Replacement of Myosin During Development of Cardiac Hypertrophy

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ABSTRACT

Metabolism of cardiac myosin was studied in unoperated rabbits and after banding of the ascending aorta. Incorporation of amino acid into myosin was determined 2 to 18 days after bandoing or sham operation by injecting \(^{4}\text{H-lysine (0.5 mCi/kg)}\) four hours before the animals were killed. The same dose of \(^{4}\text{H-lysine was injected 24 hours before surgery, and disappearance of isotope from myosin was measured after 3 to 16 days. Incorporation of \(^{4}\text{H-lysine during a four-hour labeling period was studied in unoperated animals to serve as a baseline. Disappearance of labeled myosin also was studied in unoperated animals, some of which received daily injections of unlabeled lysine to reduce isotope reincorporation. Specific radioactivity of free lysine and myosin was determined in left ventricular samples from each animal. The experimental results indicate that (1) the half-life of cardiac myosin is normally about six to eight days; (2) synthesis of left ventricular myosin was greater after banding, reaching a maximum increase on the tenth day after operation; and (3) the decrease in specific radioactivity of myosin in rabbits with coarctation was slower than in sham-operated animals. The experimental results were analyzed by a computerized simulation of myosin metabolism which allowed the rate constant for myosin degradation to be calculated on successive days after coarctation. The best fit to the observed changes in myosin content was obtained when degradation was increased in parallel with the increase in synthesis; when degradation was assumed to be unchanged from sham-operated animals or was decreased, myosin content was overestimated. These calculations suggest that the slower disappearance of myosin in banded animals may be the result of preferential reutilization of \(^{4}\text{H-lysine arising from protein catabolism. It is concluded that the myosin content is increased during hypertrophy, primarily as a consequence of increased synthesis.

KEY WORDS

protein degradation
coarctation of aorta

\(^{4}\text{H-lysine}
protein turnover
aortic stenosis

protein synthesis
radioactive myosin

Cardiac hypertrophy induced by work overload has been used extensively as a model for studies on the pathogenesis of heart failure. Following an increase in work load, the heart undergoes a series of metabolic adjustments, including changes in the production and breakdown of myocardial proteins. According to most workers who have examined this problem, amino acid incorporation into myocardial proteins is increased. However, there is no unanimity of opinion as to the effects on protein degradation. It has been reported that degradation of myocardial proteins is increased,\(^1\) unchanged,\(^2\) and decreased\(^3\) in response to augmentation of work load.

Interpretation of the data presented in many of the studies on metabolism of myocardial proteins is uncertain for one or more of the following reasons:

1. The myocardium is composed of several cell types, including muscle cells, fibroblasts, and vascular endothelium, all of which may alter their synthesis of proteins to varying degrees in response to work overload. About 75% of the cells in the heart are fibroblast or vascular endothelium, and only about 25% are muscle cells.\(^4\) This important fact has frequently been overlooked because the muscle cells are large and occupy about 75% of the volume of the myocardium.

2. Measurements of protein synthesis generally have been made without adequate consideration of the effects of changes in organ weight or variations in specific radioactivity of precursor amino acids.

3. Degradation of myocardial proteins usually has been inferred from their isotope decay curves without adequate consideration of the influence of changes in protein synthesis or recycling of labeled amino acids.

We have studied the replacement of left ven-
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tricular myosin after aortic coarctation in rabbits as a model for alterations in myocardial protein metabolism in response to the stress of chronic work overload. In this presentation I briefly summarize the data indicating that cardiac myosin is rapidly replaced during development of hypertrophy as the result of both increased synthesis and increased degradation. These results suggest an unexpected lability of the contractile proteins within the heart which may be of importance in physiological adaptations to stress.

Methods

ANIMAL EXPERIMENTS

Experiments were performed on male albino rabbits (2.74 to 3.20 kg). Coarctation was produced in 71 rabbits by placing an aluminum band around the ascending aorta so as to reduce the diameter by 65%. Sham operations were performed in 54 animals by placing a loose fitting band around the aorta.

Incorporation of amino acids into left ventricular myosin was measured 2, 3, 4, 9, and 15 days after operation by injecting 0.5 mCi/kg [3H]-lysine HCl intravenously. Four hours later, the animals were killed by a blow to the head, and samples of left ventricular muscle were taken for measurement of the specific activity of free lysine and myosin. The rate of disappearance of radioactivity from free lysine and myosin was determined on a separate group of animals by injecting the same dose of isotope one day before either sham operation or aortic coarctation. The animals were operated on the next morning and killed after 3, 5, 7, 10, or 15 days.

