CHAPTER : 8

RESPONSES EXHIBITED BY THE RAT PREPUTIAL GLAND PROTEIN METABOLISM TOWARDS CERTAIN NEUROENDOCRINE MANIPULATIONS

Lipogenesis, a unique biosynthetic potential acquired by mammalian sebaceous glands or its analogues, has turned out to be a major theme of scientific curiosity for skin biologists. A voluminous body of literature concerning sebaceous lipogenesis is available (Chapter : 2). Protein, yet another important constituent of sebaceous secretion, has unfortunately been overlooked by the workers. Only some reports are available on the skin and/or sebaceous gland protein metabolism (Beaver, 1960; Dangelo and Munger, 1964; Giegel et al., 1971; Sherins and Bardin, 1971; Ozegovic and Milkovic, 1972; Potter et al., 1979; Wheatley et al., 1979; Curtis and Cowden, 1980; Mesquita and Coimbra, 1981; Bladon et al., 1984; Masaaki et al., 1984; Baldon et al., 1985). Surprisingly enough, hormonal influences on protein metabolism of sebaceous glands have been centered mostly on preputial glands (Giegel et al., 1971; Sherins and Bardin, 1971; Ozegovic and Milkovic, 1972; Jose and Coimbra, 1981).

To be more precise, adrenal steroids have been implicated in inhibition of protein synthesis in a sebaceous analogue (Milkovic et al.; 1964; Ozegovic and Milkovic, 1972) and also in a variety of tissues including liver (Breuer and Frank, 1964; Mishunina and Mestechkina, 1981), diaphragms (Weinshelbaum and

Wool, 1961) and muscles (Kostyo and Redmond, 1966; Millward et al., 1976a, Rannels et al., 1978; Odedra and Millward, 1982). Perhaps, this inhibitory influence of adrenal steroids on protein synthesis is perplexing since chemically closely related androgenic steroids have been well documented for anabolic action (Kochakian 1964; Mainwaring and Wilee, 1972; Liang and Liao, 1975; Rajalakshmi and Prasad, 1976). Hence, it would be worthwhile to look into this matter by evaluating protein content of the preputial glands of castrates vis a vis adx castrates.

Barring few reports (Borghi, 1940; Velick and English, 1945; Wheatley et al., 1961; Lipkin et al., 1965; Wheatley et al., 1967; Grigor et al., 1970; Wheatley et al., 1971; Im and Hoopes, 1974; Peters and White, 1976; Gumenyuk et al., 1979) hardly any attempts appear to have been made in correlating the role of amino acids concerning alterations of protein, lipid and carbohydrate metabolism in skin components. Amino acids in skin have been analysed (Satsangi et al., 1961; Melancon et al., 1972; Razmakhnin et al., 1979). Metabolism of amino acids in sebaceous glands as related to transaminase activity levels in skin derivatives has not been adequately studied (Velick and English, 1945; Tickner et al., 1961; Hassan and El-Hawary, 1963; Adachi et al., 1967; Im and Hoopes, 1974; Peters and White, 1976; Raab and Gmeiner, 1976; Dikshith et al., 1978). Similarly hormonal influences on transaminase activity levels in skin components have never been known to be dealt with in desirable details earlier. Hence, an attempt has been made here in this direction by

evaluating the responses exhibited by transaminases and total protein content of the preputial glands under the influence of neuroendocrine manipulations. Keeping above ideas into consideration, two key enzymes of amino acid metabolism, glutamate-oxaloacetate-transaminase (Aspartate aminotransferase - GOT:EC 2.6.1.1) and glutamate-pyruvate-transaminase (Alanine aminotransferase - GPT :EC 2.6.1.2) of the preputial glands of male rats were studied after different neuroendocrine manipulations as outlined in methodology. Total protein content was assayed quantitatively in both preputial glands and the sebum as well.

