CHAPTER : 5

NEUROENDOCRINE REGULATION OF CERTAIN METABOLIC FEATURES OF THE RAT PREPUTIAL GLAND

There have been numerous studies on laboratory mammals and humanbeings to demonstrate that androgenic steroids are potent stimulators of growth of sebaceous glands as well as the rate of sebum production (De Graaf, 1943; Hamilton and Montagna, 1950; Haskin et al., 1953; Lasher et al., 1954; Ebling and Skinner, 1967; Pochi and Strauss, 1974; Shuster and Thody, 1974; Plewig and Lunderschmidt, 1977; Hall et al., 1983; Strauss et al., 1983). Prompt reduction in the levels of sebum production in rats, men and other animal models after surgical androgen deprivation has also been reported (Emanuel, 1936; Pochi and Strauss, 1969; Thody and Shuster, 1970 a; Yee, 1981). Even antiandrogen treatments have been shown to reduce sebum production rates (Ebling, 1973; Lunderschmidt and Plewig, 1977; Ebling et al., 1981; Taam et al., 1982). Thus, no contradictions need be entertained regarding the unequivocal influence of the gonadal androgens in the maintenance of growth as well as secretory activity of sebaceous glands. Adrenal glands are known to be another significant source of androgens in the absence of gonadal steroids (Howard and Kitay, 1972; Sanford et al., 1978; Huseby and Dominiquez, 1979; Peczely and Daniel, 1979; Santen et al., 1980). Indeed, a postive effect of many adrenal steroids on growth and sebum production of

mammalian sebaceous glands has been reported; including dehydroepiandrosterone (DHEA) and androstenedione (Kim and Hermann, 1968; Chakraborty et al., 1970; Faredin et al., 1970; Ramasastry et al., 1970; Hay and Hodgins, 1973; Ebling, 1974; Shuster and Thody, 1974; Cooper et al., 1976; Pochi et al., 1977; Agache and Blanc, 1982; Hsia et al., 1983; Marynick et al., 1983; Sawaya et al., 1984). Further, adrenalectomy of intact and castrated mammals, including men, has been found to result in substantial atrophy of sebaceous glands (Lorincs, 1963) and reduction in the rate of sebum production (Ebling et al., 1970; Thody and Shuster, 1970 a, 1971 a; Pochi and Strauss, 1974).

Most of the above mentioned reports dealt with steroidal influences over either the growth of sebaceous gland, sebum excretion rate (SER) and/or rates of lipogenesis. Nature and regulation of energy metabolism in the cutaneous components has been reported in special articles (Decker, 1971; Wheatley, 1974). However, barring few reports (Falls et al., 1960; Freinkel, 1960; Adachi and Yamasawa, 1967; Mier et al., 1967; Takayasu and Adachi, 1970; Ziboh et al., 1970; Michael and Hoopes, 1974), little is known about the quantitation of metabolic pathways in sebaceous gland or its analogues making provisions for energy demands.

While endocrine regulation of sebaceous gland or its analogues is fairly well exploited, practically only a hazy image seems to crop out of the literature relevant to neuronal regulation of functions of sebaceous analogues (Cerutti, 1934;

Starling, 1936; Serrati, 1938; Melczer and Deme, 1942; Miescher and Schonberg, 1944; Nexmand, 1944; Savill, 1944; Hodgson-Jones et al., 1952; Rothman and Herrmann, 1953; Kligman and Shelley, 1958; Harville, 1971; Burton et al., 1973; Burton and Shuster, 1973; Wheatley and Brind, 1981). Combined histological (Vyas, 1978), histochemical (Ambadkar and Vyas, 1982), quantitative (Ambadkar and Vyas, 1981b) as well as in-vitro studies (Ambadkar and Vyas, 1980) have provided clues to the effect that preputial lipogenesis is well dominated by neuronal elements. However, it is not known as to what extent such elements are involved in the regulation of metabolic pathways making provisions for energy demands of the preputial gland. Hence, a study was undertaken to deal with this issue.

