CHAPTER : 3

ACTION OF CHRONIC ISOPROTERENOL ADMINISTRATION ON CERTAIN DEHYDROGENASES IN THE PREPUTIAL GLAND OF MALE ALBINO RAT

Evaluation of lipogenesis, a prominent biochemical feature of preputial gland metabolism, has been attempted to a certain extent in the previous chapter. The biosynthetic potential acquired by the preputial gland would naturally demand enough energy as well as the required precursors and cofactors. This could be brought about through anaerobic and/or aerobic reactions and could be either stepped up or toned down according to the hormonal microenvironment at the cellular level. Importance and involvement of carbohydrates as the precursors for lipogenesis in human skin and sebaceous glands have been well recognized (Nasr, 1965; Adachi and Yamasawa, 1967; Hugh et al., 1969). The same holds true for other tissues viz., liver and adipose tissue; both actively engaged in lipogenesis (Gibson et al., 1958; Ashemore and Weber 1959; Lynen, 1961; Weber et al., 1961; Margolis and Vaughan, 1962; Martin and Vagelos, 1962; Robinson et al., 1963). Observations made in the previous chapter have shed some light on the possible biochemical routes involved in the production of cofactors which could be considered as an index for lipogenesis. However, biochemical pathways associated with production of lipid precursors like α-glycerophosphate and acetyl coenzyme A have not been studied in the context of IPR therapy as has been employed

here probably for the first time to counteract increased preputial lipognesis; akin to acne vulgaris. Hence, a study directed towards this line was initiated.

α - glycerophosphate dehydrogenase (α-GPDH) and β- hydroxybutyrate dehydrogenase (BDH) catalyze the reactions to produce lipogenic precursors like α-glycerophosphate and acetyl Co-A, respectively.

The α-GPDH catalysis is an important step in the reduction of dihydroxyacetone phosphate with $NADH_2$ ensuring a steady supply of α-glycerophosphate and NAD which are of prime importance in the continuity of the glycolysis (Kornberg and Pricer, 1952; Kennedy, 1953: Duve et al., 1962). A stimulatory influence on fatty acid esterification has been ascribed to α-glycerophosphate (Tzur et al., 1964; Howard and Lowenstein, 1965). BDH catalyses the oxidation of β-hydroxybutyrate and acetoacetate forming acetyl Co-A, which could be utilized for either energy production or lipid synthesis.

A histochemical evaluation of BDH and α-GPDH in preputial gland was taken up in normal as well as IPR treated male albino rats so as to understand contribution of such reactions towards lipogenesis, if any.

MATERIALS AND METHODS

Neuroendocrine manipulation in the male albino rats essentially remains the same as has been described in the first chapter.

For the histochemical localization of enzymes, the preputial gland was kept on a chuck of the cryostat microtome maintained at -20°C. 9 - 12 μ thick sections were cut and processed for histochemical demonstration of α-GPDH and BDH activities employing the method of Ogata and Mori (1964). The sections were post-fixed and mounted in glycerine jelly. A few sections incubated in the respective substrate-blank media and few other sections, treated with boiling water prior to incubation, served as controls.

OBSERVATIONS

Both the dehydrogenases depicted high reactivity at the periphery and showed gradual decrements towards the centre of the preputial acini in a normal rat. Irrespective of the distribution pattern, the intensity of the enzyme activity of BDH was more intense than that of α -GPDH (Fig. 1-2).

Chronic IPR therapy resulted in decreased intensity of both the dehydrogenases to a considerable extent (Fig. 3-4) without much change in the distribution patterns.

DISCUSSION

The observations noted herein reveal a higher activity of BDH and a relatively lower activity of α -GPDH in the preputial acini of normal rats. Reversible α-GPDH catalysis is known to produce α -glycerophosphate which can serve as an important

EXPLANATIONS FOR FIGURES

Figs. 1 to 4 Photomicrographs of sections of rat preputial glands showing different enzyme activity. 150×.

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

Fig. 2 α-GPDH activity in the section of the preputial gland of normal rat.

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

Fig. 4 α-GPDH activity in the section of the preputial gland of isoproterenol treated rat.

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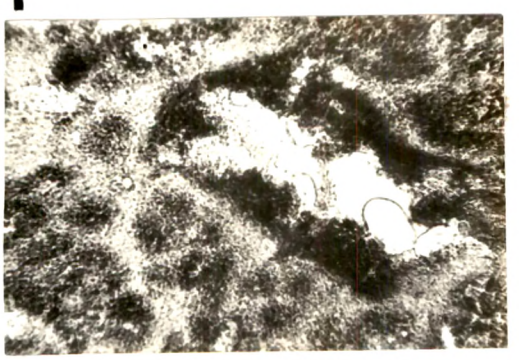

