Myosin isozyme changes in the heart following constriction of the ascending aorta of a 25-day old rat

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When a constricting band is placed around the ascending aorta of young (25-day old) rats, all chambers of the heart eventually produce hypertrophy. Both the left and right ventricles show strong shifts toward an isozyme pattern in which V3 is predominant, similar to that seen in models where hypertrophy is induced in adult rats. The hypertrophied atria however, show no detectable change in the native myosin isozymes or the light chain subunits.

Myosin isozyme *Light chain* *Cardiac hypertrophy* *Adaptation*

1. INTRODUCTION

Cardiac muscle retains a great potential for adaptation to stress throughout the life of the rat. This inherent plasticity permits the heart to rapidly respond to an increased work load by increasing its mass (cardiac hypertrophy). Such a hypertrophy may be produced either physiologically, (as a result of exercise) or pathologically (either naturally or after experimental manipulation). Large increases in ventricular mass without any corresponding increase in the numbers of myocytes (hyperplasia) can take place within 2–3 days [1] after constricting the ascending aorta of the adult rat. However, this same type of stress (aortic stenosis) in 3-week old rats not only produces a dramatic increase in muscle mass, but also stimulates myocyte DNA synthesis and presumably cell division [2,3]. In adults, in addition to increased cell size and number, the contractile properties of the heart are also altered, and in particular the myosin isozyme composition is modified [4–6].

The various ventricular myosin isozymes are called V1, V2 and V3, as defined by their relative speed of migration during electrophoresis of the native protein. Both V1 and V3 are thought to be homodimers of one of two different heavy chain isozymes, and V2 would appear to be a heterodimer [7]. In rats, the V3 isozyme is the predominant form in the fetal heart but by 3 weeks after birth the V1 isozyme is the only one found [8–10]. Beyond 2–3 months of age, V2 and V3 begin to reappear and their amounts increase with age. In adult rats, cardiac hypertrophy is accompanied by a pronounced shift in the myosin isozyme composition toward the V2 and V3 isoforms [5] which also results in decreased cardiac muscle contractility [11]. The V1 species has the highest level of ATPase activity and V3 the lowest, and changes in the relative amounts of these two isozymes are closely correlated with the altered contractile properties [12]. The V3 form which reappears in adult hearts is indistinguishable from the fetal V3 form by several biochemical and immunochemical criteria [13,14].

Since cardiac hypertrophy in young animals is accompanied by DNA synthesis and probably myocyte division in addition to cell enlargement, it is possible that the change in myosin isozyme pattern is different from that occurring during hypertrophy in the adult. We have investigated this possibility by examining the myosin isozyme com-

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position after producing cardiac hypertrophy in young (25-day old) rats.

2. MATERIALS AND METHODS

2.1. Cardiac hypertrophy

Cardiac hypertrophy was produced by placing a constricting band of 0.02 inches (0.51 mm) around the ascending aorta of 25-day old rats [3]. The same operation was performed on sham control animals except that no band was placed around the ascending aorta. At various times after the operation, the rats were killed, the hearts placed in PBS at 4°C and dissected into the left ventricular wall plus septum, right ventricular wall, and atria. These tissue samples were rapidly frozen in liquid nitrogen and stored at −80°C.

2.2. Electrophoretic techniques

Electrophoresis of native myosin isozymes was performed according to [8] as modified in [4]. Two-dimensional electrophoresis was carried out as in [15] and used to investigate myosin light chain composition. The isoelectric focusing was done using pH 5–7 ampholites (LKB). The second dimension slab gel was composed of 12.5% polyacrylamide and 0.34% bisacrylamide.

3. RESULTS AND DISCUSSION

3.1. Analysis of native myosin isozymes

Placement of a constricting band around the ascending aorta produces an increased workload on the heart, particularly in the left ventricle, and results in cardiac hypertrophy. As early as 2 days after constriction the left ventricle begins to enlarge and by two months the average increase in left ventricular weight is 80%. By two months after banding, the right ventricle increases an average of about 40% although increases of as much as 100% have been observed. Finally, the atria undergo a weight increase of about 200%, although at least two atria were enlarged as much as 10-fold. The enlargement of chambers of the heart not directly affected by the stress plus the fact that animals have lived as long as a year after banding suggest that overall compensation to a decreased performance in the left ventricle is taking place in these hearts. Few models of cardiac hypertrophy in the rat produce such dramatic changes in chambers not directly affected by the primary stress.

