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EFFECT OF 3,5,3'-TRIIODOTHYRONINE INDUCED CARDIAC HYPERTROPHY ON CYTOSOLIC PROTEIN KINASES

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SUMMARY

The protein kinase activity of right and left ventricular cytosol fractions has been determined in a group of 3,5,3'-triiodothyronine (T_3) treated and control rats over a period of 7 days.

The ventricular weights of the T_3 treated group had increased much more quickly than the control group by the third day. By the seventh day the rate of growth of the ventricles was the same as the controls. The protein kinase activity of the T_3 treated group increased significantly after three days. After seven days of treatment the protein kinase activity returned to control levels.

INTRODUCTION

The rate of growth of a tissue may possibly be controlled in the cytosol by the phosphorylation of factors involved in protein synthesis such as ribosomes (1, 2) or elongation factors (3). A repeated dose of 3,5,3'-triiodothyronine (T_3) causes hypertrophy of the right and left ventricles of the heart (4). The myocardial nuclear protein kinase activity has been shown to increase with T_3 treatment, but no such effect was shown in the cytosol (5). We now report the effects of intraperitoneal T_3 injections on the cytosol cAMP-dependent protein kinases of the right and left ventricles.

MATERIALS AND METHODS

3,5,3'-triiodothyronine, cAMP and histone f_1 were obtained from Sigma (London) Ltd. [γ-^{32}P]- ATP was obtained from the Radiochemical Centre.

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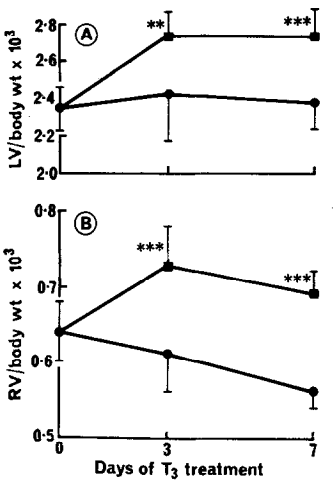

Figure 1. Development of cardiac hypertrophy in T_3-treated rats
Intraperitoneal injections of T_3 were given daily as described (Methods). The points shown are the mean observations from groups of 10 animals ± standard deviation. A, left ventricle/body weight ratio, B, right ventricle/body weight ratio. ●——● controls, ■——■ T_3 treated. Statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

All other chemicals were supplied by British Drug Houses Ltd. Male Sprague-Dawley rats (180 - 200 g) were used.

Experimental procedure

Thirty rats were injected daily intraperitoneally with 15.6 µg T_3/100 g body weight. Thirty control animals were injected with distilled water. Groups of ten animals were killed after zero (no injection), 3 and 7 days of treatment. The hearts were removed and divided into right ventricle and left ventricle plus interventricular septum. The ventricles were homogenized (in .25 M sucrose, .02 M Tris, .002 M EDTA, pH 7.4) for 2 x 10 seconds with an UltraTurrax homogenizer. The homogenate was centrifuged at 150,000 x g for 1 hour. The supernatant fraction (cytosol) was decanted and passed through G 75 Sephadex to remove endogenous ATP.

Protein kinase assay

The protein kinase reaction medium contained, in a final volume of 0.25 ml, 40 mM Tris/HCl pH 7.4, 5 mM $MgCl_2$, 600 µg histone f_1, 10 - 25 µg cytosol protein, [γ-^{32}P]-ATP 35 µM containing 20 µCi ^{32}P, with additions of 1 µM cAMP incubated for five minutes at 37°C. The reaction was linear up to 10 minutes incubation. Protein kinase activity was expressed as pmoles ^{32}P transferred per mg protein per hour incubation at 37°C. The concentration of protein was determined by the method of Lowry et al. (6) modified by Hartree (7).

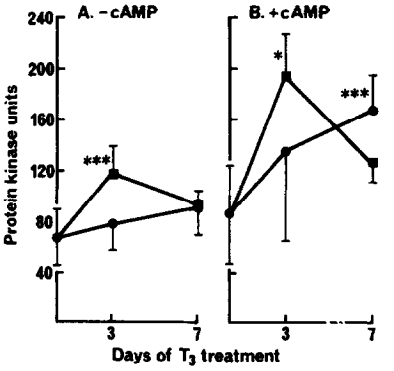

Figure 2. Right ventricular cytosolic protein kinase activity of T_3-treated rats

A and B show the protein kinase activity without and with cAMP respectively. Protein kinase activity is expressed as pmoles ^{32}P transferred per mg protein per hour incubation at 37°. ●——● controls, ■——■ T_3 treated. Statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

RESULTS

T_3 had no effect on the body weights of the animals, which increased by 15% over the experimental period in both the T_3-treated and the control groups.

