Spatiotemporal Relation Between Gap Junctions and Fascia Adherens Junctions During Postnatal Development of Human Ventricular Myocardium

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Background The growing postnatal human heart maintains electromechanical function while undergoing substantial changes of cellular topology and myocardial architecture. The capacity for growth and remodeling of ventricular myocardium in adaptation to the hemodynamic changes of early infancy later declines. This decline is associated with changes in electromechanical properties of the myocardium, which suggest that the electrical and mechanical interactions between the myocytes may change in an age-dependent manner. Thus, reduction in the capacity for myocardial growth and adaptability may relate to age-dependent alterations in the patterns of the intercellular junctions that mediate electrical and mechanical coupling. We therefore examined the hypotheses that (1) age-dependent changes in the distribution patterns of gap junctions and fascia adherentes, the intercellular junctions responsible, respectively, for electrical and mechanical coupling, accompany postnatal development in the human heart; and that (2) such changes continue into the first few years of childhood. Further, the spatial relation between the two types of junction, for which a close association has been hypothesized as necessary, was explored.

Methods and Results Ventricular myocardial gap-junction distribution was investigated in 23 pediatric surgical patients (4 weeks to 15 years old) by quantitative immunohistochemical localization of the principal cardiac gap-junctional protein, connexin43, using confocal microscopy. Immunolocalization of fascia adherens junctions by labeling N-cadherin, and correlative immunogold and standard electron microscopy, were performed in parallel. In the neonate, connexin43 gap junctions have a punctate distribution over the entire surface of the ventricular myocytes. With advancing age, gap junctions become progressively confined to the transverse terminals of the cell, ie, toward the distribution within the intercalated disk characteristic of the adult ventricle. The transversely arrayed proportion of gap-junctial label showed a linear increase with age ($R = 0.88, P < 0.01$), reaching the adult pattern at about 6 years, and the fascia adherens junctions showed a similar progression. Electron microscopy confirmed the changing pattern of junctional contacts and demonstrated that initially gap junctions and adhering junctions are frequently not closely adjacent but become increasingly so with maturation of the intercalated disk.

Conclusions Changes in the spatiotemporal patterns of the intercellular junctions responsible for electrical and mechanical coupling are closely coordinated in postnatal human ventricular myocardium and continue to about 6 years of age. Over this period there is a close and increasing association between the gap junctions and fascia adherens junctions. These changes in the distribution of intercellular electrical and adhering junctions may parallel the changing functional requirements of the ventricle, from a distribution that facilitates the remodeling necessitated by rapid growth and changing hemodynamics to that of the relatively stable and rapidly conducting adult myocardium. These age-related changes may also diminish the ability for appropriate myocardial remodeling in response to physiological, pathological, or surgical hemodynamic alterations. (Circulation. 1994;90:713-725.)

Key Words • myocardium • intercellular junctions • morphogenesis • heart defects, congenital

Gap junctions are membrane specializations that permit passage of ions and small molecules between the cytoplasmic compartments of abutting cells. In myocardium, as the low-resistance pathways for electrical propagation, gap junctions also are a determinant of myocardial conduction velocity.

Gap junctions are essential for normal tissue development and growth, and morphogenetic signals are thought to be among the small molecules that diffuse intercellularly via gap junctions. Coordination of the development and growth of the cardiac chambers in adaptation to the physiological hemodynamic changes of infancy is presumed to be guided by patterns of flux and gradients of these molecules.

In the adult heart, gap junctions are intimately associated with the membrane structures responsible for cell-to-cell adhesion and mechanical coupling, the fascia adherens junctions, within the mature intercalated disks that lie at the transverse abutments between cardiac myocytes. Although morphologically distinct fascia adherens junctions are characteristic of myocardium alone, transfection studies on cultured tumor cells indicate that the expression of cell adhesion molecules (of
which N-cadherin, the principal protein of the fascia adherens junctions, is an example) is essential for the establishment of functional gap junctions. It has been hypothesized that the close association between the fascia adherens junctions and the gap junctions that exist in the mature cardiac intercalated disk, is, therefore, a requirement for gap-junctional function in myocardium. However, the sequence of changes in the patterns of myocyte interaction that must accompany the alterations of myocardial architecture during growth, and that produce the mature intercalated disk, are poorly understood, and it is therefore not known when the close association between the intercellular junction types first occurs.