MATERIALS AND METHODS

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

For enzyme assay of transaminases the glands were homogenized in chilled distilled water and processed for quantitation as per method described in Sigma Technical Bulletin No. 505. GOT activity is expressed as Karmen units/mg protein/60 minutes. GPT activity is expressed as Karmen units/mg protein/30 minutes.

The sebum was gently squeezed out of the preputial gland on coverslip. Protein content was estimated in the tissue as well as the sebum by colorimetric micromethod of Lowry et al., (1951).

OBSERVATIONS

Total protein content :

Preputial Gland : Castration alone gradually reduced the protein

content of the preputial gland. Contrary to this, simultaneous adrenalectomy of castrates increased the total protein content of the gland significantly. TP therapy showed measurable increments in the total protein content as compared to 120 hours C. However, IPR treatment to male albino rats reduced the preputial gland total protein content to a significant extent.

Sebum : Castration reduced the total protein content of the sebum to a significant level 120 hours postoperatively. However, adx of castrates increased the protein level significantly. Replacement therapy of 120 hours C with TP increased the total protein content to a significant extent. However, IPR therapy to male albino rat didn't reduce the protein content to any significant extent.

Transaminase levels :

Preputial gland of the normal animals exhibited high GOT activity. The enzyme activity showed a significant increase 48 hours postcastration. However, it decreased to normal level 120 hours postoperatively. Simultaneous adrenalectomy of castrates increased the enzyme activity 120 hours postoperatively. Replacement therapy of 120 hours castrates with TP could bring the values back to normal levels. The drug therapy showed significant elevations of the enzyme activity.

GPT activity was not much affected by endocrine manipulations. However, neuronal manipulation with IPR increased the GPT levels to a significant extent.

TABLE : I

QUANTITATIVE LEVELS OF TRANSAMINASES AND PROTEIN CONTENT IN THE PREPUTIAL GLAND FROM THE NORMAL, C , Adx-C, TP INJECTED AND IPR-TREATED MALE ALBINO RATS. GOT ACTIVITY IS EXPRESSED AS KARMEN UNITS/MG PROTEIN/60 MINUTES. GPT ACTIVITY IS EXPRESSED AS KARMEN UNIT/MG PROTEIN/30 MINUTES. THE AMOUNT OF PROTEIN IS EXPRESSED AS MG PROTEIN/100 MG OF FRESH TISSUE OR SEBUM WEIGHT AS THE CASE MAY BE.

Mean Value ± S.D.

Experimental group	Protein in the gland	Protein in the sebum	GOT	GPT
Normal	37.71 ± 7.34	52.7 ± 4.6	25.82 ± 5.77	29.82 ± 9.62
24 hrs C	35.2 ± 3.5	44.8 ± 5.8*	31.4 ± 8.1	27.3 ± 8.2
48 hrs C	35.2 ± 5.2	52.6 ± 3.5	36.5 ± 10.1*	30.05 ± 8.1
120 hrs C	31.3 ± 5.2	44.5 ± 6.5*	27.2 ± 7.05	28.6 ± 7.4
24 hrs Adx-C	48.69 ± 5.8*	65.06 ± 10.35*	39.4 ± 9.04*	37.9 ± 8.8*
48 hrs Adx-C	49.5 ± 9.9*	65.08 ± 7.85*	33.2 ± 9.2	36.5 ± 9.5
120 hrs Adx-C	53.84 ± 7.6*	70.05 ± 4.3*	43.2 ± 10.3*	33.3 ± 7.07
120 hrs C, TP injected animals	50.08 ± 8.9*	68.01 ± 12.5*	31.4 ± 6.08	32.08 ± 7.8
IPR treated animals	20.7 ± 3.2*	50.06 ± 7.6	65.1 ± 16.1*	43.1 ± 10.2*

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

DISCUSSION

Results obtained indicate that preputial glands and its secretory products in male rats are rich in total protein content. It is apparent that protein constitutes the major component of sebum. The protein levels in the gland decreased to a non-significant extent 120 hours post-castration. However, the total protein content of sebum decreased significantly 120 hours post-operatively. Thus, when the male rats are deprived of the substantial androgenic pool, the protein anabolic activities of the preputial glands received a major set-back. This observation gains support from the literature (Giegel et al., 1971; Sherins and Bardin, 1971). Contrary to this, replacement therapy of 120 hours castrates with TP raised the total protein content of both the gland and sebum as well. It is pertinent to note here that replacement therapy with TP has been shown to induce protein anabolic action in sebaceous analogues (Giegel et al., 1971; Sherins and Bardin, 1971; Mesquita and Coimbra, 1981).

