CHAPTER : 6

INFLUENCE OF NEUROENDOCRINE MANIPULATIONS ON THE ACTIVITIES OF CERTAIN HYDROLYTIC ENZYMES IN THE RAT PREPUTIAL GLAND

The preputial glands are having a unique mode of holocrine secretion and obviously depict physiological autolysis. There is a continuous disintegration of lipid-laden mature cells, contributing to the formation of the secretory material. This needs a high rate of mitosis so as to replace the cells lost during the process. Accordingly, physiological autolysis in holocrine secretion may be influenced by acid hydrolases mediated through lysosomal enzymes (Palay, 1958; de Duve, 1959a and 1961; Bell, 1971). Lysosomal proliferation and subsequent rupture have a primary role in programmed autolysis in sebaceous glands (Lazarus et al., 1975). It has been reported that lysosomal hydrolytic enzymes appear to participate in holocrine secretion in sebaceous glands (Brandes et al., 1965).

Previous studies from this laboratory have shed some light on the contribution and neuroendocrine regulation of acid phosphatase towards the secretory mode typical of sebaceous glands in the preputial gland of male rats (Ambadkar and Vyas, 1981b). Although acid phosphatase activity is considered as the hallmark of lysosomal hydrolases, a large number of hydrolases have been shown to be present in lysosomes (De Duve and Wattiaux, 1966; Tappel, 1968a; Barrett and Dingle, 1971; Barrett, 1972).

In addition, non-parallel changes in the activity levels of certain lysosomal enzymes have been reported in the rat ventral prostate (Helminen et al., 1972), parotid glands (Robinovitch, 1973; Johnson and Sreebny, 1977) and epididymis (Mayorga and Bertini, 1981; Weiss and Gossrau, 1981) after neuroendocrine manipulations. In the light of these reports, it was thought desirable to investigate the action of neuroendocrine manipulations on the activity levels of yet some other lysosomal hydrolases, viz., 5'-nucleotidase (Novikoff, 1963; Coffey and Pletsch, 1971; Werb and Cohn, 1972b; Widnell, 1972), lipase and non-specific esterase (de Duve and Wattiaux, 1966; Tappel, 1968a; Barrett and Dingle, 1971; Barrett, 1972) in the preputial gland of male rats.

MATERIALS AND METHODS

Neuroendocrine manipulations in the male albino rats essentially remain the same as have been described in the fifth chapter.

A : Quantitative assay :

The glands were weighed and homogenized in chilled distilled water. 5'-nucleotidase (EC 3.1.3.5) was estimated quantitatively employing the method described by Jafri and Mustafa (1976). Inorganic phosphate released was estimated according to the method of Fiske and Subba Row (1925). The readings were taken at 660 μm

on Klett-Summerson photoelectric colorimeter. The enzyme activity has been expressed as mg of phosphorus released/100 mg protein/1 hr.

B : Histochemical localization :

The preputial gland was kept on a chuck of the cryostat microtome maintained at -20°C. 9-12 μ thick sections were cut, pre-fixed and processed for histochemical localization of non-specific esterase activity employing the method of Burstone (1962). Lipase activity was detected by the modified method of George and Ambadkar (1963). Histochemical localization of both lipase and non-specific esterase was studied in the preputial glands from normal, 120-hours castrated and IPR-treated male rats only.

OBSERVATIONS

A : Enzyme Assay :

Quantitatively, glands of normal rats showed significant activity levels of 5'-nucleotidase enzyme. The activity levels of the enzyme showed non-significant decrement after 24 and 48 hours of endocrine manipulations but attained significantly low value by 120 hours post-operatively. However, in case of adx-C the reduction in enzyme activity was more obvious even by 24 and 48 hours of manipulations. Nevertheless, the lowering of

TABLE : 1

ACTIVITY LEVELS OF 5'-NUCLEOTIDASE IN THE PREPUTIAL GLAND FROM NORMAL, C, Adx-C, TP-INJECTED AND IPR-TREATED MALE RATS. THE ENZYME ACTIVITY IS EXPRESSED AS mg OF PHOSPHORUS RELEASED/100 mg PROTEIN / 1 HR.

