Transmural reentry during acute global ischemia and reperfusion in canine ventricular muscle

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Wu, Jiashin, and Douglas P. Zipes. Transmural reentry during acute global ischemia and reperfusion in canine ventricular muscle. Am J Physiol Heart Circ Physiol 280: H2717–H2725, 2001.—Coronary occlusion and reperfusion produce tachyarrhythmias. We tested the hypothesis that variations in transmural activation after global ischemia and reperfusion were responsible for arrhythmias. We arterially perfused 36 isolated transmural wedges from canine left ventricular free walls. After ≥100 min of stabilization, the artery was occluded for 25 min, followed by reperfusion at various flow rates. We recorded 256 channels of fluorescent action potentials on transmural surfaces from preocclusion to >15 min after reperfusion. During endocardial pacing at 300 ms, ischemia of ≥570 ± 165 s (n = 34) produced 1:1 endocardial conduction and then 2:1 and 4:1 block as the wave fronts conducted toward epicardium. Transmural reentry appeared after 535 ± 146 s of ischemia (n = 31). Further ischemia caused epicardial inactivation and eliminated reentry (n = 24). During reperfusion, tissues progressed through sequences of epicardial inactivation and reappearance of activation with 1:1, 2:1, and 4:1 conduction; both sustained and nonsustained reentry occurred. We conclude that heterogeneous activation responses to endocardial pacing during acute ischemia provide the substrate for initiating reentry, suppressed reentry during further ischemia, and caused reentry during reperfusion.

During reperfusion, return of activation and unidirectional block could once again cause reentry that disappeared with the development of 1:1 conduction. Such changes, if present, might account in part for why some arrhythmias begin and then disappear during acute ischemia, only to return during reperfusion.

METHODS

Hearts from mongrel dogs were harvested after pentobarbital sodium (30 mg/kg body wt) anesthesia 5 min after intravenous injection of 5,000 units of heparin in accordance with institutional guidelines. The hearts were quickly Langendorff perfused with an ice-cold hyperkalemic cardioplegic solution (Tyrode solution, as shown below, with KCl increased to 15 mmol/l). Forty-nine transmural wedges were isolated from the left ventricular free walls of the hearts with the use of techniques reported previously (32). Each wedge contained either the first or second diagonal branch of the left anterior descending (LAD) coronary artery or a branch of the circumflex coronary artery along the wedge (with a diameter of >1 mm). The wedges were 20–30 mm long (along the artery), 4–7 mm thick (across the artery on epicardium), and 14–20 mm tall (transmural) (see Fig. 1). Two plastic cannulas were inserted into the openings of the artery in each wedge; the proximal cannula was for perfusion, and the distal cannula was for arterial pressure monitoring. Major arterial leaks in the wedges were closed with silk sutures. The wedges were placed in a tissue chamber surrounded by a 37°C water jacket (Radnoti; Monrovia, CA), perfused at an arterial pressure of 40–50 mmHg with 37°C oxygenated Tyrode solution [containing (in mmol/l) 128.0 NaCl, 4.69 KCl, 20.1 NaHCO3, 0.41 NaH2PO4, 1.18 MgSO4, 11.1 dextrose, and 2.23 CaCl2, gassed with 95% O2-5% CO2], and paced at or near the endocardium at a cycle length (CL) of 1,000 ms for ≥10 min before experimental recordings. The wedges were stained with a fluorescent dye, 4-(2-(6-(dibutylamino)-2-naphthalenyl)-1-(3-sulfopropyl)-hydroxide (di-4-ANEPPS; Molecular Probes; Eugene, OR), at ~2 μmol/l in Tyrode solution for ~10 min. A 256-channel optical mapping system with a photodiode array camera (C4675, Hamamatsu) coupled to a Nikon photo lens (focal length: 50 mm, aperture: 1.2) recorded fluorescent action potentials on the transmural cut surface at a rate of 1,000 samples per channel per second (32). Typical transmural recording from wedge preparations are shown in Figs. 2–5.

