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Progesterone Metabolism by Guinea Pig Intrauterine Tissues

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Progesterone metabolism by guinea pig amnion, chorion, myometrium, and endometrium was studied at the following gestational stages. Day 45 represents mid-gestation, about 5 days before strong chorion interaction between the entire surface of the chorion and the uterus; days 57–58, 1–2 days after chorion attachment, and 2–3 days before the onset of pubic symphysis relaxation; days +1–+6, 1–6 days after the onset of pubic symphysis relaxation, i.e. within 1 week of parturition. The high metabolic activity of chorion exceeded that by amnion at all stages. Metabolism by endometrium and myometrium was always low. Conversion of progesterone by amnion significantly decreased ($P<0.05$) between days 57–58 and days +1–+6. Progesterone metabolites produced by chorion and amnion were identified by TLC, HPLC, and capillary GC/MS. Both tissues converted progesterone to three major products during 60-min incubations. These were 5α-pregnane-3,20-dione, 3α-hydroxy-5α-pregnan-20-one, and 3β-hydroxy-5α-pregnan-20-one. The metabolite pattern differed between the two tissues. Three-minute incubations with chorion resulted in a significantly higher proportion of 3α-hydroxy-4-pregnen-20-one ($P<0.01$) and 5α-pregnane-3,20-dione ($P<0.025$) than at 60 min. The production of 3β-hydroxy-5α-pregnan-20-one by chorion decreased ($P<0.05$) between days 50–51 and 57–58. The ratio of 3α-hydroxy-5α-pregnan-20-one to 3β-hydroxy-5α-pregnan-20-one increased ($P<0.05$) between days 45 and post-relaxation. The marked conversion of progesterone by chorion, or the formation of one or more of its metabolites, may serve to influence uterine function prior to delivery.

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INTRODUCTION

It is generally accepted that parturition in non-primates is preceded by a fall in maternal blood progesterone. Although true for the ewe [1], rat [2], and others [3], this has not been consistently observed in the guinea pig [4,5]. In humans, plasma progesterone levels have been shown to increase throughout pregnancy [6]. It has been reported that progesterone does not suppress myometrial contractility in the guinea pig [7], even though nuclear receptor binding decreases approx. 1 week pre-partum, about the time of pubic symphysis relaxation [8]. This fall in progesterone nuclear receptor binding, without a concurrent fall in estrogen nuclear receptor binding, may reflect a local increase in the activity of estrogen relative to that of progesterone. This situation is known to lead to elevated collagenase activity [9] which precedes cervical dilation. The possible alteration in the pattern of steroid hormone activity could be due to local withdrawal of progesterone, perhaps through its metabolism by intrauterine enzymes. For this reason we have investigated the degree of conversion of progesterone by guinea pig amnion, chorion, endometrium, and myometrium. This study has led to the identification of several progesterone metabolites and to speculation that these might, by their own actions, contribute to the processes leading up to parturition.

MATERIALS AND METHODS

Animals and tissue preparation

Guinea pigs of the English shorthair variety were bred and housed in our animal quarters before being killed by CO_2 asphyxiation, as approved by the Canadian Council for Animal Care. Gestation was monitored as described previously [10] with day 0 being that on which a vaginal plug was found or when the previous delivery occurred.

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Abbreviations: 3α-OH-5αP-20-one, 3α-hydroxy-5α-pregnan-20-one;; 3β-OH-5αP-20-one, 3β-hydroxy-5α-pregnan-20-one;; 20α-OH-4P-3-one, 20α-hydroxy-4-pregnen-3-one;; 3α-OH-4P-20-one, 3α-hydroxy-4-pregnen-20-one;; 5αP-3,20-dione, 5α-pregnane-3,20-dione.

Day +1 indicates the day of detectable pubic symphysis relaxation, normally 6–7 days (+6–+7) prior to delivery [8]. Tissues were rapidly removed and rinsed with cold saline. All subsequent preparatory steps were carried out at 0–4°C including the separation of endometrium from myometrium and the blotting of tissues on filter paper before weighing.

Chemicals and biochemicals

[1,2,6,7-^{3}H]Progesterone (109 Ci/mmol), [1,2-^{3}H]progesterone (53.0 Ci/mmol), and [4-^{14}C]progesterone (57.2 mCi/mmol) were purchased from New England Nuclear (Dorval, Quebec). These were purified on columns (16×1 cm) of Sephadex LH 20 (Pharmacia Inc., Baie d'Urfe, Quebec) with heptane–chloroform–ethanol (10:10:1, by vol). Unlabelled steroids were purchased from Steraloids (Wilton, NH) or Sigma Chemical Co. (St Louis, MO). 3α-Hydroxy-4-pregnen-20-one (3α-OH-4P-20-one) was prepared as described previously [11]. Other chemicals and solvents were of appropriate analytic grade and were purchased from BDH Inc. (Toronto, Ontario) or Fisher Scientific Ltd (Toronto, Ontario). *N*-methyl-bis-trifluoroacetamide (MBTFA) was obtained from Pierce (Rockford, IL).

Incubations

Pieces of whole amnion, myometrium, chorion, and endometrium (each 0.5 g wet wt) from various stages of gestation [8], were incubated for 3 or 60 min at 37°C, with gentle shaking, in 2 ml of Krebs Ringer phosphate buffer, pH 7.4, with progesterone as substrate. Initial experiments used a mixture of 2 nM unlabelled and 2 nM [1,2,6,7-^{3}H]progesterone. Later incubations utilized either 2.5 μM [4-^{14}C]progesterone or a mixture of 2.5 μM unlabelled progesterone with 0.5 μCi [1,2-^{3}H]progesterone. Duplicate incubations were performed whenever possible. Blanks were prepared by boiling tissues for 10 min in saline and incubating as above. Tissues were removed after incubation and extracted for 1 week with 5 ml methanol at −20°C. Methanolic extracts were evaporated under N_2 at 40°C and partitioned between diethyl ether and H_2O. Incubation media were extracted with ether. All ether extracts were evaporated under N_2, dissolved in methanol, and stored at −20°C.

