Calmodulin Kinase Inhibition Prevents Development of the Arrhythmogenic Transient Inward Current

Yuejin Wu, Dan M. Roden, Mark E. Anderson

Abstract—Although it is widely accepted that afterdepolarizations initiate arrhythmias when action potentials are prolonged, the underlying mechanisms are unclear. In this study, we tested the hypothesis that action potential prolongation would raise intracellular calcium and thereby activate the arrhythmogenic transient inward current (I\text{ti}). Furthermore, given that I\text{ti} can be activated by sarcoplasmic reticulum Ca\textsuperscript{2+} release, we tested the hypothesis that inhibition of calmodulin (CaM) kinase would prevent I\text{ti}. Isolated rabbit ventricular myocytes were studied with whole-cell–mode voltage clamp. Stimulation with a prolonged action potential clamp, under near-physiological conditions, increased [Ca\textsuperscript{2+}]. I\text{ti} was reproducibly induced in 60 of 60 cells, but I\text{ti} was not seen with the use of a shorter action potential waveform (n = 12). I\text{ti} was associated with a secondary elevation in [Ca\textsuperscript{2+}]. When [Ca\textsuperscript{2+}] buffering was enhanced by dialysis with BAPTA (20 mmol/L, n = 9), no I\text{ti} was present. The Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger was likely responsible for I\text{ti}, because I\text{ti} was inhibited by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger inhibitory peptide XIP (10 μmol/L, n = 6), but not by an inactive scrambled peptide (10 μmol/L, n = 5) or by the Cl\textsuperscript{−} current antagonist niflumic acid (10 to 40 μmol/L, n = 9). Activator Ca\textsuperscript{2+} from the sarcoplasmic reticulum was essential for development of I\text{ti}, because it was prevented by pretreatment with ryanodine (10 μmol/L, n = 6) or thapsigargin (1 μmol/L, n = 6). Two different CaM kinase inhibitory peptides (n = 16) and a CaM inhibitory peptide (n = 4) completely suppressed I\text{ti}. These results are consistent with the hypothesis that CaM kinase plays a role in arrhythmias related to increased [Ca\textsuperscript{2+}]. (Circ Res. 1999;84:906-912.)

Key Words: calmodulin kinase  ■  sarcoplasmic reticulum  ■  transient inward current  ■  Ca\textsuperscript{2+}-activated chloride current  ■  Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger

Calmodulin (CaM) kinase is a ubiquitous serine/threonine kinase that has recently been shown to modulate the release of activator Ca\textsuperscript{2+} during excitation-contraction coupling in ventricular myocytes.\textsuperscript{1} CaM kinase is activated by increased [Ca\textsuperscript{2+}]\textsuperscript{2}; once activated by Ca\textsuperscript{2+} bound to CaM, CaM kinase can undergo a series of autophosphorylation events to become Ca\textsuperscript{2+}-independent.\textsuperscript{3} We have recently found that this Ca\textsuperscript{2+}-independent component of CaM kinase activity increases during action potential prolongation and early afterdepolarizations (EADs) in isolated rabbit ventricle.\textsuperscript{4} CaM kinase acts at key sites for [Ca\textsuperscript{2+}] homeostasis and is known to increase L-type Ca\textsuperscript{2+} current\textsuperscript{5-7} and facilitate the uptake\textsuperscript{7} and release\textsuperscript{9} of Ca\textsuperscript{2+} by the sarcoplasmic reticulum (SR) in ventricular myocytes. Thus, CaM kinase activity could be important for the development of [Ca\textsuperscript{2+}] overload arrhythmias.