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In small mammals, developmental changes in myocardial gap-junctional proteins are reported, and although differences in the maturity at birth of these species prohibit extrapolation of these findings to the human heart, the possibility of changing distribution in postnatal human myocardium is indicated by changes in the electrical propagation through the growing myocardium; the duration of ventricular depolarization increases by only about 20% despite a 16-fold increase in heart weight in the first few years of life. Increasing conduction velocity reported in maturing Purkinje tissue and atrial myocardium, in which there is an associated increase in the anisotropy of conduction, is also likely to occur in maturing ventricular myocardium. Recent advances in pediatric cardiac surgery are overcoming the problems of performing early procedures on small, immature infants that previously carried a prohibitive high mortality rate. Despite the residual technical problems, evidence is emerging that reconstructive surgery for a variety of congenital cardiac defects carried out early in infancy, or even neonatal, life may result in better cardiac function, with fewer late postoperative arrhythmias than if delayed until later childhood. Although the benefits may be due largely to earlier correction of the inappropriate volume or pressure loading of the ventricle, as is often assumed, benefit also may be attributable to an age-dependent reduction in the ability of the myocardium to remodel in response to altered load. This reduction in adaptive potential is associated with alterations of myocardial mechanics and electrical conduction properties, which continue through the first few years of human childhood and which correlate better with age than with body size. When taken together, these functional changes suggest that the electrical and mechanical interactions between the myocytes of the maturing myocardium may indeed change in an age-dependent manner in the rapidly growing postnatal human heart, establishing the adult pattern later in childhood.

To test the hypothesis that the mature patterns of distribution of gap-junctional communication and mechanical coupling by fascia adherens junctions are not established until several years into human childhood, with a close relation between the sites of electrical and mechanical cell interaction being maintained throughout this period, confocal microscopy was used to detect immunolabeled junctions through volumes of intact human infant myocardium. The distribution of the principal cardiac gap-junctional protein, connexin43 (Cx43), and N-cadherin was investigated in parallel to establish the temporal and spatial relations of the two junction types in postnatal cardiac development. This knowledge may advance understanding not only of the mechanics of intercalated disk development but also of the changes of electromechanical function and capacity for remodeling of the young human heart, which may be relevant to optimizing the timing of pediatric cardiac surgery.

Methods

Human Myocardial Samples

Right ventricular (RV) myocardial samples were taken at the time and from the site of initial ventriculotomy, within 3 minutes of establishing cold cardioplegic cardiac arrest, from 20 patients (4 weeks to 15 years), undergoing reparative RV surgery for congenital anomalies (tetralogy of Fallot in 15 and double outlet right ventricle in 5).

To verify that RV myocardium from these abnormal hearts was representative of “normal” myocardium, RV samples were also obtained from three normal pediatric transplant donor hearts (ages 25, 48, and 68 months; maintained in cold cardioplegic solution) at the time of routine biopsy immediately before implantation. All specimens obtained were divided immediately and placed in the appropriate fixative for the imaging techniques used (detailed below). Immunohistochemical examination was carried out to determine the distribution of Cx43 gap junctions throughout the whole tissue, and correlative electron microscopic examination with and without immunogold labeling was carried out in parallel. Immunolabeling of cadherins was performed on tissue sections adjacent to those labeled for Cx43, on four of the ventricular myocardial specimens spanning the age range of the entire series. All specimens had been exposed to cold cardioplegic solution, which produces no detectable disruption of the plasma membrane or intercalated disks, a finding confirmed in control tissue exposed to cardioplegic for up to 4 hours.

