Spatial heterogeneity of action potential alternans during global ischemia in the rabbit heart

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Spatial heterogeneity of action potential alternans during global ischemia in the rabbit heart. Am J Physiol Heart Circ Physiol 285: H2722–H2733, 2003. First published August 7, 2003; 10.1152/ajpheart.00369.2003.—Cardiac ischemia causes beat-to-beat fluctuation in action potential duration (APD) alternans, which leads to T wave alternans and arrhythmias. Occurrence of APD alternans that is out of phase at two sites is especially important, but most APD alternans studies have involved rapid pacing of normal myocardium rather than ischemia. To determine the spatial features of APD alternans during ischemia, blood-perfused rabbit hearts were stained with 4-[β-[2(di-n-butylamino)-6-naphthyl]vinyl]pyridinium (di-4-ANEPPS) and imaged with a high-resolution camera. Hearts were perfused with oxygenated Tyrode solution at 37°C for staining and then switched to a 50:50% blood/Tyrode mixture. Hearts were paced from the right ventricle at 3/s, and made ischemic by stopping flow for 6 min. Images of 10,000 pixels were obtained at 300 frames/s. Motion artifact was controlled by immobilization and by manual selection of undistorted single-pixel records. Upstroke propagation and conduction isochrones were displayed by computerized image processing. APD alternans was demonstrated in six of seven hearts, and was out of phase in different regions of the image in three hearts. The largest spatial variation in the onset of depolarization to 50% repolarization (APD50) was 155%. This caused beat-to-beat reversal of repolarization. An alternans map could be constructed for well-immobilized portions of the image. There were discrete regions of APD alternans that is out of phase at two sites is especially important, but most APD alternans studies have involved rapid pacing of normal myocardium rather than ischemia. To determine the spatial features of APD alternans during ischemia, blood-perfused rabbit hearts were stained with 4-[β-[2(di-n-butylamino)-6-naphthyl]vinyl]pyridinium (di-4-ANEPPS) and imaged with a high-resolution camera. Hearts were perfused with oxygenated Tyrode solution at 37°C for staining and then switched to a 50:50% blood/Tyrode mixture. Hearts were paced from the right ventricle at 3/s, and made ischemic by stopping flow for 6 min. Images of 10,000 pixels were obtained at 300 frames/s. Motion artifact was controlled by immobilization and by manual selection of undistorted single-pixel records. Upstroke propagation and conduction isochrones were displayed by computerized image processing. APD alternans was demonstrated in six of seven hearts, and was out of phase in different regions of the image in three hearts. The largest spatial variation in the onset of depolarization to 50% repolarization (APD50) was 155%. This caused beat-to-beat reversal of repolarization. An alternans map could be constructed for well-immobilized portions of the image. There were discrete regions of APD alternans separated by a boundary, as occurs with intracellular Ca2+ concentration alternans. Pixels as close together as 1.1 mm showed an APD alternans that was out of phase. The out-of-phase APD alternans was not due to conduction alternans, as shown by upstroke intervals and conduction isochrones. This contrasts with rapid pacing, where a causal relationship appears to exist. These new observations suggest distinct mechanisms for the genesis of arrhythmias during ischemia.

4-[β-[2(di-n-butylamino)-6-naphthyl]vinyl]pyridinium; myocardium; fluorescence imaging; arrhythmias

T wave alternans precedes lethal ventricular arrhythmias in experimental animals and also in humans (34). T wave alternans occurs reliably during coronary artery occlusion in large in vivo hearts, and is known to closely precede the onset of ventricular fibrillation (VF) (3, 17). Beat-to-beat fluctuations in action potential durations (APD) are known to be the cause of T wave alternans. A widely used method for studying APD alternans is to record from single cells or fibers at stimulation rates >300 per min (6, 11). This can also be done in two-dimensional myocardial syncytia that are produced by endomyocardial cryoablation of the left ventricle (28, 29). In this preparation, rapid pacing can produce APD alternans that is out of phase at two different sites on the ventricular surface. Out-of-phase APD alternans is more arrhythmogenic than synchronized alternans (17, 28, 29) and may therefore be an important mechanism of sudden death in ischemic heart disease. However, this chain of events needs to be substantiated through further animal research.

