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EFFECTS OF DIHYDROTESTOSTERONE TREATMENT ON ADRENAL GLAND FUNCTION AND MORPHOLOGY IN ADULT FEMALE GUINEA-PIGS

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Summary—The effect of chronic treatment of female guinea-pigs with dihydrotestosterone (DHT) on growth and function of the adrenal gland and, in particular, on the reticular zone is described. Two groups of 6 young adult, female guinea-pigs were treated with DHT (1 mg/kg dissolved in peanut oil and injected s.c.) for 30 and 60 days. Two other groups of animals, treated only with oil, were used as controls. At the end of treatment, animals were killed and adrenal glands were quickly removed. Plasma levels of pregnenolone, dehydroepiandrosterone (DHA) and its sulfate (DHA-S), 17α-hydroxyprogesterone, androstenedione, testosterone, estradiol, 11-deoxycortisol, androstenedione, DHT and 3α-androstanediol were determined by R.I.A. following celite microcolumn chromatography.

Animals treated for 30 days showed only elevated DHT and 3α-androstanediol plasma levels, whereas animals treated for 60 days also showed increased values of pregnenolone (251 ± 62 vs 193 ± 51 ng/dl; $P < 0.05$), DHA-S ($12{,}046 \pm 4110$ vs 2780 ± 888 ng/dl; $P < 0.001$) and slightly increased values of DHA (110 ± 31 vs 86.5 ± 55.4). In the 30-day-treated animals no histological changes were observed, but in the 60-day-treated group the total size as well as cell volumes of the zona reticularis were significantly increased.

Normal estrous cycles were observed in the 30-day-treated animals whereas the 60-day-treated animals showed a progressive acyclicity during the second month of treatment.

These results indicate that in guinea-pigs, prolonged treatment with DHT induces a growth of the zona reticularis of the adrenal gland associated with increased levels of 5-ene steroids, particularly DHA-S. The mechanisms inducing these modifications are probably mediated by a DHT effect at the hypothalamic–pituitary level. A direct effect of DHT on the zona reticularis, however, cannot be excluded.

INTRODUCTION

The effects of androgens on adrenal gland function and morphology are not well understood. The experimental data are inconsistent, showing an adrenal atrophic effect of androgen treatment in some cases [1–3], and, in a few cases, a trophic action limited to the reticular zone [4]. The choice of androgen and dosage administered, as well as the animal species and its sex could explain these differences.

No data are available from studies using androgens exclusively produced by target tissues. Some evidence of the possible effects of peripherally produced androgens on adrenal gland function are suggested by our results obtained in hirsute women [5]. These results suggested that hirsutism, in some cases, may be an evolving syndrome, involving first androgen metabolism in skin, and then the adrenal gland.

The present investigation is an attempt to evaluate the effects of chronic treatment with an androgen exclusively produced at target tissue level, on growth and function of the adrenal gland. DHT treatment has been chosen because DHT cannot be converted to testosterone (T) and is not aromatizable to estrogens. The guinea-pig has been chosen because its adrenal gland is very large, with a particularly well-developed and distinct zona reticularis, which in adult animals may comprise 35–60% of the whole gland [6, 7]. Furthermore, in the guinea-pig, the estrous cycle is longer than in other laboratory animals, permitting a more accurate evaluation of the effects of treatment on vaginal smears.

Finally, the guinea-pig is similar to humans and unlike other rodents in its adrenal secretion of cortisol, having in its adrenal cortex an active 17-hydroxylase system, which is required also for the production of androgens and estrogens [8].

A protocol was therefore designed to evaluate if chronic DHT (1 mg/kg) treatment of guinea-pig could induce morphological and functional

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Abbreviation and trivial name: 3α-androstanediol (3-Ad): 3α,17β-dihydroxy 5α-androstane.

modifications of the reticular zone of the adrenal gland, supporting the possible relationship between the increased availability of DHT and reticular zone function and growth.

EXPERIMENTAL

Study protocol

The study was performed on 24 young (12–14 weeks) female guinea-pigs (Atromin–Rieger, Bolzano, Italy), each weighing between 450 and 500 g. The animals were divided at random into 4 groups (6 animals each); two as controls and two for treatment purposes. Animals were maintained under standard conditions of light (6.00–18.00 h) and temperature (18°C) on a diet of fresh vegetables and tap water *ad libitum*. Following a period of acclimatization of at least 7 days, vaginal smears were checked in each animal to monitor the estrous cycle. Treatment was started on the first day of proestrous. The animals in the 2 treatment groups received daily s.c. injections of 1 mg/kg body wt of dihydrotestosterone (Sigma Chemical Company, St Louis, Mo., U.S.A., A8380) dissolved in peanut oil (Sigma Chemical Company, St Louis, Mo., U.S.A., P2144). The control animals received an equal amount of oil alone. During treatment vaginal smears were taken every 2–3 days in each animal. The animals of two groups (treated and controls) were killed by decapitation after 30 days treatment. The remaining animals (treated and controls) were killed in the same way after 60 days. Blood was collected in a heparinized glass tube and immediately centrifuged; plasma was stored at −20°C until assayed. Adrenal glands and ovaries were removed quickly, washed and fixed in 3% buffered (0.1 M cacodylate, pH 7.4) glutaraldehyde for 1 day. The method of fixation used was immersion, instead of perfusion, because of the need to collect blood without anesthesia.

