Stimulation of Cardiac Myosin Adenosine Triphosphatase in Thyrotoxicosis

Eugene Morkin

There is one malady which I have in five cases seen coincident with what appeared to be enlargement of the heart... The malady to which I allude is enlargement of the thyroid gland.

C.H. Parry

THE striking cardiovascular manifestations of thyrotoxicosis have attracted the interest of clinicians and researchers for many years. Caleb H. Parry (1785) probably was the first to note an association between enlargement of the thyroid and enlargement or palpitations of the heart. It is now known that excess thyroid hormone, either produced endogenously or administered exogenously, produces hypermetabolism and thus places greater demand on the heart for tissue perfusion. The resulting increases in coronary blood flow, myocardial oxygen consumption, total cardiac output, and work are well documented (Rowe et al., 1956; Graettinger et al., 1959). In addition to the requirements placed on the heart by the peripheral tissues, the metabolism of the heart itself is directly stimulated by thyroid hormone. Oxygen consumption (Skelton et al., 1970) and protein synthesis (Michels et al., 1963; Cohen et al., 1966) are elevated in excised cardiac tissue in response to thyroxine.

The metabolic changes in thyrotoxicosis are accompanied by augmentation of the contractile performance of the heart (Buccino et al., 1957). The basis for these effects of thyroid hormone is unclear, but new biochemical evidence has appeared recently that may explain the augmentation in myocardial performance, that is, an increase in myosin ATPase activity. Preliminary reports (Thyrum et al. 1970; Kuczynski, 1973) of increased cardiac myosin ATPase activity in thyrotoxic animals have been confirmed and extended (Yazaki and Raben, 1975; Katagiri et al., 1975; Banerjee et al., 1976). In addition, there now is evidence that this increased activity is the result of a change in myosin isozymes (Flink and Morkin, 1977). Before discussing these new findings, it may be helpful to review the effects of excess thyroid hormone on the performance of the heart, as well as the structure of myosin and its role in the contraction process.

Effects of Thyrotoxicosis on Myocardial Performance

In cat papillary muscle, Buccino et al. (1957) found that the most notable effect of thyroid hormone was an increase in the velocity of shortening and in the rate of tension development. These findings occurred in the presence and absence of intact epinephrine stores and over a wide range of temperatures and contraction frequencies. The inotropic response to norepinephrine in muscles varied inversely with the level of the thyroid hormone effect and allowed muscles from hyperthyroid, euthyroid, and hypothyroid hearts to reach a common level of isometric tension, regardless of the thyroid state. Similar findings were later reported by Pannier (1968). In rat papillary muscle, Korecky and Beznak (1971) recorded small increases in rate of tension development and shortened time to maximum tension, but noted that maximum tension decreased as the time-to-peak tension became more abbreviated. The authors concluded that the species variation in the mechanical response to thyroxine was related to the intrinsic heart rate. In species with fast heart rates, like the cat, the lowered tension was the result of failure of contraction velocity to increase in proportion to the decrease in the duration of contraction. In species with slower heart rates, such as the rat, the effect of thyroxine on the velocity of contraction was more prominent than on force. Similar studies on the force-frequency relationship were made by Murayama and Goodkind (1968) in the guinea pig, and again it was observed that the changes were independent of norepinephrine stores. Taylor et al. (1969) obtained...
the same results in the dog, and similar responses have been found in hyperthyroid human subjects (Amidi et al., 1968; Grossman et al., 1971).

These changes in cardiac performance are not dependent on the development of cardiac hypertrophy. An increase in both heart weight and heart weight-body weight ratio occurs frequently during the first few weeks that thyroid hormone is administered to experimental animals (Banerjee et al., 1976; Korecky and Beznak, 1971). Even though precordial hyperactivity may give the impression of cardiac enlargement, the absolute heart weight usually is normal or only slightly increased in chronic experiments on animals (Taylor et al., 1969) and in man with uncomplicated thyroid hyperfunction (Friedberg and Sohval, 1937). Nevertheless, the speed of contraction is increased and the time of tension development is shortened in both early (Buccino et al., 1967; Pannier, 1968; Korecky and Beznak, 1971; Murayama and Goodkind, 1968) and well-established thyrotoxicosis (Taylor et al., 1969; Amidi et al., 1968; Grossman et al., 1971). Clinical experience suggests that cardiac function may be maintained at above normal levels for many years, unless there is advanced age or coincident cardiac disease (Hurzhal, 1940; Sandler and Wilson, 1959).

