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In vitro metabolism of progesterone by canine hair follicle cells

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Abstract Dermal papilla cells (DPC) and dermal fibroblasts (DFB) derived from hair follicles from two different body sites (head, flank) of four male, castrated beagle dogs were incubated for 24 h with radioactive progesterone (P4). Thin-layer chromatography was used for separation and autoradiography for identification of the radioactive metabolites. In DFB the main metabolites were cortisol and 4-pregnene-11β-ol-3,20-dione, whereas in DPC they were 5α-pregnane-3,20-dione and cortisol. The highest percentage of metabolism of P4 was found in DFB of the head. Smaller amounts of other metabolites were found in both cell types of both locations.

INTRODUCTION

Our previous investigation about androgen metabolism in primary cell cultures of dermal papilla cells (DPC) and dermal fibroblasts (DFB) of hair follicles from male, castrated beagle dogs revealed a marked metabolism of dehydroepiandrosterone (DHEA).[1] We were therefore interested to see whether progesterone could serve as a precursor for the local metabolism of androgens or whether it is metabolized into other compounds.

METHODS

Experimental animals, preparation of hair follicles and the cell culture conditions for DPC and DFB have been described in our previous publications.[1,2]

Hair follicles were obtained from skin biopsies of four healthy, castrated male beagle dogs, also as described previously.[1] From these preparations primary cell cultures were gained and used after three passages, when the cells were still morphologically identical to their origin.[1]

As soon as the cells became confluent (about 1 million cells), they were incubated with 1 mL of Amniomax complete C100 medium containing 10 million decays per min (dpm) of radioactive-labelled ^{3}H-progesterone (NET-381, [1, 2, 6, 7–^{3}H (N)] – progesterone, specific activity: 3848 GBq/mmol, 37 MBq/mL, Lot no. 3363626), for 24 h at 37 °C in 5% CO_2.

Again, the procedure of extraction of steroid metabolites, thin layer chromatography (TLC) and autoradiography have been described in our previous publication.[1]

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After extraction of the metabolites from the incubation medium with 3 × 5 mL of diethyl ether, they were spotted on TLC plates, which had been prewashed with methanol. The TLC plates were developed in n-hexane: ethyl acetate (1.5 : 1, v : v) until the solvent front reached 17 cm. The various metabolites of P4 could be separated clearly by one-dimensional TLC according to the matching ratio to front (Rf) values of the relevant standards. These were run as a mixture of high concentrations (20 μL of a solution of 1 mg mL^{-1}) at the left and right outer lane of the plate. After autoradiography for 3 weeks at –20 °C, the standards were made visible by spraying a mixture of sulphuric acid (1 mol L^{-1}) and methanol (v/v, 1/1) onto the outer lanes of the TLC plate and heating it at 115 °C. The radioactive P4-metabolites were allocated according to the matching dark spots on the X-ray film. The relevant areas were cut out of the TLC plate and eluted with 2 × 5 mL of acetone to quantify the metabolites. The tentative identification of the P4-metabolites was achieved by matching Rf-values of the relevant standards. No attempt was made to prove identity by immunoassays or other analytical methods (gas liquid chromatography, high performance liquid chromatography), as the concentration of the radioactive metabolites was too low for this.

As there were small amounts of radioactive metabolites present in the control incubations (medium with all supplements, but without cells) their percentage was calculated and subtracted from the values of the corresponding cell incubations.

Statistical analysis

Arithmetic mean and standard deviations were calculated from the 7–12 replicates of each cell-incubation of both locations (head, flank) and both cell types (DPC, DFB). Samples from the two cell types (DPC and DFB) were compared, as were samples from the two sites (head and flank) by analysis of variance (ANOVA) on ranks and pair-wise multiple comparison procedures (Dunn's method) using a statistical

Table 1. Percentage (mean ± SD) of metabolites of ^{3}H-progesterone in the medium of incubations with dermal papilla cells (DPC) or dermal fibroblasts (DFB)

			DPC		DFB	
	Steroid		Head	Flank	Head	Flank
Rf	Abbr.	Full name	Mean ± SD*	Mean ± SD*	Mean ± SD*	Mean ± SD*
0.44	P4	4-pregnene-3,20-dione (progesterone)	42 ± 5.4a,c	49.11 ± 13.3a	23.5 ± 12.5b	28.4 ± 9b,c
0.64	5α-P4	5α-pregnane-3,20-dine	23.7 ± 3.5a	23.4 ± 5.9a	9.6 ± 2.2b	12.7 ± 4.9b
0.4	5α-P5	5α-pregnane-3β-ol-20-one	0 ± 0a	5.1 ± 4.1a	3.0 ± 1.9b	4.5 ± 1.5b
0.15	11β-OH-P4	4-pregnene-11β-ol-3,20-dione	3.2 ± 1.7a	3 ± 1.6a	18.2 ± 7.5b	12.8 ± 5.7b
0.11	17α,20α-P4	4-pregnene-17α,20α-diol-3-one	1.5 ± 0.9a	2.6 ± 1a,c	7.3 ± 3.1b	3.7 ± 1b,c
0.04	Cortisol	4-pregnene-11β,17α,21-triol-3,20-dione	12.6 ± 4.7a	8.6 ± 6.8a	24.3 ± 8.3b	24.2 ± 7.9b
0.02	11α-OH-T	4-androstene-11α,17β-diol-3-one	5.2 ± 2a,c	4.2 ± 3.5a	12.2 ± 4.5b	10.8 ± 6.4b,c

Rf, ratio to front values after separation using n-hexane/ethyl acetate (v/v 1.5/1) as the mobile phase.
Abbr., abbreviation; *different letters in the same line indicate significant differences ($P < 0.05$) between the percentages.

software package (SIGMASTAT® Version 3.0 for Windows, Jandel GmbH, Erkrath, Germany).

