Response of myocardial connective tissue to development of experimental hypertrophy

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BUCCINO, R. A., E. HARRIS, J. F. SPANN, JR., AND E. H. SONNENBLICK. Response of myocardial connective tissue to development of experimental hypertrophy. Am. J. Physiol. 216(2): 425-428. 1969.—Myocardial collagen concentration was determined by measuring hydroxyproline in right and left ventricles of normal cats and those with right ventricular hypertrophy produced by chronic constriction of the pulmonary artery. Both in normal and hypertrophied ventricles, hydroxyproline concentration was significantly higher in the epimyocardium than in endomyocardium; and in both the epimyocardium and endomyocardium, hydroxyproline was significantly higher in right ventricular than in corresponding left ventricular specimens. In the presence of right ventricular hypertrophy, the concentration, as well as the total content of hydroxyproline, increased in both the right and the unstressed left ventricle.

collagen; hydroxyproline; myocardium

Although the synthesis of myofibrillar protein is increased in hearts subjected to a systolic overload, the extent to which connective tissue elements participate in this response has not been defined (6, 7, 13, 21, 22). In the hypertrophy of skeletal and smooth muscle the content of collagen has been shown to increase along with muscle proteins without a change in their relative proportions (8, 9, 16, 25, 26). In the normal human heart, the concentration of collagen is independent of age, except for increases in the right ventricle during the first 10 years of life (17, 19, 28). Blumgart et al. (2) measured collagen concentration in the hypertrophied human heart and found it increased in 9 of 24. Of these nine, five had demonstrable coronary disease. However, Montfort and Perez-Tamayo (17) found that the ratio of collagen to muscle protein in human left ventricles was unaltered in various types of heart disease accompanied by myocardial hypertrophy, while Oken and Boucek (19) found an increase in left ventricular collagen content in only 2 of 22 hearts with significant hypertrophy.

Since connective tissue contributes to ventricular compliance and since increases in its concentration might interfere with contractile activity, the present study was undertaken to determine the changes in connective tissue which accompany hypertrophy in an experimentally controlled setting, in the absence of such potentially modifying factors as coronary disease and ischemia. Since hydroxyproline is found only in collagen and forms a constant concentration of collagen, the concentration of collagen, and thus the connective tissue component, in myocardium could be determined by measuring hydroxyproline. When this was done in the right and left ventricles of cats with right ventricular hypertrophy produced by chronic constriction of the pulmonary artery, it was found that connective tissue does participate in the process of myocardial hypertrophy, and in fact, increases proportionately more than do the muscle proteins themselves.

METHODS

Myocardial specimens were obtained from the hearts of 17 normal adult cats (N) and 26 adult cats with right ventricular hypertrophy produced by chronic constriction of the main pulmonary artery (RVH), a procedure described in detail previously (23, 24). Animals were excluded if there was evidence of overt congestive heart failure, such as pleural effusion, ascites, a marked reduction in cardiac index, or marked elevation in right ventricular end-diastolic pressure or arteriovenous oxygen difference (24). Initial body weight of animals in both groups ranged from 1.6 to 2.9 kg, and the duration of constriction in the RVH group ranged from 3 to 90 days with most falling within 20-50 days. At the time of sacrifice, animals were anesthetized with 25 mg/kg intraperitoneal sodium pentobarbital, and the heart was rapidly excised. Atria were removed by dissecting along the atrioventricular groove, and the right ventricular wall was separated free of the septum and left ventricle; the specimens were immediately weighed, the entire septum included with the left ventricle, and frozen at -4 C within 3-5 min. The free wall of each ventricle, excluding the septum, was divided at a midplane into endocardial-myocardial
and epicardial-myocardial specimens, which were sub-
sequently minced, blotted, and weighed with water
content determined by drying to constant weight (24
hr at 100 C "in vacuo" over P2O5). Dry samples,
weighing between 5 and 30 mg, were hydrolyzed at
140 C in 4 ml 6 N HCl for 3 hr. After filtration through
Whatman no. 1 paper, aliquots were assayed for hydroxy-
proline by the method of Prockop and Udenfriend (20)
and expressed in micrograms per milligram of dry
weight. Substances found by LeRoy et al. (11) which
interfere with colorimetric assay for hydroxyproline in
plasma were not present in significant amounts in
assays of heart tissue, and thus a chromatographic step
in the assay was unnecessary. Since mammalian collagen
consists of a constant 13.4% hydroxyproline by weight
(11, 18), collagen concentration was calculated by
multiplying hydroxyproline concentration by 7.46. The
nitrogen concentration of the dried tissue was measured
by the semimicro-Keljahl technique and was found
to closely parallel dry weight, indicating that there was
no measurable change in the concentration of the non-
protein components of the heart during hypertrophy.

RESULTS

Right ventricular hypertrophy. In 26 cats chronic con-
striction of the pulmonary artery by approximately
85% resulted in a doubling of right ventricular weight
(P < 0.01), while left ventricular weight remained
unchanged (Table 1). There were no significant
changes in the water content of the hearts with RVH
(Table 2), indicating that the increase in right ven-
tricular weight observed with chronic constriction of
the pulmonary artery represented a true increase in
myocardial tissue, parenchymal and/or stromal, and
was not altered by an accumulation of interstitial or
intracellular fluid.

