Changes in collagen and elastin in rabbit right-ventricular pressure overload

Robert B. LOW,*§ William S. STIREWALT,* Philip HULTGREN,* † Elizabeth S. LOW* and Barry STARCHER†
*Department of Physiology and Biophysics, University of Vermont, Burlington VT 05405, U.S.A., and †Department of Biochemistry, University of Texas, Tyler, TX 75701, U.S.A.

Collagen content, the ratio of collagen types I and III and elastin content were measured in 5–6- and 10–12-week-old rabbits with and without right-ventricular pressure overload. Significant and equivalent hypertrophy occurred in both age groups. A 2-day pressure overload caused a fall in collagen concentration below control levels in right-ventricular tissue from the older animals, but no change in the younger ones. A 2-week pressure overload in the older animals resulted in a rise in collagen concentration, a decreased ratio of type III to type I plus III [III/(I+III)] collagens, a fall in desmosine concentration and a fall in the desmosine/hydroxyproline ratio in the right ventricle. None of these changes occurred in the younger age group. We hypothesize that the changes in connective-tissue proteins after overload in the older group may contribute to previously observed changes in mechanical performance. The divergent connective-tissue responses in the two groups suggest the importance of age in determining outcome, as well as the possibility of separate regulatory mechanisms for contractile and for architectural elements of the heart.

INTRODUCTION

The adaptation of the heart to short- and long-term hypertrophic stresses involves a co-ordinated pattern of change of metabolic, contractile and morphological properties that are important to survival (Hamrell & Alpert, 1986; Cooper, 1987; Morgan et al., 1987). We have studied this problem in right-ventricular pressure-overloaded rabbit hearts caused by banding of the pulmonary artery (Hamrell & Alpert, 1977). Changes in mechanical performance (Alpert et al., 1974) as well as myosin isoforms (Litten et al., 1982; Nagai et al., 1987) have been interpreted to indicate a slower, more economic, heart better suited to the additional work load (Hamrell & Alpert, 1986).

The connective-tissue matrix of the heart, though a small proportion of total tissue, is considered a major contributor to the active and passive properties of the organ in terms of maintaining cell–cell relationships, as well as distributing mechanical stress during the cardiac cycle (Caulfield, 1983; Cooper, 1987; Factor & Robinson, 1988; Weber et al., 1988b). There are several reports that pressure-overload hypertrophy causes a selective increase in connective-tissue elements (Hamrell & Alpert, 1986; Cooper, 1987; Weber et al., 1988a), though this has not universally been the case (Bartosova et al., 1969; Williams et al., 1982).

The purpose of the present studies was to determine what changes occur in two major connective-tissue elements, collagen and elastin, in our pressure-overload model. Significant hypertrophy occurred in 5–6- and 10–12-week-old rabbits, yet the profile of changes in connective-tissue elements was different for each age group. A preliminary report of these results has appeared elsewhere (Low et al., 1987).

METHODS

The methods for producing overload have been reported (Hamrell & Alpert, 1977). Male New Zealand White rabbits were obtained from local suppliers and maintained on standard rabbit chow and water ad libitum in the University Animal Care Facility for 2–7 days before the experiments began. Antibiotics were not included in the diet. Pressure overload was produced by placing a spiral metal band around the pulmonary artery of 5–6- and 10–12-week-old animals, which caused a constrictore to 50% (5–6 weeks) or 66%, (10–12 weeks) of the original diameter (Hamrell & Alpert, 1977; Nagai et al., 1987). In no cases were there signs of congestive heart failure, as was previously found (Hamrell & Alpert, 1977; Litten et al., 1982).

Animals were killed by intravenous injections of 0.8 ml of T-61 euthanasia solution (American Hoechst Corp.) containing a local anaesthetic, a muscle relaxant and a central-nervous-system depressant, and the hearts were rapidly removed and placed in ice-cold 0.9% NaCl. The left ventricle plus septum was separated from right ventricle, and each was weighed before being frozen in liquid N₂ for subsequent biochemical analyses.

The collagen content of ventricular tissues was measured as hydroxyproline, and protein was measured as leucine in HCl protein hydrolysates as described by Airhart et al. (1979). The method gives the correct answer when applied to known amounts of purified type I and type III collagen (results not shown).

