Electrotonic suppression of early afterdepolarizations in isolated rabbit Purkinje myocytes

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Huelsing, Delilah J., Kenneth W. Spitzer, and Andrew E. Pollard. Electrotonic suppression of early afterdepolarizations in isolated rabbit Purkinje myocytes. Am J Physiol Heart Circ Physiol 279: H250–H259, 2000.—Many studies suggest that early afterdepolarizations (EADs) arising from Purkinje fibers initiate triggered arrhythmias under pathological conditions. However, electrotonic interactions between Purkinje and ventricular myocytes may either facilitate or suppress EAD formation at the Purkinje-ventricular interface. To determine conditions that facilitated or suppressed EADs during Purkinje-ventricular interactions, we coupled single Purkinje myocytes and aggregates isolated from rabbit hearts to a passive model cell via an electronic circuit with junctional resistance (Rj). The model cell had input resistance (Rm,v) of 50 MΩ, capacitance of 39 pF, and a variable rest potential (Vrest,v). EADs were induced in Purkinje myocytes during superfusion with 1 μM isoproterenol. Coupling at high Rj to normally polarized Vrest,v established a depolarizing coupling current during all phases of the Purkinje action potential. This coupling current preferentially suppressed EADs in single cells with mean membrane resistance (Rm,p) of 297 MΩ, whereas EAD suppression in larger aggregates with mean Rm,p of 80 MΩ required larger coupling currents. In contrast, coupling to elevated Vrest,v established a depolarizing coupling current during late phase 2, phase 3, and phase 4 that facilitated EAD formation and induced spontaneous activity in single Purkinje myocytes and aggregates. These results have important implications for arrhythmogenesis in the infarcted heart when reduction of the ventricular mass due to scarring alters the Rm,p-to-Rm,v ratio and in the ischemic heart when injury currents are established during coupling between polarized Purkinje myocytes and depolarized ventricular myocytes.

Purkinje-ventricular junction; injury current; membrane resistance

EARLY AFTERDEPOLARIZATIONS (EADs) likely cause triggered activity associated with acquired (3, 8) and congenital (37) long Q-T syndrome and other inherited ventricular arrhythmias (15). EADs occur as oscillations during phase 2 or 3 of the action potential (5, 6). Experimentally, phase 2 EADs are induced by interventions that primarily increase the Na+ window current, such as sea anemone toxin (2, 9), or by interventions that increase the Ca2+ window current, such as BAY K 8644 (24) or isoproterenol (34, 37). By comparison, interventions that primarily decrease K+ currents, such as cesium (3, 6) or quinidine (7, 32), induce EADs during phases 2 and 3.

In vitro, EADs are more easily induced in Purkinje fibers than ventricular muscle (8, 23). This may be attributed, in part, to the higher membrane resistance (4) in Purkinje myocytes (Rm,p) than in ventricular myocytes (Rm,v) such that a smaller net increase in inward current is required to initiate EADs in Purkinje myocytes. Furthermore, electrotonic interactions between Purkinje and ventricular myocytes at Purkinje-ventricular junctions may promote EAD formation. For example, in papillary muscle-Purkinje fiber preparations from guinea pigs (29) and dogs (30), prolonged Purkinje cell action potential duration (APD) caused “secondary plateaus” similar to phase 3 EADs in junctional action potentials that reached threshold and triggered ventricular activation (30). Conversely, electrotonic interactions may suppress EAD formation at Purkinje-ventricular junctions. In dogs with inherited ventricular arrhythmias, EADs formed in Purkinje fibers distant from Purkinje-ventricular junctions rather than at the junctions (15) because electrotonic interactions likely suppressed EAD formation by shortening Purkinje cell APD at the junction (31, 41). Although the precise conditions that promote either EAD formation or suppression at the Purkinje-ventricular interface are unknown, one important factor may be the Rm,p-to-Rm,v ratio (Rm,p/Rm,v), because it will determine the relative effects of electrotonic currents on the Purkinje and ventricular membrane potentials.

Ischemia and infarction introduce additional electrophysiological alterations that promote EAD formation and arrhythmogenesis (10, 22). In particular, the flow of “injury current” between normal and depolarized ischemic cells across the ischemic border zone may generate ectopic activity by inducing either EADs or
spontaneous activity in the normal cells (5, 13, 20, 21). In one experimental approach to studying injury current, isolated rabbit (39) and guinea pig (27) ventricular myocytes were coupled via an electronic circuit to a model depolarized cell. Although the injury current significantly modulated APD and increased cellular excitability, no EADs or spontaneous activity was observed in the isolated ventricular myocytes (39). However, because EADs are more easily induced in Purkinje than in ventricular myocytes through pharmacological intervention (8, 23), it is likely that injury current would induce EADs preferentially in Purkinje myocytes as well. Injury current across the Purkinje-ventricular interface is particularly relevant because acute ischemia differentially depolarizes ventricular myocytes (10, 22).

