Transmural reentry triggered by epicardial stimulation during acute ischemia in canine ventricular muscle

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Received 5 November 2001; accepted in final form 8 July 2002

Wu, Jiashin, and Douglas P. Zipes. Transmural reentry triggered by epicardial stimulation during acute ischemia in canine ventricular muscle. Am J Physiol Heart Circ Physiol 283: H2004–H2011, 2002. First published July 11, 2002; 10.1152/ajpheart.00965.2001.—Ischemia depresses tissue excitability more rapidly in the ventricular epicardium than in the endocardium. We hypothesized that this would provide the substrate for transmural reentry originating in the epicardium. We mapped transmural conduction in isolated and perfused wedges taken from canine left ventricles during acute ischemia while pacing alternately between the epicardium and endocardium. Ischemia reduced conduction velocity more in the epicardium than in the endocardium. We observed that the epicardial-initiated activation penetrated the ventricular wall transmurally while failing to conduct laterally along the epicardium, then conducted laterally along the endocardium and midmyocardium, and reentered the epicardium in 9 of 16 wedges during epicardial stimulation after 600 ± 182 s of ischemia. Endocardial stimulation applied immediately before or after the epicardial stimulation initiated activation that spread quickly along the endocardium and then transmurally to the epicardium without reentry in six of the nine wedges. The transmural asymmetric conduction was not observed in four separate wedges after the endocardium was removed. Therefore, ischemia-induced transmural gradient of excitability provided the substrate for reentry during epicardial stimulation.

METHODS

Transmural wedges of canine ventricular muscle were prepared using procedures similar to those in our earlier studies (26, 27). In brief, we harvested hearts from adult mongrel dogs after intravenous injection of heparin sodium (5,000 units) and pentobarbital sodium anesthesia (30 mg/kg body wt) by following National Institutes of Health and institutional guidelines and quickly Langendorff perfused the hearts with an ice-cold hyperkalemia cardioplegic solution (Tyrode solution as shown below with 15 mmol/l KCl). The time interval between the heart harvest and initiation of Langendorff perfusion was <30 s. We isolated transmural wedges from the free wall of the left ventricle. Each wedge contained a section of coronary artery (which was either a branch of the left anterior descending artery or a branch of the circumflex artery, with an inner diameter of ≥1 mm) on the epicardial surface along the length of the wedge. The wedges were 4–7 mm wide (across the artery), 10–20 mm from the epicardium to the endocardium, and 20–30 mm in length.

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along the length of the artery (27). There were 1- to 2-mm epicardial distances between the transmural recording surfaces and the perfusion artery. Two plastic cannulas were inserted into the basal and apical openings of the artery in each chamber and secured with silk sutures. The tissues were perfused with 37°C oxygenated Tyrode solution composed of (in mmol/l) 128.0 NaCl, 4.69 KCl, 1.18 MgSO4, 9.41 NaH2PO4, 20.1 NaHCO3, 2.23 CaCl2, and 11.1 dextrose (gassed with 95% O2-5% CO2) through the basal cannula (in the basal opening of the artery) at an arterial pressure of 40–50 mmHg, which was monitored with a pressure transducer attached to the free end of the apical cannula. Major arterial leaks in the wedges were closed with silk sutures. The wedges were pinned to the bottom of a 37°C water jacket-warmed tissue chamber (Radnoti; Monrovia, CA) with the observation surface up (one of the transmural cut surfaces), and paced at the endocardium at a cycle length (CL) of 1,000 ms for ≥60 min of recovery before experimental recordings. We stained the wedges with a membrane-binding fluorescent dye, di-4-ANEPPS (Molecular Probes; Eugene, OR), at ~4 μmol/l in the Tyrode solution for ~10 min. This dye emits a membrane potential-modulated red fluorescence when it is illuminated by a green light (wavelength: 520 ± 25 nm). A 256-channel optical mapping system (with 256 photon sensors in 16 × 16 arrangement; Hamamatsu) recorded the fluorescent action potentials (AP; after a long-pass optical filter of >590 nm) in an observation area of 19.5 × 19.5 mm on the transmural cut surface at the rate of 1,000 samples-channel−1-s−1 (Ref. 26, Fig. 1).

