Developmental Changes in the Human Cardiac Isomyosin Distribution: An Immunohistochemical Study Using Monoclonal Antibodies

Patrice Bouvagnet, Sylvie Neveu, M. Montoya, and Jean J. Leger

With monoclonal antibodies (Mab) specific for myosin heavy chain (MHC) isozymes, we have investigated the isomyosin content of atrial, ventricular and conductive fibers of 19 human fetuses (ranging from 14–36 weeks of gestation) and 3 newborns (2 days–2 weeks). In addition, the conduction system of 2 human adult hearts was studied. The fetal atrium is composed mostly of β-MHC during the first 23 weeks of gestation. β-MHC is already expressed as traces at 14 weeks of gestation, and its expression increases progressively until birth, resulting in a great augmentation in β-MHC. During this course, β-MHC always predominates in certain areas (the crista terminalis and the interatrial septum) but not in other areas (the auricles). Preceding birth, the fetal ventricle is composed mostly of β-MHC. From 14 weeks of gestation to birth, α-MHC is expressed in very rare fibers. Then, after birth, a large number of fibers simultaneously synthesize α-MHC. The AV node and His bundle system were labelled with anti-α and anti-β Mab in fetal, newborn, and adult hearts with a double gradient of distribution: spatial (a higher proportion of α-containing fibers in the AV node than in the distal portion of the bundle of branches) and temporal (a higher proportion of α-containing fibers at a given point in fetal development than in the adult heart). One of the twenty-five hearts studied had an isomyosin distribution pattern not accorded to its age. Interestingly, it was clinically diagnosed as having idiopathic hypertrophic cardiomyopathy. (Circulation Research 1987;61:329–336)

In the developing mammalian embryo, the primordial cardiac tube begins to pulsate about 4 weeks after conception. The pulsation results from the development of an early contractile apparatus of which myosin is a major component. Whether or not there is a primordial contractile apparatus common to cardiac and skeletal muscle is controversial. 1,2 Nevertheless, a developmental polymorphism of the cardiac myosin heavy chain (MHC) isoforms has been documented by different observers (for a review see Swynghedauw). 4 Since the isoform content of cardiac fiber is correlated with the contractile properties in rat hearts, 5,6 this variation in the expression of different MHC isoforms could correspond to an adaptation to evolving hemodynamic conditions. This plasticity of MHC during development has already been studied in some avian and mammalian hearts but so far not in the human heart.

The purpose of this study was to investigate the isomyosin heavy chain pattern of atrial, ventricular, and conduction fibers in the human heart during the fetal and neonatal life. Different sets of monoclonal antibodies (Mab) specific for different human isoforms as previously documented 7–10 were used to analyze the MHC content of cardiac fibers by immunofluorescence.

Materials and Methods

Tissue Sources

Human hearts were obtained within 1 hour after death (Maternity of Montpellier and Nimes, and Newborn Department of Montpellier, Hôpital Saint Charles) or from renal transplant donors (Nephrology and Urology unit, Hôpital Saint Charles, Montpellier). Removal of human tissue was processed according to French laws.

Nineteen fetal hearts, ranging in age from 14–36 weeks of gestation, were obtained after spontaneous or therapeutic abortions. Heart F2 had Potter’s syndrome; hearts F7, 9, and 12 had trisomy 21. The other fetus hearts had no malformation as detected prior to abortion and at anatomic examination (Table 1).

Three newborn hearts, N1–N3, were also studied. The N1 infant was premature (28 weeks of gestation) and was admitted in critical condition secondary to severe respiratory distress syndrome and hypothermia at birth. He died 4 days after birth. N2 was a full-term newborn who died at 48 hours because of multi-system failure secondary to severe anoxia during delivery. N3 was a full-term newborn who had a small interventricular communication as demonstrated at angiography and necropsy. He suffered from cardiac insufficiency and hypoglycemic tendency. He died from septicemia 2 weeks after birth (Table 1).