The purpose of this study was to determine conditions that suppressed or facilitated EAD formation at the Purkinje-ventricular interface. We coupled single Purkinje myocytes or Purkinje cell aggregates that were isolated from rabbit hearts and superfused with isoproterenol to a model ventricular cell with a variable junctional resistance ($R_j$) and rest potential ($V_{\text{rest,v}}$). Our results indicated that EAD suppression occurred preferentially in single myocytes rather than aggregates coupled at high $R_j$ because $R_{m,p}$ was higher in single myocytes. Additionally, depolarization of $V_{\text{rest,v}}$ introduced an injury current that enhanced EAD formation and initiated spontaneous activity in both single cells and aggregates. These results suggest that Purkinje-ventricular interactions tend to suppress EAD formation at Purkinje-ventricular junctions except in two pathologically important conditions: 1) infarction, which severely reduces the ventricular mass and, thus, the mismatch between $R_{m,p}$ and $R_{m,v}$ that might otherwise suppress EADs in neighboring Purkinje myocytes, and 2) ischemia, which differentially depolarizes ventricular myocytes and generates an injury current that can induce EADs or spontaneous activity.

**METHODS**

**Cell isolation.** Single Purkinje myocytes were isolated from rabbit hearts as described previously (18, 19). Briefly, isolated hearts were perfused via the aorta with nominally Ca$^{2+}$-free Tyrode solution for 8–10 min, with enzyme solution containing 0.1 mM Ca$^{2+}$ for 18–20 min, and with 0.1 mM Ca$^{2+}$ Tyrode solution for 5 min. Purkinje fibers were dissected from both ventricles and placed in a small bath containing fresh enzyme solution, where they were agitated with 100% O$_2$. The cell dissociation process required 15–60 min and yielded single Purkinje myocytes and aggregates of two or more myocytes. After collection, Purkinje myocytes were stored in 0.1 mM Ca$^{2+}$ solution for use later that day.

**Solutions.** Nominally Ca$^{2+}$-free Tyrode solution contained (in mM) 126 NaCl, 5.4 KCl, 5.0 MgCl$_2$, 22 glucose, 1.0 Na$_2$HPO$_4$, 20 taurine, 5 creatine, 5 sodium pyruvate, and 24 HEPES, with pH adjusted to 7.4 with NaOH. The enzyme solution had the same composition, except it also contained 1 mg/ml collagenase (type II, Worthington Biochemical, Freehold, NJ), 0.1 mg/ml protease (type XIV, Sigma Chemical, St. Louis, MO), and 0.1 mM CaCl$_2$.

The normal bathing solution during the experiments contained (in mM) 126 NaCl, 5.4 KCl, 1.0 MgCl$_2$, 1.0 CaCl$_2$, 11 glucose, and 24 HEPES, titrated with 13.0 mM NaOH (pH 7.4). EADs were induced in the myocytes by superfusion with 1 μM isoproterenol (Sigma Chemical). The pipette (internal) solution contained (in mM) 10 NaCl, 113 KCl, 0.5 MgCl$_2$, 10 HEPES, 5.0 K$_2$ATP, and 5.5 dextrose, with pH adjusted to 7.1 with 11 mM KOH.

**Electrical recordings.** Purkinje myocytes were placed in a glass-bottomed, temperature-controlled bath (36°C) and continuously bathed with normal solution at 1–2 ml/min. Cell images were viewed on a 17-in. monitor using a Panasonic charge-coupled device television camera (GPCD60, Matsushita Communication Industrial, Tokyo, Japan) with a ×40 objective lens in the microscope (Diaphot, Nikon, Tokyo, Japan), with which it was usually possible to distinguish individual cells within a Purkinje cell aggregate. Transmembrane potentials were recorded with an Axoclamp 2B amplifier system (Axon Instruments, Foster City, CA). Suction pipettes were made from borosilicate glass (no. 7052, outer diameter 1.65 mm, inner diameter 1.20 mm; A-M Systems, Everett, WA). Pipette series resistance was compensated before cell attachment, and pipette capacitance was minimized by maintaining a low level (1 mm) of solution in the bath. Myocytes were stimulated with intracellular current injection. To promote EAD formation, we paced the myocytes at cycle lengths between 2 and 7 s. The stimulus duration was 3 ms, and the stimulus magnitude was −1.1 times the current threshold. The Purkinje transmembrane voltage ($V_{\text{m,p}}$) was digitized at 4 kHz with a 12-bit analog-to-digital converter (Digidata 1200A, Axon Instruments) and recorded with a computer using pCLAMP 6 software (Axon Instruments). Diastolic $R_{m,p}$ was estimated in single myocytes and aggregates by applying small hyperpolarizing, constant-current pulses of 200–400 ms in duration. Note that $R_{m,p}$ changes over the course of the action potential. As an estimate for $R_{m,p}$ during repolarization, we used diastolic $R_{m,p}$ to compare the responses to coupling between single myocytes (high diastolic $R_{m,p}$) and aggregates (lower diastolic $R_{m,p}$).