The wedges were subjected to a preconditioning period of ischemia for 5–8 min [no irreversible damage (6, 14)] after the tissue was placed in the chamber and had been perfused with Tyrode solution for >30 min, as we have done previously (26, 27). We employed preconditioning ischemia to reduce the individual differences among the tissues in response to the inevitable ischemic insults during the process of heart removal and wedge isolation. The preconditioning was followed by ≥60 min of perfusion with Tyrode solution at an arterial pressure between 40 and 50 mmHg and then another episode of global ischemia of ≥20 min. We reduced the bath to a level lower than the top of the tissue to facilitate optical mapping during the periods of ischemia and data recording, but maintained the level to cover the tissue at all other times. We covered the opening between the optical lens of the mapping system and the tissue chamber with glass coverslips or plastic wrap as much as possible to minimize the loss of heat and moisture, whereas the electrodes and light paths were not interfered with. We visually inspected the tissues during and after each episode of ischemia and verified the moistness of the exposed surfaces of the tissues. We examined the tissue viability by the strength of muscle contraction and by the systolic arterial pressure after 1 h of recovery perfusion, by the evenness of perfusion, and by the signal-to-noise ratio and duration of the fluorescent APs. Only healthy wedges were included in RESULTS. These are the same techniques that we have employed successfully in the past (26, 27).

Isolated and arterially perfused ventricular wedges are mature experimental preparations that have been used successfully by several groups (1, 3, 21–23, 26–30). Wedges prepared and verified with the above procedures are stable. In a preparation tested previously (27), the velocity of transmural conduction slowed only ~3% during 131 min of continuous perfusion. We observed reproducible reductions in the velocity of transmural conduction during two sequential 8-min episodes of ischemia separated by 60 min of reperfusion in 12 wedges (27). We also showed previously (26) that wedges prepared with the above procedures had transmural dispersions of AP duration of ~20 and ~23 ms at pacing cycle lengths of 1,000 and 2,000 ms, respectively, which are similar to the measurements in intact ventricles (4, 10, 13).

We studied two groups of wedges. Group A contained 16 normal intact wedges. Group B contained four wedges that had the endocardium removed either chemically (in one wedge) with repeated endocardial applications of phenol solution (11) or surgically (in the other three wedges) by shaving off a 0.5- to 1.0-mm surface layer.

Using two pair of platinum bipolar electrodes, we paced the wedges continuously at a CL of 1,000 ms at the endocardium during the tissue recovery period and alternately between the endocardium and epicardium at a pacing CL of either 300 ms (11 wedges) or 600 ms (5 wedges) during the period of testing and data recording. All pacing stimuli were 2 ms in duration at twice the diastolic current threshold of activation, which was assessed at the beginning of the testing and data-recording sequences. Arterial perfusion pressure and fluorescent APs were recorded at segments of 1.2 s immediately before (as control) and during the second episode of ischemia (repetitively at 1-min intervals). Each recording segment was initiated 100 ms before the onset of a stimulus after ≥8 preceding pacing stimuli at the specified CL. Pacing continued during each recording segment. We determined the time of activation at the maximum rate of AP depolarization at each site of recording. The isochrone maps of activation time were constructed with linear spatial interpolations among the sites of observation at the specified times (26, 27). Unidirectional block of conduction is defined as the conduction that failed or was decremental along one direction, whereas it was successful along the opposite direction. Reentry is defined as a path of wave front exhibiting such unidirectional conduction and completing a circular loop.

The conduction velocities at the endocardium, subendocardium, and epicardium in each wedge (e.g., Figs. 2J and 5E) were represented by the means ± SD of the local velocities at the recording sites inside the respective regions (as indicated in Figs. 2G, 5A, and 5C). We calculated the velocities of conduction at each recording site from the times of activation at three sites, the local, a horizontal neighbor, and a vertical neighbor (e.g., times of activation t0, t1, and t2 at the sites a
where $V$ is the velocity of local conduction and $ds$ is the distance on tissue surface corresponding to the centers of neighboring photon sensors (same as the side length of a square area in Fig. 1B). A single site (e.g., site $a$) could have multiple local velocities, depending on the available combinations of the local and two neighboring sites (e.g., $a$-$b$-$c$, $a$-$d$-$c$, $a$-$b$-$e$, and $a$-$d$-$e$ for site $a$ in Fig. 1B). Only the first endocardial row of the recording sites was used as the local sites for the calculation of endocardial velocity of conduction because Purkinje fibers are most likely covered only by the
first endocardial row. The subendocardial velocities of conduction were calculated with the use of the recording sites at the second row (1.2 mm from the endocardial row) as the local sites and the endocardial row as the vertical neighbor sites. The epicardial velocities of conduction were calculated for all combinations of the neighboring sites in the first three rows from the epicardium. The means ± SD of the velocities in each wedge represented the average and heterogeneity of the velocity of conduction in the spatial resolution of 1.2 mm (i.e., the separation between neighboring sites, \( ds \)).

