Repolarization Inhomogeneities in Ventricular Myocardium Change Dynamically With Abrupt Cycle Length Shortening

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Background. **In single heart cells, abrupt changes in stimulation rate elicit complex alterations in repolarization. The effects of rate change on dispersion of repolarization, however, have not been well characterized.**

Methods and Results. **To determine the effects of abrupt cycle length (CL) shortening on spatial inhomogeneity of repolarization in a syncytium of ventricular cells, 124 action potentials were simultaneously recorded from Langendorff-perfused guinea pig hearts using high-resolution optical mapping with voltage-sensitive dye. The distribution of ventricular action potential durations (APDs) mapped during each cardiac cycle was used to calculate mean APD and repolarization dispersion index (DI), defined as the variance of the distribution. After abruptly shortening CL from 500 to 300 msec, mean APD declined exponentially in normoxic controls (by 23±3 msec, $p<0.0001$). This response was characterized by beat-to-beat oscillations of APD that were synchronized at all ventricular sites. After 30 minutes of hypoxia, mean APD decreased from 175.0±13.3 to 76±25.7 msec. However, during hypoxia, abrupt CL shortening lowered mean APD by only an additional 6±6 msec, and APD oscillations were no longer synchronized throughout the ventricle. In controls, beat-to-beat DI decreased significantly (−51.0±6.8%, $p<0.01$) by the sixth post-CL shortening beat and then recovered (by 15–20 beats). In contrast, DI failed to decrease during hypoxia (+7.1±23%). Two mechanisms for the transient decline of DI in controls were identified: synchronous APD oscillations and transient diminution of the apical-to-basal ventricular APD gradient.**

Conclusions. **These data demonstrate that inhomogeneity of ventricular repolarization, as measured by DI, changes dynamically with CL shortening. Furthermore, the hypoxic ventricle does not attenuate DI after abrupt CL shortening and thereby lacks a physiological response expected to diminish vulnerability to arrhythmias. (*Circulation* 1991;84:1333–1345)**

Abrupt shortening of cycle length (CL), whether spontaneous (e.g., ventricular premature beats)[1] or associated with programmed cardiac stimulation,[2] may provoke reentrant arrhythmias. Dispersion of tissue refractoriness is thought to be a prerequisite for reentry[3,4] as it may cause wave fronts propagating from the site of premature stimulation to encounter unidirectional block, conduction slowing, and fractionation from which reentrant eddies may arise. The precise timing of premature stimuli relative to the extent of spatial inhomogeneity of repolarization is critical to the initiation of reentry. In this context, tissue inhomogeneity is often viewed as an intrinsic property of myocardium and therefore not dependent on CL change per se. However, if one or more premature stimuli were to alter already existent inhomogeneities of myocardial repolarization, the probability of initiating reentry with premature stimuli would also be altered. Despite these implications for reentry, the precise effects of abrupt CL shortening on spatial inhomogeneity of repolarization within ventricular myocardium are not well understood.

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On the single cell level, repolarization is clearly sensitive to perturbations in CL. An abrupt increase in stimulation frequency results in progressive shortening of action potential duration (APD) in atrial,[5] Purkinje,[5,6] and ventricular muscle fibers[5–10] as APD decreases toward a steady-state value that is a function of the new stimulation rate. The characteristics of this response vary in different cardiac tissues. For example, after CL shortening, APDs oscillate from beat to beat in ventricular muscle and Purkinje fibers but not necessarily in atrial tissue.[5,9] Furthermore, APD oscillations of ventricular muscle and Purkinje cells may be asynchronous because muscle fibers often exhibit initial APD prolongation and Purkinje fibers often exhibit APD shortening on the beat immediately after the stimulation rate increase.[5,7,9,11] Although experimental confirmation is lacking, asynchronous APD oscillations between neighboring cells would be expected to promote spatial inhomogeneity of recovery and hence have been suggested as a factor responsible for the initiation of reentry by trains of premature stimuli.[5,9,12] Similar observations have been extended to human myocardium[13,14] with the suggestion that oscillations of refractoriness initiated by CL shortening may be an important mechanism of arrhythmogenesis[8,9,12] in some circumstances and of arrhythmia resistance[15] in others.

Direct experimental recordings of APD inhomogeneities have been limited by the small number of sites available for simultaneous recording of APD with conventional microelectrode techniques and the limited precision and spatial resolution of nonsimultaneous refractory period mapping. In this investigation, multisite, high-resolution, optical action potential mapping with voltage-sensitive dyes was used to study the aggregate cellular electrophysiological response of the intact ventricle to abrupt CL shortening. Our primary goal was to determine how beat-to-beat changes in APD at a single ventricular site affect the time course of myocardial recovery, particularly with regard to spatial inhomogeneity of repolarization. We sought to characterize time-dependent and dynamic properties of repolarization within the oxygenated, perfused guinea pig ventricle. Because hypoxia disrupts restitution of cellular repolarization[16] and has been shown to affect the phase, duration, and magnitude of APD oscillations that follow an abrupt change in stimulation frequency,[8] the effects of hypoxia on the APD response to CL shortening were also examined.

Methods

Experimental Preparation

Ten guinea pigs (300–400 g) were anesthetized (30 mg/kg i.p. nembutal) and decapitated, and their hearts were rapidly (within 60 seconds) excised and perfused via an aortic cannula with oxygenated (95% O_2–5% CO_2) Krebs-Ringers solution containing (mM) NaCl 130, $NaHCO_3$ 12.5, $MgSO_4$ 1.2, KCl 4.75, dextrose 5, and $CaCl_2$ 2.5 (pH 7.40, 32–34°C). The

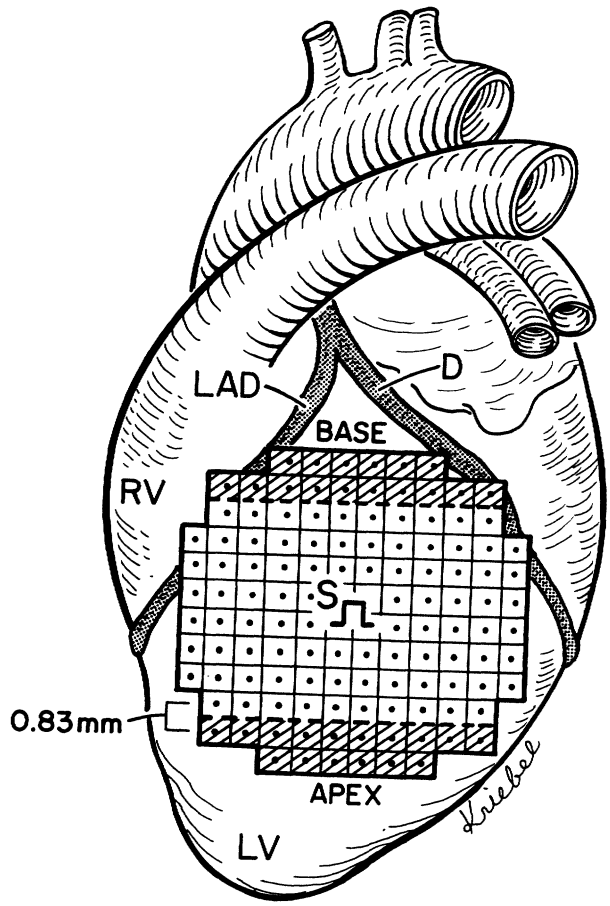

FIGURE 1. *Schematic of isolated heart preparation with photodiode mapping array shown superimposed on anterior left ventricle (LV). Basal and apical ventricular sites were defined as two rows within array (hatched regions) oriented closest to cardiac base and apex, respectively. LAD, left anterior descending coronary artery; D, diagonal coronary artery; RV, right ventricle; S, stimulus site.*

Langendorff perfusion pressure was fixed at 70 mm Hg by adjusting the height of the perfusion bath. Hearts were then stained with voltage-sensitive dye di-4-ANEPPS (Molecular Probes, Eugene, Ore.) by direct infusion into the coronary perfusate for 5 minutes at a final concentration of 10 μM.