Changes in the myosin isozyme composition of the left ventricle can be observed in a few hearts as early as 7 days after banding. However, at 15 days after constriction of the aorta (40 days old), all left ventricles examined contained the V2 and V3 isozymes, in addition to V1 which was still the major form (fig. 1A). Normally the V1 isozyme is the only form detectable by electrophoresis in cardiac muscle of control rats until 2 to 3 months of age (fig. 1B) which is in agreement with [9,10]. The amounts of V2 and V3 in the left ventricle of experimental rats continue to increase throughout the period investigated and by two months after banding V3 is the predominant species in several hearts.

The right ventricle also shows increases in the amounts of V2 and V3. Densitometry of a gel of myosins from the right ventricle of a 23-day banded rat (48 days old) clearly shows 3 peaks (Fig.2). These changes can be observed in the right ventricle as early as 15 days after banding, showing that, although the primary stress is on the left ventricle, biochemical modifications and perhaps changes in contractile performance also occur on the right side of the heart.

We also examined the myosin isozymes in the atria since 8 out of 9 left atria had at least 100% hypertrophy after 2 months of banding. Surprisingly, no clearcut changes in isozyme content were observed (fig.3) even in the atria with the greatest hypertrophy. Atria from both control and banded hearts had one major band and possibly a second minor band which was not clearly separated from the major one.

3.2. Analysis of myosin light chain subunits

The myosin light chains of control and hypertrophied ventricles were indistinguishable as determined by two-dimensional electrophoresis (fig.4A,B). However, it has been reported that in hypertrophied human atria the ventricular light chains are present in addition to the atrial forms [16]. This possibility was therefore examined in highly hypertrophied rat atria. The atria from banded animals showed the two atrial light chains which are distinct from the ventricular forms [17,18]. A small amount of the ventricular LC1 subunit could be seen in the hypertrophied atria; however, small amounts were also present in the

