CHAPTER XI

LOCAL AND SYSTEMIC PHOSPHODIESTERASE ACTIVITY IN RELATION TO TAIL REGENERATION IN THE SCINCID LIZARD, MABUYA CARINATA UNDER ALTERED FUNCTIONAL STATUS OF THYROID

In a previous study (Chapter VI) possible alterations in cAMP content during tail regeneration was attempted by evaluating temporal alterations in the quantitative levels of phosphodiesterase. Based on the alterations in phosphodiesterase activity, two specific phases of elevated cAMP content was inferred; once during the immediate post-autotomy periods and once during the late differentiation-growth phases of tail regeneration. Involvement of cAMP in metabolic alterations (Rall et al., 1957; Drummand et al., 1969; Beriz et al., 1977; Ishibashi and Catton, 1978), division, differentiation etc. (Thomas et al., 1973; Berridge, 1975; Friedman, 1976; Miller, 1977; Taban and Cathieni, 1978; Carlone and Foret, 1979; Kosher and Savage, 1980) and its regulation via adenylate cyclase by many hormones are well documented (Klainer et al., 1962; Sutherland et al., 1965; Pastan and Katzen, 1967; Krishna et al., 1968; Levey and Epstein, 1968; Levey et al., 1969; Zor et al., 1969; Irwin, 1974; Sperling et al., 1980). However, phosphodiesterase mediated alterations in cAMP content has

received scant attention, while hormonal control of phosphodiesterase activity has been totally overlooked. It was this perspective which motivated the present investigations on alterations in phosphodiesterase activity under different functional status of thyroid. Besides enabling to establish the influence of thyroid hormone on phosphodiesterase, the study, it was thought, may also provide some clue to the inhibitory action of hypothyroidism on tail regeneration in Mabuya carinata. To make the study more complete, and as significant systemic support in supplementing the local efforts has been demonstrated (Kinariwala, 1977; Chapter VI), the analysis was extended to liver and skeletal muscle as well in addition to the regenerate.

MATERIALS AND METHODS

Healthy Mabuyas of both sexes obtained from Hyderabad, India and allowed to get acclimated to the laboratory conditions were kept on insect diet. Animals were subjected to chemical thyroidectomy and thyroxine replacement therapy as described in Chapter I. Tails were autotomized by pinching off at about 1.5-2.0 cms distal to the vent. Animals were then sacrificed under mild anaesthesia at fixed intervals of 3, 5, 7, 10, 12, 15, 25, 40 and 60 days post-autotomy.

Liver, skeletal muscle and normal or regenerating tail, as the case may be, were quickly removed and homogenized in

ice-cold redistilled water. A 2% homogenate was prepared for liver and skeletal muscle whereas in the case of tail a 4% homogenate was found satisfactory. Quantitative estimation of cAMP phosphodiesterase activity, inorganic phosphate and protein content were done according to the methods described in Chapter VI.

For each day and each tissue specified a total of five to seven estimations were made. The mean and standard deviation were obtained and students' 't' test was used to determine statistical significance.

RESULTS

Figs. 1-3 and Table 1 represent the alterations in cAMP phosphodiesterase activity in the regenerate, liver and skeletal muscle under euthyroidic, hypothyroidic and thyroxine replaced conditions while Table 2 depicts the percentage modulations during various phases of tail regeneration. In general, phosphodiesterase activity in all the three tissues tended to be higher in PTU treated animals as compared to the euthyroidic controls and intermediate in T4 replaced animals in the pre-autotomy condition. Even during various periods of tail regeneration animals in the B group depicted higher levels of enzyme activity in all the three tissues. Subsequent to tail autotomy there was a

Table 1. Quantitative levels of cAMP phosphodiesterase in the regenerate, liver and skeletal muscle during tail regeneration under euthyroidic, hypothyroidic and T4 replaced conditions in M. carinata.

(Values are expressed as µg phosphorus liberated/mg protein/min.)