Two adult normal hearts whose atrial and ventricular
Table 1. Heart Tissue Sources, Age, Weight, and Diagnosis

<table>
<thead>
<tr>
<th>Hearts</th>
<th>Gestation (week)</th>
<th>Heart weight (g)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>14</td>
<td>0.9</td>
<td>TA</td>
</tr>
<tr>
<td>F2</td>
<td>14</td>
<td>0.8</td>
<td>TA</td>
</tr>
<tr>
<td>F3</td>
<td>15</td>
<td>2.7</td>
<td>TA</td>
</tr>
<tr>
<td>F4</td>
<td>17</td>
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<td>TA</td>
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<tr>
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<td>18</td>
<td>2.8</td>
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</tr>
<tr>
<td>F7</td>
<td>19</td>
<td>2.7</td>
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<tr>
<td>F8</td>
<td>20</td>
<td>3.4</td>
<td>TA</td>
</tr>
<tr>
<td>F9</td>
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<td>3.6</td>
<td>Trisomy 21</td>
</tr>
<tr>
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<td>22</td>
<td>4.3</td>
<td>TA</td>
</tr>
<tr>
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<td>22</td>
<td>4.3</td>
<td>Toxoplasmosis</td>
</tr>
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<td>22</td>
<td>4.2</td>
<td>TA</td>
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<tr>
<td>F13</td>
<td>23</td>
<td>5.4</td>
<td>Trisomy 21</td>
</tr>
<tr>
<td>F14</td>
<td>23</td>
<td>—</td>
<td>Death in utero</td>
</tr>
<tr>
<td>F15</td>
<td>24</td>
<td>5.4</td>
<td>Rupture of membrane</td>
</tr>
<tr>
<td>F16</td>
<td>27</td>
<td>5.7</td>
<td>Placenta praevia</td>
</tr>
<tr>
<td>F17</td>
<td>28</td>
<td>7.1</td>
<td>Twin pregnancy</td>
</tr>
<tr>
<td>N1</td>
<td>29</td>
<td>5</td>
<td>Third trimester hemorrhage</td>
</tr>
<tr>
<td>F18</td>
<td>31</td>
<td>10.2</td>
<td>Uterine rupture</td>
</tr>
<tr>
<td>F19</td>
<td>36</td>
<td>17</td>
<td>Death in utero</td>
</tr>
<tr>
<td>N2</td>
<td>FT</td>
<td>25</td>
<td>Anoxic delivery</td>
</tr>
<tr>
<td>N3</td>
<td>FT</td>
<td>22</td>
<td>Septicemia</td>
</tr>
</tbody>
</table>

FT, full term; TA, therapeutic abortion.

Fetal Atrium

All fetal atrial fibers of hearts F1–F14 were stained intensely and homogeneously with the anti-α Mab.

Tissue Section Processing

Serial cryostat sections were processed for indirect immunofluorescence as follows. Sections were incubated for 30 minutes at 37°C with the appropriate dilution of monoclonal antibody. After rinsing twice in phosphate-buffered saline solution, the sections were incubated for 30 minutes at 37°C with fluorescein-labelled rabbit or goat anti-mouse IgG (Nordic Laboratories, Tilburg, The Netherlands). Controls were performed by incubating sections with either a cell culture supernatant free of Mab or containing a Mab specific to renin (gift from B. Pau, Clin-Midy, Av. Prof. Blayac, Montpellier). Slides mounted with elvanol were allowed to dry overnight and examined under a Leitz Orthoplan microscope with epifluorescence optics. Samples containing the conduction tissue were thoroughly cut and every twentieth section was labelled by Masson-Goldberg staining. These were then examined under a Leitz Orthoplan photomicroscope to identify the conduction tissue.

Results

The specificity of the Mab prepared from different human cardiac MHC has been previously established through the use of concomitant immunofluorescence staining, western blots of denatured and native myosins, and radioimmunossays of atrial and ventricular tissues from rat, rabbit, bovine, and human hearts. Briefly, the Mab are designated as anti-α and anti-β Mab since they crossreact with mammal ventricular α- and β-MHC, respectively. The specificity of the Mab has been assessed by immunofluorescence staining since many fibers in the hypothyroid rat ventricle and in the human adult atrium do not react at all with anti-α Mab. Because these fibers are intensely labelled with anti-β Mab, they must contain β-MHC, which does not crossreact with the anti-α Mab. Similarly, in the young rat ventricle and in the human adult atrium, anti-β Mab does not even slightly stain some fibers intensely labelled by anti-α Mab.

The anti-β Mab stain very few fibers in human adult, normal ventricle. These rare β-containing fibers were also labelled with any anti-β Mab, but unlike the predominant fiber type, they presented a specific alkaline labile ATPase activity (Bouvagnet et al and P. Bouvagnet, manuscript in preparation).

The 24 human hearts under study have been classified a priori according to their age: 19 are representative of different stages of fetal life between 14 and 36 weeks of gestation (Table 1), 3 are newborns (2 days–2 weeks), and 2 are young adults.