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We studied two groups of preparations: group A and group B. In group A (n = 36 wedges), we tested the impact of ischemia on wedges that were first subjected to an initial episode of preconditioning global ischemia without causing irreversible damage (6, 18). This was achieved by stopping the perfusion pump for 7–9 min after the tissue was placed in the chamber and had been perfused with Tyrode solution for >40 min. The first occlusion was followed by >60 min of perfusion at 40–50 mmHg of arterial pressure, and a second episode of global ischemia of 25 min was then created. Arterial pressure dropped to zero within a few seconds after a valve was closed in the perfusion tubing and the perfusion pump was stopped. The tissues were reperfused at the end of the second episode of ischemia at full, one-half, one-fourth, or one-eighth of the original flow rate instead of at a constant perfusion pressure. Finally, in one wedge preparation, we tested the impact of ischemia on wedges that were first subjected to an initial episode of global ischemia without causing irreversible damage (6, 18). This was achieved by stopping the perfusion pump for 7–9 min after the tissue was placed in the chamber and had been perfused with Tyrode solution for >40 min. The first occlusion was followed by >60 min of perfusion at 40–50 mmHg of arterial pressure, and a second episode of global ischemia of 25 min was then created. Arterial pressure dropped to zero within a few seconds after a valve was closed in the perfusion tubing and the perfusion pump was stopped. The tissues were reperfused at the end of the second episode of ischemia at full, one-half, one-fourth, or one-eighth of the original flow rate instead of at a constant perfusion pressure, which could not be controlled fast enough for the rapid reperfusion recovery in tissue excitability. The perfusion system was opened briefly to release the buildup pressure before the reperfusion at a one-half or lower flow rates.

We studied this preconditioned preparation because we (16, 25) have shown previously that the activation changes during the first occlusion are much greater than those of the second, whereas changes in the second are similar to the third, and the third is similar to the fourth; therefore, the response to the second occlusion was studied. This preparation may be important in replicating the conditions of a patient with angina before an acute myocardial infarction.

Group B (n = 12 wedges) was used to study the effects of two sequential episodes of ischemia on activation. In the sequential episodes of ischemia, we compared the results of the first with those of the second episodes. Each episode of arterial occlusion lasted ~8 min after >60 min of recovery perfusion with Tyrode solution at 40–50 mmHg of arterial pressure. Finally, in one wedge preparation, we tested the stability of the preparation by preparing it in the same manner as for groups A and B but not occluding the coronary artery.

A bipolar platinum electrode with 1 mm of tip separation was placed at the endocardium to pace each wedge continuously at a CL of 1,000 ms before and 300 ms during data recording. A second bipolar stimulating electrode (same as the first one) was placed at the epicardium in six wedges of group A. All stimuli had the same duration of 2 ms with a constant current at twice the diastolic threshold for activation before ischemia, which was in between 0.35 and 0.5 mA.

The site of stimulation was constant throughout the experiments. Fluorescent action potentials and the arterial perfusion pressure were recorded (1.2-s samples) immediately before the second episode of arterial occlusion (as control), during the second episode of arterial occlusion for group A, during both episodes of arterial occlusion for group B at 1-min intervals, and also during the first 15 min of the subsequent reperfusion for group A (repeated every 3–5 s for the first several minutes, increased gradually to every minute when the changes between recordings slowed). In six wedges (group A), we also recorded responses during epicardial stimulation after each recording of endocardial stimulation before and during ischemia. The bath of the perfusion efflux in the chamber was removed during the periods of ischemia and data recording but was maintained at a level higher than the top of tissue at all other times. After the solution was drained during the episodes of ischemia, a thin layer of solution was maintained on the tissue surface by the capillary effects of surface muscle fibers, and by 80–90% coverage of the opening of the tissue chamber. We visually checked the tissues every few minutes during experiments and found no dry surfaces in any of our tissues at any moment.

Action potential duration (APD) was derived from the time difference between the maximum rate of rise of fluorescent action potential (activation) and the peak of the second-order derivative of fluorescent action potential (repolarization), as previously reported (32), while the wedges were immobilized by global ischemia. The contraction of cardiac muscle became reduced and then disappeared within a few minutes of global ischemia and recovered at a much slower rate on reperfusion than the recovery of electrical activity. We calculated transmural velocity of conduction by dividing the spatial difference with the activation time delay between a subepicardial site and a subendocardial site along a transmural line across the region, where wave fronts of conduction were in general parallel to both the epicardium and endocardium, and passed through the region of early activation caused by endocardial stimulation. We identified sustained reentry when reentry continued with a CL ≤ 300 ms (pacing CL) throughout one or more 1.2-s recording sequences (≥4 cycles). All other episodes of reentry were identified as nonsustained reentry.

