Species-Specific Differences of Myosin Content in the Developing Cardiac Chambers of Fish, Birds, and Mammals

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ABSTRACT

Key morphogenetic events during heart ontogenesis are similar in different vertebrate species. We report that in primitive vertebrates, i.e., cartilaginous fishes, both the embryonic and the adult heart show a segmental subdivision similar to that of the embryonic mammalian heart. Early morphogenetic events during cardiac development in the dogfish are long-lasting, providing a suitable model to study changes in pattern of gene expression during these stages. We performed a comparative study among dogfish, chicken, rat, and mouse to assess whether species-specific qualitative and/or quantitative differences in myosin heavy chain (MyHC) distribution arise during development, indicative of functional differences between species. MyHC RNA content was investigated by means of in situ hybridisation using an MyHC probe specific for a highly conserved domain, and MyHC protein content was assessed by immunohistochemistry. MyHC transcripts were found to be homogeneously distributed in the myocardium of the tubular and embryonic heart of dogfish and rodents. A difference between atrial and ventricular MyHC content (mRNA and protein) was observed in the adult stage. Interestingly, differences in the MyHC content were observed at the tubular heart stage in chicken. These differences in MyHC content illustrate the distinct developmental profiles of avian and mammalian species, which might be ascribed to distinct functional requirements of the myocardial segments during ontogenesis. The atrial myocardium showed the highest MyHC content in the adult heart of all species analysed (dogfish (S. canicula), mouse (M. musculus), rat (R. norvegicus), and chicken (G. gallus)). These observations indicate that in the adult heart of the vertebrates the atrial myocardium contains more myosin than the ventricular myocardium. Anat Rec 268:27–37, 2002. © 2002 Wiley-Liss, Inc.

Key words: myosin heavy chain; heart morphogenesis; cardiac performance

The early stages of heart development are essentially similar between different vertebrate species (Icardo, 1996; Fishman and Chien, 1997). The cardiac crescent fuses in the midline of the antero-posterior embryonic axis to give rise to a straight cardiac tube. At this stage, myosin isoforms are first expressed and the cardiac patterns of expression are different—at least in avian and rodent species (for review, see Franco et al., 1998). In mice, rats, and humans, two myosin heavy chains (MyHCs) are expressed along the tubular heart: aMyHC shows an postero-ante-rior gradient, whereas βMyHC displays an antero-poste-rior gradient (De Groot et al., 1989; Moorman and Lamers, 1994). In chicken, a single βMyHC-like (VMHC1) gene has been reported with essentially the same developmental pattern as its rodent homologue (Bishaba and Bader, 1991). In contrast, two aMyHC-like genes have been reported: CC2SV mRNA, which shows a similar pattern to the mouse aMyHC mRNA (Oana et al., 1995), and AMHC1 mRNA, which is restricted to the future atrial

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myocardial cells at the tubular heart stage (Yutzley et al., 1994). Recently, a new MyHC isoform (CMHC1) was cloned, which is expressed in both the atrial and ventricular myocardium (Croissant et al., 2000). Moreover, the neonatal skeletal MyHC is transiently expressed in the embryonic chicken heart, predominantly in the primary myocardium and ventricular conduction system (Machida et al., 2000). At the protein level, it has been suggested that up to five different MyHC isoforms are expressed in the chicken heart, each one having a specific expression pattern within the atrial and ventricular myocardial components (Evans et al., 1988; De Jong et al., 1988). At present, no information is available concerning myosin composition and distribution in the dogfish heart.

With further development, five different morphological and functional areas are present in the embryonic heart of birds and mammals: the inflow tract, atria, atrioventricular canal, ventricle, and outflow tract (Moorman and Lamers, 1994). Each of these regions represents a distinct transcriptional domain (Franco et al., 1997; Kelly et al., 1999). Concomitant with the formation of a regionalised embryonic heart, MyHC isoforms become confined to distinct compartments (Lyons, 1994; Franco et al., 1998). αMyHC becomes restricted to the atrial/inflow tract myocardium, whereas βMyHC becomes restricted to the ventricular/outflow tract myocardium. Coexpression of both isoforms is observed in the “primary myocardium,” i.e., the inflow tract, atrioventricular canal, and outflow tract (for reviews, see Moorman and Lamers, 1994; Franco et al., 1998).

