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The functional significance of altered tension dependent heat in thyrotoxic myocardial hypertrophy*)

Über die funktionelle Bedeutung der veränderten spannungsabhängigen Wärmeproduktion bei der thyreotoxischen Herzhypertrophie

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With 3 figures and 1 table

Summary

Enlargement of the heart secondary to 14 daily injections of L-thyroxine (0.2 mg/kg) in the male albino rabbit results in an increase in actin-activated myosin ATPase, velocity of unloaded shortening and rate of isometric tension development. The contribution of the altered myosin to contractile efficiency is assessed by making temperature measurements on right ventricular papillary muscles from these hearts during isometric contraction. Measurement of tension dependent heat per unit of tension is derived from rapid myothermal measurements of initial heat, tension independent heat and isometric force. The tension dependent heat measurement is used to evaluate the crossbridge cycling *in vivo* and the contribution of the changes in the pattern of crossbridge cycling to the efficiency of tension development. In the thyrotoxic heart the peak twitch tension is 72% of normal while the tension dependent heat per unit tension is 175% of normal. This *in vivo* measurement correlates well with changes in *in vitro* actin-activated myosin ATPase measurements. Analysis of the data in terms of a model derived from rapid enzyme kinetics and mechanical transient analysis indicates that crossbridges from the thyrotoxic hypertrophy myosin cycle faster and stay attached to actin for a shorter period of time than normal myosin. This alteration in cycling pattern may be the basis for the inverse relationship between myosin ATPase activity and the efficiency of tension development.

When the heart enlarges in response to a thyroid stress there is an increase in actin-activated myosin ATPase activity (1, 2). It is important to know if the in vitro change in the splitting of ATP by actomyosin is a reflection of the in vivo behavior of the myosin molecules when they are present in thick filaments associated with actin in an organized pattern. Furthermore analyses which provide data relating to the *in vivo* splitting of ATP by actomyosin during tension development should provide infor-

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mation concerning the myosin crossbridge cycling pattern and its contribution to the energetics of tension development.

The strategy is to evaluate the intracellular behavior of myosin by using rapid myothermal methods on normal rabbit hearts and those with thyrotoxic hypertrophy. With these techniques the initial heat can be partitioned into a tension dependent and a tension independent portion. The former is a measure of ATP splitting by actomyosin during tension development and reflects myosin crossbridge cycling. It thus should provide information about *in vivo* myosin ATPase activity and energetics during tension development.

Methods

Animal model

All experiments are performed on right ventricular papillary muscle preparations from male albino rabbits. Thyrotoxic hypertrophy is induced by 14 daily injections of L-thyroxine (0.2 mg/kg) after which the rabbits are sacrificed, the hearts removed, placed in oxygenated Krebs-Ringer, and the papillary muscle is isolated and mounted on the thermopile as described below.

Myothermal and force measurements

Right ventricular papillary muscles are excised and mounted on vacuum deposited thermopiles as previously described (3). The cut end of the muscle is attached to an isometric force transducer while the tedinous end is anchored to a stationary hook. Close contact with the thermopile elements is insured by a tether. The thermopile assembly is placed inside a chamber allowing the muscle to be bathed in oxygenated Krebs-Ringer solution or surrounded by moist gas. The entire myothermal assembly is placed in a 70 liter constant temperature bath where equilibration is carried out at 21 °C for two hours. The muscle is stimulated at 0.2 Hz and the

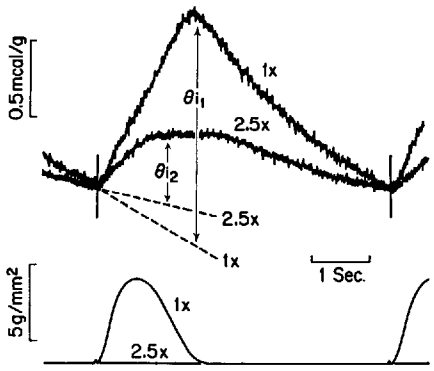

Fig. 1. Temperature and force changes in a control papillary muscle incubated in normal Krebs solution (1X) and hyperosmotic mannitol-Krebs solution (2.5X). The upper trace is the temperature and the lower trace the force records. The stimulus marker is indicated in the temperature record by the vertical line preceding the temperature change and in the force record by the deflection preceding the force change. See text for a description of the method used to calculate the initial, tension dependent and tension independent heats.

experiments are accepted only if the muscle heat and tension performance are stable over the entire experimental period and isometric twitch tension is at least four times greater than resting tension.

