CHAPTER : 4

EFFECT OF ISOPROTERENOL ADMINISTRATION ON ACTIVITIES OF LDH AND MDH IN THE PREPUTIAL GLAND

Dehydrogenase catalyzed reactions bring about oxidoreduction of metabolic intermediates in a sequential fashion constituting well defined metabolic pathways involving catabolism of carbohydrates, proteins and lipids. Some of the dehydrogenases may also indirectly facilitate the synthetic reactions concerning these metabolites. Further, by their precisely synchronized activities, they also bring about controlled interconversions between these metabolites and thus play crucial roles in bringing about adaptive metabolic alterations and energy transformations as per the requirement of tissues. Activities of specific dehydrogenases, apart from controlling the operation of anaerobic or aerobic pathways of metabolism, also generate intermediates useful in the interconversion of various metabolites.

Pyruvate, one of such intermediates, by virtue of its strategic position in the metabolic patterns, has acquired a great deal of importance in physiology of mammalian skin (Lorenz, 1965; Gumenyuk et al., 1979). During stressfully low oxygen levels, a tissue in question may resort to conversion of pyruvate to lactate through the mediation of lactate dehydrogenase (LDH); oxidizing cytosolic NADH, which otherwise is normally oxidized via the malate-aspartate shuttle. Hence, anaerobiosis could prove to be an optimal pathway towards

meeting the basic energy demands. However, pyruvate molecule is more prone normally to enter the TCA cycle through acetyl coA when oxygen supply is adequate, thus favouring oxidative catabolism of carbohydrates. At times when carbohydrate supply is not a limiting factor, excess pyruvate molecules formed would be diverted towards lipogenesis through greater turnover of acetyl coA molecules. However, during starvation, a net breakdown of proteins and certain aminoacids and their conversion to pyruvate is usually accompanied by breakdown of lipids.

Usually, pyruvate is converted to oxaloacetate, whenever TCA cycle intermediates are in short supply. Formation of pyruvate from oxaloacetate by reversal of this process is unlikely since the intracellular concentration of oxaloacetate is very low. Instead, another route could be adopted; oxaloacetate could be reduced to malate through malate dehydrogenase (MDH) intramitochondrially. Malate could be converted into pyruvate in the cytoplasm through mediation of malic enzyme (ME). Thus, an alternate metabolic route could be adopted by tissues wherein pyruvate could be replenished cyclically from oxaloacetate as well as malate (Wheatley et al., 1973). Obviously, LDH catalyzing the reversible reaction between pyruvate and lactate could shed some light on such a multiple role of pyruvate metabolism. Additionally, MDH, a representative of TCA cycle could aid in unfolding such an aspect.

A voluminous body of literature is at hand pertaining to the roles played by LDH and MDH in the metabolism of sebaceous gland or its analogues including the preputial gland of male rats

(Hashimoto et al., 1962; Michael, 1965; Marois and Salesses, 1967; Lucas, 1968; Ziboh and Hsia, 1969; Santos et al., 1974; Wheatley, 1974; Ambadkar and Vyas, 1979; Sato et al., 1981, Freinkel, 1983). However, there are no reports on variations in the levels of such enzymes under the influence of chronic IPR therapy. Hence, a study was initiated to delineate the patterns of these enzymes after IPR therapy so as to evaluate their roles in preputial gland metabolism of male rats.

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 cryostate microtome maintained at -20°C. 9-12 μ thick sections were cut and processed for histochemical demonstration of LDH and MDH 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 a few other sections, treated with boiling water prior to incubation, served as controls.

OBSERVATIONS

Both the dehydrogenases depicted higher activity at the periphery and showed decrements towards the central parts of the preputial acini of normal untreated male rats. Irrespective of distribution pattern, the intensity of LDH was greater than that of MDH (Fig. 1 & 2).

EXPLANATIONS FOR FIGURES

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

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

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

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

Fig. 4 MDH activity in the section of the preputial gland of isoproterenol treated rat.

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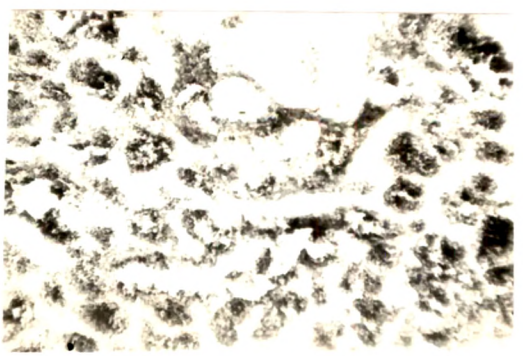

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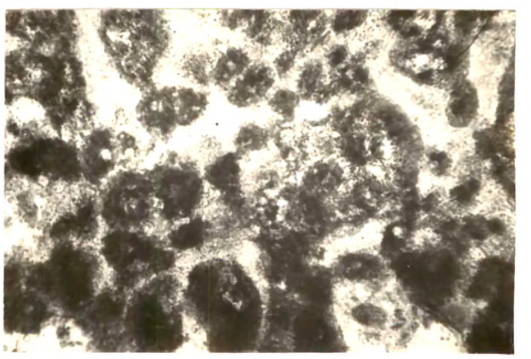

