Downregulation of Immunodetectable Connexin43 and Decreased Gap Junction Size in the Pathogenesis of Chronic Hibernation in the Human Left Ventricle

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Background—The regional wall motion impairment and predisposition to arrhythmias in human ventricular hibernation may plausibly result from abnormal intercellular propagation of the depolarizing wave front. This study investigated the hypothesis that altered patterns of expression of connexin43, the principal gap junctional protein responsible for passive conduction of the cardiac action potential, contribute to the pathogenesis of hibernation.

Methods and Results—Patients with poor ventricular function and severe coronary artery disease underwent thallium scanning and MRI to predict regions of normally perfused, reversibly ischemic, or hibernating myocardium. Twenty-one patients went on to coronary artery bypass graft surgery, during which biopsies representative of each of the above classes were taken. Hibernation was confirmed by improvement in segmental wall motion at reassessment 6 months after surgery. Connexin43 was studied by quantitative immunoconfocal laser scanning microscopy and PC image software. Analysis of en face projection views of intercalated disks revealed a significant reduction in relative connexin43 content per unit area in reversibly ischemic (76.7±34.6%, P<.001) and hibernating (67.4±24.3%, P<.001) tissue compared with normal (100±30.3%); ANOVA P<.001. The hibernating regions were further characterized by loss of the larger gap junctions normally seen at the disk periphery, reflected by a significant reduction in mean junctional plaque size in the hibernating tissues (69.5±20.8%) compared with reversibly ischemic (87.4±31.2%, P=.012) and normal (100±31.5%, P<.001) segments; ANOVA P<.001.

Conclusions—These results indicate progressive reduction and disruption of connexin43 gap junctions in reversible ischemia and hibernation. Abnormal impulse propagation resulting from such changes may contribute to the electromechanical dysfunction associated with hibernation. (Circulation. 1998;97:651-660.)

Key Words: junctions, gap myocardial contraction hibernation

The term “hibernating myocardium” describes a state of persistently impaired myocardial function at rest due to severely reduced coronary blood flow that is reversible after successful revascularization.1,2 Previous studies on pathophysiological mechanisms in hibernating human tissue have focused on the identification of pathognomonic myocardial structural changes. Histological and electron microscopic examination of biopsy material obtained from hibernating regions reveal a variable degree of fibrous tissue replacement, with a proportion of the myocytes showing depleted contractile material, numerous and small mitochondria, and irregular nuclear envelopes.3,4 Although early studies suggested that such altered myocytes characterized the hibernating state3 and could explain the delay in recovery in contractile function after successful revascularization,5 more recent reports have described them in equal frequency in both hibernating and nonhibernating ventricular segments.6,7 Similar cellular features have also been described in ventricular specimens from patients with dilated, nonischemic cardiomyopathy7 as well as in rat cardiac myocytes after a short period of unloading.8 It can therefore be hypothesized that these are secondary features of cells contracting against a low afterload and are not characteristic of hibernation.

Two PET-based studies have shown that the prognosis in medically treated patients with ischemic heart disease and impaired ventricular function is poorer when there is additional evidence of hibernating myocardium, but is significantly improved by successful revascularization.9,10 The majority of deaths in these studies were sudden, possibly implicating...
arrhythmias as being a more common feature in the presence of myocardial hibernation. This combination of segmental wall motion abnormalities and clinically important ventricular arrhythmias could plausibly result from severe disruption in conduction of the depolarizing wave front, leading to asynchronous contraction of individual myocytes and predisposing to reentry arrhythmias. Coordination of the electrical and mechanical events depends on both intercellular passive resistivity and active membrane ionic properties, which together determine propagation of the action potential and recovery of excitability. Gap junctions, clusters of transmembrane channels at the intercalated disk, form the passive low-resistance intercellular pathways. The constituent proteins of gap junctions are connexins, of which connexin43 is the predominant isofrom in working human ventricular myocardium. Disruptions of the distribution and tissue content of connexin43 gap junctions leading to impaired intercellular conduction have previously been reported to predispose to reentry arrhythmias in infarct border zones and in reversibly ischemic myocardium.

