CHAPTER : 7

A STUDY OF CERTAIN NEUROENDOCRINE MANIPULATIONS ON CYCLIC AMP PHOSPHODIESTERASE LEVELS IN THE RAT PREPUTIAL GLAND

Since the discovery of cyclic 3', 5'-adenosine monophosphate (cAMP) by Sutherland and his colleagues (Rall et al., 1957), it has gained wide recognition as an essential link in the chain of events that constitutes specific cellular responses at molecular levels to various endocrine stimuli that impinge upon the cell in vivo. An authoritative account of this important area of investigation may be found in a review article by Robison and Sutherland (1972). Normal expressions of a variety of hormonal effects which depend on cAMP have been shown to get facilitated in the presence of steroids (Maickel et al., 1967; Senft et al., 1968; Handler et al., 1969; Shaeffer et al., 1969; Zepp and Thomas, 1976; Lee and Reed, 1977; Rousseau, 1977; Fernandez and Saggerson, 1978; Zepp and Thomas, 1978; Tolone et al., 1979; Marone et al., 1980; Durant et al., 1983; Elks et al., 1983). Androgens have been shown to facilitate activities of many enzymes involved in carbohydrate metabolism, by increasing cAMP levels in the accessory sex organs (Singhal and Valadares, 1968; Singhal et al., 1968; Santti and Villee, 1971; Singhal et al., 1971 and Mangan et al., 1973). Similar enzymatic stimulation is also shown to occur in the uterus by administration of estrogen (Barker and Warren, 1966;

Hilf et al., 1972). These studies generally have shown steroids to stimulate adenylate cyclase levels within the cells. On the other hand, there are reports which show that androgens do not stimulate adenylate cyclase levels (Rosenfeld and O'Malley, 1970; Liao et al., 1971; Mangan et al., 1973; Sanborn et al., 1980). It, therefore, appears likely that the enzymic activity level of cAMP-specific phosphodiesterase (cAMP-PDE) is a crucial factor vis a vis detectable quantities of intracellular levels of cAMP at a given time and hence in the steroidal modulations of cellular functions (Djoseland et al., 1980); especially when stimulation of adenylate cyclase activity by a hormone is a doubtful candidate for being considered in the intricate mechanism of steroidal influences on cellular responses.

The investigation initiated in this laboratory concerning response of the preputial gland to some neurodynamic agents left certain doubts unresolved. One of such unanswered questions relates to probability of elevation of cAMP levels of the gland in response to IPR therapy (Vyas, 1978, Ambadkar and Vyas, 1981b, 1982). Intracellular levels of cAMP are dependent upon the intricate balance between the activity of adenylate cyclase, which synthesizes cAMP and the degradative enzyme cAMP-specific phosphodiesterase. At any given time the prevailing intracellular concentration of cAMP in any tissue will depend on the activity of cAMP-PDE (Butcher and Sutherland, 1962). Taking this fact into consideration, it was thought desirable to assay alterations in cAMP-PDE activity in the preputial gland as influenced by various endocrine manipulations.

MATERIALS AND METHODS

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

For quantitative assay, the preputial glands were weighed and homogenized in chilled distilled water. The activity of cAMP-PDE was estimated according to the method of Butcher and Sutherland (1962). The inorganic phosphate thus released due to enzymatic action was estimated by the method of Fiske and SubbaRow (1925). The readings were taken on Spectronic-20 at 720 μm. The enzyme activity is expressed as μg P released/mg protein/30 minutes.

OBSERVATIONS

Table I shows that the removal of testis increased cAMP-PDE activity significantly in the preputial gland by 24 hours post-operatively. The levels remained high upto 120 hours post-castration. Total androgen deprivation by means of simultaneous adrenalectomy of castrates was observed to hold the cAMP-PDE level close to that of normal at 24 hours, but at later two intervals, it was similar to that of castrates alone. However, replacement therapy of 120 hours castrates with 0.1 mg TP could bring the values back to normal levels.

IPR therapy decreased the enzyme activity to a significant extent as compared to normal values.

TABLE : I

ACTIVITY LEVELS OF cAMP-PDE IN THE PREPUTIAL GLAND FROM NORMAL, C, Adx-C, TP-INJECTED AND IPR-TREATED MALE RATS. THE ENZYME ACTIVITY IS EXPRESSED AS μg OF PHOSPHORUS RELEASED/MG PROTEIN/30 MINUTES. THE VALUES GIVEN HERE REPRESENT MEAN OF AT LEAST 12 REPLICATES.

Mean Value ± S.D.