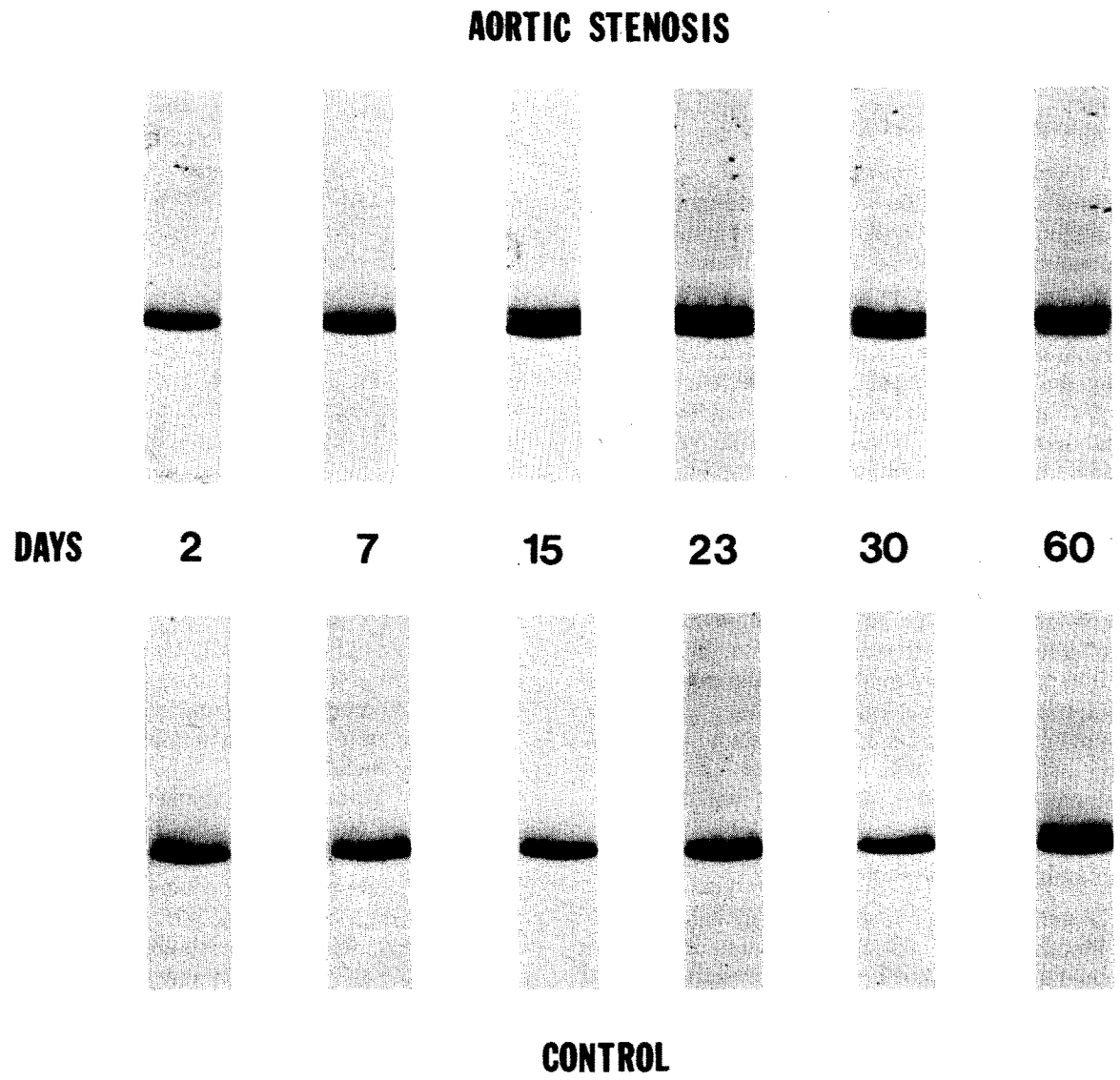

Fig. 1. Native myosin electrophoresis of ventricular samples following aortic stenosis. The number of days refers to days after banding; i.e., 2 days actually represents a 27-day old rat.

control atria (not shown) as in [16,18] for humans. Thus the light chain composition of control and banded atria were similar.

4. CONCLUSION

These experiments demonstrate that aortic stenosis in young rats results in hypertrophy of all chambers of the heart. This is accompanied by modification of the myosin isozyme composition, resulting in the accumulation of the V3 form in the left and right ventricles. These early changes are interesting in light of the fact that authors in [20] report a normal physiological function in the right ventricle of young rats despite a 2-fold hypertrophy. Hypertrophy of the atria does not result in any detectable changes in myosin isozyme content. The possibility that the shift towards the V3

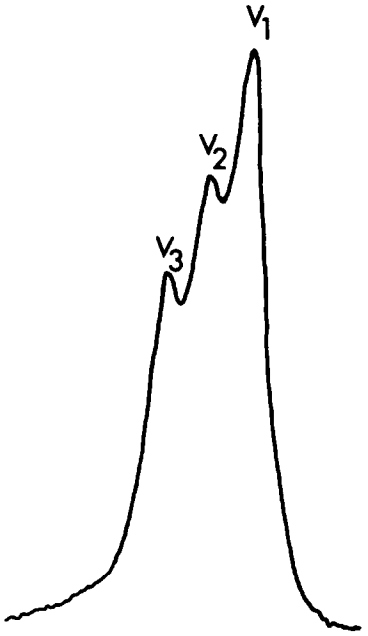

Fig. 2. Densitometric scan of a gel from the right ventricle of a 23-day banded rat. V_3 represents the slowest migrating species on the gel while V_1 the fastest.

Fig. 3. Native myosin electrophoresis of atrial samples following 2 months of aortic stenosis. Both gels contain two bands, though the second (arrow) does not separate readily from the major species. Aortic stenosis (AS); Sham operated control (CON).

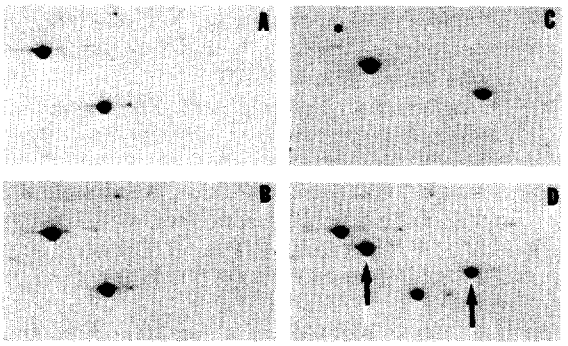

Fig. 4. Myosin light chain analysis from atria and ventricles using 2-dimensional electrophoresis. The gels are presented with the basic end to the left: (A) control ventricle; (B) hypertrophied ventricle; (C) hypertrophied atria; (D) mixture of atria and ventricle. Note the small amount of ventricular LC_1 present in the atrial sample (★). Arrows point out the atrial light chains in the mixture.

isozyme in the ventricles may also result in diminished contractile activity, as in the adult [12], is presently being examined.

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