**Myosin Structure and Function**

According to current views, myosin from mammalian striated muscle consists of two large subunits, each having a molecular mass of about 210,000 daltons. The two heavy chains twist together to form an α-helical rod region, referred to as the “tail” of the molecule, and separate into two globular “head” regions. Each head region also contains a number of noncovalently attached low molecular weight subunits, referred to as light chains. In ventricular cardiac myosin, there are two light subunits with a molecular mass of about 22,000 daltons (light chain 1) and 18,000 daltons (light chain 2), respectively. These light subunits may be involved in regulation of myosin ATPase activity (Wagner and Weeds, 1977). Light chain 2 contains the high affinity Ca$^{2+}$-binding site of myosin (Werner and Oplatka, 1974). Limited digestion with trypsin cleaves the myosin molecule approximately two-thirds of the way along the tail into two fragments, heavy meromyosin (HMM), consisting of the heads attached to a shortened tail, and light meromyosin, consisting of the remainder of the tail. Further digestion of HMM releases the individual heads (S-1) of molecular mass about 62,000 daltons. Both the ATPase and actin-binding properties are present in S-1.

It is now known that myosins isolated from fast and slow skeletal muscles and atrial and ventricular cardiac muscle represent a family of isozymes (Sarkar et al., 1971; Long et al., 1977; Nakamura et al., 1971; Huszar and Elzinga, 1972). Although these myosins are similar in overall dimensions and enzymatic properties, they differ in the composition of both their light and heavy chains. These observations support the view that, with increasing physiological demand for specialized muscle structure and function, a large number of structural genes coding for various heavy and light chains of myosin have evolved. In mature striated muscle, there are at least ten functionally related but different genes for myosin light chains and a minimum of four genes coding for myosin heavy chains. Genes coding for additional isozymes of myosin may be expressed in developing skeletal muscle and heart (Sreter et al., 1975; Pelloni-Müller et al., 1976).

Myosin plays a central role in the contraction process. The sarcomere, which is the fundamental contractile unit of cardiac muscle, consists of an interdigitating array of thick and thin filaments. The thin filaments are constructed from repeating globular actin monomers that are arranged like a double strand of pearls twisted on their long axis. The three troponin components and tropomyosin, proteins that are involved in the regulation of contraction, lie in a longitudinal groove on the thin filament. In the thick filament, the tails of the myosin molecules twist together to form a rigid backbone, from which the globular heads of myosin project outward and contact the actin filaments. The projections, or crossbridges, are now known to move and are believed to cause the sliding between the thick and thin filaments during contraction. The contraction cycle is thought to be initiated when calcium released during electrical excitation is bound by one of the troponin components (troponin-C). This produces a conformational change along the thin filament that allows myosin and actin to interact. The crossbridges are set in motion and the concurrent splitting of ATP furnishes the energy for myocardial contraction.

Beginning with Bárány (1967) it has been appreciated that there is a close correlation in different types of skeletal muscles between the maximum velocity of shortening and myosin ATPase activity. Although mature ventricular myocardium is composed of a single fiber type, myosins isolated from the heart of different species vary considerably in ATPase activity. The ATPase activity of common laboratory animals, arranged in order of decreasing activity, is rat, guinea pig, dog, and rabbit (Delcayre and Swynghedauw, 1975; Yazaki and Raben, 1974). In accordance with Bárány's hypothesis, the ATPase activity from different species correlates well with the maximum velocity of myocardial muscle shortening (Delcayre and Swynghedauw, 1975).