Accordingly, two key enzymes of oxidative metabolism, succinic dehydrogenase (Succinate: (acceptor) oxidoreductase EC 1.3.9.9) and Mg^{++} ATPase (ATP phosphohydrolase EC 3.6.1.3) were studied in the preputial glands of male rats after different endocrine manipulations and replacement therapy. Concomitantly, the activity levels of Na^{+}-K^{+} ATPase (EC 3.6.1.4) and fructose 1,6 diphosphate aldolase (EC 4.1.2.13) were also assessed in the preputial glands after different endocrine regimes as outline below.

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 (Adx) was performed via dorsolateral route, together with castration. Animals were kept under standard laboratory conditions with food and water provided ad libitum. Adx-castrates (Adx-C) were provided with glucose saline instead of plain drinking water. Castrated (C) and Adx-C rats were sacrificed at intervals of 24, 48 and 120 hours post-operatively. Some of the 120 hours C were treated with single intramuscular injections of 0.1 mg per head testosterone propionate (TP) dissolved in 0.5 ml of tributyrin.

Isoproterenel therapy was performed in yet another group of male rats as has been described in first chapter.

For quantitative assays the glands were weighed and homogenized in chilled distilled water. Succinic dehydrogenase (SDH) was measured quantitatively according to the method of Kun and Abood (1949). Enzyme activity was expressed as µg formazan formed/mg protein/30 min.

Quantitative estimation of ATPase was carried out employing the method as described by Umbreit et al., (1957). Inorganic phosphate released was estimated by the method of Fiske and SubbaRow (1925). Readings were taken at 660 µ m on Klett-Summerson photoelectric colorimeter. The enzyme activity has been expressed as mg of phosphorus released / 100 mg protein/10 min.

FDP-aldolase activity was estimated quantitatively employing the method described by Bruns and Bergmeyer (1963). Triose phosphates liberated were trapped by 2,4 dinitrophenyl hydrazine-HCL. The readings were taken at 540 μm on Klett-Summerson photoelectric colorimeter. The enzyme activity has been expressed as μ mol. of FDP cleaved/mg protein/1 hr.

OBSERVATIONS

As was apparent from the values represented in Table 1, it could be seen that 24 hours after castration, the activity levels of all four enzymes were perceptibly reduced (maximum in case of Na^+-K^+ - ATPase) whereas there were no signs of any variation after 24 hours of castration-adrenalectomy. 48 hours after castration, however, recovery was apparent, least being in case of Na^+ K^+-ATPase. In the case of 48 hours Adx-C, except for Na^+ K^+-ATPase the enzyme activity levels remained almost normal. Both castration and adx-castration were seen to reduce all parametric values significantly by 120 hours. An important point that emerged was that in case of Na^+K^+ - ATPase and SDH, the effects of two operations were seemingly additive. Replacement of hormone in case of 120 hours C was almost completely reparative.

Chronic IPR treatment was found to lead to reduction to the tune of 1/3 or more in case of all the four enzyme activities. The least affected was Na^+ K^+ - ATPase and the maximally affected was SDH.

TABLE : 1

ACTIVITY LEVELS OF CERTAIN ENZYMES IN THE PREPUTIAL GLAND FROM THE NORMAL, C, Adx-C, TP INJECTED AND IPR-TREATED MALE RATS. MEAN VALUE ± S.D.