We used an electronic circuit to couple a Purkinje myocyte or aggregate to a parallel resistance-capacitance circuit with a variable voltage offset. This resistance-capacitance circuit represented a passive ventricular cell with $R_{m,v}$ of 50 MΩ, input capacitance of 39 pF, and a variable rest potential ($V_{\text{rest,v}}$). The input resistance and capacitance were within reported ranges of membrane resistance and capacitance for normal rabbit ventricular myocytes (4, 18). Figure 1 shows a diagram of the electronic circuit used to couple the Purkinje myocyte to the model cell. As previously described (18, 38), this circuit included two amplifiers with variable gain to compute the voltage difference between the Purkinje myocyte and the model cell. That output was sent to voltage-to-current converters with fixed gain to simultaneously supply equal and opposite coupling current to the myocyte and model cell. The magnitude of the coupling current was simply the membrane voltage difference divided by $R_j$, where the coupling current was defined as positive when it repolarized the Purkinje myocyte. $R_j$ was determined by the gains of the converters and amplifiers and could be varied from 0 to 2,000 MΩ in our system.

After establishing a pipette attachment to the Purkinje myocyte or aggregate, we recorded several intrinsic action potentials while the myocytes were bathed in normal solution. We maintained this pipette attachment and switched the superfusate to a 1 μM isoproterenol solution. EADs typically formed within 1 min of superfusion. To study coupling induced suppression of EADs, we recorded $V_{\text{m,p}}$ and the
coupling current. We first recorded five uncoupled action potentials; we then coupled the myocyte at \( R_j = 1,000 \, \text{M} \Omega \) and \( V_{\text{rest,v}} = -80 \, \text{mV} \) initially and immediately recorded the next five action potentials. We then repeated this procedure for several values of \( R_j \) and \( V_{\text{rest,v}} \) to determine the conditions under which EADs in the Purkinje myocytes were suppressed by coupling.

Data analysis. Isoproterenol induced single and multiple phase 2 EADs. To quantify EAD characteristics, we measured EAD amplitude of a single action potential as the mean amplitude of all EADs fired during that trace. EADs were defined as depolarizations during repolarization that were >3 mV in amplitude (35). Additionally, we measured APD as the time of 90% repolarization. Because the action potential configuration recorded from a single myocyte demonstrated beat-to-beat variability, we averaged EAD amplitude and APD for several uncoupled and coupled action potentials at each \( R_j \) for every myocyte and aggregate. Thus the summary statistics reflect the means ± SD of all recorded traces. Statistical significance was established using a paired Student’s \( t \)-test to compare means before and after coupling, where \( P < 0.05 \) was considered statistically significant.

Additionally, we characterized the extent of electrotonic interactions between the Purkinje myocyte and \( V_{\text{rest,v}} \) by peak coupling current and the total repolarizing charge supplied by the coupling current during coupling to \( V_{\text{rest,v}} = -80 \, \text{mV} \). That charge was the integral of the coupling current between the time of activation of the Purkinje myocyte and the time of 90% repolarization. Whereas peak coupling current provided a measure of Purkinje-ventricular interactions during phase 1 repolarization, the repolarizing charge provided a measure of Purkinje-ventricular interactions throughout the action potential. Similarly, in experiments with elevated \( V_{\text{rest,v}} \), where the coupling current provided sustained depolarizing current to the Purkinje myocyte, we calculated the total depolarizing charge as the temporal integral of negative (inward) coupling current over the course of the action potential.

RESULTS

EAD suppression in single Purkinje myocytes. Superfusion with 1 \( \mu \text{M} \) isoproterenol induced EADs of varying number and amplitude that were completely suppressed by coupling at \( R_j = 1,000 \, \text{M} \Omega \) and \( V_{\text{rest,v}} = -80 \, \text{mV} \) in single Purkinje myocytes. Figure 2 shows the action potentials (A) and coupling currents (B) recorded shortly before and immediately after coupling in one experiment. The uncoupled Purkinje action potential demonstrated four EADs with mean amplitude of 10.3 mV and APD of 438 ms. During coupling, EADs were suppressed and APD was shortened to 89 ms. EAD suppression was a direct consequence of the supplied coupling current (Fig. 2B). Peak coupling current occurred on activation of the Purkinje myocyte when the potential difference between \( V_{\text{m,p}} \) and \( V_{\text{rest,v}} \) was largest. This initial outward current caused a large drop in \( V_{\text{m,p}} \) and little change to the potential of the model cell (not shown) because \( R_{\text{m,v}}/R_{\text{m,p}} \) was high (190 \( \text{M} \Omega/50 \, \text{M} \Omega = 3.8 \)). As a result of the initial decrease of \( V_{\text{m,p}} \) to ~41 mV, voltage-dependent inactivation of L-type Ca\(^{2+}\) current likely suppressed EAD formation, whereas the sustained repolarizing charge of 4.1 pC supplied by the coupling current virtually eliminated phase 2 of the action potential and shortened APD.

