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In vitro metabolism of dehydroepiandrosterone and testosterone by canine hair follicle cells

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Abstract The metabolism of radioactive dehydroepiandrosterone (DHEA) and testosterone was studied in 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. Thin layer chromatography was used for separation, and autoradiography for identification of the radioactive metabolites. DHEA was metabolized mainly to 11α-OH-testosterone and only to a minor extent to 11α-OH-androstenedione and another unidentified metabolite. The highest percentage of metabolization of DHEA was found in DFB of the head. Testosterone was metabolized only to a minor extent (less than 10%) to 5α-dihydrotestosterone and epiandrosterone and there was no significant difference between either the two cell types or the two locations. These results clearly show that the metabolization of androgens in canine DPC and DFB is different from that observed in cells from the human hair follicle.

Keywords: dehydroepiandrosterone, dermal fibroblasts, dermal papilla cells, dog, hair follicle, metabolism, testosterone.

INTRODUCTION

Many questions concerning endocrine causes of canine alopecia still exist.[1,2] The pathomechanism of sex hormone-associated alopecia in dogs is completely unknown and no explanation of the occurrence of predilection areas (such as the flank) have been found. The dermal papilla (DP), a specialized structure situated at the base of the hair follicle, is important for the induction of follicular development and the maintenance of hair growth.[3] Steroids may act via the DP, affecting the other follicular components indirectly.[4,5] Therefore, the culture of dermal papilla cells (DPC) is a valuable tool for hair growth investigations of many species. Canine DPC were successfully isolated and serially cultivated *in vitro*.[6] These cells have been shown to be morphologically and functionally different from canine dermal fibroblasts (DFB). Using immunohistochemistry it has been shown that dermal papillae from different body sites of the dog contain androgen receptors, but there was no difference in the percentage of receptor-positive cells between predilection areas of alopecia and other locations.[7] However, androgens are considered to be one of the most important regulators of human hair growth and they induce different responses of hair follicles, depending on the body location. Testosterone-responsive alopecia on particular body sites of the dog has also been described.[2] Differences in local androgen metabolism may be responsible for these different androgen sensitivities. In human dermal papillae of the beard, the activity of the enzyme 5α-reductase was three times higher than that in the occipital scalp papillae.[8] Although several previous studies have investigated the mechanism of action of androgens, the process of regulation of hair growth by these hormones is still unclear. The present study was designed to elucidate the influences of androgens on canine hair growth and to find a reason for hair loss occurring only at predilection areas. Therefore, we investigated the metabolism of radioactive labelled dehydroepiandrosterone (DHEA) and testosterone by dermal fibroblasts and dermal papilla cells derived from follicles from two different body sites of four dogs.

The rationale for choosing DHEA was the information in the literature that this weak androgen may serve as a precursor for testosterone or oestrogens. Hoffmann *et al.*[9] reported that in balding young men the elevated serum levels of DHEA (and DHEA-sulphate) could play an important role in the formation of 5α-dihydrotestosterone (DHT) in hair follicles.

METHODS

Experimental animals

Four castrated male beagle dogs, aged between 3 and 5 years, were used to eliminate diurnal changes in plasma steroid concentration and to prevent plasma steroid variations associated with the female sexual cycle.

The dogs were housed in indoor-outdoor runs at the Clinic of Small Animal Internal Medicine, University of Veterinary Medicine, Vienna, fed a standard commercial dog food once daily, and given water *ad libitum.*

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Castration of the dogs was performed 2–4 months prior to the skin biopsy.

Two weeks before biopsy, the hairs in the target area were clipped to synchronize all follicles in the anagen phase of the hair cycle.[10]

Skin biopsies (8 mm diameter) were taken from the flank (predilection area for cutaneous manifestations of endocrine diseases) and the head (not affected in such disorders) under general anaesthesia and the wounds closed with sutures.

Permission for animal experimentation had been obtained from the Federal Ministry for Education, Science and Culture (GZ 68.205/5-Pr/4/2001).

