Current topics

16 กุมภาพันธ์ 2549 9.00-12.00 น

โดย นางสาวฐิติกานต์ ชูกิจรุ่งโรจน์ 4836206 SIPS/M

อาจารย์ที่ปรึกษา: ผศ.ดร. พญ. วัฒนา วัฒนาภา

KvLQT1 Modulates the Distribution and Biophysical Properties of HERG

A NOVEL α - SUBUNIT INTERACTION BETWEEN DELAYED RECTIFIER CURRENTS


From the Research Center, Montreal Heart Institute, Departments of Medicine, Anesthesiology, and Pharmacology, University of Montreal, and **Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec H3T 1C8, Canada


Introduction

- IK is a key repolarizing current in the heart
- Loss-of-function mutations in the subunits encoding IKr & IKs destabilize repolarization and cause potentially life-threatening arrhythmias (Barhanin et al 1996 & Abbott et al 1999)

Physiological Role of IK and Subunit Interactions

- IK is a key repolarizing current in the heart
- Loss-of-function mutations in the subunits encoding IKr & IKs destabilize repolarization and cause potentially life-threatening arrhythmias (Barhanin et al 1996 & Abbott et al 1999)
Mutations in genes encoding KvLQT1 (KCNQ1), minK (KCNE1) and HERG (KCNH2) cause distinct congenital long QT syndromes (Barhanin et al 1996 & Splawski et al 1997)

In addition I_{Kr} and I_{Ks} share some behaviors such as susceptibility to suppression in native systems by cell-isolating enzymes (Yue et al 1996)

Expressed I_{kr} is different from native currents:
- Deactivation of native I_{kr} outward current is faster than deactivation of the current carried in Chinese hamster ovary (CHO) cells by HERG with or without MiRP1 because of a larger proportion in the fast phase (Weerapura et al 2002)

Functional interaction between I_{Kr} & I_{Ks}
- Action potential is prolonged
- Prevent excess repolarization delay

Objective
- Whether molecular interactions exist between the corresponding α-subunits, HERG and KvLQT1
- Whether co-expression of KvLQT1 with HERG produces currents different from those resulting from HERG or KvLQT1/minK expression alone
- Whether there is evidence of physical interactions between HERG and KvLQT1 proteins

Methods & Results

Patch clamp
**Data analysis**

- Data are presented as the mean ± S.E.
- A two-tailed $p < 0.05$ by Student's *t* test was considered statistically significant.

**Electrophysiological Evidence for HERG-KvLQT1 Interaction**

- $V_{1/2}$ = half maximal activating voltage
- No difference in the voltage dependence of activation of cells expressing HERG alone or co-expressed with KvLQT1.
Reversal potential ($E_{\text{rev}}$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean $E_{\text{rev}}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERG ($n=8$)</td>
<td>$-72.8 \pm 1.2$</td>
</tr>
<tr>
<td>HERG + KvLQT1 ($n=8$)</td>
<td>$-72.6 \pm 1.0$</td>
</tr>
</tbody>
</table>

"Co-transfection of KvLQT1 increased HERG current ($I_{\text{HERG}}$) densities"

- Tail $I_{\text{HERG}}$ densities: HERG + KvLQT1 > HERG only

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- Tail $I_{\text{HERG}}$ densities: HERG + KvLQT1 > HERG only

"Inactivation time constants were similar in both groups"

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inactivation time constant at $+10$ mV (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERG ($n=8$)</td>
<td>$2.9 \pm 0.3$</td>
</tr>
<tr>
<td>HERG + KvLQT1 ($n=8$)</td>
<td>$2.9 \pm 0.6$</td>
</tr>
</tbody>
</table>

"No differences in steady-state inactivation voltage-dependence"

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$V_{1/2}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERG ($n=8$)</td>
<td>$-86 \pm 10$</td>
</tr>
<tr>
<td>HERG + KvLQT1 ($n=8$)</td>
<td>$-82 \pm 18$</td>
</tr>
</tbody>
</table>