In this study, we investigated the hypothesis that progressive disruption in myocardial gap junctional organization during the transition from normal to reversibly ischemic to hibernating states may underlie wall motion abnormalities and increasing arrhythmogenicity. Connexin43 gap junction distribution was compared in documented hibernating, reversibly ischemic, and normally perfused segmental biopsies from patients by quantitative immunofluorescent microscopy.

Methods

Patient Selection

Principal entry criteria for the study included a history of previous myocardial infarction and subsequent established left ventricular dysfunction with dyspnea as the predominant symptom for at least 6 months. All patients had angiographic three-vessel coronary artery disease (defined as >70% luminal diameter stenosis) and left ventricular ejection fraction <35%. Patients with significant valvular disease, uncontrolled atrial fibrillation, permanent pacemaker in situ, or uncontrolled hypertension were excluded.

Surgery was indicated on clinical grounds for each individual patient; this was either for improvement of symptomatology (dyspnea and/or angina) or improvement in prognosis.

Imaging

Before surgery, all patients underwent stress/redistribution and separate-day rest/redistribution 201TI single photon emission CT. The stress procedure was performed by infusion of adenosine 140 μg · kg⁻¹ · min⁻¹ coupled with 25 to 75 W of ergometer exercise for 6 minutes. For both rest and stress studies, 80 MBq of thallium was injected, and images were acquired immediately and again after 4 hours of redistribution with an IGE Optima dual-headed gamma camera. Data were processed with REMP–Hanning filtering, and transaxial tomograms were reoriented into the vertical and horizontal long-axis and short-axis planes. Scanning was repeated after surgery to confirm successful revascularization. MRI was performed with a 1.5-T system (HPQ, Picker International Inc.). Cine gradient echo images were obtained in the vertical and horizontal long-axis planes and in the basal and apical short-axis planes. Preoperative images were acquired at rest and during infusion of dobutamine 5 to 10 μg · kg⁻¹ · min⁻¹. After surgery, only resting images were acquired. Scans were conducted <3 months before CABG and between 3 and 6 months (mean, 4.8 months) after surgery. Images were analyzed by two independent observers for the thallium studies and by two others for the MRI. Single photon emission CT images were graded for thallium uptake on a 5-point scale (4 = normal, 3 = slightly reduced, 2 = moderately reduced, 1 = severely reduced, 0 = absent). Wall motion on cine-MRI was also graded on a 5-point scale (4 = normal, 3 = mild hypokinesia, 2 = severe hypokinesia, 1 = akinesia, 0 = dyskinesia). Coronary territories were assigned as anterior, septum and apex to the left anterior descending coronary artery, lateral to the circumflex artery, and inferior to the right or circumflex artery depending on dominance. A nine-segment left ventricular model (basal and apical parts of the septum, anterior, lateral, and inferior walls, together with the apex) was used for segmental classification as follows: The normally perfused classification was defined as preoperative MRI wall motion grade ≥2 and thallium uptake grade 4. Reversibly ischemic was preoperative MRI wall motion grade ≥2 and improvement in thallium uptake by at least one grade on stress/redistribution images. These segments did not show any improvement in contractile characteristics after successful revascularization. Hibernating was defined as preoperative MRI wall motion grade ≤1 and improvement in contractation of at least one grade recorded with postoperative MRI. (Late rest/redistribution thallium uptake grade ≥2 and/or improvement by at least one wall motion grade with low-dose dobutamine infusion was used to predict hibernation before surgery, directing biopsy sites). Infarcted myocardium showed preoperative MRI wall motion grade ≤1 and late rest/redistribution thallium uptake ≤1, with no recovery of wall motion after surgery.

Twenty-one patients with more than two of nine ventricular regions provisionally classified as hibernating (likely to improve in function with revascularization) proceeded to CABG. Per-operative transmural biopsies representing each of the four classes were taken from each patient with a metal cork-borer of 2-mm ID; the surgeon was directed by a map of the nine-segment left ventricular model described. This study was approved by the local Ethics Committee, and all patients gave written consent using a specifically designed consent form.