Several approaches have been used to study the effects of ischemia on the cardiac action potential. The oldest approach is to use “floating” microelectrodes (10, 14). A more recent approach has been to use monophasic action potentials, which produce more stable recordings (18, 21). Both of these techniques reveal the development of APD alternans in the ischemic left ventricle after 2–4 min. However, the number of simultaneous recordings that can be obtained with these techniques is small, and very little is known about the spatial distribution of APD alternans during ischemia.

It is possible that APD alternans could be studied during ischemia with fluorescent potentiometric dyes such as 4-[β-[2(di-n-butylamino)-6-naphthyl]vinyl]pyridinium (di-4-ANEPPS). These dyes can be used in conjunction with high-resolution imaging cameras to obtain recordings from hundreds or thousands of pixels simultaneously (24). Fluorescence imaging has been used to map Ca2+ transient alternans during ischemia in the blood-perfused rabbit heart (30), and marked spatial heterogeneity was found. Optical methods have also been used to study the mechanism of electrical defibrillation in the presence and absence of ischemia in hearts taken from small mammals (4, 13, 15). For these studies, fully intact hearts were used, which were stained with di-4-ANEPPS and treated with the pharmacological motion blocker diacetyl monoxime (DAM). Pharmacological motion blockers are undesirable for studies of ischemia-induced APD alternans because
they may prevent alternans (12), either through reduction in myocardial oxygen consumption (1) or primary effects on the ventricular action potential (23, 25).

The objective of this study is to characterize the spatial distribution of APD alternans during brief periods of stop-flow ischemia in fully intact, blood-perfused rabbit hearts paced at physiological rates and not exposed to a motion blocker. The specific goals are to determine how often APD alternans occurs during the first 6 min of ischemia, and the phase relation of alternans when it is detected at more than one location. We have been able to demonstrate APD alternans in a series of such hearts and have found marked spatial heterogeneity across relatively short distances on the heart surface. These findings are similar to results of calcium imaging with rhod-2 in the same experimental model. In addition, we show that ischemia can produce marked APD alternans, including spatially out-of-phase alternans, with no beat-to-beat variation in conduction time (Δ𝑡 ≤ 1 ms). This is in contrast with studies (5, 11, 28, 36) involving rapid pacing variation in conduction time (Δ𝑡 > 1 ms).

METHODS

The investigation conforms to the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85–23, Revised 1986). New Zealand White rabbits of either gender (Kralek Farms; Turlock, CA), weighing 2–3 kg, were anesthetized with pentobarbital (50 mg/kg). The heart was quickly removed and mounted on a Langendorff apparatus and perfused with oxygenated Tyrode solution. The temperature of the heart was maintained at 35 ± 1.5°C. The heart was pushed from behind by a flat plunger with a set screw so that the surface of the left ventricle was stabilized against a heated glass plate. Heating of this plate limited the fall in temperature during ischemia (measured with a small thermistor) to 1.2°C at 2 min and 2.1°C at 4 min. During ischemic trials and control images, the heart was paced from the right ventricle at a rate of 3/s by a coiled pacing electrode. Ischemia was created by stopping blood flow to the heart for 6 min. Ischemic trials were preceded by 2 min of preperfusion with blood, which was diluted 50% with Tyrode solution and heparinized (50 U/ml). The heart was stained with 4-[β-[2(di-n-butylamino)-6-naphthyl]vinyl]pyridinium (di-4-ANEPPS) by a bolus injection of 0.2 mg into a 25-ml bubble trap containing Tyrode solution before perfusion with blood. Illumination was provided by a 2-W diode-pumped solid-state laser (Spectra-Physics) with a 532-nm single-line output. The laser light was directed into a beam expander and onto the surface of four 6-mm fiber bundles, which were pointed at the heart surface. Fluorescence was collected with a 12-bit digital camera fitted with a 600-nm long-pass filter and a 25-mm 0.85 numerical aperture video lens (model CA-D1-0256T, Dalsa). Digital images of the heart surface were taken at a resolution of 100 × 100 pixels at 300 frames/s for 2 s. The resulting image subtended a square region of heart surface ~2.5 cm × 2.5 cm, so that each pixel was 0.25 mm × 0.25 mm. The images were stored to a disk and processed with MatLab software, which allows interrogation of specific pixels for display of fluorescence versus time. The pixels were referenced by an x-y coordinate system where 0,0 is the top left corner of the image and 100,100 is the bottom right corner. A series of 600 images was acquired every 30 s during ischemia, and a shutter was used to minimize bleaching. No images were obtained <2 min after a change in pacing rate.