Myosin was isolated by the method of Katz et al.4 For measurement of specific radioactivity, freshly prepared myosin was precipitated with trichloroacetic acid, washed in ether twice, dried, and hydrolyzed in 6 N HCl. Free lysine was extracted from tissue and plasma in 1% picric acid as described by Tallan et al. 4

The specific activity of lysine in tissues and myosin was determined by high voltage electrophoresis according to the technique of Blackburn. 5 Electrophoresis was performed on Whatman 3 MM paper using pyridine-acetate buffer (pH 6.5) at 3 kV for two hours. The paper was stained with cadmium-ninhydrin reagent and the spots eluted in methanol. The absorbance was measured at 505 μm in a Gilford model 200 spectrophotometer, and the radioactivity was determined by liquid scintillation counting.

CALCULATIONS

Incorporation of lysine into total left ventricular myosin during a four-hour labeling period, normalized for body weight, was used as an index of myosin synthesis:

\[ \epsilon = a_m n_1 M/W \] (1)

where \( \epsilon \) = index of myosin synthesis (dpm/kg), \( a_m \) = specific radioactivity of lysine incorporated into myosin (dpm/μmole), \( n_1 \) = lysine content of myosin (μmole/μmole), \( M \) = myosin content of the left ventricles (μmole), and \( W \) = body weight (kg). The concentration of lysine in myosin (82 μmole/10^6 g) and the concentrat-

tion of myosin in the ventricle (45 mg/g wet weight) were taken to be constant.

The experimental data can be used to make a kinetic analysis of myosin replacement. Considering the LV as a whole, changes in radioactivity can be written as:

\[ \frac{dC}{dt} = m_1 a_1 - k M n_1 a_m \] (2)

where \( C \) = radioactivity of myosin (dpm), \( \nu \) = myosin synthesis (μmole/day), \( k \) = rate constant for myosin degradation (day^{-1}), and \( a_1 \) = specific radioactivity of free tissue lysine (dpm/μmole).

The rate of change of the amount of myosin in left ventricles can be shown as:

\[ \frac{dM}{dt} = \nu - k M \] (3)

In equations 2 and 3 the rate of myosin synthesis, \( \nu \), the rate constant for myosin degradation, \( k \), and the amount of myosin, \( M \), were all taken to be functions of time. The hearts of unoperated animals at time zero were assumed not to be growing, so that \( m_0 = k_0 M_0 \). The rate constant for myosin degradation at time zero, \( k_0 \), was taken to be 0.00 day^{-1}, allowing \( m_0 \) to be calculated from equation 3. On subsequent days during the experiment, myosin synthesis was taken to be proportional to measured values of the index of myosin synthesis, \( \epsilon \). Variations with time in the rate constant for myosin degradation, \( k \), were chosen to give the best fit to the observed changes in myosin content. Solution of these equations was facilitated by use of MIMIC, a computer language particularly suitable for solving differential equations. Experimentally determined values for \( a_1, \nu \), and \( M \) were entered directly into the computer, and values at intermediate times were obtained by linear extrapolation.

RESULTS

LEFT VENTRICULAR PRESSURES AND WEIGHT

The changes in left ventricular systolic and diastolic pressures after aortic coarctation are shown in the upper part of Figure 1. Left ventricular peak systolic pressure increased on the average from 97 to 163 mm Hg by the fourth day after operation. The average end-diastolic pressure was elevated (11 mm Hg) after 15 days in rabbits with aortic coarctation.

The ratio of left ventricular weight to body weight is shown in the lower part of Figure 1. There was a small decrease in this ratio during the first four days after surgery, mainly as a consequence of a small (6%) decrease in body weight. In the group of animals with aortic coarctation, the ratio of left ventricular weight to body weight increased to a maximum on about the twelfth day. There was no change in water content of the left ventricle in banded animals.

ESTIMATION OF MYOSIN HALF-LIFE

Figure 2 shows the decay of labeled cardiac myosin in two groups of unoperated rabbits. One
group was labeled with $^4$H-lysine, and the other group received, in addition, daily injections of unlabeled lysine to reduce reincorporation of isotope. In animals receiving a “chase” with unlabeled lysine, the disappearance of free $^4$H-lysine was accelerated about two times, and the decay of labeled myosin was much more rapid. Although myosin decay in these “chase” experiments is not a simple exponential curve, a half-life for myosin of 6.2 days is obtained if only the first part of the disappearance curve is considered. This value corresponds reasonably well with the half-life of 7.5 days for cardiac myosin obtained by correction of myosin decay for isotope reutilization. The decay of myosin in unoperated animals having been established, it proved possible to calculate changes in myosin degradation during development of cardiac hypertrophy by measuring changes in myosin synthesis and content.