RESULTS

Figure 2 demonstrates a typical sequence of the transmural asymmetrical patterns of conduction during acute ischemia in a group A wedge. Before ischemia, activation spread rapidly in all directions from either the epicardial or endocardial site of stimulation (Fig. 2, A and D). Acute global ischemia depressed the velocity of conduction more in the epicardium than in the endocardium (Fig. 2, J and K). Although the epicardial and transmural conductions were slowed, rapid lateral endocardial conduction remained because the endocardium was less sensitive to the first 9 min of ischemia (Fig. 2, A–K). The differential rates of transmural conduction depression during acute ischemia provided the substrate for asymmetrical patterns of transmural propagation conducive to reentry. The reduced sensitivity of endocardial conduction to ischemia caused the epicardially initiated activation to conduct rapidly along the endocardium after penetrating the ventricular wall (Fig. 2, B and C). Because of less ischemia-induced depression of conduction in the endocardium compared with the epicardium and midmyocardium after >200 s of ischemia, wave fronts arrived at the right edge of the mapping area earlier in the endocardial site than in the midmyocardial site during epicardial stimulation (Fig. 2, B and C; see Fig. 2G for the locations of the sites). The epicardial site at the right edge of the mapping area was still activated earlier than the midmyocardial site up to 390 s of ischemia (Fig. 2, A and B), due to a shorter route of conduction from the epicardial site of stimulation to the lateral epicardial site than to the midmyocardial site. Therefore, epicardial stimulation initiated a wave front that arrived at the right edge of the mapping area earlier in both the epicardial site and endocardial site than in the midmyocardial site during the time window of 200–390 ms of ischemia (Fig. 2B). After 505 s of ischemia, lateral epicardial conduction became decremental (Fig. 2C), whereas conduction from the epicardial site of stimulation to the endocardium was preserved due to the gradient of increased tissue excitability along the way (Fig. 2, B and C). Because conduction along the endocardium and subendocardium was well preserved (Fig. 2, H–K), activation then spread along the endocardium and subendocardium quickly to both sides of the wedge (Fig. 2, B and C) and reentered the epicardium. The less depressed endocardial and midmyocardial regions (Fig. 2, H–K) were more capable of activating the “downstream” epicardial tissue than the neighboring severely depressed “upstream” epicardial tissue. Endocardial stimulation immediately before or after the above epicardial stimulation (Fig. 2, B and C) produced wave fronts that spread rapidly along the endocardium and then moved slowly toward the epicardium (Fig. 2, E and F) without reentry.

Figure 3 demonstrates a single loop of transmural reentry initiated by subepicardial stimulation after 801 s of global ischemia in another group A wedge. The data segment started 100 ms before the onset of a stimulus (defined as time 0, after −20 preceding stimuli) during continued subepicardial pacing at a cycle length of 300 ms. The earliest recorded activations were at the beginning of the data segment (−100 ms) at the region around site E, indicating that they were initiated by a prior stimulus applied at −300 ms or earlier. The stimulus applied at 0 ms synchronized the activation wave front to the site of stimulation (as shown by the increased separation between the isochrone lines on the epicardial side of the site of pacing). Similar to the wedge shown in Fig. 2, activation conducted transmurally to the endocardium and subendocardium but failed to conduct laterally along the subepicardium. Activation then conducted laterally in the subendocardium and reentered the epicardium, forming a closed-loop reentrant pathway. The early activation at site E reentered the same site after passing through the route along E-A-B-C-D-E and then terminated at site A.

Both the epicardial and endocardial thresholds of activation before ischemia were the same, 0.7 ± 0.3 mA, in the 16 wedges in group A. Delayed midmyocardial conduction compared with the endocardial and epicardial conduction after epicardial stimulation (e.g.,
along the right edge of Fig. 2B) was observed after 345 ± 166 s of ischemia in 6 of 16 group A wedges. The reentry conduction pathway from the epicardial site of stimulation to the endocardium, then laterally along the endocardium and subendocardium and back toward the epicardium (e.g., Fig. 2C), was observed in 9 of 16 wedges after 600 ± 182 s of ischemia. Reentry continued for >1 loop in four of nine wedges after 708 ± 230 s of ischemia at CLs of 328 ± 106 ms. Endocardial stimulation, applied immediately before or after the epicardial stimulation that initiated reentry, caused activation that conducted rapidly along the endocardium and then moved slowly to the epicardium without reentry (e.g., Fig. 2, E and F) in six of the above nine wedges. We observed unidirectional block of conduction [similar to our previous observations (27)] at the border of the regions having different rates of responses (i.e., 1:1 endocardial and 2:1 and/or 4:1 epicardial responses) in response to the endocardial stimulation initiated in the epicardium to block laterally in the epicardium.

