Adrenal 5 α-Reductase Activity in the Rat: Effects of Gonadectomy: Some Cofactor and Substrate Requirements[1] (35550)

JULIAN I. KITAY,[2] MARY D. COYNE,[3] AND NANCY H. SWYGERT

Departments of Internal Medicine and Physiology, University of Virginia School of Medicine, Charlottesville, Virginia 22901

Adrenal corticosterone (Cpd B) secretion by the rat is decreased after gonadectomy in either sex and restored by replacement with estradiol or testosterone (1). This response to castration has been shown to be related to enhancement of the capacity of the adrenal gland to convert Cpd B to 3β,5α-tetrahydrocorticosterone (Cpd R) and other 5α-reduced metabolites (2, 3). The process is likewise reversed by gonadal hormone replacement. The present study demonstrates that the previously observed increment in reduced steroid metabolite formation is explained by increased adrenal 5 α-steroid reductase activity. Some aspects of cofactor and substrate requirements are also presented.

Materials and Methods. Male or female rats of the Sherman strain, obtained from Camm Research Institute, Wayne, New Jersey, were used in all experiments. The animals were maintained under standardized conditions of light (14 hr/24 hr) and temperature (22 ± 0.5°) on a diet consisting of Purina Laboratory Chow and water *ad libitum*. Gonadectomy was performed at 24–28 days of age with no further manipulation (except removal of wound clips). The rats were killed by decapitation 8 weeks later, and adrenal studies were performed. Final body weight exceeded 190 g.

Whole adrenal homogenates were prepared in 0.154 *M* KCl using glass homogenizers with Teflon pestles. Unless indicated otherwise, they were incubated for 60 min at 37° under 95% O_2 + 5% CO_2 in a reaction mixture containing KCl, 0.154 *M*; $NaHCO_3$, 20 × 10^{-3} *M*; Ca^{2+} 1.25 × 10^{-3}*M*; glucose-6-phosphate 3.3 × 10^{-3} *M*; NADP, 10^{-3}*M*, NADH, 10^{-3} *M*; and 5 to 20 mg of adrenal tissue/ml. Corticosterone or other steroid substrates, 10 or 15 μg in 3 μl of ethanol, was added as indicated, and final volume was 0.5 ml. Incubates were extracted with chloroform and steroid production was measured in aliquots by fluorescence (4) or by ultraviolet (UV) absorption at 240 mμ in methanol. Previous studies showed that the two methods give equivalent results (3). Adrenal 5 α-reductase activity was also assayed in cell fractions according to the method of Tomkins (5). Although the technique was described for liver, preliminary studies established that a pH optimum of 6.4 also applied to adrenal tissue. Modifications were trivial and included the substitution of corticosterone as substrate and a final incubation volume of 0.25 ml. Adrenal cell fractions were prepared by homogenization in 0.25 *M* sucrose supplemented with 0.05 *M* Tris–maleate buffer, pH 6.4; and 0.02 *M* nicotinamide. After initial centrifugation for 10 min at 700*g*, the supernatant was respun for 15 min at 5000*g*. The sediment (mitochondrial fraction) was resuspended and washed × 2 with buffer before incubation. The original 5000*g* supernatant was then centrifuged at 15,000*g* for 15 min. The resulting supernatant was then spun at 105,000*g* for 30 min. The final supernatant (cytosol fraction) was used unchanged. The sediment (microsomal fraction) was resuspended and centrifuged again at 105,000*g* for 30 min before use. In some experiments, corticosterone-4-^{14}C, 0.025 μCi,

[1] This investigation was supported in part by a U.S. Public Health Service grant (AM-03370) from the NIH.

[2] U.S. Public Health Service Research Career Development Awardee (AM-K3-13960).