One mechanism for arrhythmias due to [Ca\textsuperscript{2+}] overload is activation of a transient inward current (I\text{ti}).\textsuperscript{10} In non–voltage-clamped cells I\text{ti} causes cell membrane depolarization resulting in delayed afterdepolarizations (DADs) and triggered arrhythmias.\textsuperscript{11} Under near-physiological conditions, I\text{ti} may be due to the electrogenic Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger operating in forward mode,\textsuperscript{12} but results of other experiments performed under less physiological conditions (eg, in the absence of extracellular Na\textsuperscript{+} or after Na\textsuperscript{+}/K\textsuperscript{+} ATPase inhibition) suggest that this current could also be due to a Ca\textsuperscript{2+}-activated cation nonselective current (I\text{CAN})\textsuperscript{12-16} or a Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} current (I\text{ClCa})\textsuperscript{16-19}

Many conditions such as ischemia and tachycardia\textsuperscript{20} may be associated with increased [Ca\textsuperscript{2+}]. Prolongation of action potential repolarization, a therapeutic action of a variety of antiarrhythmic agents,\textsuperscript{21} is also associated with arrhythmias in cardiomyopathy\textsuperscript{22} and congenital long-QT syndromes. Whereas the ionic mechanisms underlying action potential prolongation in these settings are becoming better understood, those underlying the accompanying afterdepolarizations and triggered arrhythmias are less well understood. In these studies, we present evidence that the elevated [Ca\textsuperscript{2+}], which follows action potential prolongation, can activate I\text{ti} due to Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity and that this activation is dependent on CaM kinase. These results suggest that this CaM kinase–dependent mechanism may trigger arrhythmias arising when action potentials are prolonged.

Materials and Methods

Preparation of Isolated Ventricular Myocytes

Isolation of rabbit ventricular myocytes was performed as previously described.\textsuperscript{5} New Zealand White rabbits of either sex (2 to 3 kg) were

---

Received October 1, 1998; accepted February 1, 1999.

From the Departments of Medicine (Y.W., D.M.R., M.E.A.) and Pharmacology (D.M.R., M.E.A.), Vanderbilt University, Nashville, Tenn.

Correspondence to Mark Anderson, Vanderbilt University Medical Center, 315 Medical Research Building II, Nashville, TN 37232-6300. E-mail mark.anderson@mcmail.vanderbilt.edu

© 1999 American Heart Association, Inc.

Circulation Research is available at http://www.circresaha.org
euthanized with a pentobarbital overdose (50 mg/kg, IV) after heparin infusion (150 U/kg, IV). The collagenase-containing solution was prepared in nominally Ca\(^{2+}\)-free solution containing 60 U/mL collagenase type I from Worthington Biochemicals and 0.1 U/mL type XIV protease from Sigma. Myocytes were dispersed by gentle agitation and then maintained in standard saline solution containing 1.8 mM/L CaCl\(_2\). Myocytes were studied within 8 hours after dispersion. Only rod-shaped quiescent myocytes with clear striations from the left ventricle were studied.

**Solutions**

The solutions for preparation of isolated ventricular myocytes were described previously. The bath solution contained (in mmol/L) NaCl 140.0, HEPES 5.0, glucose 10.0, KCl 5.4, CaCl\(_2\) 2.5, and MgCl\(_2\) 1.0. The pH was adjusted to 7.4 with 10 N NaOH. The intracellular solution contained (in mmol/L) potassium aspartate 120.0, HEPES 5.0, KCl 25.0, disodium ATP 4.0, MgCl\(_2\) 1.0, disodium phosphocreatine 2.0, and sodium GTP 2.0. The pH was adjusted to 7.2 with 1 N KOH. In some experiments, the CI\(^-\) current antagonist niflumic acid was added to the bath solution for a final concentration of 10 to 40 \(\mu\)mol/L. The SR Ca\(^{2+}\) release channel blocker ryanodine (10 \(\mu\)mol/L) or the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase antagonist thapsigargin (1 \(\mu\)mol/L, Calbiochem) were included in other experiments. Thapsigargin was added as a DMSO stock solution with a final DMSO concentration of 0.0001 vol %. Unless otherwise noted, all chemicals were from Sigma.

