Journal of Steroid Biochemistry, 1971, Vol. 2, pp. 173–177. Pergamon Press. Printed in Great Britain

PLASMA NEUTRAL STEROID SULPHATES AND THE MENSTRUAL CYCLE

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(*Received* 12 *February* 1971)

SUMMARY

The peripheral plasma concentrations of the monosulphates of androsterone, epiandrosterone, dehydroepiandrosterone, 5-androstene-3β,17β-diol, pregnenolone and 5-pregnene-3β,20α-diol as well as of the disulphates of 5-androstene-3β,17α-diol, 5-androstene-3β,17β-diol and 5-pregnene-3β,20α-diol did not differ significantly, when samples obtained on the 10th and 22nd day of the menstrual cycle in a series of nine normal females were compared. It can be concluded that normal ovaries have little effect on the plasma concentrations of these compounds. In eight of the nine samples drawn in the latter half of the menstrual cycle, 5α-pregnane-3β,20α-diol was present in mono- or disulphate-conjugated form in concentrations considerably exceeding those reported for progesterone. In addition, sulphate-conjugated 5α-pregnane-3α,20α-diol and 5β-pregnane-3α,20α-diol were identified in a pool of plasma from blood drawn on the 22nd day of the menstrual cycle.

INTRODUCTION

THE secretion of several unconjugated C_{19} (androstenedione, testosterone, dehydroepiandrosterone) and C_{21} (progesterone, 17α-hydroxyprogesterone, 20α-hydroxy-4-pregnen-3-one) steroids by normal human ovaries has been clearly demonstrated (see [1]). These hormones are chiefly secreted at the phase when the ovary bears a ripe follicle or a corpus luteum[1]. In contrast, the secretion of sulphate-conjugated neutral steroids by normal human ovaries in the follicular or luteal phase seems to be negligible, as revealed by simultaneous analyses of plasma from the ovarian vein and peripheral blood[2]. In certain pathological conditions and after treatment with human pituitary follicle-stimulating hormone, significant ovarian secretion of dehydroepiandrosterone sulphate has been reported to take place[3]. It therefore seems that the increase in the urinary excretion of several neutral steroid conjugates observed in normal females in the latter half of the menstrual cycle (see [4–6]) is due to ovarian secretion of these compounds or their precursors as unconjugated steroids, which are then conjugated in other tissues.

The factors determining the plasma concentrations of sulphate-conjugated C_{19} and C_{21} steroids are insufficiently known. As regards the ovaries, it has been reported that the concentrations of androsterone and dehydroepiandrosterone sulphates are independent of the phase of the menstrual cycle[7]. Recently, it was suggested that there is a biphasic pattern in the peripheral plasma concentrations of testosterone, androstenedione and dehydroepiandrosterone sulphate during the menstrual cycle[8]. In addition to the sulphate-conjugated steroids named above, a number of C_{19} and C_{21} steroids have been shown to be present as sulphates in human peripheral plasma (see [9, 10]). The aim of the present study was to investigate whether the plasma concentrations of the conjugates vary in relation to the menstrual cycle.

MATERIAL AND METHODS

The subjects studied were nine females with regular menstruation. Their average age was 24·9 (20–30) yr and the mean duration of their cycles was 27·9 (26–29) and menstrual bleeding 4·6 (3–6) days.

Blood samples were obtained at 8 a.m. on the 10th and 22nd days of the menstrual cycle. The heparinized blood was centrifuged immediately and the plasma stored at −20°C until the specimens were analysed in random order.

Solvents were as previously described[11].

Determination of neutral steroid mono- and *disulphates* was carried out as previously described[11]. Briefly, the method is as follows. After acetone/ethanol extraction of 3–5 ml of plasma, mono- and disulphate fractions of neutral steroids are obtained by chromatography on Sephadex LH-20. The sulphate conjugates are cleaved by solvolysis and purified on silicic acid. After addition of a known amount of stigmasterol as internal standard, the steroids are converted to trimethylsilyl ether derivatives and analysed by gas–liquid chromatography (GLC) on QF-1 and SE-30 liquid phases. The quantification of 5α-pregnane-3β,20α-diol will be described under "Results and Discussion".

GLC and gas chromatography–mass spectrometry were performed as previously described[9].

RESULTS AND DISCUSSION

The concentrations of neutral steroid mono- and disulphates determined on the 10th and 22nd days of the menstrual cycle are given in Table 1. As repeatedly demonstrated[7–9, 11], there are great differences in the peripheral plasma concentrations of these compounds between individual subjects. However, in the same subject the concentrations of the individual steroids were very similar in samples collected at intervals of 12 days (Table 1). Therefore it can be concluded that the concentrations of the plasma sulphate-conjugated neutral steroids determined are not dependent on the phase of the menstrual cycle, with one exception. It was observed that in most of the samples collected in the latter phase of the menstrual cycle, a compound was present which could not be found in the samples taken in the earlier phase of the cycle. This compound gave a mass spectrum typical of pregnane-3,20-diol di-TMS ether (for reference spectra see [12, 13]) and the values for the relative retention time (RRT) of this derivative showed it to be 5α-pregnane-3β,20α-diol[13]. In the latter phase of the menstrual cycle, it has been found only as the disulphate in urine[6], whereas in blood plasma it is present as both mono- and disulphate (Table 1). In pregnancy blood [14] it is present as a mono- and a disulphate and is a metabolite of progesterone (see [15]).