![Fig. 1. Schematic of the electronic circuit used to couple Purkinje myocytes or aggregates to the model resistance-capacitance cell. The membrane potentials recorded by the Axon Instruments amplifier were sent to additional amplifiers to compute the voltage difference (\( \Delta V_m \)). That output was sent to voltage-to-current (\( V \rightarrow I \)) converters to supply equal and opposite coupling current to the Purkinje myocytes and the model cell. The model cell was a parallel resistance-capacitance circuit with variable voltage offset (\( V_{\text{rest,v}} \), \( R_j \), junctional resistance; \( R_{\text{m,v}}, \text{ventricular membrane resistance; } V_{\text{m,p}}, \text{Purkinje membrane potential.} \)](image)

![Fig. 2. Early afterdepolarization (EAD) suppression in a single Purkinje myocyte during coupling to \( V_{\text{rest,v}} = -80 \, \text{mV} \). Solid traces show uncoupled \( V_{\text{m,p}} \) (A) and coupling current (B). Dashed traces show \( V_{\text{m,p}} \) and current during coupling at \( R_j = 1,000 \, \text{M} \Omega. \)](image)
EADs were suppressed and APDs shortened during coupling in all seven single Purkinje myocytes. Figure 3 summarizes the coupling-induced changes in EAD magnitude (A) and APD (B) in these cells. The average EAD magnitude of all uncoupled action potentials was $12.1 \pm 4.9$ mV, whereas the average EAD magnitude of all coupled action potentials was, of course, 0 mV. Marked changes in Purkinje cell APD also occurred on coupling. The mean APD recorded from all traces dropped from $623 \pm 165$ to $84 \pm 33$ ms on coupling. Although an $R_j$ of 1,000 MΩ represents severe uncoupling, the relatively small coupling current significantly shortened APD and suppressed EADs because $R_{m,p}$ averaged 297 MΩ in these single Purkinje myocytes.

**EAD suppression in Purkinje cell aggregates.** When aggregates of two or more Purkinje myocytes were coupled to $V_{\text{rest,v}} = -80$ mV, lower $R_j$ values were required to suppress EADs. Figure 4 shows the action potentials and coupling currents recorded before and after coupling in a Purkinje cell pair with $R_{m,p} = 150$ MΩ. The uncoupled Purkinje action potential demonstrated one EAD with amplitude of 13.4 mV and APD of 922 ms (Fig. 4A). On coupling at 1,000 MΩ, APD shortened but EADs were still present. As we decreased $R_j$, APD and EAD amplitude were further reduced, but EADs were not completely suppressed until we lowered $R_j$ to 250 MΩ. A larger peak coupling current and more repolarizing charge were required to abolish EAD formation in the Purkinje pair than in single Purkinje myocytes because $R_{m,p}/R_{m,v}$ was smaller in the Purkinje pair. At $R_j = 1,000$ MΩ, peak current was 0.09 nA (Fig. 4B), comparable to that delivered to a single Purkinje myocyte (Fig. 2B). EADs were not completely suppressed in the Purkinje pair, however, until peak current reached 0.31 nA and total repolarizing charge was 56.3 pC during coupling at 250 MΩ.

In one larger Purkinje cell aggregate, EADs were not suppressed at any $R_j$. Figure 5 shows action potentials and coupling currents recorded when a Purkinje cell aggregate (effective $R_{m,p} = 45$ MΩ) was coupled to $V_{\text{rest,v}} = -80$ mV. APD was significantly shortened and EAD amplitude was reduced at all values of $R_j$, but EADs were not suppressed by coupling. Even at $R_j$ as low as 50 MΩ, small EADs averaging 5.4 mV in amplitude still formed, although peak coupling current and...
total repolarizing charge delivered to the aggregate reached 1.4 nA and 97 pC, respectively.

Figure 6 summarizes the coupling-induced changes in EAD magnitude (A) and APD (B) in eight aggregates. These data are divided into two groups. In the first group, mean $R_{m,p}$ was 224 MΩ. Coupling shortened APD by 63% and suppressed EADs. In the second group, $R_{m,p}$ averaged 80 MΩ. As a result, coupling at $R_j = 1,000$ MΩ reduced EAD magnitude by only 12% and APD by 29%. Reduction of $R_j$ further decreased EAD magnitude and shortened APD, and the average $R_j$ required to suppress EADs in the second group of aggregates was 250 MΩ.

Facilitation of EAD formation by “injury current.” In contrast to coupling-induced suppression at $V_{rest,v} = -80$ mV, coupling single myocytes to depolarized $V_{rest,v}$ resulted in less EAD suppression. Figure 7 shows the action potentials and coupling currents recorded from a single Purkinje myocyte during coupling to $V_{rest,v} = -60$ mV (A and B) and $V_{rest,v} = -50$ mV (C and D). At 1,000 MΩ and $V_{rest,v} = -60$ mV, EADs were not suppressed because the smaller peak coupling current (0.08 nA) induced less phase 1 repolarization and provided less repolarizing charge than during coupling to $V_{rest,v} = -80$ mV. However, at 500 MΩ peak current doubled, providing sufficient repolarizing charge to suppress EADs.

