CHAPTER : 2

INVOLVEMENT OF β-ADRENERGIC AGONIST IN REGULATION OF LIPOGENESIS IN THE PREPUTIAL GLAND OF RAT

A plethora of literature is available on biochemical aspects and hormonal regulation of sebaceous secretion. Problems of sebaceous gland innervation and its possible significance were full of disputes, confusions and contradictions. However, it was convincingly shown that rat preputial gland, a sebaceous analogue, is supplied by both adrenergic as well as cholinergic nerve fibres (Ambadkar and Vyas, 1981a), despite previously reported conflicting views on the subject by Rothman (1954), Boecke (1934) and Montagna (1963) citing the work of Hurley et al., (1953), Hellmann (1955), Thies and Galente (1957) and Winkelmann (1960).

The literature pertaining to secretory innervation of sebaceous glands was also full of discrepancies. Contradictory observations had been reported by workers on the basis of their studies on patients with seborrhoea and various neuronal disorders (Starling, 1936; Serrati, 1938; Nexmand, 1944; Savill, 1944; Hodgson-Jones et al., 1952; Kligman and Shelley, 1958). It was conclusively shown that extrusion of pre-formed sebum in the preputial gland is under the control of α-adrenergic system (Ambadkar and Vyas, 1980).

The literature relevant to effects of drugs influencing sebaceous gland secretion is scanty (Cerutti, 1934). Melczer and Deme (1942) reported an increase in secretion of sebaceous glands after pilocarpine injections, but others could not find such effects (Rothman and Herrmann, 1953). Miescher and Schonberg (1944) found no change in lipid levels of sebaceous glands after atropine, pilocarpine and acetylcholine administration. Harville (1971) reported striking reduction in sebum secretion with L-Dopa therapy. Burton et al., (1973), Burton and Shuster (1973) and Wheatley and Brind (1981) reported L-Dopa to reduce the rates of lipogenesis. Additionally, on the basis of a report from our laboratory (Ambadkar and Vyas, 1980), Wheatley and Brind (1981) opined that norepineprine may play a role in stimulating the sebaceous gland to replenish its secretion. Conversly, adrenal neurohormones have been reported to inhibit sebaceous lipogenesis (Wheatley et al., 1971). β-adrenergic receptors have been shown to be implicated in regulation of population dynamics of the preputial acini (Vyas, 1978). Isoproterenol, a β-adrenergic agonist, has been reported to induce certain histochemical (Ambadkar & Vyas 1982) as well as biochemical changes (Ambadkar and Vyas, 1981b) in the preputial gland of male rats, which are similar to those that follow androgen deprivation. Precisely these observations from our laboratory prompted us to make use of such an agonist for newer avenue of inhibition of sebum synthesis which could prove to be clinically beneficial for acne vulgaris and related disorders of

increased sebaceous lipogenesis. Hence, a study was undertaken to understand lipid metabolism of the preputial gland of male rats as affected by isoproterenol administration.

Since lipids and lipid derivatives constitute the major components of the preputial gland secretion, metabolic activities centered around lipids could be considered to take precedence over others. The metabolic patterns of a tissue are characterised by its enzymic profiles. In this context, the enzymes *viz.*, glucose-6-phosphate dehydrogenase (G6PDH), isocitrate dehydrogenase (ICDH) and malic enzyme (ME) have assumed greater significance in lipid synthesizing tissues like mammary gland (Abraham and Chaikoff, 1959), adipose tissue (Gibson *et al.*, 1958; Hollifield and Parson, 1961; Weber *et al.*, 1961), sebaceous glands (Hershey, 1959; Katz and Rogenstad, 1966; Michael and Hoopes, 1970; Snyder and Malone, 1970; Freinkel, 1983; Abalain *et al.*, 1984) and liver (Brady and Gurin, 1952; Kadenbach *et al.*, 1964; Goodridge, 1968 a,b,c). All these studies have shown that G6PDH, ICDH and ME are directly involved in lipogenesis. In this light, it would be interesting to study these enzymes of the preputial gland of male rats and to look for the possible influence of isoproterenol administration on behaviour of the enzymes.