**Electrophysiology**

**Current Clamp**

Cells were stimulated at 0.1 Hz in current clamp mode with 1.0- to 2.0-nA pulses of depolarizing current (1.25 times threshold) for 3 to 4 ms at room temperature (20°C to 23°C). Action potentials were low pass filtered at 2 kHz and sampled at 2.5 kHz with a 12-bit analog to digital converter (Digidata 1200 B) from Axon Instruments. Representative short (APD\(_{50}\)=194 ms; APD\(_{90}\)=217 ms) and long (APD\(_{50}\)=422; APD\(_{90}\)=561 ms) action potential waveforms were digitized and stored for application as voltage commands for most experiments using pClamp 6.03 (Figure 1).

**Voltage Clamp**

Isolated myocytes were studied with whole-cell–mode voltage-clamp configuration at 32°C on a heated stage (Warner Instrument Corp) using an amplifier (Axopatch 200B, Axon Instruments), as previously described. Micropipette electrode resistance was 1.5 to 3.5 MΩ when micropipettes were filled with the intracellular solution. Membrane currents were low pass filtered at 2 kHz and sampled at 6.7 kHz. The digitized signals were stored for later analysis with pClamp 6.03 (Axon Instruments) and Sigma Plot (Jandel Scientific). Cell membrane capacitance (167±2.2 pF) was measured using the integral of the current transient after a 10-mV hyperpolarizing step from a holding potential of –80 mV or a 10-mV depolarizing step from a holding potential of 0 mV.

**I\(_c\), Measurement**

After a dialysis-and-stabilization period of ≥5 minutes, cells were repetitively (≥50 times) depolarized at 0.5 Hz with either a long or a short action potential command waveform (Figure 1). The presence or absence of oscillatory currents after completion of repolarization (I\(_c\)) was noted during the first 50 depolarizations, and peak I\(_c\) was measured as the difference between the current at the onset of the oscillation and the current at maximum excursion of the oscillation. Oscillatory currents were also induced at different test potentials following conventional conditioning steps (0.5 Hz) from –80 to +10 or +30 mV (Figure 2D).

**[Ca\(^{2+}\)]\(_i\), Measurements**

Fluo 3

[Ca\(^{2+}\)]\(_i\) was monitored to compare the effect of long and short action potential waveforms on [Ca\(^{2+}\)]\(_i\), by including the pentapotassium salt of the fluorescent Ca\(^{2+}\) indicator Fluo 3 (Molecular Probes) in the pipette solution (100 μmol/L) as previously described, with minor modifications. Voltage signals were low pass filtered at 50 Hz before analysis. Steady-state fluo 3 [Ca\(^{2+}\)]\(_i\) transients were integrated using pClamp 6.03.

**Indo 1**

The pentapotassium salt of the ratiometric Ca\(^{2+}\) indicator indo 1 (100 to 300 μmol/L) was dialyzed into cells to verify a secondary increase in [Ca\(^{2+}\)]\(_i\) during I\(_c\). Excitation light was band pass filtered (360±20 nm) and reflected with a dichroic mirror (375 nm, Omega Optical) to the myocyte on the inverted microscope stage (Nikon). Emission light (ie, >375 nm) was reflected through an adjustable window to exclude extraneous fluorescence, split with a second dichroic mirror (455 nm), and represented as the ratio of 405±20/500±20 nm. Photobleaching was limited to 10% of peak output by an electronically controlled shutter device (Uniblitz) for both indo 1 and fluo 3 experiments.

**Inhibitory Peptides**

In separate experiments, cells were dialyzed for ≥5 minutes with inhibitory peptides against CaM kinase, protein kinase A (PKA), and...
protein kinase C (PKC) before the experimental protocol was initiated. CaM kinase was inhibited with the following peptides (20 μmol/L): 273-302, 291-317, and AC3-I (KKAL-HRQEAVDCL). Inactive control peptide sXIP did not prevent In.