MEAN VALUE ± S.D.

Experimental group	5'-Nucleotidase
Normal animals	3.27 ± 0.73
24 hrs C	3.03 ± 0.6
48 hrs C	3.17 ± 0.89
120 hrs C	1.93 ± 0.49*
24 hrs Adx-C	2.88 ± 0.92
48 hrs Adx-C	2.59 ± 0.72
120 hrs Adx-C	1.86 ± 0.37*
120 hrs C, 0.1 mg TP injected	3.07 ± 0.5
IPR-treated animals	1.78 ± 0.39*

* Significantly different from the normal at the level $P < 0.001$.

enzyme activity by 120 hours was almost of the same degree as that attained after 120 hours of castration alone. Similar degree of reduction was observed after IPR therapy. However, replacement therapy of 120 hours castrates with 0.1 mg TP could bring the values back to normal levels (Table : 1).

B : Histochemical Study :

I - Lipase :

The sebaceous acini of the normal preputial gland showed patchy localization of the enzyme activity. The lipid material in the central duct exhibited high activity. There was a virtual lack of the enzyme activity in the extra-acinar regions of the gland, meaning thereby that the enzyme is active where lipid hydrolysis is predominent (Fig : 1).

Castration resulted in diminished activity of lipase in the preputial acini (Fig : 2). The lipid material in the central duct, too showed reduced lipase activity.

IPR therapy reduced the lipase activity to a greater extent. There was a virtual lack of lipase activity in the preputial acini and duct system as well (Fig : 3).

II - Non-specific Esterase :

The sebaceous acini and the central duct of the normal preputial gland exhibited considerable non-specific esterase activity. The gland substance remained non-reactive (Fig : 4).

EXPLANATIONS FOR FIGURES

Figs. 1 to 6 Photomicrographs of sections of rat preputial glands showing different enzyme activity. 150X.

Fig. 1 Lipase activity in the section of the preputial gland of normal rat.

Fig. 2 Lipase activity in the section of the preputial gland of 120 hours castrated rat.

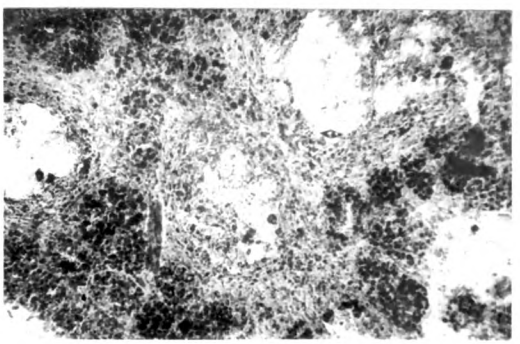
Fig. 3 Lipase activity in the section of the preputial gland of isoproterenol treated rat.

Fig. 4 Non-specific Esterase activity in the section of the preputial gland of normal rat.

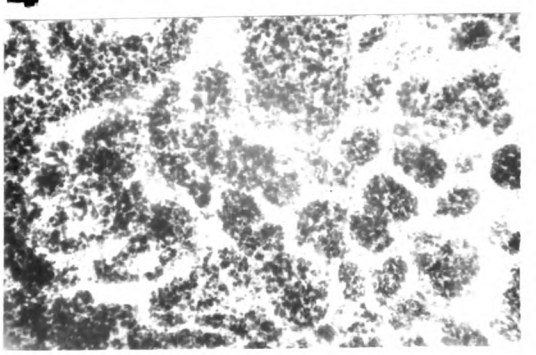
Fig. 5 Non-specific Esterase activity in the section of the preputial gland of 120 hours castrated rat.

Fig. 6 Non-specific Esterase activity in the section of the preputial gland of isoproterenol treated rat.

