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PROTEIN SYNTHESIS IN WORK-OVERLOADED MYOCARDIUM

R. Zak, T. Dowell, A. F. Martin, and M. Rabinowitz

Departments of Medicine and Biochemistry, The University of Chicago

Pritzker School of Medicine, and Argonne Cancer Research Hospital

(operated by the University of Chicago for the United States

Atomic Energy Commission), Chicago, Illinois 60637 U.S.A.

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An increased work load applied to the myocardium is a stimulus for rapid cardiac enlargement, which in the adult, consists of hypertrophy of muscle cells and hyperplasia of connective tissue cells. The extent of cardiac enlargement is determined by the severity of the work overload and by the abruptness and length of time it is applied (1). As judged by traditional indicators of protein synthesis, such as amino acid incorporation into proteins (2, 3) or tissue ribonucleic acid contents the heart is one of the least active of all organs (Fig. 1), yet pressure overload produced by severe narrowing of the lumen of the ascending aorta in adult rats leads to an accumulation of 4.24 mg of cardiac myosin per day per g of heart in the first 4-5 days. The increment in myosin content is considerably greater during acute work overload than during any period of normal myocardial growth (Fig. 2). Increased labeling of myosin with ^{14}C -lysine can be detected as early as 3 hours after aortic pressure is raised in the perfused heart (4) and actual accumulation of myocardial protein can be detected after 24 hours of work overload (5).

The mechanisms regulating the response of cardiac synthesis processes to altered work load are poorly understood. In this review the following questions will be discussed: (1) What is the pattern of accumulation of cell components in the enlarging heart? Is the basic cell architecture preserved or do some cell components increase disproportionately? (2) What are the characteristics of protein synthesis and degradation in the enlarging heart? (3) What are the possible mechanisms coupling work-overload with biosynthetic processes?

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1. Pattern of accumulation of myocardial components in the overloaded heart

The experimental data concerning developing cardiac hypertrophy, during the first few days after the imposition of a work overload indicate that various components of the cardiac cell increase proportionately although several examples of preferential accumulation of myocardial constituents have been described. The results will be presented according to the method of analysis.

a. Enzymatic assays. Increases in respiratory enzyme activities have been reported in pressure overload (6, 7) and aortic insufficiency (8). However in a recent extensive study, Dart and Holloszy (9) found that, after construction of an A-V fistula, the enzymes of the mitochondrial respiratory chain (cytochrome oxidase, succinic oxidase, succinic dehydrogenase), various enzymes of the citric acid cycle (both mitochondrial and sarcoplasmic), rate-limiting enzymes of glycolysis and glycogenolysis (phosphorylase, hexokinase, phosphofructokinase, pyruvate kinase, lactate dehydrogenase) as well as activities of adenylate and creatine kinases all increase in parallel with other components of the myocardial cell. Differences in results may be related to the type and severity of the stimulus used to produce cardiac enlargement.

b. Chemical analyses. Isolation and quantitation of myocardial cytochrome c, coupled with low temperature spectrophotometric measurement of the ratio of cytochromes b, c, and a in isolated mitochondria give a measure of total mitochondrial cytochrome mass. The ratio of the different cytochromes remained constant after aortic constriction indicating a parallel change in inner mitochondrial membrane cytochromes. Left ventricular cytochrome c concentration ($\mu\text{moles/mg dry weight}$) was unchanged one day after aortic constriction but decreased 3 days after surgery. Total ventricular cytochrome c increased in both time periods. It would appear that inner mitochondrial membrane cytochromes accumulate in parallel with other cell components one day after aortic constriction, but that by 3 days a decrease in inner mitochondrial membrane cytochromes is already apparent (10).

