Beat-to-beat repolarization variability in ventricular myocytes and its suppression by electrical coupling

MASSIMILIANO ZANIBONI,¹ ANDREW E. POLLARD,² LIN YANG,³ AND KENNETH W. SPITZER ⁴

¹Department of Evolutive and Functional Biology, University of Parma, Parma, Italy 43100; ²Department of Biomedical Engineering and Cardiac Rhythm Management Laboratory, University of Alabama at Birmingham, Alabama 35294; ³Department of Cardiology, First Teaching Hospital, Xian Medical University, Xian, China 710061; and ⁴Department of Physiology and Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, Utah 84112-5000.

Zaniboni, Massimiliano, Andrew E. Pollard, Lin Yang, and Kenneth W. Spitzer. Beat-to-beat repolarization variability in ventricular myocytes and its suppression by electrical coupling. Am. J. Physiol. Heart Circ. Physiol. 278: H677–H687, 2000.—Single ventricular myocytes paced at a constant rate and held at a constant temperature exhibit beat-to-beat variations in action potential duration (APD). In this study we sought to quantify this variability, assess its mechanism, and determine its responsiveness to electrotonic interactions with another myocyte. Interbeat APD₉₀ (90% repolarization) of single cells was normally distributed. We thus quantified APD₉₀ variability as the coefficient of variability, CV = (SD/mean APD₉₀) × 100. The mean ± SD of the CV in normal solution was 2.3 ± 0.9 (133 cells). Extracellular TTX (13 µM) and intracellular EGTA (14 mM) both significantly reduced the CV by 44% and 26%, respectively. When applied in combination the CV fell by 54%. In contrast, inhibition of the rapid delayed rectifier current with L-691,121 (100 nM) increased the CV by 100%.

Spatial and temporal dispersion of APD is arrhythmogenic. Temporal dispersion of action potential duration (APD) evident as S-T alternans is frequently followed by ventricular arrhythmias (16, 30). Detailed epicardial mapping in dogs subjected to coronary artery occlusion (16) suggested increased amplitude and discordance of S-T alternans as effective markers for occurrence of ventricular fibrillation. Naturally occurring alternans (short-long-short cycle lengths) precede spontaneous ventricular tachycardia in patients (14). The short-long-short sequence has also been used for clinical (7) and experimental (8) arrhythmia induction. Proposed mechanisms by which APD alternans influence arrhythmogenesis include alterations in the time course for membrane ionic currents (25) and intracellular calcium handling evident as contractile strength (24).

Although naturally occurring temporal APD variability in isolated myocytes has been noted (15, 18), it is typically not seen during microelectrode recordings from multicellular ventricular preparations at normal pacing frequencies. One possible explanation of this difference is that, in situ, intrinsic beat-to-beat variations in repolarization are suppressed by electrotonic interactions with neighboring cells (17). To test this hypothesis, we first quantified temporal dispersion of repolarization properties in isolated guinea pig myocytes. Pharmacological interventions to block ion channels that operate during the action potential plateau suggested that variability resulted from stochastic behavior of late sodium current (late I₊ Na), delayed rectifier current (Iₖ), and variations in the intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) transient. The influence of electrical coupling on dispersion was then quantified through the use of the coupling clamp approach of Tan and Jouyner (34). This approach represents the simplest possible cell-to-cell contact, which ensured analysis of APD dispersion without a need to consider complicating issues that arise from inhomogeneities in gap junction distribution or in transmembrane potential dispersion caused by activation sequence (45).

Electrical coupling reduced beat-to-beat variations in action potential duration. Of significance to arrhythmogenesis, we also found that triggered activity thought to induce extrasystoles and tachyarrhythmias under conditions that prolong action potential duration (27) was suppressed by coupling.

Preliminary accounts of this work have been published in abstract form (32, 41).

METHODS

Cell isolation. We chose guinea pig ventricular myocytes because the regional differences in action potential configuration are less than in other species (20, 37). Hearts removed
from adult guinea pigs (~400 g) anesthetized with pentobarbital sodium (40 mg/kg ip) were rapidly attached to an aortic cannula and perfused at 37°C with the following sequence of solutions: Ca2+-free (no added Ca2+) solution for 5 min to remove the blood, low-Ca2+ (0.1 mM) solution containing 1 mg/ml type II collagenase (Worthington, Freehold, NJ) and 0.1 mg/ml type XIV protease (Sigma, St. Louis, MO) for 20 min, and enzyme-free solution containing 0.1 mM CaCl2 for 5 min. The left ventricle was then minced and shaken for 10 min in the low-Ca2+ solution. Myocytes were stored at room temperature in the control solution. All experiments were performed within 2–6 h after isolation. All myocytes used in this study had well-defined striations and did not spontaneously contract.