**Myosin Metabolism in Hypertrophy**

The experimentally measured and computed changes in myosin metabolism during development of LV hypertrophy are shown in Figure 3. Myosin synthesis, $\nu$, was lower than normal two days after operation, then increased to a maximum on the tenth day (top panel). LV myosin content, $M_t/M_o$, increased about 22% over the course of the experiment (middle panel). The best fit to the observed change in myosin content was obtained when myosin degradation, $k$, was increased in parallel with the increase in myosin synthesis (bottom panel). On the tenth day $k$ reached a maximum of about 0.20 day$^{-1}$, which would correspond to a half-life of 3.3 days ($t_{1/2} = 0.69/k$).

Myosin replacement can be calculated from changes in its synthesis and degradation. As shown in Figure 4, more than 90% of the myosin originally present in the left ventricle is replaced within 15 days after aortic banding.

Since changes in degradation of proteins are usually inferred from their isotope decay curves, it is of interest to compare the experimentally measured and calculated decay of labeled myosin after aortic banding. Experiments in sham-operated rabbits in which $^4$H-lysine was injected one day prior to operation have shown that LV myosin radioactivity reaches a maximum by the second day after operation and then decreases monotonically. In rabbits with aortic coarctation, incor-

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Figure 1: Left ventricular pressures (above) and left ventricular weight/body weight ratios (below) following aortic coarctation (open circles) and sham operation (closed circles).

Figure 2: Disappearance of radioactivity from left ventricular myosin in the rabbit. Closed circles = unoperated animals labeled with $^4$H-lysine (0.5 mCi/kg); open circles = unoperated animals that were labeled with the same dose of $^4$H-lysine and received, in addition, 0.36 μmole/kg nonradioactive lysine each day (lysine "chase").

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poration of $^3$H-lysine increased until the fifth day after banding before beginning to decrease, as shown in Figure 5. The myosin decay curve observed in these animals does not agree with the curve obtained from equation 2. Also, it cannot be explained by slower disappearance of free $^3$H-lysine from banded animals since the disappearance of $^3$H-lysine was virtually the same in banded and sham-operated animals. Possibly, it may be the result of preferential recycling of lysine with high specific radioactivity incorporated into protein early in the experiment and subsequently degraded (see Discussion).

**Discussion**

The results indicate that the half-life for cardiac myosin is normally six to eight days and that, in response to moderate pressure overload, the half-life may shorten to only three days. These values for myosin half-life are much shorter than values of 28 to 30 days obtained for myosin from skeletal muscle. In the past these longer values for myosin half-life in skeletal muscle have led to the erroneous impression that the contractile proteins are relatively inert in all muscles. These results warrant further consideration regarding (1) the relationship of changes in myocardial protein metabolism to cardiac function, (2) evidence of increased amino acid recycling in banded animals, and (3) mechanisms which may initiate increased replacement of myosin and other myocardial proteins.

**Protein Metabolism and Cardiac Functions**

Rapid replacement of myosin may have implications regarding myocardial function. Myofilibrillar, actomyosin, and myosin ATPase activities have been noted to be lower in hypertro-
Phosphorylated and failing hearts, and it has been suggested that this decrease in enzymatic activity may underlie the diminished mechanical performance of such hearts. Considerable evidence has accumulated to indicate that myosins from different muscle types differ in subunit composition and enzymatic activity.\textsuperscript{16,18} The enzymatic activity in skeletal muscles and heart also is subject to physiological and experimental regulation that may involve changes in subunit composition.\textsuperscript{17,18} A possible explanation for the decrease in myosin ATPase activity in ventricles subjected to pressure overload is that the myosin normally present is replaced by a species with lower enzymatic activity. This might represent an adaptive mechanism to allow increased wall tension to be sustained at a lower energy cost. However, no alteration in myosin structure has so far been identified in chronically overloaded hearts.\textsuperscript{19}