Periods of tail regeneration in days	TAIL			LIVER			MUSCLE		
	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)
N	3.81 ±0.08	4.71 ±0.26	4.16 ±0.12	1.35 ±0.03	5.02 ±0.19	3.37 ±0.61	2.39 ±0.12	3.66 ±0.66	3.15 ±0.15
3	2.47*@ ±0.08	2.36*@ ±0.25	2.18*@ ±0.15	1.24@ ±0.02	4.72 ±1.19	4.15 ±0.04	1.49*@ ±0.03	2.91 ±0.89	2.32* ±0.42
5	2.71*@ ±0.23	5.85* ±0.47	2.46*@ ±0.12	1.51 ±0.11	4.52 ±1.31	2.86 ±0.44	2.02 ±0.62	4.60 ±0.47	1.71*@ ±0.08
7	3.09 ±0.07	6.13@ ±0.59	2.87*@ ±0.43	1.56* ±0.09	4.81* ±0.08	3.71 ±0.38	2.00* ±0.12	6.55@ ±0.77	2.31@ ±0.22
10	3.47 ±0.11	5.47** ±0.21	4.21 ±0.17	1.29 ±0.16	4.79 ±0.18	4.04 ±0.15	2.33 ±0.14	4.89** ±0.43	1.94*@ ±0.06
12	3.57* ±0.09	6.38*@ ±0.31	4.78 ±1.01	1.34 ±0.05	4.75 ±0.16	1.88@ ±0.51	2.78 ±0.24	1.93@ ±0.66	2.09*@ ±0.08
15	3.36@ ±0.14	7.31*@ ±0.41	3.94 ±0.14	1.39 ±0.05	3.27*@ ±0.29	2.24* ±0.68	2.15 ±0.41	4.09 ±0.15	3.26 ±0.45
25	3.97 ±0.34	4.95* ±0.93	3.62 ±0.42	1.53 ±0.23	5.75*@ ±0.26	4.19@ ±0.15	1.59* ±0.37	4.17 ±0.24	2.47** ±0.26
40	2.41*@ ±0.06	5.27* ±0.15	2.21*@ ±0.13	0.93*@ ±0.04	6.91*@ ±0.73	2.67 ±0.34	1.24** ±0.46	5.22*@ ±0.12	2.44** ±0.29
60	3.38* ±0.22	5.29* ±0.21	3.99* ±0.08	1.21 ±0.06	4.19*@ ±0.12	2.48* ±0.21	2.17 ±0.32	4.37 ±0.39	2.98 ±0.12

* $P < 0.01$; ** $P < 0.005$; @ $P < 0.0025$; @ $P < 0.001$; *@ < 0.0005

N - Normal (Pre-autotomy state)

Table 2. Percentage modulations in cAMP Phosphodiesterase content in the regenerate, liver, skeletal muscle during tail regeneration, under control euthyroidic, hypothyroidic and T4 replaced conditions in M. carinata.

(Values are calculated against the respective normal values)

Periods of tail regeneration in days	TAIL			LIVER			MUSCLE		
	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)	Control Group (A)	PTU Group (B)	PTU + T4 Group (C)
N	0%	0%	0%	0%	0%	0%	0%	0%	0%
3	-35%	-50%	-48%	- 8%	-40%	+23%	-38%	-21%	-26%
5	-29%	+24%	-41%	+11%	-10%	-15%	-15%	-26%	-46%
7	-19%	+30%	-31%	+16%	- 4%	+10%	-16%	+79%	-27%
10	-10%	+16%	+ 1%	- 5%	- 5%	+20%	- 3%	+34%	-38%
12	- 6%	+35%	+15%	- 1%	- 5%	-44%	+17%	+35%	-37%
15	-12%	+55%	- 5%	+ 3%	-35%	-34%	-10%	+12%	+ 4%
25	+ 4%	+ 5%	-13%	+13%	+15%	+24%	-33%	+14%	-22%
40	-37%	+12%	-47%	-31%	+37%	-21%	-48%	+43%	-23%
60	-11%	+12%	- 4%	-11%	-17%	-26%	- 9%	+19%	- 5%

