Pharmacological Modulation of Cardiac Gap Junctions to Enhance Cardiac Conduction
Evidence Supporting a Novel Target for Antiarrhythmic Therapy

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Background—Disease-induced alterations of cardiac gap junctions lead to intercellular uncoupling, which is an important mechanism of arrhythmogenesis. Therefore, drugs that selectively open gap junctions potentially offer a novel strategy for antiarrhythmic therapy. Because the peptide ZP123 was found to increase conductance between paired myocytes, we hypothesized that ZP123 would suppress acidosis-induced gap junction closure in the intact heart.

Methods and Results—High-resolution optical mapping was used to measure conduction velocity (CV) and action potential duration from ventricular epicardium of Langendorff-perfused guinea pig hearts at baseline (pH 7.4) and during 45 minutes of perfusion with acidic (pH 6.0) Tyrode’s solution with (n=8) and without (control, n=7) ZP123 (80 nmol/L). Acidosis produced conduction slowing transverse (29.1±0.1 to 16.8±0.2 cm/s, P<0.0001) and longitudinal (47.2±2.4 to 33.2±4.8 cm/s, P<0.0001) to cardiac fibers. Importantly, ZP123 inhibited conduction slowing during acidosis by approximately 60%. The peak effect of ZP123 was achieved after 16 minutes of acidosis, consistent with inhibition of uncoupling. ZP123 did not affect Na⁺ current in isolated myocytes, additionally affirming that preservation of CV was attributable to the compound’s action on gap junctions. ZP123 had no effect on CV in the absence of acidosis, suggesting that drug activity targets gap junctions under metabolic stress. Action potential duration heterogeneity was significantly reduced by ZP123 (6.7±0.8 ms) compared with controls (9.7±3.1 ms, P<0.05), presumably by enhancing cell-to-cell coupling.

Conclusions—These data suggest that ZP123 significantly attenuates gap junction closure during acidosis. Preservation of intercellular coupling diminished CV slowing and heterogeneous repolarization, eliminating arrhythmogenic substrates.

Key Words: electrophysiology • mapping • ischemia

Alterations in electrical coupling between ventricular myocytes play an important role in conduction delays and arrhythmogenesis in acute and chronic heart disease.1–3 The mechanisms governing uncoupling are related to multiple pathophysiological processes, including acidosis,4,5 intracellular calcium overload,6,7 and accumulation of amphipathic lipid metabolites.8 Uncoupling may cause conduction velocity (CV) slowing9,10 and heterogeneities of repolarization,11,12 both of which are key components to the electrophysiological substrate for reentrant arrhythmias. Although pharmacological enhancement of gap junction conductance is a logical and potentially novel therapeutic strategy to limit lethal arrhythmias induced by uncoupling, such therapy has remained elusive.

Recently, the novel peptide ZP123 was shown to promote electrical coupling between ventricular myocytes without changing membrane conductance.13 This peptide is closely related to the compound AAP-10,14 which enhances gap junction conductance.15 Interestingly, ZP123 was also reported to reduce the rate of inducible ventricular tachycardia during acute ischemia in dogs, suggesting an antiarrhythmic effect associated with the targeting of gap junctions.13 However, the effects of ZP123 on conduction and repolarization properties of the intact heart remain unknown.

We hypothesize that ZP123 will produce electrophysiological effects at the whole-heart level that would be predictive of a novel class of antiarrhythmic drugs that target gap junctions (ie, prevent CV slowing and inhomogeneous repolarization), that the effects on conduction would be attributable to actions on gap junctions rather than sodium channels, and that the drug does not exhibit potentially proarrhythmic side effects, such as prolongation of repolarization. The results of this study suggest that pharmacological modification of gap junction conductance...
might provide a viable and novel approach for antiarrhythmic drug development.

