Expression of Myosin Isozymes during the Developmental Stage and Their Redistribution Induced by Pressure Overload

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Cardiac muscles contain at least two isozymes — referred to as \( \alpha(\text{HC}\alpha) \) and \( \beta(\text{HC}\beta) \) — of the myosin heavy chain. The proportional ratio of these isozymes varies depending upon the developmental stage and the physiological and/or the hormonal milieu of the cell. Using monoclonal antibodies (MoAb) specific for human cardiac \( \text{HC}\alpha \) and \( \text{HC}\beta \), we have examined the expression of these isozymes in fetal through adult cardiac tissues and investigated whether isozymic redistribution occurs in pressure overloaded human ventricles. We found that 1) although \( \text{HC}\alpha \) was expressed in the atrium from the early embryonic stage, in embryonic ventricular myofibers, only \( \text{HC}\beta \) was expressed without expression of \( \text{HC}\alpha \), but some myofibers replace \( \text{HC}\beta \) by \( \text{HC}\alpha \) after birth, and 2) these \( \text{HC}\alpha \) containing ventricular myofibers were found to be decreased by pressure overload, which suggested that isozymic redistribution from \( \text{HC}\alpha \) to \( \text{HC}\beta \) also occurred in the ventricles, as well as the atrium. In addition, we also found two subtypes of \( \text{HC}\beta \) (\( \beta_1 \), \( \beta_2 \)) in the human heart. In the ventricle, both \( \beta_1 \) and \( \beta_2 \) was present in all myofibers; in contrast, some myofibers contained \( \beta_1 \) or \( \beta_2 \) or both with or without expression of \( \text{HC}\alpha \) in the atrium. \( \beta_1 \) and \( \beta_2 \) were distinctive in their expression during the developmental stage, since \( \beta_1 \) was present in the embryonic heart from the early developmental stage, whereas \( \beta_2 \) was not present in the early embryonic heart, but began to be expressed in the late embryonic stage. Therefore, there are at least three types of myosin isozymes, \( \text{HC}\alpha \), \( \beta_1 \) and \( \beta_2 \), whose expression is regulated by pressure overload and developmental stage.

MYOSIN, a major contractile protein in muscle, is composed of two 20K dalton heavy chains (HCs) and two pairs of low molecular weight light chains (LCs).\(^1\) HC largely determines myosin ATPase activity,\(^2\) which is known to be correlated with the contractile velocity of the muscle; hence, is an important subunit in contractile function. In the cardiac muscle, two types of HCs, \( \text{HC}\alpha \) and \( \text{HC}\beta \), which differ in ATPase activity (\( \text{HC}\alpha \) has a higher Ca\(^{2+}\)- and actin-activated ATPase activity than does \( \text{HC}\beta \) were found in the ventricular myocardium of laboratory animals such as rats, mice and rabbits\(^5\)–6\) The ratio of these isozymes varies according to the developmental stage or physiological and pathological state of the cardiac muscle: for example, in rats and rabbits, \( \text{HC}\beta \)

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Key Words:
- Myosin isozymes
- Heterogeneity of \( \text{HC}\beta \)
- Embryonic heart
- Pressure overload
- Monoclonal antibodies

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been accumulated with respect not only to the presence of myosin isozymes, but also to isozymic changes of myosin in the developmental and pathological states. Although a recent report from our laboratory have clearly demonstrated the presence of these isozymes in human hearts and isozymic redistribution in pressure-overloaded atrial\(^7\) it has not been determined whether similar isozymic redistribution occurs in pressure-overloaded ventricles, or whether isozymic change occur during developmental stages in human hearts. Using MoAbs specific for human HCa and HCB, we investigated the isozymic composition in pressure-overloaded human ventricles and fetal hearts. Furthermore, we demonstrated the presence of two subtypes of HCB and showed, for the first time, the distribution of these isozymes in human hearts. We discussed the physiological roles of these isozymes in the developmental and pathological state of the hearts.