Data analysis. Data are displayed from single pixel recordings, except in one instance where the software was used to average the signal from a 4 × 4 pixel array. Conduction velocity was measured by dividing the distance of propagation in a series of images by the number of frames × 3.3 ms. APD50 was measured from the onset of depolarization to 50% repolarization. Diastolic interval (DI) was measured from the point of maximum or full repolarization to the midpoint of the next upstroke (identified as a vertical line segment).

The problem of optical motion artifact was minimized in three ways. First, the heart was immobilized against a glass plate as described above. Second, ischemia produced a substantial reduction in motion as described below. Third, ex- by 10.220.33.2 on April 19, 2017 http://ajpheart.physiology.org/ Downloaded from http://ajpheart.physiology.org/
4 × 4 pixel grid. Activation times were determined at each pixel based on an adaptive amplitude threshold. The amplitude threshold was equal to 50% of the maximum action potential amplitude (APA) at each pixel during the specified frame sequence. Time 0 was the beginning of the frame in which right ventricular epicardial breakthrough first occurred. The resulting two-dimensional array of activation times was input to a contour plot function available in MatLab. This function plots contours with user-specified values based on regularly spaced intervals, which were chosen to be 10 ms. The computed isochrone contours follow the pixel grid with 1 y value per vertical pixel and 1x value per horizontal pixel.

The isochrone maps generated for individual action potentials contained a timing error of 0 to 3.3 ms because the pacing stimulus was not synchronized to the frame timer. The number of frames per stimulus pulse was programmed to be 100, but the pacing cycle length could not be specified accurately enough to ensure that epicardial breakthrough always occurred at the same point in the frame. The timing error was therefore different for each of the six action potentials in an image file.

RESULTS

Action potential propagation under control conditions and during ischemia. An example of action potentials obtained with di-4-ANEPPS in a spontaneously beating saline-perfused rabbit heart is shown in Fig. 1. The time course and morphology of the action potential are similar to those obtained with microelectrodes. Each action potential produces a fluorescence decrease of 8.5% and there is no obvious variation in APD. The mean value of APD\textsubscript{50} is 202 ± 3 ms for the three beats. At constant pacing rates, APD alternans was never seen in the absence of ischemia.

Figure 2 shows a series of 15 images of an action potential propagating across the surface of a heart that is perfused with saline and paced at 3/s. Images are obtained at 3.3-ms intervals so that the entire recording takes 50 ms. The action potential first appears on the surface of the right ventricle, near the apex, where the pacing electrode is located, and spreads as a wave across both ventricles. The velocity of propagation is 0.34 m/s, which is typical of ventricular myocardium, and the time required for complete propagation is comparable to the known ventricular activation time.

Figure 3 shows a series of 15 images of an action potential propagating in the same heart after it was perfused with blood for 2 min and rendered ischemic for an additional 3 min in the presence of blood. The pattern of propagation is unchanged from Fig. 2, and the velocity of propagation is only modestly reduced to 0.26 m/s. However, there is a reduction in image quality compared with Fig. 2 so that the borders of the propagating waveform are not as sharply defined, and the intensity of the fluorescence change during the upstroke is less. This reduction in signal quality is partly due to the addition of blood but may also be due to the deterioration in the relative fluorescence signal, which is known to occur when di-4-ANEPPS is used in ischemic hearts (15).

APD alternans during ischemia. Figure 4 shows action potentials recorded from a blood-perfused rabbit heart after 3 min of ischemia. The pixel numbered 54,69 (top) is displayed and there are six action potential upstrokes during the 2-s recording. The location of this pixel is near the apex of left ventricle. Despite the poorer image quality during ischemia, the action potential morphology is clearly visible, and there is a striking alternans of APD. The pattern of APD alternans shown here is odd action potentials are narrow, even action potentials are wide. Similar experiments were performed in seven hearts. Alternans was never observed during the first minute of ischemia but was observed consistently at 2, 3, or 4 min.

