Journal of Steroid Biochemistry, Vol. 9, pp. 643–648

0022-4731/78/0701-0643$02.00/0

PERIPHERAL AND OVARIAN VEIN PLASMA LEVELS OF 20α-DIHYDROPROGESTERONE IN WOMEN WITH NORMAL MENSTRUAL CYCLES

A. LUCISANO, S. DELL'ACQUA, A. MONTEMURRO, A. ROSSETTI, B. CINQUE, G. LAFUENTI, E. ARNÒ and A. BOMPIANI
Department of Obstetrics and Gynaecology, Università Cattolica del Sacro Cuore, Rome, Italy

(*Received* 11 *October* 1976)

SUMMARY

In seven fertile women, with normal menstrual cycles, the daily vein plasma concentrations of 20α-dihydroprogesterone, progesterone, 17-hydroxyprogesterone, oestradiol-17β and LH were measured. The patterns of progesterone and 20α-dihydroprogesterone are similar. In a second group of 4 fertile women on the 7th, 10th, 19th and 20th day of the cycle respectively, vein plasma levels of progesterone and 20α-dihydroprogesterone were measured at short intervals: the concentrations of these compounds do not seem to show rapid and relevant fluctuations. In a third group of five fertile patients, in the early follicular phase or in the luteal phase of the cycle, 20α-dihydroprogesterone and progesterone levels were measured in ovarian venous plasma. From the results obtained it appears that only the ovaries containing the corpora lutea produce and secrete important quantities of progesterone and 20α-dihydroprogesterone, whereas the concentrations of the two steroids in blood from the controlateral ovaries and in peripheral blood are similar. Finally in the three groups of patients considered a difference seems to exist in the ratio between progesterone and 20α-dihydroprogesterone, lower in the follicular phase than in luteal phase. This ratio appears to be very much higher in blood coming from the ovaries containing the corpora lutea than in peripheral blood.

INTRODUCTION

20α-dihydroprogesterone has been isolated by Zander *et al.*[1, 2] from human ripe follicles and corpora lutea. The biological activity of this compound has been shown to be one quarter to one half that of progesterone in the Clauberg and Hooker–Forbes tests[3].

Wiest[4] and Huang and Pearlman[5] demonstrated that ovarian tissue of the pseudopregnant rat was able to convert *in vitro* progesterone into 20α-dihydroprogesterone. Forleo *et al.*[6, 7] showed that 20α-hydroxysteroid-dehydrogenase activity was present also in the human ovary, through *in vitro* incubations of isolated follicular tissue and corpora lutea, and demonstrated that this reaction was reversible.

The plasma concentration of 20α-dihydroprogesterone in the follicular and in the luteal phase of the menstrual cycle has been reported by Runnebaum *et al.*[8], Mikhail[9], Saxena *et al.*[10], Van der Molen and Groen[11], Florensa and Sommerville[12], Aedo *et al.*[13]; moreover Billiar *et al.*[14] calculated its metabolic clearance rate and production rate. In several species, such as the rat, the rabbit and the guinea pig, plasma levels of 20α-dihydroprogesterone are appreciably higher than those of progesterone.

It has been suggested that in these animals a preovulatory rise of 20α-dihydroprogesterone may be of significance as a positive feedback agent, that could prolong and enhance LH secretion[15]; moreover Swerdloff *et al.*[16] demonstrated that 20α-dihydroprogesterone stimulate LH and FSH secretion in estrogen treated castrated rats. In contrast with these results are the experiments performed recently on the rabbit by YoungLai[17], who could not maintain LH secretion in animals treated with 20α-dihydroprogesterone after injections of LH-releasing hormone.

Wiest *et al.*[18] suggest that the reduction of progesterone to the biologically less active 20α-dihydroprogesterone may serve to reduce the progestational activity during the luteal phase of the cycle in the rat.

As yet, the role of 20α-dihydroprogesterone in the menstrual cycle of the human female remains to be ascertained. The purpose of the present investigation was to measure the daily plasma concentrations of 20α-dihydroprogesterone in relation to progesterone, 17α-hydroxyprogesterone and oestradiol-17β during normal menstrual cycles and to determine in follicular and luteal phase the concentration of 20α-dihydroprogesterone and progesterone in ovarian vein plasma.

