Biochemical Pharmacology, Vol. 20, pp. 803–813. Pergamon Press, 1971. Printed in Great Britain

EFFECT OF TESTOSTERONE ON THE RAT ADRENAL CORTICAL 11β-HYDROXYLATION SYSTEM*

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(*Received* 7 *April* 1970; *accepted* 19 *June* 1970)

Abstract—The influence of testosterone on the adrenal mitochondrial steroid hydroxylation system was investigated in the female Sprague–Dawley rat. After the injection of animals with testosterone for 10 days, it was possible to demonstrate impairment of progesterone metabolism in adrenal homogenates. The formation of corticosterone and 18-hydroxy-11-deoxycorticosterone was lower than in controls and there was an accumulation of 11-deoxycorticosterone (DOC). Adrenal mitochondria isolated from testosterone-treated rats showed low rates of conversion of DOC-4-^{14}C to corticosterone and 18-hydroxy-DOC, confirming the low activity of steroid 11β- and 18-hydroxylases. Rates of corticosterone formation from unlabeled DOC were also lower in adrenal mitochondria from testosterone-treated animals. Accompanying these low steroid hydroxylation activities in isolated mitochondria were decreased levels of adrenal mitochondrial cytochrome P-450. In addition, spectral studies revealed that the DOC-induced spectral change was considerably less in the adrenal mitochondria from testosterone-treated rats. The results described in this communication are consistent with the concept that, after injection into the rat, testosterone reaches the adrenal cortex and there interacts with the mitochondrial cytochrome P-450 in such a way that the natural substrate, DOC, is less able to be bound and hydroxylated. This hypothesis was confirmed by the addition of testosterone to rat adrenal mitochondria *in vitro* and the observation of inhibition of both DOC-induced spectral changes and steroid hydroxylation.

THE EFFECTS of androgens upon adrenocortical function and structure are numerous and well documented.[1,2] In a previous report, we have shown that the administration of testosterone to rats, which were uninephrectomized and given 1% saline as drinking solution, could induce hypertensive cardiovascular disease.[3] The pathogenesis of this hypertension seemed to involve impaired adrenal 11β-hydroxylation, resulting in an increased secretion of 11-deoxycorticosterone (DOC), a potent mineralocorticoid. Associated with the increased production of DOC was a decrease in the concentration of cytochrome P-450 in adrenocortical mitochondria. The following studies were carried out in an attempt to clarify the interaction of testosterone with the adrenal cortical 11β-hydroxylation system.

MATERIALS AND METHODS

Female, 35-day-old Sprague–Dawley rats obtained from the Charles River Company were used. They were individually caged and maintained in a room with 12-hr

* This work was supported by Training Grant 01500 from the National Institute of General Medical Sciences and Research Grant No. HE 06975 from the National Heart Institute.

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light and dark cycles at a constant temperature of 22 ± 1°. Rats were fed Purina Lab. Chow *ad lib.* and given tap water as drinking solution.

Testosterone was obtained from Steraloids, Inc. and checked for purity by melting point determination and thin-layer chromatography on silica gel. Experimental rats received 10 mg testosterone daily for 10 days by subcutaneous injection of a microcrystalline suspension in 0·2 ml corn oil. Controls received daily subcutaneous injections of 0·2 ml corn oil for the same length of time. Rats were sacrificed 24 hr after the final injection.

At the end of the treatment period, all rats were killed by decapitation. Adrenals were rapidly removed, carefully freed of adhering fat, weighed and pooled by group. Homogenates were prepared in 0·25 M sucrose–0·01 M tris (pH 7·4) with a Thomas tissue grinder consisting of a Teflon pestle and grinding vessel made of borosilicate glass. Appropriate volumes of buffer were added so that the final homogenate concentration was 35 mg/ml. One ml of the homogenate suspension was incubated with 0·1 μc progesterone-4-^{14}C (specific activity, 4·0 mc/m-mole) and 4·0 ml of incubation buffer (0·1 M tris-chloride, 0·005 M KCl, 0·005 M $MgCl_2$, 0·08 M NaCl, 0·05 M sucrose, pH 7·4) containing sodium malate (100 mg/100 ml). Incubations were carried out in duplicate for 10 min at 37° in air. Steroids were extracted from the incubation medium with methylene chloride and steroid analyses were performed as previously described.[4]

