Rapid Publication

Isozymic Changes in Myosin of Human Atrial Myocardium Induced by Overload

Immunohistochemical Study Using Monoclonal Antibodies

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Abstract. An immunohistochemical study using monoclonal antibodies specific for the heavy chains of either human atrial (HCα) or ventricular (HCβ) myosin was performed to clarify the distribution of each isozyme in normal as well as pressure-overloaded human hearts. In normal human ventricles, all muscle fibers were stained by a monoclonal antibody (HMC14) specific for HCβ, whereas a small number of fibers reacted with a monoclonal antibody (CMA19) specific for HCα. In contrast, in normal human atria, almost all muscle fibers were stained by CMA19, and a relatively larger number of muscle fibers also reacted with HMC14. Furthermore, in pressure-overloaded atria, muscle fibers reactive with HMC14 were strikingly increased while those reactive with CMA19 showed a corresponding decrease. The extent of this isozymic redistribution was in good correlation with atrial pressure. These results not only confirmed the existence of isoforms of myosin heavy chain in human hearts, but also demonstrated that redistribution of isomyosins could occur as an adaptation to pressure overload.

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Introduction

Evidence supporting the heterogeneity of cardiac muscle fibers with respect to myosin composition has been accumulated recently. Two distinct cardiac myosin heavy chains, HCα and HCβ, are recognized to exist in the mammalian heart, and are comprised of V_1 and V_3 as homodimers of HCα and HCβ, respectively, and V_2 as a heterodimer (1). Enzymatic (2, 3, 4), electrophoretic (5), and immunochemical (6, 7, 8) analyses have revealed (*a*) that the predominant form of ventricular isomyosin varies according to the species examined, (*b*) that HCα is the predominant isoform in the atrium, whereas HCβ is predominant in the ventricles of larger animals including humans, and (*c*) that the relative proportions of these isozymes can be changed by aging, hormonal state, and pressure overload in laboratory animals.

To clarify the existence of these isozymes in the human heart and to confirm whether or not redistribution of these isozymes occurs in the myocardium subjected to pressure overload, an immunohistochemical study using monoclonal antibodies specific for the heavy chains of either type HCα or HCβ was performed.

Methods

Myosins were isolated from human ventricles and bovine atria by a dilution technique described elsewhere (2). The light chains (I, II) were isolated from human ventricles by guanidine denaturation, as described previously (9). Hybridomas producing anti-myosin antibodies were obtained by fusion of myeloma cells (P3×63Ag8U1) with isolated spleen cells of BALB/C (male, 6w) mice immunized with bovine atrial or human ventricular myosin (0.1–0.2 ml of 1 mg/ml myosin solution administered intraperitoneally at 2-wk intervals) as HCα or HCβ antigen, respectively, essentially by the protocol of Köhler and Milstein (10).

Anti-myosin activity in the medium from hybridoma colonies was screened by enzyme-linked immunosorbent assays (ELISA)[1] according to Guesdon et al. (11). ELISA tests were also performed with light chains as antigens in order to determine whether these antibodies reacted to heavy chains or light chains. For the immunohistochemical study, cryostat sections obtained from human hearts were first incubated with anti-myosin antibodies, then treated with biotinylated goat anti-mouse IgG (Tago Inc., Burlingame, CA), and stained with fluorescein isothiocyanate-labeled avidin (EY Lab., Inc., San Mateo, CA). Specimens of human hearts were obtained from surgical operations and autopsy. Specimens obtained from surgical operations were those from patients with ischemic heart disease and valvular heart disease including mitral stenosis and/or regurgitation (MS/R) and tricuspid regurgitation (TR). The age range of these patients was from 19 to 74 years old (mean±SD; 52.0±10.5, n = 23). Cardiac catheterization had been performed in all patients within 4 wk prior to cardiac surgery, and right atrial pressure and pulmonary capillary wedge pressure were recorded in each patient. Postmortem specimens were obtained from patients without heart disease. Linear regression analysis was carried out by the conventional method of least squares.