Logically, total androgen deprivation among Adx-C should have reduced the total protein content of the preputial gland because of well documented anabolic action of male sex hormones (Kochakian, 1964; Mainwaring and Wilee, 1972; Liang and Liao, 1975; Rajalakshmi and Prasad, 1976). Surprisingly, the outcome challenges the assumption as is evident from the table. Indeed, adx of female rat has been shown to increase the total protein

content of the preputial glands and the replacement therapy with adrenal steroids has been shown to reverse the effects (Ozegovic and Milkovic, 1972). In addition, rat liver polyribosomes of four day adrenalectomized animals have been reported to exhibit a greater amino acid incorporating ability than unoperated controls (Breuer and Frank, 1964); glucocorticoid treatment decreased this incorporation. Similarly, adx has been shown to increase (2-^{14}C) histidine incorporation into protein fractions of rat diaphragms and cortisone therapy has been shown to decrease this incorporation (Wool and Weinshelbaum, 1959). These workers repeated their observation using heart slices and received the same results as mentioned above (Weinshelbaum and Wool, 1961). Adx has been shown to increase ^{14}-C alanine incorporation into proteins of brain, muscle, blood plasma and liver of rabbit (Mishunina and Mestechkina, 1981).

At this juncture, many possibilities could be taken into consideration to explain the increments met within the total protein content of both sebum and preputial glands after adx of castrated male rats. It is clearly evident from the literature cited above and the observation (Table : 1) that some of the adrenal elements might have inhibitory influence over protein synthesis in the preputial glands. Infact, corticosterone is shown to exert a net catabolic effect on skeletal muscle protein. An indisputed part of this action is the hormone's powerful suppression of protein synthesis (Kostyo and Redmond, 1966; Millward et al., 1976a; Rannels et al., 1978; Odedra and

Millward, 1982). Corticoids have also been shown to exert an inhibitory effect on preputial gland growth (Milkovic et al., 1964). It implies from these reports that the adrenal steroids among castrates would have suppressed protein synthesis in the preputial gland; when the adrenal steroidal support is removed among adx-castrates, a spurt would have occurred in the protein synthetic capacity of the preputial gland which could be reflected in the increased total protein content of the gland and the sebum (Table : 1).

Alternatively, a more plausible explanation concerning increased total protein content of the preputial gland and the sebum among adx-castrates (Table : 1) has been offered (Yip and Frienkel, 1964; Ozegovic and Milkovic, 1972). In these studies, ACTH has been shown to exert a direct stimulatory effect on preputial growth; additionally, ACTH has also been shown to exert an inhibitory effect through adrenals as shown by huge weight and protein level increases in the preputial gland of adx animals.

Yet another possibility cannot be ruled out wherein 5 α-reductase of hamster sebaceous gland has been shown to increase by 250 times after castration (Takayasu and Itami, 1981). In addition, skin and skeletal muscles have been shown to be significant extragonadal sources of 5α-DHT in castrated rabbits (Booth and Jones, 1980). Such increments in 5α-DHT content of skin could have been extended, perhaps, even in the case of adx-castrates. 5 α-DHT, thus, produced has been shown to be

involved in the restoration of secretory activities of sex accessory glands (Sherins and Bardin, 1971; Takayasu and Adachi, 1972; Dube et al., 1975). Additionally, androgens could be produced in skin (Baird et al., 1969; Kirschner et al., 1973 and Givens, 1978) even in absence of steroidal support in case of adx-C rats. These reports also could explain the increase observed in the preputial gland protein content after total androgen deprivation.