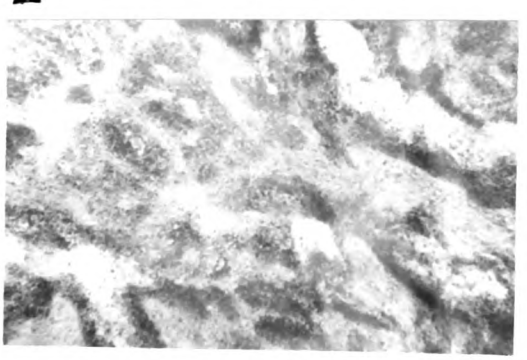
1



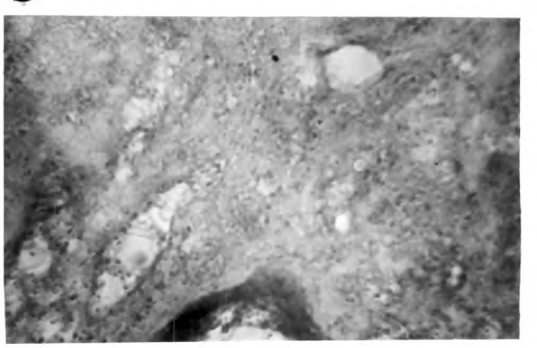
4



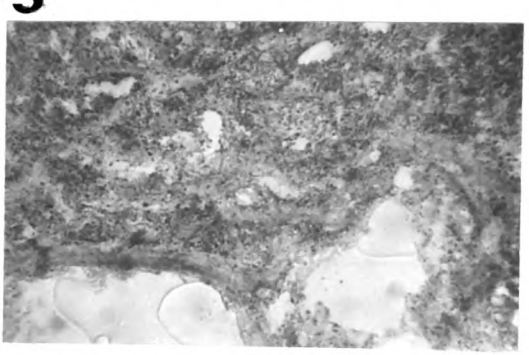
2



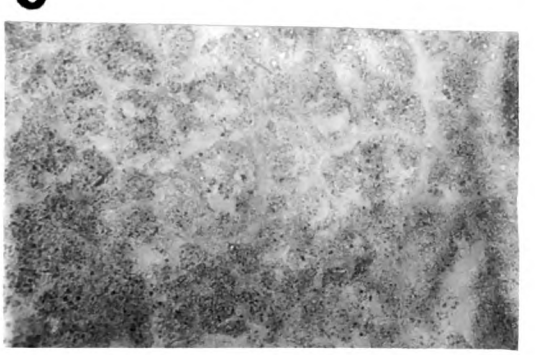
5



3



6



Castration resulted in an overall diminished activity of the enzyme in the preputial acini (Fig : 5).

IPR administration to male rats reduced the enzyme activity considerably in the acini and the duct system as well (Fig : 6).

DISCUSSION

Distribution and localization of 5'-nucleotidase has been worked out by a number of investigators in mammalian skin components (Cormane, 1962; Tabachnick and Perlish, 1967; Fukui and Miura, 1969; Moynahan et al., 1972; Klaushofer and Bock, 1974; Nizuma, 1979). However, a contradictory report pertaining to the presence of this enzyme in sebaceous glands do exist in the literature (Wohlrab, 1966). 5'-nucleotidase has been shown to be involved predominantly in the catabolism of nucleotides according to Hardonk (1968) and Hardonk and Koudstaal (1968). Goldberg (1973) has suggested that there exists a reverse correlationship between 5'-nucleotidase enzyme activity and the proliferating capacity of normal as well as tumourous cells. Contrary to this report, it was conclusively shown that histochemically demonstrable amounts of the enzyme were not restricted to non-proliferating normal tissues (Klaushofer and Bock, 1974). Hence, the function of 5'-nucleotidase can not be limited to a degradative role in nucleotide phosphate metabolism as has been hypothetised in the literature (de Duve, 1963; Novikoff, 1963; Hardonk, 1968; Hardonk and Koudstaal, 1968; Singh and Mathur, 1968; Timmer et al., 1968; Goldberg, 1973; Nizuma, 1979). Indeed, the enzyme has been shown to be involved in the transfer of nucleosides from the interstitial fluid to the cytoplasm of cells (Hoelzl and Knufermann, 1973) and hence, the enzyme has been assigned with a role in active transport processes

(Reis, 1951; Novikoff et al., 1962; Hardonk, 1968; Moynahan et al., 1972; Klaushofer and Bock, 1974; Ali et al., 1978; Guraya and Kaur, 1981).