The left ventricular weights of the control group increased at the same rate as the body weight (shown by the constancy of the left ventricle/body weight ratio, Fig. 1 A). The right ventricles of the control group were growing at a slower rate than the whole body (Fig. 1 B). After 7 days the right ventricle/body weight ratio of the controls was significantly lower ($P < 0.001$) than at day 0.

The T_3 treated animals (Figs. 1 A and 1 B) showed a significant increase in the left ventricle/body weight ratio (13%, $P < 0.005$) and right ventricle/body weight ratio (19%, $P < 0.001$) after three days, compared with the controls. Between three and seven days the ventricles of both groups were growing at virtually the same rate.

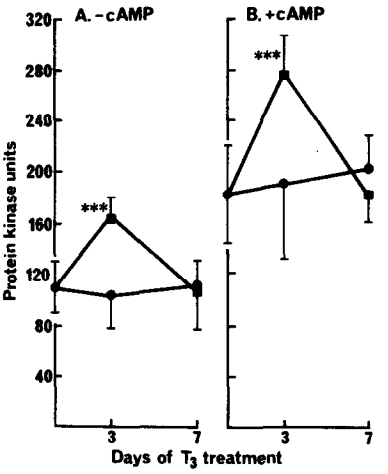

Figure 3. Left ventricular cytosolic protein kinase activity of T_3-treated rats

A and B show the protein kinase activity without and with cAMP respectively. Protein kinase activity is expressed as pmoles ^{32}P transferred per mg protein per hour incubation at 37°. ●——● controls, ■——■ T_3 treated. Statistical significance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Protein kinase activity

The protein kinase activity in the cytosol of the right and left ventricles is shown in Figures 2 and 3.

The kinase activities, both with and without cAMP, were higher in the left ventricle than in the right ventricle ($P < 0.025$). The protein kinase activity in the right ventricle of the control group increased over the seven days ($P < 0.02$) but this was not found in the left ventricle.

The effects of T_3 on the two ventricles were similar. The activities, with and without cAMP, of the T_3 treated group were significantly higher after 3 days of treatment compared with the controls. By 7 days the activity decreased to the control, or below control values. The ratio of protein kinase activity without cAMP, to activity with cAMP, did not change during the experiment.

DISCUSSION

Horwood and Singhal (8) and Byus et al. (9) have previously shown a rise in cytosol cAMP-dependent protein kinase activity during isoprenaline-induced cardiac hypertrophy. In the present study we have demonstrated an increase in the protein kinase activity of the rat myocardial cytosol in triiodothyronine-induced hypertrophy at a time when there was an increase in the rate of growth of the tissue. When the growth rate became constant the protein kinase activity returned to control values.

Protein kinase activity might have been modified by changes in the tissue concentration of cAMP (affecting the proportion of free catalytic subunits) or to a direct action of the hormone on the enzyme (as suggested by Kruh and Tichonicky (10) for the nuclear enzyme) or to changes in the rate of synthesis of enzyme protein. Since the ratio of protein kinase activity without cAMP to the activity with cAMP did not alter during the experiment, one may presume that the proportion of free catalytic subunits remained unaltered. A direct effect of the hormone seems unlikely, since the purified cytosol kinases have shown no change in activity per unit protein during T_3-induced cardiac hypertrophy (5). One is left, therefore, with the conclusion that the changes in protein kinase activity were due to changes in the rate of synthesis of enzyme protein.

Increased cytosol protein kinases may be a factor in the early development of cardiac hypertrophy. Ribosomal proteins contain covalently bound phosphate, the level of which changes under various physiological conditions, including thyroidectomy (1, 2, 11). However, phosphorylation of ribosomes in vitro has not been shown to produce any change in protein synthesizing ability (12). Stahl et al. (3) suggested that phosphorylation

of elongation or initiation factors partly control the rate of protein synthesis.

The present results confirm our previous unpublished observation that the cytosolic protein kinase activity of the left ventricle is higher than that of the right.

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