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Steroidogenic correlates of pregnancy in the rock hyrax (*Procavia capensis*)

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Abstract

In pregnant rock hyraxes isolated leucocytes metabolise both [^{3}H]pregnenolone and [^{3}H]progesterone while whole blood, erythrocytes and an erythrocyte/leucocyte mixture only metabolised [^{3}H]progesterone. Plasma displayed no tendency to metabolically convert any one of these two steroids. In whole blood [^{3}H]progesterone appears to be converted to 5α-pregnane-3,20-dione and a compound with chromatographic properties similar to that of 5α-pregnan-3α-ol-20-one. 5α-Pregnane-3,20-dione exhibited a high relative binding affinity for the uterine progesterone receptor (94%), but 5α-pregnan-3α-ol-20-one displayed very little affinity for the same receptor (0.4%). 5α-Pregnane-3,20-dione may therefore aid in the maintenance of pregnancy. Corpora lutea metabolised progesterone to 17α-hydroxyprogesterone, a compound exhibiting no progestational function because of its low relative binding affinity for the uterine progesterone receptor (2%). Progesterone appears to be the main product of the corpus luteum. However, 5α-pregnane-3,20-dione circulated at concentrations approximately 8.5 times higher than progesterone, probably due to the metabolic conversion of progesterone to 5α-pregnane-3,20-dione by the blood. We conclude that in the hyrax progesterone, produced by the corpora lutea, enters the circulation, where it is reduced to 5α-pregnanes. 5α-Pregane-3,20-dione may then be transported to the uterus where it binds to the progesterone receptor to assist in the maintenance of pregnancy. This mechanism appears to be analogous to that of the African elephant which is phylogenetically related to the hyrax, except that in the elephant the 5α-reduced metabolites are produced by luteal tissue and not the blood.

Keywords: Rock hyrax (*Procavia capensis*); African elephant (*Loxodonta africana*); Progesterone; Pregnenolone; Metabolism; 5α-Pregnane-3,20-dione; Maintenance of pregnancy

Introduction

Pregnancy in both the rock hyrax (*Procavia capensis*) and the phylogenetically related African elephant (*Loxodonta africana*) [1–5] are characterised by relatively low plasma concen-

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trations of progesterone [6–7]. Heap *et al.* [6] and Makawiti *et al.* [8] ascribed this phenomenon, as observed in the rock hyrax, to the metabolism of progesterone by erythrocytes. The low plasma concentrations of progesterone in the pregnant African elephant, however, result from the ability of the corpus luteum to predominantly convert pregnenolone to 5α-reduced progestins than progesterone [7]. 5α-Pregnane-3,20-dione (5α-DHP) down regulates the uterine progesterone receptor in the uterus of the African elephant [9], indicating that this metabolite of progesterone may play a significant role in the maintenance of pregnancy. Hodges *et al.* [7] suggested that 5α-DHP, rather than progesterone, should be considered an endocrinological marker of reproductive status in this species.

The metabolism of progesterone to other progestins by whole blood and erythrocytes has been recorded in a number of mammals [10–13]. For instance, humans leucocytes can apparently transform progesterone to other progestins, including 5α-DHP [14]. Close scrutiny of the descriptions of experimental procedures employed by Heap *et al.* [6] and Makawiti *et al.* [8] to study progesterone metabolism in the rock hyrax, however, revealed that the erythrocytes and leucocytes were not separated. The suggested metabolic conversion of progesterone by erythrocytes to 5α-pregnan-3,20-dione (5α-DHP) and in plasma to 5α-DHP (see [8]) may therefore be questioned, especially in view of the important role of luteal tissue in the formation of biologically active 5α-reduced progestins in the African elephant.