The residual homogenate preparations were centrifuged at 700 *g* for 10 min to remove nuclei, red cells, and unbroken cells, and the supernatant was centrifuged at 10,000 *g* for 10 min to obtain the mitochondrial fraction. Mitochondria were washed once with sucrose–tris chloride buffer and recentrifuged at 10,000 *g* for 10 min. Mitochondria were then suspended in the same buffer at a concentration of mitochondria equivalent to 50 mg tissue/ml (approximately 1·5 mg protein/ml).

For mitochondrial incubations with radioactive DOC, 1·0 ml of mitochondrial suspension was added with 0·15 μc DOC-4-^{14}C (specific activity, 0·65 mc/m-mole) in 0·1 ml ethanol to 4 ml of incubation buffer containing 100 mg isocitrate per 100 ml. Incubations and steroid analyses were performed as for the homogenates.

Rates of 11β-hydroxylation were determined by incubating 1·0 ml of mitochondrial suspension with 150 nmoles of cold DOC (added in 0·1 ml ethanol) in 4·0 ml of incubation buffer containing 100 mg isocitrate per 100 ml. Aliquots of incubation medium were withdrawn at 2-min intervals from the start of incubation until 10 min had elapsed and the amount of corticosterone present in each was determined fluorimetrically.[5,6]

The cytochrome concentrations in adrenal mitochondrial preparations were determined by the methods of Cammer and Estabrook,[7] using an Aminco-Chance dual wavelength scanning recording spectrophotometer. Substrate-induced spectral changes[8] and mitochondrial protoheme[9] levels were also evaluated by techniques previously reported using this same instrument. Protein assays were performed by the method of Lowry *et al.*[10]

RESULTS

Homogenate incubations

Table 1 presents the results of duplicate adrenal homogenate incubations from the control and testosterone-treated groups. It can be seen that the control homogenates

converted most of the added progesterone-4-^{14}C to corticosterone and 18-hydroxy-11-deoxycorticosterone (18-OH-DOC), the major secretory products of the rat adrenal cortex. Small amounts of radioactivity were recovered as DOC and residual progesterone. Adrenal homogenates from the testosterone-treated rats, on the other hand, while readily converting the progesterone to DOC, had a markedly reduced capacity to further metabolize this mineralocorticoid. Very little corticosterone or 18-OH-DOC was formed. These results indicate an impairment in the 11β- and 18-hydroxylation of DOC by adrenals from testosterone-treated rats.

TABLE 1. METABOLISM *in vitro* OF PROGESTERONE-4-^{14}C BY ADRENAL HOMOGENATES FROM CONTROL AND TESTOSTERONE-TREATED RATS*

Group	No. of rats	Days treated	Per cent conversion of progesterone-4-^{14}C to			
			Progesterone†	DOC	B	18-OH-DOC
Control	17	10	5·8	6·3	58·7	26·5
			4·5	5·6	64·1	30·8
Testosterone (10 mg)	17	10	2·3	77·8	11·8	5·8
			12·5	65·6	13·2	5·9

* Adrenals were removed from the two groups after 10 days of treatment. They were pooled separately and homogenized in 0·25 M sucrose–0·01 M tris-chloride (pH 7·4) to obtain a final concentration of 35 mg tissue/ml. Duplicate 1-ml samples from each group were incubated with 0·1 μc progesterone-4-^{14}C (sp. act., 4 mc/m-mole) and 4 ml of incubation buffer consisting of 0·1 M tris-chloride, 0·005 M KCl, 0·005 M $MgCl_2$, 0·08 M NaCl and 0·05 M sucrose, pH 7·4. The incubation medium contained sodium malate (100 mg/100 ml). Final volume was 5 ml. Incubations were done in air at 37° for 10 min. Percentage conversion of labeled progesterone to DOC, corticosterone (B) and 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) was measured after paper chromatographic separation of the metabolic products.[4]

† Unmetabolized progesterone.

