Brief Rapid Communication

Phosphorylation of the $I_{Ks}$ Channel Complex Inhibits Drug Block

Novel Mechanism Underlying Variable Antiarrhythmic Drug Actions

Tao Yang, PhD; Hideaki Kanki, MD, PhD; Dan M. Roden, MD

Background—$I_{Ks}$, an important repolarizing current in heart, is an antiarrhythmic drug target and is markedly increased by activation of protein kinase A (PKA; eg, by β-adrenergic stimulation). Because β-adrenergic stimulation is a frequent trigger of arrhythmias, we hypothesized that PKA stimulation inhibits drug block.

Methods and Results—CHO cells were transfected with KCNQ1 cDNA (encoding the pore-forming subunit) with or without the ancillary subunit KCNE1. $IC_{50}$ for quinidine block of basal $I_{Ks}$ was 5.8±1.2 μmol/L, versus 19.9±3.2 μmol/L. ($P<0.01$) for PKA-stimulated current. A similar >3-fold shift was apparent in the absence of KCNE1 and with the $I_{Ks}$-specific blocker chromanol 293B. The first current recorded after channels were held at rest and exposed to the drug was reduced ≈40%, and further depolarizations increased the block with a time constant ($\tau$) of 181±27 seconds. By contrast, PKA-stimulated channels displayed a <5% first-pulse block and much slower block development ($\tau$=405±85 seconds). Alanine substitution at 3 potential PKA target sites (S27, S468, and T470) generated an $I_{Ks}$ that did not increase with PKA stimulation; this mutant retained wild-type drug sensitivity that was unaffected by PKA.

Conclusions—Activation of this key intracellular signaling pathway blunts drug block. The onset of block and the data with the PKA-resistant mutant support the concept that phosphorylation of the KCNQ1 subunit directly modulates drug-channel interactions that may be especially important during β-adrenergic stimulation. (Circulation. 2003;108:132-134.)

Key Words: antiarrhythmia agents ■ signal transduction ■ ion channels

Drug block of cardiac ion channels is a dynamic process influenced by clinically important variables such as serum potassium or heart rate.1 The delayed rectifier current $I_{Ks}$ is an important mediator of cardiac repolarization; it is a target for antiarrhythmic drugs such as quinidine, azimilide, ambisilide, and amiodarone2-4; and increases with β-adrenergic stimulation5,6 (mediated by activation of the serine-threonine kinase protein kinase A [PKA]). Mutations in KCNQ1 (also known as $KvLQT1$, encoding the $I_{Ks}$ pore-forming subunit)7 that decrease $I_{Ks}$ in the congenital long-QT syndrome are associated with arrhythmias triggered by β-adrenergic stimulation.8

Recognizing the role of β-adrenergic stimulation in triggering these and other clinical arrhythmias, we reasoned—and here demonstrate—that the therapeutic effect of drug administration ($I_{Ks}$ block) is decreased by PKA stimulation. Moreover, after identifying KCNQ1 mutants that are phosphorylation resistant, we demonstrate that this inhibition is a direct consequence of modification of this protein by phosphorylation. Manipulation of the phosphorylation state thus represents a novel mechanism for modulating drug-channel interactions, with important clinical and structure-function implications.

Methods

Ion Current Recording

CHO cells, which lack endogenous K+ currents, were transfected with plasmids encoding KCNQ1 (to study $I_{KCNQ1}$) or with KCNQ1 and its ancillary subunit KCNE1 (or minK).7 generating $I_{Ks}$. Green fluorescent protein was cotransfected, and data were acquired at 22°C and analyzed as previously described.9,10 Cells were held at −80 mV and pulsed to +60 mV for 5 seconds ($I_{Ks}$) or 1 second ($I_{KCNQ1}$). Interpulse intervals were ≥15 seconds. Plasmids encoding mutant cDNAs were generated by PCR mutagenesis as previously described9 and sequenced to ensure introduction of the desired variant.

