Regulation of Kv1.3 activity in human T lymphocytes: peptide blockers and molecular interactions

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August 27, 2005, Montpellier
Simple view of an ion channel:

a hydrophilic pore allowing very rapid ion movement across the membrane (10^8 /s)
Ion Channels

- heartbeat
- secretion
- memory
- perception
- thought
- sensation
- contraction
- immunity
- movement
How do ion channels regulate the immune functions?

Ion channels are oligomeric transmembrane proteins.

Extracellular

Intracellular

voltage-sensing domain

pore

NH₂ COOH
How do we study the channels?
By measuring ionic currents using the patch-clamp technique.
How do K+ channels of T cells compare?

**Kv1.3**
- **Gating:** voltage-gated
- **Single channel conductance:** similar (~10 pS)
- **Selectivity:** K+ selective
- **Block:** different sensitivity to organic and inorganic compounds

**IKCa1**
- **Ca2+-activated**
- **Single channel conductance:** similar (~10 pS)
- **Selectivity:** K+ selective
three important problems

How does each subunit contribute to gating, specifically slow inactivation?

What kind of molecules inhibit Kv1.3 and IKCa1 channels?

Can the microenvironment of the membrane alter the channels’ behavior?
How does each subunit contribute to gating, specifically slow inactivation?

\[ \tau_i \sim 200 \text{ ms} \]

\[ \tau_i \sim 4 \text{ ms} \]

- cooperative interaction between subunits
- each subunit contributes equal free energy to inactivation
- constrains the possible physical models of inactivation

What kind of molecules inhibit Kv1.3 and IKCa1 channels differentially?
peptide toxins block the pore

Péter et al., BBRC, 1998, 242:621-625; Péter et al., BBRC, 2000, 278:34-37;
Why is it important to selectively block $K^+$ channels of T cells?

Naïve $\rightarrow$ $T_{CM}$ $\rightarrow$ $T_{EM}$

repeated antigen stimulus

$IKCa1$: 5-35/cell $\quad\quad\quad\quad\quad$ ~50/cell

$Kv1.3$: ~200/cell $\quad\quad\quad\quad\quad$ ~1500/cell

- Multiple Sclerosis
- Type-I (autoimmune) diabetes
- Rheumatoid Arthritis

proliferation of $T_{EM}$ in autoimmune diseases is selectively inhibited by Kv1.3 blockers

**Anuroctoxin is a selective, high affinity blocker of Kv1.3**

Can the microenvironment of the membrane alter the channels' behavior?

Does gating of Kv1.3 depend on the cholesterol content of the membrane?

Are Kv1.3 channels localized in specific microdomains of the membrane?
Can cholesterol modulate channel kinetics?

Let's see where the channels are!
Kv1.3 and CD3 are highly co-localized

Can we determine this relationship more precisely?
Kv1.3 and TCR/CD3 are closely associated

Does this co-localization occur during a physiological immune response?

Structured recruitment of molecules in the immunological synapse

Monks et al., Nature, 1998, 395:82-86 (Kupfer lab.)
Is Kv1.3 also in the immune synapse?

Are Kv1.3 channels in lipid rafts?

What is our model for the molecular interactions of Kv1.3 in the immune synapse?

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