KCNQ Potassium Channels: Drug Targets for the Treatment of Epilepsy and Pain
The KCNQ Gene Family

Interesting pain targets based on:
- Function
- Distribution
- Pharmacology

- The IUPHAR name for the KCNQ family is Kv7.x
KCNQ Gene Family (cont)

- **KCNQ1**
  - KCNQ1/KCNE1 (IKS) contributes to cardiac action potential repolarization. Mutation can result in Long QT Syndrome.

- **KCNQ2**
  - Forms heterotetramers with KCNQ3. KCNQ2/3 underlies the M-current that is present in several neuronal systems. Mutations in KCNQ2 cause the congenital seizure disorder benign familial neonatal convulsions (BFNC).

- **KCNQ3**
  - Expresses poorly as a homomultimer. Co-assembles with other KCNQ channels such as KCNQ2 and KCNQ5. Mutations in KCNQ3 also linked to BFNC.

- **KCNQ4**
  - Expressed primarily in inner ear. Mutation linked to one form of hereditary deafness

- **KCNQ5**
  - Expressed in nervous system and co-assembles with KCNQ3
KCNQ2/Q3 Channels - Background

From Cooper EC and Jan LY, 2003, Arch. Neurol 60(4):496-500

KCNQ2/Q3 Channel: Relationship to Neuronal M-Current


From Delmas P and Brown D, 2005, Nat. Rev. Neurosci. 8:850-862

KCNQ2 and KCNQ3 genes are co-expressed, predominantly in neuronal tissue.
Neuronal Expression of KCNQ2/Q3

qPCR studies indicate robust expression of KCNQ2 and KCNQ3 mRNA in rat dorsal root ganglia (DRG) from L4 and L5 spinal nerves.

Present at high levels in neurons including dorsal root ganglia (DRG). No significant expression in major peripheral organs.

Mutations in KCNQ2 and KCNQ3 associated with a congenital seizure disorder in humans – *Benign Familial Neonatal Convulsions*

Targeted deletion of KCNQ2 in mice increases sensitivity to chemoconvulsant induced seizures.

KCNQ channels represent molecular correlates of the neuronal M-current – control resting membrane potential, integration of synaptic inputs and spike frequency adaptation.

KCNQ/M-current activators are efficacious in animal models and human diseases associated with excessive neuronal excitability.
Flupirtine

- Drug marketed in Europe as an analgesic (Katadolon®, Asta Medica)
- Mechanism of action not demonstrated conclusively
- Positive modulator of cloned human KCNQ2/Q3 channels (Ilyin et al., Soc Neurosci Abstract 2002)
- Indirect evidence of NMDA antagonism (Seyfried et al., Europ J Pharm 2000)
Flupirtine vs Q2/Q3

EC$_{50}$ = 1.9 ± 0.5 µM

Fraction Max vs [Flupirtine] (µM)

0.0 0.1 1 10 100

0.0 0.2 0.4 0.6 0.8 1.0

[Flupirtine] (µM)

0.1 µM 0.3 µM 1 µM 3 µM 10 µM 30 µM

200 pA

2 s
In two small open-label clinical studies of refractory epilepsy, add-on flupirtine decreased seizure frequency.

- 4 of 4 patients in one study
- 8 of 9 patients in the second study

Porter, et al., Epilepsia 1983
Sheridan, et al., Neurology 1986
Flupirtine: Efficacy for Pain

韧性 models:
- Active in rat tail flick assay of acute pain
- Active in spinal nerve ligation model of neuropathic pain

韧性 Controlled clinical trials for pain:
- Effective for tumor or cancer pain
- Effective for post-operative pain Effective for pain following episiotomy
- Effective trauma pain

Retigabine

- Flupirtine analog in clinical development for epilepsy
- Activates KCNQ2/Q3, KCNQ3/Q5, KCNQ4 at concentrations from 0.1 to 10 µM
- Enhances native M-currents in dorsal root ganglion cells
- Enhances GABA-induced chloride currents in cultured rat cortical neurons (> 10 µM)

Retigabine Activates KCNQ2/3

A. Current

B. Membrane Potential

Retigabine is Antiepileptic in Animals and Man

- Activates KCNQ2/Q3 channels/M-currents
- Exhibits broad spectrum anticonvulsant activity in animals models such MES, PTZ, Kindling, audiogenic seizures (Rostock et al., Epilepsy Res 1996).
- Is efficacious in drug-resistant epilepsy in Phase II clinical trials (Bialer et al., Epilepsy Res 2001).
- May enhance GABAergic transmission in addition to M-current activation (Kapetanovic et al., Epilepsy Res 1995).
Retigabine Effects in Animal Pain Models

- Attenuates mechanical hypersensitivity and cold allodynia in chronic constriction injury model
- No effect on tail flick or on phase 1 of formalin model (acute pain)
- Reduced flinching in phase 2 of formalin model (persistent pain)
- Attenuates thermal hyperalgesia in spinal nerve ligation model
- Is active in inflammatory pain model (carrageenan), an effect blocked by KCNQ antagonist

Selective KCNQ2/Q3 Openers Identified at Icagen

- Icagen screening and medicinal chemistry have identified multiple KCNQ opener chemotypes.