EXPERIMENTAL

From seven fertile women, with normal ovulatory cycles (basal body temperature was checked daily), 7 ml of peripheral venous blood were obtained in heparinized syringes every day during the cycle at 9 a.m.

Table 1. Specificity of the antisera to progesterone-11 -hemisuccinate-bovine serum albumin (progesterone-11α -hem-BSA), 17-hydroxy-progesterone-3-carboxymethyl-oxime-bovine serum albumin (17-OH-progesterone-3-CMO-BSA),20α-dihydro-progesterone-3-carboxymethyl-oxime-bovine serum albumin (20α-OH-3-CMO-BSA) and 17β-estradiol-6-carboxymethyl-oxime-bovine serum albumin (17β-estradiol-6-CMO-BSA)

Steroids	Progesterone 11α-hem-BSA	17-OH-progesterone 3-CMO-BSA	20α-OH-progesterone 3-CMO-BSA	17β-estradiol 6-CMO-BSA
	(Cross-reaction %)			
Progesterone	100.0	6.0	0.8	—
17-Hydroxyprogesterone	1.3	100.0	<0.1	—
20α-Dihydroprogesterone	0.6	<0.2	100.0	—
20β-Dihydroprogesterone	—	—	<0.1	—
Pregnenolone	<0.2	—	<0.1	—
17-Hydroxypregnenolone	<0.2	12.5	—	—
11β-hydroxyprogesterone	17.9	—	<0.1	—
6β-Hydroxyprogesterone	<0.2	—	—	—
Pregnanediol	—	<0.2	—	—
Cortisone	<0.2	<0.2	<0.1	—
Corticosterone	<0.2	—	—	—
Cholesterol	<0.2	<0.2	<0.1	—
Estriol	<0.2	<0.2	<0.1	<0.1
Estradiol-17β	<0.2	<0.2	<0.1	100.0
Estradiol-17α	<0.2	—	—	0.3
Estrone	<0.2	<0.2	<0.1	0.8
Testosterone	<0.2	<0.2	<0.1	—
Androstendione	—	—	<0.1	—
Epitestosterone	<0.2	—	—	—
Dehydroepiandrosterone	<0.2	—	—	—
11α-hydroxyandrosterone	<0.2	—	—	—
Androsterone	—	<0.2	—	—
Androstenediol-17β	<0.2	—	—	—

In a second group of four fertile patients with normal ovulatory cycles (28–30 days) on the 7th, 10th, 19th and 20th days of the cycle respectively (calculated from the last menses) 7 ml of peripheral venous blood were obtained every 10 min for 1 h and then every h for 6 h.

In a third group of five fertile patients with normal ovulatory cycles (28–30 days), undergoing laparatomy for minor uterine surgery, in the follicular or luteal phase of the cycle an ovarian venous blood sample from both ovaries was obtained by insertion of a small needle connected to a heparinized catheter into one of the ovarian veins, isolated in the infundibulopelvic ligament. At the same time a peripheral venous blood sample was obtained. The specimens were refrigerated and, after centrifugation and separation, the plasma was stored at −20°C until processed.

All the solvents were Analar grade and redistilled before use. Cold steroids were obtained from Steroids Inc., NY, U.S.A. [1,2-^{3}H]-20α-dihydroprogesterone (Batch No. 695-095, S.A. 45 Ci/mmol), [1,2-^{3}H]-17-hydroxyprogesterone (Batch No. 531-74, S.A. 49 Ci/mmol) and and [2, 4, 6, 7-^{3}H]oestradiol 17β (Batch No. 747-037, S.A. 100 Ci/mmol) were supplied by the New England Nuclear Corporation, Boston, MA, U.S.A.; [1, 2, 6, 7-^{3}H]-progesterone (Batch 8, S.A. 84 Ci/mmol) was supplied by the Radiochemical Centre, Amersham, England. The purity of the radioactive steroids was controlled by crystallization of an aliquot to constant S.A. The preparation of antigens and antisera and the radioimmunoassays of plasma steroids were carried out, with slight modification, according to the methods of Florensa and Sommerville[12] for 20α-dihydroprogesterone, of Youssefnejadian *et al.*[19] for progesterone, of Youssefnejadian *et al.*[20] for 17-hydroxyprogesterone and of Emment *et al.*[21] for oestradiol-17β.

