Journal of Steroid Biochemistry. 1975. Vol. 6. pp. 1307 1310. Pergamon Press. Printed in Great Britain

PHYSIOLOGICAL VARIATIONS IN THE OVARIAN PRODUCTION OF 5α-PREGNANE DERIVATIVES WITH SEDATIVE PROPERTIES IN THE RAT

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(*Received* 20 *December* 1974)

SUMMARY

The ovary of the rat was found to contain and secrete several 5α-pregnane derivatives with pronounced anaesthetic properties. These steroids can be produced in quantities similar to or even larger than those of progesterone. During the oestrous cycle there was a peak in the ovarian content of 3α-hydroxy-5α-pregnan-20-one in the evening of the day of pro-oestrus and a plateau during late oestrus and met-oestrus coinciding with the periods of largest progesterone production. The cyclic fluctuations of 5α-pregnane-3α, 20α-diol followed those of 20α-dihydroprogesterone with the lowest values in pro- and di-oestrus and highest values in late met-oestrus. During pregnancy (days 5 and 14–18) the ovarian content and secretion of these two 5α-pregnanes was found to be much lower than during the oestrous cycle.

INTRODUCTION

In previous experiments we have observed that the ovary of the rat secretes a number of ring A-reduced pregnane derivatives which are probably derived from progesterone [1, 2, 3, 4]. As the rat ovary has been shown to contain a 4-ene-5α-hydrogenase [5] the presence of ring A-reduced progesterone metabolites in the ovary and ovarian venous blood was not surprising. It was however unexpected to find these reduced steroids in quantities similar to or even larger than those of progesterone itself [4]. Most prominent among them were 5α-pregnane-3α,20α-diol (allopregnanediol), 3α-hydroxy-5α-pregnan-20-one (3α-hydroxy-allopregnanolone), 3β-hydroxy-5α-pregnan-20-one (3β-hydroxy-allopregnanolone) and 20α-hydroxy-5α-pregnan-3-one. These observations have been confirmed by Ichikawa and his colleagues [6]. We also observed a three-fold rise in the ovarian secretion rates of allopregnanolone in the evening of the day of pro-oestrus following the LH release in the afternoon of this day and coinciding with the rise in progesterone secretion [2, 4]. This agrees with the observation that luteinizing hormone can stimulate the production of some reduced steroids in ovarian tissue [7]. Allopregnanediol secretion remained unchanged. The changes in the ovarian secretion rates of the steroids were accompanied by similar changes in their ovarian content [4].

In the present work the question was investigated, whether rises in progesterone production are always accompanied by rises in the production of some of these metabolites, as seen in late pro-oestrus [4], or whether in situations in which the physiological requirement for progesterone is increased, a mechanism can be called into action by which the ovarian catabolism of progesterone becomes inhibited. Experiments were therefore carried out in which the ovarian secretion rates and contents of 3α-hydroxy-allopregnanolone, allopregnanediol, progesterone and 20α-dihydroprogesterone were measured in pregnant rats. The ovarian content of the same steroids was also measured at eight different phases during the oestrous cycle of the rat. Some of the results have been communicated to the British Physiological Society [8].

METHODS

Steroid production during the oestrous cycle was studied in virgin female Wistar rats (100–150 g body weight) which were kept under artificial lighting conditions with 12 h white light (02·00–14·00 h) and 12 h red light. This time schedule was chosen in order to bring the period of late pro-oestrus into a convenient hour of the working day. Only rats which had shown at least three regular cycles of 4 days length were used. They were killed by rapid decapitation either 8 h after onset of white light or 3 h after onset of red light. The ovaries were quickly removed and frozen. Pregnant rats were of the same age and strain. The day on which a vaginal plug was found was counted as day one of pregnancy. One group of rats was killed by rapid decapitation on day 5 of pregnancy, another on day 14. The ovaries were removed and frozen. For ovarian blood collection 14–18 day pregnant rats were used. They were anaesthetized with sodium pentobarbitone (30–50 mg/kg body weight) and the right ovary and the whole uterus was removed. Venous blood from the left ovary was then collected for 30 min as described previously [4]. Total ovarian blood and homogenates of ovarian tissue were extracted with ethylacetate after the addition of tracer quantities of [4-^{14}C]-progesterone and [4-^{14}C]-pregnenolone to allow for correction of losses occurring during the chemical procedures. All steroids

were estimated by gas-liquid chromatography after separation in two paper chromatographic systems. The methods have been described previously in detail [4]. The results were corrected for losses and expressed in μg/pair of ovaries when tissue concentrations were measured or in μg/left ovary/h when ovarian secretion rates were measured.