The quantitation of myofibrillar mass based on measurements of the sequestered fraction of myofibrillar magnesium (i.e., magnesium specifically bound to thin filaments during polymerization) (5), as well as quantitation of myosin by an isotope dilution technique (see legend to table I) suggest that in the early stages of developing hypertrophy myofibrillar as well as myosin content increased in the same proportion as the total

ventricular mass. By ten days after the onset of work overload, myofibrils accumulate disproportionately more than the dry weight of left ventricle (table I).

table I
MYOFIBRILLAR AND MITOCHONDRIAL MASS OF OVERLOADED RAT HEART

Experiment	Myosin* (mg/heart)	MF Mg† (μ M/kg dr.wt.)	MF vol.† cell vol.	MIT vol.† cell vol.	MIT vol.† MF vol.
Control	24.6	--	--	--	0.67
Sham operated	25.1	1.14	0.46	0.37	--
operated(days)				--	--
1	27.6	1.18	--	--	--
5	34.5	1.07	--	--	--
10	--	1.27	0.53	0.33	--
25	--	1.37	--	--	--
50	--	--	0.57	0.30	--
60	--	1.20	--	--	--
180	--	--	--	--	0.34
360	--	--	--	--	0.23

MF = myofibril; MIT = mitochondria; MF Mg = sequestered myofibrillar magnesium.

*Aortic constriction. Myosin was quantitated by isotope dilution method as follows: purified stock myosin was reacted with 1-fluoro-2,4-dinitrobenzene (Barany, M., et al.: Biochim. Biophys. Acta 168:298, 1968) to obtain approximately 250,000 dpm/mg protein. Labeled myosin was added to known amount of myofibrils which were dissolved in Weber solution. Dissolved crude actomyosin was transferred by dialysis into 0.6 M KCl, 1 mM EGTA, 1 ml thioglycolate, 10 mM phosphate buffer pH 7.0; 5 mM Mg-ATP was then added and myosin isolated, purified (31) and dilution of ^{14}C label was determined.

†Aortic constriction, see Ref. 5.

‡Experimental hypertension, see Ref. 14.

c. Stereological measurements. Quantitative stereological techniques applied to electronmicroscopy (11) give results similar to chemical analysis. Some studies suggest a relative increase of the mitochondria/myofibril ratio in the earliest period (12, 13) but in later period of hypertrophy there is a general agreement that both the mitochondrial/myofibrillar ratio and the fractional mitochondrial volume (mitochondria/cell volume) decrease (5, 12, 14, 15). At the same time, the fractional myofibrillar volume increases (5). In recent measurements in cardiac enlargement caused by

thyroid hormone administration an increase of all cell components was found (16). In addition to mitochondria and myofibrils, the T-tubular and sarcotubular membrane systems appear to increase extensively so as to maintain both total cell surface to volume ratio and the ratio of sarcotubular membrane to myofibrillar volume at a constant level.

2. Synthesis and degradation of proteins in the overloaded heart

Significant advances to our understanding of protein synthesis have come from studies using isolated ribosomes, and soluble cofactors. Interpretation of results becomes more difficult however when protein synthesis is studied in cells or tissues because of precursor pool problems. After administration of a labeled amino acid, a complex equilibrium must be established between extracellular and intracellular spaces and between free amino acid within the cells and aminoacyl-t-RNA. After intravenous injection of labeled amino acid, blood radioactivity declines rapidly (90% in first 3-5 min.) as the label moves into the extravascular bed and subsequently into the cell. Maximal intracellular labeling is usually obtained 10 min. after injection. Penetration of label into different tissues vary considerably. There is a difference of several orders of magnitude between the rate of entry of glycine in the liver and skeletal muscle (17). Several transport mechanisms have been identified which are responsible for the widely different fluxes of different amino acids in the perfused heart (18).