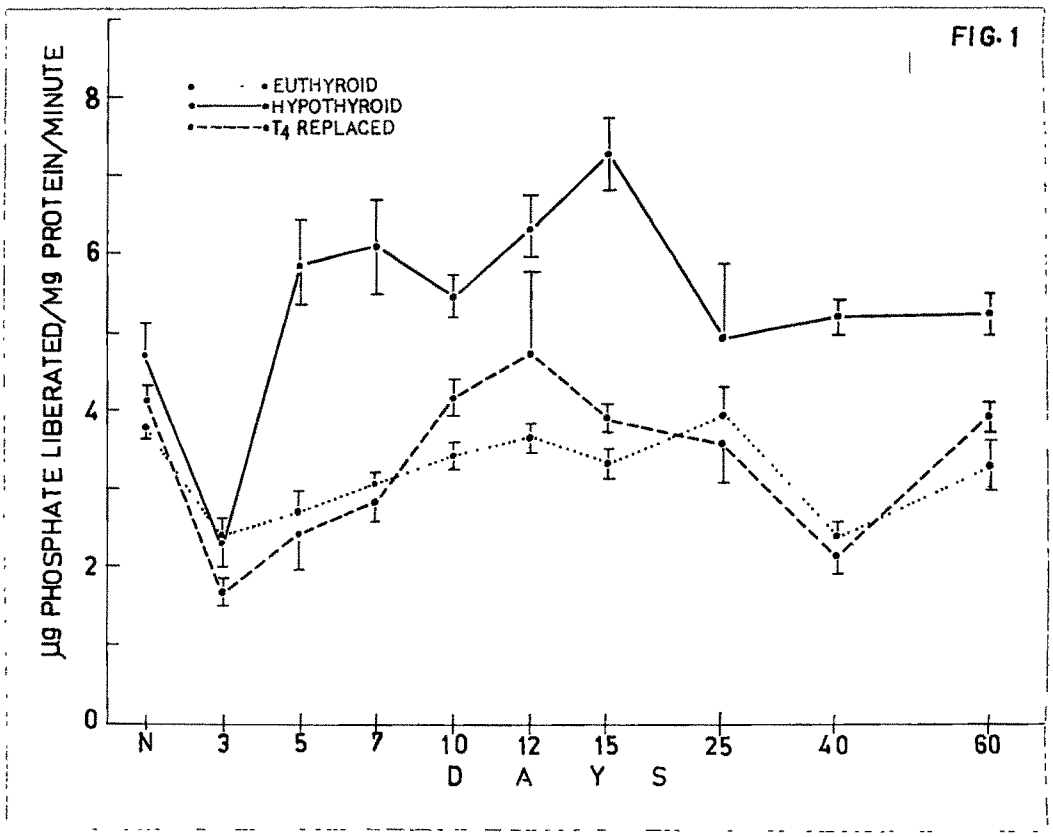

Fig. 1. Changes in cAMP phosphodiesterase activity in the regenerate during tail regeneration under control euthyroidic, PTU induced hypothyroidic and T4 replaced conditions in M. carinata.