Monoclonal Antibodies

The antibodies used in this study have been previously reported. Briefly, one fusion produced by immunization with an MHC prepared from the hypertrophied human left ventricle provided 2 sets of Mab: one set is anti-β, and the other set is anti-β’ MHC, previously designated as 1V(2A) and 2V(1A), respectively. Another fusion provided a set of anti-α Mab. This latter fusion was prepared with the free fraction of atrial human MHC collected through an anti-β MHC immunoaffinity column.

Preparation of Tissue

Hearts were rinsed with a standard solution of phosphate-buffered saline and were blotted and weighed. The whole heart or samples excised from different regions were then frozen in precooled isopentane and either studied immediately or stored at −80°C until use. Identical results were obtained under both conditions. Tissue sampling consisted of excising the auricle, a portion of the walls of both atria, the apex, a portion of the free wall, and a papillary muscle of each ventricle. The dissection of the His bundle was carried out according to either Lev and McMillan or Chomette and Hammou. Tissue sections (6–8 μm) were cut with a cryostat at −20°C and either dried for 30 minutes and processed for immunofluorescence or stored at −80°C until use. Tissues were not fixed by any treatment. Tissue-section wrapping was minimized by cutting at −22°C and at high speed. Tissue samples that contained the His bundle were carefully oriented and thoroughly sectioned.

Isomyosin distributions have been previously reported also were used in this study.

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Atrial fibers of hearts F15–F19 also were intensely labelled except for some fibers in the right atrial wall and in the interatrial septum that displayed only moderate staining. Conversely, the anti-β Mab detected only traces of β-MHC in early fetal atria (Figure 1a) but detected significantly more fibers containing β-MHC in atria F15–F19. The distribution of the labelled fibers was not homogenous since the crista terminalis contained about 2 times more β-containing fiber than the auricles. The comparison of serial sections showed that the areas rich in β-containing fibers corresponded to the areas heterogeneously stained with anti-α Mab, suggesting that fibers of these areas contained a mixture of α- and β-MHC. Thus, fetal atrium is composed mainly of α-MHC. However, very small amounts of β-MHC are expressed as early as 14 weeks of gestation. After week 24, β-MHC expression is clearly increased in well-defined areas. The left atrium apparently contains the same amount of β-containing fibers as the right atrium throughout fetal development.

Newborn Atrium

Whereas, most of the atrial fibers of the 3 newborn hearts were highly labelled with anti-α Mab, they had different patterns of reactivity with respect to the anti-β Mab. N1, the heart from a 29-week-old premature newborn (Figure 1b), contained significantly more atrial fibers labelled with anti-β Mab than the fetal atria of the same age (F16 and F18). Moreover, the pattern of β-containing fiber distribution was similar to the full-term fetal heart (F19), which is 7 weeks older. The crista terminalis of N2 had an almost equal percentage of α- and β-labelled fibers, whereas the auricles contained only few β-labelled fibers. Thus, N1 and N2, compared with fetal hearts, exhibited a significant shift toward β-MHC, particularly in certain regions. In contrast, β-MHC was not detected in the atrium of N3, which seemed to synthesize only α-MHC.

Fetal Ventricle

During the course of fetal life, the ventricle had very few fibers containing α-MHC. These rare fibers were either isolated in the myocardium surrounded by fibers that did not express α-MHC (Figure 1c) or close to the endocardium (Figure 2a and 2b). There was no difference between right and left ventricles. Conversely, all ventricular sections stained with the anti-β Mab gave the same result: all ventricular fibers were homogeneously and intensely stained. This distribution
**FIGURE 2.** a. Section of an 18-week fetal ventricle (F6) stained with anti-α Mab. Nearly all fibers are unlabelled except a bundle that corresponds to the right bundle branch close to the endocardium. b. Composite block of right atrium (upper right) and left ventricle (lower left) of a 36-week fetal heart (F19) stained with an anti-α Mab. The labelled rare ventricular fibers are located at the contact of the endocardium. These fibers and the fibers located in a at the endocardium and between the endocardium and the right bundle branch could be Purkinje fibers. Magnification, 40× and 100×, respectively. Bar, 10 μ.

pattern characterized by local and low expression of α-MHC was constant from 14–36 weeks of gestation. Fibers were not labelled with any anti-β' Mab.