Additional complications arise from the difficulties in measurement of the specific activity of the true immediate precursor in the protein synthesis. Compartmentalization of the free amino acid pool, and perhaps even of amino acyl-t-RNA pool may be present in muscle. Evidence obtained in an in vitro study of rat diaphragm (19) strongly indicates that amino acids may enter protein directly from the incubation medium without mixing with the intracellular free amino acid pool perhaps via a membrane-bound carrier system. Furthermore it is apparent that both the intracellular pool and the hypothetical carrier of amino acids (which bypasses the intracellular pool) receive amino acids from degradation of proteins, since reutilization has been shown to be important in a variety of tissues (20, 21) and probably also in the heart (18). Morgan and associates (18) on the other hand have presented evidence in the perfused heart that intracellular pools of lysine and glycine do appear to be intermediates, or are in rapid equilibrium, with the precursor of protein synthesis. In liver Henshaw and associates (22) found that the specific radioactivity of free intracellular lysine and of the lysine attached to t-RNA differ by less than 10%.

An additional difficulty is that the cells consist, not of a single intracellular pool, but of several compartments each equilibrating differently. Portugal et al. (23) have shown in rat liver that the concentration and specific radioactivity of lysine inside mitochondria is different from that in the cytoplasm. Thus several uncertainties regarding pool and reutilization make it difficult to interpret incorporation experiments in the overloaded heart. The determination of the synthesis rate requires the measurement of both the rate of incorporation of precursors into highly purified protein and the integrated specific activity of the true precursor over the entire period of the experiment. These values are at present difficult to obtain in the heart.

Rapidly induced cardiac growth has been shown to be associated with changes in the size and specific activity of the intracellular precursors. Intracellular proline concentration and specific radioactivity (24, table II) and the rate of transport of α -aminoisobutyric acid (25) have been shown to increase in the overloaded heart, indicating accelerated transport of amino acids into the cells. Changes in specific radioactivity of the actual precursor may account for some of the elevated labeling of myosin and of total noncollagenous proteins observed during the first few days after aortic constriction (24).

If one wishes to obtain information about the relative rates of synthesis of different groups of proteins in the hypertrophied heart some of the uncertainties concerning the changes in amino acid pools can be overcome by assuming that changes in pool specific activity affects labeling of each group of proteins similarly. By this approach it was established (26) that one day after aortic constriction ribosomal, nuclear, and, to a smaller degree, mitochondrial proteins, are more highly labeled than the total homogenate in hypertrophied heart. Labeling of myofibrillar and sarcoplasmic proteins is lower. The membrane fraction of hypertrophied heart showed disproportionately higher labeling 4 days after banding. In a similar approach, Florini and Danberg (27) intermixed soluble or residual proteins of ^{14}C -leucine-labeled hypertrophied hearts with corresponding fractions of ^3H -leucine-labeled sham-operated hearts. After fractionation of the proteins by isoelectric focusing the $^{14}\text{C}/^3\text{H}$ ratio was determined in each protein band. In 2-day banded rats some soluble proteins with isoelectric points in the range of pH 7-10 showed disproportionately higher labeling in the hypertrophied heart. Analyses of seven and more days banded animals

Table II

Intracellular Free Proline Concentration and Specific Radioactivity in Hypertrophied Rat Myocardium

Operation	Extracellular space			Intracellular proline pool			
	No. of rats	% hypertrophy	³⁵ S space (μl/g w.wt.)	No. of rats	% hypertrophy	Concentration (μmoles/100 ml cell H ₂ O)	Specific radioactivity (dpm × 10 ⁻³ /μmole of proline)
sham, 2 days	8		247 ± 10	5		93.64 ± 4.05	258 ± 29
banded 2 days	8	29 ± 5	307 ± 19 (p < 0.02)	4	20 ± 4	95.93 ± 5.86 (NS)*	365 ± 19 (p < 0.02)
sham, 5 days	10		270 ± 9	4		89.50 ± 5.49	395 ± 24
banded, 5 days	8	41 ± 7	315 ± 13 (p < 0.01)	5	26 ± 8	124.14 ± 8.31 (p < 0.02)	327 ± 27 (NS)*

Intracellular proline was calculated on the basis of extracellular space values obtained in sham operated animals. Per cent hypertrophy was calculated by comparison of heart wt./body wt. ratios of banded and sham operated rats.