TLC

In experiments with 4 nM progesterone, duplicate aliquots of steroid extracts, containing approx. 0.023 μCi [^{3}H] and 5 μg of unlabelled progesterone, were spotted on aluminum-backed 20×20 cm Whatman UV_{254} silica gel plates (250 μm) previously activated at 100°C for 20 min. These were run twice one-dimensionally in chloroform–ether (10:3, v/v; solvent system I) [12]. The progesterone-containing spots were visualized under UV light, scraped into counting vials, and quantified by liquid scintillation spectrometry. Recovered [^{3}H]progesterone was subtracted from the total applied to calculate the percent conversion to products. Each determination was corrected for procedural losses by running replicate [^{3}H]progesterone standards.

Aliquots of steroid extracts from the 2.5 μM substrate incubations, containing approx. 0.1 μCi and unlabelled steroids (see below), were spotted on 20×20 cm Fisherbrand glass-backed UV_{254} silica gel plates (250 μm). These were run twice in solvent system I, turned through 90°, and run two more times in hexane–ethyl acetate (5:2, v/v; solvent system II) as described previously [12]. Unlabelled carrier steroids included the following: 5α-pregnane-3,20-dione (5αP-3,20-dione, 50 μg), progesterone (10 μg), 3α-hydroxy-5α-pregnan-20-one (3α-OH-5αP-20-one, 50 μg), 3β-hydroxy-5α-pregnan-20-one (3β-OH-5αP-20-one, 50 μg), 20α-hydroxy-4-pregnen-3-one (20α-OH-4P-3-one, 10 μg), and 3α-OH-4P-20-one (50 μg). These steroid-containing spots were visualized by UV light or iodine vapour, and treated as described above. Selected samples were run in duplicate.

Autoradiography

In [^{14}C]progesterone incubations, tissue lipids in ether extracts were precipitated prior to TLC with methanol–H_2O (9:1, v/v) for 12 h at −20°C. Losses amounted to between 0.5 and 3% of total ^{14}C label. To remove additional lipid, extracts were run twice on silica gel plates of dimensions 3×7 cm (prepared in the laboratory) in solvent system II. These were scraped between the origin and the solvent front; the steroids were eluted from the silica with ether. Losses in this second step varied between 5 and 10% of total ^{14}C label. Entire ^{14}C ether extracts, purified as just described, were then spotted and run in the two-dimensional system described above without added carrier steroids. TLC plates were apposed to sheets of Kodak Medical X-Ray film (X OMAT R film) for 7-days before developing. Each radioactive zone was scraped and extracted with ether to isolate individual steroids.

HPLC

A Beckman Model 332 gradient liquid chromatograph with Model 155 variable wavelength detector and Model 171 radioisotope detector was used with a C18 column (Beckman, Ultrasphere ODS, 5 μm, 4.6×250 mm) and guard column (Ultrasphere ODS, 5 μm, 4.6×45 mm) in the solvent system methanol–H_2O (3:1, v/v) at a flow rate of 1 ml/min. Aliquots of major ^{14}C metabolites, collected as described above, were injected with appropriate unlabelled standards for identification.

GC/MS analysis

Selected ^{14}C metabolites without unlabelled carrier were injected onto a Hewlett–Packard GC-Mass Spectrometer (Model 5970A with 5790A GC and CHEMPC for DOS) with a 12.5 m (0.2 mm) cross-linked dimethyl silicone (HP Ultra-1) or a 30 m

(0.25 mm) cross-linked 5% phenol methyl silicone (HP-5MS) capillary column under conditions described previously [12].

Statistical analysis

Statistical evaluation of differences between groups was performed by analysing with Scheffe's multiple comparison test after one-way analysis of variance (ANOVA). The unpaired Student's *t*-test was used to evaluate differences between two sample means, and a one-tailed Student's *t*-test was used to make inferences about individual sample means.

RESULTS

Methodological analysis

Total recovery of radioactivity in the final ether extracts was 94.5±0.8% (SEM, n=89) for all incubations. Generally, residual tissues retained 1–2% of label after methanolic extraction. The media and tissue aqueous phases contained 1–2 and 1–5% of label, respectively. Further losses did occur during purification of extracts in the ^{14}C experiments (see Autoradiography section, above).

Recovery of [^{3}H]progesterone standard from one-dimensional TLC plates was 94±1%, (SEM, n=55). Total recovery from two-dimensional TLC plates was 91±1%, (SEM, n=73); no correction was made for recovery of individual metabolites from these. The coefficient of variation between duplicate determinations from two-dimensional TLC plates ranged from a low of 0.9 for progesterone, to a high of 8.5 for 3α-OH-4P-20-one (n=7).

4nM Incubations

Myometrium and endometrium were significantly different (P<0.01) from amnion and chorion in uptake of 4 nM progesterone. After incubation for 60 min, 27.3±7.7% of total ^{3}H was present in myometrium; 19.1±7.3% in endometrium; 48.1±5.8% in chorion; and 47.6±9.4% in amnion (n=7 for each tissue).

As shown in Fig. 1, progesterone metabolism by chorion far exceeded that found for amnion or uterus. Chorion converted almost all 8 pmol of progesterone substrate to products within 60 min. A day 58 and 60 chorion each incubated for 2 min showed 82 and 77% metabolism, respectively (not shown). This high activity led to further examination of chorion at higher substrate concentrations. Conversion of 4 nM progesterone by amnion significantly decreased (P<0.05) between days 57–58 of gestation and those following pubic symphysis relaxation. Metabolism was low for both myometrium and endometrium, although the amount detected for endometrium always exceeded that for myometrium.

Identification of ^{14}C metabolites by GC/MS and HPLC

In order to identify metabolites, tissues were incubated with 2.5 μM [^{14}C]progesterone. Typical autoradiographs of two-dimensional TLC plates for chorion, amnion, and a chorion-boiled blank are shown in Fig. 2. Metabolites are referred to by number. Autoradiographs for myometrium and endometrium did not show the presence of metabolites. Table 1 summarizes TLC, HPLC, and GC/MS information.