MATERIALS AND METHODS

Neuroendocrine manipulation essentially remains the same as described in the first chapter.

The preputial glands were obtained by excision immediately after decapitation. The glands were made free of fat and other tissues. Total lipid content of the gland was estimated by extracting the lipids with chloroform : methanol (2 :1 v/v) mixture according to the method of Folch et al., (1957).

Histochemical localization :

The preputial gland was kept on a chuck of cryostat microtome maintained at -20°C. 9-12 μ thick sections were cut and processed for histochemical demonstration of G6PDH employing the method of Ogata and Mori (1964). ICDH and ME were localized according to the method as described by Pearse (1972). The sections were post-fixed and mounted in glycerine jelly. A few sections incubated in respective substrate blank media and a few other sections treated with boiling water prior to incubation served as controls.

OBSERVATIONS

Total lipids :

Total lipid content of the gland was very high (27.02%) which showed a significant decrease at 10 days after the administration of IPR (13.3%) (Table :1).

Histochemical Study :

All the three TPN-linked dehydrogenases studied, i.e., G6PDH, ME and ICDH depict high reactivity at the periphery and show gradual decrement towards the centre of the preputial acini

TABLE : I

TOTAL LIPID CONTENT OF THE PREPUTIAL GLAND OF NORMAL AND IPR ADMINISTERED MALE RATS. MEAN ± S.D.

Experimental Group	Total lipid content mg/100 mg wet weight	Significantly different from the normal at the level 'P'
Normal Rats	27.02 ± 5.2	-
IPR treated Rats	13.31 ± 2.31	< 0.001

EXPLANATIONS FOR FIGURES

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

Fig. 1 G6PDH activity in the section of the preputial gland of normal rat.

Fig. 2 ME activity in the section of the preputial gland of the normal rat.

Fig. 3 ICDH activity in the section of the preputial gland of the normal rat.

Fig. 4 G6PDH activity in the section of the preputial gland obtained from isoproterenol treated rat.

Fig. 5 ME activity in the section of the preputial gland obtained from isoproterenol treated rat.

Fig. 6 ICDH activity in the section of the preputial gland obtained from isoproterenol treated rat.

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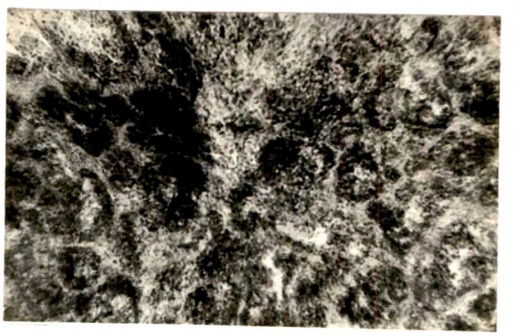

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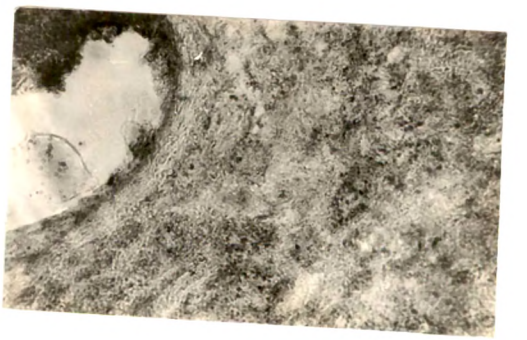

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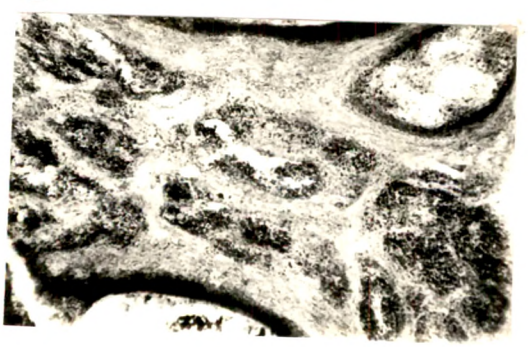