Hydroxyproline concentration (Table 3). In the normal
heart the concentration of hydroxyproline was sig-
ificantly higher in the right than in the left ventricle,
and substantially greater in the epimyocardial than in
the endomyocardial layers of both ventricles. The
dry weight concentration of hydroxyproline was found
to be increased by 39% in the right ventricular endomy-
ocardium of RVH animals and by 31% in the right

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<th>TABLE 1. Ventricular weight in normal cats and cats with right ventricular hypertrophy</th>
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<p>| TABLE 2. Water content of endocardial-myocardial |</p>
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<th>and epicardial-myocardial specimens</th>
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<td>Right Ventricle</td>
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Water content is expressed as a percentage of wet weight for endocardial-myocardial and epicardial-myocardial ventricular specimens. Figures represent the mean ±SEM with the number of experiments for each group in parentheses. There were no significant differences in water content indicating that the increase in right ventricular weight shown for RVH hearts in Table 1 represents a true increase in myocardial tissue.

| TABLE 3. Hydroxyproline concentrations in endocardial-
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Hydroxyproline concentration is expressed as μg/mg dry wt for endocardial-myocardial and epicardial-myocardial ventricular specimens. Figures represent the mean ±SEM with the number of experiments for each group in parentheses. Significant increases in hydroxyproline concentration, and thus collagen content, were produced by chronic constriction of the pulmonary artery (RVH) in both right and left ventricular specimens.

Ventricular epicardium. In addition, the dry weight con-
centration of hydroxyproline was increased by 37% in
the left ventricular endomyocardium of RVH ani-
mals and by 34% in the left ventricular epicardium.
Expressed in terms of wet tissue weight, this represented
an increase in collagen concentration from 2.4 to 3.3% in
the right ventricle and an increase from 1.4 to 2.0% of
wet weight in the left ventricle. Furthermore, in both right and left ventricles in RVH animals, as in
normal hearts, the concentration of hydroxyproline
was higher in the epicardium than in the endo-
myocardium.

DISCUSSION

The connective tissue response in the development of experimental cardiac hypertrophy has been assessed by measuring the myocardial concentration of hydroxy-
proline, an amino acid found in significant amounts only
in collagen and representing a constant 13.4% of
collagen (20). The measurement of hydroxyproline has
been shown to be both specific and reproducible and
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thus serves as a quantitative index of collagen content of tissues (11, 18, 20).

In normal hearts hydroxyproline concentration was found to be significantly higher in epimyocardial than in endomyocardial specimens, and in both the epimyocardium and endomyocardium hydroxyproline was significantly higher in right than in corresponding left ventricular specimens. Furthermore, in hearts with right ventricular hypertrophy produced by chronic constriction of the main pulmonary artery (RVH), hydroxyproline determinations in epimyocardial and endomyocardial specimens of both right and left ventricles were significantly higher than in the corresponding specimens of normal hearts. Nevertheless, within the RVH group, as in the case of the controls, epimyocardial values for the hydroxyproline were significantly greater than in the endomyocardium, and right ventricular concentrations of hydroxyproline were significantly greater than the corresponding left ventricular values.

The explanation for the increase in connective tissue and its distribution in hypertrophy remains a matter of speculation. It is difficult to account for these observations solely on the basis of limitations in coronary blood flow, hypoxia, or simple mechanical stresses, since these would favor collagen formation in endomyocardial to a greater extent than in epimyocardial areas (10, 15). However, the present findings could reflect the normal distribution of the coronary vasculature and its response to the process of hypertrophy, since collagen tissue is known to be distributed primarily between muscle fibers and along blood vessels. Coronary vessels arise from the epicardial surface and diminish in number as they penetrate to the endocardial surface (1, 3, 27). Were collagen to follow a similar distribution, the decrease in collagen from the epicardial toward the endocardium would be explained. Since there appears to be one capillary per muscle fiber in both right and left ventricles and fibers are thicker in the left (12, 27), there is a relative preponderance of vascular elements, and, hence, connective tissue in the right ventricle. Further, Meerson and co-workers (14) have provided histologic evidence that collagen fibers around blood vessels in the heart appear to increase in response to a systolic overload. From this, one might expect a greater concentration of collagen tissue in the normal and overloaded right ventricle, as observed in the present study.

Mechanisms are not available to explain why an increase in collagen should occur in the nonstressed left ventricle as well. The possibility of a circulating growth factor has not been ruled out. It is also necessary to recall that the ventricles form a functional syncytium. Further, connective tissue elements possess a greater potentiality for hyperplasia than striated muscle (4), and thus might respond proportionately more to a growth stimulus in myocardium undergoing hypertrophy. Finally, there is precedent for alterations in the left ventricles of hearts subjected to right ventricular stress, in terms of norepinephrine depletion (24) and depression of myofibrillar adenosine triphosphatase activity (3). The functional importance of the changes in the left ventricle when only the right ventricle has been stressed remain to be evaluated.

This study was presented, in part, before the American Heart Association, San Francisco, Calif., 21 October 1967 (Circulation 36 (Suppl. 2), 1967).

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