Metabolite No. 1. The relative mobilities on TLC (R_f values) in solvent systems I and II coincided with authentic 5αP-3,20-dione. Analysis by HPLC resulted in elution of radioactivity (24.35 min) with the standard (24.4 min) as shown in Fig. 3(A). Capillary GC retention time (R_t) of the concentrated HPLC peak (27.34 min) coincided with authentic 5αP-3,20-dione (27.36 min). Mass spectral analyses showed 99% similarity to the spectrum of authentic 5αP-3,20-dione, and the major identifying ions (m/z=231, 272, 298) and molecular mass (m/z=316) showed m/z +2 (233, 274, 300, and 318), indicating the presence of ^{14}C label (Table 1). Based on these criteria, metabolite 1 was identified as 5αP-3,20-dione.

Metabolite No. 2. The TLC, HPLC, and GC/MS data confirmed that this was unmetabolized [^{14}C]progesterone.

Metabolite No. 3. The TLC R_f values in systems I and II coincided with authentic 3α-OH-5αP-20-one (Table 1). Analysis by HPLC showed elution of radioactivity (34.55 min) with the standard (34.4 min) [Fig. 3(B)]. Capillary GC R_t of the concentrated HPLC peak (14.11 min; Table 1) coincided with authentic 3α-OH-5αP-20-one (14.1 min). Mass spectral analyses

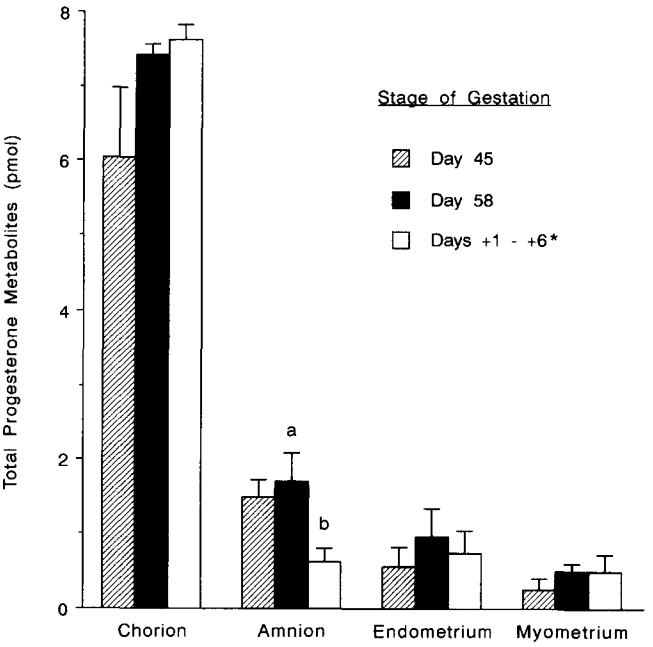

Fig. 1. Effect of stage of gestation on conversion of 8 pmol progesterone by whole chorion, amnion, myometrium, and endometrium (each 0.5 g) during 60 min incubation. Values represent the mean±1 SEM, n=3–5. Different letters denote significant (P<0.05) differences. * Refers to days following first detection of pubic symphysis relaxation.