The hearts of birds and mammals acquire separate left and right atrial/ventricular chambers during development. The timing of ventricular chamber subdivision differs substantially between avian and mammalian species. In the chick, the left and right ventricular primordia become recognisable after cardiac looping. In contrast, the formation of left and right ventricles, including the ventricular septum, occurs concomitant with cardiac looping in mice and rats. The acquisition of left and right ventricular segments is concomitant with transient differences in left and right sarcomeric gene expression (Kelly et al., 1999, Zammit et al., 2000) and transcriptional potential (Kelly et al., 1995, 1999; Ross et al., 1996; Franco et al., 1997).

Cardiac performance is directly related to conductive and contractile characteristics of cardiomyocytes. In mammals, impulse conduction is mainly achieved via gap junctional communication. Little is known about gap junction protein distribution in avian and fish hearts (Beyer, 1990; Satchell, 1991; Minkoff et al., 1993; Goudrie et al., 1993). The main determinants of contractile performance in striated muscle are differences in MyHC composition. In mammals, impulse conduction is mainly achieved via gap junctional communication. Little is known about gap junction protein distribution in avian and fish hearts (Beyer, 1990; Satchell, 1991; Minkoff et al., 1993; Goudrie et al., 1993). The main determinants of contractile performance in striated muscle are differences in MyHC composition. One can envisage that cardiac performance is related to total MyHC content, and that its regional heterogeneity contributes to differential chamber-specific cardiac performance during ontogenesis.

Currently, there is no information concerning myosin composition and distribution in the heart of primitive vertebrates, such as elasmobranchs, that maintain single atrial and ventricular chambers in the adult stage. Therefore, we selected the dogfish (Scyliorhinus canicula) as an animal model in which to examine changes in total MyHC content during the change from a single circulatory cardiac system into a double-pumping heart.

In the present study, we analysed the expression pattern profile of MyHC content in three stages: 1) an early looping stage; 2) an embryonic stage in which five morphological segments can be distinguished (inflow tract, atrium, atrioventricular canal, ventricle, and outflow tract); and 3) the adult stage.

MATERIALS AND METHODS

Embryos

Fertilised eggs and adult specimens of the spotted dogfish (Scyliorhinus canicula) were maintained in captivity under standard conditions as previously described (Gallego et al., 1997). In our experience, total length (TL) represents the most suitable parameter for describing the developmental stage of these embryos. Embryos of 20 and 40 mm TL, and adult heart samples were examined. Samples employed for in situ hybridisation were dissected in 0.1 M phosphate buffer (pH 7.3) and fixed in 4% freshly prepared formaldehyde in 0.1 M phosphate buffer (pH 7.3). After they were washed in PBS, the samples were dehydrated in increasing concentrations of ethanol and embedded in paraplast. Serial sections were cut at 7 μm, mounted onto RNase-free aminopropyltriethoxysilyl-coated glasses (for in situ hybridisation) or onto polysine-coated glasses (for immunohistochemistry) and stored at room temperature.

Fertilised chicken eggs were obtained from a local hatchery (Drost BV, Nieuw Loosdrecht, The Netherlands), incubated at 37°C in a moist atmosphere, and automatically rotated every hour. After appropriate incubation times, embryos of stages 14, 20, 24, and 30 (Hamburger and Hamilton, 1951) were isolated and processed for in situ hybridisation or protein immunohistochemistry as previously described. Adult chicken hearts were also obtained from a local hatchery and quickly processed for in situ hybridisation or protein immunohistochemistry.