As it is evident from Table No : 1, 5'-nucleotidase in the preputial gland showed initial decrements and attained the significant change among 120 hours C. It is pertinent to note the earlier reported reduced 5'-nucleotidase activity in certain accessory sex organs as a result of androgen deprivation (Krolikowska, 1966; Takuma and Kumegawa, 1977; Chalet and Catayee, 1979; Guraya and Kaur, 1981). Castration has been shown to reduce thymidine incorporation and mitotic index in hamster sebaceous glands (Frost et al., 1973). When this observation is coupled with the reduced 5'-nucleotidase activity in the preputial gland of castrates, a diminished nucleotide catabolic function could be assigned safely to 5'-nucleotidase.

When total androgen deprivation was performed by means of simultaneous adrenalectomy of castrates, this lysosomal enzyme (Novikoff, 1963; Coffey and Pletsch, 1971; Werb and Cohn, 1972b; Widnell, 1972) attained lower values earlier as compared to the values [illegible] obtained among C . The observation obviously insinuates the possibility of involvement of adrenal corticosteroids in stabilization of lysosomal membranes (Weiner et al., 1968; Symons et al., 1969; Philip and Kurup, 1978; Yavorskii, 1982). Additionally, it has been clearly shown by Booth and Jones (1980) and Takayasu and

and Itami (1981) that castration of laboratory mammals leads to highly significant rise in the 5∝-DHT metabolism of sebaceous analogues, which could be involved, in part atleast in the maintenance of the preputial gland metabolism in the absence of proper gonadal steroids. In the light of these facts, it could be suggested that the comparative maintenance of 5'-nucleotidase enzyme activity levels in case of 24 hours and 48 hours C, as against the Adx-C at similar intervals, is probably due to accelerated conversion of androgenic metabolites emanating from the adrenal crotex to 5∝-DHT by the preputial gland studied here. However, it is difficult, at present, to explain why such supplementary role of adrenal steroids does not extend to 120 hours castrates. Probably, extended absence of normal gonadal steroid level, somehow hamper the capacity of preputial glands to generate by conversion sufficient quantities of 5∝-DHT necessary. Decreased 5'-nucleotidase in the glands of Adx-C then could be attributed to withdrawal of suggested supplementary role of corticoid conversion to 5∝-DHT. It is pertinent to note here the reduced levels of 5∝-DHT in the sebaceous glands of adrenalectomized subjects (Hsia et al., 1983).

TP replacement therapy of 120 hours C could bring the value of the enzyme back to normal levels. Literature available has already pointed to the influence of sex steroids in stabilization of the lysosomal membranes (Briggs, 1973).

It is pertinent here to note that testosterone therapy to C has been reported to restore the activity levels of 5'-nucleotidase in androgen responsive organs like rat epididymis (Chalet and Catayee, 1979), mouse kidney (Kochakian and Williams, 1973) and mouse seminal vesicles (Takuma and Kumegawa, 1977).

As it is evident from the table, the enzyme activity decreased to a significant extent after IPR therapy. Such decrease in the enzyme activity can be very well explained on the basis of altered steroid metabolism as influenced by chronic IPR therapy (Ambadkar and Vyas, 1982; Chapter : 1). Alternatively, IPR has been reported to enhance cAMP levels in the gland (Chapter :7), and cAMP in its turn may cause lysosomal membrane stabilization resulting into inhibition of lysosomal enzyme discharge (Weissmann et al., 1971; Ignarro and Colombo, 1973; Ignarro et al., 1973 and 1974; Zurier et al., 1974; Busse et al., 1981; Galant and Alfred, 1981). Likewise, reduced levels of acid phosphatase have been reported in the rat preputial gland after IPR therapy (Ambadkar and Vyas, 1981b). It is worthwhile to mention here that the histological observations in the gland after IPR therapy revealed the presence of less number of disintegrating acini as well as retarded rate of cell proliferation (Vyas, 1978). Obviously, when presently reduced 5'-nucleotidase activity after IPR therapy is considered in the light of the literature cited above, a nucleotide catabolic function could be assigned to 5'-nucleotidase. Moreover, β-receptor agonist like IPR could be assigned to a role in regulation of release of lysosomal enzymes.