Exp. Group	Mg^{++}ATPase	Na^{+}- K^{+}ATPase	SDH	FDP-Aldolase
Normal Animals	1.06 ± 0.17	1.24 ± 0.27	19.58 ± 2.47	0.44 ± 0.08
24 hrs C	0.87 ± 0.34	0.63 ± 0.23**	16.47 ± 2.44	0.37 ± 0.05
48 hrs C	1.009 ± 0.29	0.79 ± 0.2**	23.81 ± 3.5	0.42 ± 0.06
120 hrs C	0.31 ± 0.07**	0.36 ± 0.11**	16.85 ± 2.5*	0.31 ± 0.08**
24 hrs Adx-C	1.04 ± 0.25	1.19 ± 0.25	19.43 ± 2.57	0.47 ± 0.12
48 hrs Adx-C	1.22 ± 0.18	0.83 ± 0.12**	23.74 ± 4.13	0.41 ± 0.09
120 hrs Adx-C	0.48 ± 0.16**	0.2 ± 0.04**	10.69 ± 2.24**	0.24 ± 0.06**
120 hrs C 0.1 mg TP inj.	0.98 ± 0.22	1.25 ± 0.07	18.56 ± 1.08	0.42 ± 0.06
IPR-treated animals	0.44 ± 0.09**	0.41 ± 0.09**	9.58 ± 1.15**	0.28 ± 0.06**

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

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

DISCUSSION

Histochemical localization and distribution of SDH has been worked out by a number of investigators in mammalian skin and sebaceous glands as well as their analogues (Formisano and Montagna, 1954; Montagna and Formisano, 1955; Argyris, 1956a and 1956b; Buno and Germino, 1958; Hashimoto et al., 1962; Montagna, 1963; Michael, 1965; Marois and Salesses, 1967; Michael and Hoopes, 1974; Santos et al., 1974; Wilfried and Neurand, 1977a; Ambadkar and Vyas, 1979; Wilfried and Neurand, 1979a). On the contrary, reports regarding ATPase activity levels in skin (Tabachnick and Perlish, 1967; Moynahan et al., 1972; Mahrle and Orfanos, 1975a) and sebaceous gland (Santos et al., 1974) are scarce. One of the primary mechanisms underlying the influence of steroid hormones on their respective target organs involves an alteration of the rate of transport of substances across the plasma membranes (Fleischmann and Fleischmann, 1952 - 1953; Metcalf and Gross, 1960; Novikoff et al., 1962, Riggs and Wegrzyn, 1966; Mills and Spaziani, 1968; Klein and Boyer, 1972; Simoni and Shallenberger, 1972). $Na^+ - K^+$ ATPase has been shown to facilitate the rate of transport of molecules across the plasma membrane against the concentration gradient (Judah and Ahmed, 1964a, Skou, 1965; Fransworth, 1972). Apparently, both Mg^{++} ATPase and Na^+-K^+ ATPase activity levels in the preputial gland of rats would be involved in the overall energy flux.

The enzyme fructose 1,6 diphosphate aldolase has been shown to be of significance in the lipid synthesizing tissues like mammary gland (Abraham and Chaikoff, 1959), adipose tissue (Hollifield and Parson, 1961; Weber et al., 1961), and skin or sebaceous analogues (Hershey, 1959; Falls et al., 1960; Mier and Cotton, 1967; Rovkach, 1967; Halprin and Fukui, 1968; Takayasu and Adachi, 1970; Santos et al., 1974; Sato et al., 1981). Precaution should be taken of not considering the presently mentioned aldolase activity as an index of "uphill" conversion of hexose (Burt, 1966) as the enzyme assaying method of Bruns and Bergmeyer (1963) employed here has been shown to indicate "downhill glycolysis". Seemingly, the enzyme activity levels in the preputial glands of rats give an indication of "downhill" glycolysis. Dihydroxyacetone phosphate could be converted into α-glycerophosphate - an important precursor of triglycerides (Kornberg and Pricer, 1953; Kennedy, 1954). Glyceraldehyde 3-phosphate, yet another triose sugar, could be incorporated into fatty acids (Howard and Lowenstein, 1965).

As is evident from table, 24 hours after castration the activity levels of all the enzymes were perceptibly reduced, maximally being in case of Na^+-K^+ ATPase. 48 hours after castration, however, trend of recovery was apparent, least being in case of Na^+-K^+ ATPase. Perhaps, recovery was achieved due to addition of androgenic steroids from the adrenals; in the absence of gonadal steroidal support. Yet another possibility canot be overlooked wherein 5α-reductase of hamster sebaceous

gland has been shown to increase 250 times after castration (Takayasu and Itami, 1981). In addition, skin and skeletal muscles have been shown to be the significant extragonadal sources of 5α-DHT in castrated rabbits (Booth and Jones, 1980). 5α-DHT thus induced could get involved in the regulation of functions of accessory sex organs in the absence of gonadal steroids. Additionally, the idea of "end organ hypersensitivity" (Lucky, 1987; Reingold and Rosenfield, 1987) may provide yet another plausible explanation to the recovery in the enzyme levels of the gland 48 hours post-castration.