Wistar rat embryos of embryonic day (E) 12.5, E14.5, E16.5, and E18.5; mouse C57BL6/J embryos of E10.5, E12.5, E14.5, and E16.5; and rat and mouse adult hearts were examined. The day of vaginal plug was taken as E0.5. Embryos were excised from the uterus and fixed either in 4% freshly-prepared formaldehyde in phosphate-buffered saline (PBS) overnight at 4°C for in situ hybridisation or in methanol : acetone : water (2:2:1) at 4°C for immunohistochemistry. Samples used for protein immunohistochemistry were handled as previously described for other species.

In Situ Hybridisation

We used a cDNA probe coding for the highly conserved ATP binding site of the human βMyHC gene (nucleotides 460–643 (Jaenicke et al., 1990)) as a general marker for MyHC content. The length of the probe is 184 nucleotides, and it shows a high homology to the same region of the MyHC genes in rat (90%), mouse (90%), chicken (88%), and carp (84%; see Fig. 1). This highly conserved homology is also shared with skeletal muscle-specific isoforms at both amino acid and nucleotide sequences (Habets et al., 1999).

The human βMyHC probe was linearised with BamHI and transcribed with T7 RNA polymerase. Complementary RNA probe was made with 35S-CTP (single-labelled)
or with 35S-UTP and 35S-CTP (double-labelled) by in vitro transcription according to standard protocols (Melton et al., 1984). Complementary RNA probes against chicken AMHC1 (Yutzley et al., 1994), chicken VMHC1 (Bishaba and Bader, 1991), rat αMyHC (Schiaffino et al., 1989; Boheler et al., 1992) and rat βMyHC (Boheler et al., 1992) mRNAs were used as positive controls on the hybridisation assays in chicken and mouse embryos. Hybridisation conditions were as described elsewhere (Moorman et al., 1995, 2000; Franco et al., 2001). Briefly, the sections were deparaffinated, rinsed in absolute ethanol, and dried in an air stream. Pretreatment of the sections was as follows: 20 min 0.2 N HCl, 5 min bidistilled water, 20 min in 2X SSC (70°C), 5 min bidistilled water, 2–20 min digestion in 0.1% pepsin dissolved in 0.01 N HCl (37°C), 30 sec in 0.2% glycine/PBS, two 30-sec rinses in PBS, 20 min postfixation in 4% freshly-prepared formaldehyde, 5 min in bidistilled water, 5 min in 10 mM DTT, and finally drying in an air stream. The prehybridisation mixture contained 50% formamide, 10% dextran-sulphate, 2X SSC, 0.1% Triton X-100, 10 mM DTT, and 200 ng/μl heat-denatured herring sperm DNA. The sections were hybridised overnight at 52°C and washed as follows: a rinse in 1X SSC, 30 min in RNase A (10 μg/ml), 10 min 1X...
protein at the 40 mm TL stage is similar to that observed at the mRNA level. MyHC sleeve of the AVC. Note that the hybridisation signal is absent in the endocardial cushion. MyHC transcripts is also observed in skeletal muscle (arrows). C: Within the ventricular myocardium, no differences are observed in the atrioventricular (avc), ventricular (v), and conal (co) myocardia. including the atrial side of the sinoatrial valve (arrowhead), as well as the caval veins, the coronary sinus, the crista terminalis, and the Thebesian valve (Rogers, 1986; Dor and Corone, 1991; Moorman and Lamers, 1994; De Rutter et al., 1995; Tasaka et al., 1996; Webb et al., 1998). The outflow tract is the myocardial region at the arterial pole of the heart lined by endocardial cushions (Franco et al., 1999); it becomes divided into pulmonary and aortic outlets by the formation of the outlet septum, and contributes to the formation of the left and right ventricular infundibuli (De la Cruz et al., 1989; Franco et al., 1997).