Results

Two clones (CMA19 and HMC14) of hybrid cells secreting anti-myosin antibodies were selected for discriminating the antigenic difference between HCα and HCβ. In ELISA tests, CMA19 reacted with the atrial myosin specifically, and HMC14 selectively reacted with the ventricular myosin. Neither CMA19 nor HMC14 reacted with light chains (Fig. 1). The immunohistochemical study using these antibodies revealed that 100% of ventricular muscle fibers were strongly and uniformly stained by HMC14, although only a small number of fibers were also stained by CMA19 (Fig. 2 *A* and *B*). In contrast, in normal human atria, >95% of muscle fibers were strongly stained by CMA19, whereas 20–60% of myofibers were stained by HMC14 (Fig. 2 *C* and *D*). The staining patterns of these fibers were highly variable in intensity among individual myofibers, and grossly divided into at least four classes: "strongly positive" (S), "positive" (P), "pseudonegative," and "completely negative."

When pressure-overloaded atria obtained from patients with MS/R or TR were stained by each antibody, a striking reversal of the staining pattern was observed (Fig. 2 *E* and *F*). The number of muscle fibers reactive with HMC14 was increased significantly, while that of fibers reactive with CMA19 showed a corresponding decrease. To demonstrate the changes in the amount of each isomyosin more quantitatively, we counted the number of the muscle fibers which reacted with each antibody. Since the fluorescence intensity of P myofibers was almost half that of S myofibers, the amount of isomyosin in P fibers was roughly estimated as half that of S fibers. As for quantitation, one S fiber was scored as one, one P fiber as 0.5, and one pseudonegative or completely negative fiber as zero. Thus, the total scores per 1,000 fibers were calculated in each specimen

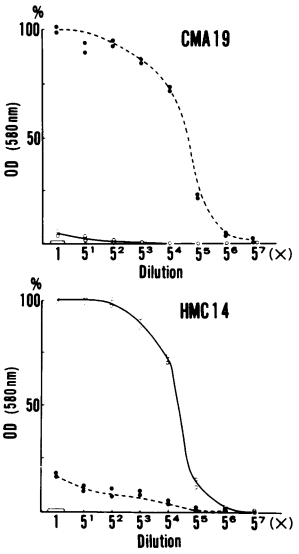

Figure 1. Reactions of each antibody with human atrial (--●--), ventricular (— ○ —) myosin, and human ventricular light chains (I + II, ⌐¬). Myosin and light chains (10–100 μg/ml in phosphate-buffered saline [PBS]; 10 mM phosphate buffer containing 150 mM NaCl, pH 7.4) were bound to each well of a 96-well microtiter plate and blocked with 1% bovine serum albumin solution. Medium (50 μl) from the hybridoma colonies was reacted with bound antigens; then, after three washings with PBS, goat biotynilated anti-mouse immunoglobulin (Vector Laboratories Inc., Burlingame, CA) was added to each well. After incubation for 1 h at room temperature, the wells were washed three times with PBS containing 0.5% Tween-20; then, avidin D-peroxidase (Vector Laboratories, Inc.) was added with H_2O_2, 4 amino-antipyrin, and phenol as substrate. Results were expressed as the percentage of maximum OD (at 580 nm). CMA19 reacted with atrial myosin specifically, and HMC14 reacted with ventricular myosin selectively. They showed no reaction with light chains. The negligible reaction between CMA19 and ventricular myosin, and the reduced amount of cross-reactivity between HMC14 and atrial myosin were explained by the existence of a very small quantity of atrial-type myosin in the ventricle, and a significant amount of ventricular-type myosin in the atrium, respectively.

and plotted against each mean atrial pressure or mean pulmonary wedge pressure (Fig. 3). It was clear that the total scores of each isomyosin were altered by pressure overload, and the degrees of HCα decrement and HCβ increment correlated well with the mean atrial pressure (HCα: $y = -28x + 847$, $r = -0.75$, $P < 0.01$; HCβ: $y = 26x + 156$, $r = 0.78$, $P < 0.01$). Furthermore, when separate calculations were carried out for a group of right or left atria, similar good correlations were also observed (total scores of HCα: right atria, $r = -0.54$, $P < 0.05$; left atria, $r = -0.79$, $P < 0.01$).

Discussion

The results presented here have clearly demonstrated the existence of two isozymes of cardiac myosin in human hearts. Furthermore, we have shown evidence that a redistribution of isozymes can occur by pressure overload even in human atrial myocardium. Our monoclonal antibodies, CMA19 and HMC14, reacted specifically with atrial myosin and ventricular myosin, respectively, without any reaction with light chains in ELISA tests; therefore, CMA19 was regarded as a monoclonal antibody specific for HCα, and HMC14 as a monoclonal antibody specific for HCβ. These results confirmed the existence of isoforms of

1. *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assays; MS/R, mitral stenosis and/or regurgitation; P, positive; S, strongly positive; TR, tricuspid regurgitation.