Last but not least, sebaceous gland, its analogues and their secretory products have been reported (Satsangi et al., 1961; Melancon et al., 1972; Razmakhnin et al., 1979; our unpublished observation) to have a large many free amino acids which could have been produced as a result of holocrine secretory mode. It is pertinent to note here that the final color obtained in protein estimation is not totally attributable to proteins only since free amino acids like tryptophan and tyrosine have been reported to develop color even in the presence of copper (Lowry et al., 1951). A possibility, therefore, cannot be overruled wherein adx of castrated rats could result in highly elevated levels of free amino acids in the gland and sebum. Probably, elevated levels of free amino acids could impart rich color to the final reaction product which may give higher values of total protein content. Obviously, this phenomenon may divert us to interprete the result wrongly. Currently, the work is in progress to unfold this issue by measuring free amino acids in the gland and sebum during different endocrine regimes as stated in methodology.

As is evident from the table, chronic IPR therapy to male rats decreased the preputial gland total protein content to a significant extent. It is important to note here that IPR therapy has been reported to depress ^{14}-C amino acid incorporation into protein of mouse epidermis (Harris and Mackenzie, 1981). IPR and cAMP have been shown to decrease collagen production by subcultured bovine corneal fibroblasts (Chao and Walkenbach, 1986). In addition, chronic IPR administration has been shown to depress protein synthesis in muscles (Jefferson et al., 1972). Even catecholamines as a whole have been reported to inhibit protein synthesis in a number of isolated tissues (Himms-Hagen, 1972). IPR therapy is shown to increase the levels of intracellular cAMP (Chapter : 7). cAMP in its turn has been shown to inhibit amino acid incorporation by rat liver ribosomes (Monier et al., 1972). It is clear from these observations that IPR must have evoked certain biochemical changes in the preputial gland which ultimately could have resulted in diminished protein content of the gland.

As is apparent from Chapter : 1, chronic IPR therapy to male rats could be expected to diminish circulating levels of testosterone which ultimately could be speculated to decrease the total protein content of the gland as influenced by chronic IPR therapy.

An interesting point that draws attention here is the nonsignificant change observed in the total protein content of the sebum after chronic IPR therapy as compared to normal values. It could be presumed here that the sebum of the gland would not

have been extruded to the exterior during the period of the drug therapy. The proteins in such sebum would have given normal values, despite of decreased levels of protein content in the preputial gland after chronic IPR therapy.

Transaminases are a group of enzymes widely distributed in living systems bringing about transfer of amino groups to α-keto acids generating the corresponding amino acid. GOT and GPT are reportedly capable of acting on almost all amino acids. This broad spectrum activity of these two amino transferase have earned them the status of major transaminases of animal tissues. Both GOT and GPT by their strategic positioning in the metabolic pathway, at the levels of oxaloacetate and pyruvate formations are pivotal in linking the metabolism of carbohydrates and lipids with that of proteins. Indeed,labelled amino acids have been shown to be incorporated into lipid components of skin derivatives (Velick and English, 1945; Bachhawat et al., 1955; Wheatley et al., 1961; Lipkin et al., 1965; Wheatley et al., 1967; Grigor et al., 1970; Wheatley et al., 1971; Kealey et al., 1986) and mammary gland (Abraham et al., 1964; Vina and Williamson, 1981a and 1981b). High transaminases in sebaceous glands (Adachi et al., 1967) have been attributed to lipid synthesis (Im and Hoopes, 1974). On the other hand, alanine has been shown to be converted into pyruvate (Peters and White, 1976) or vice-versa (Gumenyuk et al., 1979) in skin. Thus, a gluconeogenic function could be assigned to skin through mediation of transaminases. At the same time, amino acids have been shown to be incorporated into sebaceous

proteins (Giegel et al., 1971; Peters and White, 1976; Wheatley et al., 1979).

