Oxidative Mediated Lipid Peroxidation Recapitulates Proarrhythmic Effects on Cardiac Sodium Channels

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Abstract—Sudden cardiac death attributable to ventricular tachycardia/fibrillation (VF) remains a catastrophic outcome of myocardial ischemia and infarction. At the same time, conventional antagonist drugs targeting ion channels have yielded poor survival benefits. Although pharmacological and genetic models suggest an association between sodium (Na⁺) channel loss-of-function and sudden cardiac death, molecular mechanisms have not been identified that convincingly link ischemia to Na⁺ channel dysfunction and ventricular arrhythmias. Because ischemia can evoke the generation of reactive oxygen species, we explored the effect of oxidative stress on Na⁺ channel function. We show here that oxidative stress reduces Na⁺ channel availability. Both the general oxidant tert-butyl-hydroperoxide and a specific, highly reactive product of the isoprostane pathway of lipid peroxidation, E₂-isoketal, potentiate inactivation of cardiac Na⁺ channels in human embryonic kidney (HEK)-293 cells and cultured atrial (HL-1) myocytes. Furthermore, E₂-isoketals were generated in the epicardial border zone of the canine healing infarct, an arrhythmogenic focus where Na⁺ channels exhibit similar inactivation defects. In addition, we show synergistic functional effects of flecainide, a proarrhythmic Na⁺ channel blocker, and oxidative stress. These data suggest Na⁺ channel dysfunction evoked by lipid peroxidation is a candidate mechanism for ischemia-related conduction abnormalities and arrhythmias. (Circ Res. 2005;97:1262-1269.)

Key Words: sodium channels | lipid-peroxidation | isoketals | myocardial infarction

Sudden cardiac death attributable to ventricular tachycardia/fibrillation (VVT/VF) remains a major health problem, with costly implanted defibrillators as the major advance in therapy in the past decade. Conventional antiarrhythmic drugs selectively targeting ion channels that drive cardiac excitability have not yielded convincing survival benefits, and in many cases they elicit a paradoxical increase in arrhythmia risk. Moreover, growing evidence in animal models of reentrant VT implicates proarrhythmic interaction in arrhythmia risk. Moreover, growing evidence in animal models suggests that enhanced Na⁺ channel inactivation may be a common proarrhythmic gating change, although the molecular mechanisms responsible for altered inactivation in acquired and genetic arrhythmias may be entirely unique. Moreover, a molecular mechanism whereby cardiac ischemia alters Na⁺ channel function has not been identified.

Although the generation of reactive oxygen species during reperfusion of ischemic tissues has been the focus of much research, more recently it has been shown that reactive oxygen species derived from the mitochondria are generated in cardiomyocytes during ischemia. One of the major targets of oxidative modification by free radicals are lipids, which undergo peroxidation. Because cardiac ion channels are embedded in the membrane lipid bilayer, we hypothesized...
that lipid peroxidation may alter the function of these channel proteins. We reasoned that this could occur either as a result of structural alterations of the lipid bilayer by oxidative modification of membrane lipids or by the adduction of channel proteins by electrophilic short chain aldehydes produced by lipid peroxidation. In this regard, oxidative stress has been found to induce alterations in the function of a number of membrane proteins, including ion channels, enzymes, and receptors.9–11 Electrophilic short chain aldehydes produced by lipid peroxidation, such as 4-hydroxynonenal (HNE), are capable of producing some of these effects when added to cells.12–14 However, isoketals (IsoKs), highly reactive γ-ketoaldehydes formed by the isoprostane (IsoP) pathway of lipid peroxidation,15 preferentially adduct to protein lysyl residues (Figure 1) and therefore would be more attractive candidates for mediating these effects for a number of reasons. First, short chain aldehydes are scission products of oxidized fatty acids in phospholipids and thus are released from the fatty acid backbone as they are formed.16 Furthermore, short chain aldehydes are orders of magnitude less reactive than IsoKs15 and are more hydrophilic, which would permit their diffusion from the membrane before adduction to membrane proteins. Consistent with this hypothesis, short chain aldehydes, unlike IsoKs, have been detected as free unadducted compounds in biological fluids and tissues.16 The hypothesis that alteration in cardiac ion channel function by free radical-mediated lipid peroxidation is potentially caused by the formation of IsoKs is supported by our recent finding that IsoKs formed in membrane phospholipids adduct to proteins while still esterified, suggesting that membrane proteins are a preferred target for adduction by IsoKs.17 Moreover, these studies demonstrated that IsoKs dramatically inhibited the function of the delayed rectifier potassium current I\(_{\text{Kr}}\) in cultured atrial tumor cell line 1 (AT-1) myocytes.