The right atrium was excised to avoid competitive stimulation from the sinoatrial node, and the perfusion cannula was mounted on a micromanipulator. The preparation was then positioned in a Lexan chamber specifically designed for guinea pig hearts. Total immersion of the heart in the perfusate-filled chamber minimized thermal losses and motion artifact. The beating and perfused hearts were rotated about their vertical axes until the anterior surface of the left ventricle, approximately 5 mm inferior to the bifurcation of the left anterior descending and first diagonal coronary arteries, was centered in the mapping array (Figure 1). The ventricular epicardial surface was stimulated with a bipolar electrode (interpolar separation, 0.5 mm) consisting of two Tef-

lon-coated platinum wires (diameter, 0.2 mm) that were introduced through a glass capillary tube into the chamber. The stimulating electrode was positioned in the center of the optical mapping array (Figure 1). In addition, two sets of epicardial bipolar electrode pairs oriented orthogonally to each other (base to apex, left ventricular free wall to right ventricle) were used to monitor heart rhythm. Data recorded from these surface leads were amplified (×1,000) and filtered (0.1–500 Hz) with a low-noise differential preamplifier.

Two parallel perfusion systems were used to facilitate the rapid institution and reversal of hypoxia. In the normoxic system, the perfusate was gassed with 95% O_2–5% CO_2, whereas in the hypoxic system, the perfusate was gassed with 95% N_2–5% CO_2. Perfusate from both systems fed a common stopcock manifold regulator that could be switched to either the hypoxic or normoxic perfusate. The temperature of the coronary effluent was monitored with a thermister probe and maintained at 33°C with a jacketed heat-exchanging glass coil. Experiments in which temperature varied in excess of 3°C were excluded from analysis. All experiments were carried out in accordance with the guiding principles of the American Physiological Society.

Optical Mapping System

Voltage-sensitive dyes have been successfully used to measure changes in transmembrane potential in a variety of excitable cell preparations.[17,18] In sucrose-gap voltage-clamp experiments, the fractional changes of fluorescence and/or absorption of these dyes were found to vary linearly with changes in transmembrane voltage.[19] These dyes bind to the cell membrane and exhibit changes in fluorescence that reproduce the time course of cardiac action potentials with high fidelity during various ionic and pharmacological interventions.[20] The similarity of waveform characteristics of optically recorded action potentials to those of action potentials recorded by standard microelectrode techniques have been validated previously.[17,21] With a 12×12 array of closely spaced photodiodes (each 1.4×1.4 mm, with 100 μm of dead space), multiple transmembrane action potentials were recorded simultaneously from an electrically active syncytium with high spatial and temporal resolutions.

The details of the optical mapping apparatus used in the present study have been described elsewhere.[20] Briefly, hearts were uniformly illuminated by two tungsten-filament lamps oriented at a 30° angle of incidence to the surface of the preparation. Interference filters (λ=540±20 mm) were used to obtain a quasimonochromatic excitation beam. Fluoresced light emitted from membrane-bound dye was collected through a variable depth-of-field lens (Nikon 50 mm, f 1:1.4) and focused on a 12×12 element photodiode array.

The configuration of the photodiode array (Figure 1) permitted sampling of action potentials from 124 discrete recording sites. The magnification was adjusted for this study so that the entire 12×12 mapping array corresponded to 10×10 mm of ventricular myocardium, and each equally spaced photodiode element detected action potentials from a 0.56-mm^2 area of muscle. Therefore, the optical action potential measured at each recording site was a spatial sum of action potentials of the cells within a 0.56-mm^2 area and up to 200 μm in depth.[19] This degree of optical magnification resulted in a spatial resolution of 0.83 mm between individual recording sites (Figure 1).

The photocurrent from each photodiode was fed to a current-to-voltage converter, and the voltage signal was filtered (AC coupled with a 3-second time constant), amplified (×3,000), and sampled (1,000 Hz with eight-bit precision) to memory of a PDP 11/73 microcomputer (Digital Equipment Corp., Westfield, Mass.). At the time of data collection, selected analog signals were displayed on a dual-beam storage multichannel oscilloscope (Tektronix, Beaverton, Ore.). Digitized action potentials recorded from all recording sites were displayed simultaneously on a high-resolution storage cathode-ray tube terminal (model 4110A, Tektronix). Action potentials were disregarded if electrical or mechanical noise obscured the precise detection of cellular depolarization or repolarization. In these experiments, the rapid upstrokes of action potentials were essentially always distinguished from motion artifacts because they preceded the onset of visibly apparent contraction artifacts by 100–200 msec. Furthermore, less than 5% of the action potentials had repolarization phases that were not resolved because of the superposition of motion artifacts. Data were subsequently transferred to a Unix-based microcomputer (SUN Microsystems, Mountain View, Calif.) for off-line analysis.

Experimental Protocol

The epicardial surface of the left ventricle was stimulated at a site corresponding to the center of the mapping array (Figure 1) with a 2-msec rectangular pulse delivered at twice diastolic threshold current (Medtronics stimulator, Minneapolis, Minn.). Baseline stimulation at a CL of 500 msec was performed for at least 5 minutes to ensure steady-state conditions, and the stimulus CL was then abruptly shortened to 300 msec. Optical action potentials were measured simultaneously from all mapping sites during 30 consecutive cardiac cycles, five cycles at the steady-state rate (CL, 500 msec), and 25 cycles at the faster rate (CL, 300 msec).

These stimulus CLs were selected so that hearts could withstand 30 minutes of hypoxia and still have clearly measurable APDs and essentially normal patterns of depolarization (i.e., activation wave fronts). CLs of less than 300 msec (i.e., faster than the normal heart rate of guinea pigs [200 beats/min]) would elicit arrhythmias during such long hypoxic intervals. Nevertheless, this duration of nitrogen perfusion (30 minutes) was chosen to ensure that all hearts reached similar levels of hypoxia in a

quasiequilibrium state to allow meaningful comparisons between hearts.

The response to CL shortening was analyzed for 20 experiments performed in 10 animals. The stimulation protocol was first performed during normoxic perfusion in all 10 animals (controls) and then repeated after 30 minutes of hypoxia ($n=5$) or 30 minutes of continued normoxic perfusion ($n=5$). The latter group served to ensure stability of the preparation and reproducibility of baseline results over time.

Data Analysis

Because of the large numbers of action potentials (74,400) analyzed for these experiments, an automated algorithm was developed for measuring APD. APD is the difference between the time of cellular depolarization and repolarization. Depolarization time was defined as the point of maximum first derivative within each action potential, which corresponds to a point in the rapid upstroke (phase 0). For all action potentials, repolarization was defined as the time point marking the end of phase 3 and the onset of phase 4 of the action potential. This point was identified by the local maximum of second derivative measured in a window starting from the point of the minimum first derivative and ending at the onset of the subsequent cardiac cycle.[22] This corresponds to the time of greatest change in the slope of the action potential during repolarization. All automated assignments of cellular depolarization and repolarization time were reviewed by the investigators using a multichannel display program. On occasion (less than 10% of action potentials), it was necessary to correct computer-generated depolarization or repolarization times by positioning a mouse-driven cursor over the appropriate point of the action potential.

