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SYNTHESIS OF COLLAGEN, MYOSIN, NON-COLLAGENOUS
PROTEIN, AND DNA DURING EXPERIMENTAL MYOCARDIAL
HYPERTROPHY IN THE RAT¹

MASTER

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A variety of measures are available for the production of experimental cardiac hypertrophy. During and following the development of cardiac hypertrophy, a number of changes have been observed in the composition and metabolism of rodent hearts (see FANBURG, 1970). Workers in a number of laboratories have observed changes in ventricular DNA.¹ GROVE, NAIR, and ZAK (1969a), for example, noted an increase in the total DNA of the left ventricle following the induction of cardiac hypertrophy in rats by constriction of the thoracic aorta. The DNA increased in proportion to ventricular weight, so that there was no change in the DNA concentration with ventricular hypertrophy. The results of histological studies indicated that the increase in DNA resulted from proliferation of connective tissue cells. No mitotic figures were observed in muscle cells, and increased polyploidy of muscle cells following aortic banding could account for only a minute fraction of the increase. The mitotic indices of connective tissue cells increased in hearts following banding to values ten times greater than control, however. In addition, autoradiography after labeling with tritiated thymidine demonstrated that new DNA synthesis occurred almost exclusively in connective tissue cells (GROVE et al., 1969b).

Collagen is believed to be synthesized only by connective tissue cells. We have undertaken studies of the metabolism of collagen in correlation with cardiac hypertrophy as an index of the changes in the activity of connective tissue cells. Changes in the synthesis of non-collagenous protein (NCP), myosin, and DNA have also been estimated and compared with estimates of collagen synthesis during hypertrophy.

¹Abbreviations used: DNA, deoxyribonucleic acid; NCP, non-collagenous protein; TCA, trichloroacetic acid; hypro, hydroxyproline.

Some of the properties of collagen facilitate its study. Collagen is, for practical purposes, the only protein in mammalian tissue which contains the imino acid hydroxyproline (hypro). Hypro is derived from proline, which is hydroxylated after it is incorporated into procollagen, the polypeptide precursor of the collagen peptide chain (PROCKOP and KIVIRIKKO, 1968). Collagen can be extracted quantitatively from tissues into hot trichloroacetic acid (TCA) (FITCH et al., 1955). Thus, heart collagen content and radioactivity were estimated by measuring the content and radioactivity of hypro in hydrolysates of hot TCA extracts of heart (PROCKOP and UDENFRIEND, 1960).

We have confirmed the findings of GROVE et al. (1969b) and BARTOSOVA et al. (1969). The collagen content of the heart does not change during the early phases of hypertrophy induced by aortic banding and, consequently, the concentration falls. Later, the heart collagen content and concentration both increase (Fig. 1).

In a series of preliminary experiments, we measured the incorporation of radiolabeled proline into collagen hypro at times ranging from 2 to 30 days after induction of cardiac hypertrophy by aortic banding of 205-210 g female Sprague-Dawley rats (NAIR et al., 1968). Radiolabeled proline was injected intraperitoneally. Three hours later, heart collagen was extracted into hot TCA and its radioactive hypro content measured. Incorporation of radioactivity into NCP (the residue remaining after TCA extraction) was also measured (Fig. 2). Collagen hypro radioactivity was increased at each time studied. Enhanced labeling of heart components following aortic constriction could result from an increased delivery of radioactive precursor to the tissue. An increased specific activity of precursor pool and, subsequently, product would result. The percentage increase in labeling of collagen hypro exceeded the increase in labeling of

NCP (Fig. 2). Thus, it is unlikely that enhanced incorporation of radioactivity into collagen hydro resulted solely from an increase in the specific activity of the precursor pool.

The increase in heart weight resulting from aortic banding varies within a given banding procedure, and between experiments. The extent of collagen labeling varied with heart weight (Fig. 3). We wished to study the time course of labeling of collagen and NCP. In order to minimize the variations in the hypertrophic response, a large group of animals was banded on a single day. At varying times after banding, animals were injected with tritiated proline as before. Incorporation into collagen and NCP was measured. A peak of collagen labeling occurred 4 days after banding (Fig. 4). NCP labeling was maximal on about the second day, and remained elevated.

We also explored differences between the metabolism of muscle and connective tissue cells. A group of animals was banded. Two, 4 and 7 days after banding, members of the group were injected with a mixture containing tritiated thymidine (to measure nuclear DNA labeling), ^{14}C -lysine (to measure the labeling of myosin¹ and non-collagenous protein), and tritiated proline (to measure collagen labeling). The hearts were removed, weighed, and pooled according to whether they exhibited gross hypertrophy, moderate hypertrophy, or were from sham-operated animals. A peak of DNA labeling occurred in both groups of experimental animals one week after banding (Fig. 5). The curves of myosin and non-collagenous protein synthesis were very similar to each other, reaching a plateau and remaining elevated. Collagen synthesis by the moderately hypertrophied hearts reached a peak on the fourth day after banding. Collagen synthesis

¹ Myosin was extracted by a procedure to be published.

in the grossly hypertrophied hearts reached a maximum on or before the second day after banding. The time course of labeling of myosin, which is formed in the muscle cells, is distinctly different from that of the labeling of nuclear DNA and collagen, which are formed by the connective tissue cells. Also, changes in collagen labeling by the connective tissue cells in response to banding occur with a different time course than the changes in labeling of DNA.