Immunofluorescence Microscopy

Sections (10 μm) on polylysine-coated slides were dewaxed with xylene and rehydrated. Microwave treatments in different buffers and 0.1% trypsin were used for retrieval of antigenic sites masked by fixation were compared. Optimal antigen detection was found with 7 minutes of microwave oven treatment in 0.01% sodium bicarbonate at pH 7.4. Sections were blocked with 0.1% triton X-100 for 45 minutes before overnight incubation with mouse monoclonal anti-connexin43 antibody (Chemicon International Ltd) at dilution 1:1000 in 0.5% BSA in PBS at room temperature. The detection system used was biotinylated antibody/streptavidin Texas red. Image analysis was performed with the aid of Wild Heerbrugg microscope. Immunolabeling run; controls in which the primary antibody was replaced with normal mouse sera against synthetic peptides (residues 254 to 268 of rat connexin40).
proximate junctions, the binary image was further edited to separate labeled disks were inspected and compared with the corresponding easily identifiable autofluorescent lipofuscin. The binary images of the outlines, and the image was then edited by hand to eliminate spots of on a 255-point gray scale were adjusted to reduce any background cell transferred in binary format for analysis by PC image software (Foster Findlay Associates). Individual complete disks in each image were went high-pass filtering, which increased image contrast, and then was improve image quality, and the entire series was projected as a single optical sections. Each image was signal-averaged during acquisition to record images taken as a series at set intervals to ensure acquisition through the depth of tissue containing the complete disk was recorded. Multiple images taken as a series at set intervals; residues 354 to 367 of human connexin43) raised in rabbit were used for this purpose. The procedure was similar to that for connexin43, except that 0.1% trypsin was used for antigen rescue. Connexin40 antibody was diluted to 1:1000 and connexin45 to 1:50; secondary detection was with anti-rabbit Cy3 conjugate (Chemicon International Ltd). Immunostained tissue was coded and examined blind with conventional epifluorescence and subsequently confocal microscopy with a Leica TCS 4D equipped with an argon/krypton laser with the appropriate filter blocks for the fluorescent signal. All images were recorded within 48 hours of labeling with single-channel scanning.

Techniques for Quantification
Quantification of connexin43 content was performed blind on tissue finally classified as normally perfused, reversibly ischemic, and hibernating by measurement of the fluorescent label in individual intercalated disks from six fields selected by random scanning across each tissue section. En face disks seen in transversely sectioned tissue were used, because in this view, the overlap of individual gap junctions is minimized. The ×63 objective with a detector pinhole of 90 μm gave an optical section thickness of 0.5 μm on theoretical grounds. A zoom factor of 2.96 with a 512×512-pixel image was selected, yielding a pixel size of 0.1×0.1 μm, allowing proportionate representation of the smallest junctions (1 μm) detectable with this technique. Because all of the fluorescence associated with an intercalated disk was rarely seen completely in a single optical section, a series of confocal images through the depth of tissue containing the complete disk was recorded. Images were taken at 0.5-μm intervals to ensure acquisition of all gap junction label with minimal overlap between adjacent optical sections. Each image was signal-averaged during acquisition to improve image quality, and the entire series was projected as a single composite image by superimposition (Fig 1). This final image underwent high-pass filtering, which increased image contrast, and then was transferred in binary format for analysis by PC image software (Foster Findlay Associates). Individual complete disks in each image were outlined by hand for separate quantification. Thresholds for detection on a 255-point gray scale were adjusted to reduce any background cell outlines, and the image was then edited by hand to eliminate spots of easily identifiable autofluorescent lipofuscin. The binary images of the labeled disks were inspected and compared with the corresponding projection images to ensure that clearly separated juxtaposed junctions were not contiguous. If such artificial merging occurred between proximate junctions, the binary image was further edited to separate them. The total remaining “on” pixels were then automatically counted to yield the total number of gap junctions within the disk, the sizes of these individual junctions, and the total area of the disk itself. The mean gap junction size in square micrometers (total area of junctions/number of junctions in disk) and the disk content of junctions (total area of junctions/disk area) were calculated. Because there was variability in the absolute label between immunofluorescence runs, results were expressed as a percentage relative to the normally perfused tissue acting as internal normal controls for each patient.