Experimental Group	cAMP-PDE
Normal Animals	1.61 ± 0.42
24 hrs C	2.12 ± 0.43*
48 hrs C	2.46 ± 0.71*
120 hrs C	3.01 ± 0.69*
24 hrs Adx-C	1.79 ± 0.42
48 hrs Adx-C	2.12 ± 0.54*
120 hrs Adx-C	2.6 ± 0.59*
120 hrs C, 0.1 mg TP injected	1.72 ± 0.23
IPR-treated rats	0.89 ± 0.18*

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

DISCUSSION

Several workers have already reported on the distribution and localization of cAMP-PDE in skin components (Zaruba et al., 1967, 1969; Holla et al., 1972; Mier and Urselmann, 1972; Harkonen et al., 1974; King et al., 1974; Mahrle and Orfanos, 1976; Iizuka et al., 1978; Miyagawa and Eguchi, 1981). These studies are centered mostly around the enzyme responses of the skin components during various skin disorders.

As is apparent from Table No. 1, PDE activity in the preputial gland showed initial increments attaining significantly high levels among the 120 hours castrates. However, the replacement therapy with TP could bring the values back to normal levels. Similar response has been reported in case of epididymis and prostate (Holtz et al., 1981). In addition, Sanborn et al., (1980) and Holtz et al., (1981) have also shown presence of a variety of cAMP-PDE inhibitory modulator proteins responsive to androgens. Djoseland and workers (1980) have shown that variation in PDE activity is not the only specific response to androgen, but a part of modulation of overall androgenic response. Nevertheless, it is apparent from the present observation and the literature cited that even the preputial gland cAMP-PDE activity is an androgen dependent enzyme.

As far as the role of adrenal corticosteroids is concerned in the present context, it seems that only to certain extent such

steroids help maintain cAMP-PDE level more or less at normal level only upto about 24 hours post-operatively, but 48 hours onwards no such influence is observable. It, therefore, appears that in the present overall mechanism of androgenic influence on preputial gland of male rats, the adrenal steroids do not exert any additional significant role. It is pertinent to note here that adrenalectomy of rats has been shown to lead to increased PDE levels in case of muscle (Senft, 1968) and adipose tissue (Allen and Beck, 1972). Contrary to these reports, adrenal steroid therapy has been shown to decrease cAMP-PDE activity levels in rat fat cells (Vincent and Vaughan, 1972 and 1973; Schmidtke et al., 1976; Ross et al., 1977; Elks et al., 1983). It is, therefore, obvious that the preputial PDE activity is responsive to steroid hormones emanating from both testis and adrenal, in general. Elevations of PDE activity gets reflected into decrease of cAMP levels in a tissue (Butcher and Sutherland, 1962). Androgens have been shown to facilitate the activities of many enzymes involved in carbohydrate metabolism, by elevating the cAMP levels of sex-accessory organs (Singhal and Valadares, 1968; Singhal et al., 1968; Santti and Vilee., 1971; Singhal et al., 1971; Mangan et al., 1973). As against this, the presently observed facts clearly show that lack of androgens leads to elevation of cAMP-PDE activity resulting into quick hydrolysis of cAMP and hence to negation of cAMP induced changes in the metabolic patterns that were reported earlier by Ambadkar and Vyas (1975, 1979) from this laboratory.

Chronic IPR therapy to male rats would have affected the endogenous metabolism of the preputial gland in an indirect way as has been repeatedly stated (Chapter : 1). Alternatively, drug therapy might affect the preputial gland metabolism directly. Increased cAMP levels and inhibition of mitosis have been reported in epidermal components in response to IPR therapy (Powell et al., 1971; Birnbaum et al., 1976a and b; Das et al., 1978; Harris and Mackenzie, 1981). IPR therapy has been repeatedly shown to reduce cAMP-PDE levels in a number of tissues (Andersson, 1972; Hitchcock, 1973; Schwartz and Passonneau, 1974; Birnbaum et al., 1976a and b; Chiu et al., 1976; Marlene and Green, 1977; Schwartz and Costa, 1980; Makino and Tetsuro, 1981; Mallorga et al., 1981). As is evident from the table, the PDE activity levels in the preputial gland decreased to a significant level after chronic IPR therapy; meaning thereby that there could be a significant elevation of cAMP levels in the gland. Such an elevation of cAMP levels would convincingly explain stabilization of lysosomal membranes (Chapter : 6) resulting into inhibition of lysosomal enzyme discharge. Increased cAMP levels were also possibly responsible for reduced rate of cellular proliferation and subsequent lysis in the preputial acini as observed by Vyas (1978). In addition, elevated levels of cAMP reduce the activities of lipogenic enzymes as has been reported in Chapter : 2. On the basis of their work, Vyas (1978) and Ambadkar and Vyas (1981b) have postulated that stabilization of lysosomal membrane, reduction

of mitotic index and deceleration of cellular disintegration in preputial acini after IPR therapy could possibly be explained on the basis of likely increase in intracellular cAMP functions. The present investigation lends support to this contention through showing such build up of cAMP in the acinar cells due to deceleration of cAMP-PDE activity after IPR therapy.