In the course of unravelling the mechanism of the processes involved in the maintenance of pregnancy in the rock hyrax, we investigated the metabolism of [^{3}H]pregnenolone (the immediate precursor of progesterone) and [^{3}H]progesterone by whole blood, plasma, isolated erythrocytes and leucocytes, respectively, a mixture of erythrocytes and leucocytes (packed cells), and the luteal tissue of pregnant rock hyraxes. We also assessed circulating levels of progesterone and 5α-DHP, and the binding of the various progestin metabolites to the uterine progesterone receptor of the rock hyrax. The significance of the formation of these metabolic products during pregnancy in the rock hyrax is discussed with reference to similar studies completed on the African elephant.

Materials and methods

Materials

Ketamine hydrochloride (100 mg/ml) and xylazine hydrochloride (100 mg/ml) were purchased from Kyron Labs (Pretoria, South Africa) and Bayer (Isando, South Africa), respectively, while Eutha-naze was obtained from Centaur Laboratories (Bryanston, South Africa). Heparin (600 iu/ml) was acquired from Novo Nordisk (Johannesburg, South Africa). Highveld Biological (Kelvin, South Africa) supplied Hank's balanced salt solution and Eagles Minimum Essential Medium, while Ficoll-Paque was purchased from Pharmacia Biotech (Upsala, Sweden). Phosphate buffered saline (PBS) contained 0.1% w/v gelatine (pH 6.9).

[7-^{3}H]Pregnenolone (specific activity: 25 Ci/mmol) and [1,2,6,7-^{3}H]progesterone (specific activity: 94 Ci/mmol) were supplied by New England Nuclear (Boston, Massachusetts) and Amersham International (Buckinghamshire, UK), respectively. All the steroid standards: 5α-DHP, 5β-DHP, 5α-pregnan-3α-ol,20-one (5α-PO) and 17α-hydroxyprogesterone (17α-OHP), as well as β-NADPH were obtained from Sigma (St Louis, USA). Triton X-100 was acquired from BDH Chemicals (Poole, UK).

Absolute ethanol and diethyl ether were purchased from UnivAR (Saarchem-Holpro Analytic, Krugersdorp, South Africa), HPLC grade acetonitrile from Rathburn Chemicals Limited (Walkburn, Scotland), chloroform from Sky Chem (Randhart, South Africa), ethyl acetate and petroleum ether from Robert Lundie (Westgate, South Africa). All chemicals and solvents employed in this investigation were of analytical grade. Merck (Darmstadt, Germany) supplied the Kieselgel 60 F_{254} TLC plates.

Animals and sample collection

Pregnant female hyraxes used in the metabolic studies were collected from Sandton City Parks (Sandton, Gauteng, South Africa) and the Johannesburg Zoo (Johannesburg, Gauteng, South Africa). The animals were transported to the laboratory, weighed and anaesthetized (ketamine hydrochloride:xylazine hydrochloride; 1:1; 1.25 ml/kg; intramuscular). Whole blood was collected by cardiac puncture with a hypodermic syringe, transferred to glass tubes containing heparin (500 μl) and kept on ice, prior to the onset of the metabolic studies. They were then killed through an intracardiac injection of Eutha-naze solution (2 ml/kg). Corpora lutea were dissected out and kept on ice until processed.

Uteri were obtained from non-pregnant (n = 16) and pregnant (n = 8) adult female hyraxes collected at the Elandsrand Mine Reserve (27 45′ S 27 24′ E; South Africa), where they were shot as part of a population control initiative. The uteri were excised within 20 min of death, cut into smaller pieces and placed on dry ice. After wrapping the uterine pieces in aluminium foil they were snap frozen in liquid nitrogen and transported to the laboratory on dry ice. Uterine tissue samples were stored at −70°C until processed.

Isolation of erythrocytes and leucocytes from whole blood

Blood of individual animals was fractionated by isopicnic gradient centrifugation on Ficoll-Paque as described by Böyum [15]. Packed cells, isolated erythrocytes and leucocytes were thoroughly washed with PBS by repeated resuspension and centrifugation. Erythrocyte and leucocyte numbers were measured using the method of Simmons [16]. Cells were resuspended in a fixed volume (1 ml) of Eagles medium prior to the incubations.