Histology

The fixed specimens were washed and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer. Tissue specimens were then dehydrated in a graded acetone series and embedded in Epon 812. Thick sections (1 μm) were stained with toluidine blue and photographed with a Zeiss Ultraphot II. Thin sections were cut on a LKB ultramicrotome and stained with uranyl acetate and lead citrate [9] before viewing in a Zeiss EM9S2 electron microscope.

Morphometry. For each right adrenal, the volume of the whole gland and that of the three cortical zones were determined in serial 5 μm sections obtained from paraffin-embedded tissues. The volumes of the cells and nuclei of the three cortical zones were determined in serial 1 μm sections, mounted on glass slides and stained with toluidine blue.

Measurements were performed on microphotographs taken from capsule to medulla in serially cross-sectioned glands, at 30× and 250× magnification and printed at 7× photographic magnification. Volumes of whole adrenal, that of each cortical zone and that of the cells and nuclei were obtained according to Black and Russo[10] and Weibel[11], with the aid of a Zeiss Mop videoplan digitalizer planimeter. The analytical data were expressed as mean ± SD. Differences were assessed by the Student's t-test and those occurring at a level of $P < 0.05$ were considered significantly different.

Hormonal evaluation

To avoid inter-assay difference, all samples were evaluated for each hormone in one assay. Plasma levels of pregnenolone (5P), 17α-hydroxyprogesterone (17OHP), 11-deoxycortisol (S), androstenedione (A), dihydrotestosterone (DHT), testosterone (T), 3α-androstanediol (3-Ad), estradiol (E2), dehydroepiandrosterone (DHA) and its sulfate (DHA-S), and cortisol (F) were evaluated by R.I.A. as follows:

Pregnenolone, 17α-hydroxyprogesterone, 11-deoxycortisol. 5P, 17OHP and S were determined by R.I.A. following diethyl ether extraction of 1 ml of plasma and celite microcolumn chromatography (0.5 g Analytical Filter Aid celite, BDH, Rome, Italy). The three steroids were eluted sequentially from the same column using the eluting solvents isooctane for 5P, isooctane–ethyl acetate (85:15, v/v) for 17OHP, and isooctane–ethyl acetate (50:50, v/v) for S. R.I.A. of 5P was performed using a highly specific antibody (anti-pregnenolone-3-monohemisuccinate-human serum albumin, Radioassay System Laboratories Inc., Carson, Calif., U.S.A.) at a final dilution of 1:7000. R.I.A. of 17OHP was performed using a commercial kit (Bio Merieux, Charbonnieries les Bains, France). R.I.A. of S was performed using a highly purified antibody (anti-11-deoxycortisol-3-carboxymethyloxime-bovine serum albumin, Radioassay System Laboratories Inc., Carson, Calif., U.S.A.), at a final dilution of 1:15,000. Recoveries of the labeled tracers (means ± SD) were 80 ± 4% for 5P, 75 ± 4.8% for 17OHP, and 62 ± 4% for S. The intra-assay variabilities (means ± SD) were 6.5 ± 1% for 5P, 4.5 ± 0.8% for 17OHP, and 6 ± 1.1% for S.

Androstenedione, dihydrotestosterone, testosterone, 3α-androstanediol, estradiol. Plasma levels of A, DHT, T, 3-Ad and E2 were evaluated by RIA following ether extraction of 1 ml of plasma and celite microcolumn chromatography (1 g 535 celite BDH). The five steroids were eluted sequentially from the same column using the eluting solvents isooctane for A, isooctane–benzene (85:15, v/v) for DHT, isooctane–benzene (60:40, v/v) for T, isooctane–benzene (40:60, v/v) for 3-Ad, and benzene for E2. R.I.A. of A and E2 was performed using a commercial kit (Bio Merieux, Charbonniers les Bains, France). R.I.A. of DHT was performed using

a highly purified antibody (5α-dihydrotestosterone-3-*O*-carboxymethyloxime-bovine serum albumin, Biodata–Serono, Rome, Italy) at a final dilution of 1:25,000. R.I.A. of T was performed using a commercial kit (Sorin, Saluggia, Italy). R.I.A. of 3-Ad was performed using a highly purified antibody (5α-androstan-3α-diol-15-carboxymethyloxime-bovine serum albumin, Biodata–Serono, Rome, Italy) at a final dilution of 1:30,000. Recoveries of labeled tracers were 85.7 ± 9% for A, 60.2 ± 6.4% for DHT, 78.6 ± 9.6% for T, 61.5 ± 7.2% for 3-Ad and 65.3 ± 5.7% for E2. Intra-assay variabilities were 5 ± 0.9% for A, 4.3 ± 1.2% for DHT, 6.3 ± 1.5% for T, 5.1 ± 1.3% for 3-Ad and 7.5 ± 2% for E2.