Here, we link consistent changes in Na\(^+\) channel inactivation gating to oxidative stress and show that IsoKs are likely candidates for mediating this effect. The observed gating changes are similar to those associated with genetic loss-of-function Na\(^+\) channelopathies such as Brugada syndrome and also mimic loss-of-function Na\(^+\) channel gating defects seen in canine myocytes surviving in the infarcted heart. These results are in sharp contrast to previous findings that show an increase in rat cardiac Na\(^+\) current upon exposure to HNE.12 The differential effects of HNE and IsoK on Na\(^+\) channels could be attributable to their mechanisms of action: Whereas HNE is thought to exert its effect through tyrosine phosphorylation,18 IsoK is thought to exert its effect through formation of covalent adducts with lysyl residues on proteins. In addition, we show synergy between the pharmacological effects of flecainide and oxidative stress. We also find that IsoK lysyl protein adducts accumulate in cultured (human embryonic kidney [HEK]) cells on exposure to an oxidant, as well as in the epicardial border zone (EBZ) cells of the 5-day infarcted heart, where Na\(^+\) channels have been shown to exhibit enhanced inactivation.19 Our studies suggest Na\(^+\) channel dysfunction evoked by IsoKs is an important candidate mechanism for ischemia-related arrhythmias and sudden cardiac death, and it may help explain the proarrhythmic effects of Na\(^+\) channel-blocking agents during myocardial ischemia.

**Materials and Methods**

**Molecular Biology and Heterologous Expression**

Recombinant Na\(^+\) channel was prepared as previously described20 and subcloned into the expression vector pCGI (provided by David Johns, Johns Hopkins University, Baltimore, Md) for bicistronic expression of the channel protein and green fluorescent protein reporter in HEK-293 cells. cDNAs were transiently transfected into HEK-293 cells using lipofectamine (GIBCO-BRL), and were cultured in modified Eagle’s medium supplemented with 10% fetal bovine serum and 1% pen-strep in a 5% CO\(_2\) incubator at 37°C for 1 to 4 days.
Electrophysiology

Whole-cell Na⁺ currents were recorded from cells exhibiting green fluorescence at room temperature (Axopatch 200B, Axon Instruments) using electrodes of 1 to 2 mol/LΩ when filled with a pipette solution containing (in mmol/L): NaF 10, CsF 110, CsCl 20, EGTA 10, Hepes 10 (pH 7.35 with CsOH). The bath solution contained (in mmol/L): NaCl 145, KCl 4.5, CaCl₂ 1.5, MgCl₂ 1, Hepes 10 (titrated to pH 7.35 with CsOH). For HL-1 myocyte recordings, the bath solution contained (in mmol/L): NaCl 145, CsCl 4.5, MgCl₂ 1.5, CaCl₂ 1, CdCl₂ 0.1, glucose 5, HEPES 5 (pH 7.35 with CsOH). In all recordings, 75% of the series resistance was compensated, yielding a maximum voltage error of ≈1 mV. The oxidative agent tert-butyl hydroperoxide (t-BHP), and lipid peroxidation products (discussed below) were dissolved directly in the extracellular solution.

Na⁺ currents (Iₙa) were sampled at 20 KHz through an A/D converter (Digit Data 1200, Axon Instruments) and low pass filtered at 5 KHz. Data were collected and analyzed using pClamp 8.0 software (Axon Instruments). Gating kinetics were assessed using protocols described in the text and figure legends. To minimize time-dependent drift in gating parameters, all protocols were conducted at the same time point after obtaining whole-cell configuration. All results are expressed as mean±SEM and statistical comparisons were made using one-way ANOVA (Microcal Origin) with P<0.05 indicating significance. Multieponential functions were fitted to the data with nonlinear least-squares methods (Origin). The interpulse interval in all experiments was no less than 3 seconds to allow for full recovery of Na⁺ currents (full recovery occurs within 1 sec at -120 mV; see Results).

