Distribution and Three-Dimensional Structure of Intercellular Junctions in Canine Myocardium

Robert H. Hoyt, Mark L. Cohen, and Jeffrey E. Saffitz

Electrotonic coupling of cardiac myocytes at gap junctions may influence patterns of conduction in myocardium. To delineate the three-dimensional structure and distribution of intercellular junctions, we analyzed serial ultrathin sections of canine myocardium with transmission electron microscopy and disaggregated myocytes with scanning electron microscopy. Morphometric analysis of left ventricular myocardium sectioned in three orthogonal planes revealed that 80% of total gap junctional membrane occurred in large, ribbon-like gap junctions oriented transversely at cell end processes. The remaining 20% of gap junctional membrane was contained in small gap junctions located within plicate segments (interdigitating regions of cell-to-cell adhesion) of intercalated disks. In serial ultrathin sections, all gap junctions were contiguous with plicate segments. Thus, true “lateral” gap junctions do not exist in working ventricular myocytes and would not likely be able to withstand shear forces created by laterally sliding cells. Examination of serial plastic sections with light microscopy revealed complex overlapping of myocytes such that individual myocytes were connected at intercalated disks to an average of 9.1 ± 2.2 other myocytes. These observations provide an improved understanding of the extent and distribution of cell junctions and should facilitate experimental and model studies of conduction in myocardium. (Circulation Research 1989;64:563–574)

Transfer of ionic currents between cardiac myocytes occurs at gap junctions. Recent studies suggest that the extent and packing geometry of intercellular junctions may influence complex propagation patterns occurring at the microscopic level.1,2 Although few model or experimental studies of cardiac conduction have sought to resolve electrical events in detail at the cellular level, achieving this level of resolution is likely to provide insights into mechanisms of normal and abnormal conduction. The ability to specifically account for the distribution of junctional resistances in models of myocardial conduction has been limited in part by a lack of detailed quantitative data describing the configuration of cellular junctions.3,4 Although selected features of myocyte structure and interconnection such as cell branching and the specific contributions of “lateral” gap junctions facilitating side-to-side transmission have been considered important determinants of normal conduction,5–9 these structural features have not been characterized with detailed anatomic studies. Accordingly, the purpose of the present study was to delineate the three-dimensional structure and distribution of intercellular junctions in canine myocardium with rigorous morphometric analysis.

Materials and Methods

Transmission Electron Microscopy of Canine Myocardium

Hearts of five dogs anesthetized with sodium thiopental, 20 mg/kg i.v., were arrested by injection of a saturated solution of potassium chloride in vivo. Tissue was fixed for electron microscopy by retrograde aortic perfusion with cold 2% glutaraldehyde, 1% paraformaldehyde, and 2 mM calcium chloride in 0.08 M sodium cacodylate buffer (pH 7.4) and subsequent immersion of thin epicardial strips in the same fixative. Subepicardium of the anterior left ventricular free wall was selected for quantitative morphologic studies because it is composed of compact working ventricular myocardium with coherent, reproducible fiber orientation and lacks the complex trabeculae and interstitial septae typical of atrial muscle or ventricular subendocar-
dium. Fiber orientation of fixed tissue was delineated with the use of a dissecting microscope. The visceral pericardium (epicardial surface) was removed, and the tissue was cut into blocks approximately 1.0×1.0×0.4 mm to permit sectioning in three orthogonal planes as follows: 1) a plane parallel to the long fiber axis and perpendicular to the epicardial surface, 2) a plane perpendicular to the long fiber axis and perpendicular to the epicardial surface, and 3) a plane parallel to the long fiber axis and parallel to the epicardium at a depth approximately 1 mm below the original epicardial surface. Tissue blocks were washed in 0.1 M sodium cacodylate buffer, postfixed in cold 1% osmium tetroxide, washed in 0.1 M sodium acetate buffer, stained en bloc with uranyl acetate, dehydrated in a series of alcohols, infiltrated with Spurr’s resin, and flat-embedded to ensure appropriate orientation of the blocks. This processing protocol has been shown to minimize artifacts of tissue shrinkage. Fiber orientation in each plane of section was verified with light microscopy of 0.5-μm thick sections. Blocks were trimmed to include only areas of longitudinally or transversely oriented fibers. Ultrathin sections for electron microscopy (10 per block) were collected on 200-mesh copper-rubidium grids and poststained with uranyl acetate and lead citrate. A total of 15 blocks were prepared in this manner (five dogs, three planes of section each). In selected blocks, 30-50 ultrathin serial sections (approximately 100 nm in thickness) were collected sequentially on formvar-coated slot grids. Sections were analyzed with a Philips EM-200 electron microscope (Philips Electronic Instruments, Mahweh, New Jersey) and photographed with Eastman 5302 fine grain release positive 35-mm film (Eastman Kodak Co, Rochester, New York). Additional blocks were processed for light microscopy with 1% potassium ferrocyanide, a mordant for osmium, to enhance staining of the sarcolemma and intercalated disks. A set of 42 serial longitudinal 2-μm thick plastic sections were cut parallel to the epicardial surface, collected sequentially, stained with toluidine blue, and examined with light microscopy. Myocyte profiles in each section were drawn with a camera lucida device and the drawings overlaid to reconstruct intercellular connections in three dimensions. The number of myocytes connected by intercalated disks to each of 10 randomly selected cells was determined by examining cells throughout the serial sections.