After determining APD at each of 124 ventricular recording sites for 30 cardiac cycles, the average APD ($\overline{APD}$) measured during each cardiac cycle (c) was calculated as follows:

$$\overline{APD}_c=\frac{1}{124}\sum_{k=1}^{124} APD[k]_c \qquad (1)$$

where APD[k] is the APD measured at each ventricular site (k) during any cardiac cycle (c). The change of $\overline{APD}$ with CL shortening was followed as a measure of beat-to-beat aggregate myocardial APD response (i.e., $\overline{APD}_1$, $\overline{APD}_2$, $\overline{APD}_3$. . .). Initial APD (APD_0) at each site was defined as the mean of five APDs recorded from that site during baseline stimulation at a stimulus CL of 500 msec. The average of all APD_0 in the mapping array served as a measure of mean baseline ventricular APD ($\overline{APD}_0$). The final APDs (APD_{25}) measured at each recording site after 25 cycles of pacing at the shorter CL (300 msec) were averaged ($\overline{APD}_{25}$), and this value served as an index of final aggregate ventricular APD. The magnitude of $\overline{APD}$ change after CL shortening was defined as the difference between initial and final $\overline{APD}$ (i.e., $\Delta\,\overline{APD}=\overline{APD}_0-\overline{APD}_{25}$).

To assess specific responses of apical and basal ventricular cells, a similar analysis was performed using 16 preselected mapping sites that were oriented in closest anatomic proximity to either the cardiac apex or base. The most superior and inferior two rows of recording elements within the mapping array were designated as basal and apical cells, respectively (Figure 1). Beat-by-beat $\overline{APD}$ derived from these two subpopulations of ventricular sites were compared within each experimental preparation.

In addition to quantifying the overall magnitude of $\overline{APD}$ changes after CL shortening, the time course of the $\overline{APD}$ response was also evaluated. In controls, $\overline{APD}$ was measured as a function of time after CL shortening and fitted to a monoexponential as defined by the following equation:

$$\overline{APD}(t)=\overline{APD}_{25}+\Delta\,\overline{APD}\cdot e^{-t/\tau} \qquad (2)$$

where $\overline{APD}(t)$ is $\overline{APD}$ measured t seconds after the stimulation frequency was increased, $\Delta\,\overline{APD}$ is the initial-to-final $\overline{APD}$ difference ($\overline{APD}_0-\overline{APD}_{25}$), and τ is the time constant of APD change. Exponential function parameters were systematically adjusted by the Marquardt-Levenberg method until a least-squares solution was reached (RS1-BBN Research Systems, Cambridge, Mass.). The time constant (τ) was calculated for each normoxic experimental preparation and used to compare the kinetics of APD responsiveness between apical and basal ventricular cells. During hypoxia, however, $\overline{APD}$ declined in a *linear* fashion after stimulus frequency was increased and thus could not be modeled as an exponential function. Therefore, the time courses of the hypoxic and normoxic responses were compared using a response time defined as the time required after the abrupt increase in stimulation frequency to first achieve 90% of the $\overline{APD}_0-\overline{APD}_{25}$ difference. For example, if $\overline{APD}$ shortened by 90% of its baseline value ($\overline{APD}_0$) on the fifth cardiac cycle ($\overline{APD}_5$) after the sudden decrement in CL (to 300 msec), the response time would be five cardiac cycles × 300 msec/cardiac cycle = 1.5 seconds.

Oscillation time was defined as the duration of time that $\overline{APD}$ oscillations persisted after CL was shortened from 500 to 300 msec. Oscillation times measured from apical and basal ventricular sites were compared in each experiment.

Finally, the variance of the APD distribution mapped from all 124 ventricular sites during each cardiac cycle served as a beat-to-beat index of inhomogeneity of ventricular repolarization.

$$DI_c=\frac{1}{124-1}\sum_{k=1}^{124}(\overline{APD}_c-APD[k]_c)^2 \qquad (3)$$

where $\overline{APD}_c$ is the average APD measured during each cardiac cycle (c) (see Equation 1). This value,

TABLE 1. Response to Abrupt Cycle Length Shortening During Normoxic and Hypoxic Perfusions

	Baseline APD (msec)		Diastolic interval (msec)		Δ APD (msec)		Δ APD (%)		Response time (seconds)		ΔDI_{max} (%)	
Experiment	C	H	C	H	C	H	C	H	C	H	C	H
1	191	70	109	230	−29	−4	−15	−6	3.0	6.9	−49	+9
2	158	53	142	247	−23	−1	−15	−3	5.1	6.9	−62	+50
3	165	78	135	222	−20	−3	−12	−4	1.8	5.4	−43	−4
4	185	125	115	175	−22	−17	−12	−14	2.1	6.3	−51	−21
5	181	61	119	239	−21	−3	−11	−4	1.5	7.5	−51	+2
Mean±SD	176±12	77±25*	124±12	222±25*	−23±3	−6±6*	−13±2	−6±4†	2.7±1.4	6.6±0.8*	−51±7	+7±23†

Baseline APD, average action potential duration ($\overline{APD}$) measured immediately before cycle length (CL) shortening from 500 to 300 msec; diastolic interval, average interval between repolarization time of last action potential stimulated at CL of 500 msec and depolarization time of first action potential stimulated at CL of 300 msec; Δ APD, magnitude of $\overline{APD}$ change (Δ APD=$\overline{APD}_0$−$\overline{APD}_{25}$) in response to CL shortening expressed as actual value and as percent change from pre-CL shortening baseline; response time, time to achieve 90% of final APD; Δ DI_{max}, maximum change in dispersion of repolarization index (see text); C and H, control and hypoxia, respectively.
*$p<0.005$, †$p<0.05$.

referred to as the dispersion of repolarization index (DI), was calculated for each cardiac cycle that followed CL shortening (DI_1, DI_2, DI_3. . .) and was compared with the baseline DI (DI_0) measured during steady-state pacing before CL shortening.

Statistical Methods

Comparisons of $\overline{APD}_0$ with $\overline{APD}_{25}$ in response to CL shortening were made with a Student's two-tailed paired *t* test. Percent changes of $\overline{APD}$ and DI during hypoxia were compared with controls using an unpaired *t* test for populations of unequal variances. All values are expressed as mean±SD unless stated otherwise. Probability values of less than 0.05 were considered statistically significant.

Results

Magnitude of Action Potential Duration Response to Cycle Length Shortening

During oxygenated perfusion, $\overline{APD}$ decreased from 175.0±13.3 msec at baseline ($\overline{APD}_0$) to 152.8±12.0 msec ($\overline{APD}_{25}$) 25 cycles after an abrupt increase in stimulation frequency ($p<0.0001$). In the subgroup of experiments ($n=5$) in which the stimulation protocol was repeated after 30 minutes of continued normoxic perfusion, neither $\overline{APD}_0$ (172.2±9.3 msec) nor $\overline{APD}_{25}$ (155.2±9.8 msec) changed significantly ($p>0.05$). These observations demonstrated that our APD measurements were reproducible and stable under controlled experimental conditions over relatively long time periods. They also verify reproducibility of the APD response to CL shortening. Because this subgroup of continuous normoxia experiments served to confirm the stability of the preparation over time, data from these experiments were not used as controls for the hypoxia group. Instead, the prehypoxia and posthypoxia responses of each animal were compared so that each animal served as its own control.

After 30 minutes of hypoxia, $\overline{APD}_0$ (76.1±25.7 msec) measured at the baseline stimulus CL of 500 msec was significantly shorter than control $\overline{APD}_0$ measured at the same CL ($p<0.001$). The total change in $\overline{APD}$ (Δ APD) that followed CL shortening during hypoxic perfusion compared with the control response is given in Table 1. In hypoxic preparations, $\overline{APD}$ decreased minimally with CL shortening compared with controls. Even when expressed as a percent change from $\overline{APD}_0$ (Table 1), average $\overline{APD}$ decreased by only 6±4% during hypoxia compared with 13±2% in oxygenated ventricles ($p<0.01$). Therefore, the blunted APD response could not be solely attributed to reduction of absolute APD that was present during hypoxia. Results obtained in one experiment (4) did deviate from this overall trend insofar as percent $\overline{APD}$ changes were similar before and after hypoxia. There were no obvious differences in this preparation with regard to viability as judged by action potential morphology or magnitude of APD change with CL shortening during normoxic perfusion. Although hypoxia appeared to not affect the magnitude of APD change in this experiment, the time course of the APD response to CL shortening was slowed to the same extent in this experiment as in other hypoxia experiments (Table 1, see "Response time").