"Effect of HERG Co-expression on Current Carried by KvLQT1/minK"

<table>
<thead>
<tr>
<th>KvLQT1/minK + HERG</th>
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<tr>
<td>Dofetilide 1μM</td>
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"Effect of HERG Co-expression on Current Carried by KvLQT1/minK"

- Dofetilide 1μM
Co-expression of HERG did not affect the density of step or tail currents

Co-expression of HERG did not affect the activation and deactivation time constant

Conclusion: Electrophysiological interaction between HERG & KvLQT1
- HERG amplitude (fast deactivating component) ↑
- HERG deactivation time constant ↓
- HERG current densities ↑

Tissue and Cardiomyocyte Isolation
- Adult mongrel dogs
- Left thoracotomy
- Coronary artery perfusion
- Left ventricular tissue
- Left ventricular cardiomyocytes
- Immunoprecipitation
- Immunofluorescence
Confocal Microscopy

Co-localization of HERG and KvLQT1

Immunofluorescent studies of native myocytes

<table>
<thead>
<tr>
<th>Antigen</th>
<th>HERG</th>
<th>KvLQT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary antibody</td>
<td>anti-HERG raised in rabbits</td>
<td>anti-KvLQT1 raised in rabbits</td>
</tr>
<tr>
<td>Secondary antibody</td>
<td>anti-goat IgG</td>
<td>anti-rabbit IgG</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>FITC (green)</td>
<td>Cy5 (red)</td>
</tr>
</tbody>
</table>

FITC = Fluorescent isothiocyanate

Immunofluorescent studies of CHO cells

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<tr>
<td>Secondary antibody</td>
<td>anti-rabbit IgG</td>
<td>anti-goat IgG</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>TRITC (red)</td>
<td>FITC (green)</td>
</tr>
</tbody>
</table>

FITC = Fluorescent isothiocyanate
TRITC = Tetramethyl rhodamine isothiocyanate

“KvLQT1 may promote the membrane localization of HERG protein”

CHO cells

A. Transfected : HERG – IF : anti-HERG
B. Transfected : HERG + KvLQT1 – IF : anti-KvLQT1
C. Transfected : HERG + KvLQT1 – IF : anti-KvLQT1 + anti-HERG
D. Transfected : HERG + KvLQT1 – IF : anti-HERG
E. Transfected : KvLQT1 – IF : anti-KvLQT1

HERG = red
KvLQT1 = green

“KvLQT1 may promote the membrane localization of HERG protein”

CHO cells

A. Transfected : HERG – IF : anti-HERG
B. Transfected : HERG + KvLQT1 – IF : anti-KvLQT1
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D. Transfected : HERG + KvLQT1 – IF : anti-HERG
E. Transfected : KvLQT1 – IF : anti-KvLQT1

HERG = red
KvLQT1 = green
Antigen

Primary antibody

Secondary antibody

Co-immunoprecipitation of HERG and KvLQT1

CHO cells

<table>
<thead>
<tr>
<th>CHO cells</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 kDa mem.</td>
<td>CM</td>
<td>IP</td>
</tr>
<tr>
<td>155 kDa (+)</td>
<td>CM</td>
<td>IP</td>
</tr>
<tr>
<td>135 kDa (+)</td>
<td>CM</td>
<td>IP</td>
</tr>
</tbody>
</table>

CM = crude membrane extracts

IP = immunoprecipitation

Glutathione S-Transferase (GST)

Glutathione S-Transferase (GST) - C-terminal HERG Fusion Protein
Protein Purification and GST Pull Down

Glutathione-resin complex

mild elution buffer

GST - HERG

solubilized membrane sample

KvLQT1

GST Pull Down of KvLQT1

Solubilized membrane sample + GST pull down

Solubilized membrane sample + GST bead

Solubilized membrane sample

C-terminal HERG

+ KvLQT1

75 kDa

+ anti-KvLQT1

Immunoblotting of crude membrane extracts from cells transfected with KvL3.4

Solubilized membrane sample + GST pull down

Solubilized membrane sample + GST bead

Solubilized membrane sample

C-terminal HERG

+ KvL3.4

+ anti-KvL3.4

Discussion

Functional interactions between KvLQT1 and HERG

- KvLQT1 increased HERG-protein membrane localization
- KvLQT1 enhanced HERG current density
- KvLQT1 altered biophysical properties of HERG currents
- KvLQT1 co-expression with HERG did not alter the cellular distribution of KvLQT1 or change the density or properties of KvLQT1/minK currents

