Constitutively Active Adenosine Monophosphate–Activated Protein Kinase Regulates Voltage-Gated Sodium Channels in Ventricular Myocytes

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Background—Some PRKAG2 mutations in the human gene encoding for the γ-subunit of the adenosine monophosphate–activated protein kinase (AMPK) recently have been shown to cause rhythm disturbances (often fatal) in affected patients.

Methods and Results—Rat ventricular myocytes were infected with an adenoviral vector designed to express a truncated constitutively active mutant (T172D) of the AMPK α1-subunit (CA-AMPK). The human cardiac sodium channel hH1 and CA-AMPK were also coexpressed in a mammalian cell line. Patch-clamp techniques were used to measure myocyte action potentials and recombinant hH1 sodium channel currents. Our results demonstrate that action potential duration is significantly prolonged in myocytes expressing the CA-AMPK construct, leading to the production of potentially arrhythmogenic early afterdepolarizations. Recombinant sodium channel current analysis revealed that expression of CA-AMPK significantly slowed open-state inactivation and shifted the voltage-activation curve in a hyperpolarizing direction.

Conclusion—We propose that sodium channels may be substrates for AMPK, possibly contributing to the observed arrhythmogenic activity in patients with some PRKAG2 mutations. (Circulation. 2003;107:1962-1965.)

Key Words: AMP-activated protein kinase ■ protein kinases ■ adenosine monophosphate ■ ion channels ■ arrhythmia

Adenosine monophosphate–activated protein kinase (AMPK) is a serine/threonine kinase found in the majority of cell types, including cardiac tissue. It is comprised of a catalytic α-subunit and regulatory β- and γ-subunits assembled as a heterotrimer.1–5 Although all of the targets of AMPK presumably have not been identified, a crucial role of AMPK is thought to be the phosphorylation of target proteins/enzymes in the metabolic pathway that increase adenosine triphosphate (ATP) levels consequently preserving energy supplies.1 Therefore, AMPK is considered a “sensor” of metabolism.1,6

Recent evidence indicates that AMPK may also inhibit the cystic fibrosis transmembrane regulator chloride channel.7 Interestingly, a mutation has been found in the regulatory γ-subunit8 that renders the AMPK complex constitutively active via increased phosphorylation of T172 in the α-subunit.9 Patients with similar mutations present a Wolff-Parkinson-White (WPW) arrhythmogenic phenotype with ventricular preexcitation and atrial fibrillation, often resulting in the onset of fatal arrhythmias.8 In contrast to other mutations found in the AMPK γ-subunit,9 no morphological changes in the heart were observed with this novel mutation, and the underlying arrhythmogenic mechanism seems to be electrical in nature, indicating that cardiac ion channels may be substrates for AMPK.

Prolongation of the QT interval is a likely clinical correlate of the atrioventricular block and premature ventricular beats that often are observed in patients with the WPW/PRKAG2 mutation.8,10,11 Alterations in the biophysical properties of cardiac voltage-gated sodium channels have been shown to prolong the QT interval and predispose the myocardium to arrhythmogenic phenotypes such as long-QT syndrome and torsade de pointes.12,13 We therefore investigated the link between AMPK and the function of cardiac voltage-gated sodium channels.

Methods

Myocyte Isolation and Cell Culture

Rat ventricular myocytes were isolated14 and primary-cultured on fibronectin-coated coverslips. Myocytes were then infected for 8 hours with 50 plaque-forming units per myocyte/cell of the adenovirus vectors Ad.CA-AMPK15 or Ad.GFP and incubated at 37°C for 48 hours to allow for expression of CA-AMPK (and/or green-fluorescent protein [GFP]). Experiments were performed on visually identified myocytes expressing GFP.
TsA201 cells were cultured and transfected as described previously. Electrophysiological recordings were made 72 hours after the initial transfection of the human heart sodium channel (hH1) mammalian expression vector. In coexpression studies, tsA201 cells are transfected with hH1 18 to 24 hours before infection with the Ad.GFP or Ad.CA-AMPK vectors.

**Electrophysiology**

Action potentials were measured under current-clamp conditions using the perforated whole-cell recording configuration. Data were acquired digitally and analyzed using an Axopatch 200B amplifier and pClamp 8.0 software (Axon Instruments). Myocytes were bathed in the following solution (in mmol/L): NaCl 140, CaCl2 1, MgCl2 1.4, KCl 5, glucose 5 (pH 7.4).