Solutions. Isolation solution contained the following (in mM): 126 NaCl, 22 dextrose, 5.0 MgCl2, 4.4 KCl, 20 taurine, 5 creatine, 5 Na pyruvate, 1 NaH2PO4, and 24 HEPES (pH = 7.4, adjusted with NaOH). The solution was gassed with 100% O2. Control solution for cell bathing during experiments contained the following (in mM): 126 NaCl, 11 dextrose, 4.4 KCl, 1.0 MgCl2, 1.08 CaCl2, 13.0 NaOH, and 24 HEPES (pH = 7.4). Normal pipette filling solution contained the following (in mM): 113 KCl, 10 NaCl, 5.5 dextrose, 5 K3ATP, 0.5 MgCl2, 11 KOH, and 10 HEPES (pH = 7.1). The filling solution for nystatin perforated patches contained the following (in mM): 140 KCl, 10 NaCl, 0.5 MgCl2, 4.0 KOH, and 12 HEPES (pH = 7.1). Nystatin (Sigma) was dissolved in dimethyl sulfoxide just before adding it to the pipette filling solution to yield a final nystatin concentration of 600 mg/ml. Each pipette tip was first dipped into the nystatin-free pipette solution and then backfilled with nystatin-containing solution. L-691,121 (1.0 µF/cm2), and 5 was first dipped into the nystatin-free pipette solution and then backfilled with nystatin-containing solution. L-691,121 (23) was kindly supplied by M. Sanguinetti. The temperature of the solutions in the cell bath was 36 ± 0.2°C.

Electrophysiological methods. Suction pipettes were made from borosilicate capillary tubing (Corning 7052 glass). After fire polishing, the pipettes had resistances of 2–4 MΩ.

Current clamp 2A or 2B amplifier (Axon Instruments, Foster City, CA) in the bridge mode. The amplifier was also used in the continuous voltage-clamp mode to estimate membrane resistance (Rm) during action potential repolarization. The chopping frequency during voltage clamp was 7–10 kHz. Vm was measured with the disrupted patch technique, except in some experiments in which a nystatin perforated patch was used to minimize intracellular dialysis. Vm was digitized at a sampling frequency of 5 kHz with a 12-bit analog-to-digital converter (Digidata 1200 Interface, Axon Instruments). Before a cell was contacted with the pipette tip, the pipette potential was set to zero and the voltage drop across the pipette was compensated with the bridge balance. Electrical coupling between two physically separate myocytes was achieved as previously described (10, 11, 31), using simultaneous suction pipette attachments and either the coupling circuit of Tan and Joyner (34) or a resistor network. The same results were obtained with both systems. We used a junctional resistance (Rj) of 100 MΩ in all coupling experiments. Although this Rj is higher than that measured in isolated guinea pig ventricular myocyte pairs (33, 39), it is still less than the critical Rj at which conduction fails (39). In addition, we found in preliminary experiments that this Rj achieved synchrony of APD. Lower values of Rj did not induce further APD coordination. Action potentials were elicited by injecting brief (2–3 ms) depolarizing current pulses (~50% above current threshold) via the suction pipette at a rate of 0.5 Hz. In cell pair experiments, myocytes were paced simultaneously to minimize electrotonic interactions resulting from conduction delays. Diastolic membrane resistance (Rm)

and capacitance (Cm) were measured in some uncoupled myocytes using intracellular injection of small hyperpolarizing current pulses (200-ms duration). Cm was calculated as τ/Rm, where τ is the membrane time constant. Action potential duration from a series of 10 or more action potentials was calculated as the time interval between the peak maximum upstroke velocity (phase 0) and the time at 50% of repolarization (APD50). We used either MATLAB (version 5, Prentice Hall, Upper Saddle River, NJ) or CLAMPFIT (Axon) software to measure this parameter. Idetical results were obtained with either program. The data acquisition rate of 5 kHz enabled us to achieve a temporal resolution of 0.2 ms.

Statistics. Results are presented as means ± SD. Statistical analysis was performed using paired and unpaired Student's t-test. A value of P < 0.05 was considered significant.

