Direct Metabolism of 7α-^{3}H-Dehydroepiandrosteron-^{35}S-sulphate in Human Ovarian Tissue

Only recently the direct aromatization of C_{19}-steroid sulphoconjugates has been demonstrated by in vivo[1] as well as in vitro experiments[2]. Hence, it seemed of interest to assay steroidogenic tissue for such enzyme systems, capable of direct transformation of dehydroepiandrosterone (DHEA) sulphate.

For this purpose ovarian tissue, obtained from a 38-year-old female subject with collum carcinoma during the early luteal phase of her regular cycle, was incubated with double-labelled dehydroepiandrosterone sulphate. 11.5 g of tissue were homogenized in 0.2*M* phosphate buffer of pH 7.2 (5 v/w) and the suspension centrifuged for 5 min at 3000 rpm for removal of cell debris. As substrate 33.2 μg Na-7α-^{3}H-DHEA-^{35}S-sulphate with 2,510,000 dpm ^{3}H and 1,250,000 dpm ^{35}S (^{3}H/^{35}S = 2.01) were added to each of two 25 ml aliquots of the supernatant equivalent to 5 g of wet tissue for duplicate incubation. After 90 min at 37 °C the fraction of free steroids, extracted with 3 × 2 vol. methylene chloride contained a mean of 169,000 dpm ^{3}H or 6.71% of incubated ^{3}H-activity. In the fraction of steroid sulphatides and sulphates – obtained by extraction with chloroforme-methanol[3] and separated by chromatography on DEAE-Sephadex A-50[4] and thin-layer chromatography on silica gel G in chloroforme-methanol-ammonia (20:5:0.2 v/v; = system A) or (5:15:0.2 v/v; = B) – 1,230,000 dpm ^{3}H (^{3}H/^{35}S = 2.09) and 490,000 dpm ^{3}H respectively. (^{3}H/^{35}S = 2.10) could be detected, corresponding to 49.05% and 19.45% respectively of original ^{3}H-activity. By additional thin-layer chromatography on silica gel G in chloroforme-methanol-ammonia (10:10:0.2; = C) and paper chromatography in diisopropyl ether-petrol ether-t-butanol-ammonia-water (5:2:3:1:9 v/v; = D) and ethylacetate-*n*-butanol-ammonia-water (9:1:1:9 v/v; = E) the following double-labelled conjugates were isolated (Table I). Subsequently these fractions were submitted to ether solvolysis[5] and the liberated steroids separated by thin-layer chromatography on silica gel G in chloroforme-dioxane (94:6 v/v; = F), paper chromatography in propylene glycol-methylcyclohexane (G) or in the case of phenolic steroids by thin-layer chromatography on silica gel G in ethylacetate-cyclohexane (1:1 v/v; = H) and benzene-ethanol (9:1 v/v; = J). For further characterization the ^{3}H-labelled individual compounds were diluted with known amounts of the non-labelled standard and purified to constant specific activity: androstendiol and androstentriol in system H and K (paper chromatography in propylene glycol-toluene) and estradiol in system F and L (thin-layer chromatography on silica gel G in cyclohexane-ethylacetate 1:2 v/v).

17-keto steroids were converted into the 2,4-dinitrophenyl-hydrazones[6] and chromatographed in system F and M (thin-layer chromatography on silica gel G in chloroforme). The specific activity was considered constant when in the course of at least 2 chromatographic steps the variations did not exceed 10%. Quantitative analysis of ^{3}H and ^{35}S was achieved by simultaneous counting in a Packard 'Tricarb' spectrometer, Mod. 3004.

As can be seen from Table I, neutral C_{19}-steroids accounted for 95.6% of the ^{3}H-activity in the fraction of sulphoconjugates, 86.9% thereof being represented by DHEA. Ring-D hydroxylated C_{19}-steroids prevailed in the remaining portion. From the fraction of phenolic steroids, amounting to 4.4% of sulphoconjugates, estradiol and estriol were isolated and properly identified, whereas E_{x1} and E_{x2} escaped identification, E_{x1} exhibiting a polarity between that of estradiol and estriol and E_{x2} being more polar than estriol. Due to the fact that all these C_{19}- and C_{18}-steroids were derived from the sulphoconjugates with a practically unchanged ^{3}H/^{35}S ratio, the direct transformation of DHEA-sulphate to C_{19}-steroid metabolites and estrogens appears to be established also for ovarian tissue.

