Chapter - 6

EFFECTS OF CASTRATION, ADRENALECTOMY, SYMPATHOMIMETIC AND PARASYMPATHOMIMETIC AGENTS ON THE ACTIVITIES OF PHOSPHOMONOESTERASES OF THE RAT PREPUTIAL GLAND

It has been known for quite a long time that the preputial gland of rat exhibits a high acid phosphatase activity (Gutman and Gutman, 1938, Howard and Robert, 1952; Sansone et al., 1972; Michael and Hoopes, 1974); however, Montagna and Noback (1947) could notice only a little of acid phosphatase activity in this gland. Montagna and Hamilton (1947) have reported marked acid phosphatase activity in sebaceous glands of the hamster. Long term effects of castration on the phosphatases of the gland have been reported by Howard and Robert (1952). Effects of steroid hormone adminmistration on the acid phosphatase activity have been reported by Sansone et al. (1972) as well as Szego (1975). In the present study, quantitative analysis of acid and alkaline phosphatase activities of the preputial gland has been carried out to investigate the immediate effects of castration and adrenalectomy plus castration.

While endocrine regulation of preputial glands and

sebaceous glands is fairly well exploited, practically little is known about neural control of the functions of sebaceous gland or its analogues. Histological studies have provided evidence that the preputial gland is supplied with autonomic nerves (Chapter-4). No evidences from neurohistological studies reported earlier could be located regarding the secretory innervation of the gland. However, it was observed that extrusion of preformed sebum from the gland is controlled by α-adrenergic receptors (Chapter-5). Pharmacodynamic agents are useful, and in some cases almost indespensible tools, in such studies. Therefore, effects of two adrenergic agonists viz., isoproterenol (IPR) and phenylephrine (PHE) (β- and α-adrenergic agonist respectively) and a cholinergic agonist - pilocarpine (PC) on the acid and alkaline phosphatase activity of the preputial glands were studied with a view to understand role of these enzymes in the secretory functioms of these glands.

MATERIALS AND METHODS

Laboratory bred male albino rats (Rattus norvegicus albinus) weighing 140±20 gms were employed for the present investigation. Animals were castrated through scrotal incision under light ether anaesthesia. In another group

of animals adrenalectomy was performed via dorsal route, together with castration. Animals were kept under laboratory conditions with food and water provided ad libitum. Adrenalectomized-castrated animals were provided with glucose saline instead of plain drinking water. Castrated and adrenalectomized-castrated rats were sacrificed at intervals of 24, 48 and 120 hours post-operatively. Some of the 120 hours castrated animals were treated with intramuscular injections of 0.1 mg testosterone propionate/0.5 ml of tributyrine.

Isoproterenol (25 mg/Kg body wt.), phenylephrine (6 mg/kg body wt.) and pilocarpine (65 mg/kg body wt.) were administered intraperitoneally in three groups of rats, twice a day for 10 days. Animals were sacrificed 12 hours after the 20th injection of the agonist.

For quantitative estimations the glands were weighed and homogenized in chilled distilled water and the enzyme activities (both acid and alkaline phosphatases) were determined by employing P-nitrophenyl phosphate as substrate according to the method described in Sigma Technical Bulletin No.104.

Table 1

Levels of activities of acid and alkaline phosphatases in the preputial glands from normal, castrated, adrenalectomized - castrated and testosterone propionate injected rats. Enzyme activities are expressed as μ mole p-nitrophenol released/ 100 mg wet tissue wt./30 min. Mean value $\pm$ S.D.

Experimental Groups	Acid phosphatase activity	Alkaline phosphatase activity
Normal	127.97 $\pm$ 39.39	40.66 $\pm$ 12.94
24 hrs castrated	102.91 $\pm$ 39.37	42.27 $\pm$ 4.83
48 hrs castrated	69.74 $\pm$ 14.15*	43.12 $\pm$ 8.63
120 hrs castrated	54.05 $\pm$ 23.32**	38.04 $\pm$ 14.78
24 hrs adrenalectomized -castrated	92.18 $\pm$ 34.37	46.97 $\pm$ 8.57
48 hrs adrenalectomized -castrated	73.40 $\pm$ 16.43*	47.80 $\pm$ 10.23
120 hrs adrenalectomized -castrated	67.19 $\pm$ 27.53*	42.91 $\pm$ 11.46
0.1 mg Testosterone propionate injected	106.70 $\pm$ 23.76@	40.97 $\pm$ 8.24