MATERIALS AND METHODS

Production of MoAbs

Myosins were isolated from bovine atria and human ventricles by a dilution technique\(^8\) The light chains (I, II) were isolated from human ventricles by guanidine denaturation, described elsewhere\(^9\) MoAbs specific for HCa and HCB were produced by fusion of isolated spleen cells from mice immunized by myosins purified from bovine atria and human ventricles, respectively, with myeloma cell lines (P3U1) as described previously\(^17\) Anti-myosin activity in the medium from hybridoma colonies was screened by enzyme-linked immunosorbent assays (ELISA) according to Guesdon et al\(^20\)

Immunohistochemical studies

For the immunohistochemical studies, specimens were cut into 4 μm sections on a cryostat. Sections were mounted on glass slides, air-dried for 30 min, and stored at \(-20^\circ\)C until staining. For staining, cryostat sections were first incubated with antimyosin antibodies for 40 min to an hour at 37°C, then treated with fluorescein isothiocyanate (FITC)-labeled sheep antimouse IgG. The staining intensities of myofibers with each

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Abbreviations used in this paper: HCa = heavy chain; LC = light chain; ATP = adenosinetriphosphate; MoAb = monoclonal antibody; ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel; PBS = phosphate buffered saline; FITC = fluorescein isothiocyanate

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Fig. 2. Distribution of HCA and HCB in the human ventricle and atrium. A) CMA19 stained a few myofibers but HMC14, 48 and 50 stained all myofibers of the human ventricle. B) Although CMA19 stained almost all atrial myofibers, HMC14, 48 and 50 stained some myofibers. In serial atrial sections, the staining pattern of HMC14 was found to be clearly different from that of HMC48 or 50.

MoAb were divided into four classes: "Strongly positive" (S), "Positive" (P), "Pseudonegative", and "completely negative". As for quantitation, one S fiber was scored as one, one P fiber as 0.5, and one pseudonegative or completely negative fiber as 0, as described previously. Thus, the total scores per 1000 myofibers were calculated in each specimen.

Specimens
Human cardiac tissues were obtained at cardiac surgery from patients with acquired
RESULTS

Characterization of MoAbs

Four MoAbs specific for human myosin isozymes were prepared and used in this study. Of these MoAbs, one (CMA19) was specific for HCa, and the others (HMC14, 48, 50) were specific for HCβ. Fig.1 demonstrates their specificities to each isomyosin by the ELISA tests. In ELISA tests, CMA19 reacted with atrial myosin specifically, and HMC14, 48 and 50 selectively reacted with the ventricular myosin. Neither of these MoAbs reacted with light chains (I, II). The slight reactions of CMA19 with ventricular myosin and those of HMC14, 48 and 50 with atrial myosin were due to the coexistence of atrial-type myosin in the ventricle and ventricular-type myosin in the atrium, respectively. This was confirmed by immunohistochemical studies. Since a previous report from our laboratory demonstrated that atrial myosin had a higher ATPase activity than did ventricular myosin in the human heart, and we know that HCa has a higher ATPase activity than does HCβ, HCs reactive with CMA19 and HMC14, or HMC48, 50 were regarded as HCa and HCβ, respectively. These conclusions were further confirmed by immunohistochemical studies.

Immunohistochemical studies

A) Normal human hearts

Figure 2 shows the human ventricle and atrium stained by these MoAbs. Although
HMC14, 48 and 50 stained all ventricular myofibers, CMA19 stained only a small number of them (Fig. 2A). In contrast, almost all human atrial myofibers were stained by CMA19, whereas some myofibers were also stained by HMC14, 48 and 50 (Fig. 2B). Serial sections stained by HCβ-specific MoAbs revealed that the staining pattern of HMC48 or 50 was clearly different from that of HMC14. The number of muscle fibers stained by HMC48 or 50 was roughly two to three times more than those stained by HMC14. These results suggested the presence of two immunologically distinct β-type HCs in the human atrium. Since human ventricular myofiber was stained by HMC14, 48 and 50 diffusely and homogeneously, we could not eliminate the possibility that one HCβ molecule might contain epitopes detected by HMC14 as well as HMC48 or 50 in the ventricle. However, a difference of the staining pattern during the developmental stage described below should eliminate this possibility. Therefore, it was reasonable to consider that two different HCβ molecules were present in ventricular myofibers. Thus, at least three immunologically distinct myosin isozymes existed in the human heart.

B) Redistribution during the developmental stage.

Isozymic redistribution has been reported to occur during developmental stage in some experimental animals such as the rat and rabbit. Using MoAbs, we have examined the expression of these isozymes in human and calf fetal hearts. As shown in Fig. 3, HCα was already expressed in human atrial myofibers from 10 weeks gestation (10w), but was not expressed in 10w and 22w fetal ventricles except for the Purkinje-like myofibers in the subendocardial region (Fig. 3A, B). Since in adult human ventricles, HCα was present in up to 10-15% of myofibers in the epicardial region and almost 0% in the subendocardial region (Fig. 3C), the distribution of HCα in fetal ventricles was opposed to that in adult human ventricles. Thus, although HCα was present in atrial and Purkinje-like myofibers from the early embryonic stage, it seemed to be expressed after birth in ventricular working myofibers.