Collagen types I and III were quantified by a modification of the method of Laurent et al. (1981). Briefly, frozen powdered tissue was extracted repeatedly with 2% (w/v) SDS. The residue was washed with phosphate-buffered saline (0.137 M-NaCl/8.1 mm-

† Present address: Department of Physiology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501, U.S.A.
§ To whom reprint requests should be addressed.
Na₂HPO₄/1.5 mM-KH₂PO₄, pH 7.1) and suspended in formic acid for CNBr digestion. The resultant CNBr peptides were separated from the insoluble residue by centrifugation using one-dimensional SDS/polyacrylamide-gel electrophoresis under non-reducing conditions. Quantification of CB8 peptide for type I collagen and CB5 for type III was by densitometry using a Microscan 1000 automated video scanner (Technology Resources Inc.). The use of non-reducing conditions avoids the overlap of CB5 and CB9 peptides that can occur under other conditions (Chan & Cole, 1984; Turner, 1988). Background readings were taken on a gel-by-gel basis. Standards containing known amounts of types I and III collagen were included with each gel. The standards routinely used were rabbit skin type I collagen prepared as previously described (Chung & Miller, 1974; Epstein, 1974) and human placental type III collagen (Sigma Chemical Co.). The results obtained by using those standards were the same as those obtained with collagens purified from rat and human tissue (kindly given by Dr. Jill Turner). Turner (1988) has shown that there is no species variation between the ratios of CB8 and CB5 peptides for known amounts of types I and III collagen.

Desmosine was measured by radioimunoassay (King et al., 1980). Desmosine-haemocyanin conjugates were used to raise antibodies in rabbits. The titre is higher with this conjugate, whereas the sensitivity and specificity remain the same as reported previously (King et al., 1980).

The total tissue content of hydroxyproline and desmosine was calculated by using a value of 109 µmol of leucine in protein/g wet wt., as we determined for rabbit cardiac tissue.

Unpaired t tests were used to compare pressure-overloaded with control animals. Paired t tests or repeated measures of analysis of variance were used to compare data within the same heart.

RESULTS

Overall response to pressure overload

The effects of pressure overload on animal and ventricular weights (Table 1) were as reported previously (Litten et al., 1982; Nagai et al., 1987, 1988). Animal and left-ventricular weights in the pressure-overload groups were not different from control values. The degree of right-ventricular hypertrophy was slightly greater in the older-aged group in terms of absolute values, but was equivalent when normalized to total ventricular weight. The marked increases in right-ventricular mass were virtually complete within 2 days.

Collagen

Right-ventricular collagen concentration, measured as the hydroxyproline/leucine ratio, was greater than that for left-ventricular tissue for the control groups. Left-ventricular collagen concentration did not increase as a function of age in control animals. At the same time, the collagen concentration of right ventricles of the older control animals was approx. 1.5 times that of the younger ones.

Right-ventricular collagen concentration fell slightly in the 2-day period after pressure overload in the 10–12-week animals, but was significantly elevated by 2 weeks. At the same time, there were no changes in collagen concentration in the younger animals throughout the time course of our studies. Total collagen was increased in both age groups and at both times after banding, even

Table 2. Effects of pressure overload on right-ventricular collagen types

Data are expressed as means±s.d. (n). Abbreviations: I, collagen type I; III, collagen type III; LV, left ventricle; RV, right ventricle. *P<0.05 versus left ventricle. †P<0.05 versus control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Pressure overload</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV</td>
<td>RV</td>
</tr>
<tr>
<td>10–12 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.37±0.03</td>
<td>0.33±0.05*</td>
</tr>
<tr>
<td>(n=10)</td>
<td>(9)</td>
<td>(10)</td>
</tr>
<tr>
<td>PO2 (n=8)</td>
<td>0.38±0.04</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td>(8)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>PO14 (n=10)</td>
<td>0.40±0.02</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>(10)</td>
<td>(8)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Effects of pressure overload on ventricular weight and collagen