Cell pair simulations. The procedure for the computer simulations followed our previous reports (10, 11) studying Purkinje-ventricular interactions. We used the Luo-Rudy (LRd) membrane equations (22, 44) to describe the ionic currents for a single ventricular cell. Action potentials were calculated numerically from

\[
\begin{align*}
\left(V_{m1} - V_{m2}\right) / R_j &= S_m \left[C_m \left(V_{m1/dt}\right) + I_{ion1}\right] \\
\left(V_{m2} - V_{m1}\right) / R_j &= S_m \left[C_m \left(V_{m2/dt}\right) + I_{ion2}\right]
\end{align*}
\]

where Rj is measured in megohms, S_m is the membrane surface area (0.000314 cm²), Cm is the membrane capacitance (1.0 µF/cm²), and Iion is the total ionic current (µA/cm²) for the cells in each pair. Cells were arbitrarily denoted 1 and 2. Because the LRd formulation for the rapid (Ikr) delayed rectifier current underestimates an experimental report (28) and the slow (Iks) delayed rectifier current overestimates that report, we assumed nominal conductances that were 50% higher for Ikr and 50% lower for Iks than those presented by Zeng et al. (44). APD prolongation was then achieved by Ikr inhibition, whereas shortening was achieved by Iks enhancement. Complete Ikr block resulted in early afterdepolarization (EADS). Analyzed waveforms were taken from the last beat in a train of 10 action potentials paced at a 2-s cycle length. Solution times of 1–2 min were achieved on a Digital Equipment (Natick, MA) Alpha Server 2100 4/233 with 512 MB RAM.

RESULTS

Beat-to-beat variability of APD90 in isolated myocytes. To establish that beat-to-beat APD variability is a random process, we first examined recordings from cell attachments held over relatively long time intervals. Figure 1A shows an example of APD variability, which we observed despite careful control of bath temperature (36°C) and pacing rate (0.5 Hz). During 20 successive action potentials, APD90 ranged between 301 and 326 ms. Figure 1B shows the change in APD90 over these cycles. There was no obvious cycle dependence. Figure 1C shows a histogram of APD90 from 200 successive action potentials recorded in a different myocyte. APD90 was normally distributed about a mean value of 342.8 ms with an SD of 10.4 ms. A correlation coefficient of 0.92 between a Gaussian fit and the histogram indicated that APD90 variability within a single cell was a random process. We therefore normalized variability (coefficient of variability, CV) as the percentage of SD/mean APD90.
To further assess repolarization variability, we repeated this pacing protocol in 132 myocytes isolated from 19 hearts. For each cell, 10 consecutive action potentials were recorded after at least 2 min of pacing at 0.5 Hz to measure APD$_{90}$ and SD, and to calculate CV. We found that increased SD accompanied increased mean APD$_{90}$ according to the following relationship:

$$\text{SD} = (0.012 \times \text{mean APD}_{90}) + 0.75, \quad r = 0.70.$$  

However, CV changed little over this large sample size, yielding a mean of $2.3 \pm 0.9\%$ (n = 16, 3 hearts). Thus cell size does not appear to affect APD variability, at least over the range we examined.

It seems likely that beat-to-beat variability in APD results from stochastic behavior of ion channels activated during repolarization. To gain insight concerning the magnitude of the ionic current and charge displacement required to induce APD variability, the analysis shown in Fig. 3 was performed. Figure 3A shows the longest and shortest action potentials recorded from a train of 10 beats in one myocyte. Figure 3B shows the net ionic current for these action potentials, determined numerically from $I = -C_m(dV_m/dt)$. The difference between the two currents (trace d) included a rising and falling phase. Because the rising phase (shaded area) coincided with the current difference that

![Fig. 1](image1.png)

**Fig. 1.** A: action potentials recorded from 20 successive cycles in a single guinea pig ventricular myocyte showed beat-to-beat variability in action potential duration at 90% repolarization (APD$_{90}$). B: time sequence for APD$_{90}$ in the cell from A. C: histogram of APD$_{90}$ recorded from 200 successive cycles from a different myocyte with Gaussian fit to histogram data (solid line).

![Fig. 2](image2.png)

**Fig. 2.** Relationship between the coefficient of variation (CV) and mean APD$_{90}$. Action potentials were recorded with suction pipettes using either the disrupted patch (open circles) or nystatin patch (closed circles). Each point represents a different cell.

and CV = $1.8 \pm 0.7\%$ (n = 16, 3 hearts). Thus cell size does not appear to affect APD variability, at least over the range we examined.

