Differences in Response of Myosin Isozyme Transition of Ordinary and Specialized Myocardium to Overload

Kazuomi Nomoto, Issei Komuro, Makoto Kuro-o, Hidetsugu Tsuchimochi, Fumimaro Takaku, Kiyoshi Machii, and Yoshio Yazaki

To investigate the response of myosin isozyme transition in specialized myocardium to cardiac overload, we examined immunohistochemically the distribution of myosin isozymes in sinus node cells of overloaded canine atria, using the monoclonal antibodies CMA19 and HMC14, which are specific for atrial myosin heavy chain (α-HC) and ventricular myosin heavy chain (β-HC), respectively. Overloading in canine right atria was induced by artificial tricuspid valve regurgitation and pulmonary stenosis. Right atrial mean pressure rose to 15–20 mm Hg (n=4) 2 months after surgery. In the working myocardium, cardiac overload caused redistribution of myosin isozymes, α-HC to β-HC. Compared with the normal right atria, fewer myocytes were labeled with CMA19, but more were labeled with HMC14. However, the reactivity of sinus node cells with CMA19 and HMC14 was not changed between normal and overloaded right atria, indicating no redistribution of myosin heavy chain isozymes, α-HC to β-HC. These results suggest that isozymes in myosin heavy chains in the specialized myocardium are protected from overload effects by their firm cytoskeletal framework or other mechanisms. (Circulation Research 1988;62:1088–1092)

Many studies have demonstrated transitions of cardiac myosin isozymes in response to different stimuli and functional requirements. Two distinct cardiac myosin heavy chains, α-HC and β-HC, exist in the mammalian heart, and their detailed distribution has been reported in the normal human heart, including the conduction system.1 We previously demonstrated that pressure overload induced myosin isozyme redistribution, α-HC to β-HC, in human atria.2 Transition of myosin isozymes induced by cardiac overload was thought to play an important role in adaptation of the heart muscle to a demand for increased cardiac work.3-5 Although knowledge about myosin isozymic changes in the working myocardium has been accumulated, the redistribution of cardiac myosin isozymes in the conduction system has not been reported.

In the present study, we investigated immunohistochemically the response of myosin isozyme transition in the specialized canine myocardium to overload induced by artificial tricuspid valve regurgitation and pulmonary stenosis, using the monoclonal antibodies CMA19 and HMC14, which are specific for α-HC and β-HC, respectively.

Materials and Methods

CMA19 and HMC14 were obtained from cloned hybridomas as previously reported.3 Antimyosin activities in medium from hybridoma colonies were screened by enzyme-linked immunosorbent assay (ELISA).6 CMA19 reacted specifically with the canine atrial myosin, and HMC14 selectively reacted with ventricular myosin. Neither CMA19 nor HMC14 reacted with light chains of these cardiac myosins. Artificial tricuspid valve regurgitation (TR) and pulmonary stenosis (PS) in mongrel dogs (8–10 kg) were produced by open-heart surgery under general anesthesia using intravenously administered pentobarbital (25 mg/kg). To make TR, tendons of the tricuspid valve were cut with small scissors through the right auricle. PS was produced by pulmonary artery banding with a vessel loop. In this way, we obtained pressure- and volume-overloaded canine right atria. Two months after surgery, cardiac catheterization was performed in operated dogs under anesthesia, and right atrial pressure was measured. Then, the dogs were killed, and right atrial muscle tissues containing the sinus node were obtained.

Excision of the sinus node was performed according to the method described by Davies et al.7 The same procedures, including cardiac catheterization and right atrial muscle tissue preparation, were performed in unoperated mongrel dogs to obtain normal controls. Muscle blocks were embedded in Tissue Tek II O.C.T. compound (Miles Laboratories, Naperville, Illinois) and immediately frozen in liquid nitrogen. For the immunofluorescence study, cryostat sections were first incubated with anti-myosin antibodies and then treated with fluorescein isothiocyanate–labeled goat anti-mouse IgG.
To determine the amount of α-HC or β-HC more quantitatively, the percentage of labeled myofibers was calculated for each case as previously described. In brief, the staining patterns were grossly divided into at least four classes: strongly positive (S), positive (P), pseudonegative, and completely negative. As for quantitation, one S fiber was scored as 1, one P fiber as 0.5, and one pseudonegative or completely negative fiber as 0. Thus, the total scores per 400 fibers were calculated in each animal. Then, the percentage (total scores/400) was calculated.

Results

Right atrial mean pressure of successfully operated dogs was 18 ± 3.2 mm Hg (mean ± SD) (n = 4). Two months after surgery, that of normal dogs was 6 ± 1.5 mm Hg (n = 10).

We found that 100% of normal ventricular myocardial fibers were strongly labeled with HMC14, but almost no fibers were labeled with CMA19 (Figures 1A and 1B). In contrast, in normal atrial free wall, all fibers were labeled with CMA19 but only a few fibers (<1%) were labeled with HMC14 (Figures 1C and 1D). In the overloaded canine atrial free wall, myocardial fibers labeled with HMC14 markedly increased in number (mean ± SD; 30.5 ± 4.4%, n = 4), while fibers labeled with CMA19 showed a corresponding decrease (mean ± SD; 60.1 ± 7.4%, n = 4) (Figures 1E and 1F).