Immunolocalization of Gap Junctions and Fasciae Adherentes

Antibodies

Gap junctions — Cx43. The gap-junctional antibody used was raised in rabbits to a synthetic peptide matching residues 131 to 142 of the cytoplasmic loop of rat Cx43, which is homologous to human Cx43. The production and characterization of this polyclonal antisem, and its specificity for gap-junctional protein, have been described in detail in previous reports from our laboratories.

Fasciae adherentes — N-cadherin. The antibody used to detect fascia adherens was raised in rabbits against a 24-residue segment of the C-terminal of N-cadherin (the major calcium-dependent cell-cell adhesive molecule of the fascia adherens) and was kindly supplied by Dr Benjamin Geiger of The Weizmann Institute, Israel. This antibody gives specific labeling of the fascia adherens of the cardiac intercalated disk in a range of mammalian species.

Both antisera (diluted 1:10 in phosphate-buffered saline) were used in the immunohistochemical and immunogold labeling procedures detailed below.

Immunohistochemical Labeling of Tissue

The same tissue preparation and labeling procedures were used for both Cx43 and cadherin immunohistochemistry. Specimens were immediately placed in Zamboni’s fixative (2% paraformaldehyde, 0.2% picric acid, 0.1 mol/L phosphate-buffered saline, pH 7.4) for 2 hours. After fixation, all samples were washed in tap water, dehydrated in alcohol, placed in chloroform, and embedded in wax following standard histological procedures. Sections (10-μm thickness) of wax-embedded tissue were dewaxed and rehydrated. Incuba-
tion in a trypsin solution (containing 0.1% trypsin [Sigma T-8128], 0.1% CaCl₂, 20 mmol/L Trizma base, pH 7.4) then followed for 10 minutes at room temperature to re-expose antigenic sites altered by processing. The sections were washed with tap water and then treated with 0.1 mol/L L-lysine (a blocking agent) in phosphate-buffered saline containing 0.1% Triton X-100. Incubation with the primary antiserum (to Cx43 or cadherin) was carried out for 1 hour at 37°C. After the sections were washed with phosphate buffer, secondary antibody treatment with swine anti-rabbit fluorescein isothiocyanate (FITC) (Dako; 1:20 dilution) was given for 1 hour in the dark at room temperature. After final washing of the sections in phosphate-buffered saline, the slides were mounted with Citifluor mounting medium (Agar Scientific).

Control experiments were carried out for all immunolabeling techniques, using both preimmune rabbit serum instead of the primary antiserum or the second antibody only.

Microscopy of Immunolabeled Tissue

The fluorescein-labeled 10-μm tissue sections were examined by conventional epifluorescence techniques and by confocal microscopy using a Bio-Rad MRC-500 confocal laser scanning microscope. Series of confocal optical slices, with a depth of field of approximately 1 μm, were taken at steps of 1 μm through the depth of the tissue sections. These optical slices could be viewed individually, superimposed, or rotated to reconstruct the entire complement of labeled gap junctions within the three-dimensional "blocks" of tissue, as seen in figure 1A.

Phase contrast microscopy of the immunolabeled sections was used to obtain detail of tissue structure, and adjacent tissue sections were stained with hematoxylin and eosin to assess orientation, hypertrophy, and adequacy of preservation.

Analysis of Immunohistochemical Data

For each specimen, a single confocal optical slice (depth of field <1 μm) from each of three randomly selected fields (size 180×120 μm using ×60 objective lens) of longitudinally sectioned myocardium was analyzed to express the proportion of the total label present that was organized as clusters of labeled spots characteristic of the adult intercalated disk (ie, at the intercellular abutments lying transverse to the long axis of the myocytes). The total label area present in each field was defined as the total number of pixels of signal intensity greater than 100 on the 255-point gray scale, determined automatically by binarizing the image with this threshold using PC IMAGE image analysis software (Foster-Findlay Associates). When ventricular myocardium is sectioned longitudinally, the transected cell membranes of the elongated, blunt-ended abutting myocytes (and the gap-junctional label therein) are aligned either approximately parallel or perpendicular to the fiber axis in the plane of section. Labeled junctions in positions characteristic of the adult intercalated disk, defined as those junctions organized as clusters of four or more spots aligned transversely at points of intercellular abutment, then were edited from the total binary data. The remaining area of longitudinally oriented label in the binary image was counted automatically. From these data, which were acquired while blinded to the ages of the patients, the proportion of the total label present that was distributed in transverse arrays with respect to myocyte long axis was determined. Correlation of the changing proportion of label arrayed transversely with age was analyzed by least-squares regression.