The pipette solution contained (in mmol/L): KAsp 110, KCl 30, NaCl 5, HEPES 10, EGTA 1, MgCl2 1.4, MgATP 5 (pH 7.2). Action potentials were elicited by injection of current (0.5-Hz stimulus rate). In experiments on the recombinant hH1 channel, tsA201 cells were dialyzed with a pipette solution containing (in mmol/L): CsCl 120, CsOH 5, NaCl 5, MgCl2 1.4, EGTA 1, MgATP 5, HEPES 10 (pH 7.2); currents were recorded in voltage-clamp mode. Note that the recombinant hH1 currents measured varied from 1 to 8 nA in both GFP- and AMPK-infected cells; this variability therefore limits interpretation of data with regard to alterations in current density.

**Results**

The action potential duration at 90% repolarization from Ad.CA-AMPK–infected cells was significantly longer than that of control (Ad.GFP) cells (CA-AMPK, 270 ± 39 ms; GFP, 110 ± 15 ms; Figure 1, A and B). Early afterdepolarizations were often observed on the prolonged plateau of the action potential (Figure 1A-iii). These results indicate that overexpression of CA-AMPK either directly or indirectly modulates ion channel function in single myocytes. Because dysfunctional inactivation of voltage-gated sodium channels can lead to action potential prolongation and arrhythmogenesis,13,17 we studied the effects of CA-AMPK overexpression on the properties of recombinant sodium channels.

It was found that the biophysical properties of the recombinant human heart sodium channel (hH1) were altered when coexpressed with the constitutively active AMPK mutant. First, CA-AMPK induced a slowing of the inactivation process with the appearance of a persistent slowly inactivating or noninactivating current (Figure 1C and D;Figure 2, A and B). Application of the class 1c antiarrhythmic flecainide inhibited the persistent noninactivating current observed in tsA201 cells expressing hH1 and CA-AMPK (Figure 1D). Second, CA-AMPK caused a 10.4-mV hyperpolarizing shift in the voltage activation curve for hH1 (Figure 2, C and D), with no change in the voltage dependence of steady-state availability from inactivation (Figure 2E). These data indicate that CA-AMPK increases the likelihood of channel activation at more negative potentials and
slows open-state channel inactivation without affecting the availability of activation from steady-state inactivation (Figure 2F).

**Discussion**

Our findings provide the first direct evidence that cardiac voltage-gated sodium channels are regulated by overexpression of a constitutively active AMPK mutant. The observed prolongation of the action potential and early afterdepolarization formation may be accounted for by the AMPK-induced slowing of sodium channel inactivation and the increased likelihood of channel activation at more negative potentials.

Mutations in the human cardiac sodium channel have been shown to cause long-QT syndrome (LQT-3). LQT-3 mutations in sodium channels lead to a gain of function, producing a persistent noninactivating current similar to that observed during hypoxia. In a clinical setting, one useful treatment for LQT-3 patients presenting ventricular tachycardia or fibrillation with long QT involves administration of the class 1c antiarrhythmic drug flecainide. Studies on the ΔKPQ LQT-3 sodium channel mutant reveal that flecainide preferentially blocks the persistent noninactivating late current component. Results from the present study indicate that overexpression of CA-AMPK leads to a slowed sodium channel inactivation and that flecainide is similarly able to speed up the inactivation process.

AMPK is known to regulate many cellular pathways, which likely explains the variety of abnormal conditions present in patients with AMPK mutations. Findings from the present study may provide a possible electrophysiological mechanism that contributes to the WPW-like arrhythmias observed in patients with PRKAG2 mutations.

A recent report has indicated that some γ2 mutations associated with cardiac hypertrophy and WPW syndrome do not produce constitutively active AMPK in vitro. In contrast, transgenic mice overexpressing a different γ2 mutation (N488I) demonstrate cardiomyopathy and ventricular preexcitation, which is associated with an increased AMPK activity in the heart.

Recent evidence indicates that AMPK may directly regulate the activity of other ion channels such as the cystic fibrosis transmembrane regulator. Therefore, it is plausible that other cardiac ion channels may also be regulated by AMPK. However, both of these possibilities remain to be tested experimentally.

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