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THE EFFECTS OF HYPERTROPHY AND HYPOXIA ON
THE TURNOVER OF MYOCARDIAL COMPONENTS

MASTER

Murray Rabinowitz, Radovan Zak, Vaclav Aschenbrenner, and R. Albin

Departments of Medicine and Biochemistry, The University of Chicago, and
the Argonne Cancer Research Hospital (operated by the University
of Chicago for the United States Atomic Energy Commission)

Chicago, Illinois 60637 USA

Dr. Rabinowitz, Professor of Medicine and Biochemistry

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Although the adult cardiac muscle cell apparently loses its capacity for mitosis and cell division, components within the cell such as myofibrillar proteins and mitochondria turn over at relatively rapid rates in the normal heart. The cardiac muscle cell must therefore possess considerable capacity for the synthesis of nucleic acids, proteins and phospholipids in order to maintain its structural integrity. The dynamic nature of the process of continuous destruction and synthesis of cell constituents allows the heart to adapt efficiently to chronic changes in work load.

For the last few years our laboratory has been engaged in a study of the mechanisms which control the synthesis and turnover of cardiac muscle components, and the effect of hypertrophy and hypoxia on these processes. Biochemical studies of cardiac muscle are complicated by the heterogeneity of the tissue. Although we may be primarily interested in the changes that take place in the muscle cell, more than 75% of the cells in heart muscle are connective tissue. For example the increase in DNA content following experimental aortic constriction has been shown to be due to connective tissue hyperplasia and not to mitosis of muscle cells (G r o v e, N a i r & Z a k, 1969; G r o v e, Z a k, N a i r & A s c h e n b r e n n e r, 1969; M e e r s o n, 1969; M o r k i n & A s h f o r d, 1968). We therefore focused our attention initially on studies of myofibrillar proteins which are located exclusively in the muscle cell, and mitochondria, which are present in such great concentration in the muscle cell that total tissue changes reflect changes in that cell.

The turnover of muscle proteins has been measured in hearts of normal Sprague-Dawley rats. Methods have been developed for the isolation in good yield of myosin, actin, tropomyosin and troponin and for the quantitative estimation of cardiac myosin. As shown in Fig. 1, the specific activities of

cardiac myosin labeled with ^3H -leucine decline exponentially with a half-life of 10-11 days. The data have been corrected for the small increase in myosin content that occurs during the course of the experiment. The exponential decline indicates a random destruction of myosin. The results differ from those of Morkin in rabbit heart (M o r k i n, 1970a, 1970b, and personal communication) where a plateau of myosin specific activity was observed for 6-10 days followed by a rapid decline. Morkin's results were interpreted to indicate that newly synthesized myosin was preferentially retained whereas previously synthesized myosin was more readily degraded (M o r k i n, 1970a, 1970b). The reason for differences in rabbit and rat heart are still not understood. Our preliminary results also indicate that the turnover rates of actin, tropomyosin and troponin are similar to that of myosin. These results would suggest a synchronous assembly and destruction of the myofibril. The turnover rates of the myofibrillar proteins obtained in these experiments may well be too long because of reutilization of labeled precursor, ^3H -leucine. Reutilization may be of considerable magnitude at least in tissues such as liver in which synthesis of proteins is a major function. The same half-life for myosin, however, was obtained when we used ^{14}C -guanido-arginine, a precursor which is not reutilizable after degradation of the protein, at least in liver (S c h i m k e, G a n s c h o w, D o y l e & A r i a s, 1968).

Following constriction of the ascending aorta, a 20-50% increase in cardiac mass and protein content occur within 48 hours (N a i r, C u t i l l e t t a, Z a k, K o i d e & R a b i n o w i t z, 1968). The rate of RNA and protein synthesis are increased considerably (F a n b u r g & P o s n e r, 1968; K o i d e & R a b i n o w i t z, 1969; M e e r s o n, 1969; N a i r, C u t i l l e t t a, Z a k, K o i d e & R a b i n o w i t z, 1968). Myosin appears to increase proportionally to the increase in heart

weight. The myosin accumulation can be accomplished either by an increase in the rate of its synthesis, a decrease in the rate of destruction, or by a combination of both processes. The effect of aortic constriction on the turnover of myosin labeled with ^3H -leucine is shown in Fig. 2. Specific activities of myosin have been corrected for increase in content during hypertrophy. It is apparent that the rate of myosin degradation has been shut off almost completely during hypertrophy. Other evidence indicates that synthetic rates are increased. Thus myosin accumulation is due to both a decrease in the rate of destruction and to an increase in the rate of synthesis.