The number of dogs was kept low (four instead of six) in order to be able to perform the experiment within a reasonably short period of time (4 months to avoid seasonal influences) and to comply with our ethics committee, which asked us to use as few animals as possible. As the investigation was carried out with hair follicle cells grown in primary cell culture, the low number of dogs did not hamper the statistical analysis of the data.

Cell culture

The methods used for isolating and culturing dermal papilla cells (DPC) and dermal fibroblasts (DFB) have been described elsewhere.[6] Both cell types were isolated from the same skin biopsy specimen and were cultured in Amniomax complete C100 medium (Life Technologies, Eggenstein, Germany) supplemented with (for DPC) or without (for DFB) 10% inactivated (56 °C, 2 h) canine serum (Promocell GmbH, Heidelberg, Germany).

Although we knew from the literature that there is an interaction between DPC and bulb keratinocytes *in vivo* in several species (human, sheep, rat, mouse), we decided to grow DPC alone, to determine their *in vitro* capacity to metabolize androgens.

DFB were used as a control for the metabolic capacity of DPC *in vitro*. Furthermore, it could be the case that DFB would produce a metabolite which could be used by DPC *in vivo*. Both cell types were harvested after the third passage, when their morphologic appearance and their production of some indicator proteins (collagen IV, laminin) was still the same as in the original cells.[6]

The steroids were eliminated from the cell culture medium with methanol activated cartridges (Sep – Pak C18, WAT 51910, Millipore-Waters, Vienna, Austria) to obtain steroid-free culture conditions. The cells were incubated at 37 °C in a humidified atmosphere of 95% O_2 and 5% CO_2.

Metabolism of dehydroepiandrosterone and testosterone

All experiments were performed on cells in the third passage of subculture. 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-dehydroepiandrosterone (NET-814, [1, 2, 6, 7–^{3}H (N)] – dehydroepiandrosterone, specific activity: 2220 GBq/mmol, 37 MBq/mL) or radioactive labelled ^{3}H-testosterone (NET-553, [1, 2, 6, 7, 16, 17–^{3}H (N)]-testosterone, specific activity: 4544 GBq/mmol, 37 MBq/mL) for 24 h at 37 °C in 5% CO_2. Control wells with no cells were also incubated to determine whether DHEA or testosterone were metabolized in the absence of cells.

After incubation, the medium was removed with a pipette and stored at –24 °C. Only the medium was analysed, as previous studies established that less than 1% of the radioactive labelled metabolites were found in the cell pellets. The metabolites were extracted from the supernatant by the addition of 3 × 5 mL of diethyl ether to the thawed medium and by mechanical shaking for 1 h. The ether phases were transferred to a new tube, evaporated at 40 °C and the residue dissolved in 0.3 mL of toluene. The samples were split: 0.1 mL of each were spotted on each of three thin layer chromatography (TLC) plates, which had been pre-washed with methanol (100%). A mixture of 20 µg of each unlabelled steroid standard (each 20 µL of a solution of 1 mg mL^{-1}) was applied on the left and the right lane of the plate. The plates were developed in one or two different mobile phases: n-hexane/ethyl acetate (1 : 1, v/v) or toluene: ethyl acetate: chloroform (1 : 3 : 5, v/v/v), respectively. As soon as the solvent front reached 17 cm, the TLC-plates were removed and dried at room temperature in a fume hood. Thereafter, the plates were numbered with a radioactive label (different number of spots) and dried again. In the dark room the plates were packed tightly with the silica gel side facing the photosensitive side of an X-ray film (Kodak Biomax MR; Amersham Pharmica Biotech, Vienna, Austria) and thereafter left at –24 °C in the deep freezer for 3 weeks. The ratio to front (Rf) values of the dark spots on the autoradiography film were compared with the Rf-values of the steroid standards, which were run on the outermost left and right lane of the two different mobile phases, in which they were separated clearly.

