Normal Myocardial Adenyl Cyclase Activity in Hyperthyroid Cats[1] (34135)

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The relationship between the sympathetic nervous system and the cardiovascular manifestations of hyperthyroidism has aroused considerable interest and controversy. Results from studies in experimental animals (1–3) and in man (4, 5) suggest that there is an augmentation of the peripheral vascular response to infused catecholamines in thyrotoxicosis. Although it appears that the enhanced cardiac contractility seen in thyrotoxicosis may not depend on the action of myocardial catecholamines (6), other evidence suggests that some of the cardiac effects seen in hyperthyroidism reflect potentiation of beta-adrenergic actions of catecholamines on the myocardium (7–10).

Recent studies have shown that 3′, 5′-adenosine monophosphate (cyclic-AMP) is an important intracellular mediator of beta-adrenergic effects (11, 12) and that synthesis of this nucleotide is catalyzed by adenyl cyclase, an enzyme intimately related to the cell membrane in heart muscle. The degradation of cyclic-AMP is regulated by another enzyme, a soluble phosphodiesterase.

It is known that thyroid hormone may potentiate protein synthesis *in vivo* (13, 14), and it has been suggested that synthesis and steady state concentration of mycocardial adenyl cyclase may be increased in thyrotoxicosis (15). Since increased myocardial adenyl cyclase would presumably increase the heart's capacity to synthesize cyclic-AMP, augmentation of the effects of catecholamines on the heart might be anticipated in thyrotoxicosis. The present study was designed to explore this possibility by examining possible alterations in the myocardial metabolism of cyclic-AMP in thyrotoxicosis. Accordingly, the activity of adenyl cyclase and phosphodiesterase in corresponding fractions from myocardium of normal and hyperthyroid cats was determined *in vitro* under basal conditions and in the presence of known activators.

Methods. Materials. The ^{3}H-adenosine-5′-triphosphate (ATP) was obtained from Schwarz BioResearch; cyclic-AMP, dipotassium ATP, and bovine serum albumin from Sigma; Dowex (BioRad AG-50W X-4, 200-400 mesh) from Calbiochem; theophylline and epinephrine bitartrate from K & K Laboratories; and 2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]-thiophene (BBOT) from Packard. All other chemicals were of reagent grade and aqueous solutions were prepared using de-ionized and charcoal filtered water.

Procedures in animals. Hyperthyroidism was produced in seven male cats by daily administration of USP thyroid (Armour), 30 mg po, for 14 days prior to study. Protein bound iodine levels in blood from treated animals obtained at the time of study showed values greater than 20 μg/100 ml (normal: 4–8 μg/100 ml). In each experiment, a normal control animal was studied stimultaneously.

Preparation of myocardial fractions. All preparative procedures were performed at 0–4°. Animals were sacrificed following administration of 30 mg/kg pentobarbital intraperitoneally. Myocardial homogenates were prepared as follows: The heart was rapidly excised, blotted, immersed in 0.25 *M* sucrose, weighed, minced to a fine pulp with a scissors, passed through a precooled stainless steel tissue press (Harvard Apparatus Company) with 1-mm holes, homogenized in 15

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TABLE I. Adenyl Cyclase Activity in Particulate Fractions from Cat Myocardium.[a]

	Basal	Epinephrine activated[b]	Fluoride activated[b]
Control	30.8 ± 3.2 (5)	103.5 ± 11.7 (4)	108.3 ± 10.5 (7)
Hyperthyroid	26.3 ± 1.5 (6)	104.8 ± 11.0 (5)	102.0 ± 8.3 (7)

[a] Means ± standard error of the mean of the initial rate of accumulation of cyclic-AMP expressed as picomoles/mg of protein/minute. The numbers in parentheses indicate the number of experiments. Determinations were done in duplicate.

[b] The reaction medium included 10^{-4} *M* epinephrine bitartrate or 10^{-2} *M* sodium fluoride.

vol/g of 1 m*M* $MgSO_4$, 2 m*M* glycylglycine, pH 7.5, in a VirTis "45" homogenizer with two 15-sec bursts at a speed setting of 5 and subsequently by hand in a Dounce homogenizer. The crude homogenate was filtered through two layers of 60 mesh cheese cloth. A particulate fraction was obtained by centrifugation at 2000*g* for 15 min followed by two washes with 10 ml of homogenizing medium.