Protein Immunohistochemistry

Monoclonal primary antibodies against human αMYHC (Wessels et al., 1991), human βMYHC (Wessels et al., 1991), chicken αMYHC (De Groot et al., 1987), and chicken βMYHC (De Groot et al., 1987), and polyclonal primary antibody against all MYHC isoforms (L53) were used. The L53 polyclonal antibody was generated as described by Sanders et al. (1984) and its specificity was characterised by Western blot analysis. Sections were deparaffinized, hydrated in decreasing concentrations of ethanol, and rinsed in PBS. Subsequently, sections were treated for 30 min with 3% hydrogen peroxide in PBS to reduce endogenous peroxidase activity, followed by incubation in TENG-T (10 mM Tris, 5 mM EDTA, 150 mM NaCl, 0.25% gelatine, 0.05% Tween-20, pH 8.0) for 30 min, and finally incubated overnight in primary antibody. Binding of the primary antibody was detected using peroxidase-avidin/biotin-complex- or alkaline phosphatase (AP)-coupled secondary antibodies.

After application of the secondary biotinylated antibody for 2 hr, the signal was visualised by incubation with a 0.5 mg/ml diaminobenzidine (DAB) solution (sp2001; Vector Laboratories, Burlingame, CA) for 2–10 min, following the manufacturer's protocol. Incubation with secondary AP-coupled antibody was performed for 2 hr. Endogenous AP activity was inhibited by adding 5 mM levamisole in all incubation and washing solutions. After primary and secondary antibody incubations, the sections were heated for 20 min at 55°C for further blocking of the remaining endogenous AP activity. Visualisation of the AP activity was performed by incubation in NBT/BCIP (#1681451; Roche, Basel, Switzerland) solution for approximately 20 min. The specificity of the antigen-antibody reaction in different species was supported by the fact that incubations without primary antibody led to no detectable signal in all cases.

Image Analysis

Gray-scale radioactive in situ hybridisation signals were converted into colour-scaled images to easily identify quantitative differences in gene expression within different myocardial compartments, using the methodology described by Moorman et al. (2000).

Cardiac Nomenclature

In birds and mammals, the embryonic heart consists of five segments: the inflow tract, atrium, atroventricular canal, ventricle, and outflow tract. The inflow tract is the myocardial region upstream of the atrium (Franco et al., 1998); it becomes incorporated into the atrium and eventually develops into different myocardial structures, including the caval veins, the coronary sinus, the crista terminalis, and the Thebesian valve (Rogers, 1986; Dor and Corone, 1991; Moorman and Lamers, 1994; De Rutter et al., 1995; Tasaka et al., 1996; Webb et al., 1998). The outflow tract is the myocardial region at the arterial pole of the heart lined by endocardial cushions (Franco et al., 1999); it becomes divided into pulmonary and aortic outlets by the formation of the outlet septum, and contributes to the formation of the left and right ventricular infundibuli (De la Cruz et al., 1989; Franco et al., 1997).

In the dogfish, the embryonic heart shows the five segments mentioned above (Gallego et al., 1998). The inflow tract differentiates early into a well defined chamber, the sinus venosus, which remains as a single distinct entity in the adult heart (Santer, 1985; Satchell, 1991; Muñoz-Chá...
Figure 2.

Figure 3.
RESULTS

Dogfish Cardiac Development

The developmental stages of the dogfish heart are similar to the early stages observed during mouse embryogenesis (Muñoz-Chápuli et al., 1994). Early in development, the heart tube loops rightward and acquires a left-dorsal position or substantial remodelling (Muñoz-Chápuli et al., 1994; Sans-Coma et al., 1995). Thus, the heart of the adult dogfish is composed of five myocardial compartments: the sinus venosus, atrium, atrioventricular canal, ventricle, and conus arteriosus (bulbus cordis).