Mitochondrial incubations with DOC-4-^{14}C

In order to evaluate the effects of testosterone administration upon the adrenal mitochondrial hydroxylation of DOC more directly, mitochondria were isolated from control and testosterone-treated rats and incubated with DOC-4-^{14}C. The results of triplicate incubations are presented in Table 2. Control mitochondria converted most of the DOC to corticosterone and 18-OH-DOC. Less than 20 per cent of the radioactivity was recovered as unmetabolized DOC. The adrenal mitochondria from those rats treated with testosterone, on the other hand, were able to convert less than half of the added DOC to more polar compounds, again indicative of significant impairment of 11β- and 18-hydroxylase activity.

Rates of corticosterone formation

Adrenal mitochondria from control and testosterone-treated groups were also incubated in the presence of 150 nmoles of nonradioactive DOC. Aliquots of incubation medium were removed at 2-min intervals so that the rates of corticosterone formation could be determined (Table 3). It is apparent that the rate of corticosterone

TABLE 2. METABOLISM *in vitro* OF DOC-4-^{14}C BY ADRENAL MITOCHONDRIA FROM CONTROL AND TESTOSTERONE-TREATED RATS*

Group	Mitochondrial protein (mg/ml)	Per cent conversion of DOC-4-^{14}C to		
		DOC†	B	18-OH-DOC
Control	1·57	18·7	47·2	22·1
		19·1	45·9	22·8
		9·1	55·1	25·3
Testosterone (10 mg)	1·55	56·2	25·1	11·8
		52·7	27·9	14·1
		51·0	29·8	13·9

* Adrenal homogenates were obtained from the two groups as described in Table 1. Mitochondria were prepared from the 2 homogenate preparations by differential centrifugation and suspended in 0·25 M sucrose–0·01 M tris-chloride (pH 7·4) to yield a mitochondrial preparation equivalent to 50 mg adrenal tissue/ml. Triplicate 1-ml samples from each group were incubated with 0·15 μc DOC-4-^{14}C (sp. act., 0·65 mc/m-mole) and 4 ml of incubation buffer (see Table 1) containing isocitrate (100 mg/100 ml). Final volume was 5 ml. Incubations were done in air at 37° for 10 min. Percentage conversion of labeled DOC to metabolic products B and 18-OH-DOC followed paper chromatography.[4]

† Unmetabolized DOC.

TABLE 3. RATES OF 11β-HYDROXYLATION *in vitro* BY ADRENAL MITOCHONDRIA FROM CONTROL AND TESTOSTERONE-TREATED RATS*

Group	Mitochondrial protein (mg/ml)	Mean adrenal wt. (mg)	Rate of corticosterone production (nmoles/min/mg protein)	Mean rate × Mean adrenal wt. (% of control)
Control	1·57	59·7	10·53	
			11·45	
			10·97	
Testosterone (10 mg)	1·55	45·1	5·17	
			5·99	38
			5·62	

* Triplicate 1-ml samples of the two mitochondrial preparations (see Table 2) were incubated with 150 nmoles DOC in 4 ml of the incubation buffer containing isocitrate (100 mg/100 ml). Incubations were done in air at 37°. Samples were withdrawn at 2-min intervals for the determination of corticosterone by sulfuric acid fluorescence.[5,6]

production by adrenal mitochondria from testosterone-treated rats was approximately half that of controls. This observation tends to corroborate the results obtained from the incubations with progesterone-4-^{14}C and DOC-4-^{14}C.