As for HCβ, HMC14 stained ventricular myofibers strongly and homogeneously and some myofibers in the atrium from the early embryonic (Fig. 3A) through the adult stage, but HMC50 reacted very weakly with 10w fetal ventricular myofibers, and it gave a slightly stronger reaction with myofibers of the 22w fetal ventricle and strongly stained those of adult human ventricular myofibers (data not shown). This phenomenon was more marked in fetal calf ventricles.
As shown in Fig. 4, HMC50 gave no reaction with ventricular myofibers of two and five months' gestation (2M, 5M), but showed a heterogeneous staining pattern with those of 8M and reacted strongly with adult ventricular myofibers as did HMC14. HMC48 did not react with myosins of bovine hearts from the early stage to adult stage. This suggested that HC detected by HMC50 did not present in the early embryonic heart, but began to be expressed in the late embryonic heart and diffusely expressed after birth, whereas HC detected by HMC14 was expressed from the early embryonic through the adult stage. Thus, expression of myosin isozymes was regulated by developmental stages: designating HCB detected by HMC14 and HMC50 as β1, β2 respectively, in the early embryonic stage, only β1 existed; however in the late embryonic stage, β1 and β2 were present, finally after birth through the adult stage, HCα, β1 and β2 were all expressed in the ventricles, whereas in the atria, HCα was already expressed from the early embryonic stage.

C) Redistribution in Cardiac Hypertrophy

Induced by Pressure Overload

We have reported the isozymic redistribution in human atria by pressure overload, that was, HCα, a predominant myosin isozyme in the atrium was replaced by HCB in atria subjected to pressure overload. The extent of isozymic change was in good correlation with atrial pressure. We concluded that this isozymic change was a physiological adaptive mechanism to meet increased pressure overload. It is quite interesting and important to ascertain whether a similar isozymic change also occurs in the human ventricle. We obtained left ventricular papillary muscles or left ventricular myocardium from patients with valvular disease at open heart surgery and stained them with CMA19. The myofibers reactive with CMA19 were counted, and the total scores of HCα were calculated by the method as we have described previously. Total scores of HCα decreased as mean left ventricular pressure increased (Fig. 5, r = -0.68, p < 0.01). In the papillary muscles or left ventricular myocardium obtained from patients with severe aortic stenosis, HCα-containing myofibers were very scarce and we could hardly detect such a myofiber when the mean left ventricular pressure was more than 150 mmHg. This suggested that isozymic redistribution also occurred in human ventricles, but not so markedly as observed in atria since HCα-containing myofibers were much smaller in the ventricles as compared with atria.

DISCUSSION

The results presented here have clearly demonstrated 1) the presence of HCα and two subtypes of HCB in the human heart and 2) isozymic redistribution according to developmental stage and by pressure overload. Recent reports have suggested the heterogeneity of HCB in rabbit and bovine hearts by nuclease S1 mapping of mRNA or tryptic peptide mapping of myosin subfragment 110,22-25. However, not only their existence and distribution in human hearts, but also regulatory mechanisms in their expression were elucidated. Using MoAbs specific for HCB, we demonstrated for the first time the presence of two β-type myosin isozymes and their distribution in the human heart. In addition, we could determine the difference in their expression during the developmental stage.

Whether atrial β-type myosins recognized by HMC14 and 48 or 50 are the same myosins expressed in the ventricle detected by each MoAb, respectively, and whether α-type myosin recognized by CMA19 in the atrium is the same as that detected by this MoAb in the ventricle could not be clarified in this study. Since light chain I of human atrial myosin is known to show a different mobility in SDS-PAGE from that of ventricular myosins, the myosin itself recognized by each MoAb in the atrium and ventricle should not be the same. With strict accuracy, we should call the HC recognized by CMA19 in the atrium atrial
HCα, and that in the ventricle, ventricular HCβ; similarly, HCs recognized by HMC14 and HMC50 in the atrium as atrial β1, β2, and those in the ventricle as ventricular β1, β2. If there are structural differences among atrial and ventricular HCα, β1, β2, respectively, there might be at least six myosin isozymes in the human heart. But for simplicity in this paper, we call HC recognized by CMA19 as HCα and HCs detected by HMC14 and 50 as β1 and β2, respectively. HMC48 stained the same atrial myofibers as did HMC50, but did not react with HC of bovine hearts. This suggested that they were directed to different epitopes in HCB, but we could not elucidate that HMC48 detected a different myosin from that detected by HMC50. To clarify this problem, a sequential immunoprecipitation should be performed. At present, we regard HC detected by HMC48 as a β2-type myosin isozyme.