**Quantitative Analysis of Electron Micrographs**

Regions were selected for quantitative ultrastructural analysis of gap junctions according to the procedure of systematic random sampling described by Weibel. Briefly, the first entirely covered grid space in the upper left-hand corner of a technically satisfactory section in each block was identified, and a sample area from the upper left-hand corner of this grid space was photographed at a final print magnification of ×5,000. Analogous corner areas of the next four grid spaces selected left to right were photographed for a total of five ×5,000 micrographs per section. Results of a pilot study indicated that three micrographs were sufficient to minimize undersampling error. Then, areas contained in each ×5,000 sample micrograph (approximately 1,600 μm²) were examined carefully and all intercalated disks and gap junction profiles photographed in composite at a final print magnification of ×30,000. A total of approximately 4×10⁴ μm² of sample area was analyzed in each of the three planes in five animals.

The following structural components were delineated in quantitative transmission electron microscopic studies:

- **Junctional membrane**—a continuous region of specialized plasma membrane connecting two cardiac myocytes. Junctional membrane includes desmosomes (macula adherens), intermediate junctions (fascia adherens), undifferentiated regions, and gap junctions.
- **Intercalated disk**—a subset of junctional membrane containing transversely oriented interdigitating processes of adjacent myocytes.
- **Plicate segment**—a portion of an intercalated disk containing the finger-like interdigitating processes of cell adhesion. This component, named by Simpson et al., is equivalent to the "treads of a staircase" designation used by Fawcett and McNutt and others.
- **Interuplicate segment**—a portion of an intercalated disk situated adjacent to or between the plicate segments. Interuplicate segments lie in a plane parallel to the long myocyte axis and orthogonal to the plane of the plicate segments. The long axis of the interuplicate segments is oriented transverse to the long myocyte axis. The longitudinal extent of the interuplicate segments is in multiples of sarcormere length. This component is equivalent to the "risers of a staircase" described by Fawcett and McNutt. The interuplicate segment is much less convoluted than the plicate region and frequently contains a gap junction.
- **Gap junction**—a continuous region of junctional membrane characterized by narrow (20 nm) width and pentalaminar structure in uranyl acetate and lead citrate stained preparations. Slightly oblique segments of membrane continuous with the pentalaminar segments and having an electron-lucent background were considered to be gap junctional membrane. Such oblique segments could be shown to have a typical pentalaminar structure when sections were tilted with a goniometer stage.
- **Annular gap junction**—a closed circular profile of gap junctional membrane created by a plane of section through a curved gap junctional surface.
- **Oblique membrane**—a portion of the junctional membrane profile sectioned in such a plane that it cannot be reliably categorized as one of the four types of junctional membrane listed above.
- **Clear membrane**—total junctional membrane length minus oblique membrane length.
External sarcolemma—nonjunctional plasma membrane invariably lined by a glyocalyx. The external sarcolemma of two adjacent cells may be separated widely by a collagenous interstitial space or be more closely apposed (about 200 nm) to form a cleftlike structure near the intercalated disk region.