Tension dependent heat measurements

The temperature of a contracting muscle is determined by evolution of resting heat, initial heat and recovery heat. When rapid myothermal techniques are used the temperature oscillation which occurs with each beat is made up of the initial heat and the recovery heat (fig. 1). Temperature changes associated with the initial heat liberation are corrected for heat loss by the extrapolation procedures shown by the dashed line in figure 1 labeled 1X. The dashed line is obtained by translating the cool off curve which occurs upon cessation of stimulation. The corrected temperature change, θ_{il}, is converted to the corresponding heat evolution by multiplication with the effective heat capacity of the muscle plus adhering Krebs solution determined as previously described (3). The initial heat consists of the sum of the tension dependent and tension independent heats. The former represents crossbridge cycling during tension development and hence *in vivo* actomyosin ATPase activity. The latter represents the initial energy cost of excitation and the translocation of calcium during contraction and relaxation. Tension independent heat is obtained by measuring the triggerable heat output when tension is eliminated by incubating the muscle in hyperosmotic (2.5 X N) mannitol-Krebs solution (Θ_{12}, fig. 1) and multiplying this temperature by the effective heat capacity of the muscle and adhering *Krebs* solution. The tension dependent heat is the difference between initial heat and tension independent heat.

Results

General

The right ventricular heart weight : body weight ratio increased by 197% in the thyrotoxic hypertrophy (table 1). There was no significant change in the cross-sectional area of the papillary muscles or in the dry wet weight ratios of the papillary muscle and liver (table 1).

Mechanical and thermal measurements

The peak isometric twitch tension was 5.8 ± .3 (SEM) g/mm^2 for the control muscles. The thyrotoxic preparations had an isometric twitch tension which was 72% ($p < .05$) of the control. The tension dependent heat per unit of tension developed was 2.08 ± .21 and 3.65 ± .18 μCal/g cm for the control and thyrotoxic preparations, respectively (N = 8).

Discussion

Hypertrophy of the myocardium which occurs following prolonged thyrotoxic stress permits the heart to meet the additional demands for

Table 1. Thyrotoxic hypertrophy.

	RV/Body wt g/kg	X Sec RV PAP M mm^2	Dry/wet wt PAP M	Dry/wet wt liver
Normal	0.31 ± .01	0.59 ± .04	0.23 ± .006	0.28 ± .01
Thyrotoxic	0.61 ± .02	0.66 ± .06	0.24 ± .005	0.25 ± .01
	$p < .002$	N. S.	N. S.	N. S.

increased cardiac output placed on it. The change in performance is not simply the result of arithmetic addition of muscle mass. There is an alteration in the quality of performance of each muscle unit. In previous studies of pressure overload hypertrophy we reported a depression in mechanical V_{max} (4), an increase in time to peak tension (4), a depressed myosin ATPase activity (5, 6) and a depressed heat production per unit of tension (7). The present studies on thyrotoxic hypertrophy are a logical extension of the pressure overload experiments in that the changes are generally in the opposite direction. Thus the velocity of unloaded shortening and rate of isometric tension development increase while the time to peak tension decreases. In addition the actin-activated myosin ATPase from these hearts is increased (1, 2). The goal of these experiments is to use high speed myothermal measurements to analyze the contribution of the altered contractile proteins to the energetics of force development. The tension dependent heat results from the splitting of ATP during crossbridge cycling and the associated tension development. In the thyrotoxic preparation the tension dependent heat rate is 175% of control.

These results can be interpreted in terms of the rapid enzyme kinetic (8) and mechanical tension transient (9) hypotheses of crossbridge cycling rates and tension development (fig. 2). At rest myosin crossbridges and actin do not interact with each other. In the activated tension producing part of the cycle the actomyosin crossbridge system exists in two distinct states, where the actin and myosin crossbridges are dissociated (fig. 2, top) or associated (fig. 2, bottom). In the former, i.e., the dissociated or "off" state, there is a rate limiting step at arrow 1 between the two conformational states M** ADPPi and M*ADPPi. This rate limiting step probably plays the major role in determining the overall cycling rate and is characteristic of each type of myosin. In the associated or "on" state, force is believed to be generated when the crossbridge head rotates from the 90° ($AM^{*}ADPPi$) to the 45° (AM ADPPi) position stretching the compliant elements in the myosin molecule. In this scheme the length of time during which the myosin head is attached in a position less than 90° (i.e. between 90° and 45°) and the stiffness of the compliant element determine the integrated force developed during a single cycle. The time course of the

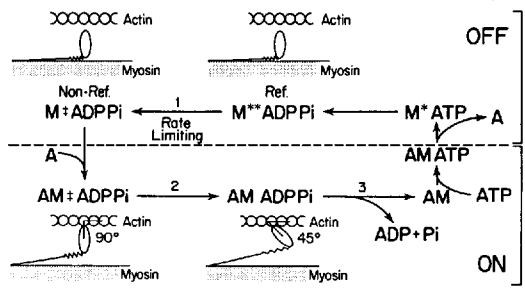

Fig. 2. Actin-activated myosin kinetics and crossbridge behavior. The following symbols are used: M, myosin; A, actin; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Pi, inorganic phosphate; M*, M**, different conformational states of myosin (redrawn from *Eisenberg* and *Hill*, 1978 (8).