Reentry by Epicardial Stimulation During Ischemia

Figure 4 shows the statistics and correlations of the ischemia-induced depression in the velocity of conduction in the endocardium (13 wedges), in the subendocardium (13 wedges), and in the epicardium (14 wedges) in group A. Before ischemia, the velocity of conduction in the endocardium was faster than in the subendocardium (P = 0.01) and in the epicardium (P = 0.00, Fig. 4A). The faster endocardial conduction could be the contribution of Purkinje fibers. The velocity of conduction in the subendocardium and epicardium were similar (P = 0.07) before ischemia (Fig. 4A).

Eight minutes of global ischemia depressed the velocity of conduction significantly (P = 0.01, 0.02, and 0.00, for the endocardium, subendocardium, and epicardium, respectively) and enhanced the transmural dispersion of the velocity of conduction, resulting significant differences among the endocardium, subendocardium, and epicardium (Fig. 4A). Eight minutes of ischemia depressed the velocity of conduction in the epicardium to 34% of the velocity before ischemia, significantly less than in the endocardium (84%, P = 0.00) and subendocardium (79%, P = 0.00, Fig. 4B). The depression of conduction velocity in the endocardium and subendocardium were similar after 8 min of ischemia (P = 0.41, Fig. 4B). The transmural dispersion in the ischemia-induced depression of conduction velocity provided the substrate for the epicardially initiated reentry and transmural conduction asymmetry as we demonstrated above, as well as provided further detail to support and extend the previous observations that ischemia depressed the activation and conduction more in the epicardium than in the endocardium.

The movies corresponding to Figs. 2C, 2F, and 3 can be viewed at http://ajpheart.physiology.org/cgi/content/full/283/5/H2008/DC1.

DISCUSSION

New observations. This study demonstrated the existence of transmural dispersion in the velocity of local conduction before ischemia in isolated canine ventricular wall. Acute ischemia enhanced the transmural dispersion with more rapid depression of excitability and conduction in the epicardium than endocardium. This differential response permitted activation initiated in the epicardium to block laterally in the epicard-
Fig. 5. Conduction in a group B wedge that had its endocardium (0.5- to 1-mm surface layer) removed. Subepicardial (A and C) or endocardial (B and D) stimuli were applied to the dotted sites at 0 ms. The numbers on the isochrone lines indicate the times of activation (in ms). The S-Endo and Epi velocities of conduction (means ± SD) in E are calculated from the activation times at the sites within the corresponding rectangular areas in A and C. Depression in the velocities of conduction after 601 s of ischemia (F) is derived from E by dividing the data of after ischemia with their corresponding mean velocities before ischemia. The recording area is 13.4 mm (transmural) × 16 mm (along the epicardium).

dium, conduct transmurally to the endocardium, travel laterally in the endocardium, and return to the epicardium to complete reentrant loops. Endocardial stimulation, applied immediately before or after the above epicardial-initiated reentry, initiated activation that conducted along the endocardium rapidly toward the epicardium without reentry. Removal of the endocardium prevented the above patterns of asymmetrical conduction and reentry during epicardial stimulation. On the basis of these observations, we conclude that ischemia-induced transmural gradient of excitability provided the substrate for reentry during epicardial stimulation.