Data are expressed as means±s.d. Abbreviations: LV, left ventricle, RV, right ventricle; PO2, PO4, PO14, 2-, 4- and 14-day pressure overload respectively. Collagen concentration is expressed as the Hyp/Leu ratio; total ventricular collagen was measured as total Hyp. *P<0.05 versus corresponding 10–12-week group. †P<0.05 versus respective left ventricle. ‡P<0.05 versus corresponding control right ventricle.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal wt. (g)</th>
<th>RV wt. (g)</th>
<th>RV</th>
<th>Hyp/Leu</th>
<th>Total Hyp (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RV + LV</td>
<td>LV</td>
<td></td>
<td>LV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RV</td>
<td>RV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10–12 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>2394±309</td>
<td>0.71±0.15</td>
<td>0.19±0.02</td>
<td>0.08±0.02</td>
<td>0.16±0.04†</td>
</tr>
<tr>
<td>PO2 (n=8)</td>
<td>1902±260</td>
<td>1.23±0.16‡</td>
<td>0.34±0.04</td>
<td>0.13±0.01†</td>
<td>0.20±0.04†‡</td>
</tr>
<tr>
<td>PO14 (n=10)</td>
<td>1920±134</td>
<td>1.39±0.22‡</td>
<td>0.34±0.02</td>
<td>0.27±0.04‡</td>
<td>0.30±0.04†‡</td>
</tr>
<tr>
<td>5–6 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=16)</td>
<td>868±266*</td>
<td>0.39±0.12</td>
<td>0.24±0.02</td>
<td>0.07±0.01</td>
<td>0.11±0.03‡</td>
</tr>
<tr>
<td>PO2 (n=8)</td>
<td>786±142*</td>
<td>0.58±0.12‡</td>
<td>0.31±0.03</td>
<td>0.12±0.01</td>
<td>0.13±0.04‡</td>
</tr>
<tr>
<td>PO14 (n=10)</td>
<td>949±133*</td>
<td>0.65±0.24‡</td>
<td>0.32±0.05</td>
<td>0.07±0.02</td>
<td>0.09±0.03*</td>
</tr>
</tbody>
</table>
in the 10–12-week animals at 2 days, when collagen concentration was decreased.

Collagen types

The percentage of type III collagen [type III/(type I + III)] was higher in the left than in the right ventricle of 10–12-week, but not 5–6-week, control animals (Table 2). The percentage of type III collagen fell in response to overload in the older animals, but there was no change in the younger ones.

Desmosine

Desmosine concentration, expressed as the desmosine/leucine ratio, was significantly higher in the right than in the left ventricle in control animals of both ages, and increased as a function of age in the right ventricle only (Table 3). The only significant difference for control animals in the relative amounts of elastin and collagen, expressed as the desmosine/hydroxyproline ratio, was between the left and right ventricles for the older animals.

The 2-week pressure overload led to a significant fall in desmosine concentration of the right ventricle from 10–12-week overloaded rabbits, but no change was observed for the right-ventricular tissue from 5–6-week animals. Overload also led to a significant fall in the desmosine/hydroxyproline ratio in right-ventricular tissue from the older animals. Total ventricular desmosine was increased in both groups.

DISCUSSION

We show that changes in collagen and elastin concentrations and in the proportions of types I and III collagen occur after right-ventricular pressure-overload hypertrophy in 10–12-week-old rabbits. Changes in connective tissue fail to occur, however, in younger (5–6-week-old) animals, despite a similar degree of hypertrophy.

The concentration and content of ventricular collagen that we calculate based on hydroxyproline agree well with those reported in the literature when converted into the same units (Buccino et al., 1969; Caspari et al., 1975; Medugorac, 1980). Our data also agree with previous findings that right-ventricular collagen concentration is greater than that for the left ventricle (Buccino et al., 1969; Caspari et al., 1975; Medugorac, 1980).

Our results for the older animals are in agreement with previous reports indicating a disproportionate increase in connective-tissue elements after pressure-overload hypertrophy (Bartosova et al., 1969; Buccino et al., 1969; Medugorac, 1980; Caulfield, 1983; Shaper, 1983; Thiedemann et al., 1983; Cooper, 1987; Weber et al., 1988a,b). An initial fall in collagen concentration immediately after overloading the heart also has been reported in the rat (Skosey et al., 1972), and in the rabbit (Turner et al., 1986), as well as in skeletal-muscle hypertrophy in the chicken (Laurent et al., 1985). Thus rapid remodelling of connective-tissue elements can be an important feature of the compensatory growth process. The overall process of cardiac hypertrophy also is rapid, taking place over a few days (Nagai et al., 1987, 1988), as again is true for skeletal muscle (Laurent et al., 1978).