Tissue viability was monitored by the arterial pressure generated by muscle contraction, by the fluorescent action potentials, by the local muscle contraction in the recording area, and by the perfusion flow rate. We could identify the perfused and nonperfused regions after the wedges were in the bath for >100 min because the perfused regions recovered and the nonperfused regions died and became uncoupled electronically from the perfused regions. The perfused regions were brighter in color, with low noise optical action potentials, by the local muscle contraction in the recording area, and by the perfusion flow rate. We could identify the perfused and nonperfused regions after the wedges were in the bath for >100 min because the perfused regions recovered and the nonperfused regions died and became uncoupled electronically from the perfused regions. The perfused regions were brighter in color, with low noise optical action potentials, and strong contractions on stimulation, in contrast to the nonperfused regions, which were darker in color and had neither contraction nor good optical action potentials. None of the well-perfused wedges had the shortening in APD characteristic of ischemic tissue. We chose mapping areas from the well-perfused wedges and discarded all other tissues.

RESULTS

A web site containing the movies of activation propagation (movies 1–10)1 is available. These clips are best viewed with a fast computer.

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1 Supplemental movie clips are available at http://ajpheart.physiology.org/cgi/content/full/280/6/H2717/DC1.
Ischemia (group A). Figure 2 demonstrates a typical sequence of the initiation and termination of transmural reentry during acute global ischemia. The rapidly changing combination of the transmural dispersion in response to pacing stimuli and velocity of conduction created a dynamic substrate in which reentry could be initiated, sustained, and then abolished. Before ischemia, the entire recording area was activated by every endocardial stimulus at either a CL of 300 ms with an endocardial-to-epicardial velocity of 0.48 m/s (Fig. 2, A and E, movie 1) or a CL of 1,200 ms with similar sequences of activation (Fig. 2F). The transmural velocity of conduction decreased with the progression of ischemia (Fig. 2A). The same endocardial-to-epicardial sequence of activation with a slowed velocity of 0.13 m/s was observed after 298 s of ischemia (Fig. 2, B and F, movie 2). After 430 s of ischemia, 2:1 and 4:1 responses occurred in the epicardial and midmyocardial regions (e.g., Fig. 2C at 520 s). Reentry appeared on the transmural surface (Fig. 2G). During 4:1 conduction, the successfully conducted impulse propagated from the endocardial site of stimulation (0 ms) counterclockwise through the right midmyocardial region (100–150 ms), the epicardial region (200–400 ms), back to the left midmyocardial region (425–475 ms), and then finally toward the endocardial region (475
ms), forming a reentry loop (Fig. 2, C and G, movie 3). The slowed conduction provided the necessary time delay (~475 ms) for the reentry to arrive at the endocardial region after the expiration of local refractory period of the original activation. Unidirectional block occurred at the border of the 1:1 and 4:1 zones, as shown in Fig. 2, J and K. Endocardial 1:1 activations were blocked from conducting into the 4:1 zone. However, conduction from the 4:1 zone to the 1:1 zone succeeded until it collided into the refractory period of another endocardial paced activation. Both the slowed conduction velocity and coexisting 1:1, 2:1, and 4:1 responses to pacing stimuli facilitated the initiation of reentry during early ischemia. However, progressive inactivation with full conduction block into the epicardial and midmyocardial regions during prolonged ischemia subsequently inhibited reentry (Fig. 2, D and H). A complete reentrant loop occurred in another wedge when the reentry cycle was shorter than the pacing CL of 300 ms (Fig. 3, movie 4).

