Effects of Postpuberal Orchiectomy on Adrenal Reductase Activity and Corticosterone Secretion in the Rat (36383)

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Many effects of gonadal hormones on the pituitary–adrenocortical axis in the rat have been described (1). Coyne and Kitay have shown that testosterone inhibits (2) and estradiol stimulates (3) the secretion of ACTH by the anterior pituitary gland. Similar effects are exerted by the gonadal hormones on hepatic steroid metabolism (4, 5) and corticosteroid-binding globulin levels in plasma (6). In addition, both testosterone and estradiol act directly on the adrenal cortex to stimulate corticosterone secretion. Replacement of rats that are hypophysectomized as well as castrated with the appropriate sex hormone increases corticosterone production by adrenal slices *in vitro* (7, 8). Recent studies have demonstrated that although total steroidogenesis in adult male rats is enhanced by prepuberal orchiectomy as a result of increased ACTH output, corticosterone secretion either declines or is unaffected (9). This apparent discrepancy represents a marked increment in the intra-adrenal metabolism of corticosterone by castrates, resulting in greater production and secretion of tetrahydro- and probably dihydrocorticosterone (10). The activity of the enzyme responsible for this transformation, 5α-reductase, is stimulated by gonadectomy and inhibited by replacement with testosterone. The present communication describes the temporal changes in adrenal 5α-reductase activity following orchiectomy after the age of puberty and their effects on steroid secretion in the adult male rat.

Materials and Methods. Male rats of the Sherman strain, obtained from Camm Research Institute, Wayne, NJ, were used in all experiments. Animals were maintained under standardized conditions of light (0600–1800) and temperature (22.0 $\pm$ 0.5°) on a diet consisting of Purina Laboratory Chow and water *ad libitum*. Orchiectomy was performed at 55–60 days of age and rats were killed by decapitation at weekly intervals thereafter.

Whole adrenal homogenates and adrenal slices were prepared and incubated as previously described (10). Incubates were extracted with chloroform and corticosterone production measured by fluorescence (11). Total steroid output was evaluated by the blue tetrazolium reaction (12) which is positive for steroids with an α-ketol side chain. Adrenal reductase activity was assayed in whole adrenal homogenates by methods already described (10). Adrenal vein blood was collected for 10 min following induction of anesthesia with sodium pentobarbital (9) and corticosterone measured by a competitive protein-binding technique (13).

Results. Body and adrenal weights. Orchiectomized rats grew at a slower rate than intact controls. Beginning at 3 weeks after gonadectomy castrates weighed significantly less than intacts of the same age (Table I). Adrenal weights increased following orchiectomy; castrates had significantly larger adrenals than controls at 2, 3 and 5 weeks after gonadectomy.

Reductase activity. Adrenal 5α-reductase activity in 1 week castrates did not differ from that in controls (Table I). Beginning at 2 weeks after orchiectomy, however, adrenal reductase activity was significantly greater in gonadectomized rats than corresponding intacts. The amount of corticosterone reduced by adrenal homogenates from castrates gradually increased each week whereas control levels were essentially unchanged.

TABLE I. Effect of Orchiectomy on Body and Adrenal Weights and Adrenal 5α-Reductase Activity.[a]

Postcastration (weeks)	Body wt (g)		Adrenal wt (mg)		Corticosterone reduced (μg/10 mg/hr)	
	Intact	Castrate	Intact	Castrate	Intact	Castrate
1	304 ± 7 (6)	303 ± 4 (6)	45.3 ± 2.9	45.7 ± 1.6	0.7 ± 0.4	1.0 ± 0.5
2	334 ± 7 (6)	317 ± 8 (6)	47.7 ± 1.9	59.6 ± 4.0[c]	1.2 ± 0.5	7.2 ± 1.3[c]
3	360 ± 8 (6)	330 ± 8[b] (6)	47.2 ± 3.2	62.3 ± 4.1[b]	0.5 ± 0.5	8.3 ± 0.7[c]
4	391 ± 12 (6)	330 ± 14[b] (6)	51.3 ± 3.0	60.6 ± 4.1	0.5 ± 0.2	9.8 ± 2.1[c]
5	406 ± 16 (6)	336 ± 22 (6)	55.6 ± 3.5	68.2 ± 3.8[b]	2.4 ± 1.1	13.6 ± 1.9[c]

[a] Values expressed as means ± SE; number of animals in each group indicated in parentheses.
[b] $p < .05$ (vs corresponding intact value).
[c] $p < .01$ (vs corresponding intact value).

Steroid production by adrenal homogenates. Corticosterone production by adrenal homogenates from castrates and controls was similar at each of the time intervals examined (Table II). Total steroid production, as measured by the blue tetrazolium reaction, however, was enhanced by orchiectomy, such that 2-week postcastration and thereafter castrates produced significantly more total steroid than intacts (Table II). As a result, the ratio of corticosterone to total steroid production declined following orchiectomy (Table II).