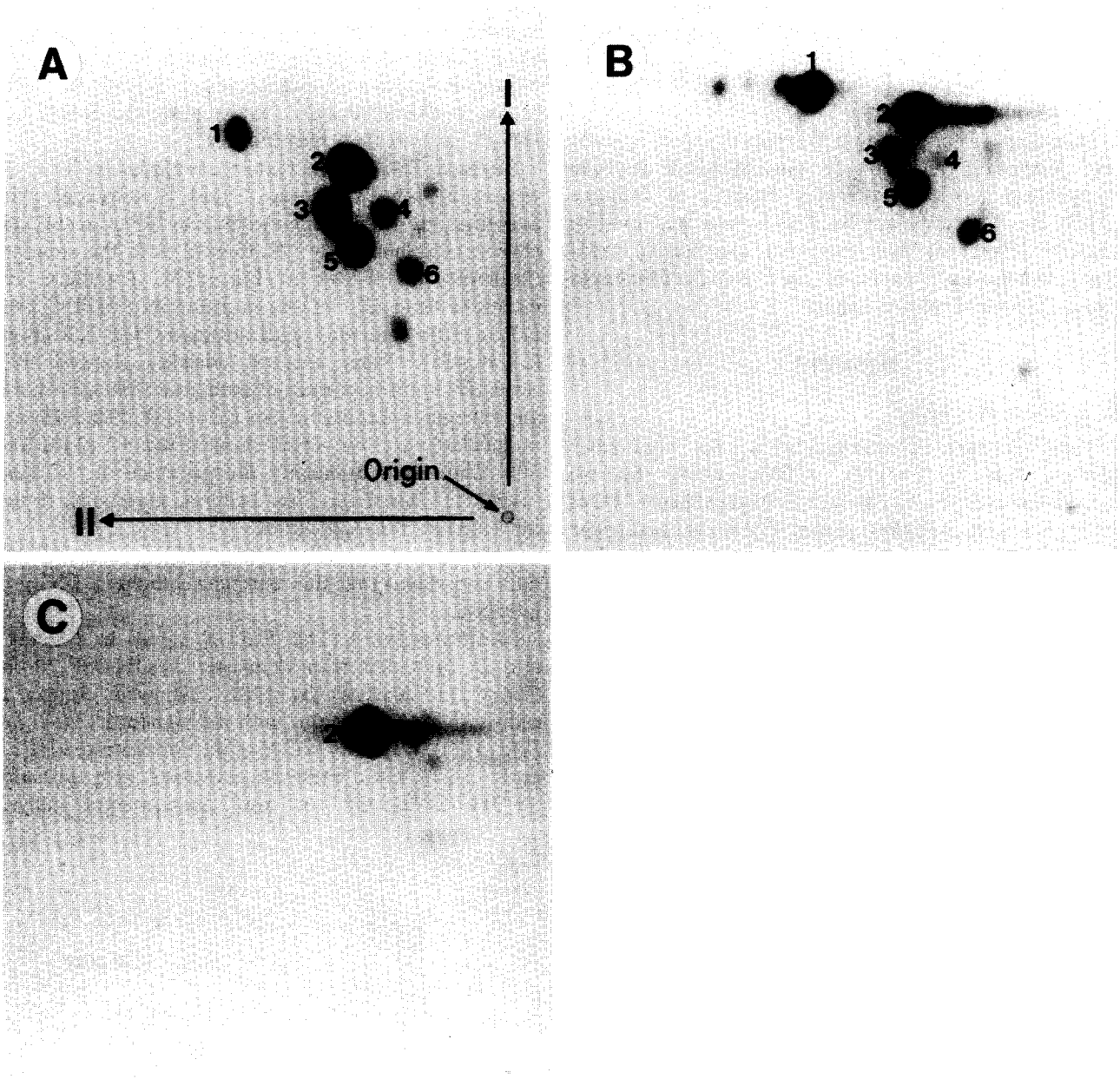

Fig. 2. Autoradiographs of TLC plates showing the products formed by incubating day 57 whole chorion (A), day 50 amnion (B), and day 57 boiled chorion (C) with 2.5 μM [^{14}C]progesterone for 60 min. The radioactive spots are identified and referred to by the numbers shown (see text).

showed 99% similarity to the spectrum of authentic 3α-OH-5αP-20-one and the major identifying ions (m/z=215, 300) and molecular mass (m/z=318) showed m/z +2 (217, 302, and 320), indicating the presence of ^{14}C label (Table 1). Based on these criteria, it was concluded that metabolite No. 3 was 3α-OH-5αP-20-one.

Metabolite No. 4. The TLC R_f values in systems I and II coincided with authentic 3α-OH-4P-20-one. Analysis by HPLC showed elution of radioactivity (23.7 min) with the standard (23.6 min) [Fig. 3(C)]. Capillary GC R_t of the concentrated HPLC peak (13.21 min; Table 1) coincided with authentic 3α-OH-4P-20-one (13.2 min). Mass spectral analyses showed 99% similarity to the spectrum of authentic 3α-OH-4P-20-one, and the identifying ions (m/z=213, 255, and 298) and molecular mass (m/z=316) showed m/z +2 (215, 257, 300, and 318), indicating the presence of ^{14}C label (Table 1). Based on these criteria, it was concluded that metabolite No. 4 was 3α-OH-4P-20-one.

Metabolite No. 5. The TLC R_f values in systems I and II coincided with 3β-OH-5αP-20-one (Table 1). Analysis by HPLC showed elution of radioactivity (27.9 min) with the standard (27.7 min) [Fig. 3(D)]. Capillary GC R_t of the concentrated HPLC peak derivatized with MBTFA (12.51 min; Table 1) coincided

Table 1. Identification of [^{14}C]progesterone metabolites by TLC and GC/MS [a]

	TLC[b]		GC peak[c]		
	R_{P1}	R_{P2}	(R_t)	*m/z* of main ions[d]	Molecular Mass[e]
5α-P-3,20-dione	108	158	27.36	231, 272, 298	316
Spot No.1 [f]	108	158	27.34	233, 274, 300	318
3α-OH-5αP-20-one	88	114	14.1	215, 300	318
Spot No.3	88	114	14.11	217, 302	320
3α-OH-4P-20-one	88	82	13.2	213, 255, 298	316
Spot No.4	88	82	13.21	215, 257, 300	318
3β-OH-5αP-20-one	79	169	12.51	215, 300, 329, 396	414[g]
Spot No.5	79	169	12.58	217, 302, 331, 398	416[g]
20α-OH-4P-3-one	69	64	19.19	230, 298	316
Spot No.6	69	64	19.15	232, 300	318

[a] Radioactive areas on the TLC plates were extracted and run on HPLC; each radioactive fraction (Fig. 3) was collected, concentrated and then run on the GC/MS.
[b] Mobilities of substances in TLC systems I and II in relation to the mobility of progesterone (×100).
[c] Retention time (R_t), in minutes, of substances in the capillary GC.
[d] *m/z* values of some of the main identifying ions determined by mass spectrometry.
[e] The molecular mass (rounded to the nearest mass unit) as determined by mass spectrometry.
[f] Spot No. refers to radioactive area on the autoradiographs (Fig. 2).
[g] MBFTA derivative.

with authentic 3β-OH-5αP-20-one derivatized with MBTFA (12.58 min). Mass spectral analyses of the derivative of No. 5 showed 98% similarity to the spectrum of authentic 3β-OH-5αP-20-one-TFA. Several major ions (*m/z*=215, 300, 329, and 396) and molecular mass (*m/z*=414) showed *m/z* +2 (217, 302, 331, 398, and 416), indicating the presence of ^{14}C label (Table 1). Based on these criteria, it was concluded that metabolite No. 5 was 3β-OH-5αP-20-one.