![Fig. 3](image3.png)

**Fig. 3.** A: action potentials with shortest and longest APD$_{90}$ from a train of 10 successive recordings. B: net ionic current for short and long APD$_{90}$ waveforms. In B, traced is difference current between two waveforms, and filled area was used to measure total charge difference between action potentials.
accelerated repolarization of the short APD₉₀ beat relative to the long APD₉₀ beat, we integrated current over this phase to measure total charge difference, in this case 5.4 pC. Mean charge from the same analysis in 13 myocytes from 3 hearts was 4.1 ± 1.6 pC. This analysis demonstrates that relatively small net charge is required to induce beat-to-beat APD variability.

It seems possible that the ability of such small charge differences to alter \( V_m \) trajectory during repolarization results from the increase in \( R_m \) that occurs during the action potential plateau. Earlier measurements of \( R_m \) during repolarization were performed with current injection (38) on multicellular preparations and are likely in error (9). In addition, \( R_m \) measurements during repolarization are apparently not available for single cardiac myocytes. Thus we measured repolarization \( R_m \), from instantaneous current-voltage (I-V) curves determined at various times during the plateau (Fig. 4). Cells were continuously paced at 0.5 Hz throughout the \( R_m \) protocol. Figure 4, top left, shows the time at which the switch from current clamp to voltage clamp was initiated. At each indicated time, three successive clamp pulses were applied. Each pulse was preceded by at least five conditioning action potentials (current clamp). The three pulses were initiated at the \( V_m \) of the action potential, and at approximately +10 mV and −10 mV above and below that \( V_m \). \( V_m \) was held constant during each clamp pulse. Figure 4, bottom left, shows the membrane current elicited by the three clamp pulses. The membrane current measured 10 ms after initiation of the clamp was used to construct I-V curves (Fig. 4, top right). \( R_m \) was calculated as the inverse of the slope of the I-V curve around the \( V_m \) of the action potential (Fig. 4, bottom right). \( R_m \) during the plateau exceeded diastolic \( R_m \) at 9 MΩ, with steady increases from 24 MΩ early (time A) to 84 MΩ (time D) measured just before the \( V_m \) zero crossing. At time E, \( R_m \) increased dramatically to ~2 GΩ as the I-V curve became less linear and its slope decreased by comparison with the curves at times A-D and F. We note that care was taken in all measurements to ensure that current injection did not move \( V_m \) to potentials negative to approximately −10 mV, where net membrane current becomes outward and slope resistance becomes negative due to inward rectifier (\( I_{\text{K1}} \)) activation (12, 29).

The same pattern of \( R_m \) changes during repolarization was observed in each of the 11 cells examined. An example of the temporal relationship between repolarization variability and \( R_m \) is illustrated in Fig. 5. The longest...
(number 1) and shortest (number 5) action potentials recorded from a train of seven are superimposed on the time course of $R_m$ determined in the same cell. The temporal disparity in the two repolarization waveforms increased as $R_m$ rose. This supports the hypothesis that high $R_m$ accentuates the effect of beat-to-beat variability in net ionic current on the trajectory of $V_m$.

Ionic mechanisms involved in APD variability. To test the hypothesis that APD variability is mediated by stochastic behavior of channels activated during the plateau, we next abolished the late (or slow) $I_{Na}$ (18, 19), which prolongs APD$_{90}$, with 13 µM external TTX in 49 myocytes. As shown in Fig. 6A, CV was significantly reduced from 2.3 ± 0.9 during control to 1.3 ± 0.4 after TTX application (P < 0.001, unpaired). Because $Ca^{2+}$ release by the sarcoplasmic reticulum (SR) is a stochastic process (5) and cytosolic $Ca^{2+}$ affects several cardiac currents (2), fluctuations in the $[Ca^{2+}]$ transient may contribute to APD variability. To test this hypothesis we blocked the $[Ca^{2+}]$ transient by intracellular dialysis with 14 mM EGTA ($n = 30$). As shown in Fig. 6B, this caused a significant reduction in the CV to 1.7 ± 0.7 (P < 0.001, unpaired). When applied in combination (Fig. 6C), TTX and EGTA reduced the CV even further to 1.0 ± 0.2 (n = 23, P < 0.001, unpaired). In contrast, the application of external ryanodine to block SR $Ca^{2+}$ release (Fig. 6D) did not significantly affect the CV (5 µM CV = 2.9 ± 0.6, n = 5; 10 µM CV = 2.0 ± 0.9, n = 11). Figure 6E shows that blockade of $I_K$ with 100 nM L-691,121 (23) to inhibit $I_K$ markedly increased CV to 9.0 ± 9.5 (n = 31, P < 0.001). This agent also frequently caused EADs (see below). It seems unlikely that fluctuations in $I_K$ were involved in APD$_{90}$ variability because the peak rate of repolarization during phase 3 was not related to CV (not shown). That rate correlates with peak $I_K$ activation (29). Taken together, these results...
suggest that stochastic variability of major ionic currents that operate during the plateau are responsible for the beat-to-beat variability in APD\textsubscript{90} observed in isolated myocytes.

