BBA Report

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Studies on the subcellular location and stability of the enzyme system involved in the biosynthesis of 5,16-androstadien-3β-ol from 3β-hydroxy-5-pregnen-20-one (pregnenolone)

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SUMMARY

The enzyme system that is involved in the conversion of 3β-hydroxy-5-pregnen-20-one (pregnenolone) to 5,16-androstadien-3β-ol(andien-β) has been assayed in homogenates and subcellular fractions of boar testis. The enzyme activity was greatest in the microsomal fraction and was retained for up to 51 days when such preparations were stored at −20°.

5,16-Androstadien-3β-ol (andien-β) is a musk-smelling steroid arising in boar testis[1,2] and submaxillary gland[3] from pregnenolone. It is excreted, conjugated as glucosiduronate, in the urine of men and women[4] and is biosynthesized in virilizing adrenocortical carcinoma tissue[5,6] and in feminizing testes[7]. Recent work[1] has suggested that andien-β may be the first 16-unsaturated C_{19} steroid to be formed from pregnenolone and that it is subsequently converted to other members of the series, two of which are now known to act as sex-attractants in the pig[8]. The enzyme system (called "andien-β synthetase" by Loke and Gower[2]) is NADPH-dependent[1] and requires O_2 for activity[9]. This communication is concerned with the subcellular location of the enzyme system in boar testis and its stability.

Solvents and materials for chromatography have been described earlier[10]. The protein content of tissue homogenates and subcellular fractions was estimated by the method of Lowry *et al.*[11], using crystalline bovine serum albumin as standard (Armour Pharmaceuticals Ltd., Eastbourne, Sussex, U.K.). Boar testes were obtained fresh at the slaughterhouse, cooled in ice and immediately transported to the Laboratory. Homogenates (10%, w/v) were prepared in sucrose (0.25 M) and subcellular fractions separated (Fig. 1) in an Omikron Refrigerated High Speed Centrifuge (Griffin–Christ, Wembley, Middx., U.K.) using a modification of an earlier method[12]. Portions of the original homogenate and of

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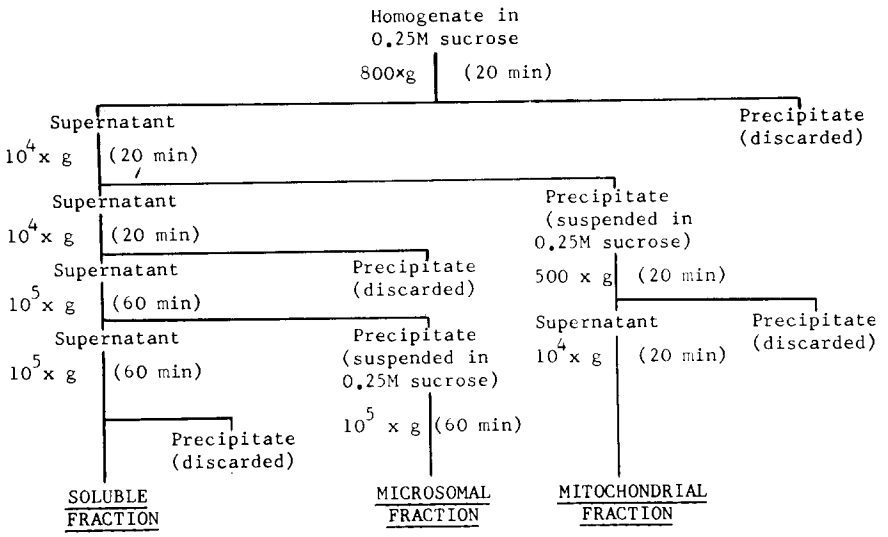

Fig. 1.

each subcellular fraction were then incubated at 37° for 10 min with [4-^{14}C] pregnenolone ($6.2 \cdot 10^5$ counts/min, specific radioactivity 55.7 mC/mmole) in 2 ml of 0.05 M Tris–HCl (pH 7.4) containing NADPH (0.4 mM). After the addition of carrier andien-β and ethyl acetate (2 ml), extraction was performed. Radioactive andien-β was isolated and characterized by thin-layer chromatography, first on Kieselgel G with benzene–ether (9:1, v/v) run twice and secondly on $AgNO_3$-impregnated Kieselgel G, using the system benzene–ethyl acetate (1:2, v/v)[1]. Earlier work[1] has shown that these procedures are sufficient to purify andien-β formed during the incubation. Other metabolites, such as 17α-hydroxypregnenolone and dehydroepiandrosterone, were separated by repeated thin-layer chromatography in benzene–acetone (4:1, v/v).

Table I shows the yields of andien-β obtained, after allowance for analytical losses[1]

TABLE I

INTRACELLULAR DISTRIBUTION OF "ANDIEN-β SYNTHETASE" IN BOAR TESTIS

[4-^{14}C] Pregnenolone (620 000 counts/min) was incubated at 37° for 10 min with each subcellular fraction in Tris–HCl buffer containing NADPH (0.4 mM). After the addition of carrier steroids, radioactive andien-β was isolated by conventional and argentation thin-layer chromatography (see text) and the yields obtained corrected for analytical losses.

	Protein N (μg)	*Andien-β formed (counts/min)*	*Specific activity (counts/min per μg protein N)*	*Yield (%)*
Homogenate	720	73 240	102	11.8
Mitochondria	48	13 230	276	2.1
Microsomes	54	88 530	1640	14.3
Soluble fraction	270	12 300	46	2.0

The bulk of "andien-β synthetase" activity was found in the microsomal fraction. Moreover, this fraction gave rise to the same pattern of metabolites, such as 17α-hydroxypregnenolone and dehydroepiandrosterone, as the original homogenate. The fact that this enzyme system is found in the microsomal fraction of boar testis implies that it is completely different from the "dehydratase" found in the soluble fraction of both boar testis and sow ovary[13]. This enzyme can also bring about the formation of 16-unsaturated steroids although in much smaller yields than "andien-β synthetase".

The stability of the "andien-β synthetase" was studied as follows: A portion of the microsomal fraction, freshly prepared from a boar testis homogenate (Fig. 1) and resuspended in sucrose (0.25 M), was assayed for enzyme activity using [4-^{14}C]-pregnenolone as substrate, as described above. The suspension was then stored at −20° and "andien-β synthetase" activity measured after 20, 51 and 72 days. The anticipated yield of radioactive andien-β was obtained from the preparation up to 51 days storage but thereafter enzyme activity was lost.

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