Analysis of Reactive Products of Lipid Peroxidation

T75 flasks of confluent HEK-293 cells were treated with 1 mmol/L t-BHP for 30 minutes at room temperature and then the oxidation reaction was quenched by placing the cells on ice and treating them with 10 mmol/L sodium borohydride for 30 minutes. Levels of total IsoK-lysyl-lactam adduct were then measured as previously described. Like t-BHP, IsoK also caused a negative shift in the voltage dependence of activation evaluated using the protocol shown in the inset. Voltage-dependence of activation over a range of membrane potentials.

Preparation of Myocardial Infarction

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (Pub. No. 85-23, 1996). Healthy mongrel dogs (12 to 15 kg, 2 to 3 years old) were used in these studies. Under isoflurane anesthesia (30 mg/kg) and sterile conditions, myocardial infarction was produced by a 2-step total occlusion of the left coronary artery using the Harris procedure. Dogs were treated with lidocaine (2 mg/kg intravenously) if multiple ventricular beats occurred at the time of the surgical procedure. Five days after surgery, a cardiectomy was performed with the dogs under sodium pentobarbital (30 mg/kg intravenously) anesthesia. Thin slices of tissue were taken from the epicardium and myocardial core of the visible epicardial border zone (EBZ) area as previously described, as well as an area remote from the infarct (left ventricular base). Tissues were quickly frozen for analysis. In some hearts, columns of tissue (from epicardium toward infarct core) were fixed using paraformaldehyde for histochemical examination.

Results

Oxidizing Agents Reduce Cardiac Na⁺ Channel Availability

We recorded Iₙa from HEK cells expressing Nav1.5 in control conditions and after 30 minutes of preincubation with a membrane-permeant form of hydrogen peroxide (t-BHP). To assess the voltage-dependence of channel availability (Figure 2A), data were fitted by a Boltzmann function (y = [1 + exp(V-V₅₀/k)]⁻¹), where V₅₀ is the half-maximal voltage and k is the slope factor. Over a range of holding potentials, channel availability was altered by preincubation with t-BHP (1 mmol/L) at V₁/₂ was hyperpolarized by 7 mV, from -86.7±1.6 mV to -93.9±1.9 mV (P<0.01). There was no effect on Iₙa decay (Figure 2A, inset). To further examine whether the gating effects induced by t-BHP resulted from generation of products of lipid peroxidation, we exposed HEK cells to an F₂-IsoP with potent receptor-mediated biological actions, 15-F₂-IsoP (8-iso-PGF₂α), and 2 reactive products of lipid peroxidation, HNE and an E₂-IsoK.
IsoK (10 μmol/L) shifted the steady-state availability curve to hyperpolarized potentials, similar to what was seen with t-BHP (Figure 2B, V_{50}; from −89.0±1.3 mV to −96.4±1.1 mV; P < 0.01). Unlike t-BHP, IsoK slightly but significantly accelerated I_{Na} delay during a depolarization pulse from −120 mV to −20 mV (Figure 2B, inset). Inactivating currents were fitted by a double exponential function, and both the fast and slow time constants were reduced. The fast time constants were 0.60±0.02 ms and 0.50±0.02 ms (P < 0.05) and the slow time constants were 3.01±0.3 ms and 2.17±0.2 ms (P < 0.05) for control and IsoK, respectively. Although the magnitude of this effect is small, it suggests that the effects t-BHP and IsoK slightly differ. However, neither HNE nor 15-F_{2t}-Iso had any detectable gating effects on I_{Na}, including I_{Na} decay, voltage-dependent activation, or the voltage-dependence of channel availability (V_{50} was −87.9±1.8 mV and −91.4±1.8 mV, for control and 15-F_{2t}-IsoP, respectively, P=NS; V_{50} was −86.7±1.6 mV and −83.7±2.1 mV, for control and HNE, respectively, P=NS).