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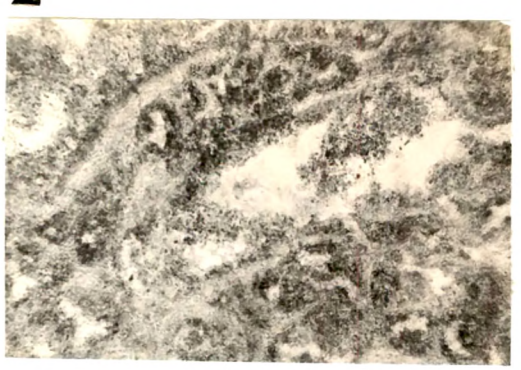

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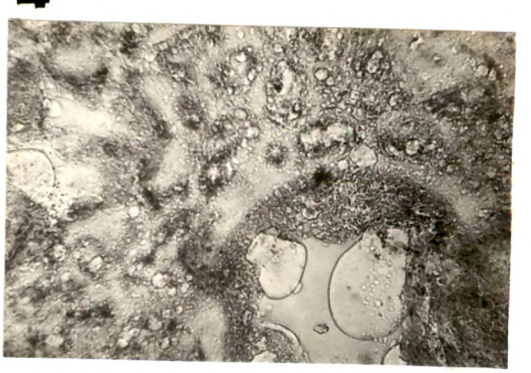

Chronic IPR administration to male rats increased the LDH activity to a considerable extent along the preputial acini without noticeable change in distribution patterns (Fig. 3). Contrary to this, MDH activity showed significant decrement (Fig. 4).

DISCUSSION

Normally, the preputial gland being holocrine in nature has to produce continuously more of cells through mitosis to replace lipid laden mature cells of the acini which are lost in the autolytic process. Thus, the mitotic index of the gland is very high (Vyas, 1978). It is worth nothing that tissues exhibiting a high mitotic index derive energy mainly through anaerobic glycolysis (Brachet, 1967). Hence, presently observed higher activity levels of LDH in the preputial gland of normal rats could be considered to resort preferntially to anaerobiosis. At the same time, in view of higher rate of lipogenesis (Chapter : 2), formation of pyruvate from lactate, the latter being obtained from circulation, has been suggested (Ambadkar and Vyas, 1979). Lactate is known to sustain a rapid rate of lipogenesis comparable to that obtained with glucose (Wheatley, 1974). Obviously, presently observed high LDH activity in the preputial gland could be considered to ensure a steady conversion of lactate to pyruvate. Thus, it seems probable that LDH may well have a dual role in the metabolism of the preputial gland. However, the problem of contribution of the LDH activity towards either lipogenesis or anaerobiosis cannot be resolved only on the basis of histochemical observations reported here.

Perhaps, the problem could be solved by assaying two distinct LDH subunits (Wilson et al., 1963; Goodfriend and Kaplan, 1964).

IPR administration resulted in a spurt in the histochemically demonstrable LDH activity. As has been stated in the previous chapters, chronic IPR therapy was noticed to have increased steroid catabolism (Chapter : 1) and as a consequence metabolic patterns of androgen-responsive preputial gland could be expected to get altered. One of the alterations is reflected in the form of enhanced LDH activity. Alternatively, IPR therapy might also directly impinge upon the endogenous metabolism of the gland as it is evident from the following discussion. IPR has been shown to induce acute hypoxia in case of myocardium (Pelouch, 1973; Ganguly et al., 1980; Bora et al., 1985). Goodfriend et al (1966) have shown that IPR induced myocardial hypoxia led to increased LDH-M synthesis. A similar response may also be expected to occur in the case of preputial gland of rat under the influence of IPR treatment. This was indeed borne out by observed spurt in histochemically demonstrable LDH in the preputial gland. This corroborates earlier assumption that anaerobiosis is probably a patent response under IPR treatment.

In addition to induction of hypoxia, toxic IPR levels have also been shown to decrease pyruvate utilization, probably by depressing the rate of oxidation via TCA cycle (Kako, 1965). Interestingly enough, andrenochrome - an oxidation product of IPR has been shown by Krall et al (1964) to drastically inhibit pyruvate oxidation in the case of rat brain. Pyruvate, thus, would