**Newborn Ventrices**

Ventricular fibers were, as in fetal ventricles, stained homogeneously and intensely with anti-β Mab. However, the pattern of newborn α-containing fibers differed from that of fetal ventricles. In the N1 ventricle, a large number of fibers were labelled with anti-α Mab, although the intensity of fluorescence was variable from one fiber to the other (Figure 1d). N2 presented less α-containing fibers than N1. In both N1 and N2, clear-cut differences were not noted between the subendocardial and the subepicardial layer nor between the right and the left ventricles. Thus, N1 and N2 expressed more α-MHC than did fetal ventricles, although the transition may not be as striking as in the atrium since all ventricular fibers were still highly labelled with anti-β Mab. The ventricle of N3 had a staining pattern clearly different from N1 and N2 since it was similar to that of fetal ventricles: only traces of α-MHC were detected. In all postnatal ventricles, no fibers were labelled with anti-β' Mab.

**His Bundle of the Fetal Hearts**

The conduction tissue of 15 of the 19 fetal hearts available was studied. AV node fibers were not different from the surrounding fibers with respect to their myosin content. They expressed, as did fibers of the crista terminalis, substantially more β-MHC than other atrial areas. AV node fibers were distinguished by their smaller size, round shape, and faint labelling. As shown in Figure 3, the His bundle displayed a heterogeneous pattern of reactivity when stained with either anti-α or anti-β Mab. Interestingly, more fibers were stained by anti-α than by anti-β Mab in the His bundle. Moreover, the bundle branches exhibited a difference in α-MHC: β-MHC ratio between the proximal and distal regions as illustrated in Figure 4.

**His Bundle in Newborns and Adults**

In newborn AV node, all fibers still expressed equal amounts of both isomyosins, whereas fibers of the His bundle and bundle branches expressed reduced amounts of α-MHC as evidenced by fibers not labelled with anti-α MHC Mab. In the adult human heart, fibers of the AV node were labelled with both sets of anti-α and anti-β Mab. Fibers of the His bundle and bundle branches primarily contained β-MHC. The staining pattern of conduction fibers was, therefore, similar to that of the surrounding ventricular fiber.

**Discussion**

This study presents the distribution of the different types of MHC in the human heart during the entire fetal period from the 14th week of gestation through the early postnatal period. Since the present work used the same Mab sets previously used on atrial and ventricular fibers of adult human hearts, the results indicate that before and after birth, both cardiac tissues probably contain the same types of MHC. No data is available to ascertain whether atrial and ventricular α- and β-MHC are actually identical at fetal, postnatal, and adult stages. Minor isomyosins were looked for by using many other Mab that originated from other fusions, particularly with Mab produced from human fetal skeletal MHC. However, the existence of other minor isomyosins in the human heart cannot be precluded because minor myosin isoforms related either to α- or β-MHC have already been detected in human hearts and in other mammal hearts. The minor isomyosin
FIGURE 3. The His bundle of a 27-week fetal heart is sectioned transversely and stained with anti-α (A) or anti-β Mab (B). The anti-α Mab stains more fibers in the His bundle (→) than does the anti-β Mab but always in a heterogeneous manner. Atrium is on the left side; ventricle is on the right side.
that was denoted as $\beta'$ (because it is related to the $\beta$-MHC), is surprisingly not expressed during fetal and perinatal life in the 22 hearts analyzed. In fact, only small amounts of this $\beta'$-MHC appear in normal adult human hearts (Bouvagnet et al, manuscript in preparation).

On the basis of data presented here and in other recent reports, the developmental course of isomyosin expression in human heart can be described. $\alpha$-MHC is expressed in the atrium during the whole fetal period. $\beta$-MHC is increasingly expressed in human atria from very low levels at 14 weeks of gestation to relatively significant levels at birth. However, this increase is not regular since the $\beta$-MHC expression rate appears augmented at about 23 weeks of gestation. Moreover, $\beta$-MHC is preferentially expressed in the same areas of the fetal atrium as in the adult atrium. This heterogeneous distribution of $\beta$-MHC has been described in the mammalian atrium as well. In fetal hearts, evidence for any clear-cut difference in $\alpha$:$\beta$ ratio between right and left atrium has not been forthcoming. In adult heart, however, more $\beta$-MHC was found in the right than in the left atrium, as previously described by Gorza et al and in opposition to Kuro-o et al. These differences may be accounted for by limitations inherent in immunofluorescence studies of tissue sections, i.e., the extrapolation of data obtained from sections to the whole organ. This discrepancy might be resolved by biochemical or immunobiochemical analysis of the whole atrium.