A linear relation ($r^2=0.95$ by linear regression) between the diastolic interval of the first prematurely stimulated action potential and subsequent APD shortening was found across all experimental preparations. As shown in Table 1, hearts with the shortest initial diastolic intervals manifested the most marked changes in APD after CL shortening. These findings suggest that diastolic interval was a common determinant of APD change during hypoxia and normoxia and may explain the seemingly anomalous result obtained during hypoxia experiment 4 (Table 1) as the change in APD observed in this preparation relative to diastolic interval was similar to that in the other hypoxia and normoxia experiments.

Despite its effects on repolarization, hypoxia did not significantly disrupt the pattern and time course of ventricular depolarization. As shown in Figure 2, the patterns of ventricular activation were similar before and after hypoxia. In this example, activation times are slightly delayed in the periphery of the map; however,

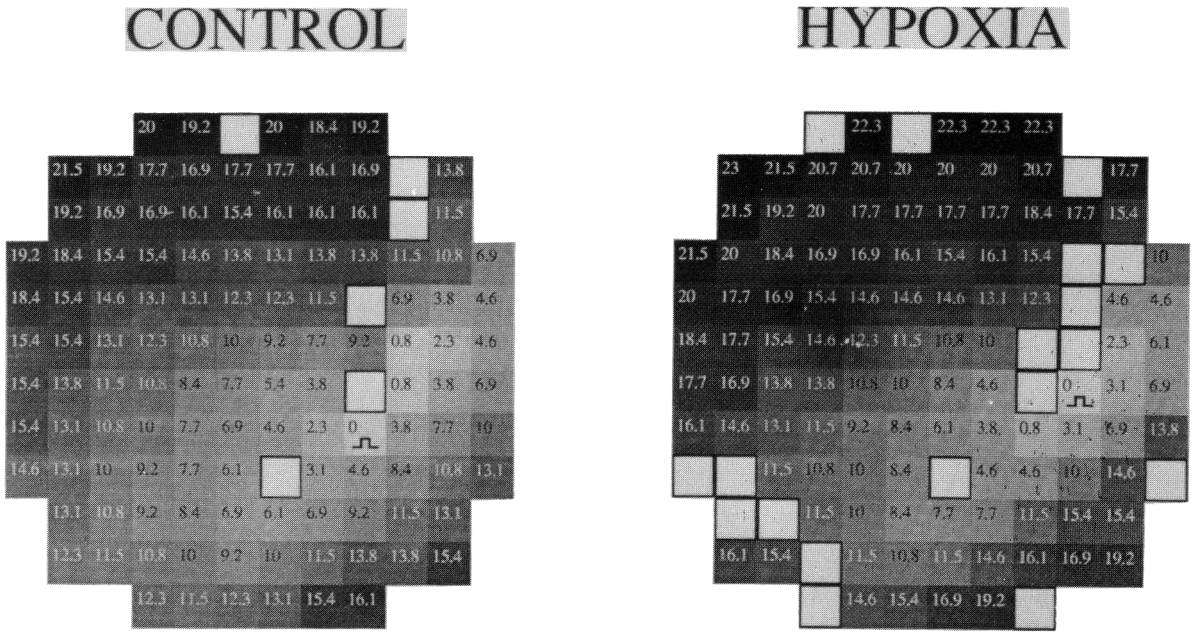

FIGURE 2. *Activation sequence maps recorded while pacing (square wave equals pacing site) an experimental preparation before (control) and during hypoxia. Depolarization time at each recording site is shown in msec. Gray scale contours demonstrate similarity of activation patterns during both experimental conditions. Sites containing suboptimal action potential tracings are indicated by empty squares.*

there are no obvious changes in the homogeneity or continuity of conduction during hypoxia.

Dynamics of Action Potential Duration Response to Cycle Length Shortening

In addition to depressing the absolute magnitude of APD shortening, the time courses of APD changes were slowed by hypoxia as the calculated response time was significantly longer after the imposition of hypoxia (Table 1). Although there was considerable variability of response time within the control group, response time was always prolonged during hypoxia relative to controls within the same experimental preparation.

A representative example of one experiment is shown in Figure 3. Beat-by-beat $\overline{APD}$ is plotted as the stimulation CL was abruptly shortened from 500 to 300 msec. As expected, absolute $\overline{APD}$ is reduced during hypoxia compared with during normoxia at both CLs. In controls, after the stimulus rate was increased, beat-to-beat $\overline{APD}$ declined rapidly in a dampened oscillatory manner. In this case, $\overline{APD}$ oscillations persisted throughout the duration of the 25-cycle recording period. By contrast, during hypoxia, beat-to-beat $\overline{APD}$ decreased slowly, and the magnitude of the overall change was relatively small. Furthermore, beat-to-beat oscillations were unpredictable and far less pronounced.

In normoxic controls, beat-to-beat oscillations of APD were present at all recording sites. Oscillations were initiated by slight prolongation (one of five experiments) or slight shortening (four of five experiments) of APD on the beat after CL shortening and followed by a brisk reduction of APD on the second

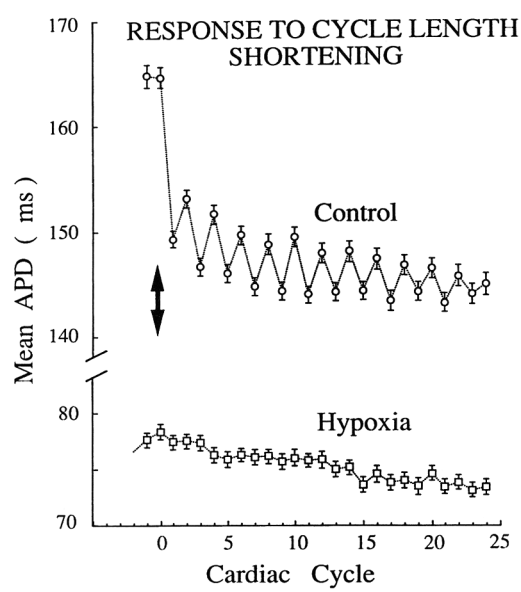

FIGURE 3. *Plots of data from representative experiment illustrating mean action potential duration (APD) within mapping array during an abrupt increase in stimulation rate. Drive cycle length is shortened (vertical arrow) to 300 msec after baseline pacing a cycle length (500 msec). In oxygenated myocardium (controls), abrupt increase in heart rate resulted in a rapid decline of $\overline{APD}$ with superimposed beat-to-beat dampened $\overline{APD}$ oscillations. Hypoxic myocardium exhibits minimal decrement of $\overline{APD}$ after increase in stimulation rate and beat-to-beat $\overline{APD}$ oscillations were greatly attenuated. Error bars indicate SEM.*