RESULTS

Ovarian steroid contents during the oestrous cycle

Figure 1 shows the amounts of progesterone and 3α-hydroxy-5α-pregnan-20-one (allopregnanolone), Fig. 2 those of 20α-hydroxy-4-pregnen-3-one (20α-dihydroprogesterone) and of 5α-pregnan-3α,20α-diol (allopregnanediol) in the ovaries at eight different phases of the cycle. It can be seen that the changes of progesterone are paralleled by those of allopregnanolone and those of 20α-dihydroprogesterone by allopregnanediol. Both progesterone and allopregnanolone increased significantly in the evening of pro-oestrus, fell in the morning of oestrus, rose again in the evening of this day and reached maximum values in met-oestrus. The rises in late pro-oestrus were, for progesterone 36% ($0{\cdot}02 < P < 0{\cdot}05$), for allopregnanolone 108% ($0{\cdot}02 < P < 0{\cdot}05$). In late met-oestrus the progesterone content was nearly double that in early pro-oestrus, the allopregnanolone content nearly 3 times that in early pro-oestrus. The ratio progesterone: allopregnanolone was more than 3 in di-oestrus and early pro-oestrus but only about 2 during the other phases of the cycle.

As can be seen from Fig. 2 the quantities of the C_{20}-hydroxy steroids contained in the ovaries were up to 3 times larger than those of progesterone. The small rise shown by both steroids in late pro-oestrus was not statistically significant. There was, however, a steady rise from early oestrus to late met-oestrus. The 20-dihydroprogesterone content in late met-oestrus was about 2·5 times that in early pro-oestrus, the allopregnanediol increased about 5 times. The

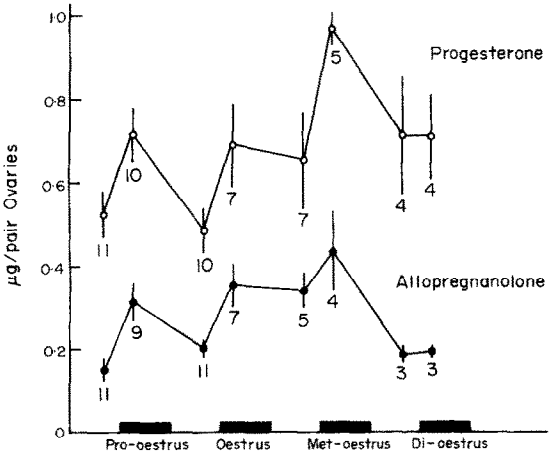

Fig. 1. Ovarian content of progesterone and 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) during different phases of the oestrous cycle of the rat. Rats kept under artificial lighting conditions: 12 h white light, 12 h red light (indicated by black bars on abscissa). Figure underneath each mean value indicates number of rats.

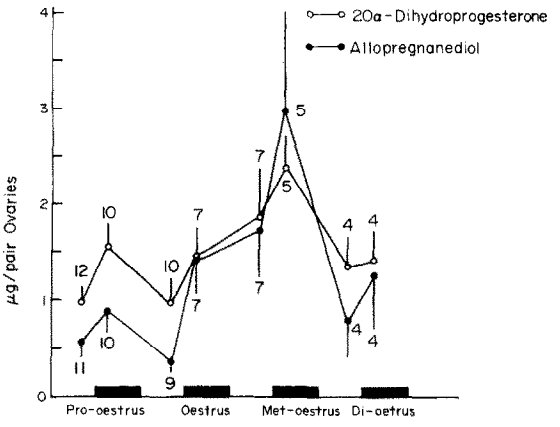

Fig. 2. Ovarian content of 20α-dihydroprogesterone and 5α-pregnane-3α, 20α-diol (allopregnanediol) during different phases of the oestrous cycle of the rat. Lighting conditions as in Fig. 1. Figure underneath each mean value indicates number of rats.

ratio dihydroprogesterone: allopregnanediol was about 1:1·7 in early di-oestrus, pro-oestrus and early oestrus and about 1:1 in the other phases of the cycle.