**Effects of Thyroid Hormone on Myosin ATPase**

Lifschitz and Kayne (1966) reported that cardiac myofibrillar ATPase activity is depressed in thyroidectomized and hypophysectomized rats and could be returned to normal by replacement with thyroid hormone, but not with growth hormone. The changes in ATPase activity in these studies
were small (about 15%), and the contribution of mitochondrial enzymes to the total ATPase activity was not evaluated. Subsequently, Thyrum et al. (1970) reported that administration of thyroxine to guinea pigs increases myosin Ca\(^{2+}\)-stimulated ATPase activity by about 30%. They also found that the helix content of myosin, as determined by optical rotation measurements, was increased from 43% to 55%, and there was an increase in the “helix-promoting” amino acids, lysine and aspartic acid, and a decrease in the “helix disturbing” amino acids, threonine and serine. They suggested that the administration of thyroxine stimulated the synthesis of a new type of cardiac myosin or a new protein which was intimately associated with cardiac myosin.

Goodkind et al. (1974) studied the relationship between changes in contractility and myosin ATPase activity in guinea pigs. Myosin and myofibrillar ATPase activities were normal on days 1–3 and increased on days 8–21. Developed tension and maximum rate of rise of tension during isometric contraction increased within 24 hours following thyroxine administration; after 8 days, the time-to-peak tension had shortened although developed tension had returned to normal. Thus, increased myosin ATPase activity after thyroxine administration appeared to correlate best with decreased time-to-peak tension, a parameter related to the velocity of contraction. The authors suggested that the initial contractile response might be an effect of thyroxine on excitation-contraction coupling.

Rovetto et al. (1972) studied the effects of hypophysectomy and replacement with thyroid hormone and growth hormone on cardiac myosin ATPase activity in the rat. In keeping with earlier observations (Lifschitz and Kayne, 1966) on myofibrillar ATPase, they found that myosin Ca\(^{2+}\)-ATPase activity decreased after hypophysectomy and could be restored to normal by thyroxine administration, but not by growth hormone. The (NH\(_4\))\(^{+}\) and K\(^{+}\)(EDTA)-ATPase activities did not vary with the hormonal status of the animal, and administration of thyroxine to euthyroid animals did not affect ATPase activity. They also observed that rat cardiac Ca\(^{2+}\)-ATPase activity was much higher than values reported for other mammalian species and that sulfhydryl modification did not enhance this activity.

Recent publications from two laboratories (Yazaki and Raben, 1975; Katagiri et al., 1975; Banerjee et al., 1976) have clarified the influence of excess thyroxine on myosin ATPase activity. These results may be summarized as follows: First, the extent of activation of myosin ATPase induced by thyroid hormone is species specific and inversely proportional to the level of activity in the euthyroid state. Thus thyroxine administration has little influence on rat cardiac myosin, which normally has high enzymatic activity, but induces a large increase in the activity of myosin from the heart of rabbit and guinea pig, which have lower basal activities. Second, treatment with thyroid hormone increases the ATPase activity of myosin that is stimulated by divalent metal cations (Ca\(^{2+}\) and Mg\(^{2+}\)), whereas the activity that is stimulated by monovalent cations (K\(^{+}\) and NH\(_4\))\(^{+}\) is unchanged. This probably can be explained by the observation of Lymn and Taylor (1971), who showed that in the presence of K\(^{+}\) and a divalent metal carbon chelator, such as EDTA, hydrolysis of ATP proceeds by a different reaction pathway than when Mg\(^{2+}\) ions are present. Considering the relatively high Mg\(^{2+}\) concentration (about 1 mM) in the cardiac muscle cell, the K\(^{+}\)(EDTA)-stimulated activity probably is of little physiological importance. Thus thyroxine seems to affect the divalent metal cation-stimulated ATPase activity that is closely linked to the function of myosin in the contraction process. Third, the Ca\(^{2+}\)-ATPase of cardiac myosin from thyrotoxic animals is resistant to stimulation by sulfhydryl modification. In cardiac myosin from euthyroid animals, this activity is stimulated 150–200% upon blocking one rapidly reacting cysteine residue per head, the so-called SHi-thiol, which is thought to be at or near the active site (Flink et al., 1977). By contrast, myosin from thyrotoxic hearts exhibits increased Ca\(^{2+}\)-ATPase activity which is not enhanced further by modifying the SHi-thiols. In addition, this myosin exhibits increased activity toward other 6-amino- and 6-oxy-substituted nucleotide triphosphates, such as CTP and UTP. This alteration in nucleotide specificity is similar to that observed after chemical modification of the SHi-thiols. This evidence suggests that the increase in activity induced by thyroxine may involve the region of the myosin head near the SHi-thiol group. However, the possibility of a simple identity in the conformational states induced by thyroxine treatment and thiol modification is excluded by the fact that the K\(^{+}\)(EDTA)-ATPase activity is markedly reduced by thiol modification but is unaffected by thyroxine treatment. Also, the SHi-thiol groups do not seem to be blocked in myosin in the thyrotoxic animal, since they are readily accessible to modification by sulfhydryl reagents, such as N-ethylmaleimide and iodoacetate (Yazaki and Raben, 1975; Katagiri et al., 1975).