Incubations

Samples of whole blood, plasma, packed cells, isolated erythrocytes and leucocytes (900 ml) or minced luteal tissue (∼20 mg) in Eagles medium (900 ml) were incubated within 20 min of collection in a shaking water bath (3 h, 37°C) with either [^{3}H]pregnenolone (∼120 000 dpm in PBS) or [^{3}H]progesterone (∼120 000 dpm in PBS) and 50 μl of the co-factor β-NADPH (2.4 mM in PBS; [8,12,14]). Controls consisting of only the incubating media and the radiolabelled precursors were included in the study.

Incubations of the blood samples were terminated by the addition of 200 μl of 10% Triton X-100 and the incubation mixtures extracted with ice-cold absolute ethanol, followed by diethyl ether. Luteal tissue incubation mixtures were similarly extracted, following homogenization (Ultra-Turrax, TP 18/10, Janke & Kunkel, Staufen, Germany). The organic solvent extracts of individual samples were combined, evaporated (N_2, 37°C) and the dried extract residues reconstituted in absolute ethanol (1 ml), prior to fractionation by

HPLC. The final recovery of radiolabelled precursors and metabolites was 86.2 ± 0.9% (mean ± SEM; n = 215).

Reverse-phase high performance liquid chromatography (HPLC)

Radiolabelled precursors and metabolites (incubation products) were separated by reverse-phase HPLC (Phenomenex Lichrosorb: 5 mm RP-18; 150 mm × 4.6 mm; Cheshire, UK). The HPLC system consisted of a 590 programmable solvent delivery module, an automated gradient controller, a 712 WISP auto-injector, a M490 programmable multiwavelength detector system (Waters, Milford, USA), and was fitted with a fraction collector (2112 Redirac; LKB, Bromma). The mobile phase consisted of 55% acetonitrile and the flow rate was maintained at 1 ml/min. Samples (80 μl) of the extract residues, reconstituted in ethanol, were injected, fractions collected at 0.5 min intervals for 30 min and the radioactivity of each fraction was measured in a scintillation counter. [^{3}H]Pregnenolone, [^{3}H]progesterone and unlabelled standards (5α-DHP, 5β-DHP, 5α-PO and 17α-DHP) were used to calibrate the column. Non-labelled standards were detected at 254 nm. Recovery of radioactivity from the column was 78.0 ± 1.7% (mean ± SEM; n = 215).

Thin layer chromatography (TLC)

Combined HPLC fractions, corresponding to peaks A, B and C on the elution profiles (Fig. 1) were extracted twice with diethyl ether (4 ml) and the extracts dried (N_2, 37°C). The extract residues were reconstituted in ethanol (100 μl) and aliquots (10 μl) applied on TLC plates together with reference standards (1 mM solution: 5α-DHP, 5β-DHP, 5α-PO and 17α-DHP). Three different mobile phase systems were employed in the identification of metabolites by TLC: (a) chloroform:ethyl acetate (9:1), (b) hexane:ethyl acetate (5:2) and (c) chloroform:absolute ethanol (9:1). Standards were detected by UV (Spectroline, TC-312A, Transilluminator 312 nm ultraviolet, New York, USA) and 70% H_2SO_4 (v/v). TLC plates were fractionated (0.5 cm fractions) and the radioactivity of each fraction measured.

Recrystallizations

Extracts corresponding to each of compounds A, B and C were obtained as for TLC, dried (N_2; 37 °C) and the residues redissolved in chloroform. Repeated crystallizations of compounds B and C, to constant specific activity, were effected following the addition of 25 mg of the corresponding unlabelled carrier steroid in 100 μl chloroform: one drop n-hexan to crystallize 5α-DHP, and the addition of methanol (1:1; v/v; methanol from BDH, Poole, UK) to crystallize 17α-DHP, respectively. Once crystals formed, a portion (∼ 1 mg) was weighed, dissolved in 50 μl chloroform and the radioactivity measured in order to calculate the specific activity (dpm/mg) of the crystallized steroids. The remaining material was subjected to recrystallization, until the specific activity of three successive recrystallizations did not differ from each other by more than 5%. Compound A could not be recrystallized to constant specific activity.