* Significantly different from the normal at the level $P < 0.01$

** Significantly different from the normal at the level $P < 0.0025$

@ Significantly different from the 120 hours castrated at the level $P < 0.005$

Table 2

Changes in activities of acid and alkaline phosphatases of the preputial gland of rats following administration of cholinergic and adrenergic agonists. Enzyme activities are expressed as μ mole p-nitrophenol released/100 mg wet tissue/ 30 min. Mean value ± S.D.

Treatments	Acid phosphatase	Alkaline phosphatase
Normal	127.97 ± 39.39	40.66 ± 12.94
Phenylephrine	95.32 ± 9.88	22.23 ± 6.27
Isoproterenol	37.64 ± 10.59*	17.34 ± 5.40
Pilocarpine	96.78 ± 9.04	25.80 ± 9.60

* Significantly different from the normal at the level $P < 0.0005$

RESULTS

Quantitatively, glands of the normal animals exhibited high acid phosphatase activity which decreased gradually after castration (Table 1). Values for the enzyme activity of castrated rats did not differ significantly from those of the adrenalectomized-castrated rats.

Drastic reduction in acid phosphatase activity was observed after IPR administration whereas PHE and PC administration did not bring about significant changes in the enzyme activity (Table 2). Acid phosphatase activity showed very wide fluctuations in the gland of normal animals, but interestingly enough, after administration of the drugs such fluctuations in the enzyme activity were conspicuously absent.

Levels of alkaline phosphatase were essentially same in the glands from normal castrated and adrenalectomized-castrated rats (Table-1). After treatment with pilocarpine, phenylephrine and isoproterenol a decrease in enzyme activity was noticeable (Table-2).

DISCUSSION

Acid phosphatase is a lysosomal enzyme and the presently

observed high acid phosphatase activity could be due to high lysosomal activity involved in cellular autolysis underlying the mode of secretion. This contention is further supported by the observation that acid phosphatase was found to be high in mature and disintegrating cells of the sebaceous gland acini (Michael and Hoopes, 1974). Brandes et al.(1965) have also reported that lysosomal hydrolytic enzymes appear to participate in holocrine secretion in sebaceous glands. Reduction in acid phosphatase activity, observed here, after castration suggested that the holocrine secretory activity of the gland was under the influence of androgens. Patterson et al. (1964) have reported that the activity of β-glucuronidase, another lysosomal enzyme, decreases in the preputial glands after castration. It is quite possible that decreased acid phosphatase activity would result in significantly reduced rates of 'sebum' production. Moreover, Szego (1975) suggested that sex steroids cause labilization of lysosomal membranes and the lysosome-hormone complex plays an important role in the synthetic activities of the glands through its influence on the nucleocytoplasmic communication. It has repeatedly been shown that 'Sebum' production decreases after castration (Ebling, 1963; 1974; Pochi and Strauss, 1974). Lipogenic enzymes also showed reduction after castra-

tion (Chapter-1). In view of these reports it is surmised that decreased 'sebum' production after castration could be due to reduction in lipogenic enzymes, and in part, also because of decreased acid phosphatase activity. Though adrenal glands influence certain enzymes involved in lipogenesis in preputial gland (Chapter-1), the removal of adrenal glands from the castrated rats did not affect acid phosphatase activity in the preputial glands (Table-1) to any significant extent. Administration of testosterone propionate restored the enzyme activity to levels more or less close to the control values. Sansone et al. (1972) have also shown that rat and mouse preputial glands possess acid phosphatase as well as β-glucuronidase activity associated with lysosomes and that the same could be increased by administration of testosterone and estradiol-17β.