be accumulated forcing the reaction towards lactate formation by inducing enhanced LDH-M synthesis. Indeed, IPR therapy has been repeatedly shown to induce synthesis of LDH-M subunits in rat C6 glioma cells (Jean and Brooker, 1974; Derda and Jungmann, 1979; Derda et al., 1980; Harrison et al., 1980; Lee and Jungmann, 1981; Miles et al., 1981; Jungmann et al., 1983) and rat myocardium (Ganguly et al., 1980; Guerra et al., 1981; Bora et al., 1985). Additionally, it has also been shown to induce cardiac lactate concentration (Seth et al., 1980, 1985). Hence, presently observed spurt in histochemically demonstrable LDH in the preputial gland could be an indication of enhanced lactate production only and not lipogenesis as the total lipid content of the gland is reduced after IPR therapy (Chapter : 2). Thus, after chronic IPR administration, a certain degree of "downhill" glycolysis may produce some NADH. However, the routes of NADH oxidation viz., pyruvate oxidation, α-GPDH activity (Chapter : 3) and malate-aspartate shuttle via MDH (present chapter), have been shown to be reduced after chronic IPR therapy. Hence, the only means of oxidizing NADH formed in this manner is by catalysing pyruvate-lactate reaction essentially involving LDH-M subunits now available in increasing quantities. Intracellular acidic pH due to increased LDH-M, perhaps, could be one of the probable reasons for decreased α-GPDH activity (Chapter:3) and FDP-aldolase activity levels (Chapter : 5), the key enzymes of Embden-Meyerhof pathway (Neely et al., 1975; Steenbergen et al., 1978).

It has been reported that micromolar concentrations of IPR inhibit glucose transport in rat adipocytes (Taylor and Halperin, 1979; Kashiwagi et al., 1983). In addition, this drug has been shown

to depress the rates of glycolysis in different tissues (Kako, 1965; Ureta et al., 1970; Seth et al., 1980; Harris and Mackenzie, 1981; Mills et al., 1984). Considering these reports on diverse effects of IPR treatment on metabolic features, an overall picture that emerges points to retardation of glucose metabolism to a significant extent. Hence, such low concentration of pyruvate would favour formation of lactate (Wilson et al., 1963) due to observed acceleration of LDH activity in the preputial gland after IPR therapy.

MDH activity was found to be slightly lesser than that of LDH in case of normal preputial glands. MDH, one of the TCA cycle enzymes, along with LDH, when viewed in the light of reported lower activities of the other two important TCA cycle enzymes viz., SDH (Ambadkar and Vyas, 1979) and ICDH (Chapter : 2), may be expected to stimulate malate cycle (Wheatley et al., 1973) and hence, lipogenesis (Levy, 1961, Brown et al., 1966; Korsrud and Baldwin, 1972; Vallivullah et al., 1985) under normal conditions. Simultaneously, the contribution of MDH towards TCA oxidation can not be ruled out.

MDH activity decreased to a greater extent in the preputial of IPR treated animals and could be taken as an indication of decrements in the rate of either lipogenesis or cellular oxidation. Indeed, IPR has been shown to exert a big jolt in rat cardiac mitochondrial MDH activity (Grieve and Williams, 1981; Guerra et al., 1981). Hence, presently observed decrement in MDH in the preputial

gland could be an indication of reduced energy metabolism. Additionally, decreased MDH could also be taken for by reduced malate cycle activity and hence, lipogenesis (Chapter : 2).

IPR therapy employed herein is expected to indulge certain metabolic features in the preputial gland which ultimately could retard energy metabolism in TCA cycle. Promotion of cardiac mitochondrial uniporter and energy metabolism have been assigned to α -adrenergic responses, whereas the response towards β -adrenergic agonists has been shown to remain inert (Otorii et al., 1977; Kessar and Crompton, 1981). IPR-induced calcium influx has been shown to lead to overstimulation of the process resulting in breakdown of cellular Ca^{++} control with concomitant loss of high energy phosphates and mitochondrial respiration (Lehninger et al., 1967; Kutsuna, 1972; Nirdlinger and Bramante, 1974; Jennings et al., 1975; Takeo and Takenaka, 1977; Steen et al., 1979; Chernysheva et al., 1980). IPR or its oxidation products have been shown to halt pyruvate oxidation (Kako, 1965; Jennings and Ganote, 1976) and oxidative phosphorylation (Krall et al., 1964; Sobel et al., 1966; Margaret et al., 1974; Nirdlinger and Bramante, 1974; Yates and Dhalla, 1975; Dhalla et al., 1978; Siess and Weiland, 1980; Hiroshi, 1981; Kuninaka, 1981a, b; Ramos et al., 1983). These reports clearly indicate that IPR therapy must have reduced the overall rates of energy metabolism in the TCA cycle, and perhaps, this is reflected in reduced MDH activity in the gland after IPR therapy.

Last but not least, studies have shown that IPR inhibits glucose transport (Taylor and Halperin, 1979; Kashiwagi et al., 1983) and even the rates of glycolysis (Kako, 1965; Ureta et al., 1970; Seth et al., 1980; Harris and Mackenzie, 1981; Mills et al., 1984). It implies from these reports that IPR therapy employed herein would have inhibited glucose uptake by the preputial acini, in addition to decreased rates of glycolysis. When these reports are considered in the light of above cited mitochondrial interactions and cellular responses challenged with chronic doses of IPR, it becomes a bit easier to understand the presently observed responses exhibited by the preputial gland inflicted by isoproterenol.