We will now turn our attention to mitochondria, which occupy about 30% of the cardiac cell volume (P o c h e & M ö n k e m e i e r, 1962), and provide almost all the energy required for muscle contractions. Mitochondria are now known to be semiautonomous organelles which have their own genetic material DNA, and their own systems for the synthesis of nucleic acids and proteins (B o r s t & K r o o n, 1969; R a b i n o w i t z & S w i f t, 1970; R o o d y n & W i l k i e, 1968). Many mitochondrial components however are synthesized in the cytosol and the assembly of a functional organelle depends on the integration of the information and synthetic processes of cytosol and mitochondria.

Our laboratory was first to demonstrate that mitochondrial DNA differs from nuclear DNA in higher organisms (R a b i n o w i t z, S i n c l a i r, D e S a l l e, H a s e l k o r n & S w i f t, 1965). Fig. 3 shows a tracing of an analysis of chick heart mitochondrial DNA and nuclear DNA by isopycnic CsCl centrifugation. The results indicate that mitochondrial DNA differs greatly in buoyant density and therefore base composition from nuclear DNA. Cardiac mitochondrial DNA is circular and has a molecular weight of about

1×10^7 daltons (Borst, Kroon & Ruttenberg, 1967; Sinclair, Stevens, Gross & Rabinowitz, 1967).

We have shown that several components of the inner mitochondrial membrane of cardiac mitochondria turn over at the similar or the same rates (Aschenbrenner, Druyan, Albin & Rabinowitz, in press; Druyan, DeBernard & Rabinowitz, 1969).

FIG. 4

Fig. 4 shows that cytochrome c and heme a labeled with ^3H - δ -aminolevulinic acid, a non-reutilizable heme precursor, turn over at similar rates with half-lives between 5-6 days. Similarly heme b also located in the mitochondrial inner membrane (Druyan, DeBernard & Rabinowitz, 1969), and cardiac mitochondrial DNA (Gross, Getz & Rabinowitz, 1969) turn over at similar rates. Outer membrane proteins and cytochrome b₅ and matrix enzymes however turn over at more rapid rates (Beattie, 1969; Brunner & Neupert, 1968; DeBernard, Getz & Rabinowitz, 1969; Marver, Collins, Tschudy & Rechcigl, 1966). The data indicate a synchronous synthesis and turnover of the inner mitochondrial membrane.

FIG. 5

The effect of cardiac hypertrophy on the synthesis and turnover of mitochondria was studied. Changes in mitochondrial mass after aortic constriction were estimated from the activity of mitochondrial respiratory enzymes in cardiac homogenate and mitochondria. As seen in Fig. 5, mitochondrial mass appears to increase proportionally greater than total myocardial proteins one day after aortic banding as indicated by the increase in homogenate specific activities of cytochrome oxidase, succinate cytochrome c reductase, and succinic dehydrogenase. Total cardiac activities of these enzymes increase to a greater extent than does total cardiac protein content. Mitochondrial enzymes

therefore appear to be synthesized more rapidly than other protein components in early hypertrophy. This preferential synthesis of mitochondrial proteins is less apparent three days after banding.

Preferential synthesis of mitochondrial proteins in early cardiac hypertrophy is also indicated by the relative incorporation of ^3H -leucine into various myocardial subcellular fractions. Fig. 6 shows that 24 hours after aortic banding the increment in incorporation of ^3H -leucine into mitochondrial proteins was greater than that into myofibrillar or supernatant proteins. It may be concluded that mitochondrial proteins are rapidly synthesized during the acute phase of cardiac hypertrophy.

To gain insight into the dynamics of synthesis and degradation of mitochondrial proteins, we also studied the effects of cardiac hypertrophy produced by aortic constriction on the turnover of mitochondrial proteins.

The rationale of the experiments was to label mitochondrial protein, or a specific mitochondrial cytochrome, several days before the experimental intervention. The effects of the experimental procedure on the specific radioactivity of that component is then measured. A decrease in specific radioactivity compared to controls reflects increased synthesis of new unlabeled material. Changes in the rate of destruction are measured by changes in the total cardiac radioactivity of that component.

The effect of aortic constriction in cytochrome c turnover was studied. Cytochrome c is known to be bound to the inner mitochondrial membrane, and to turn over at the same rate as the particulate cytochromes b and aa₃ (Aschenbrenner, Druyan, Albin & Rabinowitz, in press; Druyan, DeBernard & Rabinowitz, 1969). As seen in Fig. 7, cytochrome c content increases by about 10% after aortic

banding. After correction is made for the increase in cytochrome c content a 15-20% dilution of cytochrome c specific activity is present. The decrease in specific radioactivity, and in total cardiac cytochrome c radioactivity compared to controls, indicates an increased turnover immediately after banding. The observed decline in corrected specific radioactivity was unchanged three days after banding, indicating that the rate of destruction had returned toward normal.