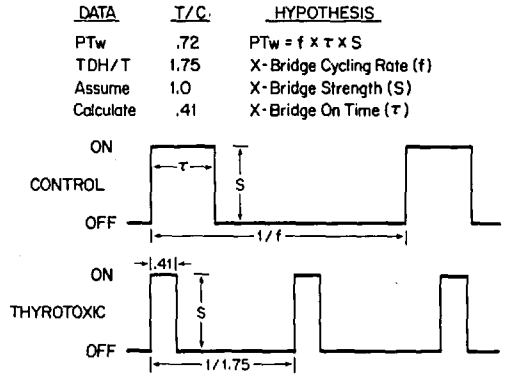

Fig. 3. The "on-off" cycle of crossbridges during activation for the control and thyrotoxic preparations (see text for discussion).

movement along arrows 2 and 3 (fig. 2) is also a characteristics of each type of myosin.

Thus the heat production associated with tension development can be analyzed in terms of "on" time and cycling rate (fig. 3). As a first approximation in quantifying the process of chemomechanical transduction consider the following model. Assume that the peak twitch is a function of the integrated rate at which the crossbridges cycle (f), average time each crossbridge is attached and in the tension producing configuration (τ) and the average stiffness of the crossbridge compliant element (S).

If the tension dependent heat is an index of the crossbridge cycling rate, and the strength of each myosin crossbridge is unity then the on time and cycling rates for the thyrotoxic preparation relative to control can be calculated. The thyrotoxic preparation develops force less efficiently than the control because of an increase in the cycling rate (f) and a decrease in the "on" time τ (fig. 3). Thus the efficiency of force development is inversely related to tension dependent heat or actin-activated myosin ATPase activity (1, 2). The ability of the heart to alter the structure of the myosin molecule synthesized in response to thyrotoxic stress appears to alter the rate limiting step of the actomyosin ATPase cycle (fig. 2, arrow 1) as well as the tension producing "on" time (fig. 2, arrows 2 and 3).

The combination of rapid myothermal measurements with high speed mechanical transient and enzyme kinetic analyses may provide additional data for testing the newer models of crossbridge behavior such as those proposed by *Eisenberg* and *Hill* (8), *Lymm* and *Taylor* (10), *Huxley* and *Simmons* (9), and *Podolsky* and *Nolan* (11).

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Zusammenfassung

Eine Herzvergrößerung beim Kaninchen als Folge von L-Thyroxin-Injektionen (täglich 0,2 mg/kg über 14 Tage) führt zu einer Steigerung der aktinaktivierten

Myosin-ATPase, der Verkürzungsgeschwindigkeit bei Nullast und der Geschwindigkeit der isometrischen Spannungsentwicklung. Durch Temperaturmessungen an rechtsventrikulären, isometrisch schlagenden Papillarmuskeln solcher Herzen wird der Beitrag des veränderten Myosins zum kontraktilen Wirkungsgrad bestimmt. Die spannungsabhängige Wärme pro Spannungseinheit ergibt sich aus schnellen myothermalen Messungen der Initialwärme, der spannungsunabhängigen Wärme und der isometrischen Kraft. Auf der Grundlage der spannungsabhängigen Wärme wird die Querbrückenkinetik *in vivo* bestimmt und untersucht, inwieweit Änderungen der Querbrückenkinetik zum Wirkungsgrad der Spannungsentwicklung beitragen. Im thyreotoxischen Herzen beträgt die maximal entwickelte Spannung 72% gegenüber den Kontrollen, während die spannungsabhängige Wärme pro Spannungseinheit 175% ausmacht. Diese *In-vivo*-Messungen stimmen mit Änderungen der *in vitro* gemessenen aktinaktivierten Myosin-ATPase überein. Eine Analyse dieser Daten anhand eines Modells, welches aus der Analyse von schneller Enzymkinetik und mechanischer Transienten entwickelt wurde, ergibt, daß der Querbrückenzyklus des thyreotoxisch-hypertrophierten Myosins im Vergleich zum normalen Myosin schneller abläuft und die Querbrücke für kürzere Zeit an das Aktin angeheftet ist. Diese Änderung des Zyklusablaufs könnte die Grundlage für eine inverse Beziehung zwischen der Myosin-ATPase-Aktivität und dem Wirkungsgrad der Spannungsentwicklung bilden.

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