Solutions and Drugs

To record $I_{KCNQ1}$, the intracellular solution contained the following (in mmol/L): KCl 110, K2BAPTA 5, K2ATP 5, MgCl2 1, and HEPES 10. The solution was adjusted to pH 7.2 with KOH (final intracellular K+ concentration ≈145 mmol/L). To record $I_{Ks}$, the intracellular solution contained 200 mmol/L KCl to minimize $I_{Ks}$ rundown. After adjustment to pH 7.2 with KOH, the solution had a final intracellular K+ concentration of ≈235 mmol/L. The extracellular solution was normal Tyrode’s, as follows (in mmol/L): NaCl 130, KCl 4, CaCl2 1.8, MgCl2 1, HEPES 10, and glucose 10, adjusted to pH 7.35 with NaOH. To stimulate PKA, a “cocktail” of 10 μmol/L forskolin+200 μmol/L 8′-bromo-c-AMP was applied to the bath. PKA response was...
Effects of PKA Stimulation (+PKA) on K⁺ Currents and Drug Block

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>S27A</th>
<th>S468A/ T470A</th>
<th>S27A+S468A/ T470A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iₖbasal</td>
<td>64±15</td>
<td>63±5</td>
<td>64±9</td>
<td>65±8</td>
</tr>
<tr>
<td>+PKA (30 minutes)</td>
<td>93±19*</td>
<td>79±5*</td>
<td>81±12*</td>
<td>66±8</td>
</tr>
<tr>
<td>Increase, %</td>
<td>44±7</td>
<td>24±3†</td>
<td>26±4†</td>
<td>1.2±0.2†</td>
</tr>
<tr>
<td>ICₕ₀ basal</td>
<td>5.8±1.2</td>
<td>7.2±0.4</td>
<td>5.9±0.4</td>
<td>5.6±1.1</td>
</tr>
<tr>
<td>ICₕ₀ +PKA</td>
<td>19.9±3.2*</td>
<td>24.4±4.2*</td>
<td>26.4±2.9*</td>
<td>5.9±1.3‡</td>
</tr>
<tr>
<td>IₖKCNQ1</td>
<td>Basal current</td>
<td>25±4</td>
<td>25±3</td>
<td>23±3</td>
</tr>
<tr>
<td>+PKA (30 minutes)</td>
<td>37±5*</td>
<td>31±3*</td>
<td>29±3*</td>
<td>25±4</td>
</tr>
<tr>
<td>Increase, %</td>
<td>48±5</td>
<td>25±3†</td>
<td>23±3†</td>
<td>1.4±0.3‡</td>
</tr>
<tr>
<td>ICₕ₀ basal</td>
<td>16.9±1.9</td>
<td>14.5±1.2</td>
<td>12.4±1.3</td>
<td>15±0.9</td>
</tr>
<tr>
<td>ICₕ₀ +PKA</td>
<td>31.9±3.3*</td>
<td>29.8±1.0*</td>
<td>26.6±1.3*</td>
<td>16.7±2.6†</td>
</tr>
</tbody>
</table>

Current indicates pA/pF at +60 mV after 1 second (Iₖbasal) or 5 seconds (Iₖdrug), ±SE; n=6 to 10 each; ICₕ₀, concentration (μmol/L) of quinidine blocking current by 50% (n=5 to 10).

*P<0.01 vs basal value.
†P<0.05 vs wild-type value.
‡P<0.01 vs wild-type value.

assessed after current amplitude stabilized after break-in; to assess the effect of PKA stimulation on drug block, data collection started >30 minutes after initial exposure to the cocktail.

Statistical Analysis

Means were compared by ANOVA, with post hoc pairwise comparisons by the Duncan test if significant differences (P<0.05) were detected. If only 2 groups were being compared, the Student t test was used. Data are expressed as mean±SEM.

Results

PKA Stimulation Blunts Drug Block

The Table shows that PKA stimulation increased wild-type Iₖ by 44±7%, and IₖKCNQ1 by 48±5% (P<0.01). However, the PKA-stimulated currents were less sensitive to drug block; the concentration of quinidine required to block Iₖ by 50% (ICₕ₀) was >3-fold higher in PKA-stimulated cells (5.8±1.2 versus 19.9±3.2 μmol/L, P<0.01). When KCNQ1 was expressed in the absence of KCNE1, the blocking potency of the drug was reduced as previously reported, but did not eliminate the PKA response of Iₖ or of IₖKCNQ1 (Table). A further search of the KCNQ1 sequence identified a second, “atypical” site (RKSPT) with two potential target residues at positions 468 and 470 in the C terminus (available at http://www.cbs.dtu.dk/services/NetPhos/ [Center for Biological Sequence Analysis, BioCentrum-DTU, Technical University of Denmark, Lyngby]). When both residues were mutated to alanine (S468A/T470A), the response to PKA stimulation was, as with S27A, blunted but not completely inhibited (Table). However, with the dual-site mutant (S27A+S468A/T470A), PKA stimulation produced no increase in either Iₖ or IₖKCNQ1.