Intercalated disk and gap junction profile lengths were measured with an electronic stylus and a digitizing tablet (Houston Systems Inc, Austin, Texas). The test-retest error using this method was less than 1%. Digitized profile lengths were stored and analyzed on an IBM PC/AT computer. The following measurements were made: 1) external sarcolemmal length, 2) intercalated disk length, 3) oblique membrane length, 4) clear membrane length, 5) gap junction length, 6) corrected gap junction length (gap junction length divided by the ratio of clear to total junctional membrane), and 7) the folding factor of the intercalated disk in longitudinal planes of section, defined as the ratio of the convoluted length of the plicate portion of the disk profile and the length of an imaginary line, parallel to the Z lines, connecting the transverse limits of the plicate segment.

Morphometric data were expressed as the ratio of membrane profile length to section area. For comparison to other data in the literature, these values can be converted to gap junction surface density (SV_{ij}) values according to the formula:

\[
SV_{ij} = \frac{8 \times \text{Corrected gap junction length}}{\pi \times \text{Section area}}
\]

A multiplication factor of two was included to account for the equal contributions of two adjacent cells to the gap junction profile. The volume fraction of myocytes in whole tissue was determined in low power electron micrographs by a standard point counting method.

Scanning Electron Microscopy of Disaggregated Adult Canine Myocytes

Scanning electron microscopy was employed to delineate the surface topography of intercellular junctions and to relate the two-dimensional structures seen with transmission electron microscopy in ultrathin sections to three-dimensional scanning images of the intact cell surface. Hearts were rapidly excised from two additional anesthetized dogs, multiple 1.0×2.0×0.2 cm patches of anterior left ventricular subepicardium were excised and placed in Krebs-Henseleit buffer bubbled with 95% O₂/5% CO₂ (pH 7.4). The tissue patches were disaggregated into a uniform population of viable, elongated myocytes according to methods developed previously. Briefly, tissue was incubated in 10 mM HEPES buffer containing 10 μM Ca²⁺ and then in multiple changes of the same buffer containing 0.05% collagenase (Type II, Worthington Biochemical Corp, Freehold, New Jersey) and 0.03% pro tease (Type XIV, Sigma Chemical Co, St. Louis, Missouri). The final disaggregation solution of HEPES-collagenase contained a high proportion of elongated myocytes. The cells were pelletized by centrifugation at 50g for 3 minutes, resuspended in HEPES buffer, pH 7.2, containing 50 μM Ca²⁺ and 1.4% bovine serum albumin, and layered onto Percoll (Sigma) to which approximately 0.1 vol NaCl had been added for a final NaCl concentration of 0.9% and final pH of 7.3. Elongated cells were pelletized selectively by centrifugation at 700g for 8 minutes and resuspended in HEPES buffer containing 50 μM Ca²⁺. Viability of myocytes was verified by exclusion of 0.5% trypan blue and demonstration of resting membrane potentials of −85 to −90 mV measured in selected cells with glass microelectrodes. Purified disaggregated myocytes were fixed in cold 1% glutaraldehyde in 0.08 M sodium cacodylate buffer, pH 7.4, for 20 minutes under direct microscopic visualization. The cells were washed in cacodylate buffer, postfixed for 10 minutes in 1% osmium tetroxide, and rewashed in buffer. No changes in cell shape or size were detected during fixation. Some fixed washed cells were mounted on glass slides for light microscopic measurements of cell length and width. The remaining myocytes were dehydrated with increasing concentrations of ethanol, mounted on polystyrene stubs, critical point dried, and sputter coated with gold-palladium. The specimens were mounted on a tilting stage, examined in a JEOL JSM-35 scanning electron microscope at 10 kV, and junctional regions photographed at a magnification of ×30–30,000.

Statistical Analysis

Morphometric data were expressed as mean±SD. Morphometric data listed in Table 1 were tested by analysis of variance for repeated measures using the General Linear Models of SAS. A value of p≤0.05 after appropriate correction for multiple comparisons was considered to indicate a significant difference. A frequency-size distribution plot of profile lengths for gap junctions in the plicate segments was constructed.

Results

Transmission Electron Microscopy

In conventional longitudinal planes of section, we typically observed 0.5–2.0 μm long gap junction profiles in the plicate and interplicate segments of intercalated disks (Figure 1). In transverse planes of section, however, we observed gap junction profiles that were much larger than those seen in the longitudinal planes and which, heretofore, have not been fully characterized ultrastructurally. These gap junctions were as long as 8 μm (Figures 2–4) and, as shown in serial 100-nm sections, were located in interplicate segments of the intercalated disk (Figure 3). The long gap junctions were continuous with narrow clefts of the external sarcolemma that often
FIGURE 1. Conventional longitudinal plane of section through an intercalated disk showing relations between the external sarcolemma (ES), plicate segments (PS), interplicate segment (IS), and multiple gap junctions (arrowheads). Magnification, ×10,000; bar, 1 μm.

extended to an adjacent capillary. The longitudinal extent of the large gap junctions was 1.3±0.8 μm, and the transverse extent was 5.1±2.0 μm, corresponding to an approximate mean surface area of 6.6 μm².