The validity of using PC IMAGE analysis of confocal immunofluorescent images to quantify gap junctions in myocardium has been verified previously by comparison with data obtained by freeze-fragment electron microscopy.

Electron Microscopy

Standard Thin Section Electron Microscopy

Samples were fixed for 2 hours with 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.3, postfixed for 2 hours in 2% osmium tetroxide (cacodylate-buffered), and dehydrated in a series of alcohols with en bloc staining in uranyl acetate at the 50% stage, before embedding in epoxy resin. Serial semithin sections (stained with toluidine blue) were examined under a light microscope, and ultrathin sections (stained with uranyl acetate and lead citrate) were prepared for electron microscopic examination.

Electron Microscopy of Immunocytochemically Labeled Thin Sections

Immunogold labeling was performed on myocardial specimens embedded at low temperature in Lowicryl K4M (Agar Scientific Ltd). Samples were fixed for 1 hour with 4% paraformaldehyde in phosphate-buffered saline, pH 7.4, and dehydrated in an ethanol series under progressive cooling down to −35°C in absolute ethanol. Infiltration was carried out with Lowicryl K4M:ethanol mixtures and then pure Lowicryl, which was polymerized with UV light while still at −35°C, and then at room temperature. Ultrathin sections were incubated with the primary antiserum (to Cx43 or N-cadherin), followed by 10 nm-gold/goat anti-rabbit complexes (BioCell) following standard procedures. All ultrathin sections were examined in a Philips EM301 electron microscope.

Results

The details of the 20 patients undergoing surgery for congenital cardiac anomalies and the 3 pediatric cardiac transplant donor patients are shown in the Table. Myocardium from all patients was labeled for Cx43. N-cadherin labeling was carried out on patients 1, 12, 17, and 22 (Table). Cx43 localization in the neonate is illustrated in Figs 1 and 2, with N-cadherin labeling of neonatal and 7-year samples in Fig 3, and Cx43 labeling of 7-year samples in Fig 4.

Neonate

A typical single confocal optical slice through longitudinally sectioned Cx43-immunolabeled RV myocardium from the 4-week-old infant is shown in Fig 1A. This image is an example of those used for determining the ratio of transverse to total label distribution. At this early stage, the punctate immunolabeling was distributed over the entire surface of the myocytes. This feature is demonstrated with even greater clarity when a number of adjacent optical slices through the same tissue field are superimposed to produce a single image consisting of data acquired through a 7-μm depth of myocardium (Fig 1B). When sectioned transversely, this same specimen has label distributed around the periphery of the cross-sectioned myocytes (Fig 1C), consistent with the longitudinal appearances. There was no detectable difference in gap-junctional organization from endocardium to epicardium across the ventricular free wall.

That this label represented intact gap junction was supported by the electron microscopic findings of gap-junctional contacts between the lateral interfaces between myocytes (Fig 2A). Annular profiles of gap-junctional membrane were observed frequently, were associated with the convoluted lateral plasma membrane, and did not appear necessarily to be a point of intercellular contact (Fig 2B).