In Table II, the composition of the free steroid fraction is depicted, indicating a significant sulphatase activity in ovarian tissue, as postulated by other authors[7]. Of the

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Table I. Double labelled metabolites of 7α-^{3}H-DHEA-^{35}S-sulphate after incubation with human ovarian tissue

Steroid	As sulphoconjugate			After solvolysis	
	dpm ^{3}H	dpm ^{35}S	(^{3}H/^{35}S)	dpm ^{3}H	% of ^{3}H-activity
Dehydroepiandrosterone (DHEA)	1,335 000	664,000	(2.01)	1,099,000	86,901
Androstendione	7,450	3,545	(2.10)	6,050	0.476
Testosterone	6,750	3,380	(2.01)	4,810	0.381
Estrone[a]	330	143	(2.30)	120	0.009
Estradiol	8,380	4,290	(1.95)	6,860	0.541
Androstendiol	77,250	37,050	(2.08)	32,600	2.580
16α-Hydroxy-DHEA				30,100	2.385
Androstentriol	106,250	50,810	(2.09)	36,900	2.915
E_{x1}				16,910	1.338
Estriol				25,200	1.998
E_{x2}				6,050	0.476

[a] Not properly identified.

98.8% of ^{3}H-activity, found in form of neutral steroids, 89.6% were isolated as DHEA, followed by androstendione with 7.5% and minute quantities of ring-D hydroxylated compounds. In the fraction of free phenolic steroids estradiol and estriol contained the major portion of ^{3}H-activity, while E_{x1} and E_{x2} were not detectable.

From these results, however, no conclusions can be drawn as to the preference of any biosynthetic pathway,

Table II. Metabolites in the fraction of free steroids

Steroid	dpm ^{3}H	% of ^{3}H-activity
DHEA	135,500	89.604
Androstendione	11,120	7.492
Testosterone	1,200	0.811
Androstendiol	1,350	0.911
Androstentriol[a]	50	0.030
Estrone[a]	45	0.030
Estradiol	1,140	0.770
Estriol	520	0.352

[a] Not properly identified.

leading from DHEA or its sulphate to androgens and estrogens in ovarian tissue. The assumption that, in the ovary, sulphoconjugated C_{19}-steroids primarily undergo hydroxylation in Ring D[8,9] prior to direct aromatization, still requires the appropriate experimental support[10].

Zusammenfassung. Nach Inkubation von menschlichem Ovarialgewebe mit 7α-^{3}H-DHEA-^{35}S-sulfat konnten als Metaboliten 5 verschiedene C_{19}- und 2 phenolische C_{18}-Steroide isoliert und identifiziert werden, die als Sulfokonjugate einen gegenüber dem Substrat unveränderten ^{3}H/^{35}S-Quotienten aufwiesen.

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Persistence of the Levator Ani Muscle in Female Rats

The levator ani muscle (MLA) has been considered as part of the genital tract of male rats. It has been generally assumed that this muscle exists only in the male rat[1]. However it could be shown that the levator ani muscle of the rat develops in both sexes from a uniform blastem. Only from the 18th day of embryological development, there occurs a progressive involution of this muscle in the female rat and the muscle cannot be found in female adult rats[2]. The homologous embryonic blastems of the levator ani in both sexes suggested that it might be possible to ensure its maintenance in female rats, if androgens are applied before or immediately at the time of birth, i.e. before the involution of the muscle anlage.

Material and methods. Experiments were undertaken to test the possibility of achieving persistence of the levator ani muscle in female rats. From 2 litters of newborn rats the female rats were selected and testosterone propionate (1 mg twice a week) was injected s.c. for the period of 1 and 2 months respectively. The muscles were weighed 1 and 2 months after birth and compared with the muscles of male untreated rats selected from the same litters. Both the absolute and relative (i.e. weight of muscle expressed as $^0/_{00}$ of body weight) weights of MLA were determined.

Results. The Table shows that testosterone treatment maintains the levator ani muscle in female rats. There is no difference in the weight of the muscles against those of the male control animals 1 month after birth, but 2 months after birth the muscles of the male control animals show a greater weight increase than those of the testosterone-treated female animals. It can also be seen that one month's treatment is not sufficient to ensure further growth of the muscle as in the muscles of animals in which treatment was continued for both months. It is, however, of interest that the relative muscle weight of the MLA appears to be stationary after cessation of treatment and these relations deserve further study.

Absolute and relative (i.e. weight of muscle expressed as $^0/_{00}$ of body weight) weights of the MLA of 1- and 2-month-old rats. The female rats received 1 mg of testosterone propionate (TP) twice weekly, beginning with the day after birth

Type of experiment	Female treated rats MLA (mg)		Male non-treated rats MLA (mg)	
	absolute weight	relative weight	absolute weight	relative weight
1-month-old 1 month TP treatment ($n = 6$)	19.6 ± 1.9	0.201	19.7 ± 1.4	0.210
2-month-old 2 months TP treatment ($n = 6$)	68.0 ± 10.6	0.321	97.5 ± 9.4	0.541
2-month-old 1 month TP treatment, 1 month cessation ($n = 5$)	38.6 ± 4.5	0.216		

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