C. Niflumic acid 10 μM

D. C, Niflumic acid did not suppress In. D, Schematic depiction of conditioning and test command voltages, applied at 0.5 Hz, used to generate the raw data tracings (E) and current-voltage relationships (F).

Figure 2. In is likely due to the Na⁺/Ca2⁺ exchanger. A through C, Data tracings from separate myocytes stimulated with the long action potential waveform (Figure 1A). A, Tracing from a myocyte dialyzed with XIP, which prevented In. B, Inactive control peptide sXIP did not prevent In. C, Niflumic acid did not suppress In. D, Schematic depiction of conditioning and test command voltages, applied at 0.5 Hz, used to generate the raw data tracings (E) and current-voltage relationships (F). E, Oscillatory currents (single arrows) in response to a range of test cell membrane potential (Vm) steps with Vm indicated next to each tracing. The large inward current present at the –80 mV test Vm was the inward rectifier, because it was abolished by addition of Ba2⁺ (0.5 mmol/L) (not shown). A small oscillatory biphasic current was present in later conditioning steps (double arrows) and was absent in XIP-treated cells. $E_{rev}$ for the oscillatory currents was not changed by reversing the order of test Vm commands (not shown). F, Current-voltage relationship of the oscillatory currents. Oscillatory inward currents were prevented by dialysis with XIP. XIP significantly reduced peak currents ($P=0.005$) at all test potentials except for –80 mV ($P=0.06$) and +60 mV ($P=0.059$) relative to control. Niflumic acid did not affect the inward currents but significantly reduced the outward current at +40 ($P=0.003$) and +60 mV ($P=0.026$) compared with control.
IC_{50}, \approx 3 \mu mol/L interaction of activated CaM kinase with substrate. The peptide AC3-C (KKALHAQERVDCL) has no inhibitory activity and was included in the pipette solution (20 \mu mol/L) in control experiments. CaM kinase inhibitory peptides were prepared using a solid-phase peptide synthesizer (Applied Biosystems) and purified by reverse-phase HPLC. The sequences were confirmed by automated sequencing. These peptides were generous gifts of Dr Howard Schuman (Stanford University, Stanford, Calif).

The competitive PKA inhibitory peptide (IC_{50}=0.2 \mu mol/L) corresponds to positions 6 to 22 of PKA and was included in the pipette solution at 10 \mu mol/L. The competitive PKC inhibitory peptide (IC_{50}=0.3 \mu mol/L) corresponds to the autoinhibitory domain positions 19 to 36 of PKC and was included in the pipette solution at 20 \mu mol/L. Both PKA and PKC inhibitory peptides were obtained commercially (Gibco-BRL). The Na^{+}/Ca^{2+} exchanger inhibitory peptide (XIP) is modeled after a putative CaM binding site on the Na^{+}/Ca^{2+} exchanger and acts to potentially inhibit Na^{+}/Ca^{2+} exchanger current in ventricular myocytes when included in the dialysate (10 or 20 \mu mol/L). Scrambled XIP (sXIP) does not affect the Na^{+}/Ca^{2+} exchanger and was included in the pipette solution (10 \mu mol/L) for the control experiments. Both XIP and sXIP were generous gifts from Dr Kenneth Philipson (UCLA, Los Angeles, Calif).

**Results**

**The Transient Inward Current Follows Stimulation With a Long Action Potential Waveform**

Stimulation using a prolonged action potential waveform reproducibly induced \(I_t\) in 60 of 60 cells (Figure 1A). In some cases, \(I_t\) followed every stimulation using the long action potential waveform, but in other experiments, \(I_t\) occurred in an alternating pattern. Stimulation with a shorter action potential waveform under identical conditions failed to elicit \(I_t\) (n=12) (Figure 1B).