Metabolite No. 6. The TLC R_f values in systems I and II coincided with authentic 20α-OH-4P-3-one (Table 1). Analysis by HPLC showed elution of radioactivity (15.96 min) with the standard (15.9 min) [Fig. 3(E)]. Capillary GC R_t of the concentrated HPLC peak (19.15 min; Table 1) coincided with authentic 20α-OH-4P-3-

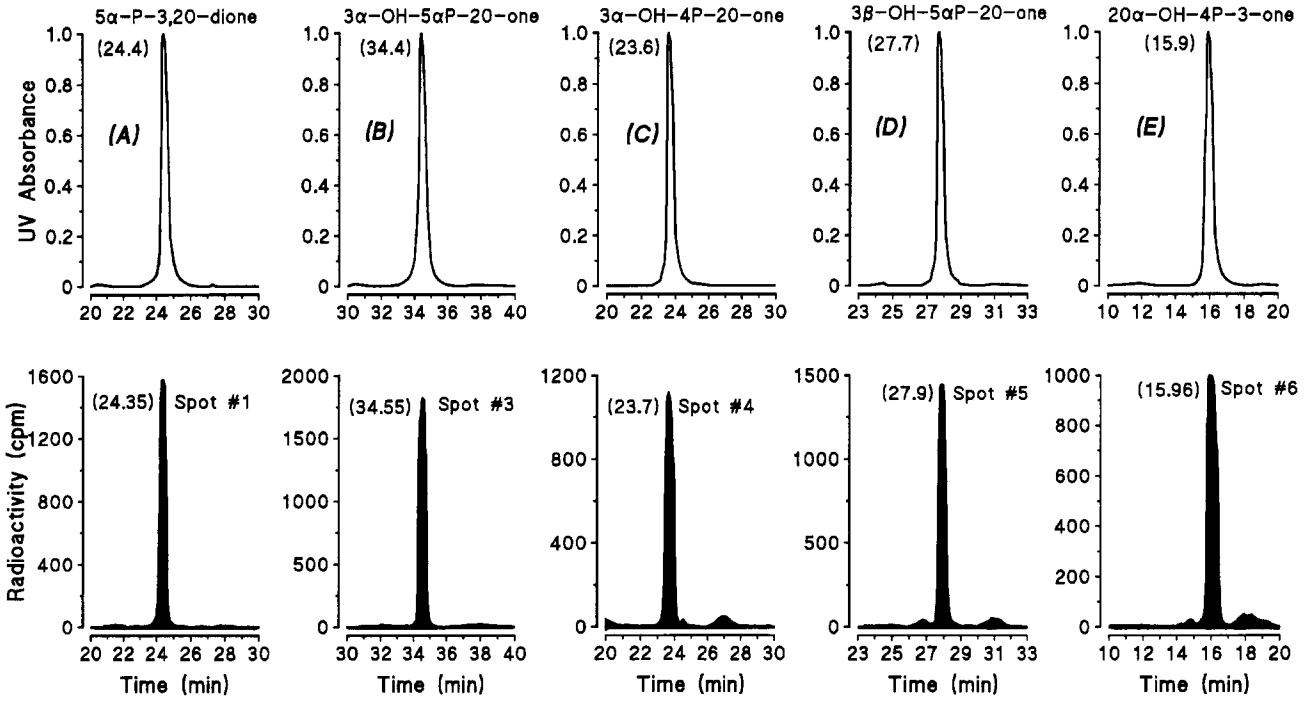

Fig. 3. Profiles of UV absorbance of authentic steroid standards (top panels) and radioactivity peaks (lower panels) of eluted TLC spots. Numbers in parentheses show retention times of peaks in minutes. Results are representative of 2–5 separate runs for each metabolite and corresponding standard.

Table 2. Relative (%) and absolute (nmol) levels of metabolites produced by whole chorion after 3 or 60 min incubation with 5 nmol progesterone[a]

Metabolite	% of total metabolites[b] (nmol metabolite×10)	
	3 min	60 min
5αP-3,20-dione	15±2 (1.1±0.2)	9±2 (1.9±0.2)
3α-OH-5αP-20-one	54±3 (5.1±2.4)	62±3 (16±5)
3β-OH-5αP-20-one	23±2 (2.0±0.9)	26±3 (7±3)
3α-OH-4P-20-one	7±2 (0.42±0.07)	1.8±0.4 (0.36±0.01)
20α-OH-4P-3-one	0.3±0.2 (0.04±0.02)	1.3±0.5 (0.24±0.04)

[a] Either [^{14}C]progesterone, or unlabelled progesterone with 0.5 μCi [^{3}H]progesterone.
[b] Values are mean % of total metabolites (or mean nmol×10) ± SEM produced by chorion from 4 guinea pigs between days 45 and 57 of gestation.

one (19.19 min). Mass spectral analyses showed 98% similarity to the spectrum of authentic 20α-OH-4P-3-one, and identifying ions (*m/z*=230 and 298) and molecular mass (*m/z*=316) showed *m/z* +2 (232, 300, and 318), indicating the presence of ^{14}C label (Table 1). Based on these criteria, it was concluded that metabolite No. 6 was 20α-OH-4P-3-one.

2.5 μM Incubations. The differences in relative and absolute quantities of individual metabolites produced by chorion after 3 or 60 min incubation are summarized in Table 2. The data show that, except for 3α-OH-4P-20-one ($P<0.05$), the pattern of metabolites was similar at these two times. In one experiment with day 57 chorion, 3α-OH-4P-20-one accounted for 28% of the metabolites after a 2 min incubation (not shown). All metabolites, except for 3α-OH-4P-20-one, showed increases ($P<0.05$) in amounts between 3 and 60 min. Considerable variation in the amount of each metabolite produced by chorion existed between the four animals depending on the animal and its stage of gestation.

When the data for Table 2 are calculated differently (not shown in tabular form), the high turnover of 5αP-3,20-dione and 3α-OH-4P-20-one as compared to other metabolites is even more evident. For these calculations, the ratio of % of total metabolites at 3 min to the % at 60 min was carried out for each individual animal prior to averaging. A ratio of unity indicated no difference between short and long incubation times. Only the ratios for 5αP-3,20-dione (1.86±0.25) and 3α-OH-4P-20-one (3.97±0.58) were significantly ($P<0.025$) different from unity.