Cx43 immunogold labeling revealed gold particles specifically concentrated on morphologically defined gap-junctional membrane (Fig 2C), with no localized clusters elsewhere. All gap junctions seen in the immu-
Table 1: Details of Myocardial Specimens From Postnatal Human Hearts

<table>
<thead>
<tr>
<th>Patient/Heart No.</th>
<th>Age</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 weeks</td>
<td>TOF</td>
</tr>
<tr>
<td>2</td>
<td>9 weeks</td>
<td>DORV</td>
</tr>
<tr>
<td>3</td>
<td>6.5 months</td>
<td>TOF</td>
</tr>
<tr>
<td>4</td>
<td>8.5 months</td>
<td>TOF</td>
</tr>
<tr>
<td>5</td>
<td>9 months</td>
<td>TOF</td>
</tr>
<tr>
<td>6</td>
<td>12 months</td>
<td>TOF</td>
</tr>
<tr>
<td>7</td>
<td>14 months</td>
<td>TOF</td>
</tr>
<tr>
<td>8</td>
<td>15 months</td>
<td>TOF</td>
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<td>9</td>
<td>20 months</td>
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<td>10</td>
<td>24 months</td>
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<tr>
<td>11</td>
<td>25 months</td>
<td>TP</td>
</tr>
<tr>
<td>12</td>
<td>28 months</td>
<td>TOF</td>
</tr>
<tr>
<td>13</td>
<td>31 months</td>
<td>DORV</td>
</tr>
<tr>
<td>14</td>
<td>38 months</td>
<td>TOF</td>
</tr>
<tr>
<td>15</td>
<td>41 months</td>
<td>DORV</td>
</tr>
<tr>
<td>16</td>
<td>48 months</td>
<td>TOF</td>
</tr>
<tr>
<td>17</td>
<td>48 months</td>
<td>TP</td>
</tr>
<tr>
<td>18</td>
<td>57 months</td>
<td>DORV</td>
</tr>
<tr>
<td>19</td>
<td>64 months</td>
<td>DORV</td>
</tr>
<tr>
<td>20</td>
<td>68 months</td>
<td>TP</td>
</tr>
<tr>
<td>21</td>
<td>70 months</td>
<td>TOF</td>
</tr>
<tr>
<td>22</td>
<td>86 months</td>
<td>TOF</td>
</tr>
<tr>
<td>23</td>
<td>180 months</td>
<td>TOF</td>
</tr>
</tbody>
</table>

TOF indicates tetralogy of Fallot; DORV, double outlet right ventricle; and TP, cardiac transplant donor.

dsgold-labeled sections (at side-to-side and end-to-end cellular abutments and the annular forms) had associated gold particles, indicating that Cx43 was present in every gap junction visualized.

At the light-microscopical level, the distribution of immunolabeled cadherin as revealed by confocal microscopy was indistinguishable from that of Cx43 on adjacent tissue sections in each case examined (see Fig 3A). At the electron-microscopical level, however, although there was a general tendency for an association between gap junctions, fasciae adherentes, and desmosomes, with small combinations of these junction types lying adjacent to one another, gap junctions were frequently observed distant from other junction types, as illustrated by the examples in Fig 2A and 2B.

Age 7 Years

Confocal microscopy revealed that by 7 years of age, Cx43 labeling formed clusters of punctate immunofluorescence to cell terminals that were orientated transverse to the long axes of the myocytes (Fig 4), that is, in the positions characteristic of the intercalated disks of adult ventricular myocardium. The immunolabeled N-cadherin in adjacent tissue sections viewed by confocal microscopy also was confined to the positions of the mature intercalated disks but showed a greater intensity of more confluent labeling (Fig 3B) than found with Cx43 label. This difference in appearance is consistent with the greater content of fascia adherens than gap junction in the mature intercalated disk. The preponderance of fascia adherens junctions is illustrated in Fig 5, which demonstrates, by immunogold electron microscopy, that N-cadherin immunolabeling at confocal microscopy was specific for morphologically distinct fascia adherens junctions. As with the neonatal specimen (Fig 2C), immunogold labeling of Cx43 at 7 years again revealed gold particles specifically associated with morphologically defined gap junctions.