During fetal and early postnatal life, all human ventricular fibers contain $\beta$-MHC, whereas few of them contain $\alpha$-MHC. The detection of low amounts of $\alpha$-MHC in human fetal ventricular fibers is in agreement with the determination of $\alpha$-MHC by ELISA assays. This low $\alpha$-MHC expression and the fact that $\alpha$- and $\beta$-MHC are expressed simultaneously in the same ventricular fibers make difficult any structural or enzymatic comparison between different ages of human ventricular tissues by classic techniques. Currently, the physiologic role of the minor $\alpha$-MHC preferentially expressed near the endocardium in human fetal ventricles is actually unknown. This local expression contrasts with the transmural gradient of $\alpha$-MHC decreasing from the epicardium to the endocardium recently observed in adult human ventricles (Kuro-o et al; Bouvagnet et al, manuscript in preparation). Nevertheless, the endocardial location of these $\alpha$-containing fibers in the fetal ventricle suggests that they might be conduction fibers. Consequently, conduction fibers that have already acquired specific electrophysiologic properties might not express the same $\alpha$:$\beta$ ratio that is common in myocytes.

The conduction system has an ontogenic and spatial variation of isomyosin distribution. More $\alpha$-MHC is expressed in the AV node than at the distal part of the His bundle branches, and at any given point along the His bundle, more $\alpha$-MHC is expressed in the fetal than in the adult stage. This is in contrast with the nonconduction ventricular myocardium in which no significant modification of the $\alpha$:$\beta$ ratio is detected during development. As described by Kuro-o et al, we also have not detected any specific MHC isoform in human conduction fibers. When tested with our Mab, the MHC of human conduction fibers did not have a common epitope with either slow or fetal skeletal MHC, unlike the case in avian and bovine conduction fibers, respectively. Furthermore, we have not identified a primordial MHC but this possibility cannot be excluded because we have not explored fetal hearts prior to 14 weeks of gestation.

The molecular basis of the developmental plasticity...
of isomyosin expression remains to be elucidated. The question is whether there are one or more features that can trigger a switch from α- to β-MHC or vice versa. Recently, Gorza et al.23 suggested that bovine "nodal" MHC could be a specific MHC of fibers originating from the sinus venosus region. They also suggested, as have others,23 that the expression of one type of MHC could be associated with particular electrophysiologic properties. Furthermore, hypertrophy of the human atrium is associated with an α to β transition that suggests fiber stretch could be a trigger for isomyosin switch.10,28,29 Animal studies have shown evidence of thyroid hormone affecting the expression of isomyosin (see Swynghedauw4 for a review). It is not yet known whether the human heart has the same property. However, it is interesting to note that birth, during which there is a rapid rise in circulating thyroid hormone levels resulting from the hypothermia of delivery,40 is associated with the induction of substantial increase of specific isomyosin synthesis, i.e., β-MHC in the atrium and α-MHC in the ventricle. Since Chizzonite and Zak30 have clearly demonstrated the role of thyroid hormone in the induction of α-MHC expression in neonatal rat ventricle, thyroid hormone hypothetically could also induce ventricular α-MHC expression in humans, although this hormone could not account for β-MHC induction in the atrium. However, Izumo et al.12 recently reported a highly tissue-specific effect of this hormone that can either switch MHC gene expression on or off depending on the tissue where it is expressed. Insulin also is involved in the regulation of MHC expression.31 Interestingly from this point of view, the N3 newborn, who had no isomyosin switch and had a small interventricular communication, suffered from glycemic dysregulation.

To conclude, the present results show that highly specific Mab allow identification of myosin fiber content and allow study of its modification throughout the development of normal and diseased human hearts. Further investigations are, however, needed to determine the molecular basis of the regulation of the expression of cardiac isomyosins.

Note: Since the submission of this manuscript, Everett46 reported isomyosin expression in fetal hearts at 14–18 weeks and in infant hearts at 3 weeks–3 months. In the early fetal period, we observed also low expression of β-MHC in the atrium, but in contrast with Everett's report, we detected α-MHC in the ventricle at this early period. This discrepancy might be due to differences in the Mab affinities and/or specificities since with the same set of anti-α Mab, we detected unlabelled adult atrial fibers,4 whereas Everett reported that all adult atrial myocytes contained α-MHC. In this paper, the progressive modulation of isomyosin expression during fetal and perinatal life was studied, but isomyosin expression was not analyzed during the infant period that appears, according to Everett, identical to that observed during the adult period. Finally, special attention was paid to the local isomyosin expression within both the common myocardial tissue and the conduction system.

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