As seen in Figure 2C, the voltage-dependence of Na\(^+\) channel activation was not affected by t-BHP and IsoK (V_{50} control: −43.1±1.3 mV; t-BHP: 42.4±1.5 mV; and Iso-K: −41.6±1.5 mV). Although previous studies have observed a persistent inward Na\(^+\) current during oxidative stress and exposure to hydrogen peroxide (H\(_2\)O\(_2\)), we did not observe a noninactivating component during exposure to t-BHP or IsoK. This could be attributable to differences in mechanism of action (t-BHP and IsoK, but not H\(_2\)O\(_2\), are localized in the membrane) or species differences. In summary, the gating effects of t-BHP and IsoK were similar, causing a hyperpolarizing effect on voltage-dependent availability, consistent with a selective stabilization of the inactivated conformational state. This effect appears specific for IsoKs, as other products of lipid peroxidation, such as F_{2t}-IsoP and even the reactive aldehyde HNE, had no effect. The small effect of IsoK on I_{Na} decay suggests the effects of IsoK and nonspecific oxidation by t-BHP, although highly similar, are not identical, and we can speculate that t-BHP generates one or more unidentified oxidation product that counteract the small I_{Na} decay effect of IsoK.

To establish that exposure of the cells to t-BHP induced the formation of IsoKs, we measured the amount of IsoK-lysyl-lactam adduct formed on cellular proteins after incubation of HEK cells with t-BHP for 30 minutes. Exposure to t-BHP increased the levels of IsoK-lysyl-lactam adducts 4.8-fold (P < 0.006; Figure 3). Therefore, exposure of cells to t-BHP significantly increases IsoK adducts, which likely accounts for the similarity in the effects of t-BHP and IsoK on sodium channel gating.

**Synergistic Effects of Na\(^+\) Channel Blockers and IsoK**

Given the evidence that free radicals are generated under ischemic conditions in cardiac myocytes and that Na\(^+\) channel block can be proarrhythmic after myocardial infarction, we tested the combined effects of flecainide and IsoK on Na\(^+\) channel function to determine whether these effects were synergistic. We examined the steady-state availability of Na\(^+\) channels in the presence of flecainide (1 μmol/L) and
IsoK alone. Although IsoK shifted the availability curve to more negative potentials (Figure 4A, V_{1/2}: from −86.4 ± 2.1 mV to −99.3 ± 2.3 mV; *P < 0.0001), flecainide had no effect (Figure 4A). Furthermore, in the presence of IsoK, flecainide had no additional effect.

Previous studies have suggested that flecainide block requires opening of the channels (supported by our studies showing no effect of flecainide on steady-state channel availability; Figure 4A) and that the block is stabilized during inactivation.28 Therefore, to further characterize the interaction between IsoK and flecainide, a twin-pulse protocol (Figure 4B, inset) was used to evaluate recovery of Na⁺ channel function from inactivation: I_{Na} recorded in the second pulse was measured relative to that recorded during the preceding first pulse as the interpulse interval was progressively increased from 1 ms to 1 sec. Figure 4B plots the magnitude of I_{Na} in the second pulse relative to first pulse. To quantify the recovery from inactivation, data were fitted by a 2 exponential function (Table). Flecainide at physiological concentrations (1 μmol/L) had no effect on the recovery of sodium channels. This result is consistent with previous studies that showed only a modest delay in recovery from inactivation in the presence of 1 μmol/L flecainide.29 IsoK delayed both kinetic components (τ_{fast} from 12.5 ± 1.1 ms to 17.4 ± 2.2 ms, *P < 0.05; τ_{slow} from 48.7 ± 3.2 ms to 155.1 ± 32.9 ms, *P < 0.001, respectively). In the presence of flecainide, IsoK still delayed both components (time constant (τ_{fast} from 10.3 ± 2.2 ms to 29.7 ± 1.8 ms, *P < 0.0001; τ_{slow} from 64.3 ± 3.8 ms to 119.4 ± 10.1 ms, *P < 0.0001, respectively), and the effect of IsoK on the fast component was greater in flecainide than IsoK alone (P < 0.01). This suggests that flecainide and IsoK have a synergistic effect in delaying the recovery from inactivation. Moreover, the time constant analysis (Table) indicates that the enhanced delay of I_{Na} recovery in IsoK and flecainide relative to IsoK alone is attributable to a predominant effect of slowing the fast recovery time constant rather than the slow time constant.