Three-dimensional reconstructions of serial ultrathin sections showed that the large gap junctions were ribbon-shaped structures, one or two sarcomeres in longitudinal extent, situated transversely across and partly encircling end processes of myocytes in the interplicate segments of the intercalated disk. The long gap junction profiles in transverse sections were undulating, sinusoidal structures frequently curving over mitochondria (Figure 4). The transverse gap junction profiles usually made a series of obtuse angulations, but were often seen to bend at 90° or follow a linear course (Figures 2-4). In serial sections the course of the large gap junctions conformed to the contours of the adjacent plicate segment of the intercalated disk (Figure 3). Small gap junctions in plicate segments were of uniform size and disk shaped, with approximate diameter of 1.4 μm (determined as the minimum of the profile length distribution) corresponding to an approximate area of 1.5 μm².

At points where the intercalated disk reached the external sarcolemma, we often observed an overlapping lip of sarcolemma that contained a gap junction. The overlapping lip of sarcolemma effectively isolated the gap junction from the laterally adjacent cell (Figure 5) and typically contained numerous sarcolemmal invaginations. Annular gap junctions appeared to be the result of fortuitous transection of a curved surface or a bulbous projection from the surface of a large ribbon-like gap junction.

It was apparent from analysis of serial ultrathin sections that gap junctions appearing to form "lateral contacts" between ventricular myocytes in either transverse or longitudinal planes of section were, in fact, invariably associated with an adhesive plicate segment (Figure 3). Infrequently, small "lateral" gap junctions seen in longitudinal planes of section were continuous with diminutive plicate regions identified in serial sections (Figure 6). Nevertheless, the structural features characteristic of end-to-end junctions were present even in these small intercalated disks.

Morphometric Analysis of Intercellular Junctions

Morphometric analysis was performed on myocardial samples comprising 120,172 μm² of section area and containing 4,158 μm of junctional membrane length and 846 gap junction profiles. Results are listed in Table 1. Gap junctions comprised 15.1% of the total intercalated disk profile length (not including annular profiles). Of the total gap junction profile length, 70.1% was found to be in the
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FIGURE 2. Transverse section of canine myocardium showing a long gap junction profile (approximately 6 μm in length). The gap junction inserts at desmosomes (arrows) into clefts of the external sarcolemma (ES). Although the predominant course of the gap junction is linear, its length is augmented considerably by its sinusoidal configuration. Magnification, ×28,000; bar, 1 μm.

TABLE 1. Morphometric Analysis in Three Planes of Section of Intercalated Disks and Gap Junctions in Left Ventricular Myocardium

<table>
<thead>
<tr>
<th>Plane of section</th>
<th>Longitudinal perpendicular to epicardium</th>
<th>Transverse perpendicular to epicardium</th>
<th>Longitudinal parallel to epicardium</th>
<th>Average of three planes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphometric parameter</td>
<td>71.8±15.4</td>
<td>63.4±10.0</td>
<td>77.0±15.2</td>
<td>70.1±11.3</td>
</tr>
<tr>
<td>Proportion of total gap junction length (%) in intercalated disk regions</td>
<td>15.9±4.6</td>
<td>26.4±14.5</td>
<td>14.5±8.7</td>
<td>19.7±6.9</td>
</tr>
<tr>
<td>Intercalated disk length (μm×10⁻²) per μm² section area</td>
<td>12.4±14.4</td>
<td>10.2±4.4</td>
<td>8.4±4.4</td>
<td>10.2±6.7</td>
</tr>
<tr>
<td>Folding factor of intercalated disk length</td>
<td>2.4±0.1</td>
<td>. . .</td>
<td>2.3±0.2</td>
<td>. . .</td>
</tr>
</tbody>
</table>