*NS = not significant, p > 0.05.

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revealed increased labeling of some residual proteins. Identification of the proteins involved requires additional work.

The level of intracellular protein depends not only on the rate of synthesis but also on the rate of destruction. All intracellular proteins are in a state of dynamic equilibrium, constantly being synthesized and degraded as originally shown by Schoenheimer and associates (28) and Swick (29). Schimke (30) has shown for several enzymes in rat liver that the rate of degradation as well as the rate of synthesis may regulate their intracellular concentration. In the steady state, the degradation rate of a specific intracellular protein may be measured from the decline in its specific radioactivity if the following criteria are met: 1. the component is purified, 2. the component is effectively pulse labeled (i.e., the intracellular precursor rapidly disappears), 3. there is no reutilization of labeled precursors from the breakdown of intracellular proteins.

In the unsteady state, accurate quantitation of the component to be measured is also essential. In the heart, true pulse labeling is difficult to obtain and excessive reutilization may occur making it difficult to interpret such studies.

We have used the nonreutilizable precursor δ -aminolaevulate (ALA) which is a specific precursor of heme synthesis to evaluate the effect of cardiac hypertrophy on the turnover of one mitochondrial component, cytochrome c. Since ALA is not reutilized and labels the heme essentially as a pulse, and since cytochrome c is easily measured, the requirements stated above are effectively met. When mitochondria were labeled with ALA 3 days prior to aortic constriction we have shown that during the first 2-3 days after surgery cytochrome c radioactivity declines more slowly than in sham-operated rats. Thus, decreased destruction as well as increased synthesis contributes to cytochrome c accumulation. However, if care is not taken to avoid severe hypoxia during surgery an increased destruction of cytochrome may result (10). We have shown that hypoxia itself can lead to mitochondrial cytochrome destruction (31).

Study of the turnover of myofibrillar proteins is complicated by the probability that the reutilization of labeled amino acids falsely prolongs the calculated half-life. The radioactivity of myosin in the normal heart decays with an apparent half-life of 11 days. Half-lives of actin, tropomyosin and troponin are similar, those of mitochondria are 6.5 days, of sarcoplasmic proteins, 5.2 days and of membranes, 3.5 days (32). After aortic constriction there is a decrease in the decay rate in total

radioactivity of myosin prelabeled with ^3H -leucine, a result similar to that described above for cytochrome (33). It cannot be stated with certainty at the present time, however, whether this is due to a decrease in the rate of destruction or an increase in reutilization of precursors, as suggested by Kimata and Morkin (34), or to both processes.

3. RNA content and synthesis in heart overloading

In general, tissues that are most active in protein synthesis have the highest RNA content. Considerable increases in both RNA content and synthesis are well documented in all experimental models of cardiac overload. There is a linear relationship between the degree of cardiac hypertrophy and the elevation of RNA per unit mass. Furthermore, muscles that are most active and have the highest oxidative metabolism have the highest RNA content. Heart is at the top of the scale, followed by tonic skeletal muscle, and phasic muscles are the lowest (35-40). The relationship between contractile activity of muscle and RNA content is also apparent in the comparison of wild and domesticated animals (41). Breast muscle of flying birds has a higher RNA content than that of nonflying birds. Leg muscle of nonflying birds on the other hand has a higher RNA content than breast muscle.

The accumulation of RNA is reflected in 3-4 fold enhancement of the incorporation of precursors into ribosomal and transfer RNA, corrected for changes in precursor pool size and specific activity (42, 43). Of interest is the expansion of the uridylic acid pool (43) possibly the result of the observed increase in uridylyl transferase activity (44). Analysis of pulse-labeled newly synthesized RNA revealed equal incorporation of label into ribosomal and t-RNA with no evidence for the presence of high specific activity m-RNA (43, 45). However changes in the types of m-RNA synthesized are suggested by finding changes in the labeling pattern of sarcoplasmic proteins separated by isoelectric focusing 2 days after aortic constriction (27). The stimulation of ribosomal RNA synthesis is reflected also in elevated activity of nuclear polymerase (46, 47) and in enlargement of the nucleoli (6, 48).