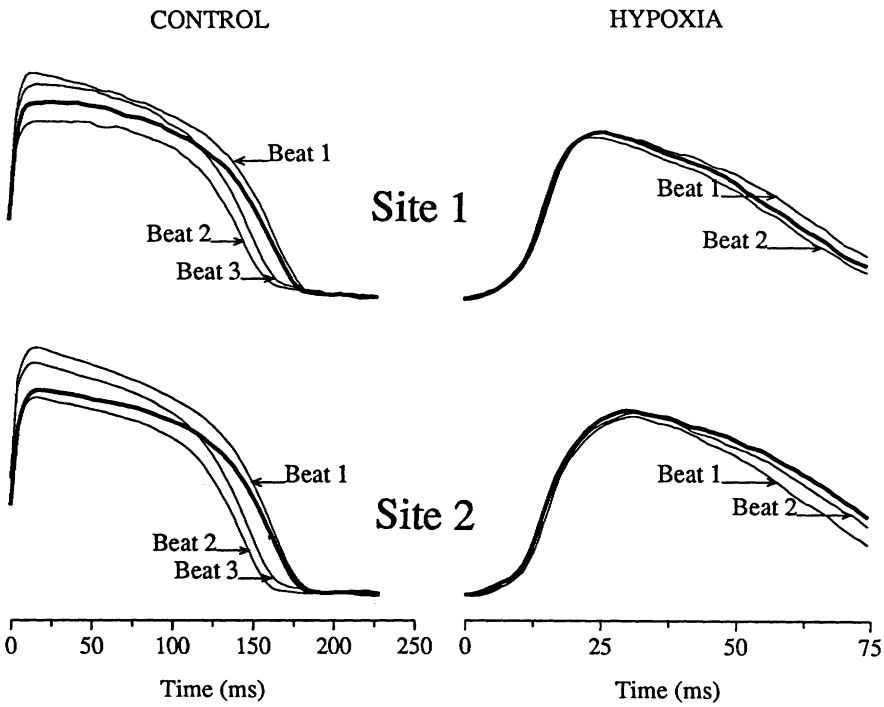

FIGURE 4. *Simultaneous recordings of action potentials from two ventricular sites separated by 8 mm. Change in action potential morphology at each site in response to an abrupt shortening of stimulation cycle length (CL) is shown before (control) and after hypoxia. Bold action potentials were recorded during baseline stimulation at a basic CL of 500 msec. Signals labeled beat 1, beat 2, and beat 3 were first, second, and third action potentials, respectively, recorded immediately after stimulation rate was increased (CL, 300 msec). Oscillations of APD are synchronized in controls but not during hypoxia (see text). Vertical scale of action potentials recorded during hypoxia has been doubled for clarity.*

beat and then by persistent long-short-long alternating intervals that typically dampened by the 20th to 25th cardiac cycle. Comparisons between the baseline action potential and action potentials recorded for three beats subsequent to an abrupt increase in stimulation rate are shown in Figure 4. Action potentials recorded from two distant sites within the mapping array (separated by 8 mm) under normoxic (control) and hypoxic conditions are illustrated. During normoxic perfusion, APD prolongs slightly after the CL of stimulation is reduced (beat 1), but by the second and third beats, marked reductions of APD are evident. Note that despite their spatial separation, the two myocardial recording sites exhibit APD oscillations that are synchronized in phase (both have long-short-long sequences). Synchronized oscillations were consistently observed at all ventricular sites during normoxic perfusion. In contrast, when the same cells were rendered hypoxic, APD oscillations decreased in magnitude and were no longer synchronized in phase (Figure 4). Note that action potentials recorded from these sites exhibit simultaneous long-to-short (site 1) and short-to-long (site 2) APD oscillations immediately after CL was shortened from 500 to 300 msec (Figure 4).

Effect of Cycle Length Shortening on Repolarization Inhomogeneity

A beat-to-beat index of repolarization inhomogeneity, DI, was calculated during baseline stimulation and during each of 25 cardiac cycles that followed the abrupt increase in stimulation frequency. The results of one representative experiment are shown in Figure 5. During normoxic perfusion (control), DI decreased immediately after the stimulation rate was increased. This response was transient in nature, however, as DI reached a nadir by the sixth post-CL shortening beat and then returned to its baseline value by the 22nd postshortening cardiac cycle (Figure 5). A sharp but transient decrease of inhomogeneity (i.e., greater homogeneity of repolarization) was observed in all normoxia experiments. DI achieved a minimum value by the third to sixth post-CL shortening beat and recovered to baseline within 25 beats in all normoxia experiments. By contrast, after 30 minutes of hypoxia, DI failed to decrease with the change in CL and actually increased slightly (Figure 5).

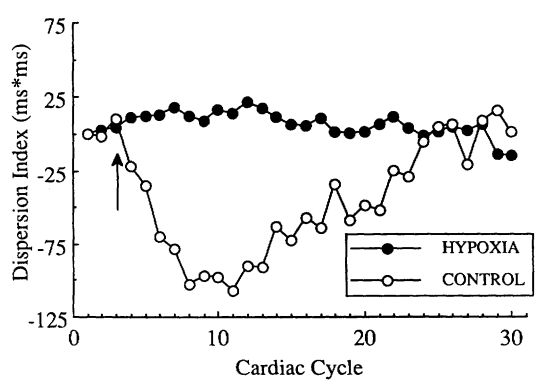

FIGURE 5. *Plots of dispersion of repolarization index (DI) measured during each cardiac cycle shown as stimulation cycle length (CL) is shortened from 500 to 300 msec (vertical arrow). Values plotted are change of DI relative to DI during baseline stimulation at basic CL (500 msec). DI decreases rapidly in oxygenated myocardium (control) as a transient response to CL shortening and returns to baseline values by 26th cardiac cycle. DI fails to decrease in hypoxic ventricle.*

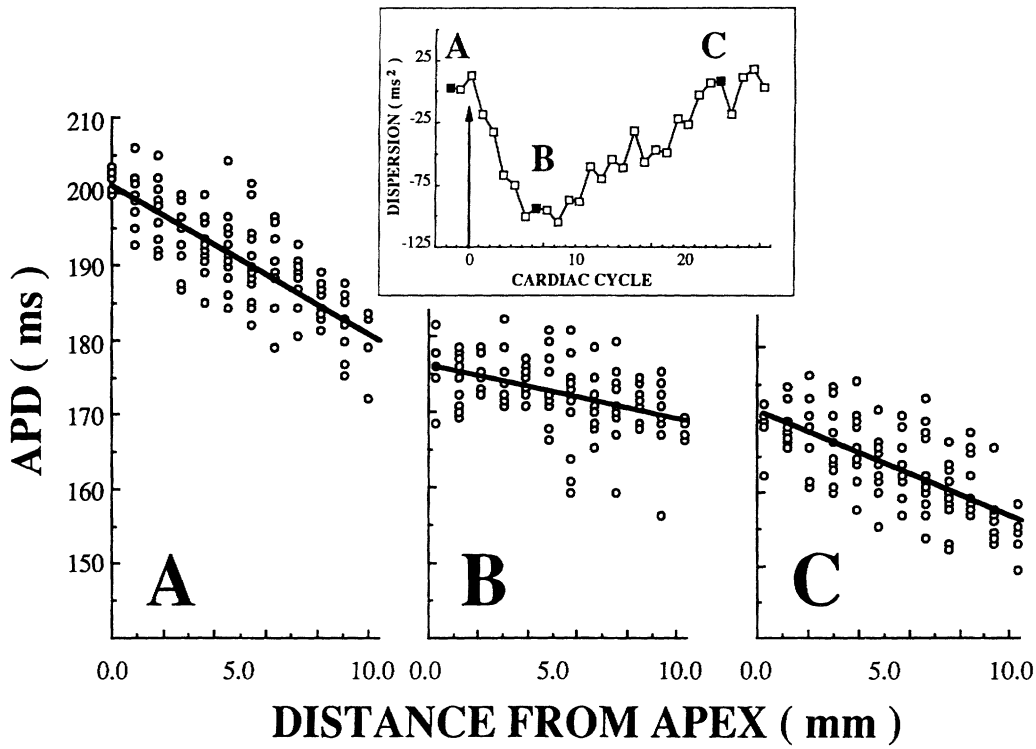

FIGURE 6. *Plots of action potential duration (APD) recorded from each mapping site as function of site's distance from cardiac apex shown during three phases of response of dispersion index (inset, Figure 5) to sudden cycle length (CL) shortening. Apex-to-base APD gradient, as indicated by slope of regression line, is shown during baseline steady-state stimulation at a CL of 500 msec (gradient, −2.0 msec/mm,* panel A*), after abrupt CL shortening to 300 msec as dispersion reaches a nadir (gradient, −0.4 msec/mm,* panel B*), and as dispersion recovers to baseline (gradient, −1.7 msec/mm,* panel C*).*

The maximum change in DI (Δ DI_{max}) was defined as the maximum percent change from baseline DI measured after the stimulation rate was abruptly increased. When considering all experiments (see Table 1), mean DI decreased by 51±7% (Δ DI_{max}=−51±7%) in controls but increased by 7±23% (Δ DI_{max}=+7±23%) during hypoxia ($p<0.01$).