Since the administration of testosterone produces adrenal atrophy (Table 3), a correction was made for the differences in adrenal mass between control and testosterone-treated groups by multiplying the mean rates of 11β-hydroxylation per milligram of adrenal mitochondrial protein by the mean adrenal weights. (The mito-

chondrial protein per unit weight of adrenal tissue was virtually identical in both groups.) If this value represents a crude index of the total 11β-hydroxylating capacity *in vivo* of the adrenals in each group, then it can be seen (Table 3) that the 11β-hydroxylating activity of adrenals from rats receiving testosterone was reduced to an even greater extent than is reflected by the rates of 11β-hydroxylation alone.

Adrenal cytochrome levels

Adrenal mitochondrial cytochrome levels in control and testosterone-treated groups are reported in Table 4. The levels of cytochromes b and c + c_1 were unaffected by testosterone treatment. However, the concentration of cytochromes a + a_3 in mitochondria from the testosterone-treated group was double that of controls.

TABLE 4. ADRENAL MITOCHONDRIAL CYTOCHROMES, PYRIDINE HEMOCHROMOGEN AND ADRENODOXIN LEVELS IN CONTROL AND TESTOSTERONE-TREATED RATS*

Group	Cytochrome conc. (nmoles/mg protein)				Pyridine hemochromogen (nmoles/mg protein)	Adrenodoxin (% of control)
	a + a_3	b	c + c_1	P-450		
Control	0·05	0·10	0·18	1·84	1·81	
Testosterone (10 mg)	0·10	0·13	0·19	1·03	1·08	60

* For the assay of respiratory chain cytochromes by the methods of Cammer and Estabrook,[7] a 6-ml aliquot of the mitochondrial suspension was divided equally between two cuvettes, placed in the Aminco-Chance spectrophotometer, and a baseline of equal light absorbancy recorded. Potassium cyanide (0·5 mM) and sodium succinate (33 mM) were then added in that order to one cuvette and anaerobic–aerobic difference spectra recorded. For the assay of cytochrome P-450, sodium dithionite was then added to both cuvettes and carbon monoxide was equilibrated with one cuvette. Difference spectra were again recorded. Spectrophotometric wavelengths used for the measurement of cytochrome levels were: a + a_3, 605–625 nm; b, 562–575 nm; c + c_1, 552–540 nm; P-450, 450–490 nm. Millimolar extinction coefficients used were: a + a_3, 16; b, 20; c + c_1, 19; P-450, 91. Protoheme was measured as the pyridine hemochromogen.[9] Adrenodoxin was measured by electron paramagnetic resonance using a Varian E-3 spectrometer with the quantitation depending on the height of the $g = 1{\cdot}94$ signal.

The absorption band due to the reduced cytochrome P-450 carbon monoxide complex was considerably smaller in the adrenal mitochondria from the testosterone-treated rats than that from controls. The level was approximately 60 per cent of that in controls. In order to be certain that the apparent reduction in mitochondrial cytochrome P-450 level was not the result of an interaction between testosterone and the cytochrome P-450, interfering with its assay, mitochondrial protoheme was measured as the pyridine hemochromogen (Table 4). It can be seen that the pyridine hemochromogen levels were in fact almost identical to those of cytochrome P-450 in each group, supporting the conclusion that there was a true decrease in the amount of cytochrome P-450 in the adrenal mitochondria of testosterone-treated rats.

A reduction in the concentration of adrenal mitochondrial adrenodoxin was also apparent in the androgen-treated rats (Table 4), which paralleled rather well the per cent decrease in cytochrome P-450.

DOC-induced spectral changes

It has been shown that the addition of DOC to bovine adrenocortical mitochondria produces a difference spectrum, with an absorption maximum at 385 nm and a minimum at 420 nm.[8] This observation has recently been confirmed for rat adrenal mitochondria.[11]

Adrenal mitochondrial aliquots from the control and testosterone-treated groups were titrated with DOC and the magnitude of the induced spectral change($\Delta A_{385-420}$) was calculated for each DOC addition. It is evident (Fig. 1) that the difference spectrum induced by DOC was considerably smaller in the mitochondria from androgen-treated rats than in those from controls.

