Binding of Cyproterone Acetate to Plasma Proteins[1] (37283)

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Cyproterone acetate (CA) (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3,20-dione-17α-acetate) is a potent antiandrogen with high progestational activity (1). Even when administered concurrently with androgens, CA generally reduces the size of male accessory sex organs. Its effect is on target tissues responsive to androgens (2). In the rat CA reduces the retention of testosterone in seminal vesicles and glans penis but not in brain, muscle or plasma (3). In brain, however, Stern and Eisenfeld (4) found the uptake of testosterone to be antagonized by the nonesterified form, cyproterone. Neumann, Elger and VonBerswordt-Wallrabe (5) suggested that CA attaches to androgen receptor sites, preventing binding of endogenous androgens to target tissues. In support, competition between testosterone and cyproterone has been demonstrated for binding sites on seminal vesicular macromolecules *in vitro* and *in vivo* (6).

Several plasma globulins possess specific binding affinities for steroid hormones. Sex hormone-binding globulin (SHBG) in human late pregnancy plasma (7) has been shown to specifically bind C_{19} steroids and to react slightly with C_{18} steroids but not with C_{21} steroids (8). Corticosteroid-binding globulin (CBG) in dog plasma (9) is specific for C_{21} steroids. We tested the possibility of receptor sites for CA, a C_{21} steroid derivative, on the two plasma globulins. Consideration is also given to competition between CA and representatives of other classes of steroids for binding sites on SHBG and CBG.

Materials and Methods. To test the displacement of ^{3}H-testosterone from SHBG 1,2-^{3}H-testosterone[3] at 1.3 $\times$ 10^7 dpm was added to 100 ml of 0.3% pooled human late pregnancy plasma as outlined by Murphy (9) and displaced with authentic testosterone, estradiol-17β (E_2) or CA[4] at concentrations of 0, 1, 2, 3 or 4 ng/tube. The assays employing 1 ml of the SHBG preparation were run in duplicate or triplicate. Florisil (magnesium silicate, Sigma Chemical Co.) was used to separate free from protein-bound steroid according to Neill *et al.* (10).

To determine the displacement of ^{3}H-estradiol-17β (^{3}H-E_2) from SHBG 6, 7-^{3}H-E_2 at 1.7 $\times$ 10^7 dpm was added to 100 ml of 0.3% SHBG. The labeled steroid was displaced with authentic E_2, testosterone or CA at 0, 1, 2, 3 or 4 ng/tube. Assays using 1 ml of the SHBG preparation were run in duplicate as above.

Displacement of ^{3}H-corticosterone from CBG was tested by adding 1,2-^{3}H-corticosterone at 11 $\times$ 10^6 dpm to 100 ml of 2.5% dog serum (9). The labeled steroid was

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[3] All radioactive steroids from Amersham/Searle Corp.

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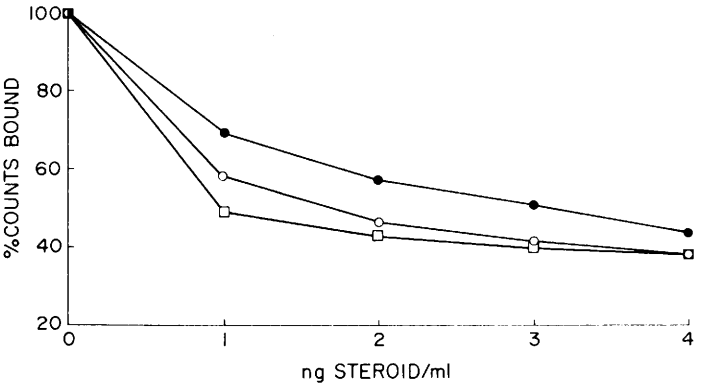

FIG. 1. Displacement of ^{3}H-testosterone from sex hormone-binding globulin (SHBG) by estradiol-17β (●), cyproterone acetate (CA) (□), and testosterone (○).

displaced with authentic progesterone, corticosterone or CA at 0, 0.5, 1, 2, 3 or 4 ng/tube as above.

Results and Discussion. CA was as effective as testosterone in displacing ^{3}H-testosterone from SHBG and E_2 was less effective (Fig. 1). Cyproterone, however, reportedly has less than 5% the binding affinity of testosterone for a testosterone-binding globulin preparation (11). The close similarity between the CA and testosterone displacement curves plus the fact that E_2 displaces relatively less bound testosterone suggests that there is a greater similarity between binding sites for testosterone and CA than between testosterone and E_2.