* p≤0.05.
interpacite segments of intercalated disk regions (including large gap junction profiles), 19.7% was observed in the plicate segments, and 10.2% occurred in annular gap junctions. Gap junctions in plicate regions were numerous, but because each had an approximate surface area of only 1.5 \( \mu \text{m}^2 \), their aggregate length was modest. The total length and number of gap junctional membrane profiles were greater in transverse than longitudinal planes of section (Table 1). In addition, the longest gap junction profiles were encountered in the transverse plane. The distribution of gap junctional profiles in the two longitudinal planes of section was similar. The folding factor of the plicate segments measured in 10 samples (two longitudinal planes of section in each of five dogs) was 2.3±0.2 \( \mu \text{m}/\mu \text{m} \). The volume fraction of myocytes in myocardium was 0.92±0.02 \( \mu \text{m}^2/\mu \text{m}^3 \), and the surface density of external sarcolemma was 0.26±0.06 \( \mu \text{m}^2/\mu \text{m}^3 \). These data are consistent with previously published values.\textsuperscript{19,20}

**Scanning Electron Microscopy**

As viewed with the scanning electron microscope, intact disaggregated canine ventricular myo-

\[ \text{FIGURE 3. Transverse sections selected from a series of 30 consecutive sections (each approximately 100 nm thick) through an intercalated disk. Upper left panel (section 7): A long gap junction profile, delineated by the open arrows, is situated between plicate segments 1 and 2. A gap junction between a second pair of adjacent cells is indicated by the solid arrow. Upper right panel (section 15): The open arrows delineate the edge of plicate segment 4 and a contiguous segment of sarcolemma that have contours and location identical to the long gap junction shown in the previous panel. Arrowheads indicate the left and lower margins of plicate segment 4. Plicate segment 5 has replaced the gap junction identified by the solid arrow in the previous panel. Lower left panel (section 22): Arrowheads indicate a gap junction folded at a 90° angle and situated at the corresponding left and lower edges of plicate segment 4 shown in the previous panel. The cross denotes the position of plicate segment 6 which appears subsequently in the lower right panel (section 29). Magnification (each panel), x5,000; bar, 2 \( \mu \text{m} \).} \]
FIGURE 4. Upper panel: Scanning electron micrograph of the intercalated disk of an isolated myocyte viewed end-on. Note the fingerlike projections of plicae and the characteristic folded contour (between arrows) of the interplicate segment (step-off) between two offset levels of adjacent plicate regions. Magnification, ×4,000; bar, 1.0 μm. Lower panel: Transmission micrograph in transverse plane of section of intact myocardium through an intercalated disk at the level of the interplicate segment. An end process from an adjacent plicate segment is apparent in the upper left hand corner. Although they are from different cells the large gap junction (between arrows) in the lower panel has a folded contour closely resembling that of the interplicate segment in the upper panel. Magnification, ×5,000; bar, 1 μm.

cytes were elongated, multi-ended, generally non-branching cells (Figure 7). Columnar end processes arose from the central cell body and were seen to subdivide into subsegments offset from one another by multiples of sarcomere length. At higher magnification, it was apparent that each subsegment was capped by a highly convoluted plicate membrane delineated by a rounded or angular contour. Situated between the subsegments was a narrow shelf region, equivalent to the interplicate segment, that formed the base of the adjacent plicate segment. The location and folded shape of this shelf region indicated that it corresponded to the site of the two-dimensional profiles of long gap junctions observed in transverse sections (Figures 4 and 8). Transmission electron microscopic studies of disaggregated canine myocytes showed only partially intact patches of gap junctional membrane indicating, as has been previously reported,\textsuperscript{21} that the junction is usually damaged during separation of cells. High magnification views of the shelf region characteristically revealed smooth elongated membrane patches and filamentous attachment sites, presumably representing remnants of the cytoplasmic or extracellular faces of the long gap junctions found in the interplicate segments of intercalated disks (Figure 8).

