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PREGNANOLONES, PREGNENOLONE AND PROGESTERONE IN THE HUMAN FETAL TISSUES OF EARLY AND MIDTRIMESTER PREGNANCY

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SUMMARY

Endogenous tissue levels of progesterone (determined by radioimmunoassay), pregnenolone and 3α/β-hydroxy-5α/β-pregnan-20-ones (determined by gaschromatography and electron capture detection) were measured in different human fetal tissues of the 11th to the 24th week of pregnancy. Progesterone was the predominant unconjugated steroid in all samples. 3β-sulfoxy-5-pregnen-20-one was isolated in considerable amounts from most fetal organs. The saturated metabolites were present at different concentrations, demonstrating a differentiated distribution of ring-A-reductases and of sulfotransferases. The qualitative and quantitative distribution of the epimeric steroids may reflect hormonal action not only of progesterone but also of the so called metabolites.

INTRODUCTION

It has been estimated that the human fetus metabolizes 30–40 mg of progesterone per day at term of pregnancy [1, 2]. From perfusion experiments [3, 4] with radioactive labelled progesterone it is known that among other compounds ring-A-saturated metabolites are formed in fetal tissues. The different organs demonstrate characteristic metabolic features as lung, brain, kidneys and skin form 5α-saturated steroids in contrast to the liver which shows 5β-reductase activity [5–8].

The aim of the present study was to determine the endogeneous concentration of presumptive metabolites of progesterone in fetal tissues because they may give a closer approximation of metabolic activity than *in vitro* experiments.

EXPERIMENTAL

Ten human fetuses (2–29 cm crown–feet length, 11–24 weeks of pregnancy) obtained at legal termination of pregnancy or at abortuses ($n = 4$) were investigated. From nine fetuses, tissue samples of skin, brain, lungs, liver, kidneys and intestine were dissected. One fetus (2 cm length, week 11 of pregnancy) was processed *in toto*. In four cases additional samples of placenta (Nos. 1–5) and amniotic fluid (Nos. 2–5) were obtained.

Tissues samples were blotted, weighed and homogenized after addition of two volumes of distilled water using an Ultra-Turrax. The following radioactive labelled steroids were added to the homogenates: [^{3}H]-progesterone (3000 c.p.m.), [^{3}H]-3β-hydroxy-5α-pregnan-20-one (20,000 c.p.m.) and [^{3}H]-3α-Sulfoxy-5β-pregnan-20-one (20,000 c.p.m.). Extraction, separation and quantitative determination of the free and sulfoconjugated steroids was performed as described previously [9].

RESULTS

Progesterone (*Table 1*)

This was the predominant steroid in all tissues analysed reaching levels up to 4.8 μg/g tissue. The highest values were measured in the placenta and in fetal kidneys. In the other organs the hormone was more evenly distributed. The lowest concentrations were observed in the samples of amniotic fluid.

Pregnenolone and pregnenolone sulfate (*Table 2*)

These were determined at high levels in the tissue. The sulfoconjugate was not found in the placenta. In the other organs the mean concentrations of pregnenolone sulfate and of free pregnenolone were about the same with the exception of the liver which contained mainly pregnenolone sulfate. The free compound was not found in amniotic fluid; low concentrations were determined in brain and liver tissue.

5β-Saturated pregnanolones

3β-Hydroxy-5β-pregnan-20-one could not be isolated from any sample. 3α-Hydroxy-5β-pregnan-20-one (Table 3) was not present in skin and in kidneys. From intestine it was found only as the free steroid. In the liver the concentration of the sulfoconjugate was lower than that of the free compound. In fetal lung tissue, only sulfoconjugated material was detected with the exception of two samples containing a very low amount of free steroid. In brain the free

For abbreviations and trivial names see the preceding paper [9].

Table 1. Progesterone (ng/g)

Fetus	Placenta	Amn. fluid	Skin	Brain	Lung	Liver	Kidney	Intestine
1	4869.7	—	473.9	775.9	1032.2	947.7	1677.0	1050.4
2	—*	31.5	285.9	398.6	284.3	156.9	819.5	934.3
3	3021.9	70.6	266.2	269.3	216.9	131.8	609.7	465.1
4	1343.6	35.4	128.3	92.9	135.9	106.9	201.0	292.7
5	—	17.5	119.8	96.8	140.3	71.7	297.8	278.9
6	—	—	79.0	42.8	152.3	84.5	194.5	197.6
7	—	—	71.2	71.7	177.4	59.2	275.6	282.9
8	572.9	—	152.7	87.5	145.2	17.3	283.8	33.0
9	—	—	394.6	30.5	293.2	68.8	188.6	167.3
M	2452.0	38.8	219.1	207.3	286.4	191.6	505.3	411.4
SD ±	1908.7	22.6	144.2	244.9	286.1	285.3	489.5	350.2

* No sample available.

