Effect of Inhibition of Δ^5,3β-Hydroxysteroid Dehydrogenase upon Pituitary and Serum Radioimmnoassayable Luteinizing Hormone in the Adult Male Rat (35337)

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Two synthetic steroid analogs (2α-cyano-4,-4,17α-trimethylandrost-5-en-17β-ol-3-one [cyano-ketone); 17β-hydroxy-4,4-17α-trimethyl-androst-5-en-(2,3*d*)-isoxazole (isoxazole)] are potent selective inhibitors of 3β-hydroxysteroid dehoydrogenase and of Δ^{5-4}-3-ketosteroid isomerase (1). In the mature rat these analogs block adrenal glucorticoid synthesis and secretion. Adrenocortical, ovarian, and testicular interstitial cell hyperplasia are also demonstrable in these steroidogenic tissues after the administration of the inhibitors (2). Adrenocortical hyperplasia results from stimulation by adrenocorticotropic hormone secreted in response to the blockade in glucocorticoid synthesis produced by these analogs (3–5). In the present communication we report that the cyano-ketone increases pituitary and serum levels of radioimmunoassayable luteinizing hormone (LH) in the mature male rat, with a concomitant fall in plasma testosterone, suggesting that the interstitial cell hyperplasia produced by this compound may be secondary to increased gonadotropin secretion.

Materials and Methods. Adult male rats (13 weeks, 250 g, Charles River Breeding Laboratory, Wilmington, Mass.) received intramuscular injections of the cyano-ketone in dimethylsulfoxide or diluent alone. The cyano-ketone was administered at a dose of 10 mg/kg daily for 2 days and the animals were sacrificed by exsanguination 2 hr after the last injection (Table I) or for 8 days (days 1, 2, 3, 4, 7, 8, 9 and 10) and the animals were sacrificed 96 hr after the last injection (Table II). In another experiment the animals received the cyano-ketone at a dose of 60 mg/kg daily for 3 days with sacrifice 48 hr after the last injection (Table II). The adrenals and testes were flash-frozen at —80° and stored at —20°. The pituitaries were homogenized in 0.01 *M* sodium phosphate–0.15 *M* sodium chloride, pH 7.5, centrifuged (4°, 2000 rpm) and the supernatant was stored at —20°. Serum was stored at —20°.

3β-Hydroxysteroid dehydrogenase activity was determined in adrenal and testicular homogenates as previously described (6). Serum testosterone concentrations were measured by modifications of a competitive-protein-binding assay (7, 8). The modifications were the application of a more sensitive standard curve previously described for the assay of Δ^4-androstenedione in plasma (8). Serum and pituitary immunoassayable LH levels were determined by double antibody radioimmunoassay, employing reagents supplied by the Hormone Distribution Officer, NIAMD (9, 10). Data are expressed in terms of NIH-Rat LH-RP-1 (Biological activity: 0.03 NIH-LH-S1). The assay was sensitive to 6.25 ng of standard. The intra-assay coefficient of variation was 3.5%. All specimens from one experiment were measured in the same assay in order to eliminate

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TABLE I. Effect of Cyano-ketone on Testicular and Adrenal 3β-Hydroxysteroid Dehydrogenase Activities and Serum Testosterone Concentrations.[a]

Group	Dose (mg/kg)	No. of doses	No. of animals	Body wt (g)	Wt (mg/100 g of body wt)			3β-hydroxysteroid dehydrogenase (μg/min/mg of protein)		Serum testosterone[b] (μg/100 ml)
					Pituitary	Adrenal	Testicular	Testis	Adrenal	
Control	—	—	8	243.5 ±12.7	2.9 ± 0.3	18.4 ± 5.1	1.15 ± 0.04	0.11 ± 0.06	2.12 ± 0.5	2.3 ± 0.3
Cyano-ketone	10	2	8	242.8 ± 5.0	3.4 ± 0.3[c]	26.3 ± 3.2[c]	1.16 ± 0.04	0.03 ± 0.16[c]	0.60 ± 0.15[c]	1.54 ± 0.10[c]

[a] Mean ± one SD.
[b] Corrected for recovery using 1-2 ^{3}H-testosterone as tracer (mean recovery 70 percent).
[c] $p < 0.01$ (versus controls).