Dehydroepiandrosterone. Plasma levels of DHA were evaluated by R.I.A. following diethyl ether extraction of 1 ml of plasma and celite microcolumn chromatography (0.5 g Analytical Filter Aid celite, BDH, Rome, Italy), using the eluting solvent isooctane–ethyl acetate (92:8, v/v). R.I.A. was performed using a highly specific antibody (anti-dehydroepiandrosterone-15β-carboxyethylmercapto-bovine serum albumin, Radioassay System Laboratories Inc., Carson, Calif., U.S.A.), at a final dilution of 1:35,000. Recovery of labeled tracer was 79 ± 13 (SD) %. Intra-assay variability was 4.5 ± 1.3 (SD) %.

Cortisol and dehydroepiandrosterone sulphate. Plasma F and DHA-S were determined directly by R.I.A. using for the former a commercial kit (Biodata–Serono Rome, Italy) and for the latter a commercial kit (Bio, Merieux, Charbonnieres les Baines, France). The intra-assay variabilities were 3.2 ± 1 (SD) % for F and 4.5 ± 2 (SD) % for DHA-S.

Reagents and solvents for extraction and chromatography were of analytical grade and were used without further purification. Each batch was tested before use for solvent blank determination.

Statistical analysis

Student's *t*-test was used to compare values in treated animals with those in the controls and values in 30-day-treated animals vs 60-day-treated animals.

Results are expressed as mean ± SD. Differences occurring at a level of $P < 0.05$ were considered statistically significant.

RESULTS

No differences were observed between the two control groups.

30-day-treatment

Results obtained following 30 days of treatment demonstrated persisting cyclicity of the estrous cycle as indicated by vaginal smear evaluation and no changes in adrenal gland morphology.

Body weights increased in both treated and control animals, reaching 635.8 ± 51 g and 632.5 ± 10.6 g, respectively, vs basal values of 497.2 ± 28 g and 491.7 ± 10.4 g. The increased body weight was not significantly different between the two groups.

No histological differences were observed between the treated and control animals both in the adrenal glands and in the ovaries.

Hormonal patterns in treated animals showed values corresponding to those in control animals with the exception of plasma DHT and 3-Ad, which were significantly higher ($P < 0.001$) in treated animals than in controls (Fig. 1).

60-day-treatment

Results obtained after 60 days of treatment showed a progressive acyclicity of estrous cycle, as demonstrated by the persistence of vaginal smear in metestrous.

No statistically significant differences in body weight were observed. Body weights increased in both

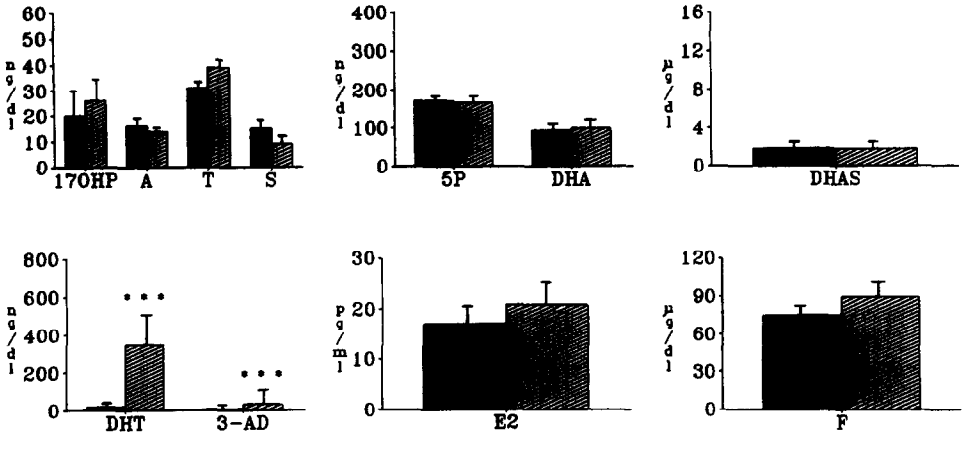

Fig. 1. Hormonal pattern in 30-day-treated (hatched bars) and control animals (filled bars). 17OHP: 17α-hydroxyprogesterone; A: androstenedione; T: testosterone; S: 11-deoxycortisol; F: cortisol; 5P: 5-pregnenolone; DHA: dehydroepiandrosterone; DHA-S: dehydroepiandrosterone sulfate; DHT: dihydrotestosterone; 3-AD: 3α-androstanediol; E2: estradiol. ***$P < 0.001$.

treated and control animals, reaching 759 ± 18.1 g in treated animals and 750 ± 8 g in controls vs basal weights of 498 ± 23 and 488 ± 10 g, respectively.