In the sinus node, the labeling with these antibodies was different from that of working myocardial fibers of atrial free wall. In normal dogs, all sinus node cells were strongly labeled with CMA19, but almost no cells were labeled with HMC14. In the normal working myocardium surrounding the sinus node (crista terminalis), all myocytes were labeled with CMA19, but a considerable proportion of them (mean ± SD; 32.8 ± 6.6%, n = 10) were also labeled with HMC14 (Figures 2A and 2B).

However, in the overloaded atrial myocardium, the number of myocytes of crista terminalis labeled with CMA19 was markedly decreased (mean ± SD; 4.6 ± 1.4%, n = 4), and those labeled with HMC14 were correspondingly increased (mean ± SD; 80.3 ± 6.8%, n = 4). In contrast, in the sinus node cells, the reactivity was similar to that of normal controls; all were labeled with CMA19 but none were labeled with HMC14 (Figures 3A and 3B). These results are summarized in Table 1.

Discussion

We observed differences in myosin isozyme transition to cardiac overload between ordinary and specialized myocardium.
In the ELISA test, CMA19 and HMC14 reacted specifically with atrial and ventricular myosin heavy chains, respectively, without any reaction with the light chains. In the normal right atrial canine free wall, the myocytes mainly contained α-HC, with β-HC being present in a very small amount. However, in the crista terminalis, almost all fibers contained α-HC but 32.8 ± 6.6% of them also contained β-HC. In the sinus node, all myocytes contained α-HC but not β-HC. In human atria, more than 95% of the myofibers contained α-HC, and 20–60% of them contained β-HC. In the crista terminalis, almost all myocytes contained both α-HC and β-HC. In the sinus node, all myocytes contained α-HC, but only 4.7% of them contained β-HC.12 Thus, there are marked differences between the proportions of α-HC and β-HC in human and canine atrial myocardium, including the sinus node.

Evidence has been accumulated that pressure overload induces myosin isozymic changes.2–4 Pressure overload caused the redistribution of myosin isozymes in the ordinary atrial myocardium. In the present study, the redistribution of myosin heavy chain isozymes, α-HC to β-HC, is shown to occur in the canine atrial myocardium due to cardiac overload, as observed in the human atria.2 However, in the sinus node in overloaded atria, the reactivities of the myocytes with two antibodies were similar to those in normal atria. The redistribution of myosin isozymes, from α-HC to β-HC, according to cardiac overload was not seen in sinus node cells. Our results suggest that myofibers of cardiac conduction systems are resistant to overload with respect to myosin isozyme transition.

Although Gorza et al8 reported the presence of the embryonic skeletal type myosin heavy chain in the...
bovine nodal conduction tissue, Bouvagnet et al\(^9\) reported no specific heavy chain isoform in human conduction fibers; we could not find such a unique myosin heavy chain isoform either. Even if it exists, its amount must be so small that it should not play an important role in the absence of the redistribution of myosin heavy chain isoforms due to overload in the conduction system.

A variety of biochemical, structural, and immunocytochemical studies have shown that myocytes of the con-

<table>
<thead>
<tr>
<th></th>
<th>Mean right atrial pressure (mm Hg)</th>
<th>Free wall (%)</th>
<th>Crista terminalis (%)</th>
<th>Sinus node cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 10)</td>
<td>6 ± 1.5*</td>
<td>α-HC 100</td>
<td>α-HC 100</td>
<td>α-HC 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-HC &lt;1</td>
<td>β-HC 32.8 ± 6.6*</td>
<td>β-HC 0</td>
</tr>
<tr>
<td>Overloaded (n = 4)</td>
<td>18 ± 3.2*</td>
<td>α-HC 60.1 ± 7.4*</td>
<td>α-HC 4.6 ± 1.4*</td>
<td>α-HC 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-HC 30.5 ± 4.4*</td>
<td>β-HC 80.3 ± 6.8*</td>
<td>β-HC 0</td>
</tr>
</tbody>
</table>

*Values are mean ± SD.
duction system are quite different from ordinary myocardial fibers, especially anatomic and histological organization.10-13 The well-developed cytoskeletal framework with specialized myofibers and the abundance of connective tissues around them may protect the sinus node cells from the effects of overloading. On the other hand, the mechanism of gene expression encoding myosin heavy chains in the specialized myocardium may be different from that in the ordinary myocardium under an overloaded condition. Further examination is necessary to know what factor plays an important role in the absence of transition of myosin heavy chain isoforms in specialized myocardium under cardiac overload.

Acknowledgments

We wish to thank Professor S. Naoe, Department of Pathology, University of Toho, Tokyo, Japan, for giving us helpful suggestions to make sections of the canine conduction systems.

References


Key Words • myosin isozyme • monoclonal antibody • specialized myocardium • overload
Differences in response of myosin isozyme transition of ordinary and specialized myocardium to overload.
K Nomoto, I Komuro, M Kuro-o, H Tsuchimochi, F Takaku, K Machii and Y Yazaki

doi: 10.1161/01.RES.62.6.1088

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/62/6/1088