In living muscle, MgATP is hydrolyzed by myosin under the stimulating influence of actin. To establish firmly the physiological significance of the increased ATPase activity of myosin from the hearts of thyrotoxic animals, it is therefore necessary to examine the activity of natural or synthetic actomyosin from such hearts. Initial attempts to measure the ATPase activity of cardiac myofibrils or actomyosin from thyrotoxic animals yielded conflicting results. The Mg\(^{2+}\)-ATPase activity of myofibrils from guinea pig hearts was found to be elevated (Goodkind, 1974), whereas the actomyosin ATPase activity from rabbit hearts was reported to be normal (Kuczynski, 1973). The reason for this
discrepancy is uncertain, since the Ca\textsuperscript{2+}-ATPase activity of purified myosin is elevated in both species. Possibly, it may be related to the variability in purity and activity encountered when myofibrils and actomyosin are prepared from heart muscle.

Banerjee and Morkin (1977) studied the Mg\textsuperscript{2+}-ATPase activity of purified cardiac myosin from thyrotoxic rabbits (myosin-T) in the presence of skeletal actin. Their results indicate that in the presence of a saturating actin concentration, the ATPase activity of myosin-T is almost 100% greater than in myosin from normal hearts (myosin-N). After modification of the SH\textsubscript{2}-thiols, the actin-activated ATPase activity of myosins from normal and thyrotoxic hearts is similar. These results further support the idea that the conformation of the enzyme around these thiol groups is probably essential to the increase in activity induced by thyroid hormone. These workers also examined the activity of both myosins in a mixture containing actin and a complex of troponin and tropomyosin, which confers calcium sensitivity on the system. They observed that reconstituted actomyosin-containing myosin from thyrotoxic hearts exhibited the expected increase in activity upon addition of CaCl\textsubscript{2}, thus indicating that the site(s) on myosin-T that participates in the regulation of actin activation is intact.

Further investigations on the steady state kinetics of actin activation of myosin from normal and thyrotoxic hearts were carried out with the HMM subfragment (Banerjee et al., 1977). This subfragment contains both of the enzymatically active head regions of the myosin molecule and is completely soluble under all ionic conditions of interest. The actin-activated ATPase of HMM from normal (HMM-N) and thyrotoxic (HMM-T) hearts shows a simple hyperbolic dependence on actin concentrations (Fig. 1). The results were analyzed by means of a double reciprocal plot (Fig. 2), from which two parameters of actin activation can be derived. The reciprocal of the ordinate intercept is the maximum rate of ATP hydrolysis at infinite actin concentration (\(V_{\text{max}}\)). The reciprocal of the intercept on the abscissa represents the apparent dissociation constant for actin as an activator (\(K_{\text{app}}\)). The reciprocal of \(K_{\text{app}}\) can be used to represent the apparent affinity for actin as an activator. Under physiological conditions (0.15 M KCl, 1 mM MgCl\textsubscript{2}), \(V_{\text{max}}\) for the actin-activated rate of MgATP hydrolysis by HMM-T is about twice the normal value. In addition, the \(K_{\text{app}}\) is about one-half the value found with HMM-N, indicating an increase in the affinity for actin. The salt dependency of \(V_{\text{max}}\) and \(K_{\text{app}}\) for HMM-T is markedly abnormal, resembling the changes in these parameters seen with the single-headed (S-1) myosin subfragment. These alterations in the interaction between HMM-T and actin suggest that there may be a loss of cooperativity between myosin heads.