EAD formation in single Purkinje myocytes was facilitated by coupling to more depolarized $V_{rest,v}$. For example, EADs were not suppressed during coupling to $V_{rest,v} = -50$ mV for resistances >250 MΩ (Fig. 7C). Additionally, EAD magnitude increased with coupling at $R_j = 1,000$ and 500 MΩ because the repolarizing coupling current (Fig. 7D) promoted a lower takeoff potential for EADs. This, in turn, likely enhanced EAD magnitude relative to that at higher $R_j$ values. However, when peak current reached 0.14 nA during coupling at 250 MΩ, EADs were suppressed.

Such injury current facilitated EAD formation in 71% of the single Purkinje myocytes and in all of the aggregates. In the single Purkinje myocytes, EADs were typically suppressed by coupling at 1,000 MΩ until $V_{rest,v}$ was depolarized to values between −60 and −40 mV. Coupling to the depolarized cell facilitated EAD formation in the sense that mean EAD magnitudes during coupling ranged from 63% to 146% larger than EADs in the uncoupled action potentials. Similarly, depolarization of $V_{rest,v}$ to −70 mV facilitated EAD formation in the Purkinje cell aggregates, whereas further depolarization of $V_{rest,v}$ reduced the $R_j$ value required to suppress EADs.
Induction of spontaneous activity by “injury current.” Injury current also induced spontaneous activity in Purkinje myocytes coupled to depolarized $V_{\text{rest,}v}$, whereas no spontaneous activity was observed in the uncoupled Purkinje action potentials. Figure 8 shows action potentials from a Purkinje cell aggregate before and after coupling to $V_{\text{rest,}v} = -60 \text{ mV}$ at 50 MΩ. As previously described, the uncoupled Purkinje action potential demonstrated several EADs and a prolonged APD (Fig. 8A). Immediately after coupling, the rest potential of the Purkinje aggregate depolarized to $-55 \text{ mV}$, and the paced response was followed by several spontaneous action potentials that fired at a frequency of 2.5 Hz. After the next paced action potential at 14 s, the spontaneous activity slowed to a frequency of 1.6 Hz. By the third paced action potential after coupling, spontaneous activity had ceased.

These spontaneous action potentials resulted directly from the coupling current. Although peak depolarizing current (Fig. 8B) only reached 0.05 nA, the sustained current supplied 10.4 pC of depolarizing charge over 275 ms of diastole. As phase 4 depolarization slowed in subsequent beats, more depolarizing charge was provided. For example, after the paced action potential at 14 s, the coupling current supplied 27.8 pC of depolarizing charge between action potentials. By comparison, the stimulus current used to elicit action potentials in this aggregate was 2.0 nA in magnitude, providing 6.0 pC of depolarizing charge.

Fig. 7. Facilitation of EAD formation in a Purkinje myocyte during coupling to $V_{\text{rest,}v} = -60 \text{ mV}$ (A and B) and $-50 \text{ mV}$ (C and D). Action potentials and coupling currents for Figs. 2 and 7 were recorded from the same Purkinje myocyte. In A, moderate depolarization of $V_{\text{rest,}v}$ reduced the outward coupling current and resulted in less EAD suppression. In C, further depolarization of $V_{\text{rest,}v}$ significantly increased mean EAD magnitude from $8.3 \pm 2.2 \text{ mV}$ (uncoupled) to $13.5 \pm 2.3 \text{ mV}$ (1,000 MΩ) to $20.5 \pm 3.8 \text{ mV}$ (500 MΩ).

Fig. 8. Induction of spontaneous activity in a Purkinje cell aggregate during coupling at 50 MΩ to $V_{\text{rest,}v} = -60 \text{ mV}$. Action potentials (A) and coupling currents (B) are shown over a 28-s period before (first panel, time 0) and immediately after coupling (last 3 panels). Each panel shows a 1.6-s window of time after the stimulus (cycle length = 7 s). Solid arrows in A indicate spontaneous action potentials induced by the coupling current. Hatched regions in B indicate the period of time over which depolarizing charge supplied by the coupling current was calculated. Action potentials for Figs. 5 and 8 were recorded from the same Purkinje cell aggregate.
EAD suppression during coupling

We observed this coupling-induced spontaneous activity in all single Purkinje myocytes and aggregates. Typically, spontaneous activity included either a single action potential or several action potentials that fired at progressively slower rates until all spontaneous activity ceased. When coupled to $V_{\text{rest, v}}$, between $-60$ and $-50$ mV, spontaneous Purkinje action potentials were only elicited with $R_j \leq 250 \, \Omega$. Further depolarization of $V_{\text{rest, v}}$ reduced the coupling strength required to elicit spontaneous activity such that coupling to $V_{\text{rest, v}} = -30 \, \text{mV}$ at $1,000 \, \Omega$ would often elicit spontaneous action potentials. Spontaneous activity occurred at the fastest rates when coupled at low $R_j$ to very depolarized $V_{\text{rest, v}}$.