Data obtained were finally pooled into the three clinical groups (normally perfused, reversibly ischemic, and hibernating myocardium) to obtain a distribution plot of results for each group and for subsequent statistical analysis using ANOVA. Secondary analysis was performed between the three groups by use of Bonferroni’s correction to Student’s t test. Data are expressed as mean±SD; secondary t test significance is expressed as P.

Results
Patients
Twenty-one patients were selected for the study: 91% male; median age, 61 years (range, 40 to 71 years); mean NYHA grade, 2.8; mean ejection fraction, 23%. Of the 21 patients initially biopsied, full follow-up investigations were completed in 15, allowing final classification of tissue. Four patients did not survive to follow-up, and the remaining 2 withdrew from the study. The demographic and clinical details of the 15 patients are shown in Table 1.

General Histology and Ultrastructure
Ventricular biopsies from all four clinical groups showed variable degrees of fibrosis, occurring both in the interstitial spaces and as separate islands of connective tissue representing local infarction. Tissue classified as infarcted showed extensive fibrous tissue replacement. The myocytes themselves varied from a normal appearance at light microscopy to pathological cells severely depleted of myofibrillar proteins (Fig 2). These cells showed evidence of glycogen accumulation by periodic acid–Schiff staining of histological sections (not shown). On transmission electron microscopy, the pathological cells revealed consistent features of reduced myofibrillar content, glycogen accumulation, numerous and small mitochondria, and irregular nuclear envelopes (Fig 3).

Distribution of Connexin Label
Low-power views of connexin43 immunolabeling revealed a normal distribution of label in the tissue away from zones of scarring, the fluorescence being confined to typical intercalated disks (Fig 4). In infarcted tissue, there was disruption of this normal pattern, as reported previously, with degenerative myocytes partially embedded in scar tissue having gap junctions distributed widely over their surface membranes. Reversibly ischemic, hibernating, and normally perfused (acting as internal normal control tissue) biopsies were examined in greater detail at higher magnification in tissue distant from such disrupted zones to determine connexin43 distribution in individual intercalated disks. Disks seen en face in transversely sectioned areas of tissue showed that in the control tissues, there was a normal distribution of gap junction label, with small central gap junctions surrounded by extensive larger spots of label in the disk periphery (Fig 5A). Disks from reversibly ischemic biopsies had a similar pattern of connexin43 labeling.
but demonstrated a decrease in the amount of label compared with the control disks in the same staining run (Fig 5B). The hibernating disks also showed a diminution of label, although in this case there was a striking loss of the larger peripheral gap junctions (Fig 5C). Endocardial and epicardial areas of clearly oriented full-thickness biopsies were compared to investigate any differences that might reflect the recognized variation in perfusion and degree of scarring present in these two areas, but none were apparent. There were no immunodetectable changes in other connexin isoforms in the samples examined; connexin40, present in arteriolar endothelium, was not detected in myocytes, and connexin45, which is expressed only in very low amounts in the heart, was found not to be detectable in fixed specimens with the antibody used. Label was absent in the controls in which the primary antibody was omitted. Tissue autofluorescence was low after microwave treatment, and highly fluorescent lipofuscin granules ubiquitous in human tissue could easily be distinguished from specific label.

Transmission electron microscopy of representative intercalated disks from each tissue class revealed preservation of the basic ultrastructure of the disk; desmosomes and fasciae adherentes occupied the transverse portions of the disks, with gap junctions situated in the zones parallel to the long axis of the cells (Fig 6). This structure was maintained regardless of the degree of loss of contractile material in a given myocyte. There was no evidence of gap junction internalization at the disk margin or of isolated junctions in nondisk membrane. These findings were consistent with the described pattern of connexin43 immunolabeling being confined to the intercalated disks in areas distant from infarcts.

