Biochemical Aspects of Experimental Cardiac Hypertrophy

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CARDIAC hypertrophy is an adaptive growth of the heart to an increased load.^{1,2} Cardiac synthetic processes are finely controlled by as yet poorly understood mechanisms so as to respond to physiological changes. This presentation is concerned with biochemical changes on the cardiac hypertrophy induced by aortic constriction of adult rats. In this type of hypertrophied heart, the left ventricular pressure was increased about 50 mmHg above that of sham operated rats. The left ventricular end-diastolic pressure was also significantly elevated. Maximum dp/dt was unchanged, however, Vmax derived from intracardiac pressure recordings on assumption of two component model was decreased. These animals did not show signs of congestive heart failure, however, growth of the body was slightly retarded.

Protein and Nucleic Acid Synthesis in Cardiac Hypertrophy

Fig. 1 shows the heart weights after sham operation and after aortic constriction. Within the first several days, heart weights in sham operated rats declined by about 5% and they took 3–5 days to return to their preoperative level. In rats with constricted aortas, the regression in heart weights did not occur. The fastest growth rate of the heart was achieved between 5 and 10 days postoperatively. When corrected for body weight, aortic constricted rat hearts were 40% increased above that of sham operated before 5–7 days and remained thereafter. This cardiac hypertrophy was reversible: on release of constriction, the heart weights returned to control level within 7 days. In aortic constricted rats amino acid incorporation into protein began to increase after 2–6 hours after operation.³ Morkin *et al.*⁴ showed that

the synthesis of myosin in the aortic constricted rabbit was increased at 2 days after operation and reached a peak at 5 days. Labelling of collagen, which is one of the specific protein of connective tissue was at its peak at 2–4 days.⁵ The increased rate of protein synthesis during developing cardiac hypertrophy appears to be primarily due to an increase in the number of myocardial polyribosomes. Increased RNA concentration per gram of heart was observed at 2 days and total RNA remained elevated for weeks.⁶ The concentration of DNA on which RNA is transcribed was remaining at the same level and total DNA was increased after several days. This increase in DNA and cell proliferation occurred primarily at interstitial cells. Cardiac enlargement is consisted of hypertrophy of muscle cells and hyperplasia of connective tissue. Fanburg and Posner⁶ showed that RNA synthesis started to increase between 4 and 8 hours after operation and reached a peak level at 8 hours. When actinomycin which inhibits transcription of DNA was given to the rats, the increase in RNA synthesis did not occur.⁶ Nair *et al.*⁷ showed that RNA polymerase activity reached a peak value at 2 days, with the earliest detectable change around 12 hours. Kako *et al.*⁸ showed a biphasic response of the enzyme activity at 4 and after 16 hours.

Pyrimidine Nucleotide Synthesis in Cardiac Hypertrophy

Pyrimidine nucleotides, nucleic acid precursor, are synthesized through de novo pathway in which orotic acid is intermediary. The "salvage" pathway in which uridine is an intermediary is available for reutilization of the nucleic acid degradation (Fig. 2). Radioactive orotic acid

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Fig.1. Heart weights in rats after aortic constriction and sham operation at various times after operation. Values are mean $\pm$ SE for more than 14 hearts at each point. Open circles indicate sham operated rats and solid circles indicate rats with aortic constriction.

Fig.2. Pathways of pyrimidine metabolism. UR: uridine, U: uracil.

Fig.3. Labeling of RNA in vitro and in vivo.

Fig.4. Time course of change in uridine kinase activity of heart extracts after aortic constriction. The numbers of matched sets of animals used are noted in parentheses. $\pm 1\text{SE}$ is indicated by vertical lines.

Fig. 5. Time course of change in cardiac ornithine decarboxylase activity after aortic constriction and sham operation at $5 \mu\text{M}$ of 1-ornithine. $\pm 1\text{SE}$ is indicated by vertical lines. Open circles indicate sham operated rats and solid circles indicate rats with aortic constriction.

Fig. 6. Effect of actinomycin D on cardiac ornithine decarboxylase activity at 4 hours after operation at 0.1mM of 1-ornithine. Open symbols indicate rats with aortic constriction. Actinomycin D was injected intravenously at indicated time from operation. Squares indicate $80 \mu\text{g}/100\text{g}$ and triangles indicate $200 \mu\text{g}/100\text{g}$ of actinomycin.

incorporation into RNA was considerably lower for rat heart than for rat liver in vivo and in vitro.⁹ Radioactive uridine was incorporated into heart RNA to a greater extent than orotic acid, and the labelling with uridine in the heart exceeded that in the liver (Fig. 3). Extracts of heart showed little enzymatic conversion of orotic acid to pyrimidine nucleotides. Uridine kinase appeared to be rate limiting in the "salvage" pathway. Aortic constriction produced an increase in uridine kinase activity at 24 hours with a peak at 2 to 6 days after operation (Fig. 4). Koide and Rabinowitz¹⁰ showed a 50% increase in uridine nucleotide pools at 48 hours after operation. The increase in uridine kinase may regulate the increase in nucleotides. The "salvage" pathway appears to play an important role in pyrimidine nucleotide synthesis in the heart and uridine kinase may be regulatory in this pathway during cardiac hypertrophy.