After autoradiography the left and right lanes of the TLC-plates were cut as 3 cm stripes and sprayed (in a fume hood) with an equal mixture of concentrated sulphuric acid and methanol.[11] The stripes were heated on an oven until the spots of the steroid standards appeared in their individual colour. For documentation these stripes were sealed with a plastic sheet (to avoid oxidation) and stored at room temperature.

The Rf-values were calculated individually for all spots on each TLC-plate according to the standards.[11] The corresponding spots were excised from the TLC-plate and the radioactivity eluted with 2 × 5 mL of acetone. After evaporation of the acetone, 6 mL scintillation fluid was added, the counts per min (cpm) were recorded and the decays per min (dpm) calculated.

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 deviation were calculated from the 8–12 replicates of each cell incubation of

both locations (head, flank) and both cell types (DPC, DFB). The statistical evaluation of the results was performed by the Student's *t*-test or the Mann–Whitney rank sum test, respectively, using a statistical software package (SIGMASTAT® Version 3.0 for Windows, Jandel GmbH, Erkrath, Germany).

RESULTS

In order to facilitate the understanding of the conversion of androgens in peripheral tissues a schematic drawing of DHEA and testosterone metabolism by the respective enzymes is provided in Fig. 1. The major pathway described in the literature is from DHEA via androstenedione to testosterone and further to 5α-dihydrotestosterone (DHT). Another pathway leads from DHEA via androstenediol to testosterone.

The androgen metabolites shown in Fig. 1 represent the known metabolites found in endocrine tissue (such as testicle, ovary, placenta and adrenal cortex), as well as those found in the skin (hair follicle). The particular metabolic pathway was derived from the quantitative presence of metabolites, which could be identified in our study.

The average recovery of radioactivity after ether extraction was 81.4 ± 18.4%. From these data we concluded that there were only negligible percentages of conjugated steroid metabolites present in the medium after 24 h.

The separation of relevant androgen metabolites of ^{3}H-DHEA and ^{3}H-testosterone could be achieved by TLC using two different mobile phases (Table 1). As shown in Table 1, the main androgen metabolites DHEA, androstenediol, androstenedione, testosterone and 5α-dihydrotestosterone (see Fig. 1) could be clearly separated by TLC.

If two particular metabolites had similar Rf-values in one system (e.g. DHEA and DHT in mobile phase 'a'), they could be separated in the other system (e.g. DHEA and DHT in mobile phase 'b').

Although we expected oestrogens to be produced from androstenedione or testosterone, they could not be found in our incubations. As the oestrogen standards had Rf-values greater than 0.60 (oestrone: 0.77; oestradiol-17α: 0.66; oestradiol-17β: 0.61) in the mobile phase 'a' and as no radioactive metabolite showed such a Rf-value, it could be concluded that no oestrogens were formed during this incubation time.

The percentage of ^{3}H-DHEA in Tables 2 and 3 or of ^{3}H-testosterone in Tables 4 and 5 refers to that amount of added hormone, which was not metabolized and remained in its original form.

In the culture medium from the control dishes incubated without cells, there was limited metabolization of dehydroepiandrosterone (0.38–1.36%) to 11α-OH-

Table 1. List of steroids with their ratio to front (Rf)-values after separation using two different mobile phases

Abbreviation	Steroid	a	b
DHEA	Dehydroepiandrosterone	0.55	0.28
Δ5diol	5-androstene-3β,17β-diol	0.45	0.16
Δ4dione	Androstenedione	0.60	0.39
T	Testosterone		0.18
DHT	5α-dihydrotestosterone	0.57	0.61
11α-OH-Δ4dione	4-androstene-11α-ol-3,17-dione	0.16	0.04
11α-OH-T	4-androstene-11α,17β-diol-3-one	0.05	
5α-Ad	5α-androstan-3,17-dione		0.84
Epi-A	5α-androstan-3β-ol,17-one	0.51	
Epi-T	4-androstene-17α-ol,3-one	0.41	0.15
11β-OH-Δ4dione	4-androstene-11β-ol-3,17-dione	0.31	
Androsterone	5α-androstan-3α-ol,17-one		0.60

a, n-hexane/ethyl acetate (v/v 1/1); b, toluene/ethyl acetate/chloroform (v/v/v 1/3/5).