Methods

Microelectrode Studies in Isolated Myocytes
Guinea pig cardiac myocytes were dispersed enzymatically. Resting and action potentials were recorded using the Amphotericin B-perforated patch technique in current-clamp mode at 31°C. Action potentials were evoked by repetitive square pulses of current (3-ms duration, twice threshold amplitude) at 1000-ms cycle length. Action potential duration (APD) was measured at 90% repolarization (APD90). Na+ currents (INa) were recorded by ruptured-patch whole-cell voltage clamp at room temperature. Patch pipettes (0.9 to 1.4 mol/L Ω resistance) were filled with (in mmol/L) CsF 120, EGTA 11, MgCl2 2, and HEPES 10; pH 7.2. External solution was (in mmol/L) NaCl 20, N'-methyl d-glucamine 120, CsCl 5.0, MgCl2 1, CaCl2 2, NiCl2 1; glucose 10, and HEPES 10; pH 7.35. Ionic current density (pA/pF) was calculated from the ratio of current amplitude to cell capacitance.

High-Resolution Optical Mapping in Langendorff-Perfused Hearts
As described in detail elsewhere,19,20 adult guinea pig hearts were perfused as Langendorff preparations with oxygenated Tyrode’s solution at 31±1°C. The right atrium was removed to avoid competitive stimulation from the sinoatrial node. Hearts were stained with the voltage-sensitive dye di-4-ANEPPS (15 mmol/L) and then positioned in a chamber such that the recording area was centered over a 14.2×14.2-mm region of left ventricular epicardium, 5 mm lateral to the left anterior descending coronary artery, halfway between the apex and base. The epicardial surface of the left ventricle was stimulated with a unipolar electrode placed in the center of the recording region.21 Gentle pressure was applied to the posterior surface of the heart with a movable piston to stabilize the heart against the imaging window. Contraction artifacts were eliminated with diacetyl monoxime (10 mmol/L). Cardiac rhythm was monitored via 3 silver disc electrodes fixed to the chamber in positions corresponding to limb leads I, II, and III.

Briefly, action potentials were optically recorded from 256 sites (0.89-mm interpixel resolution) on the anterior epicardial surface of the ventricle, as described previously.19–21 Fluorescence was excited with a 270-W tungsten-halogen light source (filtered 514±20 nm) and transmitted to a 16×16-element photodiode array detector through a tandem-lens imaging system (emission filter >610 nm). Photocurrent from each photodiode underwent current-to-voltage conversion, amplification (×2000), and bandpass filtering (0.1 to 500 Hz) and was multiplexed and digitized (1000 samples/sec per channel) with 12-bit precision.

Experimental Protocol

Isolated Myocytes
Isolated myocytes from guinea pig hearts were randomized into 2 groups, ZP123 and no drug. Action potential recordings were made from 7 unique cells in each group, whereas 6 different cells per group were used to measure INa. In both groups, cells were first patched and superfused in the control solution given above. In the ZP123 group, bath solution was then changed to include 80 mmol/L ZP123. In both groups, the bath solution was then made acidic (pH 6.8). Recordings were obtained 10 minutes after each solution change to allow equilibration of the bath solution.

Langendorff Hearts
As shown in Figure 1, Langendorff hearts were randomized to 2 groups, ZP123 (n=8) and no drug (n=7). In both groups, hearts were allowed to equilibrate for 18 minutes while being perfused with normal Tyrode’s solution (baseline). Hearts were then perfused with either 80 mmol/L ZP123 (ZP123 group) or deionized water vehicle (no drug group) for 12 minutes. Subsequently, the perfusion buffer was made acidic (pH 6.0) for 45 minutes in both groups. Action potential maps were obtained during constant cycle-length stimulation (400 ms) at 4-minute intervals during each experimental phase (Figure 1, arrows).

Data Analysis
Data analysis was performed using custom software designed for the analysis of optically recorded action potentials. Action potential depolarization and repolarization time were determined using predefined criteria, as described previously.19–20 APD was calculated during each intervention as the time interval between local depolarization and repolarization. APD dispersion was defined as the standard deviation of all APDs measured in the mapping area. CV was calculated with respect to fiber orientation using an average of velocity vectors along the conduction path, as described previously.10 Comparisons were made between groups using the Student t test, except where noted otherwise. All values are reported as mean±SD. P<0.05 was considered significant.