In the control group, histological examination of the whole adrenal gland showed a mean volume of 142 ± 8.4 mm^3 and revealed a small medullary region and a larger cortical region. In the cortical region three distinct zones could be detected: the outer zone, zona glomerulosa, comprising about 10% of the whole gland; the middle zone, zona fasciculata, comprising about 55%; and the inner zone, zona reticularis, comprising about 25%.

The adrenal glands of the treated animals showed a mean volume of 150.8 ± 10.1 mm^3. In the cortical region a different distribution of the three zones was observed. The zona glomerulosa showed no significant change compared to controls, whereas the zona reticularis showed an increased volume, comprising about 37–40% of the whole gland and the zona fasciculata was decreased to about 45% of the control volume.

Histological examination revealed a typical cortical morphology in both control and treated animals. Some differences between the two groups were observed, however.

Zona glomerulosa. The narrow zona glomerulosa of both control and treated animals, observed by light microscopy, showed only few small polyhedral cells characterized by an indented nucleus (Plate 1A).

On morphometrical analysis, glomerulosa cells of treated animals showed a reduced mean volume compared to controls. No differences were observed in the mean volume of the nucleus (Table 1).

Electron microscopic observations showed that glomerulosa cells, in both control and treated animals, were characterized by spheric and ovoid mitochondria with lamellar cristae, rough and smooth endoplasmic reticulum, free ribosomes, polysomes and few lipid droplets, peroxisomes and lysosomes (Plate 1C, D).

Although the glomerulosa cells showed a slight, but significant change in mean volume following experimental treatment, no qualitative differences in the arrangement and morphology of their cellular organelles were observed.

Zona fasciculata. This zone of radiating cords was characterized by larger cells, with round nuclei and numerous lipid droplets, in both control and treated animals (Plate 1B). In this zone, as in the zona glomerulosa, morphometrical analysis showed a significant reduction of the mean volume of the cells of the treated animals compared to controls. No differences were observed in the mean volume of the nuclei (Table 1).

By electron microscopy the cells showed a greater amount of smooth endoplasmic reticulum membranes than in the zona glomerulosa. Zona fasciculata cells also possessed mitochondria with rectolineal or contorted cristae, lysosomes and peroxisomes (Plate 1E, F). No qualitative differences were observed in the ultrastructural morphology of cellular organelles, although the fasciculata cells of the treated animals showed a significant reduction in the volume density.

Zona reticularis. The zona reticularis cells of treated animals differed substantially between the two groups. When observed by light microscopy the control cells exhibited a polyhedral shape with cytoplasm containing sparsely distributed lipid droplets. The cells were arranged in clusters surrounded by a complex and tortuous network of sinusoids (Plate 2A). In the zona reticularis of treated animals (Plate 2B), the cells appeared hypertrophic and very closely packed in voluminous clusters, so that the sinusoids appeared compressed and showed a reduced area (or caliber) compared to controls (Plate 2A).

On morphometric analysis a significant ($P < 0.001$) increase of cellular and nuclear volumes in treated animals was found (Table 1).

Under electron microscopy the zona reticularis cells of control animals had rounded, slightly irregular nuclei. In the cytoplasm, mitochondria with tubular and lamellar cristae and well developed smooth endoplasmic reticulum arranged in closely packed patches of membranes and tubular areas were identified. Polysomes, peroxisomes and lysosomes were also present, while lipid droplets were scarce (Plate 2C). In treated animals, on the other hand, zona reticularis cells appeared as larger elements with a rounded, more voluminous nucleus with numerous nucleoli, but ultrastructural observation did not show substantial differences with control animals (Plate 2D).

As far as the ovaries were concerned, observation by light microscopy revealed a large number of follicles in various stage of atresia, few growing follicles and absence of corpora lutea.

In the 60-day treatment group evaluation of the hormonal pattern (Fig. 2) revealed significantly increased values of 5P (251 ± 62 vs 193 ± 51.1 ng/dl, $P < 0.05$) and DHA-S (12,046 ± 4110 vs 2780 ± 800 ng/dl, $P < 0.01$); or slightly increased values of DHA (110 ± 30.8 vs 96.5 ± 55.4 ng/dl), and significantly ($P < 0.01$) reduced levels of E2 (7.9 ± 2.1 pg/ml) compared with the control group and with the 30-day treatment group. The remaining steroids showed no differences with respect to control and to 30-day-treated animals. DHT and 3-Ad were significantly higher in the 60-day-treated group than in the control group, but no difference was seen with respect to the 30-day-treated group.