When APD alternans from pairs of pixels is compared, four results are possible: 1) neither pixel shows alternans, 2) only one pixel shows alternans, 3) both pixels show alternans of the same phase, or 4) both pixels show alternans of the opposite phase. Examples of result 3 are shown in Figs. 5 and 6, whereas result 4 can be seen by the comparison of Figs. 5 and 6. In Fig. 5, the 12 contiguous pixels are from the same heart as Fig. 2–4. These 12 pixels all show alternans with the first and odd APD prolonged, and the second and even APD briefer. In contrast, the recordings shown in Fig. 6, which are taken from the same series of images as Fig. 5, show first and odd APD narrower, and the second and even APD wider. The four pixels in each column in Figs. 5 and 6 represent a distance of 1.00 mm, whereas the three pixels in each row represent a distance of 0.75 mm. The distance between corresponding pixels in Figs. 5 and 6 is 3.9 mm.

The pattern of action potential alternans in Fig. 5 is primarily APD alternans, with relatively little variation in amplitude (APA). However, in Fig. 6, there is
more prominent variation in APA, which is particularly marked in the top row of pixels. APA alternans was observed with floating microelectrodes in blood-perfused ischemic pig hearts in <5 min of ischemia (10), and is presumably a prelude to 2:1 conduction block. Figure 7 shows an alternans map that was constructed for the experiment in Figs. 3–6. For this plot, 342 pixels in an 19 x 18 array have been evaluated. Pixels are plotted whenever alternans is present and the signal-to-noise ratio is sufficient for the pixel to be evaluated. Alternans is present in two mutually exclusive regions. Forty-one pixels, all located in the bottom right of the map, show alternans with the first APD short, whereas 42 pixels, all located in the top left of the map, show alternans with the first APD long. These regions appear to be separated by a boundary. Pixels with opposite phase APD alternans are as close together as 1.1 mm. This type of map is called a qualitative alternans map because no measurements of the difference in APD are shown.

The 12 pixels that comprise Fig. 5 have the coordinates x = 43–46 and y = 56–59 in Fig. 7 and are located at the top left, whereas 11 pixels that comprise Fig. 6 have the coordinates x = 54–56 and y = 67–70, and are located near the bottom right. With the use of the action potential propagation images in Fig. 3, and conduction isochrones derived from Fig. 3 (see below), it is possible to project the propagation of the upstroke onto the alternans map in Fig. 7. The general direction of propagation is from the bottom left to the top right. As a result, the boundary between the two regions showing alternans (xs and solid circles) is nearly perpendicular to the direction of action potential propagation. The arrival of the action potential at the pixels in Figs. 5 and 6 is nearly simultaneous, with the upstrokes in Fig. 5 preceding those in Fig. 6 by 7 ms. In Fig. 8A, recordings from two selected pixels in Fig. 7 have been superimposed. Trace 1 shows the first and odd APD long. Trace 2 shows the second and even APD long. The distance between these pixels is 4 mm. As a result of the out-of-phase alternans, there is reversal of

Fig. 2. Propagation of a control action potential across the surface of a rabbit heart stained with di-4-ANEPPS. Images consist of 10,000 pixels and are taken at intervals of 3.3 ms. Pixels are assigned a color based on the change in fluorescence from the previous image. Propagation of the action potential upstroke produces a yellow/orange wavefront, with yellow being the largest signal change and orange being an intermediate signal change. Pixels with stable (dV/dt = 0) are shown as dark red, violet, or dark blue. Pixels starting to repolarize (dV/dt < 0) are light blue. The pacing electrode is located at the apex of the right ventricle, and the earliest epicardial breakthrough occurs near the lower left corner of the figure (top image, first column), which corresponds to the right ventricular apex. The action potential spreads progressively from the lower left portion of the figure to the top right. The entire sequence of 15 images (top of first column to bottom of third column) takes 49.5 ms. The calculated conduction velocity is 0.34 m/s. Circles identify specific pixels. The coordinates of the white pixel are x = 45 and y = 84, with 0,0 being the upper left corner of each frame.
the repolarization sequence from beat to beat. For the first and third beat pairs, the mean APD<sub>50</sub> is 224 ms for trace 1 and 88 ms for trace 2. For the middle beat pair, the APD<sub>50</sub> is 122 ms for trace 1 and 133 ms for trace 2. The largest beat-to-beat variation is therefore 84% (trace 1), whereas the largest spatial variation is 155% (first and third beat pairs). This marked alternation in repolarization from beat to beat should produce dispersion of refractoriness and T wave alternans on an ECG.