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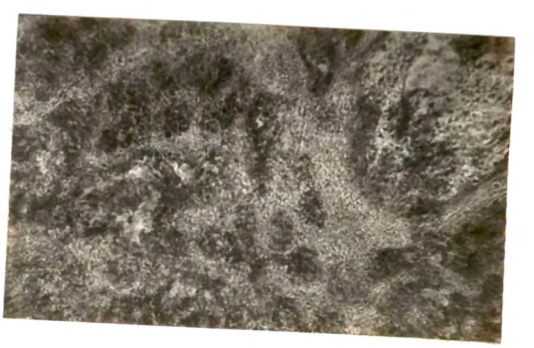

of male rats (Fig. 1, 2, 3). Cells of the duct system also show moderate enzyme activities. The interacinar substance too show enzyme activities. Among the dehydrogenases studied, G6PDH is the most reactive enzyme followed by ME and ICDH in the preputial acini of normal male rats.

Isoproterenol administration results in a drastic fall in the activities of the dehydrogenases without much change in their distribution patterns. Cells lining the duct system show feeble activity. The inter-acinar substance show diminished activity (Fig. 4, 5, 6).

DISCUSSION

Results obtained indicate that preputial gland of male rats is rich in lipids. The total lipid content of the gland decreased significantly after chronic exposure of male rats to IPR. Pfompt reductions in the levels of sebum production have been reported repeatedly in rats, men and animal models after surgical androgen deprivation (Emanuel, 1936; Ebling, 1963; Hamilton and Mestler, 1963; Pochi and Strauss, 1969; Thody and Shuster, 1970a; Andre and Chassagne, 1977) or anti-androgen treatment (Ebling, 1973; Lunderschmidt and Plewig, 1977; Ebling et al., 1981). Contrary to these, testosterone has been well documented to exert stimulatory effects on lipogenesis in sebaceous analogues (Huggins et al., 1955; Freinkel, 1963; Archibald and Shuster 1969; Pochi and Strauss, 1969; Takayasu and Adachi, 1970; Thody and Shuster, 1971a; Ebling, 1974; Pochi and Strauss, 1974;

Shuster and Thody, 1974; Andre and Chassagne, 1977; Mukherjea, 1977; Bhattacharya and Chowdhury, 1978, Mesquita and Coimbra, 1981). These reports imply that total lipid levels of the preputial glands of male rats are prone to undergo escalations according to availability of the steroidal support. Significant reduction in the total lipid content of the gland as a sequel to chronic exposure of male rats to IPR in the present investigation clearly indicates prompt reduction in the circulating level of testosterone. This observation insinuates two possibilities. IPR administration would have affected the endogenous metabolism of the gland either indirectly or directly. The indirect influence of the drug therapy could be achieved by disturbance in the steroid metabolism of liver, kidney, testes, adrenal and blood plasma and hence, it would have exerted influence over the steroid and general metabolism in the preputial gland itself (Ambadkar and Vyas, 1981b and 1982). These possibilities have been dealt with in details in the first chapter.

Alternatively, IPR therapy employed herein might have some direct impact on the endogenous metabolism of the gland itself. A voluminous body of literature is at hand pertaining to the influence of IPR on the lipid metabolism in a variety of tissues. It is, therefore, reasonable to speculate that IPR therapy must have inhibital preputial lipogenesis. IPR has been reported to inhibit lipogenesis in chicken hepatocytes and adipose tissue explants <u>in vitro</u> (Campbell and Scanes, 1985). It is also shown