Activation spread from the site of endocardial stimulation along both the endocardium and toward the epicardium before the initiation of reentry during ischemia in all wedges (e.g., Fig. 2, E and F). In response to endocardial pacing at a CL of 300 ms, we observed one or more completed reentrant loops in 19 of 36 wedges after 704 ± 278 s of ischemia and incomplete reentry in another 12 wedges. The initial times at which both incomplete and complete reentries resulted were after 535 ± 146 s of ischemia in the 31 of 36 wedges. We also noted 2:1 and/or 4:1 propagated responses in the midmyocardial and epicardial regions, along with 1:1 responses in the endocardial region after ≥570 ± 165 s of ischemia, in 34 of 36 wedges (e.g., Fig. 2C). All reentry loops were formed by successful transmural conduction along a pathway with a high rate of stimulation response (e.g., 1:1 response) bordered by regions that exhibited lower rates of stimulation response (e.g., 2:1 and 4:1 responses). Propagation then returned toward the site of pacing through the 2:1 and/or 4:1 regions, where direct conduction from the site of pacing had been blocked previously. The coexistence of two or more zones of stimulation responses in the epicardial and midmyocardial regions were observed in 33 of 36 wedges. Sustained reentry with a CL ≤ 300 ms (shorter than or equal to pacing CL) occurred after 747 ± 312 s of ischemia in 10 of 36 wedges. Sustained reentry lasted 561 ± 314 s and terminated after 1,214 ± 227 s of ischemia in 8 of 10 wedges, when epicardium and midmyocardium inactivation blocked reentry (e.g., Fig. 2D). In two other wedges, fibrillation continued for 20 and 25 min of ischemia, respectively, when the experiments were terminated. Thus, although reentry could be initiated easily during the early stage of acute ischemia, reentry was also easily terminated during further ischemia, when the ischemia-induced inactivation caused bidirectional conduction block.

In six wedges, we alternated endocardial and epicardial pacing during ischemia to test whether the lack of an epicardial response was due to local inexcitability, prolonged refractoriness, or failure of conduction. We found that epicardial pacing produced the same response ratio in the whole wedge as did the epicardial activation propagating from the endocardium; e.g., if the epicardial response to endocardial pacing was 4:1, pacing the epicardium directly at the same CL produced a 4:1 response in the whole tissue. Thus it is likely that reduced epicardial excitability and/or prolonged refractoriness were responsible for the varying block during endocardial pacing. This is consistent with a greater sensitivity of the epicardium than the endocardium to the effects of ischemia (15).

Reperfusion (group A). Figure 4 demonstrates a typical sequence of the initiation, maintenance, and termination of fibrillation during reperfusion at one-half the preischemia flow rate. At the end of 25 min of ischemia, most of the epicardium and midmyocardium were inactive, and only the endocardial region could be activated with decremental conduction (Figs. 4, A and G). After 25 s of reperfusion, activation in the epicardial and the midmyocardial regions reappeared with APDs as long as 370 ms and a 4:1 response in the epicardium, although the endocardial recording sites still registered a 1:1 response at a CL of 300 ms (Figs. 4B and 5B). Activation propagated from the endocar-
Fig. 4. The initiation, maintenance, and termination of reentry during reperfusion at one-half of the preischemia flow rate after 25 min of global ischemia. The grids of the action potential mosaics (A–F) separate the fluorescent signals (1.2 s, normalized to their respective peak-to-peak amplitudes in B–F) from neighboring sensors. The wave fronts of activation in G and I–K are shown as isochrone lines at the times indicated by the numbers (in ms, movies 4–9). The thick line in G indicates the site of conduction block. The enlarged traces of action potentials in H demonstrate unidirectional block of conduction (as indicated by the solid arrows) in the framed region in B. The dashed arrows (I–K) indicate the direction of propagation. The thick lines with one dashed side and one solid side in I and J separate consecutive loops of reentry. K: ●, site of endocardial pacing. The recording area was 19.5 mm wide × 17 mm transmural. *Data obtained from a subepicardium site and a subendocardium site are shown in Fig. 5.
Fig. 5. The fluorescent action potential (A), action potential duration (APD) recovery (B), and the average activation CL over −1 s (C) in the same sequence of reperfusion as shown in Fig. 4. Data were obtained from a subepicardium site (Sub-Epi) and a subendocardium site (Sub-Endo), as indicated in Fig. 4, A–F (*).