Mechanisms for Change in Repolarization Inhomogeneity

Dynamic modulation of APD gradients. To fully evaluate the underlying basis for the rate-induced decrease in spatial inhomogeneity observed in controls, it was necessary to examine regional differences in the APD response to CL change. During steady-state pacing, dispersion of repolarization was in part due to an apex-to-base gradient of APD. This gradient was due to progressive lengthening of APD from the ventricular base to apex. Furthermore, the magnitude of the APD gradient was sensitive to transient increments in stimulation frequency. This phenomenon is depicted graphically in Figure 6, in which APD measured at each ventricular site was plotted during three distinct time points of the DI response to CL shortening (same experiment as Figure 5). Time point A (Figure 6A) corresponds to baseline stimulation at a CL of 500 msec, point B (Figure 6B) occurs when DI reaches a nadir in response to abrupt CL shortening to 300 msec, and point C (Figure 6C) coincides with recovery of DI to baseline. Beat-by-beat DI relative to baseline DI is shown in the figure inset. The bottom (most apical) row of the mapping array served as the zero reference, and the APD measured at each site was plotted as a function of its distance from the reference row (distance from apex). The slope of the linear regression line derived from the relation of APD versus distance from apex was used to estimate the magnitude of the apex-to-base APD gradient. At baseline (Figure 6A), a steep apex-to-base APD gradient is evident. As stimulus frequency is increased and DI reaches a minimum, the APD gradient also diminishes, as evident by the less steep regression line in Figure 6B. Therefore, the reduction of DI (i.e., APD inhomogeneity) was in part due to transient reduction of the apex-to-base APD gradient. By the 24th cardiac cycle, DI returns to its baseline value and the APD gradient is also restored near baseline, even though absolute APDs have shortened substantially from their baseline values (Figure 6C).

The basis for the maintenance and the rate-induced change of the apex-to-base APD gradient was examined by comparing repolarization properties of ventricular apical and basal tissue sites. Baseline APD ($\overline{APD_0}$) was longer at apical sites (181±13 msec) than at basal sites (168±13 msec), as was the final APD ($\overline{APD_{25}}$) measured 25 cycles after the abrupt decrease in stimulation CL (apical $\overline{APD_{25}}$,

TABLE 2. Differential Responses of Apical and Basal Cells to Cycle Length Shortening

	Δ APD (msec)		τ (seconds)		Oscillation time (seconds)	
Experiment	Apex	Base	Apex	Base	Apex	Base
1	−26	−26	0.4	0.9	1.5	1.8
2	−22	−25	1.9	3.5	3.9	0.6
3	−23	−22	0.2	1.3	7.5	5.7
4	−25	−19	0.6	0.7	7.5	5.1
5	−24	−20	0.8	1.0	3.0	0.9
Mean±SD	−24±2	−22±3	0.8±0.6	1.5±1.2*	4.8±2.4	2.7±2.1*

Apical and basal ventricular cells are compared with regard to time course of action potential duration (APD) changes that follow an abrupt increase in stimulation frequency. Beat-by-beat mean APD was fit to a monoexponential ("Results") with the magnitude of response Δ APD and time constant τ shown for each experiment. Oscillation time indicates time that APD oscillations persisted after stimulus cycle length was shortened.

*$p<0.05$.

157±11 msec; basal $\overline{APD}_{25}$, 146±14 msec). The magnitudes of $\overline{APD}$ shortening in response to the increase in stimulation frequency ($\overline{APD}_0-\overline{APD}_{25}$), however, were nearly identical at the apex and base (see Δ APD, Table 2).

Time-dependent changes in beat-to-beat $\overline{APD}$ were modeled as a monoexponential decay from the pre–CL shortening baseline (see Equation 2). The magnitudes (Δ APD) of exponential responses were similar for apical (22±3 msec) and basal (21±4 msec) tissue sites and closely fit the experimentally measured values (see Δ APD, Table 2). However, ventricular apical and basal myocardium differed significantly with regard to the time constant of $\overline{APD}$ change. As shown in Table 2, the time constant (τ) was significantly slower in basal than in apical sites. The transient reduction of the apex-to-base APD gradient observed after the increase in stimulation rate could thus be attributed to more rapid lowering of longer APDs in the ventricular apex compared with a slower reduction of shorter APDs in the ventricular base.

The role of the APD gradient in modulating dynamic changes in dispersion are demonstrated graphically in Figure 7. The exponential decline of APD within apical and basal myocardium is derived from Equation 2 using experimentally measured exponential parameters (Δ APD, τ, in Table 2), and the resultant apex-to-base difference is used to estimate time-dependent changes of the APD gradient (Figure 7). Note that the APD gradient achieves a minimum value within two to six beats after cycle length is shortened, and its time course closely coincides with the experimentally measured response of dispersion (DI) to CL shortening (see control response in Figure 5). This supports the notion that differential responsiveness of apical and basal tissue was an important factor for dispersion change in these experiments.

Synchronous APD oscillations. The presence of synchronized APD oscillations was also an important factor for dispersion change after CL shortening. During normoxic perfusion, the time of most rapid decline of dispersion coincided with a period when

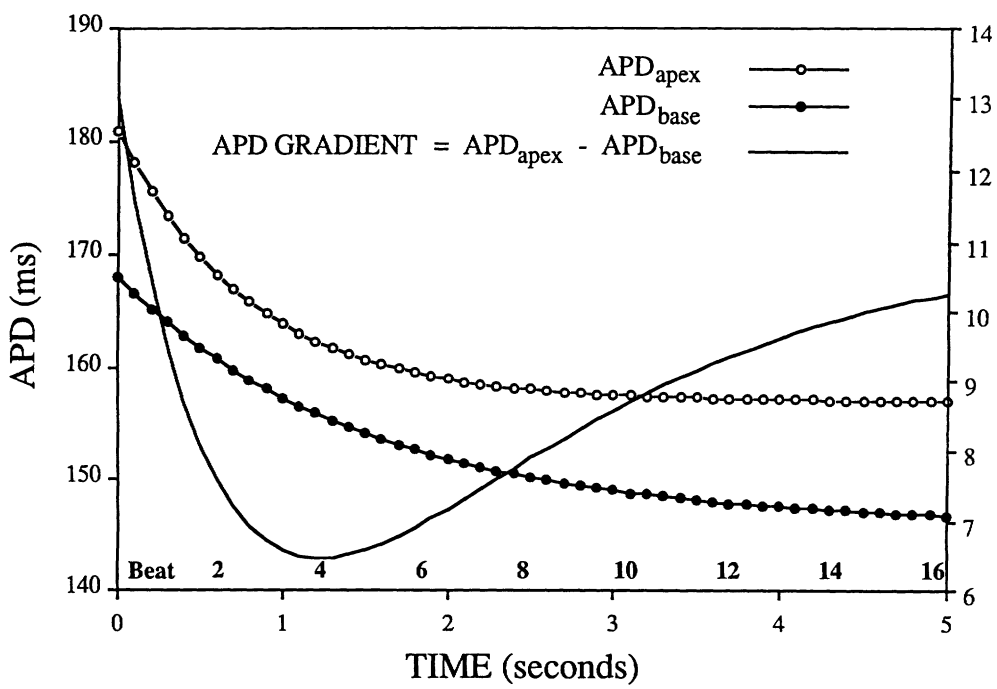

FIGURE 7. *Plots of time-dependent change of apex-to-base action potential duration (APD) gradient are predicted from difference between apical and basal APDs. Effects of abrupt cycle length shortening on APD within apical and basal myocardium are derived from Equation 2 (see text) using experimentally measured exponential parameters (Table 2, Δ APD, τ). Note similarities between time-dependent change in APD gradient and time course of dispersion change (Figure 5, control).*