Castration is seen to reduce all parametric values significantly by 120 hrs in the preputial gland. Castration has been repeatedly shown to reduce the state of mitochondrial respiration and the mechanism of active transport in a number of androgen-dependent glands (Edelman et al., 1963; Javery et al., 1963; Irena, 1966; Fransworth, 1968; Ahmed and Williams-Ashman, 1969; Fransworth, 1970a; Doeg et al., 1971 ; Wilson and Villee, 1973; Anja et al., 1977; Sinowatz et al., 1977; Brooks, 1978, 1979) and a sebaceous analogue (Ambadkar and Vyas, 1979). The literature cited here is, thus, helpful in explaining the reduction evident in the activity levels of SDH, Mg^{++} ATPase and $Na^{+}K^{+}$ ATPase in the preputial gland following 120 hours of castration.

Castration has been reported to reduce the rates of certain glycolytic enzymes including aldolase in hamster costovertebral glands (Takayasu and Adachi, 1970) and α-GPDH in rat preputial

gland (Ambadkar and Vyas, 1979). Obviously, presently observed decrements in the preputial FDP-aldolase could be considered as an index of decreased energy metabolism as well as altered rates of lipogenesis (Ambadkar and Vyas, 1975).

When total androgen deprivation was achieved in case of Adx-C, both SDH and Na^+ K^+ ATPase reduced considerably at 120 hours; as compared to the levels obtained 120 hours after castration alone. Obviously, adrenal steroids are somehow involved in the maintenance of respiratory and transport functions in the preputial glands. It is quite pertinent to note here that adrenal steroids have been shown to be involved in the regulation of respiratory and transport functions in a variety of rat tissues including intestinal mucosa (Suzuki, 1981; Suzuki et al., 1983), brain and liver (Liljeroot and Hall, 1965; Yakobson et al., 1971; Yadav and Singh, 1980 a, b), kidney (Jorgenson, 1968; Liu et al., 1972; Geloso and Basset, 1974; Kernich and Liu, 1980), thymus (Deschaux and Guibert, 1981), testis (Vallivullah et al., 1985), heart (Hegyvary, 1977), the rabbit heart (Marver, 1984) and the toad bladder (Kirsten et al., 1968, 1970; Geering et al., 1982) as well as the rabbit kidney (Kelvin et al., 1981). Further, DHEA or its sulphate have been shown to be metabolized by skin and sebaceous analogues to testosterone, DHT and androstenedione (Cameron et al., 1966; Kim and Herrmann, 1968; Oertel and Treiber, 1969; Chakraborty et al., 1970; Faredin et al., 1970; Fazekas and Lanthier, 1971; Fazekas and Sandor, 1973;

Hay and Hodgins, 1978; Hsia et al., 1983; Simpson et al., 1983; Sawaya et al., 1984). The influence of DHEA and DHEAS on sebaceous gland secretory activity has been shown by a number of workers (Ramasastry et al., 1970; Drucker et al., 1972; Sizonenko and Paunier, 1975; Cooper et al., 1976; de Peretti and Forest, 1976; Pochi et al., 1977; Agache and Blanc, 1982; Marynick et al., 1983). Thus, literature cited here lends support to the assumption that adrenal steroids regulate the overall metabolic patterns of the preputial gland which are ultimately reflected in reduced levels of SDH, aldolase, Mg^{++} ATPase and Na^{+} K^{+} - ATPase 120 hours post operatively among Adx-C.