### Isoketal Adducts Accumulate in the EBZ of the Infarcted Heart

To establish the relevance of these findings with regard to the myocardium, we first examined the effects of t-BHP and IsoK on native Na⁺ channels in HL-1 atrial myocytes. Figure 5A shows the current-voltage relation (normalized to cell capacitance) during control and after exposure to t-BHP and IsoK obtained using the protocol shown in Figure 2C. Incubation of t-BHP (2 mmol/L) for 1 hour and IsoK (10 μmol/L) for 30 minutes decreased sodium current density (control: −163 ± 25 pA/pF; t-BHP: −87 ± 12 pA/pF, *P < 0.05 versus control; IsoK: −91 ± 14 pA/pF, *P < 0.05 versus control). As in the recombinant system, t-BHP and IsoK did not affect voltage-dependence of activation (Figure 5B) (V_{1/2}: −34.2 ± 2.2 mV in t-BHP, P = NS; −35.4 ± 1.4 mV in IsoK, P = NS), but shifted channel availability (Figure 5C) to hyperpolarizing potentials (V_{1/2}: −73.6 ± 1.4 mV in control; −82.8 ± 1.7 mV in t-BHP, *P < 0.01; and −82.0 ± 1.7 mV in IsoK, *P < 0.01). The inset in Figure 5B shows the effect of t-BHP and IsoK on current decay in HL-1 cells. Unlike HEK-293 cells, both products had no effect on current decay (see figure legend for time constants).

Prior studies of Na⁺ channel function in cells from the EBZ of the 5-day infarcted canine heart reveal changes in Na⁺ channel gating not unlike those observed in the present study with IsoK.19 Therefore, we explored whether IsoK adducts are increased in vivo in canine hearts after infarction. Dogs were subjected to ventricular infarction according to the method of Harris,23 and after 5 days, transmural sections from the EBZ of the infarct and the remote area were excised and fixed in paraformaldehyde (see Materials and Methods). We determined the location of isoketal adducts using an anti-isoketal adduct single-chain antibody (D11 ScFv) that we previously showed to be highly specific to IsoK and to exhibit no cross-reactivity with other lipid peroxidation products such as HNE, 4-oxononanal, and 15-F_{2t}-isoprostane.30 Only trivial immunoreactivity was present in remote sections of cardiac tissue (Figure 6A). In contrast, intense immunoreactivity (red color) was present in the myocardial core and in the EBZ of the infarcted heart (Figure 6B). Preincubation of D11 ScFv with a small peptide RKDVY adducted with synthetic IsoK ablated immunoreactivity (not shown).

To confirm the finding of IsoK adducts present in EBZ cells, we measured the amount of IsoK-lysyl-lactam adduct present in tissue sections taken from the core and epicardium of both remote and EBZ regions of the infarct using electrospray tandem mass spectrometry. Levels of IsoK-lysyl-lactam adduct were increased strikingly in the epicardium and core regions of the EBZ (Figure 7) compared with remote regions of the same ventricles. These results clearly demonstrate that the formation of IsoKs are significantly increased in the EBZ of infarcted hearts, where they are positioned to adduct to Na⁺ channel proteins and alter channel function. To compare

### Kinetic Parameters for Recovery From Inactivation

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>A₁</th>
<th>τ_{fast}</th>
<th>A₂</th>
<th>τ_{slow}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17</td>
<td>0.77±0.03</td>
<td>12.5±1.1</td>
<td>0.23±0.03</td>
<td>48.7±3.2</td>
</tr>
<tr>
<td>IsoK</td>
<td>9</td>
<td>0.79±0.04</td>
<td>17.4±2.2*</td>
<td>0.21±0.04</td>
<td>155.1±32.9†</td>
</tr>
<tr>
<td>Flecainide, 1 μmol/L</td>
<td>12</td>
<td>0.80±0.04</td>
<td>10.3±2.2</td>
<td>0.20±0.04</td>
<td>64.3±3.8</td>
</tr>
<tr>
<td>Flecainide+IsoK</td>
<td>8</td>
<td>0.84±0.04</td>
<td>29.7±1.8‡§</td>
<td>0.16±0.04</td>
<td>119.4±10.1‡</td>
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The data in Figure 4B were fitted to a 2 exponential function of the form y = y₀ + A₁(1 - exp(-t/τ_{fast})) + A₂(1 - exp(-t/τ_{slow})), where y₀ is the offset, A₁ is the amplitude of the fast component, A₂ is the amplitude of the slow component, and τ_{fast} and τ_{slow} are the time constants of fast and slow component, respectively. Fitted parameters are shown.