Quantification

Disk Gap Junctional Density

Differences in the extent of labeling were observed between individual immunolabeling runs, with values of percentage gap junction area per unit area of disk in the control biopsies varying between 10.4% and 13.0% (Table 2). Overall, however, there were consistent patterns in the measurements

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>EF, %</th>
<th>NYHA Class</th>
<th>Infarct Location</th>
<th>Drugs</th>
<th>Biopsies</th>
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<tr>
<td>1</td>
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<td>57</td>
<td>15</td>
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<td>ACE,D,Nit</td>
<td>MI,Hib,Hib</td>
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<td>35</td>
<td>2</td>
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<td>ACE,D,Nit</td>
<td>RI,Nor,Hib,Hib</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>60</td>
<td>15</td>
<td>3</td>
<td>Anterior</td>
<td>ACE,D,Nit</td>
<td>Hib,Hib,Mi,Hib</td>
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<tr>
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<td>ACE</td>
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<tr>
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<td>M</td>
<td>57</td>
<td>12</td>
<td>3</td>
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<td>ACE,D,Nit</td>
<td>Hib,Hib</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>40</td>
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<td>2</td>
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<td>ACE,Nit</td>
<td>Hib,Hib,Hib,Hib</td>
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<td>9</td>
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<td>64</td>
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<tr>
<td>10</td>
<td>M</td>
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<td>11</td>
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<tr>
<td>12</td>
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<td>66</td>
<td>35</td>
<td>2</td>
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<tr>
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<tr>
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<td>M</td>
<td>60</td>
<td>24</td>
<td>3</td>
<td>Inf-apical</td>
<td>ACE,D,Nit</td>
<td>Mi,Hib,Ri,Ri</td>
</tr>
</tbody>
</table>

EF indicates ejection fraction; Infarct Location, site of previous documented myocardial infarction; Ant, anterior; Inf, inferior; Drugs, patient medication (D, diuretic; Nit, nitrate, and Dig, digoxin); Biopsies, classification of biopsies (Mi, infarcted; Hib, hibernating; Nor, normally perfused; and Ri, reversibly ischemic).
between the three groups. When expressed as relative values, mean gap junction density per unit area of disk was significantly reduced compared with control (100±30.3%) in both reversibly ischemic (76.7±34.6%; \( P < .001 \)) and hibernating (67.4±24.3%; \( P < .001 \); ANOVA \( P < .001 \)) tissues. These last values were not significantly different from one another on secondary analysis (\( P = .069 \)) (Fig 7). The findings confirmed the visual impressions described above.

**Size of Immunolabeled Gap Junctions**

The mean gap junction size of control tissue was 0.21±0.06 \( \mu \text{m}^2 \) (Table 2), yielding a mean gap junctional diameter of 0.53 \( \mu \text{m} \) (assuming a circular structure to a single gap junction). If mean junctional size of the diseased tissues was expressed as percentage relative to control, there was a significant reduction in size to 87.4±31.2\% (\( P < .001 \)) in reversibly ischemic tissue, and a further significant reduction in hibernating...
tion to 69.5±20.8% (P = .012; ANOVA < .001). This last value reflects the loss of peripheral large junctions observed on examination of the disks from the hibernating tissue, with maintenance of the frequency distributions of area measurements of the smaller-size individual junctions from the different tissue classes (Fig 8).

Discussion
Evidence implicating alterations in connexin43 gap junctions in arrhythmogenesis in different models of human heart disease has steadily accumulated.20–22,26,29,30 The novel findings of the present study are that (1) a reduction in connexin43 gap junctions occurs in hibernating myocardium beyond that seen in reversible ischemia, and (2) a specific feature characterizing hibernating myocardium is a loss of the population of large gap junctions at the disk periphery. These changes seen by laser scanning immunoconfocal microscopy were subsequently confirmed by quantification with PC image analysis. The use of quantitative immunofluorescence for measurement of gap junction size has been previously validated with a polyclonal anti-connexin43 antibody in rat left ventricular tissue by comparison of measurements from immunoconfocal (0.53 μm) and freeze-fracture (0.57 μm) electron microscopy.28 In the present study, quantification of images obtained with a different primary antibody yielded a mean gap junctional diameter of 0.53 μm in control tissue, in accordance with the published data, indicating that the technique reflects true structural measurements. We elected to study connexin43 content in individual intercalated disks. Previous work has shown a lack of significant changes in intercalated disk number per myocyte in ischemic human myocardium,22 indicating that the density of gap junctions in the existing disks closely reflects the tissue content of connexin43 in the ventricle as a whole.