FIG. 8 From these data, and from the turnover rate of cytochrome c, estimations of the rate of destruction and synthesis of cytochrome c can be made (Fig. 8). It appears that rate of destruction has increased considerably during the first 24 hours after banding. This increased rate of destruction is compensated by a three-fold increase in synthesis rate. Three days after banding the rates of destruction and synthesis have returned toward normal. It is of interest that our results on the synthesis and turnover of myosin and mitochondria conform closely to Meerson's electron micrographic findings reported several years ago (Meerson, Zaletayeva, Lagutchev & Pshennikova, 1964) that mitochondrial degenerative changes and an increased ratio of mitochondrial/myofibrillar volume is present after aortic constriction.

Increased mitochondrial destruction early after aortic constriction was not observed in all experiments, and may represent a pathological response to the severe abrupt rise in left ventricular pressure. The true physiological response may well be a shut-off in the rate of destruction similar to that which occurs with myosin. This hypothesis is supported by the fact that the rate of mitochondrial destruction is greatly diminished during mitochondrial accumulation in heart after thyroid hormone administration (Gross, in press). Mitochondrial mass increases significantly following thyroid administration in hypothyroid

and euthyroid rats (G r o s s, in press; T a t a, 1964). As seen in Fig. 9, the rate of mitochondrial protein turnover is markedly reduced when thyroid hormone is given. Therefore similar to the case for myosin after aortic banding, a shut-off of mitochondrial turnover is an important factor in thyroid hormone's effect in increasing mitochondrial mass.

We have also studied the effect of hypoxia on the synthesis and turnover of various cell components. Rats were exposed to a six-hour period of 4-5% oxygen, conditions which are compatible with survival of more than 80% of the animals.

In the experiment shown in Fig. 10, mitochondrial proteins were pre-labeled with ^{14}C -guanido-arginine. The rapid normal turnover of mitochondrial proteins is apparent. After exposure of rats to a six-hour period of 4-5% oxygen, the specific activity of mitochondrial proteins was 15-20% less than controls. This decrease is indicative of synthesis of new unlabeled mitochondrial proteins, presumably secondary to increased destruction produced by hypoxia. The slope of the decay curves for hypoxic and control animals are substantially the same, indicating that mitochondria which escape destruction during the hypoxic period are not more susceptible to destruction later.

These conclusions are confirmed by experiments using cytochrome c labeled with ^3H - δ -aminolevulinic acid which is a specific heme precursor and is a non-reutilizable precursor. Accurate measurements of total cytochrome c content can also be made which facilitate interpretation of the results.

The specific activity of cytochrome c, and also of isolated purified heme aa₃ decreased by 15-30% after exposure to low oxygen atmosphere (Fig. 11). No dilution was present at the end of the six-hour hypoxic period, but it was present 6-12 hours after return to a normal atmosphere. This indicates that

mitochondria may be destroyed during hypoxia, but they are resynthesized only after return to normal oxygen atmosphere. Also seen in Fig. 11, is that hypoxia causes no decrease in myosin specific radioactivity. Rates of degradation or of synthesis of myosin has not been significantly affected by hypoxia. This indicates that mitochondria are more sensitive to the effect of hypoxia than is myosin.

FIG. 12 Hypoxia leads to a significant decrease in cytochrome c content by the end of the six-hour hypoxia period (Fig. 12). The level returns to normal six hours later indicating a brisk resynthesis. Thus, it seems clear that hypoxia results in a destruction of mitochondrial components which are rapidly synthesized after termination of the hypoxia.

FIG. 13 That mitochondrial resynthesis occurs predominantly after return to normal oxygen tension is confirmed by experiments measuring the incorporation of ^3H -leucine into mitochondrial protein during and after hypoxia (Fig. 13). Incorporation was at control levels during hypoxia, but increased 200-300% two to three hours after return to normal oxygen tensions. The level of incorporation returned to normal by 24 hours.

FIG. 14 The greater sensitivity to hypoxia of mitochondria than of some other cell components is confirmed in the experiment shown in Fig. 14. Incorporation of ^3H -leucine into mitochondria, myofibrils, membranes and soluble cell fractions three hours after return to normal oxygen tension after a six-hour period of hypoxia is seen in Fig. 14. Incorporation into mitochondria and soluble fractions were both greatly increased but no significant changes were observed in the myofibrillar and membrane fractions. Thus myofibrillar and membrane fractions appear to be resistant to destruction and subsequent resynthesis after a period of severe hypoxia.

Summary

In conclusion we have shown that myofibrillar proteins and mitochondrial components turn over rapidly within the cardiac muscle cell. Myofibrillar proteins and the inner mitochondrial membrane each appear to turn over synchronously. Acute cardiac hypertrophy results in increased synthesis of these components but mitochondrial mass increases disproportionately greater than other cell proteins early in the process. The rate of myosin destruction is decreased after aortic constriction. Similarly the increase in mitochondrial mass caused by thyroid administration is accompanied by a decreased mitochondrial turnover. However immediately after aortic constriction there may be a considerable increase in mitochondrial turnover during the phase of rapid synthesis. Hypoxia leads to a selective destruction of mitochondria, which are rapidly resynthesized following return to normal oxygen tension. Myofibrils and membranes are more resistant than mitochondria to the effect of hypoxia.