From the present results the question also arises as to whether a change in metabolism of the myocardial proteins may be related to the development of cardiac failure in chronic cardiac hypertrophy. Meerson and coworkers\textsuperscript{20} reported several years ago that incorporation of $^{35}$S-methionine into actomyosin and other myocardial proteins (count/min mg$^{-1}$) gradually decreased over many months following aortic banding. They postulated that depressed protein synthesis might be responsible for the ultimate development of cardiac failure in such hearts. This hypothesis seems unlikely since, under conditions of organ growth, measurements of protein specific radioactivity may underestimate the rate of protein synthesis. Newly synthesized proteins that are labeled after isotope injection are diluted by the greater mass of unlabeled proteins. The decrease in specific radioactivity of actomyosin and mitochondrial proteins in the animals studied by Meerson et al.\textsuperscript{20} during the stage of gradual exhaustion and failure of the myocardium (220 to 225 days after banding) was less than the increase in ventricular weight; if the greater weight and protein content of these hearts had been taken into account, protein synthesis would not have appeared to be depressed. Synthesis of contractile proteins need not be depressed for failure to occur in chronic cardiac hypertrophy. De Schryver and Gudbjarason\textsuperscript{21} found that incorporation of $^{35}$S-methionine into left ventricular myosin (count/min mg$^{-1}$) was normal in animals with chronic left ventricular failure following coarctation. If their measurements had taken into account the dilution of the specific radioactivity of newly made myosin by the greater mass of unlabeled myosin, myosin synthesis would have been increased. At present we are unable to implicate a change in protein metabolism as a primary factor in the development of heart failure.

\textbf{Influence of Amino Acid Recycling on Apparent Decay of Labeled Myosin}

The decay of labeled myosin from banded animals showed a marked increase in specific activity four days after banding, even though the specific activity of free lysine in the tissue had decreased to a low level. A simple model, such as represented in equation 2 or model 1 (Fig. 6), in which amino acids are incorporated into protein from the general intracellular pool, clearly will not explain this result. Righetti et al.\textsuperscript{22} have suggested that all amino acids derived from protein breakdown may enter a separate amino acid pool ($\lambda$-amino acid), from which they either enter the general intracellular amino acid pool or are directly reincorporated into protein. Preferential reutilization of amino acids can be represented by increas-
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![Diagram of amino acid metabolism models](image)

**Figure 6**

Schematic representation of two models of myosin metabolism. In model 1 myosin is synthesized from an intracellular amino acid pool into which amino acids arising from protein catabolism mix freely with those entering from the plasma. In model 2 amino acids arising from protein degradation may be either re-incorporated directly into protein or may be reincorporated after mixing into the general intracellular amino acid pool. The disappearance of labeled myosin after aortic banding (Fig. 5) is best explained by model 2 (see text).

The rate constant for reincorporation into protein ($\lambda_2$) with respect to the rate constant for entry into the intracellular amino acid pool ($\lambda_1$). Preferential reutilization of amino acids arising from protein breakdown during work-induced growth in skeletal muscle also may explain the decrease in decay of labeled skeletal muscle proteins made hypertrophic by tenotomy of synergistic muscles.

**INITIATION OF CARDIAC HYPERTROPHY**

The sequence of events whereby work overload produces cardiac hypertrophy is poorly understood. It is recognized clinically that pressure overload of the heart produces greater cardiac hypertrophy than volume overload. Recently, several groups of investigators have attempted to identify the mechanical factors that regulate synthetic processes within the heart. Hjalmarson and Isaksson have studied the effects of pressure and volume overload on myocardial protein synthesis in isolated perfused hearts. When end-diastolic volume was kept constant, pressure overload was found to be a better stimulus to protein synthesis than volume overload. Peterson and Lesch reported that incorporation of amino acid into rabbit papillary muscle is stimulated when the muscles are paced electrically near the peak of their length-tension curve. Development of increased active tension did not seem solely responsible for enhanced amino acid incorporation since similar changes were observed when unstimulated muscles were stretched to comparable tension. These observations are reminiscent of efforts to define the determinants of myocardial oxygen consumption. Tension development, the degree of fiber shortening, and the contractile state of heart muscle have been identified as major factors in the control of myocardial oxygen consumption. Similar mechanical factors may regulate synthetic processes within the heart.

A number of possible mechanisms for translating increased mechanical work into chemical changes might be considered:

1. An increase in tension might produce alterations in the cell membrane that activate membrane enzymes to produce some kind of intracellular messenger. Adenyl cyclase is known to be located in the cell membrane and, when stimulated, produces an increase in intracellular concentrations of cyclic AMP. Several recent reports suggest that cyclic AMP may be involved in the regulation of growth of mammalian cells. The concentration of cyclic AMP in heart muscle during work overload has not been studied.

2. A product of muscular activity might stimulate synthetic processes. Ingwall et al. have reported that addition of creatine, an end-product of muscular contraction, to cultures of embryonic muscle stimulates amino acid incorporation into myosin. It seems unlikely, however, that changes in intracellular creatine are entirely responsible for initiating muscle growth since addition of creatine had much less effect on synthesis of total proteins. Also, extracellular creatine does not seem to exchange directly for intracellular creatine in muscle so that creatine addition may not stimulate creatine accumulation during activity.