In a study of this type, extrapolations of the nature of regional tissue structure are made from assessment of only a small amount of tissue taken as a biopsy. Because pathological changes resulting from ischemic heart disease are known to be inhomogeneous, there will be an unavoidable sampling error from the examination of such biopsy material. In the present study, such errors were minimized in three ways. First, we were able to examine relatively large samples by use of a metal cork-borer, which provides 2-mm-diameter transmural cylindrical biopsies, significantly larger than a specimen from a needle biopsy. Second, concurrent tissue from an individual patient was taken, whenever possible, from hibernating, reversibly ischemic, and normally perfused segments. This allowed direct comparison of diseased tissue with internal controls of “normal” tissue collected in an identical fashion. Third, all tissue examination was performed with the observer

Figure 5. High-power en face view of control (A), reversibly ischemic (B), and hibernating (C) intercalated disks. Normal pattern of large peripheral and small central gap junctions are seen in control tissue. This distribution is maintained in biopsies from reversibly ischemic regions, with an impression of decrease in overall intensity of labeling of disk. Hibernating intercalated disks show loss of normal pattern of labeling, with absence of large peripheral junctions making disk borders harder to define. Bar=25 μm (A, B, and C).
blinded to the tissue origin of each biopsy. The consistent patterns in immunodetectable connexin43 demonstrated under these conditions indicate that the data reflect true differences between the control and disease groups.

Earlier studies emphasized myofibril depletion as a hallmark of myocytes in hibernation. However, separate assessment of tissue histology and ultrastructure from the present patient series has shown no significant differences in frequency of myofibril-depleted myocytes between the groups of biopsies, including the control tissues, suggesting that alternative pathophysiological mechanisms are required to account for the wall motion abnormalities characteristic of hibernation. Alterations to gap junctions affecting intercellular coupling are one such candidate mechanism. The present study, in accordance with previous work, revealed that in reversibly ischemic myocardium, areas distant from a zone of infarction show a loss in connexin43 gap junction content but with maintenance of their overall tissue distribution. A key finding is that progressive decrease in the density of connexin43 gap junctions and loss of the large gap junctions at the disk periphery occurred in hibernating segments. No significant changes in other connexin isoforms were apparent in working myocytes in our tissue.

A relationship between abundance of gap junctions and intercellular conduction is suggested by studies of the functional role of the gap junction in cardiac muscle, the absence of conduction in vitro between cells without gap junctions, and the findings of sparse junctions in areas of heart with low conductance velocities. A relationship between gap junctional area and conductance has also been demonstrated in newly forming connections between isolated myocytes, as well as by the finding of concomitant increases in conduction velocity and connexin expression in dibutyryl cAMP-treated cultured cardiac myocytes. Although the precise in vivo relationship between absolute number of gap junctions and conduction velocity is not known, significant cardiac conduction defects are reported in heterozygotes for a connexin43 null mutation with an ≈50% reduction in gap junction content. Our findings of 23% and 33% reduction of disk gap junction density in reversibly ischemic and hibernating segments, respectively, may therefore plausibly cause significant disruption of intercellular propagation of depolarization. The specific loss of the peripheral large gap junctions in the disks of hibernating myocardium is potentially critical.