DISCUSSION

This work provides clear evidence that in the adult female guinea-pig prolonged treatment with 1 mg/kg DHT induces growth of the zona reticularis of the adrenal gland and increased levels of plasma 5-ene steroids. The results disagree with the majority of reports in the literature which describe atrophy of

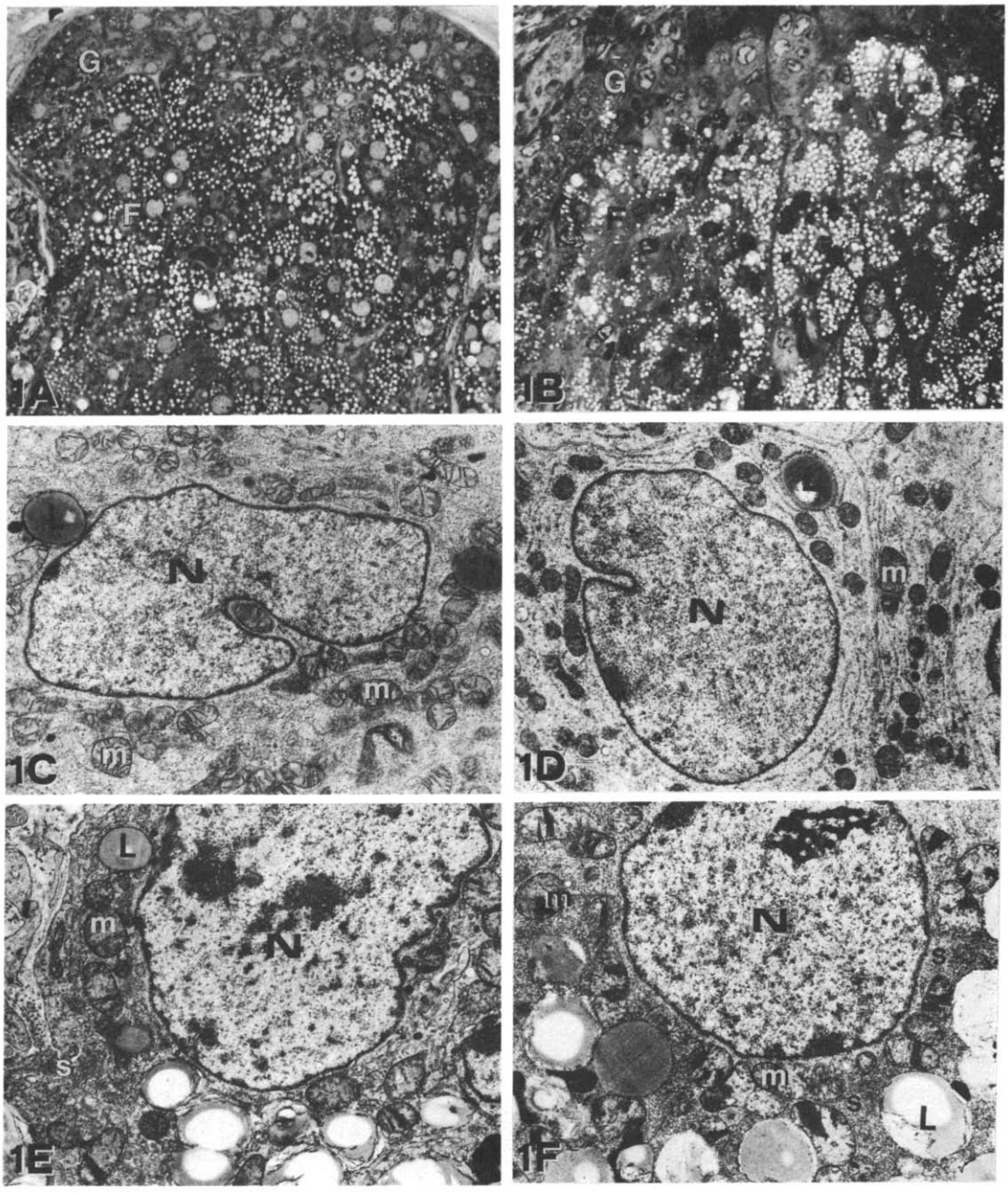

Fig. 2. Adrenal cortex, zona glomerulosa (G) and zona fasciculata (F). (A) Light microscopy. Control animal. (B) Light microscopy. Treated animal. In both, control and treated animals, zona glomerulosa is formed only by few polyedral small cells with scarce lipids droplets, while the radiating cords of the zona fasciculata are characterized by larger cells with round nuclei and abundant lipid droplets (A, B 250×). (C, D) Electron microscopy—zona glomerulosa—control animal (C), treated animal (D). The cells of both treated and control animals show indented nuclei (N), mitochondria with laminal cristae (m) and few lipid droplets (L) (C, D 6800×). (E, F) Electron microscopy—zona fasciculata—control animal (E), treated animal (D). The cells of both treated and control animals show round nuclei (N), mitochondria with rectilineal and contorted cristae (m), abundant lipid droplets (L) and smooth endoplasmic reticulum (s) (E, F 6800×).

