Biochem. J. (1973) **132**, 293–300
Printed in Great Britain

Adrenal Microsomal C_{19}-Steroid 5α-Reductase Activity in the Snell Transplantable Rat Adrenocortical Tumour 494 and the Effect of Oestradiol, Testosterone Propionate and Adrenocorticotrophin in Intact and Gonadectomized Rats

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(*Received* 15 *August* 1972)

The C_{19}-steroid 5α-reductase activity in the microsomal fraction of rat adrenal tissue under various hormonal treatments was examined. In intact control rats the activity is similar in both males and females, and after gonadectomy it is markedly increased. Treatment with oestradiol (150μg/day per animal for 7 days) or testosterone propionate (2mg/day per animal for 7 days) lowered the activity of 5α-reductase in castrated animals to approximately the values for intact animals in both sexes, and in intact animals the activity was also decreased by these treatments. The enzyme activity was also decreased by adrenocorticotrophin treatment but to a lesser extent than by the steroid hormones. The activity of the 5α-reductase enzyme in the Snell adrenocortical tumour 494 is very low when incubated as a whole homogenate, but the activity in microsomal material of the tumour was measured and unexpectedly found to be similar to that in intact controls.

The NADPH-linked C_{19}-steroid 5α-reductase enzyme of rat adrenal microsomal fractions has been shown to be active to a similar extent with respect to either androstenedione (androst-4-ene-3,17-dione) or testosterone (17β-hydroxyandrost-4-en-3-one) as substrate, and to be a different enzyme from that responsible for 5α-reduction of corticosterone (11β,21-dihydroxypregn-4-ene-3,20-dione) (Maynard & Cameron, 1973). Since the production of 5α-reduced metabolites of dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one) was significantly lower in the Snell transplantable rat adrenocortical tumour 494 (Snell & Stewart, 1959) incubated as a whole homogenate (Maynard & Cameron, 1972) a study of the relative activities in microsomal fractions from this tumour and from normal tissue was undertaken.

A C_{21}-steroid 5α-reductase enzyme has been shown to have increased activity in the rat adrenal after gonadectomy (Kitay *et al.*, 1970) and to be reversed by administration of androgen to males and of oestrogen to females. ACTH* was also shown to inhibit this enzyme (Kitay *et al.*, 1971). Androgen, oestrogen and ACTH were administered *in vivo* to ascertain whether such treatments would have similar effects on the adrenal C_{19}-steroid 5α-reductase in intact and gonadectomized rats of both sexes.

* Abbreviation: ACTH, adrenocorticotrophin.

Table 1. *Ratio of adrenal weight to body weight*

Each value represents the mean of the combined adrenal weight/body weight ratio (±S.D.) of a series of ten animals. Oestradiol-treated animals received 150μg/day per animal for 7 days; testosterone propionate-treated animals received 2mg/day per animal for 7 days; ACTH-treated animals received 0.1mg of Synacthen Depot/day per animal for 3 days and 25μg of Synacthen per animal 1h before death. * Significant difference ($P<0.001$) from control group. † Significant difference ($P<0.005$) from sham-operated group.

	Male		Female	
	Sham-operated (μg/g)	Gonadectomized (μg/g)	Sham-operated (μg/g)	Gonadectomized (μg/g)
Control	155±19.2	191±11.8†	352±38.2	324±27.0
Oestradiol-treated	283±51.7*	300±40.8*	355±31.4	433±52.7†*
Testosterone propionate-treated	133±12.8	135± 9.8	211±13.9*	190±22.8*
ACTH-treated	352±35.7*	415±44.3†*	631±76.9*	590±65.6*

Table 2. *Identification of 5α-androstane-3,17-dione isolated after incubation of adrenal microsomal fractions from untreated rats with 5.1 μCi of [7α-^3H]androst-4-ene-3,17-dione and the quantity of product formed at the times indicated*

For experimental details see the text.