Electrotonic modulation of APD\textsubscript{90} by resistive coupling. We next examined the responsiveness of APD variability to cell-to-cell electrical coupling. Figure 7 shows 10 successive action potentials recorded simultaneously from cells with intrinsically short APD\textsubscript{90} (cell 1) and intrinsically long APD\textsubscript{90} (cell 2), before and after coupling. Before coupling, APD\textsubscript{90} of cell 1 ranged from 300 to 335 ms, whereas APD\textsubscript{90} of cell 2 ranged from 452 to 494 ms. After coupling, the action potential configurations became the same, and the APD\textsubscript{90} range for both cells was limited to between 347 and 364 ms. Changes evident in these recordings reflected changes observed in all 36 cell pairs examined because CV was significantly reduced from 2.3 ± 1.2 before coupling to 1.5 ± 0.6 after coupling (P < 0.001, paired).

A second feature of these recordings that was evident in all coupling experiments was the asymmetry in the APD\textsubscript{90} response in which the intrinsically short action potential prolonged less than the intrinsically long action potential shortened (Fig. 7). Figure 8A summarizes the results from 36 cell pairs. Action potential area (AP\textsubscript{area}) measurements were included because they more accurately reflect the full time course of action potential repolarization than the APD\textsubscript{90} measurement. APD\textsubscript{90} and AP\textsubscript{area} decreases of the intrinsically long action potentials were 40 and 54% larger, respectively, than measured increases of intrinsically short action potentials. Both were statistically significant (P < 0.004, paired). Here we note that coupling initiated during the diastolic interval caused immediate changes in APD\textsubscript{90}. To gain insight concerning possible mechanisms for this asymmetrical response, we performed computer simulations in which we assumed that cell-to-cell differences in intrinsic APD resulted from differences in I\textsubscript{Kr} density. Figure 8B shows that a 25% inhibition of I\textsubscript{Kr} prolonged APD\textsubscript{90} and a 25% enhancement of I\textsubscript{Kr} shortened APD\textsubscript{90}. Coupling (R\textsubscript{j} = 100 MΩ) decreased APD\textsubscript{90} of the intrinsically long action potential cell by 25% more than it increased APD\textsubscript{90} of the intrinsically short action potential cell. With 50% adjustments, the asymmetry was even more striking because the APD\textsubscript{90} decrease was 67% more than the APD\textsubscript{90} increase. Figure 8C summarizes the simulations over a wide range of R\textsubscript{j} values. Note that in addition to the asymmetrical response of APD to coupling, marked uncoupling with an R\textsubscript{j} of > 10 GΩ was required for action potentials to achieve their intrinsic APDs.

One important consequence of the asymmetrical APD\textsubscript{90} response was the suppression of EADs in cells exposed to the I\textsubscript{Kr} blocker L-691,121. Figure 9A shows 10 successive action potentials recorded simultaneously from two myocytes during I\textsubscript{Kr} inhibition. Before coupling, cell 2 underwent APD\textsubscript{90} prolongation that was sufficient to induce EADs, whereas cell 1 exhibited normal recovery despite I\textsubscript{Kr} blockade. After coupling, EADs were completely suppressed in cell 2, and there was a slight increase in APD\textsubscript{90} in cell 1. A similar response was observed in a simulation in which a cell with complete I\textsubscript{Kr} block was connected to a cell with nominal I\textsubscript{Kr} (Fig. 9B). Coupling (R\textsubscript{j} = 100 MΩ) prolonged APD\textsubscript{90} in the cell with nominal I\textsubscript{Kr} from 192 to 237 ms and completely suppressed the EAD in the other cell. The action potentials of the two cells were identical after coupling. As in the experiments, there was much more pronounced APD shortening (762 to 237 ms) in the EAD-producing cell. Figure 9C summarizes the simulated relationship between APD and R\textsubscript{j} and shows that EADs were blocked until R\textsubscript{j} reached the 8- to 10-GΩ range.