to inhibit (^{32}P) Pi incorporation into phosphatidylinositol in rat white fat cells (Garcia - Sainz and Fain, 1980) and rat heart (Kiss and Farkas, 1975). IPR therapy is shown to inhibit prostaglandin synthesis in endometrium (Adler et al., 1981). Even cholesterol and cholesterol ester synthesis are shown to be reduced by IPR treatment of cultured human fibroblast (Maziere et al., 1983). Additionally, adrenaline, noradrenaline and phenylephrine have been shown to stimulate prostacycline (Jeremy et al., 1985) and phosphatidylinositol (Friedel et al., 1973) synthesis while IPR therapy was ineffective in both the cases. α-adrenergic agonists have been reported to enhance the labeling of phosphatidylinositol whereas the IPR therapy was nonresponsive in rat parotid slices (Michell and Jones, 1974), rat pineal cultures (Eichberg et al., 1973) and in mouse thyroid gland (Hiroya et al., 1982). Similarly, cholinergic stimulation is shown to enhance P^{32} incorporation into phospholipids of guinea pig seminal vesicles while adrenergic stimulation is shown to be non-effective in enhancing the rates of incorporation into phospholipids (Lockwood and Williams-Ashman, 1971). IPR is also shown to be inert in inducing phospholipid synthesis (Schramm, 1967). It is, thus, apparent from these reports that if IPR stimulation is not enhancing rates of lipogenesis, it is atleast chemically inert in stimulating lipogenesis.

β-adrenergic receptor agonists have been shown to regulate lysosomal proliferation and hence limit total lipid content of

the preputial gland (Chapter : 6). Moreover, it is apparent from Chapter No. 7 that IPR therapy employed herein elicited a significant increase in intracellular levels of cAMP in the preputial gland. Enough literature is available on antilipogenic role for cAMP and/or hormones known to influence intracellular cAMP levels. This has been proved by a variety of *in vivo* and *in vitro* studies of short term as well as long term duration with respect to extraneural tissues; especially liver and adipose tissue (Bricker and Levey, 1972; Lakshmanan *et al.*, 1972; Allred and Roehrig, 1973; Beg *et al.*, 1973; Klain and Weiser, 1973; Lee *et al.*, 1973; Meikle *et al.*, 1973; Capuzzi *et al.*, 1974; Brotherton and Hoak, 1980, Hopkins and Gorman, 1981; Pelech *et al.*, 1981). Obviously, the reports are self-explanatory in the present context.

High activity of G6PDH in the preputial gland is indicative of an active participation of HMP-shunt pathway. This enzyme channelizes more glucose into the direct oxidative pathway and thus could generate more $NADPH_2$ and ribose sugar. The cytosolic $NADPH_2$ thus produced could be used for synthesis of long chain fatty acids. An active participation of the shunt pathway has been reported frequently in the sebaceous analogues (Katz and Rogenstad, 1966; Michael and Hoopes, 1970; Snyder and Malone, 1970; Michael and Hoopes, 1974; Freinkerl, 1983). Though the work of sansone *et al.*, (1971) showed that the shunt pathway may be critical in sebaceous lipogenesis; Wheatley *et al.*,(1973)

presented quantitative data which suggested that operation of this pathway is not mandatory for sebaceous lipogenesis. Ziboh et al. (1970) showed that the shunt pathway may contribute 41-57% of $NADPH_2$ needed for lipogenesis in rat skin, the rest of $NADPH_2$ may arrive from the reactions catalysed by ME and ICDH. This contention supports the present study wherein both ME and ICDH show considerable activity in the preputial gland.