Equilibrium binding assays

Uterine progesterone receptors were measured by the method described by Greyling *et al.* [17]. Cytosols were incubated with a concentration series of [1,2,6,7-^{3}H]progesterone ranging

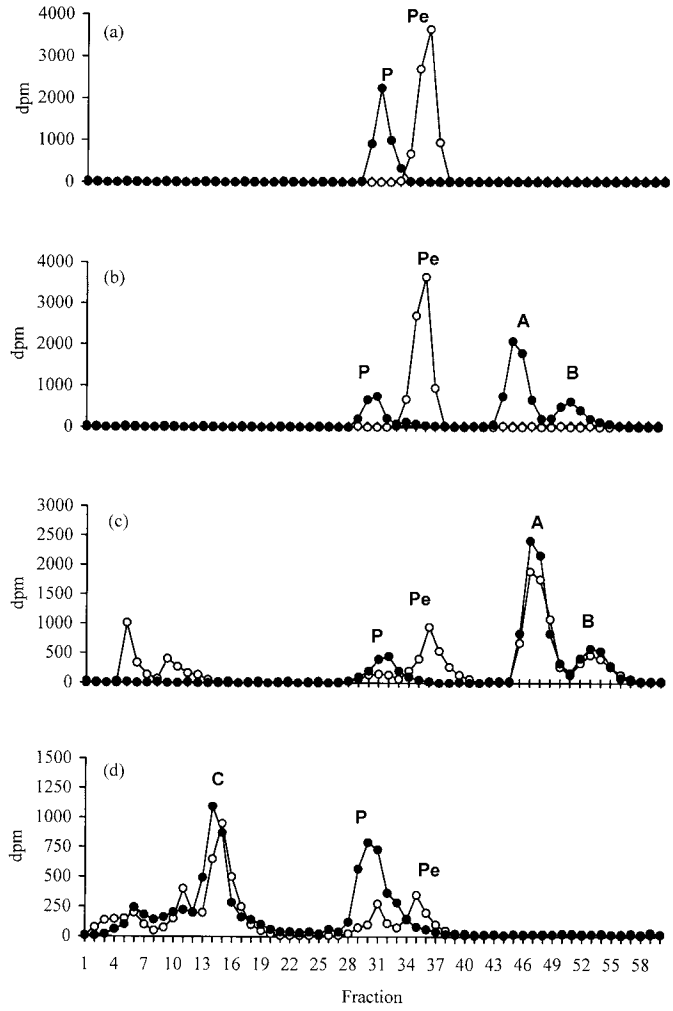

Fig. 1. HPLC elution profiles of radiolabelled metabolites obtained following incubation of (a) controls, (b) erythrocytes, (c) leucocytes and (d) luteal tissue from a pregnant rock hyrax for three hours with [^{3}H]pregnenolone (○) and [^{3}H]progesterone (●). Peaks corresponding to pregnenolone (Pe), progesterone (P), 5α-PO (A), 5α-DHP (B) and 17α-DHP (C) are labelled accordingly.

from 0.25 nM to 64 nM (for 15-point Scatchard curves), in the absence (total binding) or presence (non-specific binding) of a 1000-fold excess of unlabelled progesterone. The inter-assay coefficient of variation for the K_d value for the progesterone receptor was 19.6%, while the intra-assay coefficient of variation was 11.8%.

Competitive binding assays

Competitive binding assays were carried out according to Greyling *et al.* [17]. The unlabelled competing inhibitors of receptor binding used in the assay were 5α-DHP, 5α-PO and

17α-DHP. The intra-assay coefficient of variation never exceeded 5.0%, while the inter-assay coefficient of variation was 5.1%.