In adult human hearts, HCα was expressed in almost all atrial myofibers and in a few (up to 15% of the myofibers in the epicardial region) ventricular myofibers. In contrast, β1 and β2 coexisted in all ventricular myofibers and also some myofibers of the atrium where, however, they were not necessarily expressed simultaneously in individual myofibers. Atrial myofibers containing β2 appeared to be approximately two or three times as numerous as those expressing β1. Although we could not quantitate each isozyme accurately, we could speculate the relative quantity by counting the myofibers which express each isozyme since a MoAb is known to bind one epitope present in one molecule. Therefore, myosin isozymes expressed in the human heart in order of the relative quantity were as follows: β1 or β2 > α in the ventricles and α > β2 > β1 in atria.

The expression of these isozymes was also found to be regulated by the developmental stage in the human heart. In rat and rabbit ventricles, HCB, which is indistinguishable from HCB expressed in adult hearts, is known to be present in fetal hearts and to be replaced by HCα after birth.6–8 The result presented here that fetal human ventricles contained predominantly HCB without expression of HCα which could be expressed only after birth was consistent with other results mentioned above. But, we could demonstrate that HCB expressed in fetal ventricles was clearly different from that expressed in adult hearts. In fetal human and calf ventricles, β2 did not exist or was present in very small quantities, although β1 existed from the early embryonic stage. Therefore, although we could not find a fetal-specific myosin HC, a difference in the composition of subtypes of HCB (β1 and β2) between fetal and adult hearts was observed in this study.

The physiological role of individual HCs during development remained unknown. Since the embryonic heart beats at a higher rate than the adult heart and works against extracorporeal circulation as well as intracorporeal circulation, the mechanical load to the embryonic heart appears to be a type of "volume overload". One may expect that HCα is a physiological myosin in the fetal heart as HCα is known to be suitable for a "volume overload" because of its higher ATPase activity related to faster cardiac muscle shortening,15,16 yet this was not observed. Instead, only β1 existed in fetal hearts without expression of HCs and β2. We could speculate that β1 might have a role in such a "volume overload" in fetal hearts where myofibers should work with a lower oxygen supply compared with adult hearts. On the other hand, β2 might be a physiological myosin performing work with a sufficient oxygen supply in the adult ventricle. The accuracy of this hypothesis awaits further experiments.

Recent studies have accumulated evidence regarding isozymic redistribution induced by pressure overload in laboratory animals.13,14 In a previous report, we have shown that a redistribution of isozymes from HCα to HCB can occur by pressure overload even in the human atrial myocardium.17 In the present study, we examined whether a similar isozymic change also occurs in pressure overloaded human ventricles. Because of lower content of HCα in the ventricle than in the atrium, the isozymic redistribution was not so striking as observed in the atrium, but we could clearly demonstrate that an isozymic change from HCα to HCB also occurs in pressure overloaded human ventricles. Mercadier et al. reported no significant difference in the content of HCα between normal and hypertrophied ventricles.25 They measured HCα in extracted ventricular myosin from autopsy samples by the ELISA method. The reason for the discrepancy between our results and theirs is unknown, but probably it is because they indirectly examined the content of HCα by competitive ELISA methods using polyclonal antibodies, while, in contrast, we directly examined the distribution of HCα in ventricles using MoAbs. HCB has a low ATPase activity which relates to the low velocity of shortening; hence it has an improved econtrac-
tion for an equivalent amount of work in efficiency. Therefore, an isozymic redistribution from HCA to HCB is regarded as a compensative mechanism to adapt to increased pressure work, even in the ventricles. Since HMC14 and 50 still stained myofibers of pressure overloaded human ventricles homogeneously, as with normal ventricles, we could not elucidate an isozymic change between B1 and B2. However, since examination of human atria revealed that the degree of increment of B1 was steeper than that of B2 (data not shown in this paper), B1 might have a more important role in the pathologically hypertrophied myocardium induced by pressure overload. Further clarification of quantitative analysis in isozymic redistribution between B1 and B2 will await a new technique such as a radioimmunometric assay.

In conclusion, in the human heart, there are at least three myosin isozymes, HCA, B1 and B2. The expression of these isozymes may be regulated by at least two factors: developmental stage and pressure overload.

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