Previous studies. The mechanism of unidirectional block of conduction and transmural reentry initiated by epicardial stimulation, as shown in this study, is based on the ischemia-induced transmural gradient of tissue excitability, in contrast to the ischemia-induced transmural gradient of refractory period during endocardial stimulation that was the mechanism responsible for the reentry in our previous study (27). We reported previously (27) that the ischemia-induced transmural dispersion in tissue refractoriness and the tissue heterogeneity could block conduction unidirectionally during endocardial stimulation and cause reentry. We observed that endocardial activation could fail to conduct directly to the epicardial and midmyocardial regions where the local refractory periods were longer than the endocardial pacing cycle lengths (300 ms), resulting 2:1 and/or 4:1 responses to the pacing. However, the impulse could conduct 1:1 to other regions having refractory periods less than the pacing cycle length. Activation then conducted from the epicardium transmurally through the regions of 2:1 and/or 4:1 responses and reentered the subendocardium, where activation was originally initiated, forming reentry. We observed similar phenomena of multiple zones of tissue responses to pacing stimuli in the present study. Both our current and previous studies are complementary because acute ischemia-induced both transmural gradients in the tissue excitability and in the tissue refractory period. The spectrum of tissue heterogeneity and responses to acute ischemia provided the substrates for both types of reentry to occur when cardiac muscle was stimulated at the right time and at the right location (epicardium or endocardium).

The reason for the increased resistance of endocardial cells to ischemia compared with epicardial cells is not known, but may relate, at least in part, to the resistance of Purkinje cells to acute ischemia. Electrical uncoupling of Purkinje cells increased the sensitivity of the endocardium to the effects of ischemia (7). Indeed, in the present study, Purkinje cells contributed to the asymmetrical patterns of transmural conduction during ischemia as we observed in our wedge preparations (e.g., Figs. 2 and 3), and removing a thin layer of endocardium greatly reduced the transmural conduction asymmetry during ischemia and prevented reentry (e.g., Fig. 5).

The transmural heterogeneities in membrane AP and ionic currents could also contribute to the transmural asymmetry in conduction during acute ischemia. The shape and duration of APs are heterogeneous across the ventricular wall (2, 19, 20). Compared with endocardial cells, epicardial cells have a larger transient outward current (24), a larger ischemia-induced ATP-sensitive K⁺ channel current, and an AP that has a larger spike and dome morphology, a more pronounced rate dependency, and an increased ischemia-induced shortening (12).

Limitations. We used the wedge preparations and global ischemia protocol to focus on the contributions of the ischemia-induced depression to the initiation of reentry and to eliminate other factors not included in the scope and hypothesis of this study, e.g., the border zone of regional ischemia, which occurred clinically when a single branch of the coronary artery was blocked. Re-
Regional ischemia is a complex phenomenon that includes both the heterogeneous perfusion (the perfused and ischemic regions and the border zone) and the local responses of tissue to ischemia. The wedge preparations also limited the possible patterns of conduction. However, the contributions of transmural gradient of tissue excitability to the unidirectional block of conduction and initiation of reentry during acute global ischemia as we demonstrated in the wedges should also exist in intact heart.

Although we verified the healthiness of the wedges, as stated in METHODS, minor local damage from the surgical procedures was possible. To minimize the effects of the local damage, we perfused each wedge with oxygenated Tyrode solution for >60 min before data recording after the wedges were isolated and preconditioned. The wedges were not perfused during the period of ischemia and the tissue surface was exposed to air. Hence, a thin surface layer of tissue had access to the airborne O2 (17). However, the bulk of the wedge had no access to O2 and the effects of surface air exposure should not affect the tissue responses to ischemia in a significant way.

The optically recorded patterns of conduction were two dimensional on the surface of observation, although intramural conduction was three dimensional. To reduce the possibility of reentry in the dimension perpendicular to the observation surface, we limited the thickness of the wedge preparations to <7 mm, which was much less than the large observation surface (20–30 mm along the artery × 10–20 mm transmurally). Because of the electrotonic interaction within the tissue, it was likely that the pattern of activation within the thickness of <7 mm was similar to the observation surface.

Finally, conclusions can only be based on the region of myocardium studied. Whereas it was likely that the other regions of the ventricle would exhibit similar responses, we could not make that conclusion from this study.

Clinical implications. Normal cardiac tissue is heterogeneous (2) and its response to ischemia is even more so. The combination of tissue heterogeneities and transmural gradients of the tissue excitability and refractory period create multiple possibilities for reentry to occur during acute ischemia. Thus, depending on the nature of the heterogeneity, premature ventricular complexes originating in the epicardium and/or in the endocardium could precipitate reentry. The epicardially initiated reentry during global ischemia as we demonstrated here is a component contributing to the clinical VT and VF during acute ischemia. The understanding of the possible mechanisms of reentry initiation will provide the foundations for further research on the prevention and management of ischemia-induced VT and VF.

This research was supported by American Heart Association Midwest Affiliation Awards 9930347Z and 0256112Z and by the Herman C. Krammert Fund (Indianapolis, IN).

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