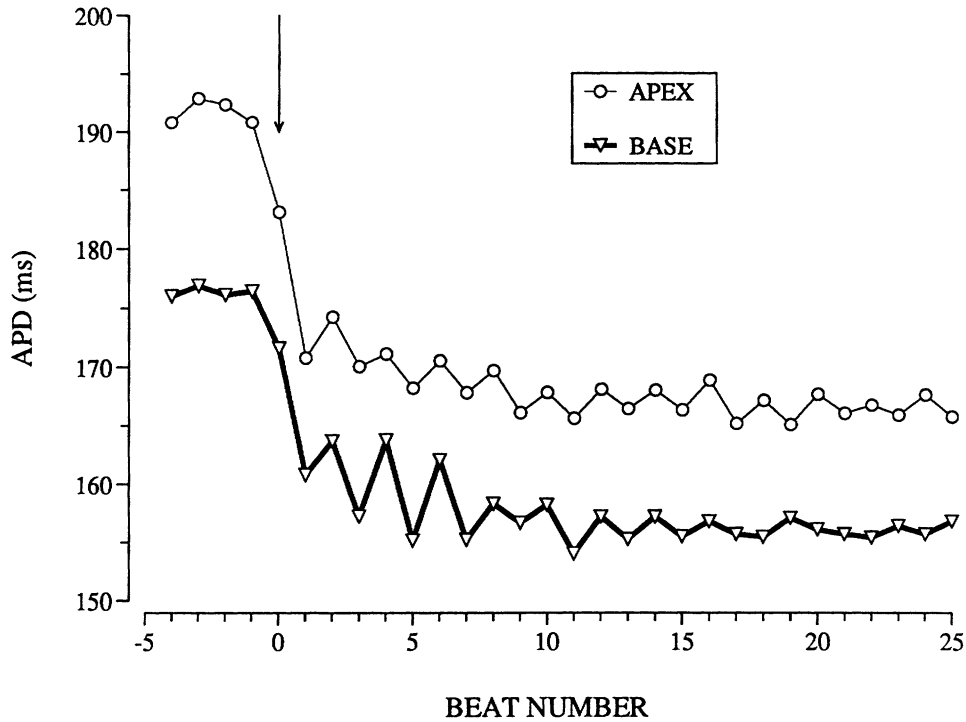

FIGURE 8. *Plots of data from representative example of mean action potential duration (ADP) changes measured in apical compared with basal ventricular myocardium after stimulation frequency was increased (arrow).*

APD oscillations were most pronounced. If these oscillations were not synchronized, the observed change in dispersion would not have occurred. For example, at baseline, the APDs of apical and basal sites differed by approximately 20 msec (Figure 6); as dispersion reached its nadir, this difference decreased to 4 msec, accounting for much of the dispersion decrease. At the same point in time, the magnitude of APD oscillations was on the order of 5 msec (Figure 3, control), which exceeds the apex-to-base APD difference. If APD oscillations of this magnitude were not synchronized, any dispersion-lowering effect of a reduced apex-to-base APD gradient would be overcome by repolarization inhomogeneity resulting from asychronous APD oscillations.

Apical and basal myocardium also differed with respect to the duration over which beat-to-beat $\overline{APD}$ oscillations persisted after stimulation frequency was increased. Oscillations were longer lasting within the ventricular apex compared with the base (Table 2). A typical response is illustrated in Figure 8. Although $\overline{APD}$ is reduced to a similar extent in the apex and base, note the slower time course for this response at basal sites (i.e., longer τ). Within the ventricular base, beat-to-beat $\overline{APD}$ oscillations were dampened by the 20th cardiac cycle, whereas $\overline{APD}$ measured from the ventricular apex continued to oscillate throughout the recording period.

Discussion

The effect of an abrupt increase in stimulation rate on spatial inhomogeneity of repolarization within the intact ventricle has not been previously reported. In this study, high-resolution action potential recordings were used to investigate the effects of rate-induced APD changes at each of multiple ventricular sites on spatial APD heterogeneity throughout the entire ventricle. The manner in which the myocardial syncytium adapts to CL shortening so as to dynamically modify the underlying electrophysiological substrate was explored. Our findings suggest that dispersion of repolarization is not a static property of myocardial tissue but rather changes dynamically with perturbations in heart rate. Furthermore, a transient reduction of dispersion appears to be a physiological response of ventricular myocardium to CL shortening in the absence of hypoxia.

Action Potential Duration Oscillations in Ventricular Cells

During normoxic perfusion, we observed a global pattern of APD oscillations (Figure 3) similar to that reported in studies examining the response of *single* ventricular fibers to CL shortening.[5-9] Hirata et al[8,9] and others[23,24] have classified oscillations that occur with initial lengthening of APD followed by short-long-short-long alternating intervals as "odd"-type alternations and oscillations characterized by initial APD shortening and subsequent long-short-long-short intervals as "even"-type alternations. In our experiments, even-type oscillations were present in some hearts (four of five) and odd-type oscillations were present in others (one of five), a finding consistent with previous reports.[5,9,24] During normoxic perfusion, however, even and odd oscillations never occurred simultaneously (i.e., APDs either prolonged

at all sites or shortened at all sites on any given beat but never shortened at one site while lengthening at another). That such a disparate response (even and odd oscillations) might *simultaneously* occur at different anatomic sites, promoting spatial inhomogeneity of recovery and hence enhancing vulnerability to reentry, has remained a point of speculation but has not been confirmed experimentally. Our results indicate that the oxygenated ventricle is resistant to such regional asynchronies; however, in the presence of hypoxia, even- and odd-type alternations of APD can occur simultaneously, even within a homogeneous population of ventricular myocytes.

The percent reductions of APD and the amplitudes of beat-to-beat APD oscillations reported in previous studies were often larger than those measured in our experiments.[8,9] These differences are most likely related to the greater range of pacing rate changes used by other investigators. We intentionally avoided extreme changes in CL and restricted our analysis to rates more likely to occur physiologically. We did not observe accentuation of APD oscillations during hypoxia as reported previously,[8] possibly because of the longer duration and perhaps more homogeneous level of hypoxia used in our experiments.

Repolarization Inhomogeneity in Normoxic Myocardium

During normoxic perfusion, beat-to-beat DI, an index of nonuniformity of cellular recovery, decreased significantly after CL shortening (Figure 5). Two causes for this transient reduction of DI were identified. The first relates to regional synchronization of APD oscillations. Although APD oscillations per se did not reduce dispersion, complete synchronization of APD oscillations was critical for maintaining the observed dispersion-lowering effect of CL shortening during normoxic perfusion. The fact that APD oscillations were not synchronized during hypoxia may have accounted for the absence of a dispersion-lowering response to CL shortening during hypoxic perfusion.

The second factor responsible for modulating dispersion was CL-induced attenuation of the normally occurring apex-to-base APD gradient. Although APD gradients have been previously implicated as a factor responsible for electrocardiographic T wave concordance,[25] repolarization inhomogeneities arising from dynamic modulation of ventricular APD gradients have not been well recognized. Dynamic change of the apex-to-base APD gradient resulted from significantly faster APD shortening in apical than in basal tissue (Table 2). Because we and others[26] measured longer APDs at apical relative to basal sites, it follows that more rapid lowering of apical APDs would cause transient narrowing of the distribution of ventricular APDs (i.e., reduced DI). Over time, APD shortening at the apex and base equalized, and the apex-to-base APD gradient was therefore restored.

