CHAPTER 10

A PRELIMINARY STUDY ON MEDIATOR ACTION OF CYCLIC AMP ELICITED BY SEX-HORMONES IN THE HEPATIC TISSUE OF RATS AS REVEALED BY cAMP SPECIFIC PHOSPHODIESTERASE LEVELS

One of the interesting developments in the field of hormone research is the finding that adenosine-3', 5'-monophosphate, cAMP, is involved as a messenger mediating cellular responses. The hormone apparently activates a membrane-bound enzyme known as adenyl cyclase of the target cells, which in its turn catalyzes the transformation of ATP (adenosine triphosphate) to cAMP. Increased cAMP, acting within the target cells, then helps in bringing about the specific effect of the hormone by activating certain enzymes/enzyme systems concerned with the response. An authoritative account of this important area of investigation may be found in a review by Robinson and Sutherland (1972). The role of cAMP in the mediation of effects of steroid hormones is not very clear. Recent studies have implicated cAMP in the mechanism of action of steroid sex hormones on the accessory sexual organs in adult male and female rats. Androgens, for example, have been shown to facilitate activities of many enzymes involved in carbohydrate metabolism, by

increasing the cAMP levels, of the seminal vesicles and ventral prostate glands (Singhal and Valadares, 1968; Singhal et al., 1968; Santti and Villee, 1971; Singhal et al., 1971; Mangan et al., 1973). Such enzymic stimulation is also known to occur in the uterus by administration of oestrogens (Barker and Warren, 1966; Eckstein and Villee, 1966; Hilf et al., 1972). On the other hand, no evidence has been obtained to indicate that adenyl cyclase activity is stimulated by androgens (Rosenfeld and O'Malley, 1970; Liao et al., 1971; Mangan et al., 1973). In direct contrast to these findings; other reports have indicated that a rise in intracellular concentration of cAMP follows the administration of steroid sex-hormone in vivo. Szego and Davis (1967) and Singhal et al. (1971) have reported increase in cAMP levels in accessory structures in ovariectomized female and castrated male rats after administration of respective sex-hormones. Several other compounds like insulin, prostaglandins, adrenergics, etc. are known to decrease cAMP levels and produce an opposite effect (Butcher, 1968). A specific cellular enzyme - phosphodiesterase - inactivates the cAMP. This enzyme, like adenyl cyclase, is present in practically

all tissues. The possibility arises, therefore, that phosphodiesterase represents a controlling step in the hormonal modulations of cellular functions. Studies reported here were carried out in order to characterize the phosphodiesterase activity of liver. This would help to establish certain information on the precise role of cAMP in the action of male sex-hormones on the hepatic tissue.

MATERIALS AND METHODS

Hepatic tissue was obtained from adult male rats (120-160 gms.) at intervals of 24, 48 and 120 hrs. after bilateral castration, and after 1, 2 and 4 hours after a single intramuscular injection of 0.1 mg TP (testosterone propionate) dissolved in 0.5 ml of tributyrin to 48 hr. castrates. Method of estimating the phosphodiesterase activity was similar to that described by Butcher and Sutherland (1962). Protein was measured by the method of Lowry et al. (1951). The estimations of glycogen and phosphorylase activity were carried out as described in Chapter-3.

RESULTS

Table I shows that there is no difference amongst the two lobes of the liver as far as the activity of phosphodiesterase is concerned. After 24 hrs. of castration the enzyme activity was decreased in both the liver lobes. At 48 hr. interval after operation an increase was observed, but the level was still below the normal. By 120 hrs. the enzyme activity of median lobe came back to normal whereas, that of the Spigelian lobe could not attain normality of phosphodiesterase activity. It remained still below normal values. Testosterone propionate depresses the enzyme activity of the liver lobes of 48 hr. castrated male rats within 4 hrs. of hormonal administration. Median lobe exhibited reduction immediately after one hour, which again increased slightly, finally settling down to reduced enzymic level. Spigelian lobe, in contrast, registered a slight elevation after one hour of hormone administration. By two hours, the influence of TP became manifest in the form of decrease in enzyme activity that was similar to that of the median lobe after four hours of injection.

Table I : Effects of TP on cAMP specific phosphodiesterase activity of rat liver along with glycogen and protein content and phosphorylase activity.