DISCUSSION

We used isolated guinea pig ventricular myocytes and computer simulations with the LRd membrane equations to examine temporal and spatial dispersion of repolarization and its modulation by electrical coupling. In these experiments coupling was achieved by an external circuit that supplied equal and opposite coupling current to both cells that was proportional to the transmembrane potential differences between the cells. Cell-to-cell interaction was therefore considered in its simplest form, independent of ionic diffusion via gap junctions or mechanical interaction between cells. In the simulations, R\textsubscript{j} was adjusted over a range of values to assess how APD\textsubscript{90} changed as the cells in a pair uncoupled from one another. The main new findings that arose from this study included demonstration that 1) beat-to-beat variability in APD\textsubscript{90} of normal guinea pig ventricular myocytes was stochastic, with normalized variability between 2 and 3% of mean APD\textsubscript{90}; 2) large increases in R\textsubscript{j} that occurred during the action potential plateau and repolarization phase are likely to have accentuated this variability because they allowed relatively small differences in ionic cur-
rents that operate during the plateau to effect relatively large transmembrane potential differences; 3) inhibition of inward plateau level ionic currents generally suppressed beat-to-beat variability, whereas inhibition of outward current enhanced variability; 4) high intracellular EGTA suppresses beat-to-beat APD variability; 5) coupling suppressed variability and induced an asymmetric response in which APD\textsubscript{90} shortening of intrinsically long action potentials was more pronounced than APD\textsubscript{90} prolongation of short action potentials; and 6) this suppression was important in preventing EAD formation.

Although beat-to-beat variations in cardiac myocyte APD have been previously noted (15, 18), our work is the first to systematically quantify this phenomenon in single ventricular myocytes and to demonstrate its suppression by TTX, EGTA, and cell coupling (41). We found that repolarization variability was random and that the CV was relatively independent of APD, yielding a mean of 2.3%. This is similar to the 2.0% reported for normalized variability of interbeat interval in spontaneously firing rabbit sinoatrial nodal myocytes by Wilders and Jongsma (40). Computer simulations by those investigators revealed that nodal beating irregularity was mediated by the stochastic open-close kinetics of inward and outward ionic currents.

Assuming that a similar mechanism accounts for beat-to-beat variations in ventricular APD, it was of interest to determine the net transsarcolemmal charge movement involved. We obtained a mean charge displacement of approximately 4 pC (Fig. 3), which is very small compared with the total charge entry via the major currents flowing during ventricular repolarization. For example, an integrated L-type calcium current ($I_{CaL}$) of 82 pC was found in paced (0.5 Hz) guinea pig ventricular myocytes subjected to action potential voltage clamp (35). Thus only small beat-to-beat variations in net ionic current are required to elicit the repolarization variability we observed.

It seems possible that this high sensitivity of APD to small changes in net current could arise from the large increase in $R_m$ that is reported to occur during repolarization in multicellular preparations (9, 38). However, repolarization $R_m$ measurements are apparently not available for single ventricular myocytes from any species. We used the “instantaneous I-V curve” technique of Goldman and Morad (9) to estimate $R_m$ during repolarization. In accord with our results in single
guinea pig ventricular myocytes, those investigators found an increased $R_m$ during repolarization in frog ventricular strips. However, in contrast to our findings, $R_m$ remained constant during the action potential plateau of their preparation. It seems unlikely that contamination of our $I-V$ curves by time-dependent changes in $I_m$ (9) influenced our results because our curves were constructed from currents measured 10 ms after clamp initiation. However, we cannot exclude the possibility of species differences in the time course of $R_m$ or the possibility that sucrose gap voltage clamping of multicellular preparations (9) may yield results different from those obtained in single myocytes with a discontinuous single-pipette voltage clamp.