4. Overload as stimulus for heart enlargement

How the mechanical activity of a muscle determines its size is not understood. Some of the possible mechanisms that have been suggested are that increased work load or wall tension results in an enhanced oxygen requirement and leads to accumulation of a metabolite that induces or de-represses RNA transcription. Another possibility is that stretching per se

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alters nuclear or plasma membrane conformation resulting in stimulus for growth. Cyclic AMP has been suggested to be involved in this process but no evidence has been forthcoming.

It is well established that both cardiac and skeletal muscle overload are accompanied by increased metabolic activity. Increased wall tension in heart results in increased oxygen consumption (49) and passive stretch of skeletal muscle leads both to increased heat production (50) and oxygen consumption (51). Cardiac enlargement follows enhanced preload or afterload, and passive stretching of the uterus (52), diaphragm (53) and muscle in organ culture (54) results in their enlargement. The relationship between metabolic activity and protein synthesis can be seen, not only in acute experiments but also in functionally different muscles. Muscles having the highest energy utilization have the highest RNA levels (35-40). Changes in muscle activity associated with domestication of animals are reflected in changes in their RNA content (41). The capacity of red muscle fibers for hypertrophy is greater than that of pale fibers (53). The uptake of α -aminoisobutyric acid (AIB) (40) and the labeling of proteins by amino acids in general parallel the RNA levels in functionally different muscles (35, 40, 56, 57). It is not clear why muscles that are more active metabolically appear to have higher rates of protein synthesis.

One possible mechanism by which increased stretch may be translated into increased protein synthesis is by altering the properties of the plasma membrane with enhancement of transport of precursors of protein and RNA synthesis. Passive stretch increases uptake of α -aminoisobutyric acid into papillary muscle (58), and the in vitro incorporation of ^{14}C -leucine into proteins is considerably increased by stretching the levator ani and extensor digitorum longus muscles (59). Similarly in cardiac hypertrophy increased uptake of AIB (25) and of proline (24), and increased intracellular amino acid concentrations (24, 60) have been noted. Increasing the concentrations of intracellular amino acids in the perfused heart leads to an increased rate of protein synthesis (18). Wannemacher and McCoy (60) found a positive correlation between the incorporation of radioactive leucine into heart proteins and the free amino acid concentration in ventricles of hypertensive rats.

It is of interest to note that nuclear DNA-dependent RNA polymerase in Ehrlich ascites tumor cells is activated by increased amino acid levels. Only synthesis of ribosomal RNA is affected (61). There are other examples for the regulation of ribosomal synthesis by amino acids (62). Amino

acyl-t-RNA has also been implicated in the regulation of protein breakdown (55).

It remains to be established if the increased transport of amino acids in the overloaded heart is involved in stimulation and regulation of biosynthetic processes or if it is a nonspecific consequence of altered membrane permeability.

Résumé

Les aspects suivant de l'augmentation du coeur produit par la surcharge cardiaque sont décrits ci-après:

1. Accumulation des composantes myocardiaques
2. Synthèse et dégradation des protéines
3. Concentration et synthèse de RNA
4. Surcharge comme stimulateur de l'augmentation du coeur.

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Figure Legends

- Fig. 1. Comparison of ^{35}S -methionine incorporation into proteins and RNA content in various organs. Data concerning ^{35}S -methionine incorporation (cpm/ μM methionine) were compiled from our data and those of Fieldberg et al. (3).
- Fig. 2. Growth of rat heart and accumulation of myosin in work overload. Myosin was quantitated as described in legend to table I.
 Δ myosin was determined over a period of 3 days.