Steroid production by adrenal slices. Steroid output by adrenal slices confirmed the results of homogenate incubations (Table III). Corticosterone production was not affected by orchiectomy. Castrates produced significantly more total steroid than intacts and therefore corticosterone represented a smaller fraction of the total steroid secreted by gonadectomized rats than by controls (Table III).

In vivo *corticosterone secretion.* Corticosterone secretion *in vivo* was unaffected by orchiectomy at 1, 3 and 5 weeks after surgery (Table IV).

Discussion. Previous studies have established that prepuberal gonadectomy of rats of either sex results in diminished corticosterone production by adrenal tissue *in vitro* after puberty (5). The effect is reversed by replacement with the appropriate gonadal hormone. Since testosterone and estradiol influence a number of components of the pituitary–adrenal axis (1), their net effect on adrenal function depends upon the outcome of multiple independent events. The apparent

TABLE II. Steroid Production by Adrenal Homogenates from Intact and Orchiectomized Rats.[a]

Postcastration (weeks)	Steroid production (μg/100 g of body wt/hr)					
	Corticosterone		Total steroid		Corticosterone/total (%)	
	Intact	Castrate	Intact	Castrate	Intact	Castrate
1	3.4 ± 0.3 (6)	3.9 ± 0.5 (6)	7.5 ± 0.8	8.2 ± 0.8	46 ± 1	42 ± 4
2	2.9 ± 0.3 (6)	2.5 ± 0.3 (6)	7.0 ± 0.6	10.0 ± 1.0[b]	40 ± 1	25 ± 1[c]
3	2.3 ± 0.3 (6)	2.5 ± 0.3 (6)	5.0 ± 0.7	10.0 ± 1.0[c]	48 ± 4	25 ± 1[c]
4	3.7 ± 0.4 (6)	3.2 ± 0.5 (6)	7.9 ± 0.8	13.4 ± 1.2[c]	46 ± 2	23 ± 2[c]
5	2.5 ± 0.2 (6)	2.1 ± 0.6 (6)	6.7 ± 0.6	13.0 ± 1.0[c]	38 ± 3	17 ± 1[c]

[a] Values expressed as means ± SE; number of animals in each group indicated in parentheses.
[b] $p < .05$ (vs corresponding intact value).
[c] $p < .01$ (vs corresponding intact value).

TABLE III. Steroid Production by Adrenal Slices from Intact and Orchiectomized Rats.[a]

Postcastration (weeks)	Steroid production (μg/100 g of body wt/hr)					
	Corticosterone		Total steroid		Corticosterone/total (%)	
	Intact	Castrate	Intact	Castrate	Intact	Castrate
1	1.0 ± 0.1 (7)	1.1 ± 0.1 (7)	2.3 ± 0.2	3.1 ± 0.3[b]	41 ± 4	35 ± 2
2	0.5 ± 0.1 (6)	0.7 ± 0.1 (6)	1.6 ± 0.2	2.7 ± 0.1[c]	33 ± 2	25 ± 3[b]
3	0.7 ± 0.1 (6)	0.7 ± 0.1 (6)	2.0 ± 0.2	2.8 ± 0.4[b]	35 ± 2	23 ± 2[c]
4	0.9 ± 0.1 (6)	0.8 ± 0.1 (7)	2.1 ± 0.2	3.3 ± 0.2[c]	40 ± 3	24 ± 2[c]
5	0.8 ± 0.1 (7)	0.7 ± 0.1 (7)	2.2 ± 0.3	3.5 ± 0.2[c]	37 ± 3	20 ± 2[c]

[a] Values expressed as means ± SE; number of animals in each group indicated in parentheses.
[b] $p < .05$ (vs corresponding intact value).
[c] $p < .01$ (vs corresponding intact value).

deficit in corticosterone production following gonadectomy is explained, at least in part, by an increase in adrenal 5α-reductase activity resulting in enhanced conversion of corticosterone to 3β,5α-tetrahydrocorticosterone and other 5α-reduced metabolites (10). The activity of this enzyme is inhibited by testosterone or estradiol. Contributing to the decline in corticosterone production after prepuberal ovariectomy is a depression in ACTH secretion (3). These factors can account for the large decrement in corticosterone secretion *in vivo* following ovariectomy (5). Orchiectomy, on the other hand, is accompanied by increased ACTH secretion (2), resulting in adrenal hypertrophy. Nevertheless, despite an increase in cholesterol side chain cleavage activity and, therefore, a stimulation of total steroidogenesis following orchiectomy, corticosterone secretion *in vivo* is reduced. This decline can be partially accounted for by an increase in the secretion of 3β,5α-tetrahydrocorticosterone (9).