the male rat adrenal gland after androgen treatment [1–3], but are consistent with effects reported in castrated male hamsters [4]. The choice of androgen, dosage used, animal species and sex may account for these differences. Our results may also explain the larger size of the zona reticularis in the normal male guinea-pig compared to the female. To our knowledge, no data are available on DHT treatment and adrenal gland growth and function in guinea-pigs. Our observation that androgens produced by target tissues may have significant effects on the zona reticularis of the adrenal gland is supported by

Table 1. Total adrenal gland volume (including medulla) (mm^3), volumes of the three zones (mm^3), of the cells and nuclei volume density (μm^3) of the adrenal cortex zones, in guinea-pigs treated for 60 days and in controls

Adrenal gland	Controls	Treated	*P*
Total volume (mm^3)	142.4 ± 8.4	150.8 ± 10.1	NS
Zona Glomerulosa			
Volume of the zone (mm^3)	15.8 ± 0.7	15.5 ± 0.8	NS
Volume of cells (μm^3)	3527.4 ± 315	3117.5 ± 168	0.01
Volume of the nuclei (μm^3)	1430.3 ± 105	1413.3 ± 148	NS
Zona Fasciculata			
Volume of the zone (mm^3)	75.9 ± 2.4	68.5 ± 1.9	0.05
Volume of cells (μm^3)	16936.1 ± 2315	15116.3 ± 1206	0.01
Volume of the nuclei (μm^3)	1575.3 ± 197	1632.7 ± 205	NS
Zona Reticularis			
Volume of the zone (mm^3)	40.1 ± 1.1	56.3 ± 3.9	0.001
Volume of cells (μm^3)	15751.8 ± 1350	20827.6 ± 2825	0.001
Volume of the nuclei (μm^3)	1518.2 ± 212	1776.7 ± 221	0.001

Mean ± SD. NS, not significant.

previous results obtained in this laboratory indicating that hirsutism, in some cases, could be considered an evolving syndrome, involving progressively, skin androgen metabolism, adrenal gland and ovary [5].

In the guinea-pig, the outer and the inner zones of the adrenal gland have a clear morphological and functional differentiation [6, 12–20]. In response to DHT treatment the outer and the inner zones showed different responsiveness. In female guinea-pigs, the treatment with DHT induced growth of the zona reticularis secondary to a significantly increased volume of its cells and nuclei, while a slight reduction in the volume of the cells of the zona fasciculata was observed, at least during the period of our observation (60 days). The increase in the volume of the cells and the nuclei of zona reticularis was associated with normal cell ultrastructure. Since electron microscopy did not reveal signs of involution or degeneration the significantly increased volume of the cells and nuclei of the zona reticularis may correlate very well with increased 5-ene steroid production, particularly DHA-S. Other authors [21] working with guinea-pig adrenals, have shown that DHA-S synthesis predominates in the zona reticularis. In addition, in guinea-pig adrenal gland homogenate DHA has been found in amounts similar to those found in human adrenal homogenate [22]. In the present study plasma DHA and DHA-S were found in much smaller amounts than the levels found in man. The presence in guinea-pig adrenal gland of a specific binding-protein, as exists for pregnenolone [23], could modulate the output of DHA and DHA-S into the circulation, thus explaining the differences between values in adrenal gland homogenate and those in plasma. Our plasma steroid results are in agreement with those of Rivarola *et al.*[24], but are not in agreement with other studies performed *in vivo* [25] and *in vitro* [8]. The differences with *in vivo* results may be explained by the younger age of the animals, their sex and different chromatographic methods performed before R.I.A. On the other hand, several criticisms [13] have been advanced to the *in vitro* study [8], focusing on the lack of formation of DHA and DHA-S from labeled pregnenolone in dispersed cells of different zones of the guinea-pig adrenal gland.

The methods used for hormonal analyses in this study have been accurately validated, and to avoid inter-assay difference, all samples were evaluated for each hormone in one assay.

Although tissue fixation by perfusion is the optimal method for the preparation of the tissue for morphometric analysis, it could not be used in our work because of the need to collect blood for hormonal evaluation without anesthesia [25]. On the other hand, the morphological modifications observed only in the zona reticularis of the adrenal gland of 60-day-treated animals and not in that of other groups (both control groups and 30-day-treated animals) support the validity of the method. 60-day-treated animals showed increased plasma values of 5-ene steroids (5P, DHA and DHA-S particularly), but no differences in DHT and 3-Ad values when compared to the 30-day group. The high levels of plasma DHT reached during the treatment indicate that the effects shown are pharmacological.