That both types of junction were confined to intercalated disks characteristic of adult ventricular myocardium was confirmed by standard thin section electron microscopy. Junctional organization within disks showed the typical arrangement, with fasciae adherentes in the plicate regions, the larger gap junctions in the interplicate regions, and smaller gap junctions and desmosomes interspersed among the fasciae adherentes in the plicate regions. A long stretch of gap-junctional membrane was frequently detectable at the edge of the disk in standard thin sections, a feature consistent with the peripheral ring of larger gap junctions in confocal reconstructions of intercalated disks viewed en face (Fig 4C).

Intermediate Ages

Between the neonate and 7 years of age, intermediate patterns of Cx43 gap junction distribution were observed (Fig 6). The pattern of immunolabeling changed progressively, from being dispersed over the entire cell surface as in the neonate to the polarized adult pattern. Thin section electron microscopy of specimens from these infants up to 7 years of age confirmed changes indicative of intercalated disk formation, with greater association than in the neonatal heart between the adhering junctions and gap junctions and fewer gap junctions seen in isolation.

When the image data are analyzed and expressed as the mean proportion of the total label present that was transversely arrayed in the three test fields from each of the entire series of hearts, the resulting plot shows a linear increase with age (Fig 7). The data points from the three transplant hearts fit this line, indicating that the samples of right ventricle from the congenitally anomalous hearts are not associated with abnormal Cx43 gap-junction distribution and verifying use of the RV myocardium from these anomalous hearts to establish the changes in ventricular myocardial junctions with maturation.

As in the 7-year-old hearts, the N-cadherin immunolabeling at confocal microscopy was more intense than Cx43 in each of the intermediate cases examined (one of which was a transplant donor), but the pattern of its distribution was otherwise similar. When plotted, the proportion of N-cadherin label that is transversely distributed shows a progressive increase with age similar to that of Cx43 (Fig 7).

Discussion

Changing Distribution of Gap Junctions in Postnatal Myocardium

The results of this study demonstrate that throughout infancy in humans, progressive and substantial changes...
occur in the distribution pattern of Cx43 gap junctions in ventricular myocardium. From neonatal ventricle, in which the gap junctions of the myocytes are spread over the entire cell surface, there is a progressive redistribution toward the adult pattern in which junctions are confined to the transversely oriented terminals of the myocytes, within the complex structure of the intercalated disk. Analysis of the immunohistochemical data shows that this change is not complete until about 6 years of age, by when most of the age-related alterations in human ventricular wall stresses, velocity of shortening, fractional shortening, and heart rate have taken place.
Although Cx43 is the principal mammalian cardiac gap-junctional protein, other cardiac connexins have recently been identified. The finding in the present study that all morphologically identifiable gap junctions were immunolabeled with gold particles when Cx43 antibody was used suggests that in working ventricular myocardium, Cx43 antibody alone localizes all gap junctions present. Importantly, this finding also suggests that if other gap-junctional connexins are present in the working RV myocardium of the human infant, it is likely that they coexist with Cx43 in the same junctions. In contrast to rat and mouse hearts, human neonatal
myocardium revealed no differences in gap-junction organization between the endocardial side and the epicardial side. This difference may relate to the less mature state of the smaller mammals at birth. In their study of structure and electrophysiology in canine atrium, Dolber and Spach showed a significant increase in conduction velocity longitudinally (parallel to the myofiber long axis), with no change in transverse velocity, from juvenile to adult myocardium, demonstrating that the immature myocardium is less anisotropic electrically. Enlargement of the myocytes did not provide adequate explanation for these alterations, which were suggested to be attributable to the changing pattern and extent of separation by connective tissue of the side-to-side interfaces between individual myocytes and groups of cells that occur in maturing atrial and ventricular myocardium. But fine connective tissue septae per se will not significantly alter intercellular coupling or conduction velocity through the tissue unless they divide points of potential gap-junctional intercellular contact. Gap junctions form the low-resistance pathway for electrical impulse propagation between myocytes and occupy only a small proportion of the cell surface, so even a substantial increase in septation may have little effect on electrical coupling. Moreover, gap junctions and the associated adhering junctions incorporate very close and tightly bound apposition of the cell membranes of abutting myocytes and are unlikely to be separated passively by spreading connective tissue. We propose that a principal event underlying alterations in the rate and anisotropy of conduction during postnatal maturation is the redistribution of gap junctions predominantly to the end terminals of the cells demonstrated in the present study, with connective tissue changes being secondary and electrophysiologically incidental.
Relation Between Gap Junctions and Fascia Adherentes

