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 in serum potassium or heart rate.1 The delayed rectifier current syndrome are associated with arrhythmias triggered by activation of this key intracellular signaling pathway blunts drug block. The onset of block and the data

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; 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 subunit7) that decrease $I_{Ks}$, are markedly increased by β-adrenergic stimulation.

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 $I_{KCNQ}$) 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 $-80 \text{ mV}$ and pulsed to +60 mV for 5 seconds ($I_{Ks}$) or 1 second ($I_{KCNQ}$). 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 $I_{KCNQ}$, the intracellular solution contained the following (in mmol/L): KCl 110, K$_2$ATP 5, MgCl$_2$ 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, CaCl$_2$ 1.8, MgCl$_2$ 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 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; 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 subunit7) that decrease $I_{Ks}$, are markedly increased by β-adrenergic stimulation.
PKA Stimulation Blunts $I_{Ks}$ Drug Block

### Results

**PKA Stimulation Blunts Drug Block**

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>S27A</th>
<th>S468A</th>
<th>T470A</th>
<th>S27A+S468A</th>
<th>T470A</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Ks}$ Basal current</td>
<td>64±15</td>
<td>63±5</td>
<td>64±9</td>
<td>65±8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+PKA (30 minutes)</td>
<td>93±19*</td>
<td>79±5*</td>
<td>81±12*</td>
<td>66±8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase, %</td>
<td>44±7</td>
<td>24±3†</td>
<td>26±4†</td>
<td>1.2±0.2†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$IC_{50}$ 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>
<td></td>
<td></td>
</tr>
<tr>
<td>$IC_{50}$ +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>
<td></td>
<td></td>
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</tbody>
</table>

KCNQ1

<table>
<thead>
<tr>
<th></th>
<th>Basal current</th>
<th>+PKA (30 minutes)</th>
<th>Increase, %</th>
<th>$IC_{50}$ basal</th>
<th>$IC_{50}$ +PKA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$IC_{50}$</td>
<td>25±4</td>
<td>25±3</td>
<td>23±3</td>
<td>25±3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37±5*</td>
<td>31±3*</td>
<td>29±3*</td>
<td>25±4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.9±1.9</td>
<td>14.5±1.2</td>
<td>12.4±1.3</td>
<td>15.4±0.9</td>
<td></td>
</tr>
<tr>
<td></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>
<td></td>
</tr>
</tbody>
</table>

Current indicates pA/pF at +60 mV after 1 second (when) or 5 seconds (when), ±SE; n = 6 to 10 each; $IC_{50}$, 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.

PKA-induced shift in drug sensitivity was also observed with the $I_{Ks}$ specific blocker chromanol 293B12 (0.69±0.12 versus 1.95±0.24 μmol/L, P<0.01).

**PKA-Resistant KCNQ1 Construct**

To determine whether modulation of drug block reflects phosphorylation of KCNQ1 or of other proteins, we first identified a KCNQ1 mutant that did not respond to PKA stimulation with an increase in current. The KCNQ1 primary sequence includes only one typical PKA target site, serine 27 (RRGS). However, mutation of this serine to alanine blunted but did not eliminate the PKA response of $I_{Ks}$ or of $I_{KCNQ1}$. A further search of the KCNQ1 sequence identified a second, “typical” site (RKSP2T) 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 $I_{Ks}$ or $I_{KCNQ1}$.

**Drug Block of PKA-Resistant Currents**

Quinidine dose-response curves were then constructed for both $I_{KCNQ1}$ and $I_{Ks}$ 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 $I_{Ks}$ 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 $\tau$ 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 ($\tau$=405±85 seconds).

**Discussion**

The data demonstrate that PKA stimulation strikingly inhibits $I_{Ks}$ drug block. One previous report suggested no effect of isoproterenol on drug block of KCNE1-mediated current in Xenopus oocytes;13 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.1,13 Our finding that phosphorylation of the target channel protein itself modulates drug block has both mechanistic implications (discussed below) and potential clinical implications; $\beta$-adrenergic stimulation, itself a potent stimulus to arrhythmias, may also blunt the arrhythmogenic effect of $I_{Ks}$ block. However, such extrapolation to the human situation awaits clinical investigation. In one study in guinea pig heart (a preparation with a particularly large...
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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