The increased activity of myosin from thyrotoxic hearts also has been demonstrated by histochemical techniques (Morkin et al., 1977). Using the pH 9.4 myofibrillar ATPase reaction of Padykula and Herman (1955), it is possible to show increased staining in dry frozen sections of papillary muscles from thyroxine-treated rabbits (Fig. 3). Although there is some variability in the intensity of the reaction, sections from thyrotoxic muscles are consistently darker than simultaneously prepared normal tissue.

The mechanism whereby thyroxine modulates the activity of myosin has remained obscure. Other workers (Yazaki and Raben, 1975; Banerjee et al., 1976) have not been able to confirm the abnormalities reported by Thyrum et al. (1970) in the amino acid composition of myosin from thyrotoxic hearts. Methylation of histidine residues or phosphorylation of the protein does not seem to be involved in the action of thyroxine (Banerjee et al., 1976). Also, the electrophoretic mobility of myosin-T light subunit does not appear to be altered (Yazaki and Raben, 1975; Banerjee et al., 1976; Goodkind et al., 1974). However, the technique used in these studies could not have detected more subtle changes in myosin structure. Recently, alterations have been found in the electrophoretic pattern of cyanogen bromide peptides of S-carboxymethylated myosin from thyrotoxic hearts (Flink and Morkin, 1977). Sodium dodecyl sulfate-urea polyacrylamide gels of myosin digests from thyrotoxic hearts contain bands of 22,000 daltons and 13,000 daltons that do not correspond to bands in digests of normal myosin (Fig. 4). Digests of myosin-N contain a band of
about 15,000 daltons that is absent from preparations of myosin-T. Also, there are marked differences between myosin-N and myosin-T in the distribution of radiolabeled cysteine-containing peptides. This change in distribution of radiolabeled thiol peptides does not involve the peptide containing the SH2-thiol group.

Proteins are cleaved by cyanogen bromide at the carboxyl terminus of methionine residues. The alterations in the electrophoretic pattern described above indicate that myosin-T must contain methionine substitutions such that CNBr cleavage results in peptides with different mobilities than found normally. These new peptides are larger in molecular mass than any of the CNBr peptides found in the two light chain species of cardiac myosin (Weeds, 1975; Leger and Elzinga, 1977), and therefore must arise from the myosin heavy chains. Additional studies will be needed to determine whether there also are subtle alterations in light chain structure.

These findings suggest that the effects of excess thyroid hormone on the contractile properties of the heart can be explained, at least in part, by the appearance of a new myosin species having greater actin-activated ATPase activity. Changes in skeletal myosin isozymes have been identified in the modification of contraction velocities that follow cross-reinnervation of a twitch muscle fiber with the motor nerves formerly supplying a tonic muscle fiber and vice versa (Buller et al., 1969; Stréter et al., 1974; Weeds and Burridge, 1975). Similar suggestions also have been made regarding changes in the activity of myosin that accompany cardiac hypotrophy induced by work overload (Swynghedauw et al., 1976), although there is as yet no definite evidence of a structurally different protein.

The appearance of a new myosin species would explain the delay of 3-5 days that has been found between the administration of thyroid hormones and the characteristic increase in myocardial
shortening velocity. The half-life of cardiac myosin is normally about 6–8 days and may be reduced to 3 days following aortic banding (Morkin et al., 1972). Although detailed observations on myosin metabolism during induction of thyrotoxicosis are not available, there is some evidence to suggest that the rate of myosin replacement may be accelerated (Wyborny et al., 1972).

Discussion

The effects of thyroid hormone on myocardial contractile performance may be explained by the appearance of a new myosin isozyme, myosin-T, that has a greater actin-activated ATPase activity than the original myosin. There is evidence to suggest that in other forms of cardiac hypertrophy, such as pressure overload, adaptations in cardiac performance also may be mediated by a change in myosin species. Thus insight gained as to the cellular and molecular adaptations that occur during thyrotoxicosis may have relevance to other forms of heart disease.

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