DISCUSSION

Previous investigators have evaluated the effects of injury current during coupling between a model depolarized cell and isolated rabbit (39) and guinea pig (27) ventricular myocytes. Severe depolarization of the model cell ($V_{\text{rest, v}} = 0 \, \text{mV}$) was required to induce EADs or spontaneous activity in ventricular myocytes bathed in isoproterenol (27). Our approach is unique in that we couple Purkinje myocytes to $V_{\text{rest, v}}$ to represent injury current flowing from ischemic myocardium to adjacent Purkinje fibers. Because $R_{\text{m, p}}$ is intrinsically higher than $R_{\text{m, v}}$, significantly less depolarization ($V_{\text{rest, v}} = -60 \, \text{mV}$) and thus less injury current was required to facilitate EAD formation or induce spontaneous activity in our Purkinje myocytes. Additionally, our results demonstrate that $R_{\text{m, v}}/R_{\text{m, p}}$ is an important determinant of EAD suppression at the Purkinje-ventricular interface during coupling to normally polarized $V_{\text{rest, v}}$. Because reduction of the ventricular mass associated with infarction (11) and regional cellular uncoupling within the ventricular mass during ischemia (10, 22) likely reduce $R_{\text{m, v}}/R_{\text{m, p}}$, our results suggest that electrotonic interactions at the Purkinje-ventricular interface promote the development of EADs and spontaneous activity during ischemia and infarction.

Purkinje-ventricular interactions and triggered activity. The Purkinje network is often implicated as the source of ventricular arrhythmias. For example, in a canine model of acquired long Q-T syndrome, El-Sherif et al. (9) showed that the first ectopic beat of tachycardias induced by anthopleurin-A resulted from EADs. In that study EADs developed in Purkinje fibers but not ventricular fibers subsequent to differential APD prolongation. Similarly, in a canine model of inherited sudden death, Gilmour and Moise (15) showed that EADs induced triggered activity in Purkinje fibers that initiated ventricular arrhythmias. Furthermore, pharmacological agents such as quinidine (32) or almokalant (1) induce EADs preferentially in canine and rabbit Purkinje fibers rather than in endocardial muscle fibers.

Taken together, these studies suggest that the development of triggered activity depends on the extent to which EADs initiated in Purkinje myocytes conduct to neighboring ventricular myocytes. Computer simulations have shown that some degree of cellular uncoupling is required for EAD formation and propagation (36, 42). At very high $R_j$, EADs form locally but do not propagate to surrounding tissue, whereas at low $R_j$, EADs are suppressed. At intermediate resistances that are one to two orders of magnitude higher than in normal tissue, EADs form and conduct. Such cellular uncoupling is normally present at the Purkinje-ventricular junction, where the junctional resistivity is relatively high (35, 40) and increases under pathological conditions (12, 14, 25). Our experimental results complement these computational studies. Although $R_j$ at the Purkinje-ventricular junction has not yet been quantified, our previous study (18) on conduction between coupled Purkinje and ventricular myocytes showed that Purkinje-to-ventricular conduction occurred with physiological conduction delay (3–6 ms) when the cells were coupled at $R_j = 50–100 \, \Omega$. In the present study, EADs in all single cells and most aggregates were suppressed during coupling at $100 \, \Omega$. However, further uncoupling to $R_j > 250 \, \Omega$ allowed EADs to form in Purkinje aggregates.

Because the high membrane resistance intrinsic to Purkinje myocytes (4) promotes EAD formation subsequent to a small increase in net inward current (8), EADs develop and conduct readily in Purkinje fibers (9, 15, 24). Differential EAD formation can occur between normal and ischemic Purkinje fibers (16). However, Purkinje-Purkinje interactions would not be expected to suppress EAD formation in otherwise normal fibers. In contrast, Purkinje-ventricular interactions may either promote or prevent EAD formation at Purkinje-ventricular junctions. In dogs with inherited sudden death, Gilmour and Moise (15) identified the site of EAD initiation as the middle of a false tendon far from Purkinje-ventricular junctions. They suggested that electrotonic interactions likely suppressed EAD formation at the Purkinje-ventricular junction because these Purkinje action potentials were probably shortened by coupling to ventricular cells. In contrast, Li et al. (29, 30) found that electrotonic interactions at the Purkinje-ventricular junction were instrumental in inducing triggered activity. EDTA preferentially prolonged APD in Purkinje fibers, which yielded “secondary plateaus” or phase 3 EADs that triggered ventricular activation. Our results suggest that Purkinje-ventricular interactions likely suppress EAD formation at the normal Purkinje-ventricular junction because $R_{\text{m, p}}$ is typically higher than $R_{\text{m, v}}$ (4, 18, 19). As a result, small coupling currents elicit larger changes in $V_{\text{m, p}}$ and smaller changes in $V_{\text{m, v}}$, which allows $V_{\text{m, v}}$ to “dominate” $V_{\text{m, p}}$ in Purkinje-ventricular interactions. For example, whereas the intrinsic resting potential, plateau potential, and APD are different in Purkinje and ventricular myocytes, Purkinje-ventricular coupling typically modulates these values such that the intermediate values of $V_{\text{rest}}$ (19), plateau potential (18, 19), and APD (29, 41) are much closer to intrinsic ventricular values. In the present study, EADs were always suppressed in single Purkinje myocytes coupled to $V_{\text{rest, v}} = -80 \, \text{mV}$.
because mean $R_{\text{m,p}}/R_{\text{m,v}} = 6$. Therefore, the coupling current significantly shortened APD and reduced the plateau potential in the single cells, whereas the model cell loaded and repolarized $V_{\text{m,p}}$. However, by patching on to larger Purkinje aggregates, we effectively decreased $R_{\text{m,p}}$ so that $R_{\text{m,p}}/R_{\text{m,v}} \approx 1$. In that case, larger repolarizing coupling current was required to suppress EAD formation.