[3] Present address: Department of Physiology, Louisiana State University Medical Center, New Orleans, La. 70112.

was also added to the adrenal homogenates. The radioactive steroid, with initial specific activity of 40 mCi/mmole, was obtained from New England Nuclear Corp. and rechromatographed before use. After incubation, an aliquot of the chloroform extract was chromatographed on a Bush B1 system (6), and the distribution of the radioactivity on the chromatogram determined with a Packard Model 7200 scanner using authentic Cpd B and Cpd R standards. Previous studies demonstrated these zones to be essentially homogeneous (3). Quantitative measurements were then made on eluates (overall recovery rate 85–90%) from each strip, with a Packard Tri-Carb liquid scintillation spectrometer.

Results and Discussion. In the first experiment (Fig. 1), whole adrenal homogenates from intact and castrate female rats were incubated for 5, 15, 30, and 60 min. Rates of metabolism of 15 μg of added Cpd B, monitored by acid fluorescence, were linear over the entire time span. Adrenal homogenates from ovariectomized rats utilized significantly more steroid at all time intervals except the 5-min point ($p < .01$). Similar results (not illustrated) were obtained with adrenal tissue from intact and castrate male rats.

The relation between adrenal tissue concentration and rates of metabolism of Cpd B was studied in the next experiment (Fig. 2). Individual whole adrenal homogenates from

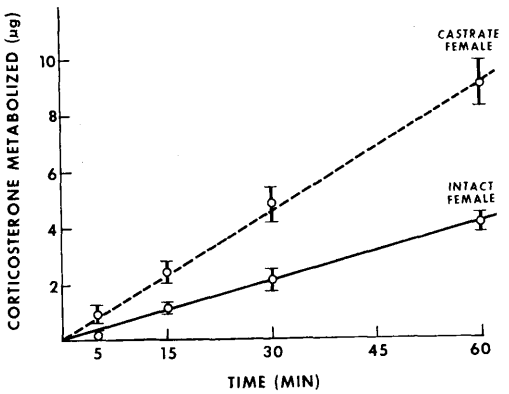

FIG. 1. Relation of Cpd B metabolism by adrenal homogenates and duration of incubation: each point represents the mean of 5 observations. The vertical lines represent the standard error of the mean. Each beaker contained 10 mg of adrenal tissue.

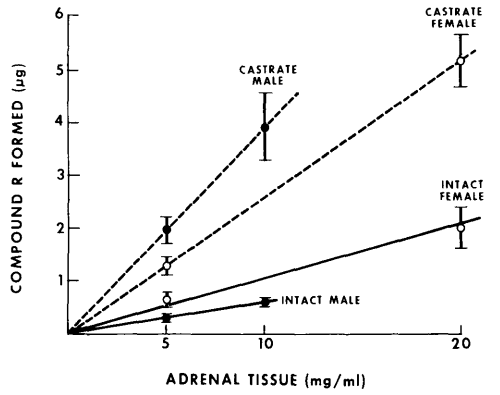

FIG. 2. Relation of Cpd R formation and adrenal tissue concentration ($N = 5$); incubation time, 1 hr.

intact and gonadectomized rats of both sexes were prepared simultaneously and incubated for 60 min with added 4-^{14}C-Cpd B as well as 15 μg of unlabeled steroid. Recovery of Cpd R was calculated from the resulting specific activity of 3700 dpm/μg of steroid. Metabolite formation was linear with all the homogenate preparations from 5 to 20 mg/ml. Gonadectomy in either sex resulted in significantly more Cpd R formed compared to the respective intact control level ($p < .001$). The amount formed by adrenal tissue from castrate males exceeds that from castrate females ($p < .01$). However, the reverse relationship (F > M) obtains with adrenal homogenates from intact rats.