Table 2. Pregnenolone and (pregnenolone-sulfate) (ng/g)

Fetus	Placenta	Amn. fluid	Skin	Brain	Lung	Liver	Kidney	Intestine
1	23 (0)	— (—)	242 (64)	8 (1)	248 (181)	7 (58)	124 (35)	19 (199)
2	— (—)	1 (29)	174 (71)	9 (15)	56 (133)	0 (108)	152 (651)	20 (136)
3	10 (0)	0 (30)	142 (66)	19 (39)	28 (215)	7 (260)	62 (684)	74 (403)
4	20 (0)	0 (21)	306 (56)	15 (22)	588 (61)	0 (134)	230 (227)	370 (632)
5	— (—)	0 (35)	66 (8)	4 (13)	68 (216)	14 (124)	50 (75)	17 (182)
6	— (—)	— (—)	98 (26)	4 (5)	54 (96)	6 (25)	55 (57)	14 (223)
7	— (—)	— (—)	117 (5)	3 (6)	46 (106)	5 (18)	31 (81)	11 (100)
8	27 (0)	— (—)	5 (109)	2 (3)	15 (41)	9 (17)	54 (39)	19 (65)
9	— (—)	— (—)	130 (65)	19 (6)	124 (139)	37 (108)	66 (74)	35 (151)
M	20 (0)	0 (29)	142 (52)	9 (12)	136 (132)	9 (102)	99 (214)	44 (232)
SD ±	7 (—)	0 (6)	90 (33)	7 (12)	183 (63)	11 (73)	85 (264)	116 (178)

Table 3. 3α-OH-5β-Pregnan-20-one and (3α-sulfoxy-5β-Pregnan-20-one) (ng/g)

Fetus	Placenta	Amn. fluid	Skin	Brain	Lung	Liver	Kidney	Intestine
1	6 (0)	— (—)	0 (0)	10 (16)	0 (188)	262 (16)	27 (0)	141 (0)
2	— (—)	3 (0)	0 (0)	54 (11)	0 (157)	242 (96)	61 (0)	189 (0)
3	7 (1)	1 (0)	0 (0)	7 (47)	0 (102)	140 (60)	66 (0)	138 (0)
4	1 (1)	4 (0)	0 (0)	5 (30)	0 (77)	101 (39)	27 (0)	124 (0)
5	— (—)	1 (0)	0 (0)	29 (49)	2 (193)	145 (15)	68 (0)	115 (0)
6	— (—)	— (—)	0 (0)	1 (8)	0 (35)	97 (29)	26 (0)	45 (0)
7	— (—)	— (—)	0 (0)	8 (13)	3 (54)	54 (19)	17 (0)	59 (0)
8	17 (117)	— (—)	0 (0)	16 (2)	0 (44)	93 (5)	51 (0)	24 (0)
9	— (—)	— (—)	0 (0)	14 (7)	0 (91)	44 (62)	23 (0)	60 (0)
M	8 (30)	2 (0)	0 (0)	16 (20)	1 (105)	131 (38)	41 (0)	100 (0)
SD ±	7 (58)	2 (—)	— (—)	16 (18)	1 (61)	77 (30)	21 (—)	55 (—)

Table 4. 3α-OH-5α-Pregnan-20-one and (3α-sulfoxy-5β-Pregnan-20-one) (ng/g)

Fetus	Placenta	Amn. fluid	Skin	Brain	Lung	Liver	Kidney	Intestine
1	13 (10)	— (—)	35 (15)	10 (0)	48 (18)	0 (15)	24 (0)	46 (45)
2	— (—)	7 (0)	32 (0)	11 (0)	16 (12)	5 (7)	23 (0)	37 (φ)
3	17 (49)	10 (0)	9 (3)	5 (0)	37 (15)	5 (12)	0 (0)	24 (ND)
4	20 (38)	16 (0)	21 (0)	4 (0)	20 (6)	6 (4)	0 (0)	21 (φ)
5	— (—)	5 (0)	24 (2)	7 (0)	19 (4)	0 (6)	15 (0)	39 (28)
6	— (—)	— (—)	37 (48)	19 (0)	39 (17)	6 (10)	102 (0)	30 (79)
7	— (—)	— (—)	20 (7)	18 (0)	18 (7)	0 (4)	19 (0)	25 (111)
8	20 (22)	— (—)	2 (4)	16 (0)	28 (4)	0 (5)	18 (0)	14 (10)
9	— (—)	— (—)	19 (20)	9 (0)	8 (9)	2 (4)	15 (0)	12 (6)
M	17 (30)	10 (0)	22 (11)	11 (0)	26 (10)	3 (8)	24 (0)	27 (35)
SD ±	3 (18)	5 (0)	12 (15)	5 (0)	13 (10)	3 (4)	31 (—)	12 (41)