Absence of conduction alternans during APD alternans. In Fig. 8A there is no variation in the timing of the action potential upstroke from beat to beat. Thus a marked variation in APD occurs during ischemia without detectable conduction alternans. A similar observation was made in a second heart, as shown in Fig. 8B. This recording shows no variation in conduction time during six consecutive action potentials despite a marked spatial difference in beat-to-beat APD alternans.

To further test for the presence of conduction alternans, the interval between action potential upstrokes
has been measured for all 115 pairs of consecutive action potentials in Figs. 5 and 6. Mean upstroke-to-upstroke interval for the 58 pairs of action potentials, in which a long APD precedes a short APD was $333 \pm 6$ ms (SD), whereas the mean value for the 57 pairs of action potentials, in which a short APD precedes a long APD was also $333 \pm 6$ ms. The mean SE was $1$ ms in each case. This observation shows that the variation in DI observed in Figs. 5 and 6 does not produce a measurable change in conduction. Because the conduction time in this experiment is $\sim 33$ ms (time from epicardial breakthrough in Fig. 3 to the first upstroke in Fig. 5) a very minor change in conduction would have been detected by this method.

It has also been suggested that ischemia-induced conduction alternans plays a necessary role in organizing spatially out-of-phase APD alternans. This possibility can be tested by the computation of conduction isochrone maps, as shown in Figs. 9 and 10. These figures are taken from the same image files as Figs. 4–6. Figure 9A shows the second beat in Figs. 4–6, whereas Fig. 9B is from the third beat. There is no evidence of conduction block in any portion of the image, and the positions of each isochrone are similar in both images.

Figure 10 shows conduction isochrones for all six beats for the specific region of the image shown in Fig. 7 (see the rectangles in Fig. 9). The position of each isochrone has been calculated at 18 specific points corresponding to the number of vertical pixels in the illustrated region. While there is a slight variation in the position of the isochrones, there is no systematic variation for odd (Fig. 10, 1, 3, and 5) and even beats (Fig. 10, 2, 4, and 6). The variation in the position of

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Fig. 5. Recordings from 12 contiguous pixels in the same rabbit heart as Figs. 3 and 4. Each of these 12 pixels shows alternans with the first and odd action potentials prolonged and the second and even action potentials briefer. The pixel in the top left corner is 43.56 (see map Fig. 7).
 Isochrones in Fig. 10 reflect a combination of true variation in conduction, frame timing errors (see METHODS), and limitations of the isochrone calculation program, which plots isochrone boundaries along the edges or between opposite corners of specific pixels. Variations that are random can be eliminated by averaging the isochrones from a series of action potentials. Mean isochrones have been constructed for the odd and even beats in Fig. 10 (not shown). The mean isochrones are generally parallel, and corresponding isochrones for odd and even beats are very close together. The mean distance between the 30-ms isochrones (measured in the x-direction) is 0.31 ± 0.19 mm (SD) on the heart surface (1.24 ± 0.76 pixels) with the even beats leading. The mean difference for the 40-ms isochrones is 0.32 ± 0.19 mm with the odd beats leading. With the use of the measured conduction velocity of 0.26 m/s, the even beats are leading by an average of 1 ms for the 30-ms isochrone, and the odd beats are leading by 1 ms for the 40-ms isochrone. Thus no localized or generalized conduction alternans could be detected for the action potentials in this region.