Lipase and non-specific esterase constitute yet another group of lysosomal enzymes (Novikoff, 1961; de Duve and Wattiaux, 1966; Tappel, 1968a; Barrett and Dingle, 1971; Barrett, 1972). Lipase has been reported in skin components (Montagna, 1955a; Nicolaides and Wells, 1957; Miraglia and Santos, 1971; Wilfried and Neurand, 1976; Athavale, 1980; Gulbenkian et al., 1980; Salinukul, 1985) and has been assigned a role in hydrolysis of sebaceous lipids. Esterase has been reported in sebaceous glands from time to time (Malaty and Bourne, 1954; Findlay, 1955; Montagna, 1955a; Montagna and Formisano, 1955; Argyris, 1956a, 1956b; Nicolaides and Wells, 1957; Montagna and Ellis, 1958; Steigleder, 1958; Montagna and Ellis, 1960; Brandes et al., 1965; Nasr, 1965; Ballantyne and Bunch, 1967; Ruddle and Harrington, 1967; Holt and Jones, 1973; Wilfried and Neurand, 1976, 1977b, 1979b; Wrench et al., 1980). Esterase has been assigned a role in cellular autolysis (Jarrett and Spearman, 1964; Brandes et al., 1965; Wilfried and Neurand, 1976). It has also been shown to be responsible for production of free fatty acids found in the skin surface lipids and sebum (Montagna, 1955a; Montagna and Formisano, 1955; Nicolaides and Wells, 1957; Nicolaides, 1963; Nasr, 1965).

Castration reduced the lipase activity in the preputial to a greater extent. Antiandrogen treatment has been reported to reduce lipase activity in the hamster flank organ (Gulbenkian et al., 1980).

IPR therapy reduced the lipase activity levels in the gland considerably. The drug has been reported to stimulate the activity levels of lipases acting on a variety of glycerides in adipose tissue (Vaughan and Steinberg, 1965; Takeo and Takenaka, 1978; Berglund et al., 1980; Khoo et al., 1980; Kawamura et al., 1981; Masahiro et al., 1981) and adrenal gland (Pittman and Steinberg, 1977). Conversely, IPR does not enhance rat myocardial lipase (Takeo and Takeneka, 1978). Obviously, the presently observed reduction in preputial lipase activity after IPR therapy could be due to decreased plasma testosterone levels (Chapter : 1) and altered glandular steroid metabolism (Ambadkar and Vyas, 1982).

Castration reduced the preputial non-specific esterase considerably. Naturally, androgens are shown to maintain esterase levels. It is quite pertinent to note that rat epididymis bears a population of holocrine secretory cells (Martan and Risley, 1963) and the levels of certain epididymal hydrolytic enzymes have been shown to be decreased after castration (Allen and Slater, 1957a; Allen and Hunter, 1960; Weiss and Gossrau, 1981). Even kidney has been reported to bear testosterone dependent esterase (Allen and Hunter, 1960; Shaw and Koen, 1963; Ruddle, 1966; Deimling and Grobarth, 1972; Wehler et al., 1972; Kochakian and Williams, 1973).

IPR therapy decreased histochemically demonstrable non-specific esterase in the preputial, contrary to reported

stimulatory influence of the drug on esterase activity levels of the adipocytes (Kawamura et al., 1981). Thus, IPR therapy reduced the activity levels of some more lysosomal enzymes in the preputial gland in addition to those reported earlier (Ambadkar and Vyas, 1981b). The drug therapy has also been reported to reduce steroid metabolism (Ambadkar and Vyas, 1982; Chapter : 1) and the rates of cellular proliferation and the process of cell disintegration (Vyas, 1978) in the preputial acini. When these reports are collectively taken into consideration, they could hint at the diminished lipid content in the preputial gland after IPR therapy (Chapter : 2). Thus, the present investigation confirms the assumption (Ambadkar and Vyas, 1981b) that β-adrenergic agonist receptors are involved in the regulation of release of lysosomal enzymes in the preputial glands of rats.