Table 3 summarizes the differences in relative and absolute quantities of products formed by chorion and amnion after 60 min incubation. Amnion and chorion each converted progesterone to three major products: 5αP-3,20-dione, 3β-OH-5αP-20-one, and 3α-OH-5αP-20-one. Chorion significantly differed from amnion in the relative percent of 5αP-3,20-dione ($P<0.005$), 3α-OH-5αP-20-one ($P<0.005$), 3β-OH-5αP-20-one ($P<0.05$), and 20α-OH-4P-3-one ($P<0.01$). In the four animals studied, 3β-OH-5αP-20-one and 3α-OH-5αP-20-one accounted for 87% of all metabolites produced by chorion; 5αP-3,20-dione and 3β-OH-5αP-20-one for 82% of all metabolites by amnion. Furthermore, chorion converted approx. 2.5 times more progesterone to product than did amnion. As a result, both chorion and amnion showed similar accumulations of 5αP-3,20-dione and 3β-OH-5αP-20-one after the incubation; however, chorion produced 13–38 times more 3β-OH-5αP-20-one than did amnion. Both chorion and amnion accumulated low amounts of 3α-OH-4P-20-one, although the amount produced by chorion exceeded that by amnion ($P<0.01$).

Figure 4 shows the amounts of 3α-OH-5αP-20-one and 3β-OH-5αP-20-one produced by chorion from different stages of gestation after incubation with 5 nmol progesterone for 60 min. These two products accounted for between 80 and 98% of all metabolites between mid

Table 3. Relative (%) and absolute (nmol) quantities of metabolites produced by incubating 5 nmol progesterone[a] *with whole chorion or amnion for 60 min*

Metabolite	% of total metabolites[b] (nmol metabolite×10)	
	Amnion	Chorion
5αP-3,20-dione	46±7 (3.3±0.8)	9±2 (1.7±0.3)
3α-OH-5αP-20-one	9±1 (0.6±0.1)	65±4 (13±3)
3β-OH-5αP-20-one	36±6 (3±1)	22±2 (4.3±0.8)
3α-OH-4P-20-one	2±1 (0.09±0.04)	2.3±0.4 (0.44±0.08)
20α-OH-4P-3-one	7±2 (0.45±0.08)	1.6±0.4 (0.27±0.04)

[a] Either [^{14}C]progesterone, or unlabelled progesterone with 0.5 μCi [^{3}H]progesterone.
[b] Values are mean % of total metabolites (or mean nmol×10) ± SEM determined with chorion and amnion from 4 guinea pigs between days 45 and 59 of gestation.

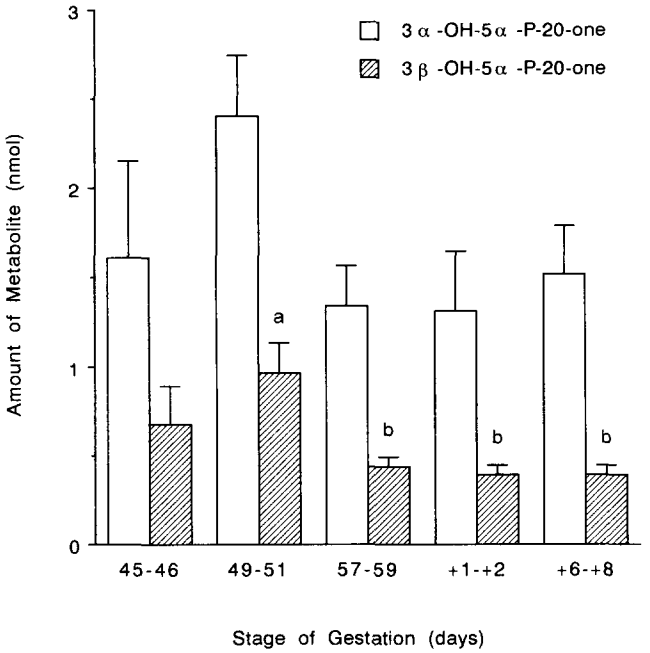

Fig. 4. Effect of stage of gestation on conversion of 5 nmol progesterone to 3α-OH-5αP-20-one and 3β-OH-5αP-20-one by chorion (0.5 g) after 60 min incubation. Values represent the mean ± 1 SEM, n=4–6. Different letters denote significant (P<0.05) differences.

and late gestation. The data show that the quantity of 3β-OH-5αP-20-one produced at days 49–51 of gestation differed significantly (P<0.05) from that produced at days 57–59. A similar pattern is apparent for 3α-OH-5αP-20-one, although no statistical differences were detected. When the data for Fig. 4 are recalculated in terms of the ratio of the 3α- to 3β-isomer (before averaging), the ratio at days 45–46 (2.28 ± 0.15, SEM, n=4) increased significantly (P<0.05) after pubic symphysis relaxation (days +1–+8) (3.61 ± 0.26, SEM, n=10). One day 50 chorion, showing very low conversion, was not included in the data for Fig. 4. An additional incubation with a day 55 chorion produced 2.85 nmol of 3α-OH-5αP-20-one and 0.94 nmol of 3β-OH-5αP-20-one. This animal was omitted from Fig. 4 because it did not match the time frames under investigation. 3α-OH-4P-20-one, 5αP-3,20-dione, and 20α-OH-4P-3-one were present in variable and low amounts with no particular pattern being evident between mid and late gestation. The most abundant of these, 5α-P-3,20-dione, accounted for 3–10% of the total metabolites produced by chorion.

DISCUSSION

It is clear from our study that guinea pig foetal membranes, particularly chorion, are capable of the *in vitro* conversion of progesterone to a number of metabolites. The degree to which this tissue metabolized 4nM progesterone was so great, even at 2 or 3 min of incubation, that it was not possible to compare quantitatively its activity either with that of the other intrauterine tissues or with itself at different stages of gestation. Attempts to employ extremely small chorion pieces led to difficulty with the replication of experiments. For this reason we ultimately utilized 500 mg pieces of tissue along with a considerably higher steroid concentration (2.5 μM) in order to achieve an upper limit of about 60% conversion. This upper limit was exceeded in 5 of the 11 animals studied between days 45 and 55 of gestation; nevertheless, a substrate concentration of 2.5 μM enabled comparison of chorion activity at different times of pregnancy.