Polyamine Synthesis in Cardiac Hypertrophy

The polyamines, spermidine and spermine, and their precursor, the diamine, putrescine, are widely distributed among mammalian tissues. Direct addition of polyamines stimulates RNA polymerase, stabilizes DNA, RNA, and ribosomes, and stimulates protein synthesis in some ribosome systems. Correlation between poly-

Fig. 7. Time course of change in max V, resting tension and developed tension of trabecular muscles after aortic constriction.

mine concentrations and increase in polynucleotide production have been observed during growth of various tissues. Caldarera et al.¹¹ showed concentration of spermine increased at 2 hours after aortic constriction with a peak at 4 days. In the rabbit, S-adenosylmethionine decarboxylase, which catalyzes the formation of spermidine from putrescine and S-adenosylmethionine was found to be increased in the right ventricle 4 hours after pulmonary constriction.¹² Ornithine decarboxylase, which catalyzes the formation of putrescine, was increased as early as 2 hours after aortic constriction of rats.¹³ Two peaks in the activity occurred — one at 4 hours and the other at 5–10 days (Fig. 5). This is similar to a biphasic response of RNA polymerase activity reported by *Kako et al.*⁸ The changes in ornithine decarboxylase activity correlated well with the subsequent hypertrophy in the hearts of rats with aortic constriction and with the regression in the hearts of sham operated rats suggesting a role for polyamines in the regulation of cardiac growth. The early increase in ornithine decarboxylase was inhibited by actinomycin given 40 minutes before operation.¹³ Actinomycin given 30 minutes after operation or even at the time of operation failed to inhibit the increase in the enzyme activity, suggesting that the transcription required for the increase occurs early after operation (Fig. 6).

Correlations between Mechanical Properties and

Biochemical Changes in Hypertrophied Cardiac Muscle

In this model of cardiac hypertrophy, depression of isotonic shortening velocity and maximum isometric force of trabecular muscles was first seen at 7 days, and persisted at 14 and 28 days.¹⁴ When matched for cross sectional area, muscles from hypertrophied heart demonstrated a depressed maximum velocity of shortening (Max V) while development of isometric tension was unaltered (Fig. 7). A good correlation between Max V and the degree of hypertrophy was noted ($r = -0.84$). The depression in Max V occurred only after cardiac hypertrophy was established at 7 days. According to Morkin et al, this coincides with about 70% replacement of total cardiac myosin by newly synthesized myosin after operation. It has been postulated that V max of contractile element is proportional to the amount of ATP hydrolyzed between cross bridges of myosin and actin. Several studies have been reported on correlation between depressed muscle shortening velocity and depressed myofibrillar ATPase or purified myosin ATPase activity. Depressed muscle velocities might be also due to changes in the active state. *Sordahl et al.*¹⁵ showed that sarcoplasmic reticulum revealed significantly decreased rates and total binding of calcium. *Kaufmann et al.*¹⁶ showed that by supra-maximal calcium activation, the shortening velocities of both the hypertrophied and the normal control heart muscles was increased to about the

same final value. These results are consistent with the concept that a disorder in the excitation-contraction coupling underlies the depressed contractile state of hypertrophied cardiac muscle. In a three component model of heart muscle, a decrease in muscle shortening velocity may be due to an increase in the stiffness of the parallel elastic component which supports a high resting tension in heart muscle. It is postulated that an increase in connective tissue indicated by the increase in collagen can be responsible for the elevation of resting tension. On the other hand, there was a significant overall correlation between collagen and max V ($r = -0.48$).¹⁴ To resolve this problem, β -aminopropionitrile, and inhibitor of collagen cross link formation was given to rats following aortic constriction. Soluble collagen in the heart increased about two-fold. Max V was still decreased while there was no increase in resting tension in hypertrophied hearts.¹⁷ Findings suggest that collagen plays a role in the altered compliance seen in cardiac hypertrophy but not in the altered contractile state.

SUMMARY

In experimental cardiac hypertrophy induced by aortic constriction of rats, the hypertrophy was established after 5–7 days. The basic biochemical changes for increasing tissue mass; increases in protein, nucleic acid, and polyamine synthesis started to occur between 2 and 8 hours followed by an increase in uridine nucleotide pools via predominance of "salvage" pathway. Although the precise coupling mechanism between mechanical stress and biochemical changes is still obscure, an interval between increased load and DNA transcription may be quite short. Some of the key enzymes regulating these processes showed a biphasic response the reason of which is not clear. The established hypertrophied heart muscle showed a decrease in velocity of isotonic shortening and an increase in resting tension. The former alteration is referred to a decrease in myosin ATPase activity and an disorder in excitation-contraction coupling mechanism, and the latter is supposed to be due to an increase in collagen in heart muscle.

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