The increased values of 5-ene with respect to 4-ene steroids suggest a decreased activity of 3β-ol dehydrogenase. In the 5-ene pathway the increased values of DHA and DHA-S, particularly, reflect increased 17α-hydroxylase, 17–20 lyase and sulfatase activities. A similar enzymatic pattern (reduced 3β-ol dehydrogenase activity and increased 17α-hydroxylase and 17–20 lyase) has been found to be characteristic of adrenal steroidogenesis during the adrenarche in man [28, 29], a process known to occur concomitantly with the development of the reticular zone of the adrenal gland, probably in response to a pituitary factor other than ACTH [30].

As far as the mechanism of DHT action is concerned, the lack of statistical differences in weight between control and treated animals of both groups excludes the possibility that the growth of the zona reticularis represents only one aspect of a generalized anabolic effect of DHT administration. It might be very interesting, therefore, to consider

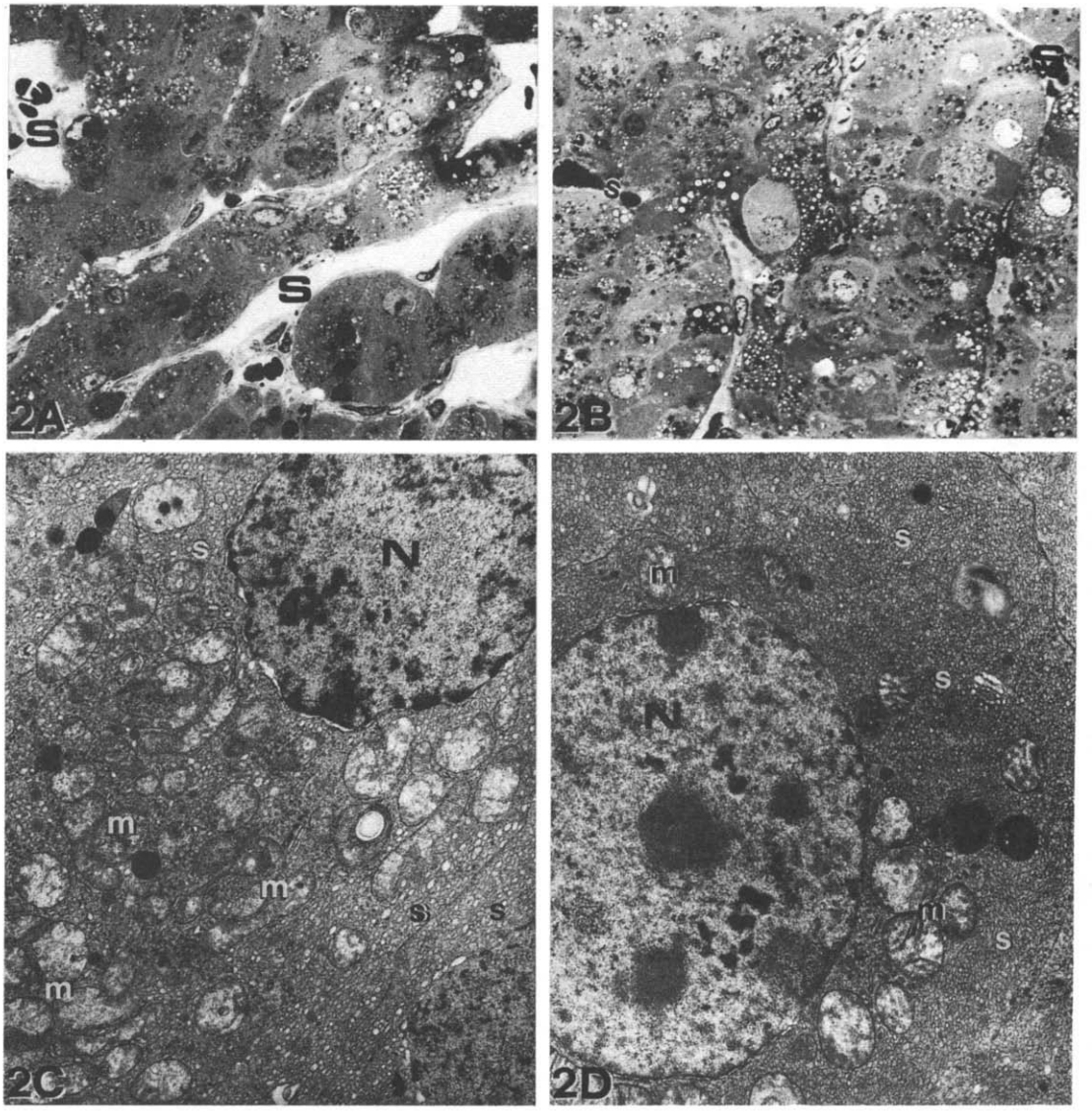

Fig. 3. Adrenal cortex, zona reticularis. (A) Light microscopy. Control animal. The cells have a polyedral aspect; their cytoplasm contain lipid droplets. Large sinusoids (S) surround the clusters of cells (400 ×). (B) Light microscopy. 60-days-treated animal. The cells appear hypertrophic and very closely packed in clusters. The sinusoids (S) appear compressed (400 ×). (C) Electron microscopy. Control animals. Note the presence of a rounded, irregular nucleus (N). In the cytoplasm mitochondria with tubular cristae (m) and well developed smooth endoplasmic reticulum (s) are present (10,700 ×). (D) Electron microscopy. 60-days-treated animal. Note the presence of rounded voluminous nucleus (N), and a large amount of smooth endoplasmic reticulum membranes (s). m: mitochondria (10,700 ×).

how DHT could induce the growth of the zona reticularis in the 60-day-treated animals. Two possible mechanisms could be considered; a direct or indirect action mediated by the hypothalamic–pituitary axis.

A direct DHT action at the adrenal level is possible because androgen receptors have been detected in total adrenal gland homogenate, both in male and female rats and not in tfm rats [31].