Recent findings suggest that tetrodotoxin-insensitive voltage-dependent sodium channels, essential for the rapid early upstroke of the cardiac action potential, have been localized to the lateral sarcolemma and may be preferentially concentrated around the intercalated disks. The peripheral intercalated disk conductance, determined by these large junctional

### TABLE 2. Results of Quantification

<table>
<thead>
<tr>
<th></th>
<th>Gap Junction Content of Disk, % (Runs 1 and 2)</th>
<th>Mean Relative Gap Junction Content of Disk, %</th>
<th>Gap Junction Size, μm² (Runs 1 and 2)</th>
<th>Mean Relative Gap Junction Size, %</th>
</tr>
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<tbody>
<tr>
<td>Normal perfusion</td>
<td>10.4±3.4</td>
<td>100±30.3*</td>
<td>0.21±0.06</td>
<td>100±31.5†</td>
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<td>13.0±3.7</td>
<td></td>
<td>0.22±0.08</td>
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<tr>
<td>Reversible ischemia</td>
<td>7.6±4.0</td>
<td>76.7±34.6*</td>
<td>0.17±0.06</td>
<td>87.4±31.2†</td>
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<td>10.3±4.0</td>
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<td>0.20±0.07</td>
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<td>Hibernation</td>
<td>7.1±2.6</td>
<td>67.4±24.3*</td>
<td>0.13±0.04</td>
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<tr>
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<td>8.7±3.1</td>
<td></td>
<td>0.16±0.04</td>
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</table>

Absolute and mean relative values of intercalated disk gap junction content and gap junction size (mean±SD). *ANOVA P<.001; †ANOVA P<.001.
plaques, may be of particular importance because of this spatial
total relationship. On theoretical grounds, intercellular conduction
depends on the reciprocal relationship between intercalated
disk resistance, determined by the absolute number of con-
nexin channels, and cell size. Although cell volume was not
directly assessed in our study, intercalated disk area, which
reflects the cross-sectional area of the corresponding myocyte,
provides an indirect measure of cell size. The finding of
progressively decreasing density of labeling in the disks in
reversible ischemia and hibernation partly reflects larger disk
sizes in these groups. Disruption in passive intercellular com-
munication22 from such changes may, in turn, predispose to the
increasing frequency of arrhythmia and sudden death associ-
ated with reversible ischemia40 and hibernation.9 Furthermore,
regional contractile dysfunction in hibernating areas may be
partly accounted for by local incoordination in contraction of
individual myocytes secondary to fragmentation of the depo-
larizing wave front.

The mechanisms by which reduced intercellular coupling
predisposes to arrhythmia may involve the interaction of the
characteristics of the action potential of individual myocytes
and the degree of intercellular coupling. Human and canine
myocytes from endocardial, epicardial, and the deep subepi-
cardial (M-cell layer) regions of normal and diseased ventric-
ular myocardium have been shown to have different action
potential durations, suggested to be due to a variable transient
outward K+ current (I_{to}).41-45 The coupling of these cells to
their neighbors in vivo masks such differences by modulating
action potential duration of each cell to reach a homogeneous
depolarization.34 A moderate reduction in such communica-
tion by disruption of gap junctions could result in an unmask-
ing of these variable action potential characteristics and hence
to slowing and inhomogeneities in the conduction of the
depolarization wave.15 This may, in turn, be manifest at the
clinical level by the establishment of potential reentrant circuits
and hence ventricular arrhythmias.14

A common feature of hibernating myocardium is the ability
to respond to inotropic stimulation with dobutamine. It is
becoming increasingly clear, however, that certain myocardial
segments are resistant to dobutamine stimulation but eventu-
ally show recovery of function after revascularization and
hence are defined as hibernating.46,47 A variable severity of gap
junctional disruption could provide a mechanism for this
phenomenon, with the dobutamine-resistant tissue being the
more severely affected.

Our data are consistent with the hypothesis that a reduction
in gap junction coupling is involved in the pathophysiology of
hibernation, possibly by unmasking local inhomogeneities in individual cell action potential durations, hence disrupting wave-front propagation, slowing conduction, and leading to loss of the local coordination in myocyte contraction. The changes at the cellular level would be manifested by nonspecific changes at the surface ECG. The disruption in intercellular conduction and investigation of excitation–contraction coupling needs further documentation by cellular electrophysiological studies. A more complete understanding of the role of cellular conduction and investigation of excitation-contraction changes at the cellular level would be manifested by nonspecific changes in the individual cell action potential durations, hence disrupting hibernation, possibly by unmasking local inhomogeneities in myocyte contraction.

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