A novel potential mechanism for I_{Kr}-I_{Ks} interaction

The molecular interaction between α-subunits HERG and KvLQT1:

- KvLQT1 was found to associate physically with HERG in both CHO cells and native cardiomyocytes and to increase membrane localization of HERG in CHO cells
- KvLQT1-induced increases in HERG membrane expression were paralleled by increased I_{HERG} density
In the presence of KvLQT1, $I_{\text{HERG}}$ deactivation was accelerated, bringing its deactivation properties closer to those of native $I_{K_r}$ (Weerapura et al. 2002). The mechanism of this interaction remains to be established more fully.

HERG interactions with other subunits:
- Suppression of minK expression reduces $I_{K_r}$ in AT1 cells and neonatal mouse cardiomyocytes (Yang et al. 1995 & Kupershmidt et al. 1999).
- HERG appears to co-assemble with exogenous minK in CHO cells and in native equine cardiac tissue, affecting $I_{\text{HERG}}$ density and gating kinetics (McDonald et al. 1997, Ohyama et al. 2001 & Finley et al. 2002).

HERG interactions with other subunits:
- MiRP1 was first described as an essential $\beta$-subunit for reconstitution of $I_{K_r}$ (Abbott et al. 1999).
- Understanding the role of MiRP1 has been complicated:
  - Ventricular MiRP1 expression is limited (Yu et al. 2001).
  - HERG co-expression with MiRP1 in mammalian cells does not make $I_{\text{HERG}}$ more $I_{K_r}$-like (Weerapura et al. 2002).
  - MiRP variants endogenous to Xenopus oocytes have contrasting effects on $I_{\text{HERG}}$ (Anantharam et al. 2003).
- Interestingly, MiRP1 is believed to interact with the C-terminal region, specifically the cyclic nucleotide binding domain of HERG (Cui et al. 2001).
- This study is the first to suggest interactions between the $\alpha$-subunits of $I_{K_r}$ and $I_{K_s}$, which may explain the inconsistent findings in the literature.

Possible role of HERG’s C-terminal to HERG membrane trafficking:
- HERG can interact with $\beta_1$-integrin; N-terminal HERG deletions could still interact with integrin (Cherubini et al. 2002).
- LQT2 mutations in the C-terminal tail affect trafficking of HERG from the ER to the cell surface (Aydal et al. 2001 & Akhavan et al. 2002).

The interactions between the C-terminal HERG with KvLQT1 may facilitate HERG membrane trafficking.

These studies were performed primarily in CHO cells. It remains to be determined whether the interactions that we observed are seen in other systems as well.

The evidence for co-localization of HERG and KvLQT1 protein in canine heart cells and the ability to co-immunoprecipitate HERG and KvLQT1 from crude left ventricular membrane preparations --> relevance to cardiac HERG-KvLQT1 interaction.
- Like Pond et al. (Roti et al. 2002), the study found HERG to localize to cross-striations compatible with the T-tubular system as well as to lateral membranes and intercalated disks.
- Other cardiac potassium channels such as Kir 2.1 and Kir 2.3 have also been localized to T-tubules (Clark et al. 2001 & Melnyk et al. 2002).
- However, the function of these protein localization to T-tubular structures in native cardiomyocytes remains unclear and needs further investigation.

- The molecular motifs involved in the HERG-KvLQT1 interaction remain to be more fully defined.
- Such information would be very useful in pointing to potential effects of mutations in HERG and KvLQT1 on the interaction defining the potential involvement of the HERG-KvLQT1 interaction in contributing to the clinical manifestations of congenital long QT syndromes.