Animals	Protein incubated (μg)	Time (min)	Sp. radioactivity of steroid isolated and derivative formed (d.p.m./nmol)		Amount of product formed (nmol)
			5α-Androstane-3,17-dione	5α-Androstane-3β,17β-diol	
Intact females	509	5	15.2	13.9	0.008
		10	28.0	27.0	0.015
		20	43.3	42.0	0.024
		30	68.1	62.9	0.037
	1186	5	63.1	59.9	0.035
		10	99.3	102	0.057
		20	202	199	0.111
		30	294	276	0.162
Gonadectomized females	557	5	177	177	0.100
		10	297	294	0.168
		20	485	489	0.276
		30	652	700	0.384
	1056	5	241	235	0.135
		10	371	379	0.213
		20	644	615	0.358
		30	763	790	0.442
Intact males	758	5	29.5	27.1	0.016
		10	56.8	55.3	0.032
		20	81.6	90.3	0.049
		30	121	121	0.069
	1867	5	31.4	33.4	0.018
		10	52.6	49.8	0.029
		20	93.4	96.3	0.054
		30	129	125	0.072
Gonadectomized males	792	5	93.4	88.3	0.052
		10	181	174	0.101
		20	293	289	0.166
		30	373	408	0.222
	1013	5	187	185	0.106
		10	352	328	0.193
		20	603	603	0.343
		30	845	797	0.467

Materials and Methods

Materials

Animals. Rats of the Sprague–Dawley strain were purchased from Fisons Ltd., Loughborough, Leics., U.K., and maintained conventionally. The Snell transplantable adrenocortical tumour 494 (Snell & Stewart, 1959), generously supplied by Dr. Katharine C. Snell, was maintained in male Sprague–Dawley rats.

Reagents. All reagents including ethanol were of analytical grade. Other solvents were of laboratory grade and were redistilled before use. [7α-^{3}H]-Androstenedione (specific radioactivity 3.1 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and its purity was checked by reverse isotope dilution.

Methods

Animals used in investigations involving hormonal administration were divided into groups of ten, gonadectomized or sham-operated under ether anaesthesia, and killed 10 days later. Oestradiol [oestra-1,3,5(10)-triene-3,17β-diol] was given intramuscularly as the free steroid at a rate of 150μg in 0.2ml of arachis oil per animal per day for 7 days before they were killed. Testosterone was administered as the propionate at a dosage of 2mg in 0.2ml of sesame oil per animal per day for 7 days before the animal was killed. ACTH was given as a long-acting preparation, Synacthen Depot (Ciba, Horsham, Sussex, U.K.), 0.1mg per animal per day for 3 days and then one injection of 25μg of Synacthen (Ciba) in water (0.1ml) on the tenth post-operative day, 1h before the animal was killed.

Animals were killed by cervical dislocation and the adrenal glands or tumour removed and placed in a Petri dish on ice. The glands were decapsulated and a 10% (w/v) homogenate was prepared in 0.25M-sucrose containing 3mM-$MgCl_2$. The microsomal pellet was collected by centrifugation of the

Table 3. *Identification of 5α-androstane-3,17-dione isolated after incubation of adrenal microsomes from oestradiol-treated rats with 5.1 μCi of [7α-^3H]androst-4-ene-3,17-dione and the quantity of product formed at the times indicated*

For experimental details see the text.