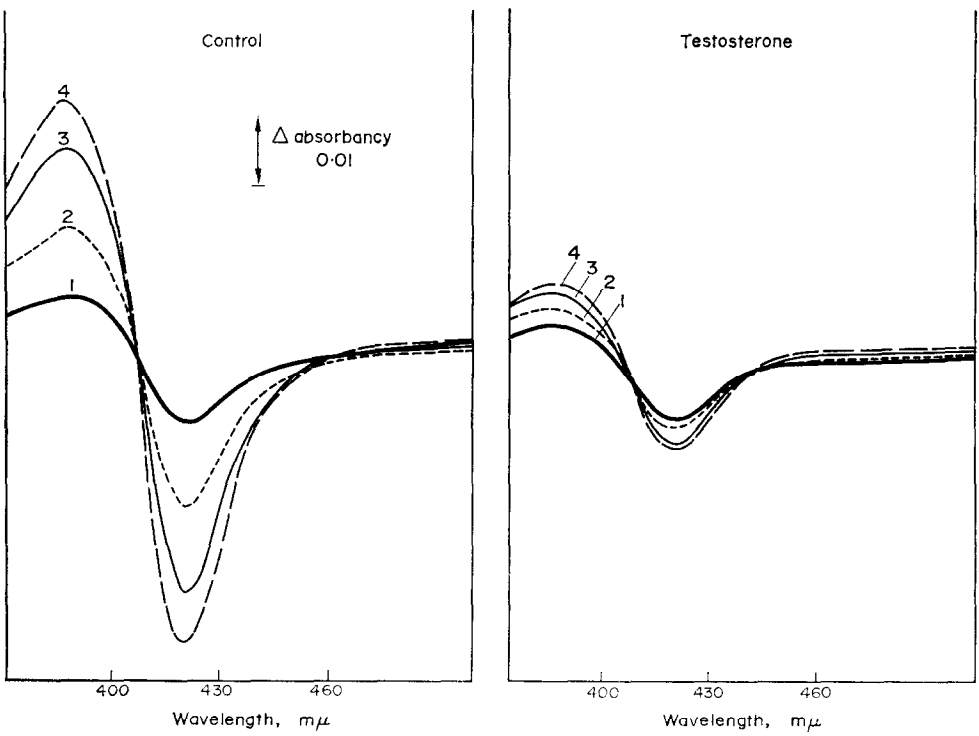

FIG. 1. Spectral changes resulting from the addition of DOC to adrenal mitochondrial suspensions from controls and rats treated with testosterone for 10 days. Spectra were obtained as described in Table 5. The resulting difference spectra have been replotted. DOC concentrations were: (1) 0·5 μM; (2) 1·0 μM; (3) 2·5 μM; (4) 4·0 μM.

Since the magnitude of the substrate-induced spectral change depends upon the concentration of cytochrome P-450 in the cuvettes, and since testosterone treatment produced a decrease in the level of adrenal mitochondrial cytochrome P-450, the induced difference spectrum for each group was evaluated per nmole of cytochrome P-450 present (Table 5). Even after correction for differences in cytochrome P-450 levels, the DOC-induced spectral changes in the mitochondria from testosterone-treated rats were still less than half the magnitude of those in controls. It therefore

appears that testosterone is capable of interacting with cytochrome P-450 in adrenal mitochondria in such a manner as to prevent its (cytochrome P-450) subsequent interaction with its natural substrate, DOC.

TABLE 5. EFFECT OF TESTOSTERONE ON THE DOC-INDUCED SPECTRAL CHANGE IN ADRENAL MITOCHONDRIA*

Group	Mitochondrial cytochrome P-450 (nmoles/mg protein)	DOC added (μg)	$\Delta A_{385-420}$	$\Delta A_{385-420}$/nmole P-450	
				Absolute	% Control
Control	1·84	1·0	0·0385	0·0140	
		2·5	0·0610	0·0222	
		4·0	0·0750	0·0273	
		9·0	0·0860	0·0313	
Testosterone (10 mg)	1·03	1·0	0·0150	0·0088	62·9
		2·5	0·0185	0·0108	48·6
		4·0	0·0205	0·0120	44·0
		9·0	0·0215	0·0125	39·9

* Adrenal mitochondrial preparations were obtained from both groups as described in Table 2. Six ml of mitochondrial suspension was equally divided between two cuvettes of the Aminco-Chance spectrophotometer and a baseline of equal light absorbancy was recorded. Successive aliquots of DOC dissolved in small volumes of ethanol were then added and difference spectra were obtained after each addition.