Intracellular localization of the site of 5α-reduction was determined by incubating cell fractions prepared with adrenal glands from castrate male rats (Fig. 3). The fractions were reconstituted in a volume equivalent to 20 mg of whole homogenate/incubation tube. The greatest part of the Cpd R recovered was obtained in the 105,000*g* sediment or microsomal fraction. Formation rate was linear with time. Approximately 10% of the overall yield of Cpd R was found in the 5000*g* sediment fraction, probably reflecting inadequate washing of the mitochondria. Negligible levels were obtained in the 105,000*g* supernatant or cytosol fraction. Similar results, limited to the 105,000*g* sediment, are presented for adrenal tissue from castrated female rats. Cpd R production is linear with time, and the yields are significantly lower

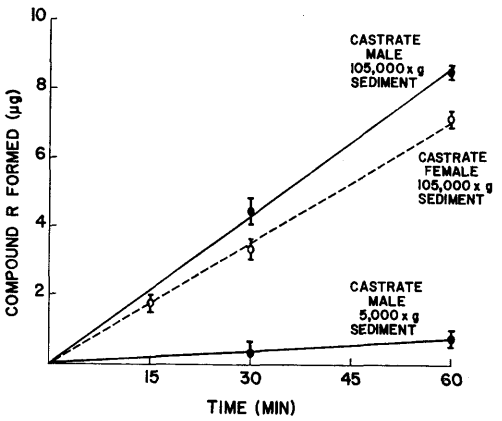

FIG. 3. Relation of Cpd R formation and adrenal cell fractions ($N = 4$); each tube contained the equivalent of 20 mg of adrenal tissue.

than those obtained in the same fraction from orchiectomized rats.

Some effects of varying cofactors are presented in Table I. Incubations were performed for 60 min, and metabolism of Cpd B was measured by acid fluorescence. In every instance, Cpd B utilization was greater ($p < .01$) in whole adrenal homogenates from castrated rats than intact animals of the same sex. Standard conditions of incubation are indicated on the first line of Table I. Omission of NADH resulted in significantly less metabolism of Cpd B by all homogenates except those from intact male rats. Omission of Ca^{2+} was without further effect. Use of NADPH in lieu of NADP + G-6-P resulted in markedly reduced activity.

Metabolism of different substrates by adrenal homogenates was compared to that of Cpd B in the final experiments (Table II). UV absorption was used to measure steroid metabolism by whole homogenates. Testosterone was utilized most rapidly (compared to Cpd B) by all the homogenate preparations; whereas aldosterone was metabolized least rapidly. Cortisol was also less effectively utilized than Cpd B in homogenates from male rats.

The data demonstrate the presence of 5α-reductase activity in the rat adrenal gland confirming previous reports (7–9). Additional discussion of reductive enzymes in the adrenal gland is presented elsewhere (3). Removal of the gonads from both male and female rats is followed by greater production of 5α-reduced metabolites and a concomitant diminution in Cpd B production (1–3). The present results indicate that the rise in production of such metabolites is explained by increased 5 α-steroid reductase activity. The conversion of Cpd B to Cpd R involves two steps: (i) 5α-reduction to 5-dihydrocorticosterone; and (ii) 3β-reduction to Cpd R. The activity of the enzyme concerned with the second step, 3β-hydroxysteroid dehydrogenase, is not increased after castration in either sex (3). Moreover, its activity in the rat adrenal cortex greatly exceeds that of the 5-reductase so that the latter is rate limiting under any of the conditions tested. Adrenal reductase activity is lower in intact males than females but increases to a higher level in the castrate male than in the castrate female. The biological significance of these

TABLE I. Relation of Cofactor Requirements and Metabolism of Cpd. B by Adrenal Homogenates.[c]

Conditions	Cpd. B metabolized (μg/10 mg of adrenal/hr)			
	Male		Female	
	Intact	Castrate	Intact	Castrate
NADP + G-6-P + NADH	1.5 ± 0.2	8.4 ± 0.3	3.1 ± 0.6	6.0 ± 0.4
NADP + G-6-P	1.2 ± 0.4	5.7 ± 0.8[a]	0[a]	4.0 ± 0.7[b]
NADP + G-6-P (0 Ca^{2+})	—	6.2 ± 0.6[b]	1.2 ± 0.8[b]	4.9 ± 0.6
NADPH	0.9 ± 0.2	2.0 ± 0.2[a]	0.1 ± 0.2[a]	1.0 ± 0.2[a]

[a] Differs from the corresponding value obtained with the addition of NADP + G-6-P + NADH: $p < .01$; [b] $p < .05$.