Animals	Protein incubated (μg)	Time (min)	Sp. radioactivity of steroid isolated and derivative formed (d.p.m./nmol) 5α-Androstane-3,17-dione	5α-Androstane-3β,17β-diol	Amount of product formed (nmol)
Intact females	806	5	10.4	9.9	0.006
		10	17.2	15.8	0.009
		20	39.8	42.9	0.024
		30	49.3	50.5	0.028
	2083	5	12.8	11.4	0.007
		10	26.8	25.4	0.015
		20	40.2	44.9	0.024
		30	83.4	80.8	0.047
Gonadectomized females	725	5	18.9	18.4	0.011
		10	39.1	36.0	0.021
		20	95.6	102	0.057
		30	145	136	0.080
	1373	5	43.5	43.7	0.025
		10	80.7	78.0	0.045
		20	168	156	0.092
		30	247	229	0.135
Intact males	1224	5	40.8	39.8	0.023
		10	74.8	83.1	0.045
		20	166	162	0.094
		30	244	264	0.144
	1934	5	20.4	19.9	0.011
		10	41.2	38.8	0.023
		20	66.7	64.8	0.037
		30	89.7	81.2	0.048
Gonadectomized males	1066	5	4.7	4.8	0.003
		10	9.2	8.7	0.005
		20	14.6	14.7	0.008
		30	23.8	23.0	0.013
	1123	5	15.8	14.1	0.009
		10	32.5	29.1	0.018
		20	73.5	69.7	0.041
		30	117	109	0.064

Table 4. *Identification of 5α-androstane-3,17-dione isolated after incubation of adrenal microsomal fractions from testosterone-treated rats with 5.1 μCi of [7α-^3H]androst-4-ene-3,17-dione and the quantity of product formed at the times indicated*

For experimental details see the text.

Animals	Protein incubated (μg)	Time (min)	Sp. radioactivity of steroid isolated and derivative formed (d.p.m./nmol) 5α-Androstane-3,17-dione	5α-Androstane-3β,17β-diol	Amount of product formed (nmol)
Intact females	1320	5	6.5	7.3	0.004
		10	13.5	14.5	0.008
		20	23.9	21.8	0.013
		30	38.9	36.4	0.022
	1142	5	10.3	10.7	0.006
		10	19.9	21.1	0.012
		20	42.3	43.9	0.024
		30	67.4	73.3	0.040
Gonadectomized females	830	5	19.8	21.2	0.012
		10	37.0	35.0	0.020
		20	86.1	84.9	0.048
		30	139.0	145.0	0.081
	1334	5	25.3	27.2	0.015
		10	53.9	51.7	0.029
		20	76.9	80.9	0.045
		30	120.0	132.0	0.071
Intact males	1238	5	7.6	8.0	0.004
		10	12.3	12.9	0.007
		20	24.5	23.3	0.013
		30	36.7	38.5	0.022
	1862	5	4.9	4.5	0.003
		10	10.0	9.2	0.005
		20	17.5	16.2	0.010
		30	25.7	24.1	0.014
Gonadectomized males	1430	5	10.6	9.8	0.006
		10	19.5	17.9	0.011
		20	44.4	42.6	0.025
		30	56.2	54.4	0.032
	1008	5	9.2	8.6	0.005
		10	17.5	17.9	0.010
		20	26.9	25.7	0.015
		30	44.2	47.0	0.026

26300$g_{av.}$ supernatant at 105000$g_{av.}$ and washed by resuspension and recentrifugation. The protein content of the pellet, after resuspension in 1.5ml of 0.25M-sucrose solution containing 3mM-$MgCl_2$, was measured by the method of Lowry *et al.* (1951), with bovine serum albumin [Sigma (London) Chemical Co., London S.W.6, U.K.] as standard. A portion of the fraction (1.2ml) was incubated with an equal volume of medium containing cofactors such that their final concentrations were: Tris–HCl buffer, pH7.4, 43.02mM; KCl, 42.82mM; $MgSO_4$, 3.16mM; ATP, 0.94mM; NADPH, 0.29mM; cytochrome *c*, 0.08mM; 5.1μCi of [7α-^{3}H]androstenedione was added per incubation mixture. Samples (0.5ml) were removed after 5, 10, 20 and 30min and added to ethanol–acetone (1:1, v/v) (5ml) containing carrier androstenedione and 5α-androstanedione (5α-androstane-3,17-dione) (300μg of each). The extraction of

the steroids, and the t.l.c. methods employed in their purification, are described in the preceding paper (Maynard & Cameron, 1973).