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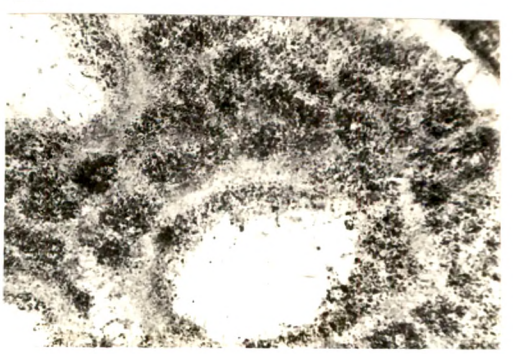

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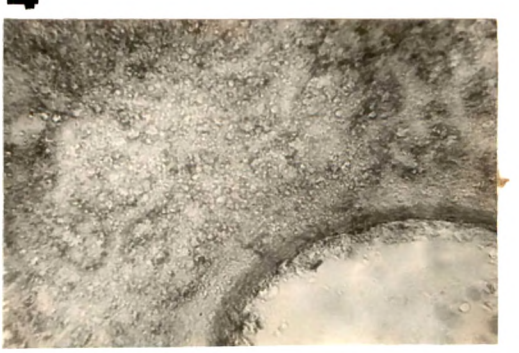

precursor for lipid synthesis. Ziboh and Hsia (1969) have ascribed a regulatory function to glyceraldehyde 3-phosphate in lipogenesis in skin. Synthesis of squalene and cholesterol as well as fatty acids, too has been reported in human preputial and abdominal skin using acetate as a precursor (Hugh et al., 1969). In addition, glucose has been implicated as a major source for lipid synthesis in adipose tissue (Winegrad and Renold. 1958). It is clear from these reports that carbohydrate moieties serve as major source of precursors for lipogenesis. Taking these reports into consideration, the presently observed activity of α-GPDH in the preputial acini of normal male rat is suggestive of the probable utilization of carbohydrate intermediates in the synthesis of lipids. Another significant aspect of α-GPDH catalysis, is the reduction of dihydroxy acetone phosphate with $NADH_2$ yielding α-glycerophosphate and NAD which are important in the continuation of glycolytic pathway (Kornberg and Pricer, 1952; Kennedy, 1953; Duve et al., 1962, Adachi and Yamaswa, 1967). α-glycerophosphate is shown to be an important precursor for phospholipid and triglyceride synthesis (Kennedy, 1953; Kornberg and Pricer, 1953; Kennedy, 1954, 1957 a,b; Rossiter et al., 1957). Besides, a stimulatory influence on fatty acid esterification also has been ascribed to α-glycerophosphate (Tzur et al., 1964; Howard and Lowenstein, 1965).

Since the reaction catalyzed by α-GPDH is reversible, presently observed higher α-GPDH activity in the preputial of normal rats could be attributed towards utilization of lipids so

as to meet the energy requirements, too (Fredrickson and Gordon, 1958; Rossiter and Strickland, 1960; Chaffee et al., 1964 & 1966; Dryer and Paulsryd, 1966). Histochemical demonstration of ∝-GPDH is considered as indicating glycerol fermentation in tissues (Pearse, 1968). Thus, the possibility of ongoing lipolysis can not be ruled out in the preputial gland of normal male rats. In fact, higher activities of lipolytic enzymes like non-specific esterase and lipase observed in the normal preputial gland (Chapter-6) would naturally entertain ∝-glycerophosphate production from the triglyceride stores. ∝-glycerophosphate thus produced could be attributed to ∝-GPDH action to enhance dihydroxy acetone phosphate levels which in turn could be speculated to stimulate energy transducing reactions. Thus, ∝-GPDH may be considered to play a strategic dual role in the preputial gland metabolism. Hence, the problem of contribution of ∝-GPDH reaction towards either lipogenesis or glycerol utilization can not possibly, decidedly explained on the basis of histochemical studies alone. The exact mechanism of the enzyme action could possibly be evaluated through use of radio-labeled compounds only.

IPR administration resulted in a drastic reduction in the histochemically demonstrable activity of ∝-GPDH. As it has been claimed repeatedly in the previous chapters, chronic IPR therapy would have affected the endogenous metabolism of the preputial gland either directly or indirectly. Likely, the indirect influence could be met with the increase in steroid catabolism and hence, the androgen responsive preputial gland metabolism could be altered (Chapter 1 and 2).