Whether or not the changes in connective tissue that we have observed in the older animals are due to increased numbers of fibroblasts, increased connective-tissue output per cell, or both (Morkin & Ashford, 1968; Skosey et al., 1972; Turner et al., 1986), remains to be determined. In any case, we do not believe that the results with the older animals are due to injury and necrosis (Meerson, 1969; Bishop & Melsen, 1976; Williams et al., 1982), based on previous morphological evaluation (Legato et al., 1984).

At least ten different collagen types exist in mammalian tissues, of which the most important on a quantitative basis are types I and III (Miller, 1976; Epstein & Munderloh, 1975; Mays et al., 1988; Weber et al., 1988a). The control values we report for collagen type III as a percentage of type I plus type III are significantly higher than those reported by others, except for Turner & Laurent (1986) and Mays et al. (1988), whose methods we used. This method avoids the variable recoveries that have been reported based on analyses of pepsin digests (Burke et al., 1977; Dawson et al., 1982; Medugorac, 1982; Weber et al., 1988a).
Dawson et al. (1982) and Medugorac (1982) have reported increases in type III collagen in left-ventricular pressure-overload hypertrophy in the rat. Weber et al. (1988a) have reported a transient increase in the proportion of type III collagen in left-ventricular pressure overload after systemic hypertension in non-human primate myocardium, which is followed by a return to normal. We report a decrease in the proportion of type III collagen 2 weeks after right-ventricular pressure overload in older animals, as Turner & Lauret (1986) have found. It may be that the response of the right and left ventricles to overload is not the same, though, again, the different results could be explained by the different models, species and/or methods that have been used.

We are not aware of published values for the elastin content of heart tissue, though the values are considerably lower than for vascular tissue (Starcher & Gallione, 1976; Brayden et al., 1983). We believe that the desmosine measurement is a true reflection of elastin, since the rate of desmosine cross-link formation is rapid (Miyoshi et al., 1976; Narayanan & Page, 1976). Noteworthy is the fall in elastin concentration 2 weeks after overload in the older animals over the same time period during which collagen concentration increases while the proportion of type III collagen falls.

Disproportionate changes in connective tissue may be causally related to changes that also occur in both the active and passive properties of the myocardium after pressure-overload hypertrophy (Thiedemann et al., 1983; Hamrell & Alpert, 1986; Cooper, 1987; Weber et al., 1988b). Particularly relevant are the studies by Hultgren & Hamrell (1985), who found a shift in the sarcomere-length–passive-stress relationship, indicating higher resting stress, using the same hypertrophy model in which we found connective-tissue changes. Studies of mechanical performance after pressure-overload hypertrophy in the younger animals that we studied, in which connective-tissue changes do not occur, would provide important insight.

Changes in collagen concentration do not always occur after pressure-overload hypertrophy (Bartosova et al., 1969; Williams et al., 1982), as indicated by our data for the younger rabbits. The lack of change in the proportions of types I and III collagens as well as in elastin further indicate the importance of age in determining the outcome. We believe these results indicate that the contribution of regulatory mechanisms such as passive stress (Petersen & Lesch, 1972; Zak, 1981; Cooper, 1987; Morgan et al., 1987) is different in the two age groups. Alternatively, changes in the relative contents of connective-tissue and of contractile elements reflect the differential rates of turnover of the two groups of proteins (Zak et al., 1979). The decrease in protein turnover that occurs with age (Zak et al., 1979) could place an age-dependence on the pattern of change after a hypertrophic stress, particularly for slowly turning-over connective-tissue proteins.

We thank Dr. Jill Bishop for thoughtful discussion, as well as Ms. Ilene Morgan, Ms. Bonnie McLeod, Ms. Sandy Kapsalis and Mr. Robert Smith for expert technical assistance, and Ms. Janice Gregoire for expert secretarial service. This research was supported by grants PHS-HL28001, PHS-HL36412 and PHS-HL14212, as well as grants from the Meadows Foundation and N.A.T.O.

REFERENCES


1989
Collagen and elastin in pressure overload


Received 14 February 1989/12 June 1989; accepted 3 July 1989