The TMS ether derivative of 5α-pregnane-3β,20α-diol is only partly separated from the corresponding derivative of 5-pregnene-3β,20α-diol on an SE-30 column and similarly, from that of androsterone on a QF-1 column (for RRT values, see [9, 13]. To obtain more exact quantification, 5α-pregnane-3β,20α-diol was measured on the QF-1 column as the TMS ether derivative after reduction of the sample with sodium borohydride. This step leads to the formation of androstane-3,17-diols from androsterone, which, as TMS ether derivatives, are eluted considerably earlier from a QF-1 column than androsterone-TMS ether or 5α-pregnane-3β,20α-diol di-TMS ether (see [9, 13]). As can be seen from the values in Table 1, the sum of the plasma concentrations of mono- and disul-

Table 1. Concentrations of neutral steroid mono- and disulphates in blood plasma of females on the 10th and 22nd days of the menstrual cycle. Values are given as μg of the free steroid/100 ml of plasma

Compound		R.U. 20*	M.L. 21	I.K. 22	L.V. 23	S.K. 25	S.O. 26	S.P. 28	K.V. 29	L.L. 30	Mean ± S.E.
Monosulphates											
Androsterone	10th	31	112	52	74	54	18	4	22	8	41·7 ± 11·7
	22nd	29	109	56	72	37	17	5	29	4	39·8 ± 11·4
Epiandrosterone	10th	9	23	17	21	22	5	3	11	5	12·9 ± 2·6
	22nd	10	26	18	16	12	5	5	16	5	12·5 ± 2·4
Dehydroepiandrosterone	10th	165	286	184	119	176	79	59	122	76	140·7 ± 13·5
	22nd	166	262	139	99	187	80	65	113	63	130·4 ± 21·9
5-Androstene-3β,17β-diol	10th	11	23	14	N.D.‡	8	6	5	4	8	9·9 ± 2·2
	22nd	7	23	11	5	11	4	7	7	3	8·7 ± 2·0
Pregnenolone	10th	13	6	10	6	7	4	3	5	3	6·3 ± 1·0
	22nd	10	7	10	7	7	5	3	7	4	6·7 ± 0·8
5-Pregnene-3β,20α-diol	10th	34	34	29	17	26	14	14	12	12	21·3 ± 3·1
	22nd	28	33	22	17	38	14	17	20	17	21·1 ± 4·2
5α-Pregnane-3β,20α-diol	10th	—†	—	—	—	—	—	—	—	—	—
	22nd	12	6	—	1	7	10	5	7	7	6·1 ± 1·3
Disulphates											
5-Androstene-3β,17α-diol	10th	22	9	29	9	13	15	22	6	22	16·3 ± 2·6
	22nd	7	9	27	6	16	5	18	8	20	12·9 ± 2·5
5-Androstene-3β,17β-diol	10th	24	47	39	25	14	14	12	20	17	23·5 ± 4·0
	22nd	14	40	38	23	13	9	9	27	15	20·9 ± 3·9
5-Pregnene-3β,20α-diol	10th	17	13	19	7	9	10	14	8	14	12·3 ± 1·4
	22nd	13	13	23	10	11	7	13	11	19	13·3 ± 1·6
5α-Pregnane-3β,20α-diol	10th	—	—	—	—	—	—	—	—	—	—
	22nd	2	—	—	—	2	6	2	5	6	2·5 ± 0·8

*Age (years).
†Not found.
‡Not determined because of interfering impurities.

phated 5α-pregnane-3β,20α-diol clearly exceeds the plasma concentration of progesterone in the latter half of the menstrual cycle[16, 17]. In one of the subjects examined (I.K., Table 1), no 5α-pregnane-3β,20α-diol mono- or disulphate was present in either of the samples investigated. It is possible that her cycle was anovulatory.

After administration of progesterone, 5α-pregnane-3α,20α-diol and 5β-pregnane-3α,20α-diol, in addition to 5α-pregnane-3β,20α-diol, were found as sulphate conjugates in blood plasma (see [15]). To detect whether endogenous progesterone is metabolised similarly, some of the samples were pooled to give 30 ml specimens of blood plasma. In the pool of samples taken on the 22nd day of the menstrual cycle, two compounds were found in both the mono- and disulphate fractions which gave mass spectra typical of pregnane-3,20-diol di-TMS ether[12, 13], and their RRT values (see [13]) showed them to be 5α-pregnane-3α,20α-diol and 5β-pregnane-3α,20α-diol.

The urinary excretion of sulphate-conjugated epiandrosterone,* dehydroepiandrosterone, 16α-hydroxydehydroepiandrosterone, 3β,17β-dihydroxy-5-androsten-16-one, as well as of total $C_{19}O_2$ 17-ketosteroids and of total 16-oxygenated ketonic C_{19} steroids, is significantly higher during the luteal phase of the menstrual cycle than during the follicular phase[6]. In the present study it is shown that there are no differences in the plasma concentrations of $C_{19}O_2$ 17-ketosteroid (androsterone, dehydroepiandrosterone and epiandrosterone) sulphates at the corresponding phases of the menstrual cycle. It is possible, therefore, that the increase in the production of the compounds that are excreted in increased amounts in urine is so slight that it is not observed with the present method in plasma samples which reflect a momentary situation. Another possibility is that the compounds excreted in increased amounts reach the kidneys in unconjugated form and are sulphated in this organ. However, the steroid sulphokinase activity of kidney tissue has been reported to be low[18].

The results of this study support the view that normal ovaries have little effect on the plasma concentrations of several sulphate-conjugated C_{19} and C_{21} steroids[2]. It remains to be investigated whether determination of the conjugates of 5α-pregnane-3β,20α-diol, which is present in blood plasma in the latter half of the menstrual cycle in concentrations considerably exceeding those of progesterone, might be useful in studies on ovulation and corpus luteum function.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the National Research Council for Medical Sciences, Finland, the Sigrid Jusélius Foundation and the Ford Foundation.

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