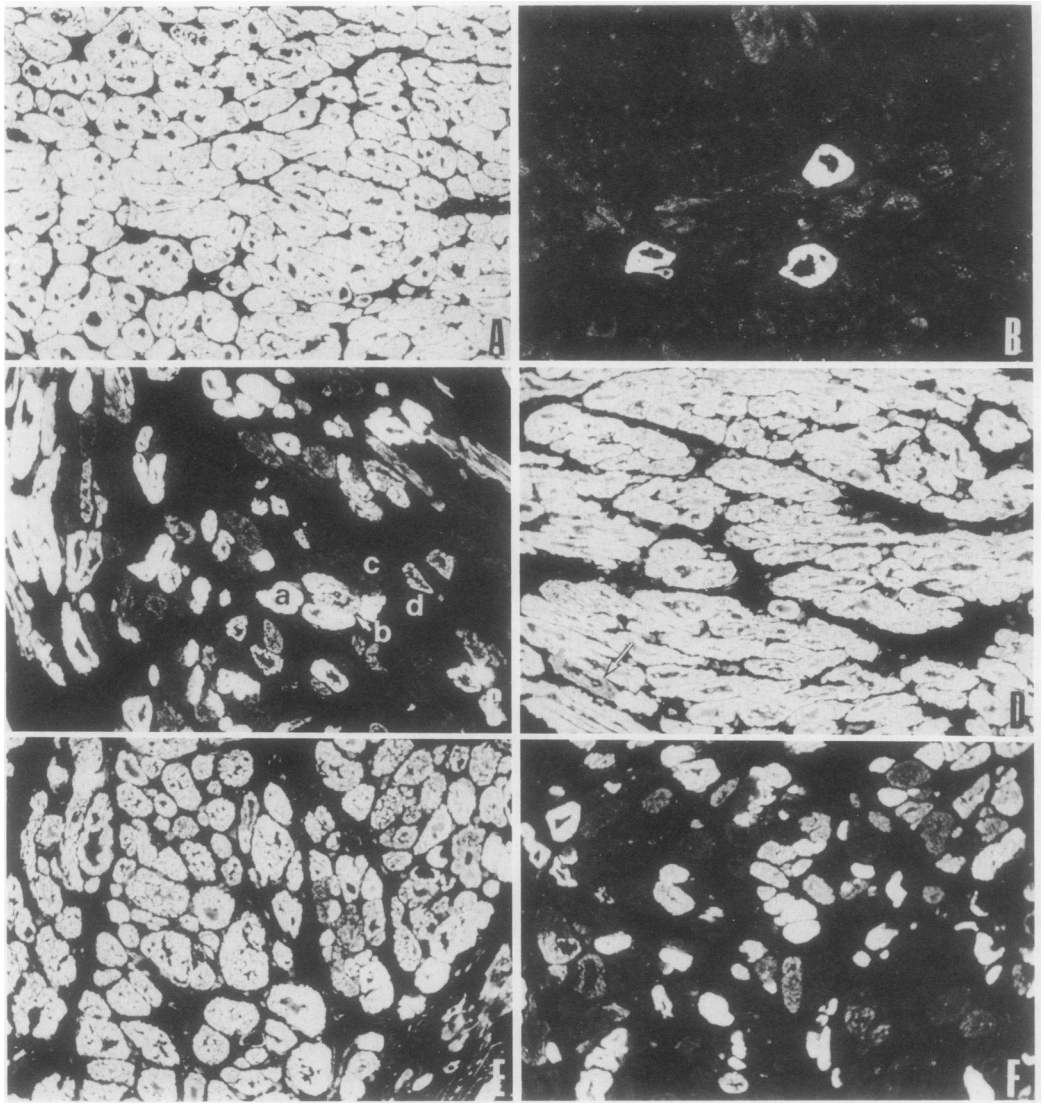

Figure 2. (*A*) Cryostat section of normal human ventricle stained by HMC14. All fibers reacted strongly and uniformly. (*B*) As in (*A*), except that the muscle fibers were stained by CMA19. A small number of fibers were reactive. (*C*) Cryostat section of normal human atrium stained by HMC14. The staining intensity was highly variable, and divided into at least four classes: (*a*) S, (*b*) P, (*c*) "pseudonegative," and (*d*) "completely negative." (*D*) As in (*C*), except that the myofibers were stained by CMA19. Almost all fibers were strongly reactive. Note a myofiber which shows a weak reaction (arrow). (*E*) Cryostat section of a pressure-overloaded atrium stained by HMC14. The specimen was obtained from a patient with MSR. Almost all fibers became reactive. (*F*) As in (*E*), except that the myofibers were stained by CMA19. Unreactive fibers were significantly increased.

myosin heavy chains even in human hearts. Further confirmation was obtained from the immunohistochemical studies of human hearts using these antibodies. In normal human atria, although HCα was a predominant myosin heavy chain isoform, the existence of a larger amount of ventricular-type myosin than expected was revealed in this experiment. Since the patients in this report who underwent surgical operation were slightly older (mean age, 52 yr. old) and the proportion of HCβ has been reported to increase with aging in rat and rabbit hearts (6, 8), the unexpected amount of HCβ in human atria might have been due to aging. The amount of ventricular-type myosin appeared to be larger in left atria than in right atria. From this view point, the previous work by Gorza et al. (12) might have shown a difference from our results. Using polyclonal antibodies, they reported that the number of fibers reactive with antibovine ventricular myosin were reduced in bovine left atria compared with right atria. The reason for this difference is unclear but might have been due to differences in the specificity of each antibody. In their study, the antibodies used were polyclonal antibodies which removed crossreactive antibodies by cross-absorption (namely, antibovine ventricular myosin was absorbed by bovine left atrial myosin), and there was