We can only speculate about the effects of an increased collagen concentration in the hypertrophied heart. It has been suggested that the collagen of blood vessels prevents excessive distension when pressure in them is increased (see HARKNESS, 1968). Collagen in the heart might similarly prevent excessive distension which would result in decreased efficiency of contraction processes (cf. SONNENBLICK, 1970).

Summary

Using appropriate precursors, we have measured the labeling of collagen hydroxyproline (hypro), non-collagenous protein (NCP), myosin, and DNA in rat hearts undergoing hypertrophy as the result of surgically-induced aortic stenosis. A peak of incorporation of radiolabeled proline into collagen hypro occurred at 4 days after aortic banding. The peak of DNA labeling (with ^3H -thymidine) occurred at 7 days. Labeling of myosin and NCP was maximal on day 4 and remained elevated. The percentage increase in collagen hypro labeling exceeded the increase in labeling of NCP and myosin. The results indicate that muscle and connective tissue cells are stimulated differently following the induction of cardiac hypertrophy.

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References

BARTOSOVA, D.; CHVAPIL, M.; KORECKY, B.; POUPA, O.; RAKUSAN, K.;

TUREK, Z. and VIZEK, M.: The growth of the muscular and collagenous parts of the rat heart in various forms of cardiomegaly. *J. Physiol., Lond.* 200: 285-295 (1969).

FANBURG, B. L.: Experimental cardiac hypertrophy. *New Engl. J. Med.* 282: 723-732 (1970).

FITCH, S. M.; HARKNESS, M. L. R. and HARKNESS, R. D.: Extraction of collagen from tissues. *Nature, Lond.* 176: 163 (1955).

GROVE, D.; NAIR, K. G. and ZAK, R.: Biochemical correlates of cardiac hypertrophy. III. Changes in DNA content; the relative contributions of polyploidy and mitotic activity. *Circulat. Res.* 25: 463-471 (1969a).

GROVE, D.; ZAK, R.; NAIR, K. G. and ASCHENBRENNER, V.: Biochemical correlates of cardiac hypertrophy. IV. Observations on the cellular organization of growth during myocardial hypertrophy in the rat. *Circulat. Res.* 25: 473-485 (1969b).

HARKNESS, R. D.: Mechanical properties of collagenous tissues. In GOULD, B. S. (Editor): *Treatise on collagen, Vol. 2, Biology of collagen, Part A*, pp. 247-310 (Academic Press, London/New York 1968).

NAIR, K. G.; CUTILLET, A. F.; ZAK, R., KOIDE, T. and RABINOWITZ, M.: Biochemical correlates of cardiac hypertrophy. I. Experimental model; changes in heart weight, RNA content, and nuclear RNA polymerase activity. *Circulat. Res.* 23: 451-462 (1968).

PROCKOP, D. J. and KIVIRIKKO, K. I.: Hydroxyproline and the metabolism of collagen. In GOULD, B. S. (Editor): Treatise on collagen, Vol. 2, Biology of collagen, Part A, pp. 215-246 (Academic Press, London/New York 1968).

PROCKOP, D. J. and UDENFRIEND, S.: A specific method for the analysis of hydroxyproline in tissues and urine. *Analyt. Biochem.* 1: 228-239 (1960).

SONNENBLICK, E. H. : Myocardial ultrastructure in the normal and failing heart. *Hospital Practice* 5: 35-43 (1970).

Figure Legends

Fig. 1. Effect of banding upon total heart collagen and collagen concentration. Open circles represent means from sham-operated animals; closed circles means from banded animals.

Fig. 2. Effect of banding upon recovery of radioactive proline as collagen hypro and in NCP. Animals were each injected with 10 μCi proline- $\text{U-}^{14}\text{C}$ on day 7 after banding, and with 50 μCi proline-2,3- ^3H on all other days. The height of the bars represents mean radioactivity recovered in hearts from sham-operated (white bars) and banded (black bars) animals. The lengths of the vertical lines represent 2 S.E. of the means. The number in each group is given at the base of the bars. Each difference is significant by Student's t test with a p value of 0.01 or less.

Fig. 3. Correlation of heart weight with collagen synthesis. Individual data from the experiments in Fig. 2 are plotted. Open circles represent observations in sham-operated animals; closed circles, banded animals.

Fig. 4. Time course of collagen and NCP synthesis after banding. The mean radioactivity recovered in sham-operated (open circles) and banded (closed circles) animals is plotted. The lengths of the vertical lines represent 2 S.E. of the means. Where comparisons between banded and sham-operated animals are possible, the differences are significant by Student's t test with a p value of 0.02 or less.

Fig. 5. Time course of synthesis of collagen, myosin, NCP, and DNA following aortic banding. Animals were injected with a mixture of ^3H -proline, ^{14}C -lysine, and ^3H -thymidine 2, 4 and 7 days after banding. Hearts were weighed and pooled according to whether they exhibited gross hypertrophy (solid triangles), moderate hypertrophy (solid squares) or no hypertrophy (sham-operated, open circles). The result from each pool of 2-3 hearts is plotted.

TOTAL HEART
COLLAGEN, MG

COLLAGEN
CONC., MG PER
100 MG HEART
(WET WEIGHT)

COLLAGEN
HYPRO RADIOACTIVITY
RECOVERED PER
100 MG TISSUE,
PERCENT MEAN
CONTROL

NCP PRO
RADIOACTIVITY
RECOVERED
PER MG PROTEIN,
PERCENT MEAN
CONTROL

COLLAGEN
HYPRO RADIOACTIVITY
RECOVERED PER
100 MG TISSUE,
PERCENT MEAN
CONTROL