Results

ZP123 Has No Effect on Ionic Currents
To assure that ZP123 has no effect on membrane currents, isolated myocytes were used to test the effects of acidosis and
ZP123 on the action potential and \( I_{\text{Na}} \). Representative action potentials recorded under current-clamp conditions during baseline and acidosis, both with and without ZP123, are shown in Figure 2. Acidosis (pH 6.8) produced a prolongation of APD\(_{90}\) compared with baseline (pH 7.35), which was expected based on inhibition of repolarizing currents by acidosis.\(^{22}\) Importantly, acidosis affected APD identically in both ZP123 and no-drug groups, indicating that ZP123 did not influence cardiac repolarization under normal or acidic conditions. Resting membrane potential and action potential amplitude were unaffected by either acidosis or ZP123 (Table in Figure 2).

ZP123 had no effect on whole-cell \( I_{\text{Na}} \) (Figure 3, top). Acidosis produced a rightward shift in the current-voltage relationship for \( I_{\text{Na}} \) to the same extent in both ZP123 and no-drug groups, reaffirming that ZP123 does not affect \( I_{\text{Na}} \), irrespective of pH. These results suggest that any effect of ZP123 on CV cannot be explained by an effect on \( I_{\text{Na}} \).

**Figure 3.** ZP123 does not affect \( I_{\text{Na}} \). Regardless of the presence of ZP123, acidosis introduces a rightward shift in the \( I_{\text{Na}} \) current-voltage relationship. This shift reduces excitability, providing another mechanism for acidosis-induced conduction slowing in the intact heart in addition to intracellular uncoupling. The lack of an effect of ZP123 on \( I_{\text{Na}} \) suggests that preservation of CV by ZP123 during acidosis in the whole heart is attributable to the drug’s action on intercellular communication rather than excitability.
ZP123 Attenuates Dispersion of Repolarization

Action potentials recorded from Langendorff-perfused guinea pig hearts are shown in Figure 4. Action potentials demonstrate no change in morphology or duration from baseline during perfusion with vehicle or ZP123, additionally indicating the absence of an effect of the drug on membrane currents. As expected, acidosis prolonged APD in hearts from both ZP123 and no-drug groups. These results were consistently observed in all hearts, producing an average 19% increase in APD during acidosis in both the ZP123 (184±14 to 218±13, P<0.005) and no drug (178±16 to 212±20, P<0.005) groups, whereas there were no differences between groups. Although ZP123 had no apparent effect on acidosis-induced APD prolongation, ZP123 significantly attenuated acidosis-induced increases in APD dispersion. As shown in Figure 5 (no-drug group), acidosis produced a nearly 2-fold increase in APD dispersion, as expected from its effect on cell-to-cell coupling.12 Pretreatment with ZP123 eliminated the acidosis-induced APD dispersion (ZP123 group). Interestingly, in the absence of acidosis, ZP123 did not effect APD dispersion, indicating that ZP123 only promotes gap junction coupling during metabolic stress.

ZP123 Enhances CV During Acidosis

Representative isochrone maps of ventricular propagation from a point stimulus demonstrate that acidosis clearly slowed CV (Figure 6A versus 6B), evident from both the relative crowding of isochrones and the increased time between action potential upstrokes during acidosis compared with baseline. Interestingly, ZP123 did not affect impulse propagation during baseline conditions (Figure 6A versus 6C) but substantially attenuated CV slowing during acidosis (Figure 6C versus 6D). The effect of ZP123 on conduction is summarized for all experiments in Figure 7. CV for each heart is normalized to its value measured just before perfusion with acidic Tyrode’s solution. In the no-drug group, CV progressively and significantly slowed during the period of acidosis, reaching a plateau after approximately 30 minutes. CV slowing was slightly more pronounced transverse to fibers (≈40%) than longitudinal (≈30%), resulting in a trend toward greater anisotropy of conduction. Both the time course of conduction changes and the changes in anisotropy are consistent with an effect of acidosis on intercellular coupling.2,3 Importantly, during acidosis, CV slowing in the ZP123-treated preparations was significantly attenuated both longitudinally and transverse to fibers (P<0.05, by 2-way ANOVA with Bonferroni/Dunn post hoc analysis). There was relative preservation of CV during acidosis in the ZP123 group, which became significant at 16 minutes and persisted throughout the duration of the experiment. These results, taken in conjunction with the absence of an effect of ZP123 on $I_{Na}$ or isolated myocyte action potentials, strongly support a mechanism of action of ZP123 on intercellular communication, presumably through the targeting of cardiac gap junctions.