TABLE II. Effect of Cyano-ketone on Serum and Pituitary Levels of Luteinizing Hormone.

Group	Dose (mg/kg)	No. of doses	No. of animals	Body wt (g)	Wt (mg/100 g of body wt)			3β-hydroxysteroid dehydrogenase (μg/min/mg of protein)		Luteinizing hormone		
										Pituitary		
					Pituitary	Adrenal	Testicular	Testis	Adrenal	Total (μg)	Concentration (μg/ng)	Serum[a] (ng/ml)
Control	—	—	12	274.4 ±18.4	2.8 ±0.3	16.2 ±3.0	1.13 ±0.09	0.08 ±0.05	2.18 ±0.45	293.6 ±75.7	36.9 ±9.5	65.3 ±6.3
Cyano-ketone	60	3	12	280.8 ±11.3	3.4[b] ±0.2	24.9[b] ±9.4	1.05 ±0.12	0.04[b] ±0.05	0.53[b] ±0.11	348.6 ±106.4	40.7 ±13.7	84.7[b] ±12.9
Control	—	—	8	347.8 ±26.0	2.78 ±0.37	— —	— —	— —	— —	382.0 ±73.1	39.9 ±7.1	81.3 ±25.0
Cyano-ketone	10	8	8	343.0 ±22.6	3.00 ±0.35	— —	— —	— —	— —	551.0[b] ±150.8	53.4[c] ±3.0	126.6[d] ±50.2

[a] Serum available in 9 animals from each group.
[b] $p < 0.01$; [c] $p < 0.02$; [d] $p < 0.05$ (versus controls).

interassay variability. Statistical evaluation was performed by the method of Student's t, or by the method of analysis of variance.

Results. Adrenal and pituitary weights were significantly greater in animals who received the cyano-ketone (10 and 60 mg/kg). Concomitantly, 3β-hydroxysteroid dehydrogenase activity of adrenal and testicular homogenates and serum testosterone concentrations were significantly lower in these analog-treated rats (Tables I, II). Serum LH concentrations were significantly higher in the cyano-ketone treated animals (Table II). Although pituitary LH content was higher in animals who received the analog at the dose of 60 mg/kg for 3 days, this difference was not significant when compared to the pituitary LH content of the control animals. Following the administration of cyano-ketone, 10 mg/kg for 8 days, there was a significant increase in pituitary LH content and concentration of the analog-treated animals (Table II).

Discussion. In adult male rat, inhibition of testicular 3β-hydroxysteroid dehydrogenase activity was followed by decrease in serum testosterone concentrations and increase in serum and pituitary immunoassayable LH levels. These data suggest that testicular interstitial cell hyperplasia observed histologically may be secondary to LH stimulation. Gay and Midgley (11) and Yamamoto *et al.* (12) reported that serum immunoassayable LH concentrations increased within 8 to 18 hr after castration of the adult male rat, but that pituitary LH content did not increase until 5 days after orchiectomy. Under the conditions of the present experiments, noting that different dosages of analog were employed, inhibition of testosterone synthesis as reflected by a fall in plasma levels was followed by an increase in serum LH concentrations prior to an increase in pituitary LH. The failure to document an increase in pituitary weight after 8 doses of cyano-ketone (10 mg/kg) is unexplained.

Although the effect of inhibition of adrenal androgen synthesis upon the regulation of LH secretion has not been considered in this discussion, the most probable site of action of the cyano-ketone in relation to its effects upon LH secretion is at the level of testicular testosterone production. Inhibition of endogenous testosterone synthesis is most probably responsible for the (compensatory) increase in LH synthesis and secretion.

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