Effects of the addition in vitro *of testosterone to adrenal mitochondria*

If the effects of testosterone upon the DOC induced spectral change and mitochondrial metabolism of DOC are the result of its interaction with adrenal mitochondrial cytochrome P-450, as opposed to an alteration in some extra-adrenal parameter which secondarily elicits a response in the adrenal, they should be duplicated when testosterone is added *in vitro* to adrenal mitochondria from untreated rats. The following study was therefore carried out to test this hypothesis. Adrenal mitochondria were isolated from normal rats as previously described and suspended in sucrose-tris buffer. To half of the mitochondrial suspension was added an amount of testosterone so that its final concentration was 2×10^{-4} M, while the other half had a similar volume of vehicle (0·1 ml ethanol) alone added. The following studies were then performed.

Metabolism of DOC-4-^{14}*C and rates of* 11β*-hydroxylation*

It can be seen (Table 6) that in the control flasks most of the DOC-4-^{14}C was converted to corticosterone and 18-OH-DOC. In the presence of testosterone, however, much of the DOC was unmetabolized ,with considerably less converted to more polar steroids.

The rates of 11β-hydroxylation in the flasks containing testosterone were correspondingly lower than those of the controls. Thus, the impairment in DOC metabolism evident after the administration *in vivo* of testosterone was also apparent when it was added *in vitro*.

TABLE 6. EFFECT OF TESTOSTERONE *in vitro* ON THE METABOLISM OF DOC BY ADRENAL MITOCHONDRIA*

Group	Per cent conversion of DOC-4-^{14}C to			Rate of corticosterone formation	
	DOC†	B	18-OH-DOC	(nmoles/min/mg protein)	(% Control)
Control	10·7	53·7	26·3	27·3	
	12·9	50·4	25·1	27·3	
Testosterone (2×10^{-4} M)	40·5	30·7	16·2	16·7	62
	41·2	28·5	14·9	17·3	

* An adrenal mitochondrial pool was prepared from control rats. Mitochondria were incubated with DOC-4-^{14}C as described in Table 2. Rates of corticosterone formation were measured as described in Table 3. Testosterone was added to flasks dissolved in a small volume of ethanol to give a final concentration of 2×10^{-4} M. The higher rates of corticosterone formation in control adrenal mitochondria (see Table 3) represent normal variation between different batches of rats, but may also reflect seasonal variation in adrenal cortical function.

† Unmetabolized DOC.

DOC-induced spectral change

Figure 2 shows the effect of testosterone at a concentration of 2×10^{-4} M upon the magnitude of the spectral change induced by DOC. No correction for protein or cytochrome P-450 concentration differences was necessary, since both mitochondrial aliquots were drawn from the same sample. It is clear that the presence of testosterone had a pronounced effect upon the substrate-induced difference spectrum, reducing its magnitude to one-third that of control.

DISCUSSION

It is well established that androgens affect adrenocortical function in the rat.[1,2] When male rats are castrated, their adrenals hypertrophy and plasma corticosterone concentrations increase.[12] On the other hand, the administration of testosterone to female rats has been shown to result in decreased steroidogenesis *in vitro*, as well as a decrease in plasma corticosterone.[1] Similarly, large doses of testosterone produce a decrease in the corticosterone content of the adrenals of male rats[13] and reduced corticosterone production by adrenal slices.[2]