Chicken Heart Development

The early stages of cardiac organogenesis in chicken are essentially similar to those observed in the dogfish heart. At H/H14, the heart can still be considered as a linear tube, with the myocardium separated from the endocardium by an acellular cardiac jelly. At this stage, thickening of the cardiac jelly can already be observed in the prospective outflow tract (conal) region and the atrioventricular canal region. In contrast to the dogfish (20 mm TL), expression of total MyHC transcripts is higher in the ventricular/conal myocardium (Fig. 3B–D). The atrioventricular myocardium displays a weaker hybridisation signal than the atrial and the ventricular myocardium (Fig. 3D). The expression of total MyHC transcripts in the conal myocardium is just slightly weaker than in the ventricular myocardium (Fig. 3C). No differences in MyHC expression were observed between the trabeculated and compact myocardial layers of the ventricles. Similar results were obtained at the protein level (Fig. 3F–H). The atrioventricular canal displays a slightly weaker MyHC protein level than the atrial and ventricular myocardia, whereas the MyHC protein level of the conus arteriosus is similar to that of the ventricular myocardium (Fig. 3F and G). Thus, according to our observations, the atrial and ventricular myocardia contain more MyHC transcripts and are flanked by myocardial segments that contain less MyHC transcripts, namely the atrioventricular canal and the conus arteriosus. At the protein level, the differences are less sharp than those observed at the mRNA level.

VENOUSOUS shows a clear hybridisation signal confined to the thin myocardial middle layer of the sinus wall (Fig. 3E and H). This observation is in line with previous reports that described a single muscular layer in the sinus venosus surrounded by connective and neural components (Saetersdal et al., 1975; Ramos et al., 1996; Gallego et al., 1997). Expression in the atrial myocardium is higher than in the ventricular/conal myocardium (Fig. 3B–D). The atrioventricular myocardium displays a weaker hybridisation signal than the atrial and the ventricular myocardium (Fig. 3D). The expression of total MyHC transcripts in the conal myocardium is just slightly weaker than in the ventricular myocardium (Fig. 3C). No differences in MyHC expression were observed between the trabeculated and compact myocardial layers of the ventricles. Similar results were obtained at the protein level (Fig. 3F–H). The atrioventricular canal displays a slightly weaker MyHC protein level than the atrial and ventricular myocardia, whereas the MyHC protein level of the conus arteriosus is similar to that of the ventricular myocardium (Fig. 3F and G). Thus, according to our observations, the atrial and ventricular myocardia contain more MyHC transcripts and are flanked by myocardial segments that contain less MyHC transcripts, namely the atrioventricular canal and the conus arteriosus. At the protein level, the differences are less sharp than those observed at the mRNA level.
Expression of total MyHC content at both the mRNA and protein level was analysed in rat and mouse embryos at heart development stages similar to those considered in the dogfish and chicken. No differences were detected between rats and mice. Similar to the situation in the dogfish heart, the expression of MyHC transcripts is homogeneous through the myocardium at both the tubular heart stage (mouse E8.5; rat E10.5) and the embryonic heart stage (mouse E10.5; rat E12.5) (Fig. 6B–D). Similar results were obtained at the protein level, using the polyclonal L53 antibody (data not shown). Control experiments designed for the localisation of αMyHC and βMyHC (mRNA and protein) displayed a regionalised expression pattern as previously described (for review, see Franco et al., 1998).

In the adult rodent heart, as in the dogfish and chicken adult hearts, expression of total MyHC content was higher in the atrial than in the ventricular myocardium (Fig. 6E). A similar pattern was observed at the protein level (data not shown).

**DISCUSSION**

A comparison of the amino acid (data not shown) and nucleotide sequences (Fig. 1) of different mammalian (human, mouse, rat, pig, and hamster), avian (chicken and....
quail), and fish (carp) isozymes corresponding to the ATP binding site of the human βMyHC gene used to localise cardiac MyHC transcripts shows that this region of the MyHC gene is highly conserved among species. Such a high homology guarantees that the hybridisation signal obtained according to our standard hybridisation conditions (Moorman et al., 1995, 2000; Franco et al., 2001) would react with all MyHC transcripts, and that it can therefore be used as a parameter to estimate total MyHC content. The pattern of expression of total MyHC protein, as revealed by using the L53 polyclonal antibody, is consistent with the data obtained at the transcriptional level.