Hormone assays and validations

Plasma progesterone concentrations were measured using the radioimmunoassay method described by Greyling *et al.* [9]. Recovery of [1,2,6,7-^{3}H]progesterone (∼20 000 dpm; specific activity 94 Ci/mmol; radiochemical purity 97.7%; Amersham International, Buckinghamshire, UK) added to 200 μl plasma was 81 and 86% (n = 2). Serially diluted plasma produced a displacement curve parallel to the standard curve ($t_5 = 1.55$, $p > 0.05$). The amounts of progesterone recovered from plasma pools did not differ significantly to the amounts added ($t_2 = 2.01$, b = 1.07, $p > 0.05$, n = 3). Mean ± SEM sensitivity of the assay was 0.029 ± 0.001 ng/ml (n = 3). Non-specific binding was 5.3 ± 0.5% (n = 3) and the intra- and inter-assay coefficients of variation were 3.2% and 16.4%, respectively.

5α-DHP plasma concentrations were determined using the amplified enzyme-linked immunoassay method similar to that described by Hamon *et al.* [18]. For validation of the assay, serially diluted samples of pooled plasma from non-pregnant and pregnant females displayed a curve parallel to the standard curve ($t_7 = 1.09$, $p > 0.05$). Plasma spiked with 5 ng 5α-DHP resulted in 76 ± 4% (mean ± SEM, n = 6) recovery. The intra- and inter-assay coefficients of variation were 3.5% (n = 4) and 13.0% (n = 3), respectively. The limit of detection was 0.09 ng/ml.

Statistical analyses

The total radioactivity in each peak in a sample was expressed as a percentage of the total radioactivity injected. The Kruskal-Wallis *H* and Mann-Whitney *U* tests were used to determine the differences in the levels of [^{3}H]pregnenolone and [^{3}H]progesterone metabolised, and levels of metabolic conversion products formed between whole blood, plasma, isolated erythrocytes and leucocytes and packed cells. Scatchard and saturation curves with the Rosenthal-correction for non-specific binding were constructed (COMBICEPT 2000CA, Packard Instrument Company, Illinois, USA). Scatchard plots and displacement curves were subjected to least square regression analysis and only data with correlation coefficients ≥ 0.9 were used for further analyses. The relative binding affinities of competitor steroids were determined using the equation of Rodbard [19]. All mean values are followed by one standard error of the mean (SEM), and significance is taken at 95%.

Results

Identification of the metabolites

The HPLC retention times of both [^{3}H]pregnenolone and [^{3}H]progesterone corresponded with their unlabelled standards (fractions 35 to 39 and 28 to 32, respectively). The retention times of the compounds in peak A (22.0 min), B (25.0 min) and C (6.5 min) corresponded to 5α-PO (23.2 min), 5α-DHP (25.0 min) and 17α-DHP (6.5 min), respectively. TLC of the compounds in the isolated fractions in the different mobile phases (a and b) confirmed that compound A had an R_f value identical to that of 5α-PO, while compound B had an R_f value

identical to that of 5α-DHP. Compound C displayed a R_f value identical to 17α-DHP on TLC in two different mobile phases (a and c). Crystallization experiments confirmed the identity of compound B as 5α-DHP (three successive crystallizations gave specific activities of 503, 490 and 493 dpm/mg steroid, respectively) and that of C as 17α-DHP (three successive crystallizations gave specific activities of 1813, 1843 and 1768 dpm/mg steroid, respectively). The identification of compound A as 5α-PO by HPLC and TLC could not be confirmed by recrystallization.