**Light Microscopy**

The length of disaggregated myocytes was 124 ± 24 μm and the width was 21 ± 6 μm (n = 50). The extent to which myocytes were interconnected via intercalated disks was determined quantitatively by analyzing camera lucida drawings of selected regions of tissue in serial 2 μm thick plastic sections cut parallel to the epicardial surface. Tissue had been processed with 1% potassium ferrocyanide to enhance staining of junctional regions. Plicate seg-
ments of intercalated disks were readily identified with light microscopy (Figure 9). Analysis of 10 randomly selected individual myocytes followed in serial sections revealed that each myocyte was connected via intercalated disks to \(9.1 \pm 2.2\) other myocytes (Figure 9). We did not observe unit bundle cell configurations in subepicardial ventricular myocardium as has been described in an endocardial strand of guinea pig cardiac muscle. 22

Discussion

In this study we have built on previous investigations of myocyte structure 13,23–27 to delineate the three-dimensional fine structure of the intercalated disk. Results of quantitative morphometric analysis of gap junction densities in compact left ventricular muscle indicate the following: 1) Cardiac myocytes are connected to one another primarily by extremely large gap junctions (up to \(8 \mu\)m in length) oriented transversely across cellular end processes at intercalated disks. These gap junctions have an approximate mean area of \(6.6 \mu\)m\(^2\) and some are \(\geq 16 \mu\)m\(^2\) in area. Although large gap junctions and their arrangements have been described in ventricular muscle, 23,25,28,29 their three-dimensional structure and distribution along with the implications of their distribution have not been characterized previously with the detail provided in this investigation. The long axes of these gap junctions lie across and partly encircle the cell body. Thus, in conventional longitudinal section planes, the large gap junctions are seen in interplicate segments and usually have a profile length of one or two sarcomeres (equivalent to the longitudinal extent of the ribbon-like gap junctions seen in transverse sections). The large gap junctions in the interplicate segments of the intercalated disk (including annular profiles) account for 80% of total gap junction profile length. Because these gap junctions have a large surface area in close proximity to local circuit currents at the external sarcolemma, it is likely that they are preferential sites of electrotonic coupling of myocytes. Small gap junctions contained in the plicate segments comprise only 20% of total gap junction profile length although they account for approximately 50% of the number of individual gap junction profiles. 2) Gap junctions have an anisotropic spatial distribution. Gap junction length per unit section area is 30–40% greater in transverse than in longitudinal planes of section. This difference is likely related to the transverse orientation of individual gap junctions and their frequency of occurrence along the cell body. This finding, not previously reported, is consistent with the high degree of anisotropy of other oriented membrane structures encountered in muscle 16 and must be taken into account in stereologic analysis of myocardium. 3) The distribution of gap junctions about the lon-
FIGURE 6. Longitudinal sections selected from a series of 34 consecutive ultrathin sections. Upper panel (section 11): An apparent small lateral gap junction is denoted by the arrow (ES, external sarcolemma). Center panel (section 15): The gap junction (above) is attached to a desmosome (arrow head). A second lateral gap junction (arrow) is apparent. Lower panel (section 18): A small plicate adhesive segment (arrowhead), now evident, is continuous with the second gap junction (arrow). Such attenuated intercalated disks occur occasionally along an end process of a myocyte but in serial sections are not found to be structurally distinct "lateral" junctions. Magnification (each panel), ×15,000; bar, 1 μm.

gittal cell axis is radially symmetric, as shown by the similar morphometric measurements in two orthogonal longitudinal planes.

The morphometric data permit calculation of the cell surface distribution of gap junctions in canine myocytes. Because the dimensions of disaggregated canine myocytes are slightly larger than the rat myocytes studied by Nash et al,9 and considering the data of Bishop and Drummond24 as well as the flattened shape of myocytes demonstrated by Nas-sar et al32 (also shown in Figure 4), we have estimated the volume of individual subepicardial canine myocytes to be approximately 20,000 μm³. The myocyte volume fraction of 0.92 μm³/μm³ measured in whole tissue indicates a cell density of 46,000 myocytes/mm³. Based on a surface density of external sarcolemma of 0.26 μm²/μm³ (see "Results") the external sarcolemmal surface area of a typical myocyte is 5,652 μm² (not including T tubules, intercalated disks, caveolae, or other surface invaginations). By averaging the morphometric data for three planes of section in Table 1, the intercalated disk area per cell is calculated to be 1,827 μm² or 24% of the total exterior cell surface. Gap junction area is 388 μm² or 5.2% of the exterior cell surface area. Twenty percent of gap junction area per cell, or 78 μm², consists of approximately 52 disk-shaped plicate segment gap junctions each about 1.5 μm² in area. The remaining gap junction area of 310 μm² is accounted for by approximately 47 large gap junctions (each averaging 6.6 μm² in area) located in interplicate segments. Thus, there are about 99 gap junctions per cell. This value is somewhat greater than that estimated for bullfrog atrial myocytes by Haas et al33 although many other features of canine and frog myocyte structure are disparate.