dium to subepicardium and then reentered the midmyocardial region, where direct conduction from the endocardial site of pacing was blocked unidirectionally (Fig. 4H, movie 5). After 36 s of reperfusion (Figs. 4C and 5A, movie 6), the epicardial and midmyocardial regions recovered further, with the longest APD at 277 ms and a CL of 459 ms. At the same time, the subendocardial region fibrillated at a CL of ⃞186 ms (Fig. 5). With further reperfusion, the APDs at the epicardial and midmyocardial regions continued to shorten (e.g., Figs. 4D and 5A, movie 7). Well-organized clockwise reentry with a single CL of 92 ms involving the entire and lasted 153 s (e.g., Fig. 4), right corner of the recording area. A new counterclockwise reentry occurred after 223 s of reperfusion and lasted 153 s (e.g., Fig. 4E and J, movie 9). By 376 s of reperfusion (Fig. 4, F and K, movie 10), fibrillation, after lasting 346 s, was terminated by the continued endocardial pacing, and 1:1 responses were observed in all recording sites. The activation propagated with the normal sequence from the endocardium to epicardium at the pacing CL of 300 ms (Fig. 5C). During reperfusion, the area exhibiting 2:1 and 4:1 responses increased initially with the return of excitability and was then replaced by expansion of the area having 1:1 responses or faster reentrant activation (Fig. 4, A–D).

Return of excitability in the subepicardial and midmyocardial areas was slower than in the endocardium. The action potentials in the subepicardial site (Fig. 4, A–F) evolved through a sequence of inactivation, an active response with long APD, and then an active response with short APD as reperfusion progressed (Figs. 4, A–D, and 5A and B). In contrast, the APDs in the subendocardial site (Fig. 4, A–F) were relatively stable between 133–154 ms (Figs. 4, A–D, and 5B) until after ⃞30 s of reperfusion. At that time, the tissue started to fibrillate, with different pathways of activation and CLs of ⃞870 ms at the subepicardial region and ⃞222 ms at the subendocardial region (Fig. 5C). The transmural difference between endocardial and epicardial CLs decreased with the progression of reperfusion (Fig. 5C).

We reperfused seven, six, eight, and six wedges at full, one-half, one-fourth, and one-eighth of the original flow rates after 25 min of ischemia and observed sustained reentry in one, four, two, and zero wedges and nonsustained reentry in the other five, two, five, and six wedges, respectively (see Table 1). As reperfusion progressed, the inactive subepicardial and midmyocardial regions reactivated, initially with 4:1 and 2:1 con-

Table 1. The dependency on reperfusion flow rate of the reappearance of AP and the 1:1 responses to the endocardial pacing stimuli in the entire region of observation and dependency of the occurrences of sustained reentry and both sustained and nonsustained reentry

<table>
<thead>
<tr>
<th>Events</th>
<th>Flow Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full</td>
</tr>
<tr>
<td>Reappearance of AP (1:1, 2:1, and 4:1) in entire region, s</td>
<td>14 ± 3(7)</td>
</tr>
<tr>
<td>1:1 Responses in entire region, s</td>
<td>22 ± 2(7)</td>
</tr>
<tr>
<td>Sustained reentry, n</td>
<td>1(7)</td>
</tr>
<tr>
<td>Earliest sustained reentry, s</td>
<td>26(1)</td>
</tr>
<tr>
<td>Termination of sustained reentry, s</td>
<td>&gt;1,044(1)*</td>
</tr>
<tr>
<td>Sustained and nonsustained reentry, n</td>
<td>6(7)</td>
</tr>
<tr>
<td>Earliest sustained and nonsustained reentry, s</td>
<td>14 ± 3(6)</td>
</tr>
<tr>
<td>Latest initiation of nonsustained reentry, s</td>
<td>27 ± 11(5)</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of wedges. Time was calculated from the initiation of reperfusion. AP, action potentials. Numbers in parentheses indicate total number of preparations. *Reentry continued at the time of experiment termination (1,044 s of reperfusion). †Nonsustained reentry continued to the end of the observation period (>2,886 s) in another wedge.
duction in epicardial and midmyocardial regions in response to the endocardial stimuli at a CL of 300 ms. An activation of 1:1 or rapid reentrant activation in the entire observation area (e.g., Fig. 4) then occurred. Reducing the rate of reperfusion slowed the recovery of the 1:1 conduction and extended the time period during which 1:1, 2:1, and 4:1 conduction coexisted (Table 1). The differences in the rates of recovery between regions created a time period having highly dispersed responses of 1:1, 2:1, and 4:1 to pacing stimuli. This provided not only unidirectional block and the chance for initiation of reentry (see Table 1 for the initial times of sustained or nonsustained reentry) but also an increased possibility for spontaneous termination of reentry due to longer CLs and APDs at epicardial and midmyocardial regions. Sustained fibrillation occurred after 93 ± 49 s of reperfusion at the one-fourth preischemia flow rate \((n = 2)\) and after 18 ± 10 s of reperfusion at the full or one-half preischemia flow rate \((n = 5)\). With further reperfusion, the full recovery restored 1:1 responses to pacing stimuli (in wedges without sustained reentry), which in turn eliminated unidirectional block. Although new reentry could no longer start after 27 ± 11, 139 ± 110, 104 ± 108, and 286 ± 183 s of reperfusion \((n = 5, 2, 4,\) and 4) for reperfusion at the above flow rates, sustained reentry or fibrillation lasted for >1,044, 243 ± 233, and 627 ± 717 s \((n = 1, 4, 2)\) of reperfusion at the flow rates of full, one-half, and one-fourth, respectively.