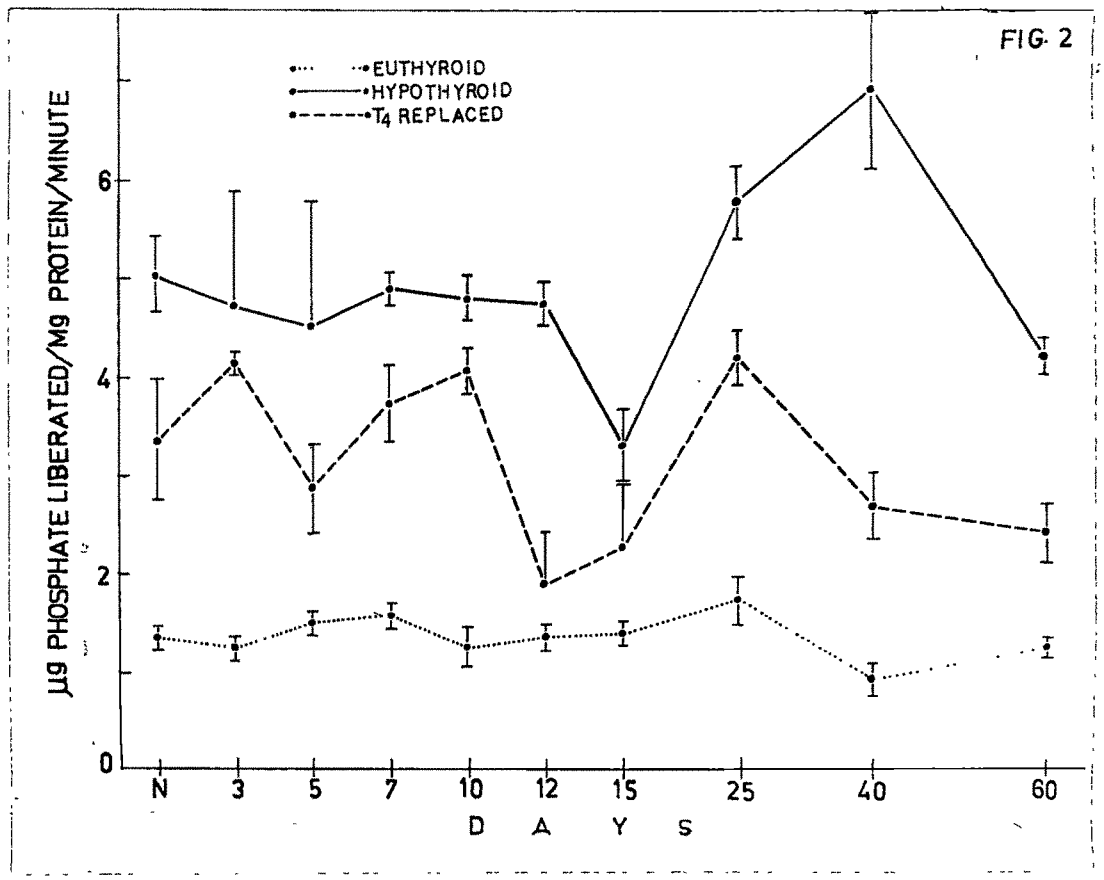

Fig. 2. Changes in cAMP phosphodiesterase activity in liver during tail regeneration under control euthyroidic, PTU induced hypothyroidic and T4 replaced conditions in M. carinata.