Although distinct MyHC isoforms are expressed in each cardiac compartment of the chicken and mammalian hearts (e.g., αMyHC and βMyHC in the atria and ventricles, respectively), the L53 antibody reacts with both the atrial and ventricular myocardium in dogfish, chicken, rat, and mouse, and can thus be considered as a pan-MyHC antibody. Furthermore, L53 antibody reacts similarly in cardiac and skeletal muscle in dogfish, chicken, rat, and mouse tissues. Although we did not perform epitope mapping of the binding affinity of this antibody, the fact that the L53 antibody reacts in all species and in all striated muscles suggests that it recognises a highly conserved epitope, which is shared by MyHC isoforms among different vertebrate species.

We have demonstrated herein the existence of regional differences in total MyHC content in different vertebrate species during ontogenesis. MyHC content is homogeneous along the myocardium in the dogfish and rodent tubular and embryonic heart stages, whereas differences in MyHC content occur at the tubular heart stage in chicken, being higher in the venous pole than in the arterial pole. However, in the adult heart, the atrial myocardium contains more MyHC than the ventricular myocardium in all species studied. This suggests that although there are differences in the MyHC content profile during ontogenesis of each species, the adult MyHC distribution pattern persists throughout the phylogenetic tree of all vertebrates. Thus, the vertebrate cardiac design implies an atrial myocardium with higher MyHC content than the ventricular myocardium in the adult heart.

Fig. 6. A: Schematic representation of an embryonic (E12.5) mouse heart. B: Whole-mount in situ hybridisation using a probe against the ATP binding domain of the human βMyHC gene corresponding to a E12.5 mouse heart. In situ hybridisation using a probe against the ATP binding domain of the human βMyHC gene in the (B–D) developing and (E) adult mouse heart in tissue sections. Panel D represents a colour scale conversion. A colour scale bar is provided. Yellow indicates low expression and blue indicates high expression. C: Expression of the MyHC transcripts in the embryonic heart (E9.0) is homogeneous along the inflow tract, and the atrial and ventricular myocardia. D: At E12.5, expression of MyHC transcripts remains similar in the atrial and ventricular myocardia as well as in the inflow tract derivatives, pulmonary veins (pv), and caval veins (lscv). E: In the adult mouse heart, expression of MyHC transcripts is higher in the atrial myocardium than in the ventricular myocardium. ao, aortic valve; ave, atrioventricular canal; cv, caval veins; ift, inflow tract; la, left atrium; lv, left ventricle; ra, right atrium; rv, right ventricle; rscv, right superior caval vein. Bar: (B) 300 μm, (C) 100 μm, (D) 240 μm, (E) 500 μm.
The species-specific differences observed during ontogenesis may be related to differential patterns of expression of the distinct MyHC isoforms (αMyHC and βMyHC) existing in avian and mammalian hearts. In rodents, opposite gradients of expression for αMyHC and βMyHC have been described as early as at the tubular heart stage (De Groot et al., 1989). In contrast, expression of AMHC1 in the chicken heart is confined to the venous pole of the heart at the tubular stage, and precedes in time the ubiquitous expression of VMHC1 in the myocardium (Yutzley et al., 1994; Yutzley and Bader, 1995). CCSV2 is more broadly expressed than AMHC1, but it is mainly confined to the atrial myocardium (Oana et al., 1995). Recently, a new MyHC isoform (CMHC1) was cloned, which is expressed similarly in both the atrial and ventricular myocardia (Croissant et al., 2000). Interestingly, neonatal skeletal MyHC is also transiently expressed in the embryonic heart (Machida et al., 2000). The analysis of MyHC protein distribution suggests the presence of as many as five protein isoforms in the developing avian heart (De Jong et al., 1987, 1988, 1990; Evans et al., 1988), in contrast to two isoforms expressed in the mammalian heart (De Groot et al., 1989; Wessels et al., 1991; Lyons, 1994; Franco et al., 1998). Several skeletal muscle-specific MyHC isoforms have been reported in bony fishes (Rohlerson et al., 1985; Gerlach et al., 1989; Chanoine et al., 1990). However, few data are available concerning the cardiac MyHC composition in teleosts and elasmobranch species (Vornanen, 1994; Yelon et al., 1999), which makes correlation with the present findings difficult. The differential MyHC content observed between atrial and ventricular myocardia during all stages of chicken cardiogenesis suggests that the distinct atrial and ventricular functional requirements may be mediated by the total MyHC content, although formally we can not exclude an isoform-specific contribution to the chamber-specific functional modulation.