also a species difference. By contrast, although the muscle fibers were composed almost exclusively of V_3 isomyosin, a small number of cells reactive with CMA19 were also observed in normal human ventricles. These results suggested that a small quantity of V_1 or V_2 also existed in human ventricles, corresponding to the recent report by Mercadier et al. (13).

The isozymic redistribution in pressure-overloaded human atria presented here may have a physiological implication. Since

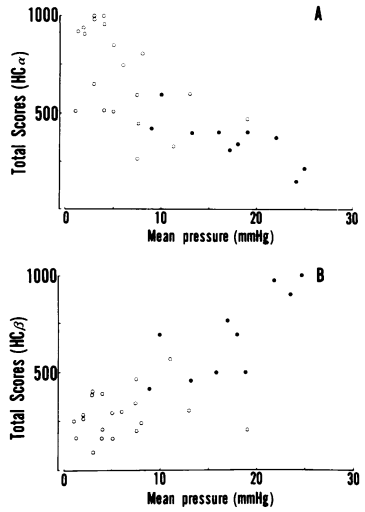

Figure 3. The total scores per 1,000 myofibers of HCα (*A*) or HCβ (*B*) plotted against the mean atrial or pulmonary capillary wedge pressure. The scores were calculated by scoring one S fiber as 1, one P fiber as 0.5, and one pseudonegative or completely negative fiber as 0. ○, right atrium; ●, left atrium.

the low ATPase activity related to low velocity of shortening improves the efficiency of contraction for an equivalent amount of work (14), HCβ appears to be a physiological myosin isozyme for performing pressure work. In mitral and tricuspid valvular disease, atrial myocytes subjected to pressure overload may promote synthesis of HCβ instead of HCα in order to improve the efficiency of contraction. With reference to the light chain, Cummins (15) reported that the ventricular-type light chain replaces the atrial-type light chain in pressure-overloaded human atria. It is reasonable to assume that such a redistribution of the light chain may have accompanied that of the heavy chain in this experiment, and the concomitant replacement of ventricular-type heavy and light chains should work against pressure overload. Thus, isozymic redistribution can be considered as a physiological adaptive mechanism to meet increased pressure overload. Since the muscle fibers are already composed almost exclusively of V_3 isomyosin in the ventricular myocardium, such a marked isozymic redistribution cannot occur by pressure overload. Therefore, the isomyosin redistribution induced by pressure overload in human hearts seems to play a more important role in the atrium than the ventricle, since the content of HCα which could be transformed to HCβ is much larger in the atrial myocardium.

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