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Murray Rabinowitz, M.D.
Department of Medicine
University of Chicago
950 East 59th Street
Chicago, Illinois 60637 USA

Figure Legends

Fig. 1.--Turnover of rat heart myosin labeled with ^3H -leucine.

Fig. 2.--Effect of aortic constriction on myosin turnover in rat heart.

Control 0—0; aorta banded ●—●.

Fig. 3.--Buoyant density of native and denatured chick mitochondrial and nuclear DNA (from Rabinowitz, Sinclair, De Salle, Haselkorn, and Swift, 1965).

Fig. 4.--Turnover of δ aminolevulinate labeled heme a (●) and cytochrome c (□) in rat heart mitochondria. For heme a, half-life was 5.9 days; for cytochrome c, half-life was 5.8 days (from Aschenbrenner, Druyan, and Rabinowitz, 1970).

Fig. 5.--Effect of banding the ascending aorta in rats on cardiac homogenate respiratory enzyme activities.

Fig. 6.--A mixture of ^{14}C -amino acids was given to control and banded rats 24 hours after aortic constriction, and the incorporation into various cardiac subcellular fractions was measured. The ratio of amino acid incorporation of banded to sham-operated animals in homogenates was taken as 1.0:

Fig. 7.--Effect of banding the ascending aorta of rats on total cardiac cytochrome c content, and on cytochrome c specific activity corrected for changes in cytochrome content. Cytochrome c was prelabeled with ^3H - δ -aminolevulinate 3 days prior to aortic banding.

Fig. 8.--Estimate of rates of synthesis and destruction of cardiac cytochrome c following constriction of the ascending aorta of rats.

Fig. 9.--Turnover of total mitochondrial proteins of rat heart labeled with ^{14}C -guanido-arginine following thyroxine administration to euthyroid rats (from Gross, 1970).

Fig. 10.--Effect of a 6-hour period of 4-5% oxygen on the specific activities of mitochondrial protein previously labeled with ^{14}C -guanido-arginine (from Rabinowitz, Aschenbrenner, Albin, Gross, Zak, and Nair, 1970).

Fig. 11.--Effect of a 6-hour period of 4-5% O_2 on the specific activities of cytochrome c, and heme a prelabeled with ^3H - δ -aminolevulinic acid and myosin prelabeled with ^3H -leucine. C-control; H-end of 6 hours of hypoxia, H+6, 6 hours after return to normal O_2 tensions (from Aschenbrenner, Zak, and Rabinowitz, 1970).

Fig. 12.--Cardiac cytochrome c content after a 6-hour period of hypoxia, and 12 and 18 hours after return to normal O_2 atmosphere (from Aschenbrenner, Zak, and Rabinowitz, 1970).

Fig. 13.--Incorporation of 1-hour pulses of ^3H -leucine into mitochondrial protein during the 6-hour period of 4-5% O_2 and after return to a normal atmosphere (from Aschenbrenner, Zak, and Rabinowitz, 1970).

Fig. 14.--Incorporation of ^3H -leucine into subcellular cardiac fractions 3 hours after return to normal oxygen tensions following a 6-hour period of hypoxia.

EFFECT OF AORTIC CONSTRICTION ON MYOSIN TURNOVER (³H LEUCINE)

TURNOVER OF RAT HEART MYOSIN (³H LEUCINE)

Changes In Respiratory Enzymes In Heart Homogenates After Aortic Banding

Relative incorporation of ^{14}C amino acids into protein of subcellular fractions of hearts of banded and control animals

Fraction	Sham	Banded	Ratio: $\frac{\text{Banded}}{\text{Sham}} \times 100$
Homogenate	1.0	1.0	
Mitochondria	0.95	1.24	130.5
Myofibrils	0.97	0.90	93.0
Supernatant	1.28	1.35	105.5

Effect of aortic banding on cytochrome c
content and specific radioactivity

Calculated rates of synthesis and degradation of cytochrome c

Effect of Thyroxine on Turnover of Mitochondrial Proteins Rat Heart (^{14}C Arginine)

Effect of Hypoxia on The Turnover of Rat Heart Mitochondrial Proteins Labeled With ^{14}C Arginine

Change in specific activity after hypoxia treatment

INCORPORATION OF ^3H LEUCINE INTO MITOCHONDRIAL PROTEIN DURING AND AFTER EXPOSURE TO HYPOXIA

INCORPORATION OF ^3H LEUCINE INTO SUBCELLULAR FRACTIONS AFTER 3 HOUR RECOVERY FROM A 6 HOUR HYPOXIC PERIOD