	Normal Animals		Sham-operated animals		Castrated Animals						48 H castrates injected with 0.1 mg TP					
					24 H*		48 H*		120 H*		1 H@		2 H@		4 H@	
	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp	M	Sp
Phosphodiesterase /µg PO_4/mg protein per 30 min.	2.34 ±0.135	2.52 ±0.098	2.45 ±0.088	2.50 ±0.102	1.17 ±0.048	1.12 ±0.082	1.69 +0.052	1.57 ±0.055	2.53 ±0.130	1.89 +0.098	1.38 ±0.187	1.88 ±0.134	1.50 ±0.130	1.52 ±0.016	1.13 ±0.079	1.14 ±0.145
Glycogen as % of fresh tissue	3.83 ±0.395	3.66 ±0.531	12.93 +0.783	8.28 +0.442	9.13 ±0.907	6.93 ±0.517	7.77 ±0.500	8.17 ±0.439	4.99 ±0.672	4.30 ±0.326	8.33 ±0.077	6.51 ±0.062	6.61 ±0.065	5.79 ±0.053	6.63 ±0.141	6.47 ±0.107
Phosphorylase /µg PO_4/mg protein per 15 min.	78.82 ±5.41	58.74 ±4.92	71.30 ±4.03	53.09 ±3.12	94.45 ±1.18	63.52 ±4.30	90.00 ±4.49	59.74 ±2.58	124.37 ± 3.04	88.74 ±5.57	17.0 ±2.05	13.3 ±2.22	21.3 ±1.69	22.2 ±2.29	17.6 ±1.75	17.7 ±1.91
Protein as % of fresh tissue.	14.41 ±1.67	17.55 ±2.73	18.42 ±3.42	16.69 ±3.10	15.59 ±0.75	17.38 ±0.69	18.24 ±0.74	19.11 ±0.58	19.45 ±0.89	20.25 ±0.80	20.96 ±1.96	21.01 ±1.43	20.44 ±0.492	21.36 ±0.756	24.16 ±0.438	24.06 +0.622

Values are means of ten different samples.
M - Median lobe of the liver.
Sp - Spigelian lobe of the liver.
*Time interval in hours after castration.
@Time interval in hours after injection of TP.

Glycogen content was found to be depleting, in comparison to sham-operated animals, after castration. Phosphorylase activity was found to be increased after castration. After TP administration, the activity of phosphorylase was very much suppressed. The apparent decreasing tendency of glycogen levels after castration was seen to be retarded due to TP administration.

Protein content was observed to be altered but slightly due either to sham-operation or after 24 hrs. of castration. There was a definite rise in the levels of protein within 48 hrs. in the liver lobes. Both the lobes retained the higher concentrations upto 120 hrs. Hormone therapy not only led to steadying influence on protein concentrations in both of the lobes but induced an increased anabolic effect after four hours.

DISCUSSION

An initial decrease in phosphodiesterase activity was observed, in the rat liver following gonadectomy, which later on returned almost to normal levels by 120 hrs.

Administration of TP led to sustained depression of enzyme activity upto 4 hrs. after injection to 48 hr. castrates. Reduction in this enzyme activity may result into increasing intracellular concentration of cAMP. An increase in cAMP level after administration of testosterone to castrated rats has been reported in case of accessory sex glands by Singhal et al. (1971). On the other hand, Jhon et al. (1973) opined that testosterone has no significant effect on cAMP in the liver, oviduct, muscle tissue and the blood of intact fowl. However, present findings obviously demonstrate that the sex-hormones most likely influence cAMP levels in liver of rats (mammals). cAMP is known to influence the metabolic activities of tissues (Beviz et al., 1971; Singhal et al., 1971; Jain, 1978). Hence, the effect of sex-hormone, on liver metabolites is logically mediated through alterations in the concentration of cAMP. cAMP has been shown to activate the phosphorylase system of mammalian liver (Drummond et al., 1969; Rindi, 1971; Krebs et al., 1966) and to decrease the glycogen synthetase activity (Lerner et al., 1968; Hers et al., 1970; Hers, and Dewulf and Stalman, 1970). This finally

results into a net decrease of glycogen content. The present study bears out these facts quite well after castration. It is clear from the table that the significant rise in the hepatic glycogen concentration brought about by operational injury (sham-operated animals) is effectively brought down under the influence of castration. In consonance with this; activity of phosphorylase was found to exhibit sustained increasing tendency as a result of castration.

Sham-operated animals exhibited an increase in the protein concentration in the median lobe but that of Spigelian was hardly altered. Castration resulted into a slight elevation of protein levels in both lobes after 24 hrs but at 48 hrs these were decidedly high. Protein levels showed increasing trends upto 120 hrs. after castration.

Replacement with TP to 48 hr. castrates vividly brought about the well known fact that androgen have an anabolic influence in general. Phosphorylase as well as phosphodiesterase activities were seen to be held under suppression by the administration of TP. Both,

the glycogen as well as protein synthesis, was seen to go on at comparatively high rates, more so for the protein. Apart from its effect on carbohydrate metabolism, cAMP has also been reported to regulate DNA synthesis (Short et al., 1975). It is known to increase RNA and protein synthesis in rat uteri (Hechter et al., 1967; Sharma and Talwar, 1970). During the course of the present investigation it was observed that castration resulted into increased levels of DNA and RNA in the rat liver (Chapter-4). Considering this information, and the present observation on decreased phosphodiesterase activity, it is logical to suggest that castration leads to increased intracellular concentration of cAMP, the latter in its turn stimulates DNA/RNA synthesis and the increased RNA concentration enhances the rate of protein synthesis, within first 48 hrs. When TP is administered to 48 hr. castrates the anabolic tendencies are sustained with the resultant increase in glycogen and protein contents alongwith concomittant depression of phosphodiesterase and phosphorylase activities. This would obviously mean that the sex-hormone, in all probability, exerts its influence via the agency of cAMP - the "secondary-hormone".