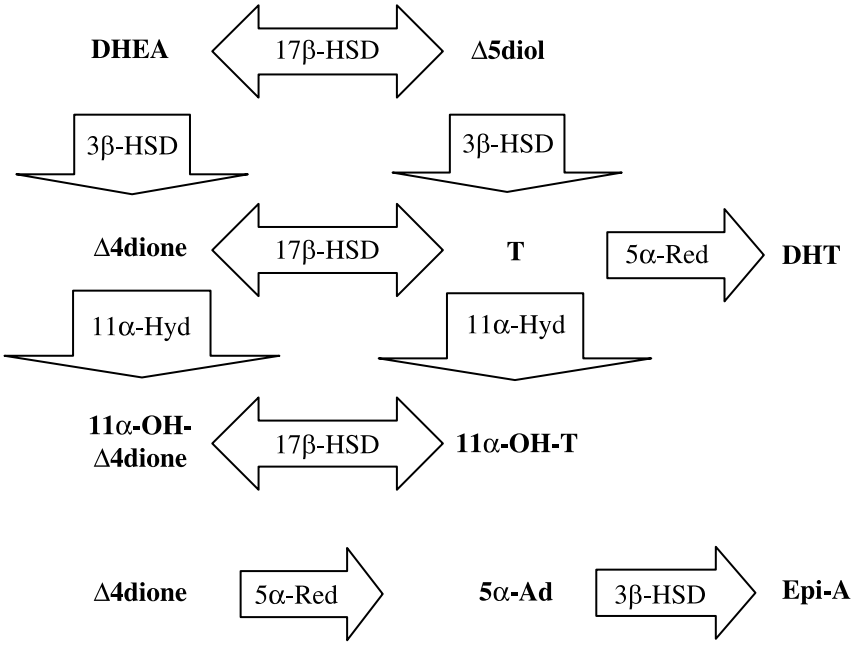

Figure 1. General pathway of the metabolism of C19 androgens in canine DPC and DFB. Androstenedione may serve as a precursor for either testosterone (upper part) or for androstanedione (lower part).

Table 2. Percentage (mean ± SD) of metabolites of ^{3}H-dehydroepiandrosterone in control incubations of medium without cells

		DHEA	11α-OH-T	11α-OH-Δ4dione
		Mean ± SD	Mean ± SD	Mean ± SD
DPC	Flank	97.16 ± 0.57	0.85 ± 0.15	0 ± 0
	Head	97.63 ± 0.38	0.84 ± 0.26	0 ± 0
DFB	Flank	96.83 ± 0.92	1.36 ± 0.36	0 ± 0
	Head	96.46 ± 0.82	1.17 ± 0.29	0 ± 0

testosterone (Table 2) and even less of testosterone (0.40–1.21%) to 5α-dihydrotestosterone and epiandrosterone (Table 4).

In the presence of hair follicle cells (DPC, DFB) the added ^{3}H-dehydroepiandrosterone (Table 3) remained mostly in its original form. However, a high percentage of metabolization was found in DFB of the head, where only 48.80 ± 7.11% remained as original. This was significantly ($P < 0.001$) less than in DFB of the flank or DPC of both locations.

The major metabolite (18.02 ± 3.92–42.23 ± 5.64%) in all incubations with ^{3}H-DHEA was 11α-OH-testosterone (4-androstene-11α,17β-diol-3-one). The percentage of this metabolite was significantly higher ($P < 0.001$) in DFB of the head than in DFB of the flank or DPC of both locations.