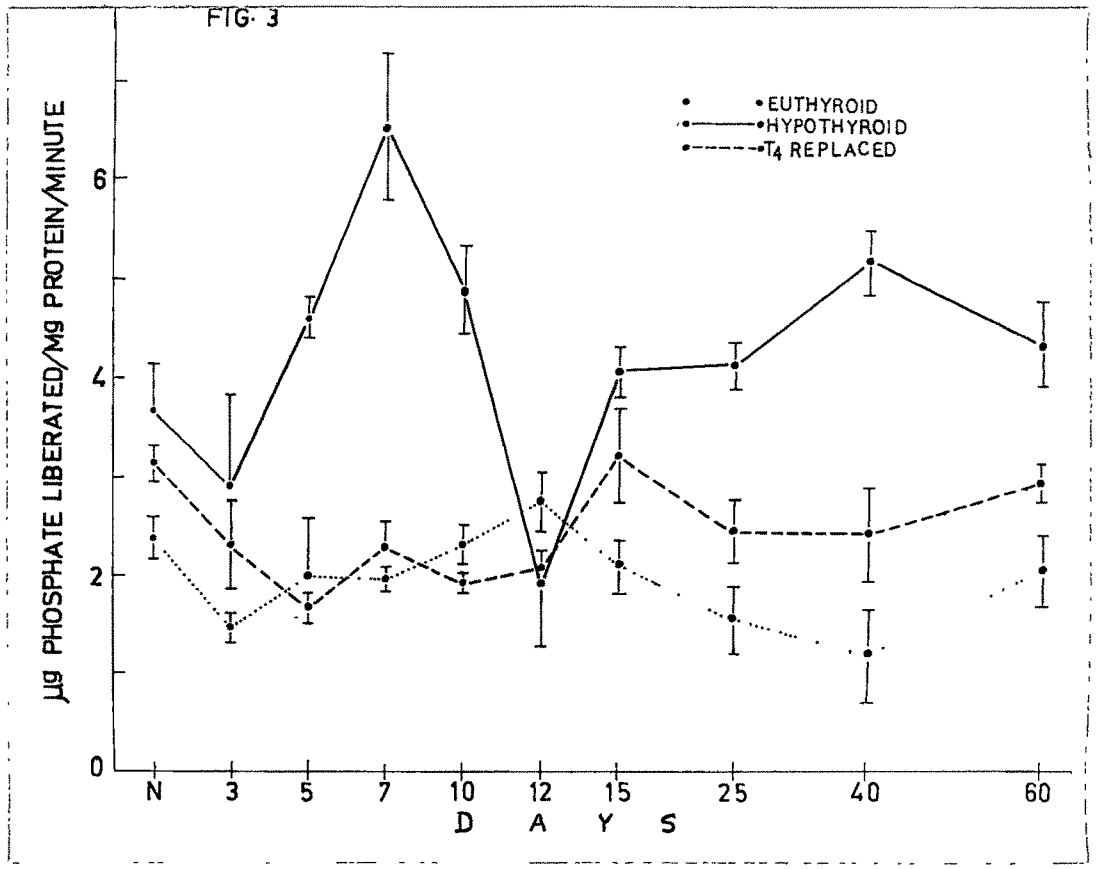

Fig. 3. Changes in cAMP phosphodiesterase activity in muscle during tail regeneration under control euthyroidic, PTU induced hypothyroidic and T4 replaced conditions in M. carinata.

generalized fall in the enzyme activity in all the three groups by about 3rd day. Pattern of alterations in enzyme activity though similar in A and C groups of animals, C group of animals tended to have slightly higher levels of enzyme activity with the hepatic tissue being most prominent. Phosphodiesterase activity in the regenerate and skeletal muscle never registered above normal levels in A and C groups while below normal levels were noted during the first 10 days post-autotomy and again during 15th to 40th days of tail regeneration. However, enzyme activity in group B animals tended to be significantly above normal levels in both the regenerate and skeletal muscle except for 3rd and 12th days in muscle and 3rd day in the regenerate whence the enzyme activity tended to be subnormal. Hepatic phosphodiesterase activity tended to be subnormal during 3rd, 10th and 40th days in group A, and 5th, 12th, 15th, 40th and 60th days in C group. Supranormal levels were recorded during 5th, 7th and 25th days in group C animals. Group B animals tended to maintain more or less the elevated pre-autotomy level except for 3rd, 5th and 15th days whence it was subnormal, and on 25th and 40th days it was above normal.