Prevalence of APD alternans during ischemia. Of the seven hearts studied, six showed APD alternans in at least some pixels, whereas one heart did not show APD alternans during ischemia. The experiment from which Figs. 2–6 were made had a better signal-to-noise ratio extending over more contiguous pixels than the other
Despite its clinical importance, there have been few studies (10, 14, 18, 21), in which action potential alternans has been directly observed during ischemia, and only one published study (18) in which sustained alternans was observed at two locations on the heart surface. That study involved the use of two monophasic action potential electrodes in the rabbit left ventricle face. Six hearts. In the six hearts that showed APD alternans, the two regions with alternans that was out of phase could be identified in three of the hearts, and in the other three, alternans of only one phase was found. However, the hearts where alternans of only one phase was found tended to have poorer signal quality with fewer interpretable pixels. Thus it is likely that most or all of the hearts had regions where APD alternans was out of phase. Qian et al. (30) found Ca$^{2+}$ transient alternans in each of the eight ischemic hearts studied with rhod-2, with regions where alternans was out of phase in six of the eight hearts. Because rhod-2 has a considerably better signal-to-noise ratio than di-4-ANEPPS, the ability to detect alternans and the completeness of the alternans maps would be better with rhod-2.

**DISCUSSION**

Despite its clinical importance, there have been few studies (10, 14, 18, 21), in which action potential alternans has been directly observed during ischemia, and only one published study (18) where sustained alternans was observed at two locations on the heart surface. That study involved the use of two monophasic action potential electrodes in the rabbit left ventricle during global ischemia, and contains one illustration of alternans that is spatially out of phase at a pacing rate of 300 beats/min. Janse et al. (14) attempted a similar experiment in open-chest pig hearts with the use of floating microelectrodes during coronary artery occlusion. They did not observe spontaneous alternans but were able to record a few beats of unstable, spatially heterogeneous APD alternans during a postocclusion rate jump that induced VF.

The present study is the first in which high-resolution optical mapping has been used to characterize the spatial distribution of APD alternans in an intact ischemic heart and its relation to conduction. This is also the first study in which a potentiometric dye has been used in a blood-perfused heart and may be the first study in which the effects of ischemia were studied with di-4-ANEPPS in the absence of motion blockers. Blood was present in these experiments because this more closely simulates clinical ischemia and because the degree of calcium transient alternans is greater in the presence of blood (38). Motion blockers were avoided because they might prevent alternans from being observed. Two laboratories have used di-4-ANEPPS to study defibrillation mechanisms in ischemic hearts treated with DAM (4, 13, 15). Ischemia still
reduced APD after DAM, but APD alternans was not reported. Concentrations of DAM that effectively block motion reduce myocardial oxygen consumption by 60% or more (1). Furthermore, DAM causes a substantial reduction in APD in the absence of ischemia, which could interfere with the development of alternans (23, 25).

The experiments reported here utilized the same experimental model and recording apparatus used by Qian et al. (30) to study the spatial characteristics of intracellular \(\text{Ca}^{2+}\) transient alternans in ischemia. In that study, \([\text{Ca}^{2+}]_i\) transient alternans was confined to specific regions of the heart surface, and multiple regions, which were out of phase and separated by a boundary, could be present in the same heart. Circumscribed regions of \([\text{Ca}^{2+}]_i\) transient alternans were as small as 5 mm and quantitative maps could be constructed using an alternans ratio.

Avoiding motion artifact without drugs. The problem of motion artifact is greater with potentiometric dyes than with long wavelength calcium indicators, owing to the much larger fluorescence change obtained with rhod-2 or Fura Red compared with di-4-ANEPPS. Ischemia produces mechanical alternans, which appears at about the same time as APD alternans in the rabbit heart. This mechanical alternans has been shown to exhibit spatial heterogeneity when studied with strain gauges having an interpin distance of 4 mm (21). Because mechanical alternans has never been studied at higher spatial resolution, some caution is needed in interpreting the di-4-ANEPPS signals shown here.

In the present study, motion artifact has been dealt with by manually selecting pixels that have undistorted action potentials with good signal-to-noise ratios. There are several indications that this strategy is satisfactory. First, action potentials that appear free of motion are restricted to the central part of the image where the heart is pressed against a glass surface. In contrast, the action potential upstroke, which precedes motion, can be mapped across most of the image (compare Figs. 9 and 4). Second, about one-half of the pixels in Fig. 6 show some degree of APA alternation, in which the taller action potential has a longer APD. Records with floating microelectrodes show the same pattern of APD and APA alternans during ischemia (10). Mechanical alternans could not affect APA because the upstroke precedes contraction (33). It follows that the APD and APA alternans in Fig. 6 are both authentic. Finally, motion artifact produces specific patterns of signal distortion, such as a prolonged action potential with a second rising phase, or a foreshortened action potential, followed by a deep undershoot (33). These patterns have been identified and excluded by manual inspection of single pixel recordings, and they disappear if a heart is treated with DAM. Thus, the extremely short action potentials observed during APD alternans (\(\text{APD}_{50} = 88\) ms), which have no undershoot, cannot be ascribed to motion (see Ref. 33, Fig. 5).