Additionally, ^{3}H-DHEA was metabolized to a smaller percentage (0.90 ± 0.51–2.34 ± 1.15%) to 11α-OH-androstenedione (4-androstene-11α-ol-3,17-dione) and another unidentified metabolite (UIM). The percentage of 11α-OH-androstenedione was significantly higher ($P < 0.017$) in DFB of the flank than in DPC of both locations, but it did not differ significantly from DFB of the head. The unidentified metabolite could be found in DFB of the head in significantly higher ($P < 0.001$) percentages than in DFB of the flank or in DPC of both locations (Table 3).

Nonmetabolized ^{3}H-testosterone was the major steroid identified in the culture medium of DPC and DFB of hair follicles of the head and the flank (Table 5). In the medium of both cell types and both body sites only the two metabolites, 5α-dihydrotestosterone (3.74 ± 0.34–5.95 ± 0.93%) and epiandrosterone (1.34 ± 0.96–3.08 ± 0.59%) were detected in small amounts. However, there was no significant difference between the two cell types or the two locations (Table 5).

DISCUSSION

Although the pathophysiology of alopecia resulting from hypothyroidism, hyperadrenocorticism or an oestrogen-producing Sertoli-cell tumour is well understood, the pathomechanism of sex hormone-associated alopecia in dogs with normal plasma concentrations of steroids or growth hormone is still unknown.[12,13] A bilaterally symmetrical alopecia in male dogs with normal, atrophic or cryptorchid testicles, or those which were prematurely castrated also exists.[2] Although no reliable results have been obtained until now, androgens are considered to be important regulators of canine hair growth. Immunohistochemical studies showed that androgen and oestrogen receptors were present in the interfollicular epidermis, outer root sheath and dermal papilla cells, which indicates that canine skin and hair follicles are target organs for these sexual steroids.[7] The percentage of androgen receptor (AR)-positive cells (but not oestrogen receptor (OR)-positive cells) in the epidermis was significantly higher in samples from the thorax and the flank. On the other hand, in the dermal papillae the percentage of OR-positive cells (but not AR-positive cells) was significantly lower in biopsies from the flank. This indicates that there is a difference

Table 4. Percentage (mean ± SD) of metabolites of ^{3}H-testosterone in control incubations of medium without cells

		T	DHT	Epi-A
		Mean ± SD	Mean ± SD	Mean ± SD
DPC	Flank	98.47 ± 0.52	1.14 ± 0.51	0.40 ± 0.02
	Head	98.48 ± 0.75	1.05 ± 0.59	0.47 ± 0.18
DFB	Flank	98.08 ± 1.02	1.21 ± 0.69	0.7 ± 0.34
	Head	98.80 ± 0.20	0.73 ± 0.21	0.47 ± 0.02

Table 5. Percentage (mean ± SD) of metabolites of ^{3}H-testosterone in the medium of incubations with DPC or DFB

		Testosterone	DHT	Epi-A
		Mean ± SD	Mean ± SD	Mean ± SD
DPC	Flank	93.25 ± 1.07	3.74 ± 0.34	1.48 ± 1.12
	Head	92.58 ± 0.83	3. ± 0.88	1.99 ± 0.71
DFB	Flank	93.39 ± 1.45	3.36 ± 1.17	1.34 ± 0.96
	Head	90.13 ± 1.44	5.59 ± 0.93	3.08 ± 0.59

DHT, 5α-dihydrotestosterone; Epi-A, epiandrosterone; mean, arithmetic mean; SD, standard deviation.