The aforementioned effects might all be logically attributed to the capacity of androgens to inhibit ACTH secretion by the pituitary gland.[14] However, other effects of of androgens upon adrenocortical function can only be explained via a direct effect at the adrenal level. For example, testosterone *in vitro* reduces adrenal protein synthesis in the rat[15] and competitively inhibits the 11β-hydroxylation of DOC by bovine adrenal mitochondria.[16] In addition, high doses of various androgens will retard or prevent adrenal involution after hypophysectomy[17,18] or treatment with adrenocortical steroids.[19] It has also been shown[20] that the simultaneous treatment of rats with ACTH and androgens does not prevent the inhibition of corticosteroidogenesis produced by androgens alone. Recent studies[1] have revealed that, although total steroid production by adrenal homogenates is enhanced by castration, corticosterone levels are reduced. These changes can be reversed by testosterone replacement. This apparent

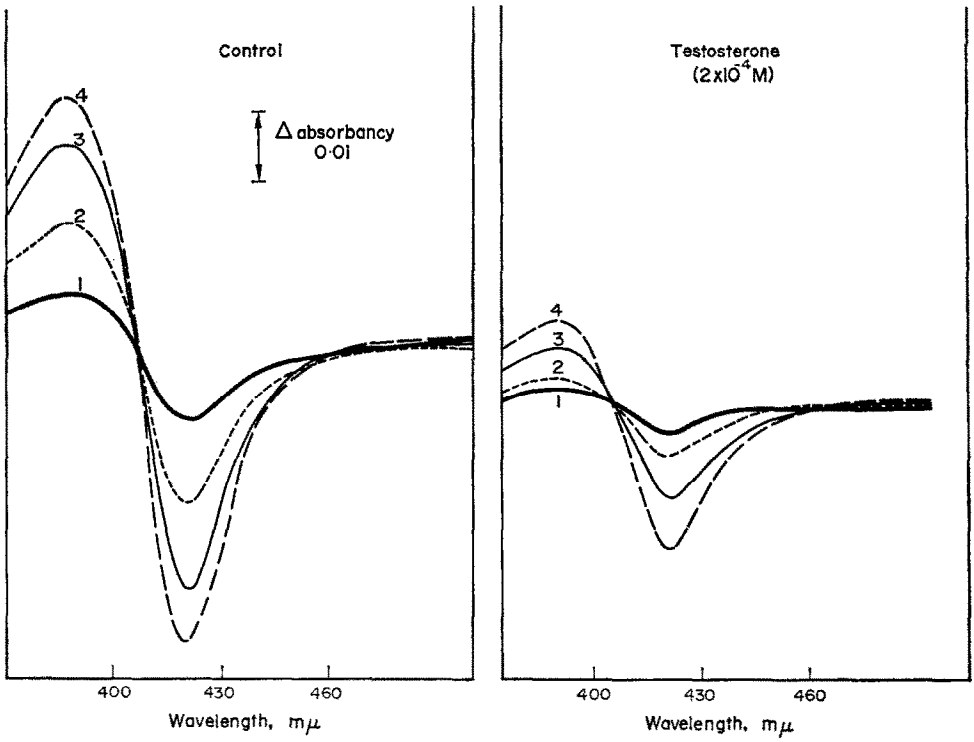

FIG. 2. Spectral changes resulting from the addition of DOC to a control adrenal mitochondrial suspension and one containing testosterone at a concentration of 2×10^{-4} M. DOC concentrations were the same as in Fig. 1.

discrepancy in total steroid and corticosterone formation has been shown to represent an increased rate in the metabolism of corticosterone by adrenals from gonadectomized male rats, resulting in an increased production of 3β,5-allotetrahydrocorticosterone.[1]

We have previously reported[3] that the administration of testosterone in large doses to properly sensitized female rats produced hypertensive cardiovascular disease. Adrenal homogenates from these rats were unable to produce substantial amounts of corticosterone or 18-OH-DOC from added tracer progesterone, resulting in a tremendous accumulation of DOC in the incubation medium. This indicated an impairment in the 11β- and 18-hydroxylation of DOC by adrenals from testosterone-treated rats, at least as measured *in vitro*. Plasma DOC levels were also markedly elevated in those rats treated with testosterone. Accompanying the decrease in the 11β- and 18-hydroxylation of DOC was a fall in the concentration of adrenal mitochondrial cytochrome P-450, the terminal oxidase for both of these hydroxylations.[21,22] The present studies were conducted in an attempt to elucidate the mechanism of this testosterone-induced decrease in DOC metabolism by adrenocortical mitochondria.