In theory, stochastic behavior of any of the channels that contribute to plateau level ionic currents could be involved in APD fluctuations. Liu et al. (19) proposed that variations in slow or late TTX-sensitive sodium current could account for beat-to-beat variations in APD observed in chick embryonic hearts. Late sodium current has also been observed in mammalian cardiac muscle, including guinea pig ventricular myocytes (15, 18). Our finding that 13 µM TTX significantly reduced the mean CV by $\sim 44\%$ (Fig. 6A) suggests that fluctuations in late $I_{Na}$ contribute to guinea pig ventricular APD variability. In contrast, the lack of any correlation between APD variability and variability in either maximum upstroke velocity or action potential amplitude suggests that the excitatory sodium current is not involved. Our TTX results are in general accord with recent work by Li et al. (18) in paced guinea pig ventricular myocytes. However, our finding of a 44% reduction in variability contrasts with the complete suppression of APD variability they observed with 1 µM TTX. Because guinea pig ventricular myocytes lack transient outward current ($I_{to}$), we did not study the response of APD variability to blockade of this current. However, it seems likely that beat-to-beat variability of $I_{to}$ will have large effects on both the trajectory of phase 1 and APD in preparations in which $I_{to}$ is large, e.g., canine ventricular myocytes (20) and rabbit Purkinje myocytes (10, 11).

We also found that buffering $[Ca^{2+}]_i$ with intracellular EGTA (14 mM) significantly reduced the mean CV by $\sim 26\%$ (Fig. 6B). This chelator concentration is insufficient to disinhibit $Ca^{2+}$-sensitive adenyl cyclase but does reduce bulk cytosolic $Ca^{2+}$ and blocks $[Ca^{2+}]_i$ transients (43). When used in combination, intracellular EGTA and external TTX significantly reduced the mean CV by $\sim 54\%$ (Fig. 6C). Our results do not reveal
the mechanism for EGTA-induced suppression of APD variability. However, \([\text{Ca}^{2+}]\), modulates several sarcolemmal currents, including \(I_{\text{Ca}}\) (42) and sodium-calcium exchange current \((I_{\text{Na,Cal}})\) (3). In addition, \(\text{Ca}^{2+}\) release by the SR is a stochastic process (4, 5, 21). Thus beat-to-beat variations in subsarcolemmal \(\text{Ca}^{2+}\) might be expected to modulate sarcolemmal channels and electrogenic transporters. In contrast to the effects of EGTA, equilibration in ryanodine to block SR \(\text{Ca}^{2+}\) release did not significantly affect CV (Fig. 6D). However, cells continued to contract in ryanodine, indicating that transsarcolemmal \(\text{Ca}^{2+}\) flux via \(\text{Ca}^{2+}\) channels and perhaps reverse \(I_{\text{Na,Cal}}\) was still present and may have been sufficient to mediate beat-to-beat variability. It is interesting that beat-to-beat APD variability was accompanied by corresponding changes in the duration of cell shortening (unpublished observation).

Whereas TTX and \([\text{Ca}^{2+}]\), buffering acted to reduce APD variability, \(I_{\text{Kr}}\) inhibition significantly increased mean CV by \(\sim 300\%\) (Fig. 6E). Thus \(I_{\text{Kr}}\) appears to normally exert a significant suppressive action on APD variability. By promoting repolarization, perhaps it acts to mask the normal stochastic behavior of the inward currents flowing during repolarization.

Although the suppression of beat-to-beat APD variability by coupling has not been systematically considered previously, our findings are consistent with mathematical modeling studies and recordings from paced and spontaneously firing cardiac preparations. For example, in two-dimensional computer simulations with APD dispersion established by random adjustments to repolarizing currents, Lesh et al. (17) showed that uncoupling was required for dispersion to be maintained. Similarly, Joyner (13) showed that APD in membrane patches representing cell aggregates moved toward intermediate values during coupling at 1–50 M\(\Omega\). Using chick cell aggregates brought into contact by micromanipulation, Clapham et al. (6) and Veenstra and DeHaan (36) showed entrainment of spontaneous activity as gap junctional contacts were established. The latter investigators also showed that entrainment was accompanied by coordinated repolarization. We have previously shown that electronic interactions coordinate repolarization when ventricular myocytes are electrically coupled to either atrioventricular nodal (31) or Purkinje (11) myocytes.