The results of the present study demonstrate that the organization of intercellular mechanical interaction mediated by fasciae adherentes also undergoes dynamic changes in human infancy. The pattern of immunolabeling for N-cadherin, as seen at the level of confocal microscopy, was qualitatively similar to that of Cx43 in each of the specimens examined, showing the same overall progression to the adult distribution confined to intercalated disks at the end-on abutments between cells. Although thin section electron microscopy con-
firmed that at the cellular level a close association between these junction types generally existed in neonatal ventricular myocytes, there also appeared to be many examples of isolated gap-junctional contacts in the absence of adjacent adherens junctions. Furthermore, although most of the gap junctions seen were demonstrably between abutting myocytes, there were numerous examples of annular gap-junctional membranes that appeared to lie beneath convoluted lateral plasma membrane and that did not appear to link adjacent cells. Although some of these annular profiles may have arisen from the plane of section through undulating membrane, the frequency of these appearances suggests that, as previously described in developing mammalian hearts, 53, 54 at least some discrete cytoplasmic vesicles of gap-junctional membrane may exist within the myocytes of neonatal ventricular myocardium. Complementary ultrastructural examination of the tissue therefore revealed a more complex situation than might have been concluded from the confocal findings alone.

The demonstration in transfection studies of cultured tumor cells that coupling by gap junctions requires the presence of calcium-dependent cell adhesion molecules 10-12 has led to the suggestion that the close association of fascia adherens junctions with gap junctions that exists in the mature cardiac intercalated disk is a requirement for gap-junctional function in myocardium. 13 Although adherens junctions may promote gap-junctional formation simply by providing close membrane apposition, the association between junction types may have a more complex explanation. 55 Transfection of communication-deficient cells with cDNA encoding cell adhesion molecules has been shown to enhance phosphorylation of Cx43, thereby increasing gap-junctional conductance. 12 The finding that N-cadherin and Cx43 distributions were closely related and changed in parallel throughout the age range examined in the present study is consistent with the hypothesis that a close association is required. However, the electron-microscopic finding of morphologically distinct iso-

lated gap-junctional contacts distant from any adherens junctions suggests that the formation (as opposed to function) of a gap junction may not require immediate adjacency of fascia adherentes.

That the distribution of gap junctions in the transplant donor hearts was close to the line of best fit for the plot of distribution with age obtained from the hearts with congenital cardiac anomalies suggests that the latter were not associated with a gross disturbance of RV myocardial Cx43 gap-junction distribution. Although an intact gap-junctional communication system has been shown to be essential for normal development, tissue differentiation, and growth, 14 a disturbance of Cx43 gap-junctional expression and organization lasting into postnatal stages of development does not appear to play a role in the formation of these anomalies.

Development of the Intercalated Disk

Early electron-microscopic studies shed considerable light on the development of the intercalated disk in small mammals. 14 What was not evident, however, was the overall distribution of the adherens junctions and gap junctions over the surface of individual cells and through intact myocardium and how the distribution pattern of these junction changes postnatally. These aspects are uniquely revealed with the aid of confocal microscopy, as applied in the present study. Early findings based on thin section electron microscopy postulated that the mechanical tension of the increasing number of myofilaments inserting into the fasciae adherentes is the explanation for the predominantly transverse orientation of these junctions within the mature intercalated disk. 14 Moreover, fully formed intercalated disks were thought to be present in humans from birth. 14 As the present study demonstrates, coalescence of the specialized intercellular junction types into the mature intercalated disk does not in fact occur until about 6 years of age. As this is long after establishment of postnatal hemodynamics, and after most of the cardiac growth and alterations of myocardial mechanical loading have taken place, 29 it seems unlikely that the fascia adherens reorientation simply occurs secondarily to mechanical tension. Alteration of the junctional organization may serve a primary functional role; the ability of myocardium to adapt and remodel appropriately to the physiological changes of infancy while maintaining electrical and mechanical coupling may depend on an appropriate pattern of intercellular adhesion and communication in the early period of rapid growth, with subsequent change to the mature adult pattern in the intercalated disk.