Discussion

In the present study, we sought to determine whether ZP123 could enhance conduction and prevent APD dispersion, both of which could prove to be antiarrhythmogenic actions during ischemia-induced intercellular uncoupling. Because it is well established that ischemia-induced cellular acidification is responsible for reducing gap junction conductance, we used an acidosis model to evaluate the efficacy of pharmacological targeting gap junctions by ZP123. Our data demonstrate that ZP123 effectively prevented a nearly 2-fold slowing of CV and prevented significant increases in APD dispersion during acidosis. Because ZP123 had no apparent effect on inward $I_{Na}$ or repolarizing currents, its actions were likely attributable to prevention of intercellular uncoupling. These actions could represent a novel mechanism for antiarrhythmic drug action through the targeting of gap junctions.

Clearly, in addition to its effect on gap junctions, ischemia produces numerous changes in cellular electrophysiology.23–25 To isolate the effects of ischemia on gap junction coupling, we used an experimental model of extracellular acidosis. Conditions of low intracellular pH are known to promote uncoupling in isolated oocytes and cell pairs.4,6 In intact tissue, it is possible to manipulate extracellular pH to modulate gap junction coupling. Although we did not measure intracellular pH, we used a similar level of extracellular acidosis found to produce uncoupling previously.3,26,27 The
data presented in this report are consistent with this time course of uncoupling, because the ZP123 effect on conduction first became significant at 16 minutes (Figure 7).

The precise mechanism by which ZP123 modulates gap junction function remains unknown and cannot be fully ascertained from our results. The significant effect on conduction during acidosis but not at baseline (Figure 6) suggests that ZP123 only affects gap junctions under conditions of metabolic stress. The preservation of conduction during acidosis suggests an effect of ZP123 related to maintenance of gap junctions in their open state, possibly by interfering with the particle-receptor binding mechanism of connexin43.4 Additional studies are required to determine whether ZP123 can open or restore function to gap junctions that have already closed. More fundamental to this discussion is the identification of the binding target for the compound. It is not known whether ZP123 interacts with membrane-bound ligands or if it crosses the cell membrane and binds directly to connexin protein complexes or signaling molecules.

ZP123 enhances CV during acidosis while having no effect on sodium channels in single cells. In addition, ZP123 eliminated the acidosis-induced APD dispersion without affecting APD on average. Slow conduction and inhomogeneous repolarization are known to produce a highly arrhythmogenic substrate. By specifically targeting gap junctions, ZP123 counteracted these arrhythmogenic effects, providing evidence for the antiarrhythmic mechanism of this compound. It is interesting to note the fact that although ZP123 had a major effect on APD dispersion during acidosis, the increase in APD during acidosis was the same both with and without ZP123. These findings provide support for ZP123 affecting gap junctions while having no effect on membrane currents.

In general, conduction slowing can be attributed to 1 of 2 mechanisms, diminished cardiac excitability or increased intercellular resistance. In the present report, acidosis produced a slowing of conduction and an increase in APD dispersion. These changes are expected during diminished gap junction coupling.9,11 However, because one cannot rule out an effect of acidosis on excitability, we performed experiments in isolated myocytes to determine the effects of acidosis and ZP123 on \( I_{\text{Na}} \) and the action potential. We found that acidosis produced a rightward shift in the \( I_{\text{Na}} \) current-voltage relationship, providing another mechanism for the
Figure 7. ZP123 inhibits acidosis-induced conduction slowing. The progressive slowing of CV during acidosis was significantly attenuated both transverse and longitudinal to cardiac fibers by ZP123. There was no significant change in anisotropic ratio, although there was a trend for ZP123 to maintain anisotropy. These data are consistent with a preservation of intercellular coupling by ZP123. *P<0.05, obtained by 2-way ANOVA with Bonferroni/Dunn post-hoc analysis.