Biochemical determinations. Protein was determined in the crude homogenate and the particulate fraction by the standard Lowry procedure (16) in which collagen is not solubilized. Corresponding control and experimental fractions were adjusted to contain equal protein concentrations.

Adenyl cyclase activity was measured by the method of Krishna *et al.* (17) exactly as previously described (18, 19). In this procedure enzyme activity is assayed by the rate of synthesis of labeled cyclic-AMP from labeled precursor ATP. The accumulated product is separated from other components in the reaction medium by barium–zinc precipitation and chromatography on Dowex-50. Since the corresponding proportions of protein and of adenyl cyclase recoverable in the particulate fraction were the same in experimental and control preparations, and since the data obtained using the particulate fraction are less likely to be influenced by ATPase activity and other tissue constituents, assays are routinely performed using the particulate fraction. In all experiments typical kinetics were observed and reactions were linear for at least 4 min (18).

Phosphodiesterase activity was measured in the crude homogeneate as previously described (18). Hydrolysis of cyclic-AMP was determined by the change in absorbance at 260 mμ of supernatant fractions of reaction media after precipitation of substrate and protein with barium and zinc. Reactions were linear for at least 15 min.

Results. The major finding in this study is that adenyl cyclase activity is not enhanced in particulate fractions from hearts of hyperthyroid cats (Table I). Similar results were observed when activity was measured using whole homogenates. Basal activity, activation by epinephrine, and activation by fluoride were similar in experimental and control preparations, and reactions from both preparations showed similar kinetics. Cyclic-AMP was accumulated linearly for 4 min and subsequently at a progressively slower rate, probably because of the interfering action of phosphodiesterase or adenosinetriphosphatase included in the particulate fraction.

Assays of phosphodiesterase in whole homogenates showed no significant differences in activity in thyrotoxic compared with control tissue. With homogenate containing 1 mg of protein in a reaction volume of 1 ml, the change in absorbance due to hydrolysis of cyclic-AMP measured after barium–zinc precipitation averaged 0.026/min in control ($N = 7$) and 0.027/min for experimental groups ($N = 8$). Thus, the data indicate that alterations in the activity of the enzymes regulating synthesis and degradation of cyclic-AMP do not occur in broken cell preparations derived from the myocardium of hyperthyroid animals.

Discussion. The evidence obtained in the present study suggests that myocardium from thyrotoxic animals is not characterized by altered capacity to synthesize or degrade cyclic-AMP. This is of particular interest since it has been reported that adenyl cyclase activity can be enhanced by thyroid hormone *in vitro* (20). However, definite effects of

thyroid hormone on the synthesis or degradation of cyclic-AMP *in vivo* have not been documented. Furthermore, addition of thyroid hormone *in vitro* may produce changes which are difficult to interpret and which may not be relevant to physiologic effects of the hormone (21).

There is considerable physiologic and pharmacologic evidence to support the findings of the present study. Augmentation of cardiac muscle contractility, reflected by increased maximum velocity of shortening has been demonstrated in myocardium depleted of catecholamines (6) and enhanced automaticity has been observed in myocardium isolated from thyrotoxic, reserpinized animals (22). In carefully controlled studies, cardiac supersensitivity to adrenergic stimulation has not been found (23) and, in man, beta-adrenergic blockade does not uniformly lower the heart rate of thyrotoxic patients (24). Thus, the present findings and those of several previous investigators support the view that the capacity to synthesize cyclic-AMP in response to catecholamines is not increased in thyrotoxic myocardium. It is interesting that even in the denervated heart, a situation in which there is characteristically altered autonomic reactivity, both adenyl cyclase and phosphodiesterase activity are normal (18).

Summary. Adenyl cyclase and phosphodiesterase activity were measured in corresponding myocardial fractions from hyperthyroid and euthyroid cats. Activity of adenyl cyclase under basal conditions or with epinephrine or fluoride as activators, and phosphodiesterase activity were the same in experimental and control preparations. Thus, the enzymes responsible for synthesis and degradation of cyclic-AMP exhibit normal activity in myocardium from hyperthyroid animals.

Addendum. While this manuscript was in preparation, an abstract reporting similar effects of norepinephrine on adenyl cyclase activity of heart particulate fractions from hyperthyroid animals appeared [Skelton, C. L., Levey, G. S., and Epstein, S. E., Clin. Res. **17**, 264 (1969)]. Results concur with those of the present investigation and demonstrate normal myocardial adenyl cyclase activity in tissue fractions derived from hyperthyroid animals.

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