Table 3. Percentage (mean ± SD) of metabolites of ^{3}H-dehydroepiandrosterone in the medium of incubations with DPC or DFB

		DHEA	11α-OH-T	11α-OH-Δ4 dione	UIM
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DPC	Flank	77.11 ± 4.26	18.02 ± 3.92	0.90 ± 0.51	1.07 ± 0.15
	Head	74.08 ± 4.31	21.13 ± 4.38	0.99 ± 0.52	1.08 ± 0.10
DFB	Flank	73.12 ± 5.16	19.40 ± 4.99	2.34 ± 1.15	1.34 ± 0.24
	Head	48.80 ± 7.11	42.23 ± 5.64	1.78 ± 0.70	1.81 ± 0.24

DHEA, dehydroepiandrosterone; 11α-OH-T, 4-androstene-11α,17βdiol-3-one; 11α-OH-Δ4 dione, 4-androstene-11α-ol-3,17-dione; UIM, unidentified metabolite; mean, arithmetic mean; SD, standard deviation.

in the percentage of receptor-positive cells between predilection areas of sexual steroid associated dermatopathies (e.g. flank) and other locations (e.g. head).

Several studies have investigated the metabolism of testosterone in subunits of human hair follicles.[14–16] Itami *et al.*[17] incubated human beard DPC and reticular DFB with 50 nM of ^{3}H-testosterone (which approximates the plasma level in adult men) for varying periods up to 10 h. More than 95% of the metabolites were recovered in the medium (as opposed to the cells themselves). In the beard DPC the formation of 5α-dihydrotestosterone (DHT) increased linearly for about 3 h. In the DFB this increase was much smaller, but lasted up to 7 h. The amount of DHT was dominant in DPC, but equivalent to that of androstenedione in DFB. The activity of 5α-reductase (which converts testosterone to DHT) was significantly higher in beard DPC than in other cells (DPC from the occipital scalp, reticular DFB). When Thornton *et al.*[14] incubated cells from hair follicles with radioactive testosterone for 2 h, DHT was formed only in beard DPC. Hamada *et al.*[15] incubated DPC from human beard and pubic hair follicles with 5 nM ^{3}H-testosterone for up to 24 h. Beard DPC secreted large amounts of DHT (55%) into the medium, in contrast to pubic DPC (8.6%). Androstenedione and 5α-androstanedione levels reached 4.4% and 9.2%, respectively, in the beard DPC, but in pubic DPC only 1.7% of androstenedione was formed.

In our study, testosterone was metabolized to DHT only in very small amounts (less than 6%). This is an important finding because it is much less than in human DPC. As no oestrogens could be identified in our incubations of DPC or DFB with ^{3}H-testosterone, the question arises if other androgens might influence hair growth in dogs.

Testosterone itself derives either directly from androstenedione or via androstenediol from DHEA. In healthy 1-year-old male dogs the plasma levels of DHEA were twice as high as those of androstenedione and half of those of testosterone.[18] Therefore, DHEA may well serve as a precursor for the local production of testosterone or oestrogens.

Schmeitzel and Lothrop[19] proposed an imbalance of adrenal steroid production as being responsible for certain forms of bilateral alopecia in Pomeranian dogs. As DHEA is produced by the adrenal gland, we were interested to check if this weak androgen could be metabolized to more potent androgens (e.g. DHT) or to oestrogens in canine hair follicles.

Dogs with a Sertoli cell tumour of the testis frequently develop symptoms of feminization and alopecia. Peters *et al.*[20] found significantly elevated levels of oestradiol-17β and significantly decreased testosterone concentrations in peripheral venous blood of dogs with a Sertoli cell tumour compared to healthy dogs. This change in the systemic concentration may be reflected in the local concentration in the hair follicles as well.

Recently, Frank *et al.*[21] published results obtained in an extensive study comprising 276 dogs of 54 different breeds. This study aimed to determine whether specific steroid hormone aberrations were associated with suspect endocrine alopecia in dogs (excluding hypothyroidism and hyperadrenocorticism). The most frequent (58% of samples) hormone elevation after ACTH-stimulation was that for progesterone. Compared with normal dogs, progesterone baseline levels were greater in Pomeranian and Siberian husky dogs; oestradiol-17β baseline level was greater in Keeshond dogs.

These results support our hypothesis that the local metabolism of steroid hormones within the cells of the hair follicle may be important for the regulation of hair growth.