Acidosis-induced conduction slowing in addition to intercellular uncoupling. However, because acidosis produced identical changes in \( I_{\text{Na}} \) in both the ZP123 and no-drug groups, this mechanism cannot be responsible for the relative preservation of CV in the ZP123 group, leaving modulation of intercellular coupling as the most likely mechanism. The data presented in this report also support the lack of any effect of ZP123 on sarcolemmal ion channels, because there was no difference in action potential parameters between the ZP123 and no-drug groups. In addition, previous studies have shown that ZP123 has a low binding affinity for ion channels, additionally indicating that this compound is unlike conventional antiarrhythmic drugs.\(^\text{13}\)

To further develop antiarrhythmic strategies based on targeting of gap junctions, it is critically important to better understand the specific effects of gap junction function and expression on the cardiac arrhythmogenesis. Clearly, spatial dispersion of repolarization promotes susceptibility to reentrant arrhythmias.\(^\text{28}\) There are at least 2 mechanisms by which enhanced intercellular coupling from pharmacological targeting of cardiac gap junctions can reduce spatial dispersions of repolarization. First, greater electrical coupling between cells reduces differences in APD between cells possessing different ionic composition across the heart by increasing electronic current flow between neighboring cells.\(^\text{11,12}\) Second, restoration of electrical coupling will reduce differences in depolarization times between cells by enhancing conduction, thereby diminishing spatial gradients of activation.\(^\text{19}\) When expressing CV in terms of activation gradients (CV=1/activation gradient), we found that increased APD dispersion accounted for a greater portion (61%) of acidosis-induced dispersion of repolarization than conduction slowing (39%). Similarly, when comparing the no-drug group with the ZP123 group, the action of ZP123 to ameliorate potentially arrhythmogenic dispersions of repolarization was attributable to a greater extent to its effect on homogenizing ADP gradients between cells rather than its effect on enhancing conduction. Because APD gradients are independent of the direction of propagation,\(^\text{19}\) the effect of ZP123 on overall dispersion of repolarization is not expected to be influenced to a large extent by propagation direction. ZP123 did not affect average APD, because the increased electrical coupling it caused between cells both reduced and prolonged APD in cells possessing the longest and shortest APDs, respectively.

There are other potentially important antiarrhythmic actions of ZP123 that should be considered. The prevention of greater than 2-fold slowing of CV by ZP123 could itself be highly antiarrhythmic (Figure 7). Uncoupling may also serve to organize and stabilize reentrant circuits by increasing the critical curvature of the excitatory wavefront. Finally, restoration of intercellular coupling may also prevent arrhythmogenic discordant alternans between cells.\(^\text{20}\) The present study may provide an electrophysiological basis for earlier observations of suppression of ventricular tachycardia by ZP123 during acute ischemia.\(^\text{13}\) Additional studies are required to determine whether ZP123 possesses antiarrhythmic properties in other disease models.

Traditional antiarrhythmic drugs target 1 or more sarcolemmal ion channels to modify the properties of conduction and repolarization at the single-cell level. The fact that most antiarrhythmic compounds not only fail to prevent arrhythmias but may actually promote arrhythmias (ie, are proarrhythmic) provides significant impetus for developing fundamentally different approaches to the pharmacological suppression of arrhythmias. Because many disease states that predispose to arrhythmias are known to substantially effect gap junction expression and function,\(^\text{1,3,29–31}\) drugs like ZP123 that target gap junctions offer the potential for a novel strategy for antiarrhythmic drug therapy without its well-recognized proarrhythmic side-effects. Because ZP123 did not affect cellular repolarization (Figures 2 and 4), it is unlikely that treatment would result in proarrhythmia from acquired LQTS. However, earlier reports by some investigators\(^\text{32}\) but not others\(^\text{33}\) have suggested that pharmacological uncoupling can organize fibrillation wave fronts, therefore suggesting, theoretically, that the restoration of intercellular coupling might destabilize reentry and be proarrhythmic. In this light, it is apparent that considerable additional study is required to determine whether such a strategy is effective and safe for clinical use.
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