The specificity of the antisera obtained was investigated with different C-21, C-19 and C-18 steroids and cholesterol, testing their ability to compete with radioactive steroids for binding sites of the antibodies. Calculation of the percentage of cross-reaction was as described by Abraham and Odell[22]. Corresponding values are listed in Table 1. Since 20α-dihydroprogesterone and 17-hydroxyprogesterone display a small cross-reaction to progesterone antiserum, progesterone was separated from 17-hydroxyprogesterone and 20α-dihydroprogesterone through a small column of Sephadex LH 20, according to the method of Florensa and Sommerville[12]. 20α-dihydroprogesterone antiserum was used at 1:25,000 dilution, progesterone antiserum at 1:8000, 17-hydroxyprogesterone at 1:15,000 and oestradiol-17β at 1:20,000.

For all the antisera used the bond was fairly good, about 50%. To measure the radioactivity an automated two channel liquid scintillation spectrometer (Packard-Tri Carb 2425) was used. The samples were placed in disposable glass vials containing 10 ml of the scintillation medium, as described by Bray[23]. All samples were stabilised for a minimum of 90 min at 2°C in the dark, and were then counted for 10 min.

For accuracy, precision, sensitivity and specificity of the methods, our results were similar to those described by other authors[12, 19–21].

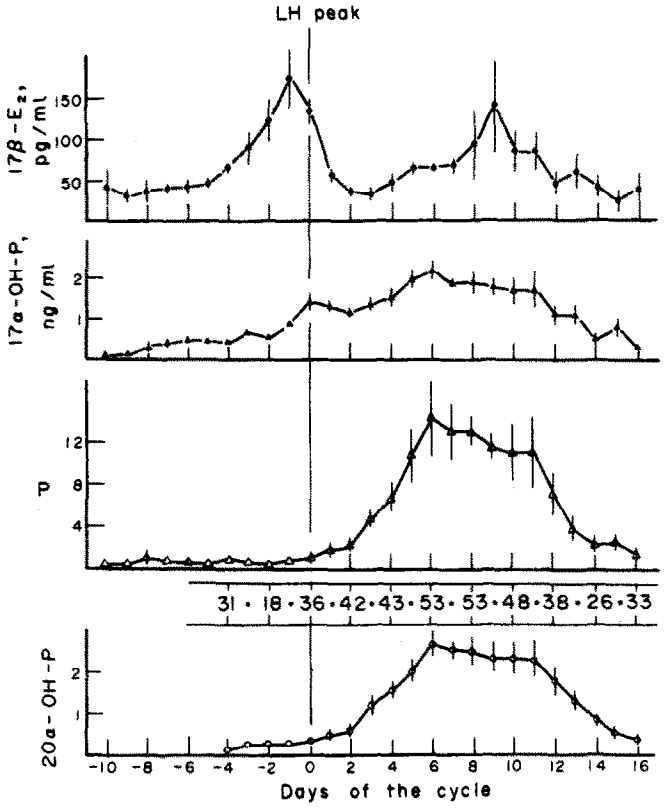

Fig. 1. Mean values of 17β-oestradiol (17β -E_2), 17-hydroxyprogesterone (17-OHP), progesterone (P) and 20α-dihydroprogesterone (20α-OHP) in the same aliquots of daily plasma samples obtained from 7 women during ovulatory cycles. The day of the LH maximum value is the reference day. P/20α-OHP ratios are illustrated between the two steroids. Vertical bars represent one standard error of the mean.

The coefficients of variation on replicate analyses ranged from 5.3% to 11.6% for 20α-dihydroprogesterone, from 7% to 9.2% for progesterone, from 7% to 10.2% for 17-hydroxyprogesterone and from 3.2% to 6.9% for oestradiol-17β.

Plasma LH was measured by specific radioimmunoassays, using the double antibody technique, according to Serra *et al.*[24]. All samples for steroids and LH determinations were assayed in duplicate or triplicate.