Ovarian steroid contents during pregnancy

Figure 3 shows the quantities of allopregnanolone, allopregnanediol, progesterone and 20-dihydroprogesterone found in the ovarian tissue of rats which were 14 days pregnant. For purposes of comparison the figure includes the observations made on rats in early pro-oestrus (clear columns), that phase of the oestrous cycle in which the ovarian contents of progesterone metabolites was lowest. It can be seen that during pregnancy only very small quantities of ring A-reduced progesterone metabolites were present in the ovaries. The allopregnanolone content amounted to approximately 10% of that in the non-pregnant rat, the allopregnanediol content was about 6%. There was also a decrease in 20-dihydroprogesterone by about 65%. These metabolites were also measured in the ovaries of nine rats on day 5 of pregnancy, a stage when progesterone secretion shows a sudden

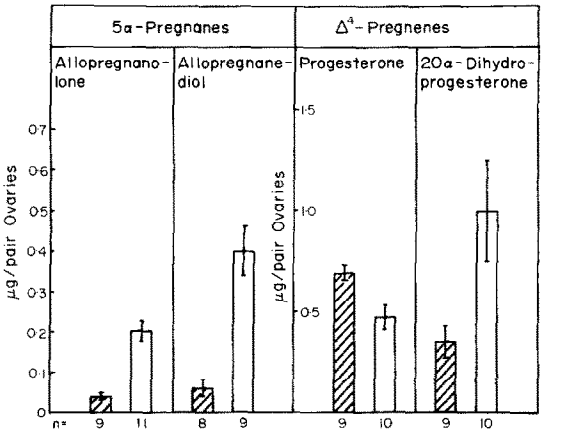

Fig. 3. Ovarian contents of 3α-hydroxy-5α-pregnan-20-one (allopregnanolone), 5α-pregnan-3α,20α-diol (allopregnanediol), progesterone and 20α-dihydroprogesterone of pregnant rats (day 14) (▨) compared with that of non-pregnant rats in early oestrus (□). (Mean values ±S.E.M.).

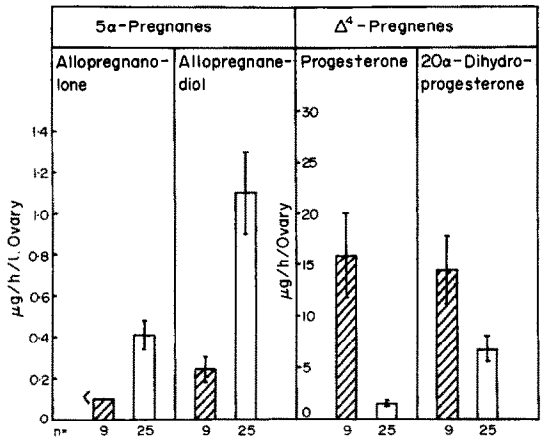

Fig. 4. Ovarian secretion rates of 3α-hydroxy-5α-pregnan-20-one (allopregnanolone), 5α-pregnane-3α,20α-diol (allopregnanediol), progesterone and 20-dihydroprogesterone of pregnant rats (day 14 or 18) (▩) compared with the average secretion during the oestrous cycle (□). (Mean values ± S.E.M.).

decrease after an initial rise [9]. In these rats, too, the quantities of progesterone metabolites were very low. Expressed as μg/pair of ovaries the values were for 3α-hydroxy-allopregnanolone 0·05 ± 0·006, for allopregnanediol 0·08 ± 0·03. The value for progesterone in the same ovaries was 0·47 ± 0·06, for 20α-dihydroprogesterone 0·39 ± 0·10.