Our data therefore suggest that apical and basal myocardium may manifest inherently different responses to CL shortening. This result may be reconciled with previous studies, which have ascribed changes in APD after CL change to offsetting influences of electrical restitution[7] and declining memory effect.[7,27] Restitution dictates that APD reduction after CL shortening is determined by the diastolic interval that precedes the prematurely stimulated action potential.[28] Although formal restitution curves were not generated, if a restitution effect were present, action potentials in the mapping array that were preceded by the shortest diastolic intervals would be expected to decrease to the greatest extent. During fixed-rate pacing, however, diastolic interval and APD are not independent (diastolic interval equals pacing cycle length minus APD); hence, restitution would similarly be expected to shorten APD to the greatest extent in cells with the longest baseline APD. APD shortening was indeed accentuated, at least transiently, at sites manifesting the longest baseline APDs (i.e., apex, Figure 7), resulting in narrowing of the APD distribution (i.e., reduced dispersion). When considering all experiments, the extent of APD shortening was related to diastolic interval (Table 1), suggesting that diastolic interval was an important determinant of APD change during both hypoxic and normoxic perfusions. Because of the aforementioned interdependence of APD and diastolic interval, we cannot determine conclusively whether regional differences in diastolic interval or differences in baseline APD were responsible for the varied APD responses elicited at different anatomic sites.

Unlike restitution, cardiac "memory" would act to maintain APD at the pre-CL shortening level.[27] Because the magnitudes of APD changes within the apex and base were identical by the 25th post-CL shortening beat, intrinsic differences in long-lasting memory are not likely to explain the distinctly different responses observed at these respective sites. However, because the time constants of APD decline within apical and basal tissue differed significantly (Table 2), differences in recovery kinetics of the ionic channels governing repolarization in apical and basal cells[25] may be a more likely explanation for dynamic modulation of the apex-to-base APD gradient.

Recently, Saitoh et al[6,28] presented data suggesting that as opposed to Purkinje fibers, APD in ventricular myocytes is mediated by factors controlling action potential shape and myocardial contractile forces rather than electrical restitution or memory. They reported changes in action potential shape after the stimulus rate was increased that were similar to those observed in our study (Figure 4). Given differences in mechanical wall stresses at the cardiac apex and base,[29] differential mechanical loading at these respective sites may have explained our observed differences in APD responses of apical and basal cells. These speculations must be made with caution, however, because our experimental preparations were

studied in an unloaded state and we did not perform detailed hemodynamic measurements of wall stress.

Repolarization Inhomogeneity in Hypoxic Myocardium

The response of DI to CL shortening changed strikingly during hypoxia as DI failed to decrease in hypoxic myocardium (Table 1). This was attributed to a decreased absolute responsiveness of APD to CL changes in addition to loss of sychronization of APD oscillations between myocardial recording sites. This finding is consistent with previous observations of changing phase of APD oscillations within a single cell as the duration of hypoxia increases.[8] The loss of APD responsiveness observed during hypoxia may have been due to hypoxia-induced APD shortening, resulting in longer diastolic intervals (Table 1) and hence a less potent stimulus for APD shortening based on restitution. We cannot, however, rule out a direct and independent effect of hypoxia on repolarization currents.

Because cellular repolarization is influenced by electrotonic coupling between cells,[30] it is possible that reduced cell-to-cell electrotonic interaction was the cellular basis for nonsynchronized APD oscillations during hypoxia. We observed asynchronous APD oscillations even between neighboring elements of the mapping array, suggesting that the spatial extent of repolarization uncoupling would be at least on an interelement recording distance scale of 0.83 mm. Studies performed with greater optical magnification may be helpful in determining whether APD oscillations are uncoupled over distances approximating the length of a cardiac myocyte. One might predict that uncoupling of repolarization over such small distances would be particularly arrhythmogenic.

Study Limitations

Because a single range of CL changes was used to compare responses during normoxic and hypoxic perfusions, we cannot determine whether our results would differ if other heart rates were used. However, APD shortening and oscillations have been elicited by a broad range of CL changes in single cells[5,8,14]; hence, one would predict our results to also apply to a wide range of CL changes. Our study did not address the effects of abrupt CL *slowing* on APD inhomogeneity. These effects may be relevant to clinical arrhythmias because short followed by long stimulus intervals may be particularly arrhythmogenic in patients undergoing electrophysiological studies.[12]

We recorded action potentials from the epicardial surface; hence, dynamic APD changes at intramural and endocardial sites and their possible contributions to repolarization inhomogeneity cannot be assessed from our data. Epicardial recordings were advantageous to our experimental design because analysis was largely restricted to a homogeneous population of ventricular myocytes without the potential confounding effects of the His-Purkinje system. However, we cannot absolutely rule out that epicardial activation distant to the stimulus site arose from His-Purkinje breakthrough even though ventricular activation maps (Figure 2) demonstrated a uniform pattern of epicardial propagation without obvious epicardial breakthrough from subendocardial sites. Our analysis was necessarily restricted to a region of the anterior left ventricular surface that was incorporated by the mapping array. We are not aware of data suggesting that APD characteristics differ significantly at other epicardial sites within the left or right ventricles.

Finally, the technique of optical mapping with voltage-sensitive dyes is particularly advantageous for making multisite, high-resolution action potential measurements. Nevertheless, it is important to emphasize that optically recorded action potentials arise from minute clusters of cells and are not necessarily identical to single-cell recordings.

Study Implications

Recent studies have confirmed that downward modulation of refractoriness within the His-Purkinje system[31] and ventricular myocardium[13] of humans follows an abrupt increase of stimulation rate. Furthermore, the response of ventricular myocardium is characterized by dampened oscillations of recovery[13] closely resembling those observed in our study. Although further study is required before our experimental results can be extrapolated to clinical arrhythmias, the data indicate that dynamic changes of APD in response to abrupt CL shortening directly alter spatial homogeneity of repolarization and therefore may alter the electrophysiological milieu for reentry.

Reentry is most often initiated by a sudden change in CL. The ability to respond to an abrupt acceleration of stimulation rate by reducing dispersion of recovery would be expected to protect against reentrant arrhythmias. Although causality remains to be established, dispersion was actively modulated in normoxic myocardium in a manner expected to diminish vulnerability to reentry, whereas this potentially protective response was absent after hypoxia. Similar phenomena have been previously demonstrated in human atria where dispersion of atrial refractoriness was reduced in response to faster pacing rates in patients who were resistant to inducible atrial fibrillation but not in patients manifesting inducible atrial fibrillation.[32] Although assessment of dispersion of recovery with endocardial catheters is less precise than our methods, these findings may represent a clinically relevant atrial counterpart of the observations we have made in the ventricle.

Dynamic changes of APD in response to CL shortening may also play a role in the termination of reentrant tachycardias. The termination of a clinical tachycardia is often successful only when multiple, closely coupled extrastimuli are introduced.[33] Oscillations of ventricular refractoriness caused by trains of rapid extrastimuli have been suggested by others[15] to terminate tachycardias by local conduction block precipitated by a large oscillation toward increased

refractoriness. However, it is also possible that a transient reduction of ventricular APD inhomogeneity, as was observed in the present study, may modify factors responsible for the maintenance of the tachycardia and therefore interrupt them. Conversely, an "abnormal" oscillatory response to a drive train, as was observed during hypoxia, may accentuate APD inhomogeneities and thus accelerate the tachycardia. Further studies of APD modulation at the time of tachycardia initiation and termination are required to fully elucidate these mechanisms.

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