**The Transient Inward Current Is Likely Due to the Na^{+}/Ca^{2+} Exchanger**

\(I_t\) was not observed in any (n=6) of the XIP-treated cells (Figure 2A), but was present in all (n=5) cells treated with sXIP (Figure 2B). In another group of cells, niflumic acid (n=9) failed to prevent \(I_t\) (Figure 2C). These experiments therefore suggest that \(I_t\) may be due to forward-mode Na^{+}/Ca^{2+} exchange current. When conventional square wave conditioning steps were used to activate the oscillatory current (Figure 2D and 2E), the reversal potential (\(E_{rev}\)) was between +20 and +40 mV (Figure 2F), which is far different from the calculated \(E_{rev}\) for Cl\(^{-}\) (~45 mV) or monovalent cations (~2 mV) under these experimental conditions. Treatment with niflumic acid only significantly inhibited outward current at +40 and +60 mV compared with control and did not affect inward current or \(E_{rev}\) (Figure 2F). XIP prevented inward current oscillations and shifted \(E_{rev}\) in a positive direction (Figure 2F). The measured \(E_{osc}\) for the oscillatory currents, the lack of effect of niflumic acid, and the suppression of \(I_t\) by XIP are all consistent with the hypothesis that the predominant mechanism underlying \(I_t\) is the Na^{+}/Ca^{2+} exchanger.

**The [Ca^{2+}]i Transient Is Greater With the Long Than the Short Action Potential Waveform**

Integrated fluo 3 fluorescence from paired experiments (Figure 1C) showed that the steady-state [Ca^{2+}]i transient with the long action potential (557±89 V×ms) was significantly greater than that measured during the short action potential (328±28 V×ms) waveform (\(P=0.04, n=5\)). These results are consistent with previous reports that show that prolonged action potential duration is associated with increased [Ca^{2+}]i and suggest that increased [Ca^{2+}]i is important for \(I_t\).

**The Transient Inward Current Is Associated With Secondary [Ca^{2+}]i Oscillations**

Myocytes dialyzed with indo 1 and exhibiting \(I_t\) (n=3) showed a secondary [Ca^{2+}]i oscillation after completion of the long action potential step. Myocytes without \(I_t\) (n=10) did not have secondary [Ca^{2+}]i oscillations. Similar observations were made in fluo 3–loaded cells. These findings provide further evidence that the \(I_t\) measured in this study is dependent on an increase in [Ca^{2+}]i.

**The Transient Inward Current Is Dependent on Activator Ca^{2+} From the SR**

Addition of BAPTA completely inhibited \(I_t\) after stimulation with the long action potential waveform in 9 of 9 cells studied. Because the release of SR Ca^{2+} stores has been linked to \(I_t\), \(I_t\) inducibility was tested after exposure to ryanodine (n=6) or thapsigargin (n=6). \(I_t\) was not inducible after exposure to either of these agents. These findings support the hypothesis that \(I_t\) is linked to increased [Ca^{2+}]i, and specifically indicates that participation of SR activator Ca^{2+} is necessary for \(I_t\) after stimulation with a prolonged action potential waveform.

**The Transient Inward Current Is Dependent on CaM Kinase**

To test whether CaM kinase is necessary for \(I_t\), cells were dialyzed with specific CaM kinase inhibitory peptides. Dialysis with AC3-I (n=11), 273-302 (n=5), or 291-317 (n=4) completely abolished \(I_t\) after stimulation with the prolonged action waveform (Figure 3). Dialysis of the inactive control peptide AC3-C (n=6) had no apparent effect and failed to inhibit \(I_t\). Like XIP, the CaM kinase inhibitory peptide AC3-I also inhibited oscillatory inward currents in response to test potentials from −80 to +20 mV (Figure 4) but, unlike XIP (Figure 2F), AC3-I did not change the \(E_{rev}\).