Data from tissue-cultured cells (33) show that when obviously distorted pixels are excluded, the largest error in the optically measured APD that can be attributed to motion is 8–9%. The amount of spurious beat-to-beat variation in APD that could be produced by mechanical alternans is even more limited because the weak and strong beats will contain the same error in varying proportions. This conclusion is supported by studies in both tissue-cultured cells and whole hearts (21), where the direction of the contraction is the same for strong and weak beats. Even if the strength of contraction during weak beats is zero, the amount of spurious beat-to-beat variation in the optical APD can be no greater than the measurement error for action potentials that accompany the strong beats. The effect
of mechanical alternans on the recordings is therefore negligible.

Absence of conduction alternans and mechanistic implications. An important observation in this study is the occurrence of marked spatial heterogeneity of APD alternans in the absence of detectable conduction alternans. Because of technical limitations, such as frame-timing errors, and the limited number of action potentials available in the image files for averaging, the occurrence of biologically significant conduction velocity alternans cannot be excluded with certainty. Nevertheless, the data suggest that spatially heterogeneous APD alternans during ischemia involves a different mechanism than occurs when rapid pacing is used to produce this phenomenon in nonischemic myocardium. There are five published reports in which spatially heterogeneous (out of phase) alternans is produced by rapid pacing. Three of these are experimental studies (11, 28, 29) and two are computer simulations of two-dimensional propagation (32, 36). In these studies, slow conduction velocities and a dependence of conduction velocity on DI are observed in conjunction with spatially out-of-phase alternans. In the study of Pastore et al. (28), out-of-phase alternans did not develop unless the pacing rate was 300/min or greater. Some of the DIs under these conditions were as short as 15–25 ms, and conduction alternans was grossly evident from QRS alternans in the ECG. In the study of Fox et al. (11), pacing rates of 600/min were needed.

In the present study, there is no detectable difference in conduction time after the short and long APDs, the pacing rate is much slower (180/min), and the DIs are much longer. The shortest DI in Fig. 8A is 80 ms, and the three shortest intervals in Fig. 8B give a mean value of 74 ± 6 ms. Furthermore, the boundary separating regions with out-of-phase alternans (which is sometimes called a node), can be almost perpendicular to the wavefront of action potential propagation, so that action potential upstrokes in pixels on either side of the boundary remain simultaneous before and during ischemia. This differs from the results of Fox et al. (11), where rapid stimulation of a Purkinje fiber at one end caused APD alternans that was out of phase at the opposite end.

While ischemia produces extracellular potassium accumulation, and a reduction in resting potential, which are known to impair conduction, we observed only moderate conduction slowing at 3 min of ischemia, with no additional slowing at short (70–80 ms) DIs. In nonischemic tissue, conduction velocity becomes maximal at DIs >50 ms (36). Because ischemia can produce postrepolarization refractoriness, changes in conduction cannot be predicted simply by measuring DI. How...
ever, the long DIs reported here are potentially consistent with the absence of conduction alternans in the isochrone maps and upstroke intervals.

The relationship between the DI and APD alternans has been studied by Koller et al. (16) during partial simulation of ischemia with high potassium. Elevation of extracellular K$^+$ to 12 mM caused marked APD alternans at long DIs, comparable to those in Fig. 8B. However, this only occurred at a pacing cycle length of 150 ms. KCl (12 mM) did not produce any APD alternans at a cycle length of 300 ms, which is 10% faster than the cycle length used here.

Both ischemia and very rapid pacing reliably induce VF, but they appear to do so by different mechanisms. For example, brief periods of ischemia induce fibrillation only in large animal hearts, whereas rapid pacing or stimulation on the T wave can induce VF in a heart of any size. Furthermore, drugs that prevent calcium overload, such as verapamil, diltiazem, or β-blockers prevent VF during ischemia (7) but do not prevent induction of VF by rapid pacing or change the electrical VF threshold under nonsimic conditions (2). The fact that ischemia can produce spatially heterogeneous (out of phase) APD alternans at slow pacing rates and long DIs is likely to be important for the genesis of VF during ischemia.