Ovarian steroid secretion during pregnancy

Figure 4 shows the amounts of 3α-hydroxy-allopregnanolone, allopregnanediol, progesterone and 20-dihydroprogesterone which were secreted by the left ovaries of rats pregnant between 14 and 18 days. The average secretion of the same steroids during the cycle as studied on a previous occasion is given for comparison. Not unexpectedly, there was a 10-fold rise in progesterone secretion during pregnancy whereas the 20α-dihydroprogesterone secretion was only doubled. In contrast, 3α-hydroxy-allopregnanolone could not be detected in the ovarian blood of pregnant rats (< 0·08 μg/left ovary/h) and the average secretion of allopregnanediol was less than one quarter of that in non-pregnant rats.

A comparison between the values given in Figs. 3 and 4 indicates that in the non-pregnant rat the quantity of progesterone contained in the ovary is similar to the quantity secreted in about 0·25 h whereas in 14 day pregnant rats it is only equal to the amount secreted in 1 min. For 20-dihydroprogesterone these figures are about 5 min in the non-pregnant and less than 1 min in the pregnant rat and for allopregnanediol about 30 min in the non-pregnant and 5–10 min in the pregnant rat.

As shown previously [1] the ovary of non-pregnant rats contains also 3β-hydroxy-5α-pregnan-20-one in quantities similar to those of pregnenolone (approx. 1 μg/pair of ovaries). These two steroids were not separated in the paper chromatographic systems used in the present experiments. As they have also the same retention time on the 3·8% S.E. 30 column of the gas chromatograph they had to be measured together. The sum of these two steroids in the ovaries of 5 or 14 day pregnant rats was found to be 0·12 ± 0·03 μg/pair of ovaries. As the activity of 5α-reductase appears to be very low during pregnancy we can assume that most of the compound measured was pregnenolone. The pregnenolone content of the pregnant rat ovary is thus considerably lower than that of progesterone (less than 20%) and the increased production of progesterone during pregnancy depends on the rate at which pregnenolone is formed from cholesterol. In non-pregnant rats the average ovarian content of pregnenolone plus 3β-hydroxy-5α-pregnan-20-one was about 0·3 μg/pair [4].

DISCUSSION

In the present experiments phase dependent variations in the ovarian content of ring A-5α-reduced pregnane derivatives during the oestrous cycle have been observed. In addition to the previously described short lasting increase during late pro-oestrus in the ovarian concentration and secretion of progesterone, allopregnanolone and 20α-hydroxy-5α-pregnan-3-one [4], there was also a prolonged rise in ovarian 3α-hydroxy-allopregnanolone and progesterone during late oestrus and met-oestrus. As changes in the ovarian content of these steroids parallel changes in their secretion rate [4] we can assume that after ovulation the peripheral blood concentration of reduced progesterone derivatives will be elevated for about 36 h. The rise in 3α-hydroxy-allopregnanolone during late pro-oestrus is probably caused by the preceding LH release as Ichikawa and his colleagues [7] have shown that LH can cause a rise in the progesterone metabolites which are not hydroxylated in position 20. This is not the case for the rise during met-oestrus as during this phase of the cycle LH secretion remains low [10]. The newly formed corpora lutea reach maximal size in met-oestrus and seem to produce "spontaneously" a larger quantity of progesterone and its metabolites. This was also observed by Anderson and his colleagues [11] who found that superfused ovaries of rats in met-oestrus release larger quantities of progesterone than during any other phase of the cycle. Although there is no evidence for a decrease in the size of the corpora lutea during di-oestrus [12] the ovarian production of pregnane derivatives is considerably reduced, and remains so until the evening of pro-oestrus when it responds to the luteotrophic hormone. The 20α-hydroxylated pregnane derivatives did not show a significant rise in late pro-oestrus which agrees with the reports that LH has a much smaller effect on the secretion of these steroids [7]. However, in late oestrus and met-oestrus these derivatives, too, increased in the ovarian tissues. The parallelism in the behaviour of 20α-dihydroprogesterone and allopregnanediol suggests that the latter is formed by ring A-reduction of 20α-dihydroprogesterone and reduction of the oxo group in position 3.