Both PKA and PKC may also participate in [Ca^{2+}]i homeostasis. To test for a possible role of these kinases in \(I_t\) development, cells were dialyzed with suprainhibiting concentrations of protein kinase A inhibitor (PKI 6-22) (n=6) and PKC 19-36 (n=5), and neither of these inhibitory peptides prevented \(I_t\) (Figure 3). These results suggest that \(I_t\) is dependent on CaM and CaM kinase, but not on PKC or PKA, under these experimental conditions.

**Discussion**

**Action Potential Prolongation, Arrhythmogenesis, and [Ca^{2+}]i**

Action potential prolongation has both antiarrhythmic and proarrhythmic consequences. Since the Cardiac Arrhythmia Suppression Trial demonstrated that potent Na^{+} channel blocking agents resulted in excess mortality, considerable effort has been expended to develop safer
antiarrhythmic agents. Most of these agents prolong action potential repolarization. With the exception of amiodarone, which has multiple actions, including CaM antagonism, all of these agents cause significant proarrhythmia. Proarrhythmia from action potential prolongation is thought to result from triggering due to EADs and subsequent functional reentry.

Action potential prolongation results in increased \([\text{Ca}^{2+}]_i\) \(^{27}\), and increased CaM kinase activity \(^{3,4}\); both of these actions have been linked to EADs. \(^{4,28}\) \(I_t\) underlies DADs and is another cause of arrhythmia due to elevated \([\text{Ca}^{2+}]_i\) and oscillatory release of SR activator \(\text{Ca}^{2+}\).\(^{11,32}\) Because CaM kinase has recently been shown to enhance SR \(\text{Ca}^{2+}\) release during excitation-contraction coupling,\(^1\) we hypothesized that CaM kinase would facilitate \(I_t\). The present findings show that a prolonged action potential waveform increases \([\text{Ca}^{2+}]_i\) under steady-state conditions and results in \(I_t\). \(I_t\) measured in response to the prolonged action potential waveform has dependence on SR activator \(\text{Ca}^{2+}\) similar to that of previously measured currents \([\text{Ca}^{2+}]_i\) overload under near-physiological conditions.

It is increasingly recognized that prolongation of action potential repolarization is an important cause of increased \([\text{Ca}^{2+}]_i\)\(^{27}\) and that it is associated with lethal arrhythmias in a variety of settings.\(^{21,22}\) Many studies have implicated EADs as an arrhythmogenic trigger when action potential is prolonged. However, most such studies use block of the rapid component of the delayed rectifier (\(I_{Kr}\)) to prolong action potentials, especially at slow rates.\(^{21}\) Action potential prolongation that persists at fast heart rates is characteristic of blockade of the slow component of the delayed rectifier (\(I_{Ks}\)), and it is now known that the most common cause of long-QT syndrome is mutations in the KVLQT1 gene, which encodes \(I_{Ks}\) components.\(^{21}\) Importantly, preliminary reports have implicated DADs as the arrhythmogenic mechanism when \(I_{Ks}\) is blocked and isoproterenol is added to the experimental preparation.\(^{33}\) The onset of torsade de pointes occurs with adrenergic stress in \(>95\%\) of patients with mutations in KVLQT1, according to preliminary reports.\(^{34}\) Our findings suggest a possible mechanism for arrhythmias due to action potential prolongation and point out possible proarrhythmic consequences of using \(I_{Ks}\) blocking agents for antiarrhythmic therapy.
The Identity of the Transient Inward Current