**Sequential ischemia (group B).** We also studied the transmural velocity of conduction before and during two sequential episodes of arterial occlusion (~8 min for the first episode and >8 min for the second episode, separated by more than 60 min). The transmural velocities of conduction were 0.40 ± 0.06 m/s \((n = 7)\) before the first episode of arterial occlusion and 0.38 ± 0.08 m/s \((n = 12)\) before the second episode. The transmural velocity ratio before the second episode versus that before the first was 0.85 ± 0.13 in five wedges having both measurements. Transmural velocity of conduction decreased during ischemia in all wedges, with similar relative reductions of transmural velocity of conduction during the first and second episodes of ischemia (Fig. 6). Thus the wedges were electrophysiologically stable between the first and second episodes of ischemia, and there did not seem to be an exaggerated electrophysiological response to the first ischemic challenge in this preparation.

In a separate wedge without arterial occlusion, the transmural velocities of conduction were 0.33 and 0.32 mm/s after 71 and 131 min of perfusion, respectively, during an endocardial pacing CL of 300 ms.

**DISCUSSION**

**New observations.** This study demonstrated how the greater epicardial sensitivity to ischemia, the heterogeneity in tissue excitability, and the conduction delay combined to provide all the necessary conditions for the initiation, maintenance, and termination of transmural reentry during global acute ischemia and subsequent reperfusion. The differential responses in the epicardial and midmyocardial regions to fixed-rate endocardial pacing at 300 ms provided sites of unidirectional block, which created the functional pathways for reentry during ischemia and reperfusion. The dynamic changes in activation during ischemia and reperfusion created “windows of opportunity” for reentry to begin and terminate. Ischemia initiated reentry, but with its progression, ongoing reentry was terminated by extensive inactivation in the epicardial and midmyocardial regions. Then, as reperfusion started, reentry again could be initiated, but as further tissue recovery restored 1:1 responses in the entire wedge, unidirectional block of conduction was eliminated, which consequently reduced the probability of initiating new reentry. However, the same reperfusion recovery could facilitate the maintenance of ongoing reentry with a decreased CL of reentry (as shown in Fig. 5C). These dynamic changes in activation permitted reentry during acute ischemia to last for a short time period but permitted the transmural reentry during reperfusion to be sustained. Reperfusion flow rate affected the tempo of recovery and also the possibility of reentry.
initiation and maintenance. We observed a higher probability of VF occurrence in four of six wedges during reperfusion at the one-half preischemia flow rate than at any other flow rate tested. We found that the first and second episodes of ischemia produced similar changes in the velocity of transmural conduction.

Previous studies. Transmural APD dispersion in our wedges during slow pacing (dispersions of ~20 and ~23 ms at pacing CLs of 1,000 and 2,000 ms, respectively, as demonstrated in Fig. 10A of our previous study [32]) under normal perfusion pressure (40–50 mmHg) was similar to in vivo observations of 20–30 ms (11, 3). Therefore, the electrophysiological properties of our wedges were similar to those found in the intact hearts (3, 11) in which M cells did not have a major effect. However, others have reported a greater transmural APD at 90% repolarization dispersion in association with significant M cell effects in arterially perfused wedges (34), similar to the observations in isolated cells (2).