3. The energy requirements of the cell may be linked to macromolecular synthesis. Since heart muscle has very limited stores of high energy phosphates, mitochondrial synthesis of ATP must be finely adjusted to myocardial oxygen requirements. Mitochondrial mass is increased during periods of prolonged increase in work load. Experiments in *Drosophila hydei* suggest that the redox state of a part, or the entire respiratory chain, or a deficiency in ATP supply may provide a signal for activation of specific genome loci. The relevance of these observations to the regulation of synthesis of mitochondria and other myocardial constituents is unknown, but these findings illustrate the possibility that changes in respiration may activate synthetic processes at the genome level.

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Discussion

Dr. M. Rabinowitz, Chicago, Illinois: There is one semantic point which we should perhaps clarify, that is, the definition of reutilization. You indicated that reutilization was the shunting of amino acids derived from the degradation of protein into the amino acyl-tRNA pool. The same amino acids entering the total amino acid pool was not considered to be reutilization. It is not yet

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absolutely established that the first process takes place, although we suspect it. Both processes in any case represent reutilization since labeled amino acids derived from protein breakdown reenter protein.

Secondly, what value did you find for myosin content in your experiments? As Dr. Meerson showed many years ago, after constriction of the ascending aorta, the percentage of the cellular volume of the myocardial cell occupied by myofibrils increases. Was this taken into account in your determination of myosin synthesis?

Dr. Morkin: I agree with your comments about reutilization. In model 2 (Fig. 6), amino acids derived from protein catabolism may be reincorporated into protein directly or via the general intracellular amino acid pool. With respect to the second question, we measured the yield of myosin since there is no method by which it can be recovered completely in pure form. The yield in sham-operated and banded animals was the same. However, Page and coworkers have reported that the myofibrillar mass per gram of tissue is increased 12 to 15% in moderate cardiac hypertrophy. If the myosin concentration is increased proportionately, our estimate of the maximum increase in degradation (Fig. 3) should be adjusted downward by about 20%.

Dr. Rabinowitz: The degree of hypertrophy may be important. Dr. E. Page's morphological studies confirmed Dr. Meerson's findings that with severe hypertrophy the proportion of the cell occupied by myofibrils increased within a very few days.

The measurement of the specific activity of amino acyl-tRNA may be rather important in this type of analysis since differences in the synthetic rate in hypertrophied animals was based on the experimental observations that specific activities of the total free amino acid pool were identical in hypertrophy and normal controls. During physiological alterations, such as in hypertrophy, it is quite possible and even likely that there may be a difference in the relationship between the specific activity of amino acyl-tRNA and the total free amino acid pool.

I shall mention a recent finding made by Dr. Ann Martin in our laboratory, in collaboration with Dr. Ira Wool, which explains an observation that was very puzzling for many years. Protein synthesis based on incorporation of labeled amino acids in diaphragms of adrenalectomized rats was always higher than in normal rats. Dr. Wool and his colleagues tried to determine changes in ribosome function, initiation, elongation, etc., and never could find any. Now, Dr. Martin finds that although the total free amino acid specific activities are the same in normal and adrenalectomized diaphragms, there is a twofold difference in the specific activities of the amino acyl-tRNA which accounts completely for the incorporation differences. I think one has to be very wary about using the specific radioactivity of the total free amino acid pool in modeling protein turnover.

Dr. Morkin: I certainly agree with you. Our measurements of myosin synthesis may be affected by nonequilibrium between the free amino acid pool and amino acyl-tRNA. However, if the reason for nonequilibrium relates to increased reutilization of amino acids derived from protein breakdown, then our measurements of synthesis would be too low. If synthesis were actually higher, our estimates of degradation would have to be increased proportionately, which would only further substantiate our conclusion that synthesis and degradation of myosin increase during hypertrophy.

Dr. Howard E. Morgan, Hershey, Pennsylvania: I would like to make one comment about the choice of a radioactive amino acid for studies of this type. Lysine transport in heart muscle is quite a slow process in contrast to the transport of leucine or phenylalanine. The fact that the exchange of radioactive amino acids across the cell membrane is slow would favor reutilization of cold amino acid derived from protein. This problem would be more acute in experiments such as Dr. Morkin described than it might be in experiments involving an amino acid whose transport rate was faster.

Dr. Eugene Braunwald, Boston, Massachusetts: Have you examined myosin kinetics following debanding, in other words, during regression of hypertrophy?

Dr. Morkin: No, we have not studied the effects of debanding.

Reference

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