DISCUSSION

In an earlier chapter (I) inhibition of tail regeneration was correlated with lack of thyroxine. Similarly phospho-

diesterase mediated phase specific alterations in cAMP content were also presumed to be positively interlinked with molecular events associated with regeneration (Chapter VI). Modulations in systemic cAMP too (liver and muscle) were correlated with metabolic responses of these tissues. The present chapter which records the basal levels and alterations in these levels of phosphodiesterase activity, during differing functional status of thyroid, has shown significantly elevated levels of phosphodiesterase activity *in loco* as well as systemically all throughout regeneration on the one hand, and temporally displaced alterations in the PTU induced hypothyroid animals. The present observations may have some relevance in the above mentioned inhibition of regeneration in the hypothyroidic condition as well as the functional involvement of cAMP in the regenerative mechanics of lacertilians. An increase in the basal levels of the enzyme by about 242% in the liver, 53% in the muscle and 24% in the tail in the PTU fed animals (group B) as compared to the control euthyroidic animals (group A) clearly imply the regulatory influence of thyroxine on cAMP phosphodiesterase. Though there are many reports regarding the regulation of cAMP content in cells and tissues by endocrine control of adenylate cyclase (Sutherland *et al.*, 1965; Pastan and Katzen, 1967; Gilman and Rall, 1968; Zor *et al.*, 1969; Irwin, 1974; Arch and Newsholme, 1976) possible regulation of cAMP

content by hormonally induced alterations in PDE activity is totally overlooked, especially as phosphodiesterase is considered to be one of the factors involved in the control of cellular cAMP content (Butcher and Sutherland, 1962; Braun and Shiozawa, 1973; Arch and Newsholme, 1976).

Persistently high increasing levels of phosphodiesterase activity observed in the regenerate as well as the two visceral organs in the hypothyroid condition throughout the present experimental period, might be considered to act directly or indirectly in inhibiting tail regeneration by abolishing crucial phase specific adaptive fluctuations in cAMP content; the varied effects of which (in loco and systemic) on tail regeneration were discussed in an earlier chapter (VI). Incidentally, the near normalisation of the levels and pattern of changes of phosphodiesterase activity in T4 replaced group C animals (very much comparable to the euthyroidic group A animals) and the earlier reported reversal of the inhibitory influence of PTU by T4 replacement (Chapter I) are favourable observations. Suppression of the metabolic responses of the liver and skeletal muscle specifically with respect to carbohydrate metabolism in PTU fed animals (Chapter VIII) may also be correlated with the currently noted elevation in phosphodiesterase activity under the PTU induced hypothyroidic state. The concept of a definite negative influence of thyroxine on PDE inferred in

the present study is further strengthened by the observed comparatively more pronounced percentage reduction in PDE activity in general in the T4 treated animals as compared to the controls (see Table 2). An examination of the data also reveals a slightly different response of the hepatic tissue as compared to the regenerate and skeletal muscle. One difference is the more or less unchanging level of phosphodiesterase activity during the first 12 days post-autotomy and the percentage alterations becoming significant only thereafter in the hypothyroid group of animals which may be explained as due to the high increment in the enzyme activity registered right from the pre-autotomy stage itself. Another is the inability of T4 given animals to attain enzyme levels typical of euthyroidic controls though there was a definite reduction in the enzyme activity from the levels characteristic of PTU induced hypothyroidism. This might possibly be due to the inability of T4 to overcome completely the PTU induced hypothyroidic effect or the dose of T4 may be insufficient. This could be inferred not only by the intermediate levels of PDE in the T4 replaced animals but also by the pattern of changes during regeneration which is a subtle admixture of the euthyroidic and hypothyroidic ones.

Though a definite negative influence of thyroxine on phosphodiesterase activity is inferred herein, the fluctuations observable in the hypothyroidic animals too during the course

of regeneration, though temporally displaced, may also indicate probable influence of other humoral or non-humoral factors (which may be purported to be regeneration associated) on phosphodiesterase. However, the markedly elevated level of enzyme activity in the pre-autotomy period as well as maximal percentage increase during certain phases of regeneration in the hypothyroid condition definitely underscore the clearcut negative regulation of phosphodiesterase activity by thyroxine. Presumably, though other factors too may be considered to have some influence on phosphodiesterase, it appears that presence of thyroid hormone is essential in modulating the enzyme activity to normal functionally potent levels.

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