Observations in serial longitudinal 2-μm thick plastic sections indicate that an individual cardiac myocyte is connected via one or more intercalated disks to 9.1 ±2.2 other myocytes. This complexity of cell apposition and interconnection is likely to influence patterns of conduction in myocardial tissue. Clerc5 considered cell branching to be crucial in transverse conduction between myocytes, but we and others have shown that normal cardiac myocytes do not usually bifurcate into major branches.34 Rather, a multiplicity of overlapping end processes distributed at various points along the myocyte cell body provide multiple input and output points at gap junctions with varying cytoplasmic path lengths between these points.

Previous reports based on assessment of two-dimensional gap junction profiles have described "lateral" gap junctions (similar to those in Figures 2 and 6, upper panel)9 considered to be sites of side-to-side electrical coupling. In the present study, results of analysis of serial ultrathin sections through individual intercalated discs show that apparent lateral gap junctions seen in two dimensions invariably connected to a plicate segment when followed in the third dimension (Figures 3 and 6). This observation indicates that even when cell bodies lie side-by-side they are connected by gap junctions in
overlapping columnar end processes of cytoplasm. Although Purkinje strands or atrial muscle may have lateral contacts,6–9 our observations indicate that true lateral gap junctions connecting adjacent working ventricular myocytes at sites separate from adhesive plicate regions do not exist. The proximity of adhesive plicate regions to all gap junctions underscores the intimate relationship between mechanical and electrical coupling in myocytes. Because of the extremely dense packing and cell-to-cell bridging of connexon proteins, gap junction membranes are stiff and, in contractile myocardium, would appear to require the mechanical stabilization afforded by adjacent plicate segments. True “lateral” gap junctions would not likely be able to withstand the shear forces between laterally sliding myocytes during contraction. Shear deformation is the ratio of lateral displacement between two points (connexons) lying in parallel planes, to the vertical separation between the planes. Hence, the narrow longitudinal extent and transverse orientation of the large gap junctions in interplicate

![Figure 7](image1)

**FIGURE 7.** Low magnification (×860) scanning electron micrograph of an isolated adult canine myocyte. The cell has multiple end processes (one of which is indicated by EP) along the cell body which subdivide to form intercalated disks (ID), each capped by fingerlike projections of plicate segments (arrows).

![Figure 8](image2)

**FIGURE 8.** Left panel: Scanning electron micrograph of one end of an isolated cardiac myocyte showing the three-dimensional topography of the intercalated disk region (×3,000). The arrow demonstrates the location of an apparent gap junction remnant on the cell surface. Right panel: Drawing to scale of the intercalated disk region shown in the left panel. The distribution and location of transversely oriented large ribbon-like gap junctions (stippled patches) are based on three-dimensional analysis of ultrathin sections of canine myocardium.
segments are features that minimize deformation with sarcomere shortening. The overlapping nature of myocyte appositions was readily apparent in serial plastic sections studied by light microscopy. Although the most extensive intercalated disks are distributed in the terminal 20% of overall cell length, a typical cell "end" may lie in the central portion of the cell body. Thus, the complex interconnections of cardiac myocytes are not well represented by regularly spaced cylinders packed end-to-end and side-to-side, a simplification frequently employed in model studies. The relation of the overall pattern of conduction to microscopic transjunctional fields is complex because cells overlap and are not strictly longitudinally or transversely situated with respect to each other. Because local potential gradients ultimately determine ion movement through the gap junctions, individual junctions may subserve either axial or lateral propagation, or perhaps not conduct at all, depending on the shape of local field potentials along a wavefront of depolarization. Thus, local transverse ion movement could occur during global longitudinal conduction. In short, the concept of a distinct functional subclass of "lateral" gap junctions is not substantiated by the anatomic data. This conclusion is supported by the data of Wittenberg et al., who found no major differences in gap junctional conductance between cell pairs coupled in side-to-side or end-to-end configurations.

Our quantitative results on the structure and distribution of cell connections suggest that two- or three-dimensional simulations of coupling resistance at a microscopic level will, of necessity, be highly complex. It will be essential to consider the arrangement of gap junctions at the intercalated disk and distribution of interconnections along the cell body, as well as the orientation of myocytes with respect to the direction of propagation.

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References


**KEY WORDS** • gap junction • intercalated disk • myocardium
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