Thus, normal preputial glands depicting high values of both the transaminases could be expected to favour protein synthesis to a greater extent since carbohydrate supply is not a limiting factor. So when anaerobiosis is predominant, the increasing pool of pyruvate could be transaminated by GPT to alanine and thence to the alanine family of amino acids. During aerobiosis, oxaloacetate molecules generated through TCA cycle could be effectively transaminated by GOT to yield aspartate and thence to aspartate family of amino acids. Amino acids thus formed could favour protein synthesis. However, the possibility of incorporation of branched chain amino acids into fatty acids can not be ruled out as discussed in above paragraph. This feat, perhaps, could be achieved by means of transaminases only. This issue of the extent of contribution of transaminase reactions towards either lipogenesis or protein synthesis, thus, warrants further investigation. Even the gluconeogenic function could be assigned to the preputial gland of normal rats.

Castration is not seen to bring about significant change in the activity levels of both the transaminases in the gland 120 hours post operatively (Table : 1). In the view of significantly decreased lipid content (Ambadkar and Vyas, 1975) and a nonsignificant decrement in the total protein content (Table : 1) of the preputial glands at 120 hours post-operatively, persistently normal values of both the transaminases in the gland hint at ongoing protein synthesis.

Adrenalectomy of castrates brought about elevations of both the transaminases in the gland 120 hours post-operatively. Once again recalling the decreased lipid content of the gland at 120 hours post-operatively (Ambadkar and Vyas, 1975), elevated levels of both the transaminases in the gland could be expected to favour protein synthesis. In fact, in the view of decreased ($^{14}C_3$) alanine incorporation into plasma glucose following adrenalectomy of rats (Dunn et al., 1969), elevated GPT levels in the preputial gland of adx-castrates could construed to favour alanine formation and hence protein synthesis. Moreover, adx has been shown to be ineffective in diminishing aspartate incorporation into glucose (Dunn et al., 1971). Keeping the possibility of gluconeogenesis aside in the gland, elevated GOT levels could be expected to favour aspartate formation and hence protein synthesis. Glucocorticoids have been shown to enhance protein catabolism by releasing amino acids from peripheral tissues (Long et al., 1940; Smith and Long, 1967). Obviously, protein anabolism could be expected to occur in the peripheral tissues in absence of adrenal steroid support as discussed earlier. Presently observed spurt in transaminases of preputial glands of adx-castrates, thus, could be an indication of protein anabolism and not proteolysis.

TP replacement therapy to 120 hours castrates elevated the activity levels of transaminases in the gland as compared to 120 hours castrates. Such elevated transaminases level could be

an indication of protein synthesis. Pertinent here is to note the stimulatory influence of testosterone therapy on the GOT levels of the rat ventral prostate (Franklin et al., 1982).

Chronic IPR therapy to male rats elevated the transaminase levels in the gland to a significant extent as compared to normal values with a co-ordinate decrement in the total protein content of the gland; indicating, thereby, either a clear-cut inhibition of protein synthesis or an elevated proteolytic effect in the gland. Chronic IPR therapy is shown to decrease total lipid content of the preputial gland (Chapter : 2). Hence, elevated transaminases level in the gland after such a therapy can not be attributed to lipid synthesis. IPR therapy is shown to decrease alanine level in muscle perfusate (Li and Jefferson, 1977). Catecholamines as a whole have been shown to decrease the circulating amino acid pool (Luck and Morse, 1933; Griffin et al., 1954). Chronic IPR therapy, a sort of physiological stress, employed in the present investigation could be expected to deplete circulating amino acid pool. Knox and Greengard (1965) have shown stress to elevate amino transferase levels. Elevated amino transferase levels in the preputial gland after chronic exposure of male rats to IPR corroborates well with reduction in the total protein content of the gland; these facts could be suggestive of proteolysis, possibly to compensate for the decreased circulating amino acid pool.