Literature regarding the effects of drugs influencing the sebaceous glands is scanty (Cerutti, 1934). There are no conclusive experimental evidences to indicate that the glands are directly under the control of autonomic nervous system. Melczer and Deme (1942) reported an increase in secretion in the sebaceous glands after pilocarpine injections, but others could not find such an effect (Rothman and Herrmann, 1953). Miescher and Schonberg (1944) found no

change in lipid levels of sebaceous glands after atropine, pilocarpine and acetylcholine administration. During the course of the present study significant decrease was observed in the free acid phosphatase activity after administration of a β receptor agonist-IPR. β-adrenergic receptor agonists are known to be important in controlling metabolism in other tissues (Ellis, 1967; Ellis, et al., 1967). The decrease in the acid phosphatase activity of the gland can very well be explained as IPR is known to elevate intracellular levels of c-AMP in tissues (Duell et al., 1971; Powell et al., 1971; Zepp and Thomas, 1976; 1978; Tsang and Singhal, 1976); and c-AMP may cause lysosomal membrane stabilization which could ultimately result in decreased free acid-phosphatase activity. Such an inhibition of lysosomal enzyme discharge has been reported in case of leucocytes under the influence of such agents as c-AMP, theophylline, PGE_1 and isoproterenol, all of which are known to increase c-AMP concentration (Weissmann et al., 1971). Ignarro et al. (1972) also reported that epinephrine, isoproterenol and c-AMP inhibits lysis of rat liver lysosomes and consequent release of acid hydrolases. However, this effect (decreased free acid phosphatase activity) could also be due to altered steroid metabolism as discussed in Chapter-8.

As described earlier, lysosomes are known to be significantly important in sebaceous gland physiology, which can be appreciated by their participation in holocrine secretion. Lysosomal proliferation and consequent rupture play a primary role in the programmed autolysis occuring in the sebaceous glands (Lazarus et al., 1975). Observed reduction in acid-phosphatase activity due to the administration of IPR, which is most active and largely a β-type agonist, is significant, since reduction in the release of lysosomal enzymes would cause reduction in the sebum secretion. Harville (1971) has reported striking reduction in sebum secretion after L-Dopa administration. Burton et al. (1973) and Burton and Shuster (1973) have also reported that L-Dopa reduces seborrhea of parkinsonia. Sebaceous gland secretion is acknowledged to be an important factor in the pathogenesis of acne vulgaris and reduction or inhibition of glandular activity can prove to be clinically beneficial.

Under these experimental conditions, phemylephrine, a purely α-adrenergic receptor agonist, lacking the characteristic catechol moiety, was completely without effects on acid phosphatase activity of the gland.

It seems evident from these observations that the

possible receptors involved in the release of lysosomal enzymes in these glands, as evident from decreased acid phosphatase activity, are of β-adrenergic type. Thus, the present investigation underlines the role of neurotransmitters in the regulation of release of lysosomal enzymes in the preputial glands of rats. It is worthwhile to mention here that the histological observations on the preputial glands of IPR treated rats revealed the presence of less number of distintegrating acini in the glands ~~of rats treated with IPR~~ (Chapter-7). These observations parallel the decreased activity of acid phosphatase reported here and lend further support to the present findings.

Pilocarpine, a parasympathomimetic agent, has been known to elevate c-GMP levels. On the basis of the in vitro studies on the hepatic tissue, Ignarro et al. (1975) have reported that pilocarpine, labilizes the lysosomal membranes by increasing c-GMP levels, leading to the release of lysosomal enzymes. Pilocarpine is also shown to increase adrenomedullary secretion (Douglas and Poisner, 1965). In the present in vivo studies no significant increase in acid phosphatase activity could be noticed following pilocarpine treatment. In this context it could be suggested that a direct effect of pilocarpine on preputial glands was perhaps

counteracted by possible simultaneous stimulation of adrenomedullary system, and thereby, the activity of acid phosphatase was not affected.

Alkaline phosphatase activity has been known to be implicated in the transport of metabolites across the cellular membranes, protein synthesis and mitotic activity (Save, 1963). Mitotic proliferation is obviously a prime requirement in holocrine glands, so that cells lost could be replaced. Additionally, these glands are known to secrete sufficient quantities of protein (Beaver, 1963). In the light of these evidences alkaline-phosphatase activity of these glands can be correlated with mitosis as well as protein synthesis. No significant change was observed in the alkaline phosphatase activity of the glamds of the castrated or adrenalectomized-castrated rats, but the decrease in alkaline phosphatase activity following neuro-transmitter agonist administration is difficult to explain at this stage.