TABLE IV. *In Vivo* Corticosterone Secretion Rates by Intact and Orchiectomized Rats.[a]

Postcastration (weeks)	Corticosterone secretion (μg/100 g of body wt/hr)	
	Intact	Castrate
1	18.4 ± 2.1 (6)	24.3 ± 2.9 (6)
3	19.3 ± 3.4 (6)	19.8 ± 3.0 (6)
5	15.0 ± 1.1 (7)	15.4 ± 2.5 (7)

[a] Values expressed as mean ± SE; number of animals in each group indicated in parentheses.

The increase in adrenal 5α-reductase activity following prepuberal gonadectomy occurs abruptly at about 50 days of age (14). Near maximal enzyme activity is attained within 1 week after its initiation. Regardless of the age at which castration is performed, the elevation in reductase activity does not occur prior to the normal age of puberty, suggesting that its appearance represents a maturational phenomenon (14). Increases in reductase activity resulting from hypophysectomy or cortisone suppression of ACTH secretion, on the other hand, can be promptly elicited at any age (14). The decrement in corticosterone production associated with gonadectomy is also not elicitable before puberty and thereafter parallels the change in reductase activity. In contrast to these observations, the present studies indicate that adrenal 5α-reductase activity is elevated significantly within 2 weeks after removal of the testes from adult rats. The response is comparable to that evoked by ovariectomy of sexually mature female rats (14). The level of enzyme activity achieved following postpuberal orchiectomy is at least equivalent to that induced by castration of immature male rats. Nonetheless, corticosterone production *in vitro* and secretion *in vivo* is unaffected by orchiectomy after puberty. This is in contrast to the decline in corticosterone output after prepuberal gonadectomy. Furthermore, our observations fail to confirm a preliminary report indicating a similar fall in corticosterone secretion following orchiectomy of

adult rats (15). The latter investigations did, however, consider more chronic effects of gonadectomy than examined in these studies.

The increment in reductase activity after postpuberal orchiectomy is associated with adrenal hypertrophy, consistent with a concomitant increase in ACTH secretion. This, in turn, can account for the elevation in total steroid production by adrenal tissue *in vitro* following removal of the testes. These changes are reflected by a steadily diminishing ratio of corticosterone to total steroid secretion following gonadectomy. The increase in reductase activity tends to offset the action of ACTH to stimulate corticosterone production. The net outcome of these opposing effects is to maintain normal corticosterone secretion rates both *in vitro* and *in vivo* after orchiectomy. The additional quantity of steroid secreted by the castrate probably represents 5α-reduced metabolites of corticosterone and other Δ^4-3-ketosteroids. Determination of the *in vivo* rates of secretion of these metabolites is needed to establish the validity of this hypothesis. Preliminary studies indicate that the secretion of 3β, 5α-tetrahydrocorticosterone increases after prepuberal gonadectomy (9).

Although corticosterone secretion per animal or per unit body weight is unaffected by postpuberal orchiectomy, net corticosterone output per unit adrenal mass is significantly reduced. Most investigators have employed the last means of expression for *in vitro* studies but one of the former for *in vivo* experiments. Clearly such an approach in the present studies would have yielded divergent findings for the *in vitro* and *in vivo* observations. A more uniform approach to the evaluation of secretory rates might similarly reduce the number of seemingly "conflicting" reports in the literature.

The relation of the age at which orchiectomy is performed to its effect on adrenal corticosterone secretion may be related to the findings of Witorsch and Kitay (14) indicating that the development of adrenal 5α-reductase activity represents a pubertal phenomenon. The essential role of the pituitary gland in the onset of puberty has been clearly defined. Furthermore, at least three pituitary factors, ACTH, growth hormone and prolactin, affect adrenal 5α-reductase activity (16). Age-dependent changes in the secretion of any of these hormones in response to castration could account for the different effects of orchiectomy on corticosterone secretion in the adult and immature animal. Maturational changes in the sensitivity of the adrenal cortex to testosterone could similarly provide an explanation for these differences. The involvement of still other mechanisms cannot at present be excluded. The effects of gonadal and pituitary hormone interactions on adrenocortical function are currently under investigation.

Summary. Gonadectomy of adult male rats resulted in hypertrophy of the adrenal glands. Total adrenal steroid (blue tetrazolium positive) production *in vitro* was similarly augmented. Corticosterone secretion both *in vitro* and *in vivo* was unaffected. The ratio of corticosterone to total steroid, therefore, gradually declined following removal of the testes. This effect is explained by a concomitant increase in adrenal 5α-reductase activity after orchiectomy, resulting in greater conversion of corticosterone to 5α-reduced metabolites.

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