Previous studies have generally concluded that the predominant inward current after a depolarizing step is due to (forward-mode) Na⁺/Ca²⁺ exchanger current in the presence of physiological solutions.12,25 However, other candidate currents include I_{CACr} and I_{CAN}.12-19 Our findings suggest that I_{AC} measured in these experiments is due to Na⁺/Ca²⁺ exchanger current for the following reasons. (1) I_{AC} is inhibited by XIP, but not by the inactive control peptide sXIP (Figure 2). (2) E_rev for the oscillatory current is approximately +30 mV, which is far different from that predicted for I_{CACr} (–45 mV) or I_{CAN} (–2 mV) but consistent with previously reported values under similar experimental conditions, in which both the Na⁺/Ca²⁺ exchanger current and I_{CACr} were present.17,36 (3) I_{AC} is not inhibited by niflumic acid (Figure 2C). Niflumic acid did reduce outward currents at positive potentials (Figure 2F), which is consistent with the described outward rectification of I_{CACr}.19 These results suggest that I_{AC} is due to the Na⁺/Ca²⁺ exchanger and that I_{CACr} is present but significant only at relatively depolarized cell membrane potentials under these conditions. One recent report in ferret ventricular myocytes found that the time course for activation of the Na⁺/Ca²⁺ exchanger current was slower than for I_{CACr}, making E_rev measurement problematic.37 Limitations to our conclusion that I_{AC} is due to the Na⁺/Ca²⁺ exchanger in these experiments are that (1) no antagonists are available to exclude participation of I_{CAN}, and (2) XIP is a CaM antagonist over the range of concentrations useful for inhibition of the Na⁺/Ca²⁺ exchanger.38 Thus, inhibition of I_{AC} by XIP could occur partially via a CaM kinase–dependent mechanism analogous to the inhibition with the CaM inhibitory peptide 291-317 (Figure 3D). It is interesting to note that XIP shifted the apparent E_rev for the oscillatory current (Figure 2F), whereas the specific CaM kinase inhibitory peptide AC3-I did not (Figure 4), which suggests that XIP effects are not solely due to CaM kinase inhibition and therefore likely reflect inhibition of the Na⁺/Ca²⁺ exchanger.

CaM Kinase Inhibition

The specific inhibitory peptides used in these experiments offer a significant advantage over other organic inhibitors, because the latter are also I_{ACr} antagonists.1,4,5 Two different peptides modeled after separate regions of the CaM kinase molecule were effective at suppressing I_{AC} (Figure 3B, 3C, and 4). A less specific inhibitory peptide with antagonist actions against CaM (and presumably a diverse array of CaM-dependent processes, including CaM kinase) was also effective at preventing I_{AC} (Figure 3D). Thus, inhibitory peptides acting at 3 different sites along the CaM kinase activation pathway were all effective in preventing I_{AC}. In contrast, inhibitory peptides against PKA or PKC did not prevent I_{AC}. Although these studies do show that CaM kinase activity appears necessary for I_{AC}, the results do not precisely define or quantify the site(s) of action of CaM kinase inhibition for I_{AC} suppression. One possibility is that enhanced [Ca²⁺], activates CaM kinase, which, in turn, augments SR Ca²⁺ uptake and release to secondarily activate I_{AC} caused by forward-mode Na⁺/Ca²⁺ exchange. These results strongly support the hypothesis that CaM kinase facilitates I_{AC} due to the Na⁺/Ca²⁺ exchanger during increased [Ca²⁺]. CaM kinase may thus be a useful antiarrhythmic drug target to prevent arrhythmias related to [Ca²⁺] overload.

Acknowledgments

This work was supported by NIH Grants HL03727 (to M.E.A.) and HL46681 and HL49989 (to D.M.R.) and a Cardiac Arrhythmia Research and Education Foundation, Inc, award (to M.E.A.). D.M.R. holds the William Stokes chair in Experimental Therapeutics, a gift of the Dai-ichi Corp.

References

Calmodulin Kinase and Transient Inward Current


23. Terracciano CM, MacLeod KT. Measurements of Ca2+ entry and sarcoplasmic reticulum Ca2+ content during the cardiac cycle in guinea pig and rat ventricular myocytes. Biophys J. 1997;72:1319–1326.


Calmodulin Kinase Inhibition Prevents Development of the Arrhythmogenic Transient Inward Current
Yuejin Wu, Dan M. Roden and Mark E. Anderson

Circ Res. 1999;84:906-912
doi: 10.1161/01.RES.84.8.906

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/84/8/906

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/