The adult dogfish heart presents five different morphological segments (Galleco et al., 1998), resembling those of chicken and rodent embryos (Moorman and Lamers, 1994; Franco et al., 1998). In the latter, the atria and ventricles are fast-conducting segments (high abundance of gap junctional proteins, mainly connexin 43) which are flanked by slow-conducting segments (absence of connexin 43), namely the inflow tract, atrium, atrioventricular canal, ventricle, and outflow tract (Van Kempen et al., 1991, 1996).

In the adult dogfish heart, a distinct atrioventricular segment is interposed between the atrium and the ventricle. The low MyHC content in the myocardium of this segment suggests that this myocardium may play a role in the coordination of the cardiac cycle. Moreover, the AVC myocardium may provide a delay in the propagation of the cardiac impulse throughout the heart, thereby allowing the integrated performance of the atrial and ventricular myocardia. However, further investigations into the distribution of gap junctional communication genes are required to verify this hypothesis.

Interestingly, a weaker expression of MyHC is observed in the sinus venosus vs. the atrium of the embryonic dogfish heart. In contrast, the expression in the inflow tract is similar to that observed in the atrium of embryonic avian and mammalian hearts. Such differences may be related to the fact that in the dogfish, the sinus venosus acts as a passive drainage pool of blood early in development and retains this function in adult life (Satchell, 1970, 1991). In contrast, avian and mammalian inflow tracts are transient structures that mainly become incorporated into the right atrial chamber, and eventually also contribute to the remodelling of the right and left inlets to the atrial chambers in the adult heart (De Ruiter et al., 1995; Tasaka et al., 1996; Webb et al., 1998; Franco et al., 2000). The weak MyHC expression in the adult sinus venosus wall of the dogfish heart is attributed to the fact that neural and connective tissues are intermingled with a thin, poorly contractile myocardial layer (Santer, 1985; Ramos et al., 1996; Gallego et al., 1997) which acts as the nodal tissue (these animals lack a morphologically distinguishable conduction system).

The observation that the conal myocardium has just a slightly weaker total MyHC content as compared to the ventricular myocardium suggests that it plays an active role in contraction during the cardiac cycle. In fact, the adult conus arteriosus of elasmobranchs is known to display a peristaltic pattern of contraction that plays a critical role in contributing an “extra systole” (Johansen, 1965) in Squalus suckleyi, and in allowing the conal vascular apparatus to function correctly (Satchell and Jones, 1967) in Heterodontus portusjacksoni. In summary, the different levels of total MyHC distribution in distant vertebrate species support the notion that the functional requirements of the avian heart diverge from those of dogfish and rodents as early as at the linear cardiac tube stage, although, curiously, there is a similar pattern in adulthood. The homogeneous expression along the embryonic slow- and fast-conducting segments argues in favour of the notion that differences in contractile properties in the distinct cardiac segments are dictated more by the MyHC isoform composition than by the total MyHC content, whereas in the adult heart, the atrium has a higher MyHC content than the ventricles, despite having a single or double circuitry. Moreover, the adult vertebrate cardiac design appears to require a higher MyHC content in the atrial than in the ventricular myocardium.

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LITERATURE CITED


tion of three heavy chains in adult lateral muscle. FEBS Lett 277:200–204.