Relation of T wave alternans to APD alternans. The present study complements an earlier study, in which T wave alternans was mapped during ischemia with the use of multiple surface electrodes. Carson et al. (3) constructed isoarea difference maps of T wave alternans in pig hearts during coronary artery occlusion. Two to four minutes of ischemia produced “hot spots” of T wave alternans 5–10 mm in diameter, where the difference between successive T wave integrals was greatest in the center of the region and declined progressively toward the periphery. Conduction time was increased by 35 ms compared with nonsimic conditions, but there was no beat-to-beat variation in conduction, nor was there alternans in the amplitude of the R wave. The absence of conduction alternans under these conditions is in strong agreement with the results obtained here. Konta et al. (17) performed similar experiments in canine hearts and found frequent instances, in which the isoarea difference contours were positive in one region and negative in an adjoining region. In their study, four of eight dogs with T wave alternans had VF, and three of these had T wave alternans that was spatially out of phase just before VF onset. Nearing and Verrier (27) confirmed this result and also described more complex alternations in the T wave just before VF. Brief episodes of global ischemia do not produce VF in isolated rabbit hearts, but this may be due to other factors, such as tissue mass or denervation.

Although ECGs were not recorded in this study, it is clear that the observed spatial heterogeneity of APD alternans would contribute to and explain arrhythmogenic T wave alternans during ischemia. Pixels with out-of-phase APD alternans are close enough to each other that observed fluctuations in APD would produce an alternating flow of current in the extracellular space from beat to beat. Spatially out-of-phase APD alternans has been correlated with T wave alternans during rapid pacing (28), so it is fairly certain that T wave alternans could have been observed in the experiments reported here. Disparities in alternans between the endocardium and epicardium may also contribute to T wave alternans as suggested by recent recordings of T wave and Ca$^{2+}$ transient alternans during rapid pacing of canine wedge preparations (19).

Figure 8A is the first known example in which stable beat-to-beat reversal of repolarization has been demonstrated in closely spaced recordings during ischemia. Any degree of spatially heterogeneous APD alternans would theoretically cause beat-to-beat reversal of repolarization, but small amounts of alternans can be overridden by regional differences in APD, which were first described with global hypoxia (35), and are shown here for ischemia (Fig. 8B). Whereas net reversal of repolarization is not necessary to explain T wave alternans, it may be more arrhythmogenic than alternans without reversal.

Possible role of [Ca$^{2+}$]i in APD alternans. As noted above, APD alternans displays spatial features in the rabbit heart that are similar to those of [Ca$^{2+}$]i transient alternans. This lends support to the proposal that calcium transient amplitude controls the duration of the action potential plateau through effects on one of several calcium-regulated ionic currents. A rise in cytosolic calcium is known to produce inward current through the sodium calcium exchanger (8, 26), as well as outward current carried by calcium-activated chloride current (39, 40), and faster inactivation of the L-type calcium current through calcium-mediated inactivation (22). Consequently, calcium transient alternans could explain either concordant APD alternans, in which the taller calcium transient accompanies the longer APD (5, 6), or discordant alternans in which the reverse is true (37). The data reported here do not specifically establish the relation between calcium transient and APD alternans. However, based on this study, and the previous report of Qian et al. (30), it is likely that [Ca$^{2+}$]i and APD alternans occur conjointly in the same cells, and that spatially heterogeneous [Ca$^{2+}$]i and APD alternans can occur in the absence of conduction alternans.

Simultaneous recordings of calcium transient and APD alternans in ischemia have been obtained by Lee et al. (21) using the Franz monophasic action potential electrode and a large fiber-optic probe. However, the spatial resolution with this method was not as good as can be achieved with imaging cameras, and the signals did not arise from the same cells. Several methods have been proposed to record calcium transients and action potentials concurrently from small isopotential regions of the heart surface (5, 9, 20). These methods may provide more specific information about the ionic currents that produce APD alternans in ischemia.

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DISCLOSURES

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