Results

Testosterone significantly ($P<0.025$) increased body weight in all groups, whereas oestradiol and ACTH had no effect. In females gonadectomy increased ($P<0.001$) body weight but this was apparently prevented by the oestradiol treatment. Oestradiol and ACTH significantly ($P<0.001$) increased the adrenal weight/body weight ratio in all cases except for the action of oestradiol in intact females (Table 1). In females testosterone significantly decreased this ratio, particularly in the gonadectomized group ($P<0.001$), but in males the effect was only slight.

To obtain duplicate values for adrenal 5α-reductase activity each group of animals was arbitrarily divided

Table 5. *Identification of 5α-androstane-3,17-dione isolated after incubation of adrenal microsomal fractions from ACTH-treated rats with 5.1 μCi of [7α-^3H]androst-4-ene-3,17-dione and the quantity of product formed at the times indicated*

For experimental details see the text.

Animals	Protein incubated (μg)	Time (min)	Sp. radioactivity of steroid isolated and derivative formed (d.p.m./nmol) 5α-Androstane-3,17-dione	5α-Androstane-3β,17β-diol	Amount of product formed (nmol)
Intact females	1493	5	48.9	47.5	0.027
		10	83.6	84.9	0.051
		20	204	189	0.111
		30	329	359	0.196
	643	5	31.0	35.9	0.019
		10	56.8	53.2	0.031
		20	118	108	0.064
		30	164	167	0.094
Gonadectomized females	1013	5	72.3	67.2	0.040
		10	137	128	0.075
		20	244	251	0.141
		30	359	332	0.196
	1723	5	236	229	0.132
		10	500	486	0.280
		20	871	821	0.481
		30	1267	1172	0.693
Intact males	3038	5	11.9	10.4	0.006
		10	16.3	17.0	0.010
		20	36.4	34.9	0.020
		30	58.4	61.0	0.034
	2050	5	9.4	9.1	0.005
		10	19.1	18.2	0.010
		20	40.3	37.2	0.022
		30	63.2	58.7	0.035
Gonadectomized males	936	5	6.3	6.0	0.003
		10	9.6	9.5	0.005
		20	18.1	17.4	0.010
		30	28.2	26.5	0.015
	1358	5	52.8	55.2	0.031
		10	98.7	106	0.058
		20	197	204	0.114
		30	290	295	0.188