[c] Each entry is the mean of 6 observations ± SE; 10 μg Cpd. B added at the outset of incubation.

TABLE II. Metabolism of Various Substrates by Adrenal Homogenates.

	Steroid metabolized (μg/10 mg of adrenal/hr)			
	Male		Female	
Substrate added	Intact	Castrate	Intact	Castrate
Expt. 1[a]				
Corticosterone	3.4 ± 0.1	7.8 ± 0.3	2.4 ± 0.4	6.3 ± 0.6
Cortisol	1.1 ± 0.5[c]	5.8 ± 0.6[d]	1.6 ± 0.7	5.1 ± 0.3
Δ^4-Androstenedione	3.0 ± 0.7	7.6 ± 0.6	4.3 ± 0.4	8.3 ± 0.6
11-Deoxycorticosterone	1.7 ± 0.1[d]	7.8 ± 0.3	4.6 ± 0.4	7.7 ± 0.9
Expt. 2[b]				
Corticosterone	1.8 ± 0.5	11.1 ± 0.7	4.6 ± 0.4	8.8 ± 0.9
Testosterone	3.9 ± 0.4[d]	13.0 ± 0.9[d]	5.4 ± 0.6[d]	11.9 ± 0.6[c]
Aldosterone	1.0 ± 0.6	5.3 ± 0.8[c]	2.3 ± 0.8[c]	4.0 ± 0.3[c]

[a] Each entry is the mean of 5 observations ± SE; 10 μg of each steroid added at outset.
[b] Each entry is the mean of 8 observations ± SE; 15 μg of each steroid added at outset.
[c] Differs from corresponding value obtained with corticosterone: $p<.01$; [d] $p<.05$.

differences is not apparent. The 105,000*g* sediment or microsomal fraction appears to contain most (if not all) adrenal reductase activity. Inclusion of NADH enhances the reaction in whole homogenates, possibly because 3-hydroxysteroid dehydrogenase activity requires this cofactor (10).

Summary. Steroid 5α-reductase activity is demonstrated in the adrenal cortex of male and female rats (F > M). Following either orchiectomy or ovariectomy, activity of the enzyme is elevated extending previous findings of increased adrenal 5α-metabolite formation in gonadectomized rats. Reductase activity in adrenal tissue from castrate males is greater than that from castrate females. NADH enhances enzyme activity in whole homogenates. Centrifugation studies demonstrate most of the activity to reside in the 105,000*g* sediment. Compared to corticosterone, testosterone is a more preferred substrate, whereas cortisol is less well metabolized and aldosterone least of all.

1. Kitay, J. I., Endocrinology **73**, 253 (1963).
2. Kitay, J. I., Nature (London) **209**, 808 (1966).
3. Kitay, J. I., Coyne, M. D., and Swygert, N. H., Endocrinology **87**, 1257 (1970).
4. Guillemin, R., Clayton, G. W., Lipscomb, H. S., and Smith, J. D., J. Lab. Clin. Med. **53**, 830 (1959).
5. Tomkins, G. M., "Methods Enzymol. **5**, 499 (1962).
6. Bush, I. E., "The Chromatography of Steroids," 437 pp. Pergamon, New York (1961).
7. Schneider, J. J., and Horstmann, P. M., J. Biol. Chem. **191**, 327 (1951).
8. Tomkins, G. M., J. Biol. Chem. **225**, 13 (1957).
9. Brown-Grant, K., Forchielli, E., and Dorfmann, R. I., J. Biol. Chem. **235**, 1317 (1960).
10. Tomkins, G. M., J. Biol. Chem. **218**, 437 (1956).

Received Jan. 4, 1971. P.S.E.B.M., 1971, Vol. 137.