In contrast to our expectations, our results have shown that DHEA is metabolized predominantly to 11α-OH-testosterone. This is an interesting observation, as we did not find any other metabolite of the common androgen pathway, although all relevant ones were checked.

However, it has to be kept in mind that after 24 h of incubation, a particular metabolite might be present in low concentration, if the reaction kinetics were in favour of the next metabolite. Further studies, using inhibitors of enzymes involved in steroid metabolism should reveal the significance of these findings.

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Résumé Cette étude s'est intéressée au métabolisme de la hydroépiandrostérone (DHEA) et de la testostérone radioactives dans les cellules de la papille dermique (DPC) et les fibroblastes dermiques (DFB) obtenus à partir des follicules pileux de deux régions différentes (tête, flanc) chez quatre Beagle mâles castrés. Une chromatographie en phase liquide a été utilisée pour la séparation et une autoradiographie pour l'identification des métabolites radioactifs. La DHEA a été métabolisée principalement en 11α-OH-testostérone et seulement en quantité minime en 11α-OH-androsténedione et autres métabolites non identifiés. La métabolisation de la DHEA était plus importante au niveau de la tête. La testostérone était métabolisée en faible quantité (moins de 10%) en 5α-dihydrotestostérone et en epiandrostérone et aucune différence significative n'a été observée entre les différents types cellulaires ou les localisations cutanées. Ces résultats montrent clairement que la métabolisation des androgènes dans les DPC et DFB du chien est différente de celle observée dans les cellules des follicules pileux de l'homme.

Resumen Se estudió el metabolismo de la dehidroepiandrosterona radioactiva (DHEA) y la testosterona en células de papila dérmica (DPC) y fibroblastos dérmicos (DFB) derivados de folículos pilosos de dos áreas corporales diferentes (cabeza y flanco) de cuatro perros Beagle macho castrados. Se utilizó la cromatografía con capa fina para la separación y autoradiografía para la identificación de los metabolitos radioactivos. DHEA fue metabolizada principalmente a 11α-OH-testosterona y sólo en una pequeña parte a 11α-OH-androstenediona y otro metabolito no identificado. El mayor porcentaje de metabolización de DHEA se produjo en DFB de la cabeza. La testosterona fue metabolizada sólo en una pequeña parte (menos del 10%) a 5α-dihidrotestosterona y epiandrosterona y no existía una diferencia significativa entre los dos tipos celulares y las dos localizaciones. Estos resultados muestran claramente que la metabolización de andrógenos en DPC caninos y DFB es diferente de la que se observa en células del folículo piloso humano.

Zusammenfassung Es wurde der Metabolismus von radioaktivem Dihydroepiandrosteron (DHEA) und Testosteron in Zellen der dermalen Papille (DPZ) und dermalen Fibroblasten (DFB) von Haarfollikeln zweier verschiedener Körperlokalisationen (Kopf, Flanke) bei vier männlichen, kastrierten Beageln untersucht. Für die Separation wurde Dünnschichtchromatographie und für die Identifikation der radioaktiven Metaboliten Autoradiographie eingesetzt. DHEA wurde hauptsächlich zu 11α-OH-Testosteron und nur zu einem geringen Ausmass zu 11α-OH-Androstendion und anderen, nicht identifizierten, Metaboliten verstoffwechselt. Der höchste Prozentsatz an Metabolismus von DHEA wurde in DFB des Kopfes gefunden. Testosteron wurde nur zu einem geringen Ausmass (weniger als 10%) zu 5α-Dihydrotestosteron und Epiandrosteron metabolisiert und es gab keine signifikanten Unterschiede, weder zwischen den beiden Zelltypen noch zwischen den beiden Lokalisationen. Diese Ergebnisse zeigen deutlich, dass der Metabolismus von Androgenen in caninen DPZ und DFB verschieden von dem in den Zellen des menschlichen Haarfollikels ist.