ANDROSTENEDIONE AND TESTOSTERONE BIOSYNTHESIS BY THE ADRENAL CORTEX OF THE HORSE

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-ABSTRACT-

An homogenate from cortical tissue of mare adrenals was incubated in the presence of tritiated pregnenolone. The (^{3}H) androstenedione and the (^{3}H) testosterone synthesized during the incubation were extracted, purified, and co-crystallized to constant specific activity in the presence of unlabeled carriers. The rate of conversion of pregnenolone to androstenedione and testosterone was of the order of 5 and 0.15 per cent respectively. The high ratio of (^{3}H) androstenedione to (^{3}H) testosterone observed in this study suggests that androstenedione is the main androgen produced by mare adrenals.

It is concluded that adrenals could contribute to the production of blood androgens in normal as well as hyperandrogenic mares.

INTRODUCTION

Testosterone has been identified by mass spectrometry in the blood and follicular fluid of the mare (1). Its presence in large amounts in the follicular fluid and the fluctuations of its level throughout the estrus cycle (2) suggest an ovarian origin for at least part of its production.

The general structure of the equine adrenal resembles that of other mammals in having a capsule, a cortex and a medulla (5). Cells from the zonae fasciculata and reticularis of the cortex have been found to have the ability to transform pregnenolone to cortisol in vitro (6), but the

ability of the mare adrenal cortex to synthesize androgen has not been investigated.

However, the administration of a synthetic glucocorticoid (dexamethasone) has been found to lower the peak level of dehydroepiandrosterone occuring during estrus in the cycling mare (3) and to reduce the level of circulating testosterone in an infertile hyperandrogenic mare (4). Ginther (3) has suggested that the occasional occurence of estrus behaviour in ovariectomized mare could result from adrenal secretion of sex steroids.

The present work was undertaken to assess the ability of the adrenal cortex of the mare to synthetize androstenedione and testosterone from pregnenolone in vitro.

MATERIAL AND METHODS

Adrenals

Mare adrenals were obtained at the local slaughterhouse within 30 min of slaying and stored at 0°C until incubation. The glands were freed from fat and other adhering tissue and the cortex was isolated by careful blunt separation from the medulla, which was discarded.

Histology

In order to ascertain the absence of medulla cells, small pieces of cortex were fixed in Bouin's solution and embedded in wax. Sections 4 µm thick were stained with Masson trichrome-staining (7).

Incubation

The cortical tissue was minced with scissors and homogeneized in Krebs-Ringer bicarbonate buffer, pH 7.4,containing 0.01 M glucose. About 5 ml of the homogenate corresponding to 2.5 g of tissue was incubated in the presence of 10 IU glucose-6-phosphate dehydrogenase and 50 µCi of 7α-(^{3}H)pregnenolone (specific activity: 10 Ci/mmol - Radiochemical Centre Amersham) in 0.25 ml propylene-1,2-glycol. Duplicate beakers were incubated at 37°C for 2 h in a metabolic incubator in an atmosphere of 95 % oxygen - 5% carbon dioxide and incubation was terminated by freezing. The material was kept at - 20°C until analysis. Experiments were performed with adrenal tissue from two mares. Each experiment included one control sample consisting of all the incubation ingredients except the tissue homogenate.

Extraction

After thawing the incubation mixture, 2.5 µmol of testosterone (Sigma) and 2.5 µmol of androstenedione (Steraloids) were added to the medium as unlabeled carriers. The pH was adjusted to 10 with 1 M NaOH and the steroids were extracted with 3 x 30 ml of diethyl ether (Merck DARMSTADT). The ether extracts were washed with distilled water and evaporated to dryness under nitrogen. The residue was used for chromatography and steroid identification.

Chromatography

Whatman N° 1 paper was used for all chromatographic steps. The solvent systems used were system I : ligroin - propylene glycol (20:80 v/v) (8,9) and system II (Bush A): petroleum ether-methanol-water (50:40:10 v/v/v) (10).

Acetylation

Acetylation was performed according to the procedure of Hammerstein et al.(9).

