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EFFECT OF CYPROTERONE ACETATE ON THE PROTEINASE ACTIVITIES IN ADULT RAT TESTIS AND EPIDIDYMIS*

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Abstract—The effects of *in vivo* and *in vitro* administration of the antiandrogen cyproterone acetate (CA) on different proteinase activities in adult male rats were studied. Acid proteinase activities increased in both testis and epididymis, but the inhibition of neutral and alkaline proteinase activities was more pronounced in epididymis than testis, after long- and short-term CA treatment. As the neutral and alkaline proteinases were more functionally involved than acid proteinase during the process of fertilization and sperm maturation, it may be concluded from our result that CA might act by inhibiting epididymal function although it has also a slight inhibitory influence on spermatogenesis.

Cyproterone acetate (1,2 α-methylene-6-chloro-pregn-4,6-diene-17α-01-3, 20-dione-17α-acetate), an antiandrogenic compound, has been claimed by several investigatiors to induce "functional sterility" in the male gonadal system [1, 2]. Several studies also indicate that although CA causes a marked reduction in the weights of accessory sex glands as well as the motility and viability of spermatozoa, it has only a slight inhibitory influence on spermatogenesis [3–7]. Although a number of papers have been published dealing with clinical [3, 4, 8] and experimental studies [5, 6] of the effects of CA in relation to reproductive endocrinology and physiology, much less attention has been paid to the effects of CA on enzyme systems connected with spermatogenesis and sperm maturation phenomena.

Previous observation made from this laboratory indicates the role of proteinases in rat testis and epididymis during spermatogenesis and maturation processes, and that neutral (pH 7.5) and alkaline (pH 8.8) proteinases are more functionally involved in the process of fertilization [9]. The present paper describes the *in vivo* and *in vitro* effects of CA on the proteinase activities in epididymal and testicular spermatozoa in male albino rats.

MATERIALS AND METHODS

Male albino rats (CIBA strain, 100–120 g body wt) were used throughout the experiment. CA (lot No. 50402, Schering AG) was injected intramuscularly at a dose of 50 mg/kg which is equivalent to 5 mg/rat (in a suspension of benzyl benzoate–olive oil, 1:1) daily for 10, 20 and 30 days. The control group of animals received equivalent amount of the vehicle suspension. The animals were killed 24 hr after the administration of the final dose. After decapitation, testis and epididymis were quickly removed and homogenized in ice-cold physiological saline to a 10% homogenate. The homogenates were centrifuged (0–4°) at 1500 *g* for 15 min and the supernatants were used for the enzyme assay. For *in vitro* experiment, the 1500 *g* supernatants were incubated at 37° for 30 min with CA at a concentration of 5, 10 and 20 mg per g of tissue weight basis. Proteinases were assayed as described by Nag *et al.* [9] using acid-denatured haemoglobin as substrate. Protein was estimated according to the method of Lowry *et al.* [10]. Specific activity of the enzyme was expressed as change in optical density at 570 nm/mg of protein/hr.

RESULTS AND DISCUSSION

Results of both *in vivo* and *in vitro* experiments (Table 1) indicate that in CA-treated animals, there is more marked inhibition in the neutral and alkaline proteinase activities in epididymis (57–85 and 62–100 per cent) than the testis (18–28 and 15–36 per cent). Acid proteinases, on the other hand, are increased in both testis (166–208 per cent) and epididymis (195–230 per cent). Maximum changes either in the inhibition or stimulation of the enzyme activities are generally observed after 20 days treatment. In view of the fact that acid proteinases, which are generally lysosomal enzymes, are involved in the hydrolytic degradation and removal of proteins and peptides from the cellular environment, whereas alkaline and neutral proteinases generally being arylamidase or trypsin-like [11], are more involved in the turnover and synthesis of free amino acids and smaller peptides, it may be concluded that CA exerts its effects not only by increasing cathepsin-type breakdown, but also by preventing transport and turnover of amino acids and thereby preventing storage and maturational phemomena.

It has already been pointed out by several investigators that androgens can influence certain changes in "effective concentrations" of some hydrolytic enzymes [12–14]. Therefore another possibility can not be ruled out that after CA treatment, decreased activities of neutral and alkaline proteinases may be due to the decreased accumulation of androgen in the target organs. As the CA-induced inhibition of

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Table 1. *In vivo* and *in vitro* effects of cyproterone acetate upon the proteinase activities of rat testis and epididymis

Treatment and dose of CA		Sp. act. at different pH Testis 4.8	Testis 7.5	Testis 8.8	Epididymis 4.5	Epididymis 7.5	Epididymis 8.8
In vivo (5 mg/rat/day)							
Control	10 days	1.26	0.55	0.63	1.00	1.30	0.78
Treated		2.10	0.45	0.53	2.30	0.19	0.16
Control	20 days	1.20	0.49	0.48	1.18	1.20	0.68
Treated		2.50	0.35	0.21	2.20	0.46	N.D.
Control	30 days	1.10	0.47	0.62	1.10	0.95	0.53
Treated		1.90	0.37	0.40	0.83	0.40	0.20
*In vitro** (mg/100 mg tissue wt)							
Control		0.45	0.82	0.62	1.17	0.93	0.94
0.5		0.85	0.62	0.52	1.33	0.72	0.81
1.0		0.64	0.36	0.43	1.38	0.28	N.D.
2.0		0.83	N.D.	0.40	2.19	0.08	N.D.

Values are the mean of five separate determinations. The assay mixture contained (final vol 1.0 ml), 0.05 M respective buffers, 2 mg acid-denatured haemoglobin (substrate) and 200–300 μg of protein equivalent and incubated at 37° for 30 min.

* Conditions as given in Materials and Methods section.

N.D.—Not detectable under the same experimental conditions.

neutral and alkaline proteinases is more pronounced in the epididymis than in testis, it may be additional evidence supporting the idea that the inhibitory effect of CA is more prominent on maturation and fertilization processes than on the spermatogenesis. Thus the result of this study confirms the working hypothesis that the antiandrogenic effect of CA is due to its effect on the maturational processes in the epididymis by selective inhibition of androgen action than the testicular spermatogenesis [15–19].

Although the exact mechanisms of the inhibitory effect of CA on the maturation of spermatozoa remains unknown, it may be speculated that (i) CA, by its well-known blocking action of FSH [20], decreases androgen supply in the epididymis and/or (ii) CA might have its action at the level of the function of androgen-binding protein (ABP) probably by degradating ABP [20–22]. Whether the CA-induced activation of acid proteinase may inactivate the ABP by proteolytic breakdown, thereby preventing the ABP to function properly in the subsequent maturational process in the epididymis, remains to be established. Further studies are in progress to elucidate the biochemical mechanism of action of CA during long- and short-term administration on the formation and maturation of spermatozoa.

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