Direct Action Upon Avian Target Organs by the Antiandrogen Cyproterone Acetate [1]

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ABSTRACT At ten days of age, intact male chicks had significantly larger combs than intact female chicks. Castration at four days of age eliminated the sex difference in growth of the comb. Growth of the comb, as a result of the secretion of endogenous androgens by the testes, was inhibited by inunction of the comb with small dosages of cyproterone acetate (Cyp A).

Combs of other chicks were divided surgically into posterior and anterior portions of equal weight, thus providing two separate androgenic target organs in the same animal. The local action of Cyp A was investigated by inunction of this compound on the anterior or the posterior portion of the comb, along with simultaneous application of testosterone propionate to both portions. Small dosages of Cyp A antagonized the effect of androgen on the portion of the comb inuncted with Cyp A without affecting growth of the other target organ in the same animal. It was concluded that Cyp A exerted antiandrogenic actions directly upon target organs and was effective topically in doses too small to act systemically.

The posterior portion of the comb, in comparison with the anterior, was more responsive both to the androgen and to the antiandrogen.

Cyproterone acetate [2] (Cyp A) is a potent antiandrogen that has strong progestational activity but lacks androgenic and estrogenic properties (Lerner, '64). Cyp A counteracted the stimulation provided by exogenous androgens (Neumann and Kramer, '64), and did so without increasing destruction of androgens by the liver (Wollman and Hamilton, '66). Cyp A also antagonized the action of endogenous androgens in dosages which did not appear to inhibit the secretion of interstitial cell-stimulating hormone (Hamada, Neumann and Junkmann, '63; Junkmann and Neumann, '64; Neumann, '66). On the basis of this indirect evidence, the site of action has been hypothesized to be target organs (Junkmann and Neumann, '64; Neumann, Richter and Günzel, '65; Neumann and von Berswordt-Wallrabe, '66; Wollman and Hamilton, '66, '67; Bloch and Davidson, '67).

The present study was designed to ascertain if Cyp A acted directly upon androgenic target organs. The comb of the chick, which affords an external site for assay of locally-applied androgens and antiandrogens (Dorfman, '62; Lerner, Bianchi and Dzalzkalns, '63), was divided surgically into posterior (P-comb) and anterior (A-comb) portions to provide two separate androgenic indicators in the same animal. Cyp A was inuncted on one portion of the comb which served as a primary target organ, while the other portion of the comb served as a secondary target to reflect systemic effects of Cyp A. Previously, studies have been made of gross, histologic and chemical responses of the P- and A-combs to various dosages of androgens administered topically and systemically (Wollman and Hamilton, '68). The present study employed a small dosage of testosterone propionate (TP), which had been found to be effective when inuncted directly on the comb.

MATERIALS AND METHODS

Body weight was recorded for two to three-day-old male White Leghorn chicks (Hall Brothers Hatchery, Wallingford, Conn.) and their head feathers were cut. The chicks were placed in a temperature-controlled brooder (33–37° C) and sup-

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[2] Cyproterone acetate (1,2 α-methylene-6-chloro-Δ^{6}-17 α-hydroxyprogesterone acetate) was supplied through the courtesy of Berlin Laboratories, Inc., New York.

plied with chick Purina Startena and water *ad lib.*

In order to determine whether endogenous androgens were present in young chicks, studies were made of ten-day-old chicks which received no exogenous steroids, comparing weights of the comb in females, castrate males and sham-operated males. Orchiectomies and sham operations were done at four days of age.

Operations on the comb were done on the third day after hatching in other groups of chicks. Posterior (P-comb) and anterior (A-comb) portions of equal weight were prepared as described previously (Wollman and Hamilton, '68).

To ascertain if Cyp A were inhibiting stimulation by endogenous androgens, both portions of the combs of some chicks were inuncted with the vehicle containing graded dosages of Cyp A. The combs of other chicks were inuncted with only the vehicle.

To test the local effect of Cyp A against exogenous androgen, each of the two portions of the comb was inuncted on the left side with 10 μg of TP given in seven subdivided daily doses. Graded dosages of Cyp A were applied simultaneously to the right side of the P-comb in several groups of chicks and to the right side of the A-comb in other groups. The vehicle alone was applied to the right side of portions of the comb not treated with Cyp A.