Detection and identification of steroids

Testosterone and androstenedione were detected on paper chromatograms using a short wave (254 nm) ultraviolet lamp. Pregnenolone was visualized by antimonium trichloride (Merck, Darmstadt) in chloroform (20:100 v/v), after heating to dryness at 90°C for 4 min. Radioactive areas were located on chromatograms using a radiochromatogram scanner (Packard). The zones corresponding respectively to pregnenolone-androstenedione and to testosterone were eluted with dichloromethane-methanol (1:1 v/v). The chemical purity of the labeled steroid was checked by co-crystallisation to constant specific activity in acetone after addition of 25-30 mg unlabeled carrier steroids. Radiochemical homogeneity was considered satisfactory when a difference of no more than 10 % was obtained between the specific activities of the crystals in two successive crystallizations and between the specific activities of the crystals and the mother liquor in the last crystallization. Androstenedione and testosterone recoveries were estimated before the addition of unlabeled carrier for co-crystallization by measuring their maximal absorption in methanol at 240 nm as well as at 230 and 250 nm. A corrected absorbancy was calculated by means of the Allen formula (11).

RESULTS

Histological examination of the material used for incubation confirmed that it was composed of adrenal cortex only, without any contamination by the medulla cells.

Chromatography of the incubated extract in the ligroin-propylene

glycol system for 48 h yielded two main radioactive zones. Zone I, containing testosterone and 17-hydroxyprogesterone was eluted, acetylated and chromatographed in system II for 4 hours to separate the testosterone acetate from 17-hydroxyprogesterone. Zone II, containing androstenedione and pregnenolone was eluted, acetylated and chromatographed in system II for 4 hours to separate the androstenedione from pregnenolone acetate.

The data relative to co-crystallization with additional unlabeled steroids are presented in table 1. The specific activity of (^{3}H) androstenedione was constant after three cristallizations (experiment 1) and

TABLE 1

Co-crystallizations of (^{3}H) androstenedione and (^{3}H) testosterone acetate after additions of unlabeled carriers.

		Specific activities (dpm/mg)					
	(Starting activities)	1°CRYST		2°CRYST		3°CRYST	
		C	ML	C	ML	C	ML
D4 I	11500	9300	14400	8700	10500	8500	9200
II	15900	14000	15800	14600	14700	-	-
Ta I	1350	1210	1640	1240	1250	-	-
II	1430	1310	1500	1310	1350	-	-

Specific activities of crystals (C) and mother liquors (ML) in dpm/mg (Cryst : crystallizations , D4 : Androstenedione , Ta : Testosterone Acetate, I and II : experiments 1 and 2).

two crystallizations (experiment 2). The recoveries of the unlabeled carrier were respectively 62 % and 50%. The calculated rates of conver-

sion of (^{3}H) pregnenolone to (^{3}H) androstenedione were 4.8 % and 5.1 %. In the two experiments, the specific activity of testosterone acetate was constant after two crystallizations. The recoveries of the unlabeled carrier were 64 % and 44 %. The rates of conversion of (^{3}H) pregnenolone to (^{3}H) testosterone were 0.13 % and 0.15 %.

DISCUSSION

The whole adrenal cortex (zonae glomerula, fasciculata and reticularis) was used in the present experiment. While certain studies attribute androgen production to the zona reticularis, recent results tend to support the concept that probably both zonae fasciculata and reticularis are important and differences may only be quantitative (12).

The ability of the mammalian adrenal cortex to convert pregnenolone into steroids of the C_{19} group has been investigated in the rat by Askari (13). The incubation of rat tissue with (^{3}H) pregnenolone yield a higher (^{3}H) androstenedione to (^{3}H) testosterone ratio in males than in females (=4 to 9) leading Askari to conclude that, in the rat, male adrenals synthesize more androstenedione than female adrenals. The very high ratio obtained in our experiment (=33) suggests that in the mare, as in other mammals, androstenedione is the main androgen produced by the adrenals. However, it is not possible to draw any firm quantitative conclusion from the our results since the endogenous steroids pools could not be evaluated.

In conclusion, the incubation of tissue homogenate has made it possible to ascertain the ability of the mare adrenal cortex to convert pregnenolone into androstenedione and testosterone in vitro. As in other mammals, the adrenals could contribute to the production of these circu-

lating androgens in normal or hyperandrogenic mares.

Trivial and systematic names of steroids

androstenedione: 4-androstene-3,17-dione
dehydroepiandrosterone: 3β-hydroxy-5-androsten-17-one
dexamethasone: 9-fluoro-11β,17,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione
17-hydroxyprogesterone: 17-hydroxy-4-pregnene-3,20-dione
pregnenolone: 3β-hydroxy-5-pregnen-20-one

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