ACTIVATION OF CARDIAC ADENYL CYCLASE BY THYROID HORMONE

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SUMMARY

The thyroid hormones, thyroxine and triiodothyronine, increased the conversion of $AT^{32}P$ in the particulate fraction of cat heart homogenates. The concentration of thyroxine that produced half of the maximal response was 5×10^{-7} M and of triiodothyronine was 6×10^{-7} M. The increase in cyclic 3', 5' - $AM^{32}P$ was not due to an inhibition of phosphodiesterase, indicating a direct effect of both hormones on the activation of adenyl cyclase in myocardial tissue, a finding that may explain some of the cardiac manifestations of the hyperthyroid state.

INTRODUCTION

The mechanism responsible for the hyperdynamic circulatory state of patients with hyperthyroidism has been the subject of considerable speculation (Waldstein, 1966). Since some of the cardiac manifestations of hyperthyroidism resemble those caused by excessive adrenergic stimulation and certain anti-adrenergic drugs diminish these manifestations, it has been suggested that some of the effects of excessive thyroid hormone are mediated through the sympathetic nervous system (Canary et al. 1957; Gaffney et al. 1961). More recent studies, however, have cast doubt on this hypothesis (Wilson et al. 1964; Cairoli, 1966). Alternatively, it is possible that thyroid hormone may exert a direct effect on the heart.

Since the potent positive inotropic and chronotropic effects of norepinephrine and glucagon are accompanied by activation of adenyl cyclase, it has been postulated that activation of this enzyme may be responsible for mediating the cardiac effects produced by these hormones (Sutherland and Robison, 1966; Levey and Epstein, 1968). This investigation demonstrates that like norepinephrine and glucagon, thyroxine and triiodothyronine activate adenyl cyclase

in the particulate fraction of cat heart homogenates.

MATERIALS AND METHODS

L-Thyroxine Lot Number 16B-1660 was obtained from Sigma; triiodothyronine from Smith, Kline, and French; adenosine 5'-triphosphate disodium salt (ATP) from P-L Biochemicals; $AT^{32}P$, 600-800 mC_i/mMole from International Chemical and Nuclear Corporation, ^3H-cyclic 3', 5'-AMP, $1C_i$/mMole from Schwarz Bioresearch; and Dowex 50W-X8, 100-200 mesh from Calbiochem.

Left ventricular muscle was obtained from normal cats, and a single cat was used for each experiment. After anesthesia with pentobarbital, 25-35 mg per kg intraperitoneally, the heart was quickly excised. The left ventricle was dissected free of endocardium and epicardium and approximately 220 mg of left ventricular muscle was homogenized in 4.5 ml of cold 0.25 M sucrose with a motor-driven homogenizer at 1° C. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4° C and the supernatant decanted; the particles were washed with cold 0.25 M sucrose and resuspended and recentrifuged at 10,000 rpm for 10 minutes. The washed particles were resuspended and rehomogenized in the cold 0.25 M sucrose. Adenyl cyclase was assayed by a recently developed method (Krishna et al, 1968; Rodbell, 1967). The particulate fraction, containing 0.07 to 0.10 mg protein in a total volume of 0.06 ml, was incubated at 37° C for 3 minutes with ATP, 1.6 mM; $AT^{32}P$, 2.5 to 3.5 x 10^6 cpm; theophylline, 8mM; $MgCl_2$, 2mM; tris-Cl, 21 mM, (pH 7.7); human serum albumin 0.8 mg/ml; and hormone concentrations stated in the text. The incubations were started by adding particles, which had been kept at 1° C, to the other components which were at 23° C. Thyroxine or triiodothyronine were added to the particles just before the incubations were initiated. After 3 minutes the incubations were stopped by adding 0.1 ml of a solution containing 4 μmoles of ATP, 1.25 μmoles of cyclic 3', 5'-AMP, and 0.15 μC of of ^3H-cyclic 3', 5'-AMP and boiled for 3 minutes. The ^3H-cyclic 3', 5'-AMP served to determine the recovery of cyclic 3', 5'-AMP during the procedure; recovery ranged from 30 to 35 per cent. After boiling, 0.4 ml of water was added, the precipitate removed by centrifugation and the supernate applied to a 0.5 x 2.0 cm Dowex-50

column. The column was washed with 3.0 to 6.0 ml of water, and the eluate was collected and precipitated with 0.17 M $ZnSO_4$ and 0.15 M $Ba(OH)_2$ and the cyclic 3', 5'-AM^{32}P and ^{3}H-cyclic 3', 5'-AMP counted in a liquid scintillation spectrometer. The presence of cyclic 3', 5'-AMP was confirmed by thin-layer chromatography in a solvent system containing a mixture of n-Butanol, acetone, acetic acid, 5% ammonia, and water (7:5:3:3:2). Protein was determined by the method of Lowry et al. (1951).

For the determination of phosphodiesterase activity ^{3}H-cyclic 3', 5'-AMP (200 picomoles) was added to each reaction mixture which contained approximately 0.06 mg of protein. Incubation conditions were identical to those noted above except that AT^{32}P was omitted. Thyroxine or triiodothyronine were present at 5 x 10^{-6} M. After boiling, the precipitate was removed by centrifugation; the supernatant was mixed with 3.4 ml of water followed by 0.2 ml of 0.17 M $ZnSO_4$ and 0.2 ml of 0.15 M $Ba(OH)_2$. After centrifugation, 3 ml of the supernatant was added to 17 ml of Bray's solution and the radioactivity measured in a liquid-scintillation spectrometer.