The greater conversion of progesterone by chorion than by amnion and the decrease in progesterone metabolism by amnion preceding parturition appears to be similar to that reported for the human [13]. Unlike the situation in the human [13], in the guinea pig the pattern of metabolites produced by these two membranes differed, mainly with respect to the predominance of 3α-OH-5αP-20-one in chorion and 3β-OH-5αP-20-one in amnion. Chorion production of 3β-OH-5αP-20-one was significantly higher at 50 days than at later times of gestation. Although the formation of 3α-OH-5αP-20-one by chorion appeared to decrease near parturition, the ratio of the 3α- to the 3β-isomer increased significantly between mid (day 45) and late gestation (days +1 to +8). The possible importance of this finding in relation to an alternative synthetic pathway for 3α-OH-5αP-20-one is discussed later.

Most recent studies, particularly associated with the central nervous system, have drawn attention to the production of 3α-OH-5αP-20-one from progesterone via the intermediate 5αP-3,20-dione [14]. This may also hold true in guinea pig chorion since 5αP-3,20-dione accounted for a higher percentage of metabolites after 3 min as compared to 60 min incubation. An additional pathway through the allylic steroid 3α-OH-4P-20-one has been described for rat granulosa cells [15] and porcine ovaries [16]. It might be expected, on the basis of its structure, that this steroid would exhibit a considerable rate of turnover. Our data support this since the relative contribution of this compound to the metabolite pattern in guinea pig chorion was even more striking than that of 5αP-3,20-dione at short incubation times. Although our extraction conditions were not necessarily optimal for the isolation of 3α-OH-4P-20-one [17], this alternative pathway may account for the observed increase in the 3α/3β isomer ratio between mid and late gestation. The possible importance, if any, of the two pathways for the formation of 3α-OH-5αP-20-one by guinea pig chorion is unclear at present.

It is interesting to note that the metabolites formed by guinea pig chorion and amnion included virtually no water-soluble forms. This is in strong contrast to the situation where the estrogen molecule is concerned. Estrone and estradiol are subjected to marked sulphation in the chorion by a highly specific estrogen sulphotransferase. The sulphated forms are subject to hydrolysis by

a sulphatase. Both sulphotransferase and sulphatase activities are highly dependent upon the stage of gestation and it has been speculated that these provide a means of regulating active estrogen availability in the intrauterine compartment [18][19]. There appears to be no such mechanism for the progesterone metabolites.

Neither guinea pig endometrium nor myometrium exhibited appreciable *in vitro* conversion of progesterone. This is in contrast to the results of studies with human and rat uteri. In rat, 5αP-3,20-dione and 3α-OH-5αP-20-one were the major products [20–22]. Of the products identified, human uterus formed mainly 20α-reduced compounds [23,24], and the pattern of metabolites was claimed to change with the phase of the menstrual cycle [25]. An overall increase in progesterone metabolism toward term in rat uterus [21] may provide a means of withdrawing progesterone from its sites of action. The guinea pig uterus possesses little ability to perform this function. The chorion, however, which becomes increasingly interactive over its surface with the uterine wall between days 50 and 55 of gestation, possesses marked ability to metabolize progesterone. It may be that this latter activity serves to influence the amount of progesterone available to the uterus. The contributing effects of extremely high levels of guinea pig progesterone binding protein [4] on intrauterine progesterone availability is unknown. The metabolites of progesterone, notably 3α-OH-5αP-20-one and 3α-OH-4P-20-one, could possibly influence uterine function prior to delivery. Steroids of this type are known to affect chloride ion channels in brain cells, through their interaction with the $GABA_A$ receptor [26–28], and to possess anaesthetic [29] and anxiolytic [30,31] properties. Recently 3α-OH-4P-20-one has been shown to affect cytosolic Ca^{2+} concentrations by interaction at the level of the Ca^{2+} channels and intracellular Ca^{2+} mobilization [32,33]. Such actions on ion flux are well known to regulate neural and muscular activity and may be involved in regulating the electrical communication between uterine muscle cells at term [34]. It remains to be established whether the progesterone metabolites produced by the guinea pig foetal membranes exert these functions in the uterus.