ME together with MDH could be helpful in bringing about the cyclic conversions between pyruvate, malate and oxaloacetate and thus maintaining a sufficient enough pool of citrate which in turn could stimulate lipogenesis by fixation of CO_2 in the presence of acetyl CoA carboxylase (Brady and Gurin, 1952; Gibson et al., 1958; Brown et al., 1966). ME has been assigned a role in the production of $NADPH_2$ necessary for lipogenesis (Lee and Lardy, 1964; Brown et al., 1966; Goodridge and Ball, 1966; Goodridge, 1968 a, b and c; Lunass et al., 1968). The step of TCA cycle catalyzed by ICDH occupies a central position in the intermediary metabolism and provides substrate and co-factor for a variety of synthetic and energy yielding pathways and could be rate limiting, too (Kornberg and Pricer, 1951; Lowenstein, 1961; Brady and Gurin, 1952; Baker and Newburgh, 1963; Kadenbach et al., 1964).

IPR administration resulted in a drastic fall in the activities of G6PDH, ME and ICDH without much change in their distribution patterns. Such a change in the activities of these enzymes would result in reduced availability of $NADPH_2$. This attenuated supply

of $NADPH_2$ may contribute to observed reduction of total lipid content in the gland after chronic IPR exposure (Table : 1). At this juncture, it is quite pertinent to note the influence of IPR on the activity levels of some of the important lipogenic enzymes in a variety of tissues. IPR, db cAMP and theophylline have been shown to reduce the levels of functional mRNA for G6PDH, ME and fatty acid synthetase during adipose differentiation of 3T3 cells in vitro (Spiegelman and Green, 1981). This report in particular lends support to presently observed reductions in the total lipid content and activities of G6PDH, ME and ICDH in the preputial gland of male rats as affected by IPR therpay. In recent years, IPR has been repeatedly shown to suppress the activity levels of certain lipogenic enzymes, viz., ATP citrate lyase and acetyl CoA carboxylase, the enzymes involved in channelizing acetyl CoA towards long chain fatty acid synthesis (Correze et al., 1982) and the enzyme fatty acid synthetase (Weiss et al., 1980; Gaben et al., 1984) as such in differentiating rat adipocytes. As has been discussed in the first chapter, IPR is a potent inducer of a variety of mixed function oxidases. During maximal rates of mixed function oxidation of aminopyrine, rate of acetyl unit synthesis is shown to be depressed by about 40 % in the perfused rat liver (Thurman and Scholz, 1973). Obviously, the report does not need much explanation in the present context.

In addition to its direct action on lipid metabolites and lipogenic enzymes, IPR has also been shown to be implicated in

regulation of certain metabolic features which ultimately could be expected to alter the overall rate of lipogenesis in the gland. Studies have shown IPR to inhibit glucose transport in rat adipocytes (Taylor and Halperin, 1979; Kashiwagi et al., 1983). In addition, IPR has been assigned with depressent effects on the rates of glycolysis (Kako, 1965; Ureta et al., 1970; Seth et al., 1980; Harris and Mackenzie, 1981; Mills et al., 1984) and amino acid incorporation into proteins (Harris and Mackenzie, 1981). The effects of IPR administration might be expected to induce a fall in tissue concentration of intermediates of the glycolytic pathway (Seth et al., 1980) or beyond and may thus lead to decreased fatty acid synthesis. These reports are self-suggestive in the present context as related to effects of IPR on preputial lipogenesis.

Before concluding, one is tempted to note that IPR therapy employed in the present studies elicited a spurt in the cAMP levels of the preputial gland (Chapter :7) which was mainly responsible for regulation of lipogenesis, as cAMP and agents known to trigger cAMP content have been implicated in the regulation of a number of glycolytic (Ureta et al., 1970) and lipogenic enzymes (Rudack et al., 1971; Lakshmanan et al., 1972; Allred and Roehrig, 1973; Beg et al., 1973; Klain and Weiser, 1973; Lane et al., 1973; Meikle et al., 1973; Capuzzi et al., 1974 Goodridge, 1975; Volpe and Vagelos, 1976). These reports clearly indicate that the total lipid content decrement in the preputial gland as a sequel to chronic exposure of male rats to IPR could

possibly be due to direct action of the drug therapy on the gland metabolism as such, in addition to the indirect influence of the drug as has been discussed in the first chapter.