The metabolism of [^{3}H]pregnenolone and [^{3}H]progesterone

Only isolated leucocytes were able to metabolise [^{3}H]pregnenolone (Table 1), with the fraction of [^{3}H]pregnenolone metabolised ranging from 18.0 to 89.0% ($n = 7$). Isolated leucocytes metabolised [^{3}H]pregnenolone to progesterone, 5α-PO and 5α-DHP (Fig. 1; Table 2), the principal metabolite being 5α-PO, which was present at significantly higher levels than progesterone and 5α-DHP ($p < 0.05$). The incubation of [^{3}H]progesterone with whole blood, isolated erythrocytes and leucocytes, and packed cells resulted in the formation of both 5α-PO and 5α-DHP (Tables 1 & 2). Plasma alone did not metabolise [^{3}H]progesterone.

Isolated leucocytes were able to metabolise a significantly larger fraction of progesterone than whole blood, erythrocytes and packed cells ($p < 0.05$). Erythrocytes displayed a similar level of [^{3}H]progesterone metabolism to packed cells ($p > 0.05$), whilst the metabolism of this precursor was significantly suppressed in whole blood ($p < 0.05$). 5α-PO appeared to be the principle metabolite derived from progesterone by isolated erythrocytes and leucocytes, and packed cells ($p < 0.05$), while whole blood formed similar amounts of 5α-PO and 5α-DHP ($p > 0.05$).

The number of erythrocytes ($5.0 \pm 0.5 \times 10^9$ cells/ml, $n = 8$) in hyrax blood was approximately 500-fold higher (556 ± 59, mean ± SEM ; $n = 8$) than the number of leucocytes ($9.6 \pm 1.2 \times 10^6$ cells/ml, $n = 8$).

A large fraction of [^{3}H]pregnenolone (61.0 to 100%; $n = 8$) was metabolised by luteal tissue (Table 1), and progesterone and 17α-DHP were the two principal metabolites in the

Table 1
Metabolism of [^{3}H]pregnenolone and [^{3}H]progesterone by blood and luteal tissue

	Mean ± SEM percentage of steroid metabolised (no. of replicates)	
Incubation substrate	Pregnenolone	Progesterone
Control	0 (8)	5.0 ± 2.9 (3)
Whole blood	1.9 ± 1.2 (8)	41.3 ± 7.3 (3)
Plasma	0.6 ± 0.6 (8)	0.3 ± 0.3 (3)
Erythrocytes	0.6 ± 0.3 (8)	86.0 ± 5.0 (3)
Leucocytes	60.6 ± 9.8 (7)	99.2 ± 0.4 (3)
Packed cells	0.2 ± 0.2 (5)	90.5 ± 5.5 (3)
Luteal tissue	90.9 ± 4.9 (8)	37.5 ± 27.5 (2)

The percentage (mean ± SEM) of [^{3}H]pregnenolone and [^{3}H]progesterone metabolised during three hour control, whole blood, plasma, isolated erythrocytes and leucocytes, packed cell and luteal tissue incubations, for pregnant female rock hyraxes (sample sizes are given in parentheses).

Table 2
Formation of metabolic products by blood and luteal tissue

Incubation substrate	Precursor[a] (no. of replicates)	Mean ± SEM percentage of metabolite formed				
		Prog	5α-PO	5α-DHP	17α-OHP	Other
Leucocytes	P_5 (7)	10.1 ± 2.3	30.6 ± 6.6	8.6 ± 1.9	—	11.3 ± 3.5
	P_4 (3)	—	66.3 ± 4.5	16.7 ± 4.2	—	16.2 ± 6.3
Whole blood	P_4 (3)	—	15.0 ± 7.7	19.2 ± 5.0	—	7.2 ± 3.6
Erythrocytes	P_4 (3)	—	66.7 ± 24.2	15.0 ± 13.1	—	4.3 ± 2.6
Packed cells	P_4 (3)	—	73.5 ± 25.1	12.7 ± 8.1	—	4.3 ± 7.5
Luteal tissue	P_5 (8)	15.1 ± 4.1	—	—	46.0 ± 3.2	29.8 ± 4.1
	P_4 (2)	—	—	—	44.0 ± 22.0	15.5 ± 7.8

The percentage (mean ± SEM) of metabolites formed after three hour incubations of leucocytes and luteal tissue with [^{3}H]pregnenolone, and after incubations of whole blood, isolated erythrocytes and leucocytes, packed cells and luteal tissue with [^{3}H]progesterone, of pregnant rock hyraxes (sample sizes are given in parentheses).