Alternatively, IPR therapy might have some direct influence on the endogenous metabolism of the gland. It is pertinent to note here that IPR therapy has been shown to retard reductive synthetic and energy transducing reactions including α-GPDH in the heart of exercised as well as sedantary rats (Mitova et al., 1983). The report is self-suggestive in the present context wherein IPR therapy has been shown to alter lipid metabolism (Chapter 2) and oxidative phospharylation (Chapter 5) too, in the preputial gland of male rats. In addition to its direct action on α-GPDH activity in the preputial, IPR has also been shown to be implicated in the regulation of certain metabolic features which ultimately could be expected to reduce the α-GPDH activity. The drug could be expected to induce an intracellular acidic pH due to increased LDH activity in the preputial gland (Chapter : 4). Intracellular acidic pH could be expected to reduce α-GPDH activity, (Neely et al., 1975; Steenbergen et al., 1978). The drug has been assigned with depressant effects on the rates of glycolysis (Seth et al., 1980; Harris and Mackenzie, 1981; Mills et al., 1984). Such effects might be expected to induce a fall in the tissue concentration of intermediates of glycolytic pathway (Seth et al., 1980) and may thus lead to decrease the tissue concentration of dihydroxyacetone phosphate. The reports, thus, do not need much explanation.

Whatsoever may be the cause, decreased α-GPDH activity in the preputial gland of IPR-treated animals can not be accounted for by decreased glycerol fermentation (Pearse, '68) and hence

altered energy metabolism. This assumption is based on the fact that increments in the levels of lipase and non-specific esterase is a bsic requirement in fulfillment of enhanced lipolysis. This basic requirement is not satisfied in the preputial gland of IPR-treated animals (Chapter : 6), contrary to a prominent lipolytic function assigned to IPR treatment (Spiegelman and Green, 1981). Thus, lipolysis and β-oxidation of fatty acids to meet the energy demands can not be entertained in the preputial gland after IPR treatment. It is, therefore, obvious to speculate the decreased α-GPDH activity in the preputial gland after IPR therapy as a consequence of decreased phospholipid metabolism (Schramm, 1967; Lockwood and Williams-Ashman, 1971; Eichberg et al., 1973; Fridel et al., 1973; Michell and Jones, 1974; Kiss and Farkas, 1975; Garcia-Sainz and Fain, 1980; Hiroya et al., 1982) per se rather than the involvement of the same in energy transducing reactions through production of dihydroxyacetone phosphate which in turn could have been oxidized to completion via Embden-Meyerhof pathway and the TCA cycle.

In general, BDH activity is high in the preputial of normal rats. The enzyme catalyzes the first step in the oxidation of acetoacetate and β-hydroxybutyrate and forms acetyl Co-A which could be an important source of readily available metabolic energy. In normal preputial gland acini, glucose uptake is not a limiting factor. So the tissue concentration of the intermediates of glycolytic and TCA cycle could be well maintained. In this condition, some oxaloacetate could be made available to acetyl Co-A for

citrate condensation. Thus, diversion of acetyl-Co-A towards TCA cycle (Lehninger and Greville, 1953) can not be ruled out as the tissue levels of oxaloacetate is not a rate-limiting factor. Indeed, higher BDH activity in the sebaceous gland has been correlated with energy production (Wheatley, 1974). In addition, higher BDH activity in lipid rich breast muscle of pigeon (Cherian, 1967) and fish skeletal muscle (Bokdawala and George, 1967) has been attributed towards energy transducing reactions. At the same time, BDH catalyzed reaction could be considered to favour acetyl Co-A synthesis which in turn could be diverted towards fatty acid synthesis, too (Nicolaides, 1963., Wilson, 1963). The same role has been attributed to BDH catalyzed reaction in the developing brain (Williamson et al., 1971; Weng et al., 1973; Edmond, 1974; Patel, 1975; Patel et al., 1975; Patel and Owen, 1976, 1977; Yeh et al., 1977).

BDH activity decreased to a greater extent in the preputial gland of IPR-treated animals and could be considered as an indication of decrements in rates of either lipogenesis or energy transducing reactions in TCA cycle. Studies have shown to inhibit glucose transport at micromolar concentration of IPR (Taylor and Halperin, 1979; Kashi Wagi et al., 1983). Even the rates of glycolysis and amino acid incorporation have been shown to be hindered by IPR therapy (Kako, 1965; Ureta et al., 1970; Harris and Mackenzie, 1980; Seth et al., 1980; Mills et al., 1984). It implies from these reports that IPR therapy employed in the present investigation would have inhibited glucose uptake by the preputial acini, in addition to decreased rates of glycolysis. Apparently, tissue

concentration of oxaloacetate could be expected to be diminished. Hence, the diversion of acetyl Co-A produced by BDH catalyzed reaction towards oxidations in TCA cycle could be expected to be altered. Obviously, acetyl Co-A could not be attributed towards TCA oxidations. Thus, only one possibility is left behind wherein acetyl Co-A could be expected to be channelized towards lipid synthesis. Contrary to our speculations, IPR has been shown to suppress the activity levels of ATP-citrate lyase and acetyl Co-A carboxylase in rat adipocytes (Correze et al., 1982). Hence, observed decrements in histochemically demonstrable BDH in the preputial gland of IPR-treated rats could neither be awarded totally to decreased total lipids (Chapter : 2), nor be fully considered as being involved in retarded energy metabolism. Perhaps, this puzzle, too, could be resolved only with the use of labeled compounds.