The global ischemia used in this study created uniform ischemia but depressed the excitability more in the midwall and epicardium than in the endocardium. This differs from in vivo observations during LAD occlusion, where collateral blood flow in the ischemic region preserved epicardial activation (4). Reentry has been found in many models (1, 7, 14, 17, 28–30). The present study demonstrated reentry initiation and termination during acute global ischemia and reperfusion due to the unidirectional block of conduction caused by the spatiotemporal dispersion in tissue responses to stimulation. The latter resulted from a higher sensitivity to effects of ischemia of the canine epicardium and midmyocardium compared with the endocardium (8).

We observed ischemia-induced reduction in the velocity of transmural conduction (Figs. 2A and 6), which was suggested as a cause of increased transmural delay of activation (4). Previously, the reduction in the velocity of conduction during ischemia was observed in the epicardium of isolated porcine hearts (10, 13) and in the endocardium of canine hearts (12). The results of this study support previous suggestions (5) that VF during reperfusion after acute ischemia was most likely caused by intramural reentry of excitation. Our data were also consistent with an earlier study (19) showing that arrhythmias with characteristics of transmural reentry occurred in 33% of the isolated guinea pig hearts during 15 min of simulated ischemia and in 75% of the hearts during early reperfusion with regular Tyrode solution. In addition, our data also support another finding (20) showing that rapid pacing tended to initiate reentry during acute ischemia but terminated ongoing reentry during reperfusion.

Reperfusion arrhythmias after global ischemia, as we demonstrated, are different from those after regional ischemia in an intact heart because ventricular arrhythmias during reperfusion after regional ischemia are more likely (75%) not to be reentrant and are initiated at the border of the ischemic region, as demonstrated in feline hearts (22). However, the border region between acutely ischemic and normal tissues is able to host transmural reentry (24). We chose to study global ischemia to investigate the changes that probably occur within the core of the ischemic region in hearts with coronary occlusion.

Limitations. Extensive surgery was performed to isolate transmural wedges from canine ventricular free walls. Even though we verified the healthiness of the wedges, as shown in METHODS, minor local damage from the cuts was possible. To minimize the effects of surgical damage, we perfused each wedge with oxygenated Tyrode solution for >100 min before data recording. Although the wedges were not perfused during the period of ischemia, a thin layer of the tissue surface might have a limited supply of O2 diffusing from the surrounding air (26). However, because the bulk of the wedge had no direct access to O2, the effects of surface exposure to air should not affect the tissue responses to ischemia qualitatively. It might have been possible for a reentry loop to occur within a wedge thickness of 4–7 mm (the dimension that could not be observed because it was perpendicular to the surface observation area). However, the occurrence of such reentry should be much less frequent than in the large surface area (20–30 mm along the artery × 14–20 mm transmurally). Also, it was likely that the pattern of activation within the thickness was similar to the surface layer due to the electrotonic interaction within the tissue. Most of the data presented in this study were obtained during rapid pacing at a CL of 300 ms. However, the electrical activity of cardiac tissue is rate dependent (10a). Thus it is possible that the observed changes in transmural conduction might have been different, or at least may have occurred at a different time course, at slower activation rates. Finally, conclusions can only be based on the region of myocardium studied. While it is likely that the other regions of the left ventricle would exhibit similar responses, we cannot make that conclusion from this study. In addition, as mentioned, the preparation lacked the border zone with normal myocardium that is ordinarily found in the intact heart with coronary occlusion.

Clinical implications. Although regional ischemia in the heart is clinically far more common than global ischemia, this study isolated the factors contributing to arrhythmia development by concentrating on global ischemia, which would represent the core of a transmural ischemic region in the intact heart.

The mechanism of reentry initiation by the spatiotemporal dispersion in the transmural responses to endocardial activation during acute ischemia supports the notion that a rapid ventricular rate can contribute to the initiation of reentry and VF during acute ischemia. The widespread conduction block and suppression of reentry during severe ischemia could also explain the spontaneous termination of reentry during continued ischemia. Transmural reentry during reperfusion, as we demonstrated, is one of the possibilities for the clinically observed reperfusion arrhythmias (9). The reduced occurrences of sustained reentry during reperfusion with slower flow rates, as observed in this study, support the earlier observations that reperfusion arrhythmias could be prevented when reperfusion oc-
curred gradually instead of abruptly (21, 27, 33) and may help explain the relatively low incidence of serious ventricular arrhythmias during reperfusion in humans.

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