CA was less capable of displacing ^{3}H-E_2 from SHBG than testosterone or E_2 (Fig. 2). Displacements by the latter two steroids were very similar, indicating that similar binding sites may be shared by E_2 and testosterone. Generally, displacement of ^{3}H-E_2 from the protein was less complete than displacement of ^{3}H-testosterone (Fig. 1). It has been shown (8) that E_2 will maximally displace 20% of labeled testosterone from SHBG. CA will displace over 65% of labeled testosterone from SHBG but a minimal amount of ^{3}H-E_2. This differential displacement suggests that E_2 occupies binding sites that differ from those of CA. Our explanation of this enigma is that testosterone and E_2 occupy similar but not identical sites on SHBG whereas CA and testosterone interact on the same site.

CA (a C_{21} steroid), unlike progesterone or corticosterone, does not displace ^{3}H-corticosterone (Table I). None of the C_{21}

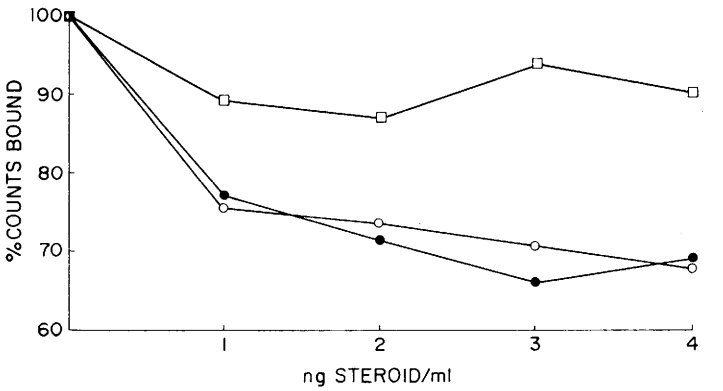

FIG. 2. Displacement of ^{3}H-estradiol-17β from sex hormone-binding globulin (SHBG) by estradiol-17β (●), cyproterone acetate (CA) (□), and testosterone (○).

TABLE I. Displacement of ^{3}H-Corticosterone from Corticosteroid-Binding Globulin by Progesterone, Corticosterone and Cyproterone Acetate.[a]

Concn (ng/tube)	Test steroids [% counts bound (av)]		
	CA	Progesterone	Corticosterone
0	100	100	100
0.5	104	88	73
1.0	102	84	52
2.0	98	72	50
3.0	—	66	48
4.0	92	59	47

[a] Determinations were in duplicate.

steroids as tested by Horton, Kato and Sherins (8) displaced testosterone from SHBG. With respect to its binding to blood proteins, CA behaves as a C_{19} steroid rather than a C_{18} or C_{21} steroid. This behavior would support its action as an antiandrogen in competing for androgen-binding sites in blood and other tissues. It may also explain its potency as a progestin, because it is not bound to plasma progestin-binding proteins, rendering it free to act progestationally at other sites.

Summary. Cyproterone acetate (CA), a potent antiandrogen with progestational properties, was tested for binding affinity against sex hormone-binding globulin (SHBG) and corticosteroid-binding globulin (CBG). CA readily displaced ^{3}H-testosterone but not ^{3}H-estradiol-17β from SHBG indicating the possibility of more than one binding site on SHBG. CA did not displace ^{3}H-corticosterone from CBG. CA thus behaves as a C_{19} rather than a C_{21} steroid in binding to plasma proteins.

1. Hamada, H., Neumann, F., and Junkmann, K., Acta Endocrinol. 44, 380 (1963).

2. VonBerswordt-Wallrabe, R., and Neumann, F., Excerpta Med. Found. Int. Congr. Ser. **n133**, 581 (1966).

3. Whalen, R. E., Luttge, W. G., and Green, R., Endocrinology 84, 217 (1969).

4. Stern, J. M., and Eisenfeld, A. J., Endocrinology 88, 1117 (1971).

5. Neumann, F., Elger, W., and VonBerswordt-Wallrabe, R., Ger. Med. Monthly. 12, 182 (1967).

6. Stern, J. M., and Eisenfeld, A. J., Science 166, 233 (1969).

7. Murphy, B. E. P., Can. J. Biochem. 46, 299 (1968).

8. Horton, R., Kato, T., and Sherins, R., Steroids 10, 245 (1967).

9. Murphy, B. E. P., J. Clin. Endocrinol. Metab. 27, 973 (1967).

10. Neill, J. D., Johanssen, E. D., Datta, J. K., and Knobil, E., J. Clin. Endocrinol. Metab. 27, 1167 (1967).

11. Vermeulen, A., and Verdonck, L., Karolinska Symp. Res. Meth. Rep. Endocrinol. 2nd Symp. 239 (1970).

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