*P < 0.05; †P < 0.001 vs control; ‡P < 0.0001 vs flecainide 1 μmol/L; §P < 0.001 vs IsoK.
the amount of IsoK adduct formed in HEK-293 cells to that formed in ischemic tissue, we estimated the IsoK adduct per mg protein by normalizing the adduct formation (from Figure 3) to amount of protein found in our typical preparations of HEK-293 cells (87.8 ± 0.6 mg protein per flask). Using this estimate, we find that the IsoK adducts formed in HEK-293 cells (35.4 pg lactam/mg protein) is comparable to those measured from infarcted tissue (Figure 7).

Discussion

Free-radical scavengers, or agents that prevent free radical production, have been shown to reduce the incidence of arrhythmias caused by ischemia in both experimental animals and humans. This strongly suggests that the generation of free radicals in the myocardium can induce an arrhythmic substrate, but the downstream underlying mechanisms have not been fully elucidated. Although functional changes have been described in multiple ion channels and transporters, Na\(^+\)/H\(^+\) channel dysfunction appears to play a key role under these conditions: Arrhythmias in the remodeled ischemic myocardium often evolve from sites of slow conduction near the border zone, which displays rate-dependent slowing and facilitated reentry because of Na\(^+\) channel blockade. In the 5-day healing infarcted heart, cells of the EBZ show post-
repolarization refractoriness,\textsuperscript{19} which is critical for initiation and perpetuation of ventricular tachycardias in this substrate.\textsuperscript{36} Additional studies identified that delayed recovery from Na\textsuperscript{+} channel inactivation in EBZ cells contributed to post-repolarization refractoriness and enhanced use dependence.\textsuperscript{19,37,38} In the present study, we found that acute exposure of HEK cells to an oxidant (t-BHP) mimics oxidative stress in myocardial ischemia by evoking a reduction of Na\textsuperscript{+} current in both heterologously expressed channels and cardiac myocytes. For reasons discussed previously, we thought that IsoKs were an attractive candidate for mediating these effects of oxidation on Na\textsuperscript{+} channel function. Our results clearly support this notion, as incubation of HEK cells with IsoKs mimicked the effects of t-BHP on Na\textsuperscript{+} channel function, whereas another reactive product of lipid peroxidation, HNE, was ineffective. In addition, we demonstrated the formation of IsoK protein adducts in HEK cells after exposure to t-BHP. Finally, we demonstrated by immunohistochemistry that IsoK adducts are selectively increased and localized to the EBZ cells of infarcted canine hearts.

CAST\textsuperscript{+} demonstrated that the administration of flecainide or encainide to suppress premature ventricular contractions increased both cardiac and arrhythmic mortality, contrary to the expectation that these drugs might reduce mortality. In experimental models of myocardial infarction or ischemia-reperfusion, flecainide had proarrhythmic consequences attributable to conduction block or delay attributable to reduced Na\textsuperscript{+} channel availability.\textsuperscript{1,39–41} Computer models support the hypothesis that proarrhythmic mechanisms of Na\textsuperscript{+} channel blockade are associated with the reduction of Na\textsuperscript{+} channel availability.\textsuperscript{42} In our study, flecainide potentiated the delay of recovery from inactivation caused by IsoK (Figure 4B). Studies in the intact EBZ preparation have shown this synergistic effect of flecainide on conduction abnormalities of this arrhythmic substrate.\textsuperscript{1,43}

This study has focused on IsoKs in the EBZ of the healing infarcted heart. We did not include data on acute ischemic substrates, where ion channel changes are quite dynamic and difficult to assess. Our data are compelling in that they show that IsoKs persist in the cell membrane of the infarcted heart and may contribute to severely remodeled ion channels (eg, Na\textsuperscript{+} channels) in the cells of this arrhythmic substrate. Given recent studies that suggest strong familial components in the risk for cardiac arrest during myocardial ischemia and infarction,\textsuperscript{44} it will be important to assess whether arrhythmia risk is influenced by inherited changes in the complex milieu of gene products that coassemble with Na\textsuperscript{+} channels in the cell membrane. Additionally, the identification of lipid peroxidation products as potent modifiers of cardiac Na\textsuperscript{+} channel gating dysfunction during oxidative conditions raises the possibility of developing antiarhythmic agents targeted for action specifically during conditions of myocardial ischemia. Protection of the Na\textsuperscript{+} channels from the downstream effects of lipid peroxidation deserves further evaluation as a new therapeutic approach for the prevention and treatment of sudden cardiac death.

\textbf{Acknowledgments}

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