Ventricular Growth

Many functional differences between neonatal and adult myocardium are directly related to the immaturity of neonatal tissue components, 46-59 a factor contributing to the limited ability of the neonatal heart to cope with various acute metabolic and hemodynamic stress es. 22, 60, 64 Although most of these systems rapidly mature in early postnatal life, ventricular mechanics in the infant remain different from those of the adult 27, 33-35, 62 and are more able to adapt appropriately to progressive changes in hemodynamics. 22, 28-31, 53, 64 such as those that accompany the physiological circulatory changes and substantial growth in this period.
Cardiac myocytes retain the capacity for mitotic division for about the first 6 months of postnatal life in humans, during which time hyperplasia accounts for a significant proportion of the ventricular growth. The cellular basis of myocardial growth, therefore, depends on age, but a longstanding and hitherto unanswered question is how the heart can maintain its overall geometry and pumping function while growing rapidly.

In 1974, Richter referred to the heart as a “topological manifold” in which changes in the surface area and configuration of any cardiac myocyte must affect the cell surface configurations of its neighbors. At that time, he made the largely correct assumption that the adaptive processes of myocardial growth are governed by intercellular information transfer via gap junctions. Richter postulated that although considerable configu-
rational changes occurred between individual myocytes, there were "invariants in the surface topology" of the specialized intercellular junctions, which would maintain the same position with respect to each other, the cell surface, and the surface of adjacent cells throughout growth. Thus, he postulated that gap junctions not only played an important part in intercellular communication governing patterns of growth, but also with this growth, they were the unvarying points of contact and coupling between cells. By demonstrating that the positions of the junctions change markedly with postnatal development, the present study also demonstrates that this postulate is incorrect. In terms of the "topological manifold," however, the infantile junctional pattern may be the means by which the massively growing heart, undergoing hyperplastic as well as hypertrophic enlargement, can maintain the required communicating and adhering contacts between the component myocytes despite the configurational changes.

**Potential Implications for the Timing of Surgery in Children**

The changing pattern of intercellular coupling and adhesion demonstrated in this study is likely to be associated with alterations in the functional anisotropy of the tissue, including the directional ease and rates of intercellular diffusion of the small molecules regulating growth, tissue differentiation, and maturation. The arrangement of communicating and adhering intercellular junctions that exists at birth may best fulfill the requirements of ventricular myocardium undergoing growth and adaptive remodeling, permitting the substantial and rapid changes in myocardial architecture during this period. Progressive change toward the arrangement in the adult ventricle throughout early childhood may not only best fulfill the more stable requirements of the older ventricle but may also render the myocardium less able to adapt appropriately to altered hemodynamics and may be of relevance to the timing of cardiac surgery. Corrective surgery for a variety of congenital cardiac anomalies, when performed early in infant or even neonatal life, may result in better long-term cardiac performance, both mechanical and electrical, than if delayed. Although this may, in part, be due to reducing the duration of exposure of the myocardium to the adverse hemodynamics before correction, it is likely the age-dependent reduction in the ability of myocardium to remodel is an important factor. Even when early preparative surgery is performed to improve the hemodynamic loading of cardiac chambers before a delayed definitive repair, thereby reducing but not eliminating the requirement for later myocardial adaptive remodeling, the long-term results from such two-stage repairs may not be as good as early immediate correction.

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