The ovarian progesterone secretion in 14 day pregnant rats was at least 10 times larger than the average

secretion during the cycle. However, unlike during the oestrous cycle, this rise was not accompanied by a simultaneous rise in the secretion of allopregnanolone. On the contrary, the reduced steroid could no longer be detected in ovarian blood. Similarly, the ovarian blood contained only traces of allopregnanediol although the secretion of 20-dihydroprogesterone was somewhat higher in pregnant than in non-pregnant rats. This lack of ring A-reduction in the pregnant rat ovary could be taken as a safeguard to ensure the supply of adequate quantities of progesterone required to maintain pregnancy up to about day 17 [13].

This could be achieved by several mechanisms. It is possible that in the corpus luteum of pregnancy progesterone is formed at a site remote from the enzyme and is quickly eliminated from the cell by an active transport mechanism. In this context the experiments of Mason [5] are of interest in which he found that the ability of PMS stimulated ovaries to reduce testosterone was only about 10% of that of non-stimulated ovaries. Mason suggested that this lack of enzyme activity may be due to either the presence of an inhibitor or to the lack of enzyme synthesis due to change in cell type.

The interpretation of results obtained on rats anaesthetized with sodium pentobarbitone may require some care because this drug was reported to inhibit the conversion of pregnenolone to progesterone [14]. However in the sodium pentobarbitone anaesthetized rat from which either ovarian or adrenal venous blood had been collected the ovarian content of progesterone and its metabolites was rather high [15].

The possible physiological significance of the ovarian secretion of ring A-reduced pregnanes is still obscure. In a previous paper [4] the possibility was discussed that these steroids might have an endocrine function and suggested that such a function might be due to their depressant activity on the central nervous system. The minimum anaesthetic dose of 3α-hydroxy-5α-pregnan-20-one in the rat was found to be 2·5 mg/kg i.v. [16] whereas that of progesterone is at least 60 mg/kg (i.v.) and that of sodium pentobarbitone 30 mg/kg (i.v.). It can be calculated that during met-oestrus the total amount of pregnane derivatives secreted by the ovaries of one rat in 24 h can exceed the anaesthetic dose. Thus modulations in the function of certain brain regions by the activity of the ovaries during the oestrous cycle in the rat seem unavoidable. It is well known that progesterone can be metabolized to 5α-pregnanes in the liver. However, in the liver the metabolites are also conjugated and do no longer easily penetrate the blood brain barrier. The significance of the secretion of sedative steroids by the ovaries lies in the fact that a large portion can reach the central nervous system without having to pass through the liver. During pregnancy the supply of these steroids from the ovaries has nearly ceased. However the quantities of progesterone to be metabolized in the liver are so large that enough of the ring A-reduced metabolites may remain unconjugated to satisfy the needs of the central nervous system.

Progesterone can also be metabolized directly in the brain [e.g. 17, 18]. These studies were only done with radioactive precursors and no data are available to decide on the quantities of reduced metabolites produced from endogenous sources. The weak anaesthetic effect of progesterone may be partly due to this intracerebral metabolism. Thus, the central actions of progesterone showed a latency of 5–15 min after its intravenous injection whereas allopregnanolone acted immediately [19].

Like the ovary, the adrenal gland can also form 5α-reduced metabolites from its major secretion product corticosterone. It was suggested [20] that this provides a mechanism for controlling the output of biologically active steroid hormones.

While this manuscript was in preparation a paper by Ichikawa and his colleagues [21] appeared in which they also describe a decrease in the secretion of reduced progesterone metabolites in pregnant rats and increased ovarian blood concentrations during met-oestrus.

Acknowledgements—I would like to thank Mrs. Urma Godden and Mr. D. Blatchford for their excellent technical assistance.

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