Structural Mechanisms of Two Pore Domain Potassium (K2P) Channel Gating

Alistair Mathie
Medway School of Pharmacy
University of Kent

Royal Society Industry Fellow
The voltage-gated-like (VGL) ion channel “chanome”

(from Yu & Catterall 2004, Science STKE 253, 15)
15 mammalian K2P channels in 6 families

6 families of K2P channels

K2P channels are dimers
K2P structure (TREK1)

Based on KcsA

Based on KvAP
K2P structure (TREK1)

Based on KcsA

Based on KvAP

Based on TRAAK (Brohawn et al 2012)
K2P structure (TREK1)
Themes

- Physiological Roles of K2P channels
- How do regulatory compounds act on K2P channels to produce their effect?
- Do K2P channels have a single gate at the selectivity filter?
- Regulation of TREK1 isoforms
Development of Resting Membrane Potential and $I_{KSO}$ in cerebellar granule neurons (CGNs) with time in culture

K2P channel expression in the *mouse* cerebellum

$\text{IK}_\text{SO}$ is blocked by extracellular acidification

TASK-3 KO mice have a reduced $I_{KSO}$ and a compromised neuronal excitability.

TASK3 is selectively blocked by Zinc

**TASK3**

**TASK1**
E70 in the M1/P1 loop and H98 at the selectivity filter have a role in zinc regulation of TASK3 channels.
E70 & H98 contribute to TASK-3 zinc sensitivity

K2P structure

Based on KcsA

Based on KvAP

Based on TRAAK (Brohawn et al 2012)
Muscarinic ACh receptor activation inhibits $I_{KSO}$

How does M₃ receptor activation inhibit TASK channels?

Does $G_{\alpha q}$ inhibit TASK-3 directly?

Constitutively active $G_{\alpha q}$ ($G_{\alpha q}^*$) inhibits TASK-3 current. So too does a further mutated form of $G_{\alpha q}$ ($G_{\alpha q}^*,R256A,T257A$) which does not activate PLC.

A region of 6 amino acids of TASK channels is critical for transducing their regulation by $G_{\alpha_q}$-coupled receptors.

K2P channels have gate(s) which may control their activity.
Does A237 in TASK3 regulate the bundle crossing gate?

M2 (107-133)  KAFCMFYAVLGIPLTLVMFQSLGERMN
M4 (221-247)  VAFSMYILVGLTVIGAFLNLVVLRFL

A237T in TASK3 occludes modulation by muscarine

Emma Veale – unpublished data
Do different regulators of TASK3 act through different gates?

zinc?

Gαq?
The Pore Structure and Gating Mechanism of K2P Channels

Dr Stephen J. Tucker
Long chain QAs can block K2P channels from intracellular side

Use TPenA and THexA as probes of pore structure

Piechotta et al (2011) *EMBO Journal*
The QA ion binding site in TREK-1.
Use high-affinity pore blocker to probe gating mechanism

State-dependent accessibility of QA ion to its blocker site?
State-dependent accessibility of QA to its blocker site?

Helix bundle crossing does not restrict access of QA to blocker site when the pH gate is closed (at pH 8.0)
Mutation of W275 blunts regulation of TREK1

Bagriantsev et al 2011 & 2012 EMBO J
Gating of TREK1:

Selectivity Filter acts as Primary Gating Mechanism
K2P channel gating

Closed?

Open?

Open/closed?
Enhancement of TREK channels by Fenamates

Enhancement of TREK1 by BL-1249 (1 μM)

BL-1249 (1 μM)

Current (pA)

Time (s)

TREK1 % Change

1000 pA

200 ms
Enhancement of TREK1 by BL-1249 (1 μM)

Effect of BL-1249 (1 μM) on TREK1_Y284A
Important residues that influence fenamate action in TREK1

TMs 1 and 2

TMs 3 and 4 (rotated 90°)
Alternative Translation Initiation in Rat Brain Yields $K_{2P}2.1$ Potassium Channels Permeable to Sodium

Dierk Thomas, Leigh D. Plant, Christina M. Wilkens, Zoe A. McCrossan, and Steve A.N. Goldstein

Department of Pediatrics and Institute for Molecular Pediatric Sciences, Pritzker School of Medicine, University of Chicago, 5721 South Maryland Avenue, Chicago, IL 60637, USA
Enhancement of TREK1_1-41Del by BL-1249
Enhancement of TREK1_1-41Del by FFA
Enhancement of TREK1_1-41Del_Y284A by FFA
Fenamates AND point mutations which alter TREK1 gating at the selectivity filter “overcome” N terminus truncation
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• Regulation of TREK1 isoforms
# K2P channel - colleagues

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