[a] P_5 is [^{3}H]pregnenolone, P^4 is [^{3}H]progesterone.

luteal extracts (Fig. 1; Table 2). 17α-DHP appeared to be present at significantly higher amounts than progesterone ($p < 0.05$; Table 2).

Relative binding affinities of the uterine progesterone receptor for competitor ligand

The K_d value of progesterone for the uterine progesterone receptor obtained from both saturation curves and 15-point Scatchard plots was 1.78 ± 0.11 nM (mean ± SEM, n = 8). The K_d and relative binding affinity (RBA) values for 5α-DHP (2.00 nM and 94% respectively) were similar to those of progesterone (Fig. 2). Both 17α-DHP (K_d = 105.76 nM; RBA = 2.0%) and 5α-PO (K_d = 281.28 nM; RBA = 0.4%) exhibited very weak binding to the progesterone receptor (Fig. 2).

Plasma concentrations of progesterone and 5α-pregnane-3,20-dione

Plasma progesterone concentrations in pregnant females ranged from 0.42 to 5.91 ng/ml (mean ± SEM; 1.60 ± 0.62 ng/ml; n = 8) and that of 5α-DHP from 3.50 to 9.57 ng/ml

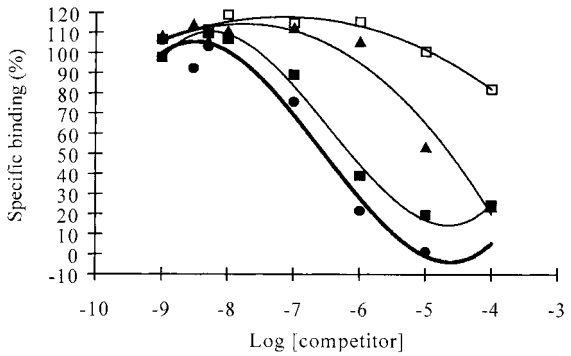

Fig. 2. Displacement curves to illustrate the competition of various natural progestins to the binding to the uterine progesterone receptor in the rock hyrax: progesterone (●), 5α-DHP (■), 5α-PO (▲) and 17α-DHP (□).

(mean ± SEM; 7.39 ± 0.80 ng/ml; n = 7), thereby exceeding those of progesterone by four to 17 times (mean ± SEM 5α-DHP:progesterone ratio; 8.5 ± 1.7; n = 7).

Discussion

The present study clearly demonstrated that whole blood, isolated erythrocytes, isolated leucocytes, and packed cells of the female rock hyrax display the ability to metabolise progesterone. However, a report by Makawiti *et al.* [8], concluding that the plasma of the hyrax can metabolise progesterone, could not be corroborated. Isolated leucocytes appear to be the only component of blood that could metabolise both pregnenolone and progesterone. It can be speculated that this unique ability of the leucocytes may play an important role in protecting the maternal and foetal tissues from the detrimental effects of high levels of progesterone [8]. On the other hand, leucocytes may serve as targets for progesterone, in order to down regulate their immunocompetency in pregnant females as a mechanism of protecting the foetus against the immune system of the mother, as has been suggested for humans by Scully *et al.* [14]. The apparent discrepancy in the ability of whole blood and packed cells to metabolise progesterone may result from the presence of enzyme inhibitors in hyrax plasma which interfere with pregnenolone and progesterone metabolism [12]. Another explanation for the observation could be that some form of competition may arise between the binding of progesterone and pregnenolone by steroid binding proteins in the plasma and/or some high affinity binding protein in, or on the surface of erythrocytes [12]. However, Gombe, Heap & Sale [20] failed to demonstrate the presence of such a putative high affinity progesterone binding globulin in rock hyrax plasma.