The magnitude and symmetry of electronic interactions between two coupled cells depends on \(R_m\), the difference in \(V_m\) between the cells, and the \(R_m\) of each cell (31). During action potential repolarization \(V_m\) and \(R_m\) continuously change. We found that electrical coupling elicited asymmetrical changes in ventricular repolarization characterized by greater APD shortening than lengthening (Figs. 7 and 8). This effect was not related to conduction delays because the cells were simultaneously paced. We have previously observed asymmetrical changes in diastolic potential when ventricular and AV nodal myocytes were electrically coupled (31). This effect is attributable to the large difference in diastolic \(R_m\) between the two cell types. The cell with the highest \(R_m\) will exhibit the largest change in \(V_m\) for a given coupling current. Differences in \(R_m\) also provide the most likely explanation for coupling-induced APD asymmetry. Time-varying \(R_m\) during the plateau phase of the cell with the longest APD (source cell) is much greater than the diastolic \(R_m\) of the other cell (sink). During coupling, the loading action of the short APD cell accelerates repolarization in the source cell, thus forcing its \(V_m\) into the range for inward rectifier current \((I_{\text{K1}})\) activation. We have observed a similar mechanism to promote ventricular repolarization when single Purkinje and ventricular myocytes are electrically coupled (11).

The magnitude of asymmetrical repolarization was even more pronounced during coupling of cells exposed to \(I_{\text{Kr}}\) blocker L-691,121. This occurred at a relatively high \(R_m\) (100 M\(\Omega\)) and illustrates the extreme sensitivity of ventricular APD to electrical loading. Tan and Joyner (34) observed similar sensitivity when real ventricular myocytes were coupled to a well-polarized passive RC model cell. EAD initiation by \(I_{\text{Kr}}\) decrease has been proposed as a mechanism for triggered arrhythmias in long Q-T syndrome (1, 27). In this study, we provide experimental confirmation, in part, for the model prediction of Saiz et al. (26) that electrical coupling suppresses EAD formation. Those investigators completed cell pair simulations with the LRd phase II membrane equations in which adjustments to \(I_{\text{Cal}}\) and \(I_{\text{K}}\) were implemented in one cell from the pair to initiate EADs. EADs were suppressed at relatively low coupling resistance, transmitted with modest uncoupling, and blocked with further uncoupling. Our results demonstrate that EADs are readily suppressed on a one cell-to-one cell basis, even when coupling is at relatively high \(R_m\). Thus in vivo transmission of EADs to normal surrounding cells will require a large number of EAD-producing cells and elevated \(R_m\).

In contrast to beat-to-beat variability in APD, we have not observed similar fluctuations in the conduction delay during propagation from one myocyte to another (unpublished observation). However, it seems likely that beat-to-beat APD variability would induce conduction velocity variability in premature beats that are triggered during repolarization.

It is important to consider our results in the context of certain limitations. This work does not unambiguously identify the individual contributions to the CV of each channel involved in repolarization. In this regard experiments and simulations along the line of those described by Wilders and Jongma (40) for variability in pacemaker firing would be of considerable interest. In addition, although we used pharmacological interventions to block specific currents and buffer intracellular calcium, each intervention altered action potential trajectory from control. It is therefore probable that the time course for each current that operates during the plateau changed from control during the interventions. This may have secondarily reduced or increased \(\text{APD}_{\text{wp}}\). In addition, seal leakage current may have changed randomly during the recorded cycles. Although we expect that such leakage currents primarily introduced background noise in our recordings, we cannot rule out
the possibility that they contributed to our findings by providing small charge fluctuations during the action potential plateau. However, it seems unlikely that such leakage currents would be sensitive to EGTA or TTX. Despite these limitations, this work demonstrated at the most fundamental level the importance of gap junctions to synchronize intrinsic differences in action potential repolarization and thus reduce both spatial and temporal heterogeneities in contraction and refractoriness.

M. Zaniboni, A. E. Pollard, and L. Yang contributed equally to this study.

This study was supported by National Heart, Lung, and Blood Institute Grants HL-42873 and HL-54024, the Nora Eccles Treadwell Foundation and the Richard A. and Nora Eccles Harrison Fund for Cardiovascular Research, National Science Foundation/National Young Investigator Award BES-9457212, and by a Whitaker Foundation Special Opportunity Award to University of Alabama Biomedical Engineering.

Address for reprint requests and other correspondence: K. W. Spitzer, Nora Eccles Harrison Cardiovascular Research and Training Institute, 95 S. 2000 E., Univ. of Utah, Salt Lake City, UT 84112-5000.

Received 11 May 1999; accepted in final form 19 October 1999.

REFERENCES


35. Terracciano, C. M., and K. T. MacLeod. Measurements of Ca\textsuperscript{2+} entry and sarcoplasmic reticulum Ca\textsuperscript{2+} content during the cardiac cycle in guinea pig and rat ventricular myocytes. Biophys. J. 72: 1319–1326, 1997.