RESULTS

The mean values of daily oestradiol-17β, 17-hydroxyprogesterone, progesterone and 20α-dihydroprogesterone plasma levels in 7 normal cycles, in which the day of the LH peak is the reference day, are illustrated in Fig. 1. Between progesterone and 20α-hydroprogesterone mean values, the daily ratio of these two compounds is reported.

The mean oestradiol-17β levels began to rise 4 to 5 days before the LH peak, but the mean 17-hydroxyprogesterone levels rose only one day before. The mean peak of oestradiol-17β concentration occurred one day prior to the mean LH peak, whereas the mean mid-cycle 17-hydroxyprogesterone maximum occurred on the day of the LH peak. The oestradiol-17β dropped to early follicular phase levels 2 days after the LH peak, whereas the 17α-hydroxyprogesterone concentration remained relatively constant.

The levels of both 17-hydroxyprogesterone and oestradiol-17β began to rise 3 to 4 days after the LH peak.

The mean progesterone and 20α-hydroxyprogesterone levels began to rise immediately after the LH peak and the patterns of both compounds were very similar until the next menses. Also considering the low levels of these compounds during the follicular phase, it seems that the mean ratio between progesterone and 20α-dihydroprogesterone is higher in the luteal phase than in the follicular phase.

The levels of progesterone and 20α-dihydroprogesterone in five representative individual cycles are illustrated in Fig. 2. It is possible to observe that the patterns of the compounds through the cycle are rather different from one patient to another.

In all the cases of the second group the results obtained for both progesterone and 20α-dihydroprogesterone were quite homogeneous: the fluctuations during the time considered, calculated as percentage deviation from the mean, never exceeded 20%. Also in this group of patients a difference seems to exist

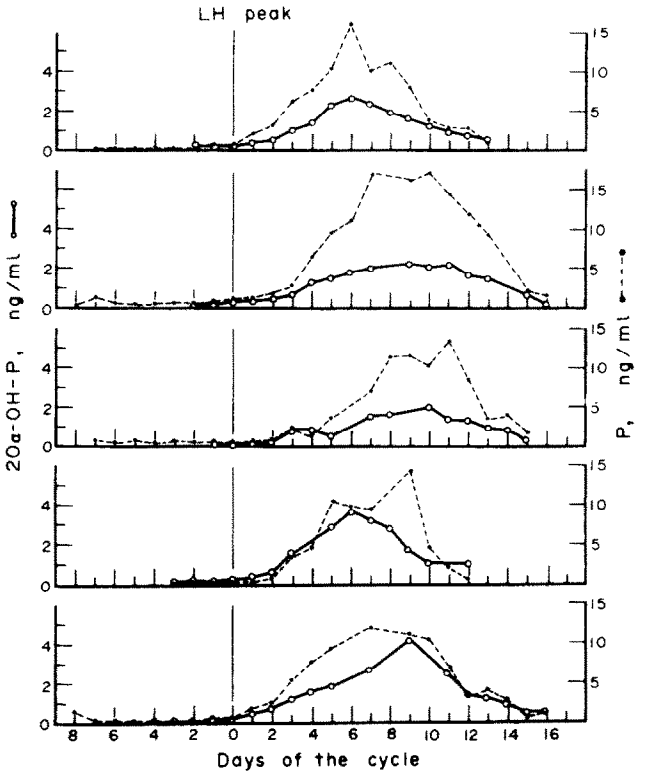

Fig. 2. Daily plasma concentrations of 20α-dihydroprogesterone (20α-OHP) and progesterone (P) in 5 individual subjects through an ovulatory cycle.

in the mean ratio between progesterone and 20α-dihydroprogesterone in the early follicular phase (1.2) and in the luteal phase (5.2).

Table 2 illustrates the progesterone and 20α-dihydroprogesterone concentrations in peripheral blood and in blood coming from both ovaries during early follicular phase and the luteal phase.

