for the rise in adrenal progesterone during late pro-oestrus.

It has been suggested that an increased release of adrenal progesterone during the early afternoon of pro-oestrus might facilitate the release of LH on this day [15, 16]. Although there is little doubt that the main function of adrenal progesterone is that of a precursor steroid, the fact remains, that progesterone is secreted by the adrenal gland in quantities which are sufficient to maintain in ovariectomized rats plasma progesterone concentrations similar to the lower values observed during the oestrous cycle [17]. Adrenal progesterone must therefore be taken into consideration when attempts are made to interpret the events of the oestrous cycle. This is emphasized by the significant changes in adrenal progesterone concentrations during the cycle observed in the present experiments. In order to know how much progesterone is actually secreted by the adrenal gland during the cycle a method will have to be devised by which adrenal venous blood can be collected from an unstressed female rat.

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