Table 5. 3β-OH-5α-Pregnan-20-one and (3β-sulfoxy-5α-pregnan-20-one) (ng/g)

Fetus	Placenta	Amn. fluid	Skin	Brain	Lung	Liver	Kidney	Intestine
1	52 (1)	— (–)	40 (0)	96 (0)	137 (13)	168 (0)	168 (0)	44 (311)
2	— (–)	1 (0)	5 (0)	46 (0)	62 (5)	55 (24)	79 (0)	25 (54)
3	45 (4)	1 (0)	13 (18)	22 (0)	0 (4)	1 (0)	0 (0)	17 (73)
4	41 (2)	1 (0)	14 (0)	20 (0)	44 (3)	0 (20)	11 (0)	0 (0)
5	— (–)	1 (0)	11 (8)	58 (0)	106 (66)	79 (66)	98 (0)	74 (191)
6	— (–)	— (–)	30 (32)	157 (0)	321 (11)	35 (11)	420 (12)	138 (313)
7	— (–)	— (–)	2 (3)	84 (0)	122 (2)	29 (74)	96 (0)	49 (159)
8	98 (3)	— (–)	14 (31)	82 (0)	117 (6)	58 (18)	139 (8)	43 (79)
9	— (–)	— (–)	17 (26)	34 (0)	128 (6)	25 (18)	43 (0)	29 (42)
M	59 (3)	1 (0)	16 (13)	67 (0)	115 (6)	34 (27)	117 (2)	47 (136)
SD ±	26 (1)	0 (–)	12 (4)	44 (–)	90 (3)	26 (25)	126 (5)	40 (116)

and sulfoconjugated compound was determined at similar concentration.

3α-Hydroxy-5α-pregnan-20-one (Table 4)

This was determined as free steroid from all tissues at similar concentration. The sulfoconjugate was not found in amniotic fluid, brain and kidney. In the other tissues, the sulfate occurred in amounts about equal to those of the free compound.

3β-Hydroxy-5α-pregnan-20-one (Table 5)

This was distributed unequally in the different fetal organs. Skin, liver and intestine contained similar amounts of free and conjugated steroid. The amount of sulfate was low in placental tissue and lung. It was detected in two samples of kidneys at low concentration. In brain it was not detected.

The smallest fetus (2 cm length, 11th week of pregnancy) analysed in toto contained 499 ng/g progesterone, 17 ng/g 3α-5α-P and 19 ng/g 3α-5α-P sulfate. 3α-5β-P and 3β-5α-P were present as sulfoconjugate only at concentrations of 20 and 28 ng/g respectively. Pregnenolone (4 ng/g) was found mainly as sulfoconjugated material (32 ng/g).

DISCUSSION

The results obtained from experiments in which saturated metabolites of different structures have been isolated from fetal tissues compare well to the results of *in vitro* experiments using radioactive labelled progesterone [3–5, 6]. Saturated metabolites of progesterone were isolated from meconium [10], liver [11] and bile [12]. The main part was sulfoconjugated with exception of steroids isolated from bile which were found as glucuronides [12]. The same authors isolated pregnenolone sulfate from fetal adrenals, kidneys, lungs and liver in amounts equal to those found in this study. Our results show that pregnenolone is present in all fetal tissues reaching about half the levels of progesterone. Even in a fetus of the 11th week of pregnancy a significant amount was determined indicating early and considerable *de novo* synthesis of this steroid by human fetuses.

The qualitative distribution of saturated metabolites in different fetal tissues demonstrates a distinct pattern: Pregnenolone sulfate was not isolated from placental tissue, whereas it was the only sulfoconjugated steroid which could be determined in amniotic fluid of this group. Fetal skin contained no 5β-saturated metabolites and in the kidney only traces of sulfoconjugated material was found.

No 5α-reduced sulfates could be isolated from brain but high concentrations were present in the lung. 3α-sulfoxy-5β-pregnan-20-one was also found in this organ at higher levels than in the intestine where it was not detected. Assuming that the metabolites are not changed by other metabolic compartments and originate in the tissue from which they were isolated, we conclude that ring-A-reductases and sulfotransferases are distributed non-uniformly in fetal tissues.

The preferential formation of different metabolites in fetal organs is difficult to interpret. Especially when these steroids are looked upon as inactive compounds. Besides, specific effects like induction of protein synthesis in chick oviduct [13], hypnotic action [14], action at hemoglobin synthesis [15, 16], competitive and allosteric effects on fetal and placental enzyme have to be considered [17]. A regulatory system may exist based on interactions of fetal steroid-sulfates and placental sulfatases [18], which influences transplacental passage of compounds. This assumption would be supported by the existence of a feto-placental circulation of 5α-reduced pregnanolones in analogy to the known entero-hepatic circulation of these metabolites [20].

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