The indirect action, mediated by the hypothalamic–pituitary axis, seems to be more acceptable. The possibility of DHT uptake and metabolism on the neural cell of the guinea-pig has already been demonstrated [32]. The DHT effect at the hypothalamic–pituitary level could in fact explain the morphological and functional modifications both at the ovary and at the adrenal gland level.

The finding of normal cortisol, unchanged mean volume of the zona glomerulosa and slightly reduced mean volume of the zona fasciculata support the hypothesis that the isolated growth and hyperfunction of the zona reticularis may be mediated by a pituitary factor other than ACTH. The possibility that a pituitary factor(s) other than ACTH could play a role in the growth and maintenance of the zona reticularis has already been suggested in the

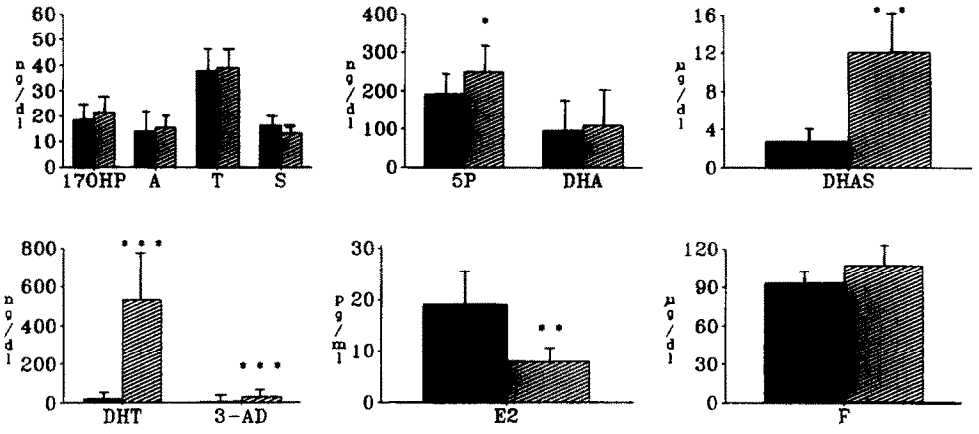

Fig. 4. Hormonal pattern in 60-day-treated (dotted bars) and control animals (filled bars). 17OHP: 17α-hydroxyprogesterone; A: androstenedione; T: testosterone; S: 11-deoxycortisol; F: cortisol; 5P: 5-pregnenolone; DHA: dehydroepiandrosterone; DHA-S: dehydroepiandrosterone sulfate; HT: dihydrotestosterone; 3-AD: 3α-androstanediol; E2: estradiol. $*P < 0.05$ $**P < 0.01$ $***P < 0.001$.

guinea-pig, reticularis cells having a low responsiveness to ACTH or to exogenous cAMP [27, 33, 34].

In conclusion, our results demonstrate that in adult female guinea-pigs, prolonged DHT treatment induced an isolated growth of the zona reticularis of the adrenal gland with increased production of 5-ene steroids and DHA-S particularly, probably through a hypothalamic–pituitary-mediated action. Further evidence for a different morphological and functional control of the outer and inner zone of the guinea-pig adrenal gland is provided by this paper.

However DHT may exert its action, our results demonstrate that the administration of an androgen, almost exclusively produced at target tissue level, such as DHT, is able to induce an isolated growth of the zona reticularis of the adrenal gland and to increase the 5-ene steroid production, particularly DHA-S. These results, therefore, further support our previous data [5] in hirsute women in which hirsutism appears to be an evolving syndrome, involving progressively skin androgen metabolism and then adrenal gland function.

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