In the last column the progesterone/20α-dihydroprogesterone ratios are tabulated. It is evident that the ovaries containing the corpora lutea produce and

Table 2. Progesterone and 20α-dihydroprogesterone concentrations in peripheral venous plasma and in ovarian venous plasma at different days of the cycle (calculated from the last menses) in 5 patients with normal ovulatory cycles. In the last column the progesterone (P)/20α-dihydroprogesterone (20α-OHP) ratios are tabulated

Day of the cycle	Plasma sample	Progesterone (ng/ml)	20α-Dehydroprogesterone (ng/ml)	P/20α-OHP Ratio
	peripheral	0.57	0.26	2.1
3rd	right ovarian	0.79	0.48	1.6
	left ovarian	1.23	0.66	1.8
	peripheral	0.61	0.32	1.9
7th	right ovarian	2.62	1.05	2.4
	left ovarian	0.60	0.33	1.8
	peripheral	10.16	2.15	4.7
	right ovarian			
19th	(corpus luteum)	518.00	6.30	82.2
	left ovarian	10.50	2.21	4.7
	peripheral	17.90	3.78	4.7
	right ovarian	12.80	4.00	3.2
22nd	left ovarian			
	(corpus luteum)	1501.00	73.20	20.5
	peripheral	4.19	1.24	3.3
23rd	right ovarian	5.09	1.17	4.3
	left ovarian			
	(corpus luteum)	1207.00	37.30	32.3

secrete an important quantity of progesterone and 20α-dihydroprogesterone, whereas the concentrations of these compounds in blood from the controlateral ovaries and in peripheral blood are similar. Also in this group of patients the progesterone/20α-dihydroprogesterone ratio seems to be higher in the luteal phase than in the early follicular phase both in peripheral and in ovarian blood and definitely very high in blood coming from the ovaries containing the corpora lutea.

DISCUSSION

The values of daily plasma concentration of 20α-dihydroprogesterone in the cycles studied agree with those obtained by Mikhail[9] Saxena *et al.*[10], Van der Molen and Groen[11], Florensa and Sommerville[12] and Aedo *et al.*[13]. The patterns of progesterone and 20α-dihydroprogesterone are similar, but also considering the low levels of these compounds in the follicular phase there seem to be a difference in the mean ratio in the follicular phase and in the luteal phase.

The values of progesterone and 20α-dihydroprogesterone found in ovarian venous blood agree with those obtained by Mikhail[9]. The ratios of these compounds in the luteal phase are, in our three cases, very much higher in the blood coming from the ovaries containing the corpora lutea than in peripheral blood. It is to be pointed out that the differences in the ratios are not in agreement with the similar values for metabolic clearance rate of the two compounds[14]. In the four patients, in which progesterone and 20α-dihydroprogesterone were measured in peripheral blood at short intervals, the concentrations of these compounds do not seem to show rapid and relevant fluctuations.

It has been suggested that in animals the preovulatory rise of 20α-dihydroprogesterone may be a positive feed-back agent, that could prolong and enhance LH secretion; moreover 20α-dihydroprogesterone seems to induce a release of LH following a priming with estrogens in castrated rats[16].

In humans, during the preovulatory phase of the cycle, 20α-dihydroprogesterone together with progesterone show a significant increase, induced by the first LH surge[25]. As yet there is no evidence that the increase of 20α-dihydroprogesterone prior to ovulation is of significance as a positive feed-back in humans.