The vehicle used for all inunctions was a 40% mixture of benzyl benzoate in castor oil, 0.5 μl (0.0005 ml) of which was applied with a 5 μl Hamilton microsyringe graduated in divisions of 0.05 μl. This small volume of inuncted material was employed inasmuch as the response obtained was seven times greater than that obtained with the larger volume of 5 μl that has been used routinely (Wollman and Hamilton, '68). Inunctions were done at a magnification of 2.75.

Chicks were sacrificed by etherization 24 hours after the last treatment. Body weights were again recorded and the two separate portions of the comb were excised and weighed as described previously (Wollman and Hamilton, '68).

A total of 347 chicks was employed in this study with 20 animals in each subgroup except where otherwise specified. Because of the correlation between weights of the comb and body, values were expressed in terms of a comb ratio, the comb weight (mg) / body weight (g). The results were analyzed by an analysis of variance (Steel and Torrie, '60) and Dunnett's multiple comparison test (Dunnett, '55). Missing values owing to death of chicks were corrected for by the method of Finney ('64).

RESULTS

Presence of endogenous androgens in male chicks. Endogenous androgens were present in young male chicks since by the tenth day of life, weights of the combs were significantly greater in sham-operated males than in castrate males ($p < .01$, table 1). The significantly heavier combs in intact male than in intact female chicks ($p < .01$) appeared to be due to testicular secretions since weight of the comb was similar in castrate males and intact females (table 1).

Inhibition of endogenous androgens by Cyp A. In intact untreated males the P-comb had developed significantly by ten days of age whereas the A-comb had grown but little. In intact males inuncted with Cyp A on both portions of the comb, stimulation of growth by endogenous androgens was inhibited significantly on the P-comb ($p < .01$), but not on the A-comb

TABLE 1

Effect of testicular secretions upon weight of the whole comb in ten-day-old chicks

Groups	no. of chicks	Comb wt (mg) / Body wt (g) ± S.E.[1]	p value
Female	7	.380 ± .021	< .01
Male (sham-operated)	12	.564 ± .039	< .01
Castrate male	8	.394 ± .021	

[1] S.E. = Standard error

TABLE 2

Effect of Cyp A on the increase in weight of the posterior (P-) and anterior (A-) portions of the comb of intact male chicks stimulated by endogenous androgens

Dosage of [2] Cyp A (μg)	Comb wt (mg) / Body wt (g) ± S.E.[1]		p value [3]
	P-comb	A-comb	
0	.391 ± .031	.296 ± .018	—
3.2	.296 ± .016	.286 ± .028	< .01
16	.277 ± .014	.245 ± .018	< .01
80	.313 ± .022	.280 ± .014	.05

[1] S.E. = Standard error
[2] Total dose, subdivided for daily application to each portion of the comb for seven days (20 chicks per group)
[3] Significance of difference in weight of the P-comb between those chicks which received the indicated dosages of Cyp A and those chicks which received only the vehicle. There was no significant difference in weight of the A-comb.

(table 2). The degree of inhibition of the P-comb did not differ with the use of a wide range of dosages of Cyp A.

Inunction of exogenous androgen. Inunction of each portion of the comb with 10 μg of TP significantly increased the comb ratio (P-comb from .335 to .853, $p < .001$; A-comb from .306 to .708, $p < .001$).

(A). *Cyp A applied to the P-comb.* With inunction of 10 μg of TP on both portions and simultaneous application of Cyp A to only the P-comb, Cyp A significantly inhibited growth of the P-comb without significantly inhibiting growth of the A-comb ($p < .05$, fig 1). The data for all dosages combined showed that the mean percentage inhibition was significantly greater in the Cyp A-inuncted P-comb than in the A-comb which was not treated with Cyp A (41.6 *vs.* 11.0%, $p < .001$). The dosage-response curve was not linear, in agreement with results obtained for the comb as a whole (Lerner, '64).

(B). *Cyp A applied to the A-comb.* With inunction of 10 μg of TP on both portions of the comb and simultaneous application of Cyp A on the A-comb alone, the highest dosage of Cyp A significantly inhibited growth of the A-comb ($p < .05$) and no dosage of Cyp A significantly inhibited growth of the P-comb (fig 1). The data for all dosages combined showed that the mean percentage inhibition was greater in the Cyp A-inuncted A-comb than in the P-comb which was not treated with Cyp A (27.7 *vs.* 12.0, $p < .01$).