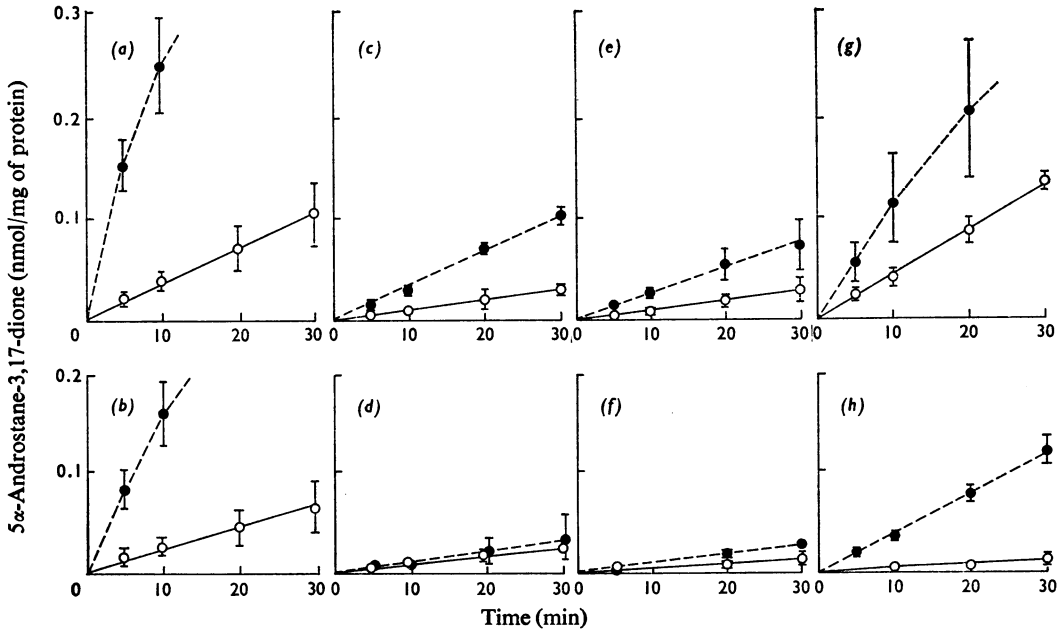

Fig. 1. *Rat adrenal C_{19}-steroid 5α-reductase activity*

Activity of 5α-reductase system in microsomal preparations of adrenal tissue taken from controls (*a*, females; *b*, males), oestradiol-treated (*c*, females; *d*, males), testosterone propionate-treated (*e*, females; *f*, males) and ACTH-treated (*g*, females; *h*, males) rats is shown, with [7α-^{3}H]androst-4-ene-3,17-dione as substrate, measured as 5α-androstane-3,17-dione produced per mg of protein. In (*a*), (*b*) and (*g*), where graphs are plotted beyond the experimental points, further results have been omitted for the sake of clarity. ●, Gonadectomized animals; ○, sham-operated animals. Points indicate the means and the vertical bars the ranges of the values determined.

Table 6. *Identification of 5α-androstane-3,17-dione isolated after incubation of microsomal preparations from the Snell tumour 494 with 5.1 μCi of [7α-^{3}H]androstenedione and the quantity of product formed at the times indicated*

For experimental details see the text.

Protein incubated (μg)	Time (min)	Sp. radioactivity of steroid isolated and derivative formed (d.p.m./nmol) 5α-Androstane-3,17-dione	5α-Androstane-3β,17β-diol	Amount of product formed (nmol)
2064	5	99.2	99.1	0.056
	10	168	179	0.099
	20	297	305	0.166
	30	309	312	0.177
1848	5	98.3	94.5	0.055
	10	152	145	0.084
	20	287	280	0.158
	30	463	447	0.259

into two sub-groups, and microsomal material from the adrenals incubated as described in the Materials and Methods section. The specific radioactivity of the isolated 5α-androstanedione and of the reduced product 5α-androstane-3β,17β-diol are shown in Tables 2–5, together with the amount of product formed at

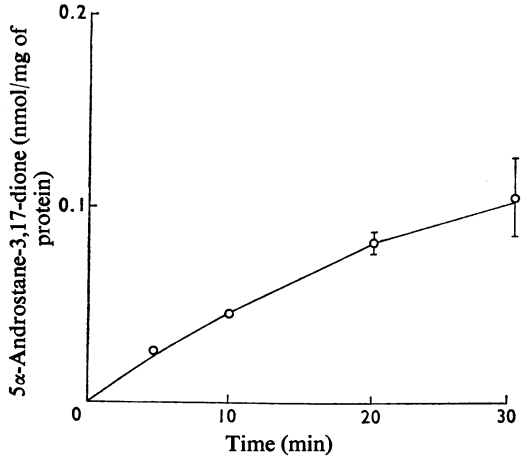

Fig. 2. *C_{19}-steroid 5α-reductase activity in Snell 494 tumour*

Activity of 5α-reductase system in microsomal preparations of the Snell tumour 494 was measured with [7α-^{3}H]androst-4-ene-3,17-dione as substrate, as 5α-androstane-3,17-dione produced per mg of protein. Points indicate the means and the vertical bars the ranges of the values determined.

each time. The rates of reaction per mg of protein are shown diagrammatically in Fig. 1. The androstenedione in the 30min samples was also analysed and the specific radioactivities of the free and reduced steroid were measured. In all of these cases more than 95% of the radioactivity added was accounted for in the two steroids examined, indicating that 5α-androstanedione was the only product formed from [7α-^{3}H]-androstenedione under these conditions.