REFERENCES

1. Zander J., Forbes T. R., Neher R. and Desoulles P.: Ueber biologisch aktive Progesteronmetaboliten im menschlichen Organismus. *Klin. Wchnschr.* **35**, (1957) 143–146.
2. Zander J., Forbes T. R., Von Munstermann A. M. and Neher R.: Δ^4-3-ketopregnene-20α-ol and Δ^4-3-ketopregnene-20β-ol, two naturally occurring metabolites of progesterone. Isolation, identification, biologic activity and concentration in human tissues. *J. clin. Endocr. Metab.* **18** (1958) 337–353.
3. Hooker C. W. and Forbes T. R.: Specificity of the intrauterine test for progesterone. *Endocrinology* **45** (1949) 71–74.
4. Wiest W. G.: Conversion of progesterone to Δ4-pregnen-20α-ol-3-one by rat ovarian tissue *in vitro*. *J. biol. Chem.* **234** (1959) 3115–3121.
5. Huang W. Y. and Pearlman W. H.: The corpus luteum and steroid hormone formation. I. Studies on luteinized rat ovarian tissue *in vitro*. *J. biol. Chem.* **237** (1962) 1060–1065.
6. Forleo R. and Collins W. P.: Some aspects of steroid biosynthesis in human ovarian tissue. *Acta endocr., Copenh.* **46**, (1964) 265–278.
7. Forleo R. and Sbiroli C.: Studies on 20α-hydroxisteroid dehydrogenase activity in human ovaries. In *The Hormonal Function of the Ovary*. Pacini. Pisa (1975) pp. 339–352.
8. Runnebaum B., Van Der Molen H. J. and Zander J.: Steroids in human peripheral blood of the menstrual cycle. *Steroids, Suppl.* **2** (1965) 189–204.
9. Mikhail G.: Sex steroids in blood. *Clin. Obstet. Gynec.* **10** (1967) 29–39.
10. Saxena B. B., Demura H., Gandy H. M. and Peterson R. E.: Radioimmunoassay of human follicle stimulating and luteinizing hormones in plasma. *J. clin. Endocr.* **28** (1968) 519–534.
11. Van Der Molen H. J. and Groen D.: In *The gas–liquid chromatography of steroids*. Memoirs of the Society for Endocrinology (Edited by J. K. Grant), Cambridge University Press, N. **16** (1967) p. 155.
12. Florensa E. and Somerville I. F.: Radioimmunoassay of plasma 20α-dihydroprogesterone. *Acta endocr., Copenh.* **22** (1973) 451–465.
13. Aedo A. R., Landgren B. M., Cekan Z. and Diczfalusy E.: Studies on the pattern of circulating steroids in the normal menstrual cycle. 2. Levels of 20α-dihydroprogesterone, 17-hydroxyprogesterone and 17-hydroxypregnenolone and the assessment of their value for ovulation prediction. *Acta endocr., Copenh.* **82** (1976) 600–616.
14. Billiar R. B., Lin T. J. and Little D.: Metabolic clearance rate and production rate of 20α-hydroxypregn-4-en-3-one. *Gynec. Invest.* **4** (1973) 148–154.
15. Hilliard J., Penardi R. and Sawyer C. H.: A functional role for 20α-hydroxy-pregn-4-en-3-one in the rabbit. *Endocrinology* **80** (1967) 901–909.
16. Swerdloff R. S., Jacobs H. S. and Odell W. D.: Synergistic role of progestagens in estrogen induction of LH and FSH surge. *Endocrinology* **90** (1972) 1529–1536.
17. YoungLai E. V.: Effect of 20α-hydroxy-4-pregnen-3-one on luteinizing hormone secretion in the female rabbit *Acta endocr., Copenh.* **84** (1977) 45–50.
18. Wiest A. G., Kidwell W. R. and Baloch J. K.: Progesterone catabolism in the rat ovary: a regulatory mechanism for progestational potency during pregnancy. *Endocrinology* **82** (1968) 844–859.
19. Youssefnejadian E., Florensa E., Collins W. P., and Sommerville I. F.: Radioimmunoassay of plasma progesterone; *J. steroid Biochem.* **3** (1972) 893–901.
20. Youssefnejadian E., Florensa E., Collins W. P. and Sommerville I. F.: Radioimmunoassay of 17-hydroxyprogesterone. *Steroids* **20** (1972) 773–788.
21. Emment Y., Collins W. P. and Sommerville I. F.: Radioimmunoassay of oestrone and oestradiol in human plasma. *Acta endocr., Copenh.* **69** (1972) 567–582.
22. Abraham G. E. and Odell W. D.: In *Immunological Methods in Steroid Determination* (Edited by F. G. Peron and B. V. Caldwell). Appleton-Century-Crofts-Meredith, New York (1970) p. 87.
23. Bray G. A.: A simple efficient liquid scintillator for

counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.* **1** (1960) 279–285.
24. Serra G. B., Caniglia R. and Barile G.: Some characteristics of the double antibody method for the assay of gonadotropins. In *Recent Progress in Reproductive Endocrinology* (Edited by P. G. Crosignani and V. H. T. James). Academic Press. London and New York. no 4 (1974) pp. 65–82.
25. Leyendecker G., Hinckers K., Nocke W. and Plotz E. J.: Gonadotropine und ovarielle Steroide im Serum während des normelen menstruellen Cyclus und bei Corpus-luteum-Insuffizienz. *Arch. Gynäk.* **218** (1975) pp. 47–64.