RESULTS

The P4-metabolites could be separated by one TLC according to the matching Rf-values of the relevant standards (Table 1), which were run on the same plate. In the culture medium from the control dishes, incubated without cells, there was only a small amount of metabolism of progesterone to 5α-pregnane-3β-ol-20-one.

Table 1 also shows percentages of metabolites of the added radioactive progesterone from DPC and DFB from the head and flank after their separation by TLC. Significant differences ($P < 0.05$, indicated by different letters in the column with the asterisk [*]) were found between the two cell types (DPC and DFB), but not between the two locations (head and flank).

In DPC the main metabolites were 5α-pregnane-3,20-dione (head: 23.7 ± 3.5%; flank: 23.4 ± 5.9%) and cortisol (head: 12.6 ± 4.7%; flank: 8.6 ± 6.8%). In DFB progesterone was metabolized mainly to cortisol (head: 24.3 ± 8.3%; flank: 24.2 ± 7.9%), followed by 4-pregnene-11β-ol-3,20-dione (head: 18.2 ± 7.5%; flank: 12.8 ± 5.7%), 4-androstene-11α,17β-diol-3-one (head: 12.2 ± 4.5%; flank: 10.8 ± 6.4%) and 5α-pregnane-3,20-dione (head: 9.6 ± 2.2%; flank: 12.7 ± 4.9%). Additionally, in both cell types, smaller percentages (less than 10%) of other metabolites were found.

DISCUSSION

Progesterone plays an important role in the metabolic pathway of corticosteroids and sexual steroids. The reduction of the double bond between C4 and C5 by a 5α-reductase results in the formation of 5α-progesterone and, further, of other 5α-pregnanes – for example, 5α-pregnane-3β-ol-20-one.

The main metabolic pathway leading from progesterone to cortisol involves a 17α-hydroxylase, a 21-hydroxylase and an 11β-hydroxylase. In addition to cortisol, one of these intermediate metabolites of progesterone (11β-OH-progesterone) has been found in our incubations of DPC and DFB from canine hair follicles. The percentage of metabolism was significantly different ($P < 0.05$) between DPC and DFB, but not between the locations (head or flank). This observation that metabolism of progesterone was different in both cell types but not in both locations points towards different functions of these cells. The fact that DPC and DFB differ in the metabolism of androgens has already been described in the literature.[3–7]

However, this is the first report of the formation of progesterone metabolites in canine hair follicle cells. Although there was no significant difference between head and flank, the marked production of cortisol in DFB from the head is interesting, considering that this location is never affected by alopecia.

Progesterone (P4) has been found to be a major inhibitor of the formation of 5α-dihydrotestosterone (DHT) in DPC derived from human scalp biopsies.[8] When human DPC were incubated for up to 48 h with ^{3}H-testosterone, the addition of P4 (1 nM) inhibited the formation of DHT by 75%. This effect was only slightly less than that of finasteride, a well-known inhibitor of the 5α-reductase. Oestrogens were less effective (e.g. 100 nM oestradiol-17β inhibited by only 60%).

Therefore the role of P4-metabolites in DPC and DFB of canine hair follicles needs further investigation.

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Résumé Des cellules de la papille dermique (DPC) et des fibroblastes dermiques (DFB) obtenus à partir de follicules pileux de deux zones cutanées (tête et flanc) chez 4 Beagle mâles castrés ont été incubés avec de la progestérone radio-active (P4) pendant 24 heures. Une chromatographie en couche mince a été utilisée pour la séparation et une autoradiographie pour l'identification des métabolites radio-actifs. Pour les DFB, les principaux métabolits étaient le cortisol et le 4-pregnene-11ß-ol-3,20-dione, alors que pour les DPC il s'agissait de 5α-pregnane-3,20-dione et de cortisol. Le pourcentage métabolique de P4 était le plus élevé dans les DFB de la tête. Des quantités plus faibles des autres métabolites ont été retrouvées dans les deux types cellulaires dans les deux zones étudiées.

Resumen Se incubaron células de la papilla dérmica (DPC) y fibroblastos dérmicos (DFB) derivados de folículos pilosos de dos áreas corporales diferentes (cabeza, flanco) de cuatro Beagle machos, castrados, durante 24 hrs con progesterona radioactiva (P4). Se utilizó la cromatografía de capa fina para la separación y autoradiografía para la identificación de los metabolitos radioactivos. En los DFB los principales metabolitos fueron el cortisol y la 4-pregnene-11ß-ol-3,20-diona, mientras que en las DPC fueron la 5α-pregnane-3,20-diona y el cortisol. El mayor porcentaje de metabolismo de la P4 fue hallada en los DFB de la cabeza. Se encontraron pequeñas cantidades de otros metabolitos en ambos tipos celulares de las dos localizaciones.

Zusammenfassung Zellen der Haarpapille (ZHP) und dermale Fibroblasten (DFB), die von Haarfollikeln zweier verschiedener Körperlokalisationen (Kopf und Flanke) von vier männlichen, kastrierten Beaglen stammten, wurden für 24 Stunden mit radioaktivem Progesteron (P4) inkubiert. Für die Seperation wurde Dünnschichtchromatographie und für die Identifikation der radioaktiven Metaboliten Autoradiographie benutzt. In DFB waren die Hauptmetaboliten Cortisol and 4-pregnen-11ß-ol-3,20-dion, wogegen es in ZHP 5α-Pregnan-3,20-dion and Cortisol waren. Der höchste Prozentsatz an P4-Metabolismus wurde in DFB des Kopfes gefunden. Kleinere Mengen anderer Metaboliten wurde in beiden Zelltypen von beiden Lokalisationen gefunden.