Clearly, $R_{\text{m,p}}$ cannot be reduced in vivo by adding Purkinje tissue. However, under pathophysiological conditions, a significant portion of the ventricular mass may be lost because of scarring after infarction (11, 22) or regional cellular uncoupling during ischemia (10, 22). These conditions likely alter the membrane resistance mismatch normally present between Purkinje and ventricular tissue such that $R_{\text{m,v}}$ approaches $R_{\text{m,p}}$. Our results suggest that such reduction of $R_{\text{m,p}}/R_{\text{m,v}}$ promotes EAD formation at the Purkinje-ventricular junction.

**Injury current.** Another mechanism by which electrotonic interactions at the Purkinje-ventricular junction may trigger arrhythmias is via the flow of injury current. At an ischemic border, depolarized cells in the ischemic myocardium provide a sustained electrotonic current to “normal” cells across the border (17, 20, 21). Because Purkinje and ventricular tissues are differentially affected by ischemia (10, 22), such an injury current may flow across the Purkinje-ventricular junction and promote arrhythmogenesis. Specifically, after acute myocardial ischemia, significant changes in Purkinje action potentials do not occur until 20–30 min after occlusion (10), whereas S-T segment elevation and depolarization of ischemic myocardial tissue occurs with 7 min of occlusion (22). Thus the voltage gradient between the normal Purkinje cells and the depolarized ventricular cells can initiate the flow of injury current that may induce Purkinje cell EADs (5, 21).

We modeled injury current in the present study by coupling Purkinje myocytes to a depolarized $V_{\text{rest,v}}$. With moderate depolarization to $-60 \text{ mV}$, reduction of the outward coupling current resulted in less suppression of EADs in single Purkinje myocytes that had previously demonstrated complete EAD suppression when coupled to the normally polarized $V_{\text{rest,v}}$. Further depolarization of $V_{\text{rest,v}}$ facilitated EAD formation as $V_{\text{rest,v}}$ approached the takeoff potential for EADs in these Purkinje myocytes. Interestingly, the number of EADs fired during coupling to depolarized $V_{\text{rest,v}}$ decreased, whereas EAD magnitude increased in Purkinje myocytes and aggregates during coupling to $V_{\text{rest,v}} \approx -50 \text{ mV}$. We considered the EAD magnitude rather than the number of EADs fired to be the “index” of facilitation because larger EADs are expected to conduct more readily and thereby elicit more triggered activity than smaller EADs (29, 30). Additionally, larger EADs typically repolarize the membrane to more negative voltages (8), and this trajectory promoted final repolarization rather than a chain of several EADs.

Joyner and co-workers (27, 38) have also used an electronic coupling system to model injury current. In those studies, injury current elicited by coupling isolated ventricular myocytes to $V_{\text{rest,v}} = 0 \text{ mV}$ did not induce EADs unless the myocytes were additionally superfused with isoproterenol, forskolin, or BAY K 8644 (27). Although all of our myocytes were bathed in isoproterenol during coupling, it is quite possible that injury current applied to Purkinje myocytes bathed in normal solution would induce EADs because of the high membrane resistance in Purkinje cells.