Table I

	Cyclic 3', 5'-AMP Accumulated (Picomoles/3 minutes)
Control	8.0 ± 0.7
Thyroxine (5 x 10^{-6} M)	13.7 ± 0.7*
Control	8.1 ± 0.7
Triiodothyronine (5 x 10^{-6} M)	13.3 ± 0.7†

Legend: The effect of thyroxine and triiodothyronine on cardiac adenyl cyclase. Each value represents the mean ± SE of 10-12 samples.

*$p < 0.01$
†$p < 0.05$

RESULTS

The thyroid hormones, thyroxine and triiodothyronine, increased the conversion of $AT^{32}P$ to cyclic 3', 5'-$AM^{32}P$ in the particulate fraction of cat heart homogenates by 70 and 65 per cent respectively (Table I). The activation of adenyl cyclase increased over the range 2×10^{-7} M to 5×10^{-6} M (Fig. 1). Higher concentrations of thyroid hormone failed to remain in solution in the molar Tris buffer used in this system for dilution of the hormone. The

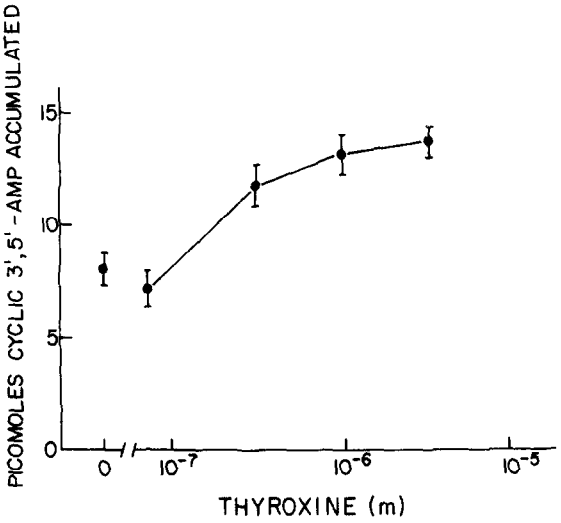

Fig. 1. The effect of increasing concentrations of thyroxine on cardiac adenyl cyclase. Each value represents the mean ± SE of 10-12 samples in 4 cats.

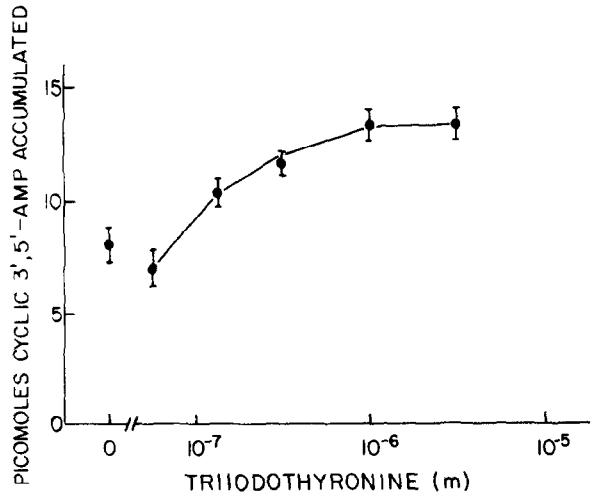

Fig. 2. The effect of increasing concentrations of triiodothyronine on cardiac adenyl cyclase. Each value represents the mean ± SE of 6-11 samples in 3 cats.

Table II

Incubation	Cyclic 3', 5'-AMP Hydrolyzed (Picomoles/3 minutes)
Control	4.0 ± 1.0
Thyroxine (5×10^{-6} M)	10.0 ± 3.0
Triiodothyronine (5×10^{-6} M)	4.0 ± 1.3

Legend: Effect of thyroxine and triiodothyronine on phosphodiesterase activity in cat hearts. The values represent the mean ± SE of 6 samples. The difference between the control and thyroxine values is not statistically significant.

concentration of thyroxine that produced half of the maximal response was 5×10^{-7} M and of triiodothyronine was 6×10^{-7} M. The increase in cyclic 3', 5'-AM^{32}P was not due to an inhibition of phosphodiesterase (Table II), indicating a direct effect of both hormones on the activation of adenyl cyclase.

DISCUSSION

These results demonstrate that thyroid hormone increases the accumulation of cyclic 3', 5'-AMP in the particulate fraction of cat heart homogenates. To ensure that this increase was due to enhancement of adenyl cyclase activity rather than to diminished degradation by phosphodiesterase, the effect of thyroid hormone on phosphodiesterase activity was also determined. This was felt to be particularly important since Mandel and Kuehl (1967) reported that a high concentration of triiodothyronine, 10^{-4} M to 10^{-3} M, inhibited phosphodiesterase in adipose tissue extracts, a change that could also explain our findings. However, we did not detect any inhibition of myocardial phosphodiesterase with a concentration of thyroid hormone up to 5×10^{-6} M, a level that maximally increased the rate of formation of cyclic 3', 5'-AMP. Thus, the increase in cyclic 3', 5'-AMP levels induced by thyroid hormone in the present study is assumed to have been caused by an increase in the activity of adenyl cyclase.

Furthermore, since only three minutes of incubation was sufficient to produce an approximately 70 per cent increase in enzyme activity, it would appear that the increased activity was due to actual activation of adenyl cyclase rather than to de novo synthesis of the enzyme. This latter mechanism was hypothesized as being responsible for the increased activity of adenyl cyclase observed in fat cells from hyperthyroid rats by Krishna, et al. (1968), since puromycin abolished the 50 per cent increase in adenyl cyclase activity produced by a two-hour incubation of triiodothyronine with fat cells.

Thus, the present investigation provides the first evidence that thyroid hormone has the capacity to activate adenyl cyclase in myocardial tissue, a finding that may explain some of the cardiac manifestations of the hyperthyroid state.

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