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REFERENCES

1. Liggins G. C., Fairclough R. J., Grieves S. A., Kendall J. Z. and Knox B. S.: The mechanism of initiation of purturition in the ewe. *Recent Prog. Horm. Res.* **29** (1974) 111–159.
2. Bartholomeusz R. K., Bruce N. W., Martin C. E. and Hartmann P. E.: Serial measurement of arterial plasma progesterone levels throughout gestation and parturition in individual rats. *Acta Endocr. (Copenh.)* **82** (1976) 436–443.
3. Pasqualini J. R. and Kincl F. A.: *Hormones and the Fetus.* Vol. I. Pergamon Press, New York (1985).
4. Illingworth D. V., Challis J. R. G., Ackland N., Burton A. M., Heap R. B. and Perry J. S.: Parturition in the guinea pig; plasma levels of steroid hormones, steroid-binding proteins, and oxytocin, and the effect of corticosteroids, prostaglandins and adrenocorticotrophin. *J. Endocr.* **63** (1974) 557–570.
5. Heap R. B. and Deanesly R.: Progesterone in systemic blood and placentae of intact and ovariectomized pregnant guinea-pigs. *Endocrinology* **34** (1966) 417–423.
6. Milewich L., Gomez-Sanchez C., Madden J. D. and MacDonald P. C.: Isolation and characterization of 5α-pregnane-3,20-dione and progesterone in peripheral blood of pregnant women. Measurement throughout pregnancy. *Gynec. Invest.* **6** (1975) 291–306.
7. Porter D. G.: The failure of progesterone to affect myometrial activity in the guinea pig. *J. Endocr.* **46** (1970) 425–434.
8. Glasier M. A. and Hobkirk R.: Nuclear receptors for progesterone and estradiol in the guinea pig uterine compartment during gestation. *Steroids* **58** (1993) 478–483.
9. Rajabi M. R., Dodge G. R., Solomon S. and Poole R. A.: Immunochemical and immunohistochemical evidence of estrogen-mediated collagenolysis as a mechanism of cervical dilatation in the guinea pig at parturition. *Endocrinology* **128** (1991) 371–378.
10. Hobkirk R. and Cardy C.: UDPGA-dependent estrogen glucuronyltransferase of guinea pig uterus: assay, temporal relationships in pregnancy and some characteristics. *J. Steroid Biochem.* **13** (1980) 1039–1045.
11. Wiebe J. P., Deline C., Buckingham K. D., Dave V. and Stothers J. B.: Synthesis of the allylic gonadal steroids, 3α-hydroxy-4-pregnen-20-one and 3α-hydroxy-4-androsten-17-one, and of 3α-hydroxy-5α-pregnan-20-one. *Steroids* **45** (1985) 39–51.
12. Wiebe J. P., Buckingham K. D., Zobell R. L. and Hertelendy F.: Metabolism of progesterone by avian granulosa cells in culture. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 113–120.
13. Milewich L., Gant N. F., Schwarz B. E., Chen G. T. and MacDonald P. C.: Initiation of human parturition VIII. Metabolism of progesterone by fetal membranes of early and late human gestation. *Obstet. Gynec.* **50** (1977) 45–48.
14. Robel P. and Baulieu E-E.: Neurosteroids: biosynthesis and function. *Trends Endocr. Metab.* **5** (1994) 1–8.
15. Wiebe J. P., de Gannes G. C. and Dallaire M. J.: Synthesis of the allylic regulatory steroid, 3α-hydroxy-4-pregnen-20-one, by rat granulosa cells and its regulation by gonadotropins. *Biol. Reprod.* **50** (1994) 956–964.
16. de Gannes G. C. and Wiebe J. P.: Synthesis of the neurosteroid, 3α-hydroxy-4-pregnen-20-one (3α-HP), by porcine granulosa and theca cells and regulation by gonadotropins. *Biol. Reprod.* (Suppl. 1) **46** (1992) No. 424 (Abstr.).
17. Wiebe J. P.: Identification of a unique Sertoli cell steroid as 3α-hydroxy-4-pregnen-20-one (3α-dihydroprogesterone:3α-DHP). *Steroids* **39** (1982) 259–278.
18. Bartlett N. R. and Hobkirk R.: Estrogen sulfotransferase and 17β-hydroxysteroid dehydrogenase activities in guinea-pig chorion through gestation. *J. Steroid Biochem. Molec. Biol.* **38** (1991) 241–247.
19. Glutek S. M. and Hobkirk R.: Estrogen sulfatase and steroid sulfatase activities in intrauterine tissues of the pregnant guinea pig. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 707–719.
20. Wiest W. G.: *In vitro* metabolism of progesterone and 20α-hydroxypregn-4-en-3-one by tissues of the female rat. *Endocrinology* **73** (1963) 310–316.
21. Howard P. D. and Wiest W. G.: Progesterone metabolism by uterine tissue of pregnant rats. *Steroids* **19** (1972) 35–45.
22. Wichmann K.: On the metabolism and subcellular distribution of progesterone in the myometrium of the pregnant rat. *Acta Endocr. (Copenh.)* (Suppl.) (1967) 116.
23. Bryson M. J. and Sweat M. L.: Metabolism of progesterone in human proliferative endometrium. *Endocrinology* **81** (1967) 729–734.
24. Bryson M. J. and Sweat M. L.: Metabolism of progesterone in human myometrium. *Endocrinology* **84** (1969) 1071–1075.
25. Collins J. A. and Jewkes D. M.: Progesterone metabolism by proliferative and secretory human endometrium. *Am. J. Obstet. Gynec.* **118** (1974) 179–185.
26. Purdy R. H., Morrow A. L., Blinn J. R. and Paul S. M.: Synthesis, metabolism, and pharmacological activity of 3α-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. *J. Med. Chem.* **33** (1990) 1572–1581.
27. Majewska M. D.: Neurosteroids: endogenous bimodal modu-

lators of the $GABA_A$ receptor. Mechanism of action and physiological significance. *Prog. Neurobiol.* **38** (1992) 379–395.
28. Tauboll E., Ottersen, O. P. and Gjerstad L.: The progesterone metabolite 5α-pregnan-3α-ol-20-one reduces K^+-induced GABA and glutamate release from identified nerve terminals in rat hippocampus: a semiquantitative immunocytochemical study. *Brain Res.* **623** (1993) 329–333.
29. Wiebe J. P. and Kavaliers M.: Analgesic effects of the putative FSH-suppressing gonadal steroid, 3α-hydroxy-4-pregnen-20-one: possible modes of action. *Brain Res.* **461** (1988) 150–157.
30. Kavaliers M., Wiebe J. P. and Galea L. A. M.: Reduction of predator odor-induced anxiety in mice by the neurosteroid 3α-hydroxy-4-pregnen-20-one (3αHP). *Brain Res.* **645** (1994) 325-329.
31. Bitran D., Purdy R. H. and Kellogg C. K.: Anxiolytic effect of progesterone is associated with increases in cortical allopregnanolone and $GABA_A$ receptor function. *Pharmac. Biochem. Behav.* **45** (1993) 423–428.
32. Dhanvantari S. and Wiebe J. P.: Suppression of follicle-stimulating hormone by the gonadal- and neurosteroid 3α-hydroxy-4-pregnen-20-one involves actions at the level of the gonadotrope membrane/calcium channel. *Endocrinology* **134** (1994) 371–376.
33. Wiebe J. P., Dhanvantari S., Watson P. H. and Huang Y.: Suppression in gonadotropes of gonadotropin-releasing hormone-stimulated follicle-stimulating hormone release by the gonadal- and neurosteroid 3α-hydroxy-4-pregnen-20-one involves cytosolic calcium. *Endocrinology* **134** (1994) 377–382.
34. Garfield R. E., Sims S. and Daniel E. E.: Gap junctions: their presence and necessity in myometrium during parturition. *Science* **198** (1977) 958–959.