The mean percentage inhibition observed in the A-comb when Cyp A was inuncted on this portion was only about one-half of that observed in the P-comb when a similar amount of Cyp A was applied to the P-comb. When one portion of the comb was inuncted with Cyp A, the more limited inhibition of growth of the inuncted A-comb was significantly different from that of the inuncted P-comb ($p < .05$).

In brief, with inunction of low doses of Cyp A on either portion of the comb, androgenic stimulation of growth was inhibited only on the portion of the comb receiving Cyp A. With application of large

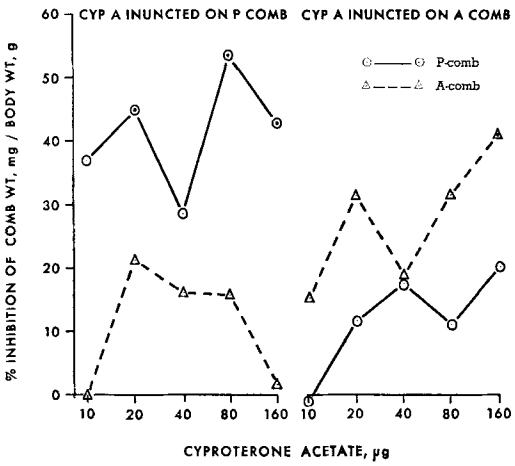

Fig. 1 Local inhibition of each of two androgen-stimulated target organs upon inunction with Cyp A. The posterior (P-comb) and the anterior (A-comb) portions of the chick comb were each inuncted with 10 μg of TP. TP and Cyp A were given as seven subdivided dosages, applied daily from days three to ten. Dotted symbols (3 circles, 1 triangle) identify group in which the percentage inhibition was significantly greater ($p < .05$) than that of the groups which received the androgen alone (20 chicks per group).

amounts of Cyp A to one portion of the comb, the inhibition of growth of that portion was significantly more marked than that of the portion not inuncted with antiandrogen. When Cyp A was applied to one portion of the comb, the inhibition was more marked when the inuncted portion was the P-comb rather than the A-comb. The greater response to Cyp A, by the androgen-treated P-comb than by the androgen-treated A-comb, was in keeping with the greater response by the P-comb than by the A-comb to stimulation by TP. The amount of inhibition which was possible seems to relate to the amount of growth which can be counteracted.

Comparison of the same portion of the comb in different chicks showed that growth of the P-comb was inhibited to a greater degree when Cyp A was applied directly to this target organ than when it entered systemic circulation after absorption from the A-comb ($p < .001$ in data for both weight and percentage inhibition, fig 1). Similarly, growth of the A-comb was inhibited to a greater degree when Cyp A was placed directly on this target organ than when the antiandrogen entered the systemic circulation by absorption from the P-comb ($p < .05$ in data for weight, $p < .02$ in data for percentage inhibition, fig 1).

DISCUSSION

Androgenic secretions in the cockerel were believed to begin in the second week of life, since a sharp increase in weight of the comb took place between days 7 and 14 (Dorfman and Stevens, '60). Yet the administration of antiandrogens during the first week of life resulted in subnormal weight of the comb (Lerner, Bianchi and Borman, '60; Lerner et al., '63). The present study showed that endogenous androgens had been present in male chicks before ten days of age (the age at which chicks were sacrificed in androgenic assays) and that the stimulation afforded by these androgens was inhibited by Cyp A.

From studies of locally-applied Cyp A, this compound had been reported to inhibit the action of androgen on the chick comb, but the possibility of systemic action was not eliminated, since only one target organ was employed (Lerner, '64; Neumann, '66). The present study showed that Cyp A can act upon a target organ in doses too small to have demonstrable systemic effects. It seems logical to presume that other target organs may also be sites where stimulation by exogenous androgen is inhibited.

The P-comb has been demonstrated to be more sensitive to androgen than the A-comb (Wollman and Hamilton, '68). This study indicates that the P-comb is also more responsive to an antiandrogen than the A-comb.

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