We observed that blood and some isolated components of blood, could convert progesterone to 5α-DHP, together with another compound, displaying chromatographic properties similar to 5α-PO. These two 5α-reduced progestins appeared to be produced in similar amounts, and we infer that one, or both of them, may play a role in maintaining pregnancy in the rock hyrax [6]. Differences in the relative binding affinities of the uterine progesterone receptor for these two progestins strongly suggest that 5α-DHP, rather than 5α-PO, may play a physiologically important role in the maintenance of pregnancy in the hyrax. In the African elephant, however, both 5α-DHP and 5α-PO have been identified as the major progestins produced by luteal tissue [7], but not by blood [21], as had been demonstrated in the hyrax. In the elephant, both 5α-DHP and 5α-PO exhibited a high relative binding affinity for the uterine progesterone receptor [17]. In this species, 5α-DHP appear to display binding properties very similar to progesterone [17] and appears to down regulate the uterine progesterone [9]. It can therefore be inferred that, in the African elephant, 5α-DHP exhibits a biochemical function normally fulfilled by progesterone in other mammals. The same type of progestational function of 5α-DHP also exists in the horse where concentrations of this progestin, rather than progesterone, correlate with reproductive status [22–25]. 5α-Pregnanes produced during pregnancy in the horse also seem to reduce placental activity prior to the onset of parturition [26]. In other species, 5α-DHP seems to support placental growth [27, 28].

Our study clearly demonstrates that the corpus luteum of the rock hyrax produces both progesterone and 17α-DHP. This is different from the African elephant, in which luteal tissue does not produce 17α-DHP, but only 5α-pregnanes [29]. Bilateral ovariectomy in pregnant

hyraxes resulted in abortion [30, 31], implying that a steroid from the corpus luteum may be important for maintaining pregnancy. Since 17α-DHP displayed a very low relative binding affinity for the hyrax uterine progesterone receptor, progesterone may be the important luteal product necessary during pregnancy. However, plasma 5α-DHP concentrations were on average 8.5 times higher than those of progesterone in pregnant rock hyraxes, suggesting that 5α-DHP is also important during pregnancy. The results from our study therefore suggest that blood plays a primary role in maintaining high circulatory concentrations of 5α-DHP, by producing this progestin from progesterone. The low circulatory progesterone concentrations found in the present investigation corroborate those reported in previous studies [6, 20] and may well result from the reduction of progesterone to 5α-DHP. Nevertheless, it is possible that a sensitive mechanism, present in target tissues and mediated by the progesterone receptor, may enable low concentrations of progesterone to maintain pregnancy [6]. Here it is of interest to note that circulating progesterone concentrations in pregnant African elephant females are also relatively low [7], whereas those of 5α-DHP and 5α-PO are 20 and 13 times higher than that of progesterone [7]. These 5α-reduced progestins display progestational characteristics in the African elephant [9, 17], this also being the case for 5α-DHP in pregnant hyraxes.

Results from our investigation suggest that blood and luteal tissue may perform complementary roles in the maintenance of pregnancy in the rock hyrax. In this species corpora lutea appear to be important in the production of progesterone which, in circulation, is metabolised to the 5α-reduced metabolites (5α-DHP and 5α-PO). 5α-DHP is then transported to the uterus where it binds to the progesterone receptor, triggering a number of important physiological effects necessary for the support of pregnancy. This mechanism of support seems to be similar to that of the African elephant, the only notable difference being the origin of the 5α-reduced metabolites of progesterone. Alternatively, the metabolism of progesterone by the blood may only be necessary to get rid of excess amounts of circulating pregnenolone and/or progesterone, and the reduced metabolites may only be metabolic intermediates in the production of biologically inactive compounds that will eventually be excreted in the urine [32]. The actual biological significance of the 5α-reduced progesterone metabolites in the pregnant hyrax is however not yet clear.

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