A similar study was performed by using the microsomal fractions from duplicate samples (300mg) of the Snell tumour 494. The specific radioactivities of the 5α-androstanedione isolated and of its reduced product are shown in Table 6. The rate of reduction of [7α-^{3}H]androstenedione per mg of protein is shown diagrammatically in Fig. 2.

Discussion

The role of C_{19}-steroid 5α-reductase in the rat adrenal is not at present clearly understood although the activity of this enzyme in the normal gland is apparently high (Maynard & Cameron, 1972). However, it may provide a means of controlling the concentrations of circulating androgens, such as testosterone, and of providing a source of biologically active 5α-reduced C_{19}-steroids.

In examining mechanisms for control of the activity of the enzyme our initial studies have investigated the effect of gonadectomy in both sexes and the administration of testosterone propionate, oestradiol and ACTH. A similar effect on the microsomal C_{19}-steroid 5α-reductase in both males and females was found under these treatments, although in all cases the activity per mg of protein was lower in males than in females.

Gonadectomy markedly elevated the activity in control animals (Figs. 1*a* and 1*b*), but this was decreased virtually to normal values by treatment with oestradiol or testosterone propionate (Figs. 1*c*, 1*d*, 1*e* and 1*f*). ACTH was not as effective in this respect, particularly in male rats (Figs. 1*g* and 1*h*). Similar results have been reported with respect to the microsomal C_{21}-steroid 5α-reductase (Kitay *et al.*, 1970, 1971), although this is probably a different enzyme system (Maynard & Cameron, 1973).

In spite of the fact that the activity of the C_{19}-steroid enzyme is decreased by both oestradiol and testosterone they have quite different effects on the weight of the adrenal glands. Thus these two effects are unlikely to be mediated by the same mechanism. It is possible that both steroids act directly to decrease the enzyme activity, but only oestradiol causes a growth of the adrenal gland, probably by increasing ACTH secretion to a small extent (Coyne & Kitay, 1969). More information is required to determine whether such a simple situation exists or whether there is a more complicated mechanism decreasing enzyme activity, perhaps through some action of prolactin or of luteinizing hormone.

The results obtained by using microsomal material from the Snell adrenocortical tumour 494 (Fig. 2) were surprising in that the activities of the C_{19}-steroid 5α-reductase were similar per mg of protein to those found in intact animals. In whole homogenates this enzyme activity was apparently considerably lower than in normal tissue (Maynard & Cameron, 1972). This may be explained in several ways. For example, the 5α-reductase activity in homogenates of whole normal adrenal tissue may be due to an enzyme in another subcellular fraction. C_{19}-steroid 5α-reductase activity different in character from that of the microsomal fraction has been shown to exist in the $105000g_{av.}$ supernatant (Maynard & Cameron, 1973) and perhaps it is this enzyme that has decreased activity in the tumour. The possibility that the decreased activity in the tumour is due to a lower concentration of microsomal material seems unlikely, since [7α-^{3}H]dehydroepiandrosterone was metabolized extensively by tumour tissue (Maynard & Cameron, 1972) and this requires a microsomal enzyme system, 3β-hydroxy steroid dehydrogenase (Beyer & Samuels, 1956). A more likely explanation is that material exists in other parts of the cell, which inhibits the microsomal activity in whole homogenates either acting on the enzyme directly or on its utilization of NADPH.

We express gratitude to the Tenovus Organisation for generous financial support and to Dr. Katharine C. Snell, National Cancer Institute, Bethesda, Md., U.S.A., for the gift of the transplantable rat adrenal tumour. We also thank Professor K. Griffiths for his greatly valued criticism and advice. This report formed part of a thesis submitted by P. V. M. for the degree of Ph.D. to the University of Wales.

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