The replacement therapy of 120 hours C with TP could bring the values of all the enzymes in the gland back to normal levels. Bulk of literature available has shown the influence of androgens on functional changes in various androgen-responsive organs (Jakubovic and Cekan, 1967; Brooks, 1976a; Anja et al., 1977; Engel et al., 1980; Pirkko, 1981; Ambadkar and Gangaramani, 1982b) including sebaceous gland or its analogues (Ebling, 1963; Strauss and Pochi, 1963; Ebling et al., 1971; Pochi and Strauss, 1974; Shuster and Thody, 1974; Plewig and Lunderschmidt, 1977; Hall et al., 1983; Abalain et al., 1984). In the light of the literature cited here and the results obtained, it is obvious that harmone replacement in case of 120 hours C did lead to reversal of castration effects in respect of all the parameters investigated.

IPR is a well known β-andrenergic agonist. Chronic IPR therapy would have affected the endogenous metabolism of the androgen-responsive preputial gland in an indirect way as has been discussed in the first chapter. Alternatively, it would have affected the gland metabolism directly. IPR administration resulted in a drastic fall in the activity levels of all the enzymes in the preputial gland. Comparatively, the reduction was maximum in case of SDH, whereas ATPases were affected to a lesser extent. IPR induced Ca^{++} 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 the mitochondrial respiration (Lehninger et al., 1967; Kutsuna, 1972; Nirdlinger and Bramhate, 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). Thus, probable altered mitochondrial oxidative phosphorylation in the preputial of IPR treated rats could be reflected in reduced activity levels of ATPases and SDH (Table 1). IPR therapy has been shown to reduce the activity levels of various ATPases in different tissues (Matsui and Schwartz, 1966; Gordon et al., 1972; Pelouch et al., 1973; Margaret et al., 1974; Pagano and Inchiosa, 1977, 1979; Takeo

et al., 1980; Sistare et al., 1982; Hettinger and Horwitz, 1983; Shimizu et al., 1985) and also to reduce the SDH activity levels (Ohlin, 1966; Tomlish and Eversole, 1972; Margaret et al., 1974; Cope et al., 1976; Woolf et al., 1976, 1977; Balakleevsii and Vismont, 1979; Steen et al., 1979; Siess and Weiland, 1980; Grieve and Williams, 1981; Mitova et al., 1983). It is conceivable from these reports that IPR therpay reduces the tone of oxidative phosphorylation and the process of active transport. This is, perhaps, reflected in reduced activity levels of both ATPases and SDH in the preputial gland.

It is evident from table 1 that IPR therapy significantly reduced the aldolase level in the preputial glands. The observation is complimentary to the decreased activities of certain lipogenic enzymes which were shown to be associated with decreased total lipid content of the gland (Chapter : 2). IPR therapy has been shown to be implicated in control of certain metabolic features which ultimately could be expected to reduce the overall rates of glycolysis in the preputial gland. IPR administration has been shown to be involved in the regulation of the rate of glycolysis (Kako, 1965; Ureta et al., 1970; Seth et al., 1980; Harris and Mackenzie, 1981; Mills et al., 1984; Bora et al., 1985) by decreasing the rate of entry of glucose into the tissue cells (Taylor and Halperin, 1979; Kashiwagi et al., 1983). Obviously, presently observed decrements in the aldolase activity after the drug therapy is in agreement with a glycolysis inhibitory role assigned to IPR therapy.

An interesting corollary needs to be cited here in support of above contention. It has been observed during the course of present work that IPR therapy resulted into increase in cellular cAMP content by inhibiting cAMP-phosphodiesterase (as discussed in Chapter : 7). As has been shown by Ureta et al.,(1970), raised intracellular levels of cAMP lead to suppression of rates of glycolysis. It implies from these observations that IPR therapy employed herein thus, exerted castration like effects on the rates of glycolysis, transport phenomenon and oxidative metabolism in the preputial glands and finds convincing support in the work done previously (Vyas, 1978; Ambadkar and Vyas, 1981b, 1982) and in the work done during the present course of investigation (Chapter 2, 3 and 4).