As in our previous experiments,[3] adrenal homogenates from testosterone-treated rats were impaired in their capacity to convert added progesterone beyond DOC. Control homogenates, on the other hand, metabolized almost all of the progesterone to

corticosterone and 18-OH-DOC with very little radioactivity recovered as DOC. Similarly, adrenal mitochondria isolated from the testosterone-treated rats showed considerably lower rates of 11β-hydroxylation of DOC than those isolated from controls. The calculated rates of corticosterone formation were consistent with the per cent conversion of DOC-4-^{14}C to more polar steroids by the same mitochondrial preparations.

A decrease in the concentration of adrenal mitochondrial cytochrome P-450 and an increase in cytochromes a + a_3 were apparent after testosterone treatment. The levels of cytochromes b and c + c_1 did not differ from those of controls. A similar change in adrenal mitochondrial cytochrome levels was reported[23] after treatment of rats with the synthetic androgen, 17 α-methylandrostenediol.

To be certain that the observed decrease in the level of cytochrome P-450 resulting from androgen treatment was real and not the result of testosterone interfering with the assay for cytochrome P-450, adrenal mitochondrial protoheme levels were also determined. The concentration of pyridine hemochromogen in each case was almost identical to that of cytochrome P-450, indicating that testosterone administration did produce a real fall in the concentration of adrenal mitochondrial cytochrome P-450. In addition the adrenals from testosterone-treated rats showed a reduction in adrenodoxin levels which paralleled very well the per cent decrease in cytochrome P-450. Thus, another component of the hydroxylating electron transport chain was affected by androgen.

When suspensions of adrenal mitochondria from testosterone-treated rats were titrated with DOC, the magnitude of the induced spectral change was considerably less than that of controls. It has been fairly well established that this spectral shift represents an interaction between DOC and cytochrome P-450 in the course of its (DOC) hydroxylation.[24–27] Therefore, the size of the difference spectrum depends upon the concentration of cytochrome P-450 and, since the amount of P-450 was reduced in the androgen-treated rats, this reduced spectrum was not an unexpected finding. However, even when the magnitude of the substrate-induced spectral change was evaluated per nmole of cytochrome P-450, it was still markedly smaller in adrenal mitochondria from testosterone-treated rats than in those from controls. This same effect was also observed when testosterone was added *in vitro* to adrenal mitochondria from untreated rats, so that it apparently is a direct effect of testosterone on adrenal cortical mitochondria and not secondary to an alteration in some extramitochondrial parameter.

The evidence available would therefore seem to indicate that testosterone acts to inhibit the metabolism of DOC by producing a decrease in mitochondrial cytochrome P-450 levels and, in addition, by interacting with cytochrome P-450, thereby reducing available binding sites on P-450 for DOC and preventing its further hydroxylation. Recent studies indicate that testosterone may itself be hydroxylated in place of DOC.[28]

Preliminary findings indicate that these effects may be of physiologic significance. In a single experiment, adrenal mitochondria from castrated male rats were found to have a somewhat higher concentration of cytochrome P-450 and greater spectral response to DOC than those from intact controls. This would be consistent with a physiologic interaction between androgen and cytochrome P-450. In addition, although direct comparisons were not made in any single experiment, the rates of 11β-hydroxylation *in vitro* and the magnitude of the DOC-induced spectral change were consider-

ably greater in adrenal mitochondria from untreated female rats than in those from males of the same strain. Of course, the effect of estrogen on these parameters must also be considered.

Acknowledgements—The authors wish to thank Mrs. J. Colby, Mrs. L. Chew and Mr. L. Joseph for excellent technical assistance.

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