Injury current may also promote arrhythmogenesis by inducing spontaneous activity in normal cells (17, 21, 26, 28). For example, Katzung et al. (26) demonstrated that the flow of injury current from a chamber with high extracellular $K^+$ concentration ($[K^+]_o$) to a chamber with normal $[K^+]_o$ induced spontaneous action potentials in the chamber with normal $[K^+]_o$. Similarly, small depolarizing currents applied to canine Purkinje fibers increased the slope of diastolic depolarization and induced repetitive action potentials while increasing the magnitude of the sustained current increased the rate of spontaneous depolarization (5, 17). Our results complement these early studies. The injury current produced by the difference in $V_{\text{m,p}}$ and $V_{\text{rest,v}}$ elicited several spontaneous action potentials in all Purkinje myocytes and Purkinje cell aggregates coupled to the depolarized ventricular cell. It is important to note that the Purkinje myocytes in our study did not demonstrate spontaneous activity before coupling, and no pacemaker current has been detected in rabbit Purkinje cells (4). However, as a result of the injury current, Purkinje myocytes and aggregates depolarized to values between $-65$ and $-55 \text{ mV}$, and the sustained depolarizing charge supplied by the coupling current triggered action potentials at a rate that depended on the magnitude of the current.

**Limitations.** Our results must be considered within certain limitations. The normal spatial distribution of membrane properties and $R_{\text{i}}$ in the heart is complex. Ischemia and infarction make that distribution more complex. Although our experimental approach was simple by comparison, it yielded important insight regarding coupling-induced suppression of EADs that would not have been readily available with syncytial preparations. For example, we were able to vary $R_{\text{i}}$ to any desired value. This allowed us to quantify the range of $R_{\text{i}}$ for which EADs were suppressed by coupling in Purkinje aggregates. Additionally, this approach revealed the importance of geometric size factors in electrotonic interactions. Because we were able to couple single myocytes and aggregates to the same model cell, we could estimate the relative cell sizes that would promote EAD formation. For example, we distinguished two to four individual cells within aggregates of mean $R_{\text{m,p}} = 80 \text{ M}\Omega$. By extrapolating to larger groups of cells, these results suggest that a group of 100 ventricular cells would suppress EADs in a Purkinje aggregate unless that aggregate included 200–400 cells. The spatial pattern of cell coupling is complex, however, and this analogy is valid only as long as
the respective Purkinje and ventricular groups are well coupled and activation delays within the groups are smaller than activation delays between the groups.

We measured diastolic $R_{m,p}$ rather than $R_{m,v}$ during repolarization. It is, of course, $R_{m,p}$ during repolarization that is the relevant measure of membrane response to electrotonic current during the plateau. Whereas diastolic $R_{m,p}$ is fairly simple to measure and requires only a single current pulse delivered while the cell is at rest, measurement of $R_{m,p}$ during repolarization is much more difficult. To determine $R_{m,p}$ at a single point in time, a current-voltage (I-V) curve must be derived by switching from current clamp to voltage clamp during successive action potentials at several levels of $V_m$. The membrane resistance can then be calculated as the inverse of the slope of the I-V curve (43). Because of the technical difficulty associated with measuring $R_{m,p}$ during the action potential, we assumed that cells with high diastolic $R_{m,p}$ would have higher $R_{m,p}$ during repolarization than cells with low diastolic $R_{m,p}$. As a result, our estimate of $R_{m,p}$ yields the minimum $R_{m,p}/R_{m,v}$ expected over the course of the action potential.

The depolarized model cell was a very simplified representation of an ischemic myocardial region. Myocytes in an ischemic region undergo a multitude of electrophysiological changes during the first 10 min of myocardial ischemia. Elevated $[K^+]_o$, acidosis, and anoxia lead to a reduction of resting potential, a decrease in action potential amplitude and upstroke velocity, and postrepolarization refractoriness (10, 22). We did not attempt to recreate these conditions or their effects on ionic permeabilities. Instead, we represented the severe depolarization and loss of cellular excitability that accompanies ischemia with a passive model cell that allowed us to vary the severity of depolarization. With this approach, we were able to vary the magnitude of the imposed injury current and quantify the current required to enhance EAD induction or spontaneous activity.

Implications. Our results suggest that Purkinje-ventricular interactions tend to suppress EAD formation at the Purkinje-ventricular junction except when the ventricular mass has been reduced. Whereas in the structurally normal heart the three-dimensional ventricular mass will “dominate” interactions with the thin layer of Purkinje tissue, cellular uncoupling within the ventricular mass can occur pathologically after infarction. In patients with recurrent sustained ventricular tachycardia secondary to healed myocardial infarcts, Fenoglio et al. (11) found that the ventricular mass was reduced by as much as 90% in surgical resections of the earliest activated sites during tachycardia. Viable Purkinje fibers with normal ultrastructure and ventricular cells with normal and abnormal ultrastructure were present in these resections. After resection, tachycardia could not be induced by electrical stimulation, suggesting that interactions between the Purkinje network and the greatly reduced ventricular mass initiated the tachycardias. In the present study, we did not reduce the ventricular mass per se, but we reduced the difference between $R_{m,v}$ and $R_{m,p}$ by studying aggregates of Purkinje myocytes coupled to a single model ventricular cell. Our results suggest that under pathological conditions that reduce $R_{m,p}/R_{m,v}$, EADs initiated near the Purkinje-ventricular junction via injury current or otherwise will not be suppressed, potentially triggering life-threatening arrhythmias.

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