Drug Block of PKA-Resistant Currents

Quinidine dose-response curves were then constructed for both IₖKCNQ1 and Iₖ in the presence and absence of PKA stimulation of these single-site and the dual-site mutants (Table). The S27A and the S468A/T470A mutants both displayed wild-type block by quinidine in the absence of PKA stimulation and preservation of the effect of PKA to abrogate drug block. However, although the dual-site mutant showed wild-type sensitivity to drug at baseline, PKA stimulation did not alter the drug block.

First-Pulse Block

Taken together, the data suggest the hypothesis that PKA generates a change in KCNQ1 conformation that modulates drug access to or egress from a blocking site. The Figure shows an initial test of this concept. The Iₖ response to slow pulsing was first assessed in the absence of drug. A high concentration of quinidine was added while cells were held at rest, and slow pulsing was then repeated. Without PKA stimulation, drug block was apparent with the first pulse and then developed with a time constant τ of 181±27 seconds. By contrast, with PKA stimulation, first-pulse drug block was nearly absent (consistent with inhibited drug access to a binding site) and developed much more slowly (τ=405±85 seconds).

Discussion

The data demonstrate that PKA stimulation strikingly inhibits Iₖ, drug block. One previous report suggested no effect of isoproterenol on drug block of KCNE1-mediated current in Xenopus oocytes; this difference may reflect unique characteristics of oocytes (versus mammalian cells), particularly with respect to drug block. Furthermore, by identifying PKA phosphorylation-resistant mutants, we show that the effect is attributable to phosphorylation of the KCNQ1 subunit itself. Previous studies have identified multiple factors, such as rate, membrane potential, extracellular potassium, and pH, that modulate the interaction between blocking drugs and cardiac ion channels in vitro. Our finding that phosphorylation of the target channel protein itself modulates drug block has both mechanistic implications (discussed below) and potential clinical implications; β-adrenergic stimulation, itself a potent stimulus to arrhythmias, may also blunt the arrhythmogenic effect of Iₖ block. However, such extrapolation to the human situation awaits clinical investigation. In one study in guinea pig heart (a preparation with a particularly large
PKA stimulation markedly alters the onset of drug block. A, Protocol used to assess the development of block. B, Five-second activating currents predrug and with the first, fifth, and 20th pulses after drug application. C, Summary data showing current at the end of the 5-second depolarizing pulse plotted relative to the value measured with the corresponding pulse at predrug baseline.

$I_{Ks}$, isoproterenol elicited afterdepolarizations more readily with $I_{Ks}$ block than with $I_{Ko}$ block; a role for other PKA-modulated currents (eg, L-type calcium current) in this situation also seems likely.

The response of heterologously expressed KCNQ1 and KCNE1 to PKA stimulation is controversial. Marx et al\textsuperscript{14} reported that no effect was seen unless the A-kinase anchoring protein yotiao was also cotransfected. However, the stimulus to PKA in that study was applied intracellularly (via dialysis through the pipette), so pre-PKA current was not recorded. It has also been controversial whether KCNQ1 alone responds to PKA stimulation. We and others\textsuperscript{15} find an effect without KCNE1, although others report that it is required; we assume that methodological details explain this difference.

The single phosphorylation site mutants each reduced response to PKA but retained wild-type drug sensitivity. We infer that the mechanism whereby PKA stimulation increases current (which is not yet well understood) differs from the drug-modulating effect. For the latter, the data in the Figure provide initial support for a model in which phosphorylation inhibits access to a binding site.

In summary, we suggest here that modulation of channel function by stimulation of intracellular signaling systems not only produces well-recognized changes in gating but also can alter the pathway by which a drug accesses a binding site and can thereby modulate channel pharmacology in a clinically relevant fashion.

Acknowledgments

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References

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