- Example: ICA-27243

- Selective for Q2/Q3 over a range of ion channels, receptors and transporters.
- Bioavailable in mouse and rat (i.p. and p.o.).
- Plasma levels sustained for > 2h in rat

Icagen, Inc. US patent 6,372,767
ICA-27243: a Selective KCNQ2/Q3 Opener

**KCNQ2/Q3**
- 10 μM ICA-27243
- 1 μM
- 100 nM
- 30 nM
- EC$_{50}$ = 0.5 ± 0.1 μM

**KCNQ4**
- 100 μM ICA-27243
- 1 μM
- 3 μM
- 30 μM
- EC$_{50}$ = 7 ± 1 μM

**KCNQ3/Q5**
- 10 μM retigabine
- 0.3 μM 3 μM
- 30 μM ICA-27243
- EC$_{50}$ > 30 μM

Experiment date prior to Aug 1999

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ICA-27243: Mechanism of Action

Control
ICA-27243 (10 µM)

Normalized tail current

Voltage (mV)

[ICA-27243] (µM)

Δ V1/2  = 15mV

Experiment date prior to Aug 1999

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ICA-27243 Hyperpolarizes the DRG Resting Membrane Potential

![Graph showing the hyperpolarization effect of ICA-27243 and XE991 on DRG resting membrane potential.](image)

- Graph shows the change in membrane potential (ΔVm) in response to different concentrations of ICA-27243 and XE991.
- The resting membrane potential is indicated as -60 mV, and the hyperpolarization effect is observed.
- The graph includes concentration vs. ΔVm for concentrations of 0.3, 1, 3, 10, and 30 µM.
ICA-27243 Inhibits Spontaneous Firing

ICA-27243, µM

0.3  0.1  1  3  10  30

20 mV

1 min
ICA-27243 Reverses Capsaicin Stimulated Excitation

Capsaicin (100 nM)

ICA-27243 (10 uM)  ICA-27243 (10 uM)

20 mV  30 sec
ICA-27243 Increases the Stimulus Threshold for Excitation in DRG Neurons

-50 mV

Control

1 µM

10 µM

Wash

Fold current

Concentration (µM)
ICA-27243 Efficacy in *In Vivo* Pain Models

**Capsaicin Induced Thermal Hyperalgesia**

- **Vehicle**
- **3 mg/kg**
- **10 mg/kg**
- **30 mg/kg**

**Chung (Spinal Nerve Ligation) Thermal Hyperalgesia**

- **Vehicle**
- **3 mg/kg**
- **10 mg/kg**
- **30 mg/kg**
<table>
<thead>
<tr>
<th>Model</th>
<th>Type of Pain</th>
<th>Species</th>
<th>Active Dose mg/kg PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve Injury (Chung)</td>
<td>Neuropathic</td>
<td>Rat</td>
<td>5 (ED$_{50}$)</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>C-fiber activation</td>
<td>Rat</td>
<td>3 (ED$_{50}$)</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Inflammatory</td>
<td>Rat</td>
<td>30</td>
</tr>
<tr>
<td>Formalin phase 1</td>
<td>Acute Chemical</td>
<td>Mouse</td>
<td>17</td>
</tr>
<tr>
<td>Formalin Phase 2</td>
<td>Persistent/Spinal Sensitization</td>
<td>Mouse</td>
<td>17</td>
</tr>
<tr>
<td>Hot plate</td>
<td>Acute Thermal</td>
<td>Rat</td>
<td>30</td>
</tr>
</tbody>
</table>

KCNQ2/Q3 activators exhibit efficacy in a wide variety of *in vivo* pain models
Conclusions: Pain

- KCNQ activators exhibit broad spectrum activity in animal pain models
  - Neuropathic pain
  - Inflammatory pain
  - Acute pain

- KCNQ activators modulate the excitability of nociceptive dorsal root ganglion (DRG) neurons.
  - Increases cellular conductance
  - Hyperpolarizes membrane potential
  - Increases the threshold for action potential firing
  - Inhibits capsaicin-induced as well as spontaneous firing

- Flupirtine, a molecule with KCNQ activating activity is effective in controlled clinical trials for pain

- KCNQ activators represent a novel class of agents for the treatment of pain.
Effects of ICA-27243 on Electrographic Seizures in Hippocampal Slice

Seizure activity evoked by 150 uA, 60 Hz, 2 second stimulus train.
Effects of ICA-27243 in *In Vivo* Anticonvulsant Assays

<table>
<thead>
<tr>
<th>In Vivo Anticonvulsant Assay</th>
<th>Species</th>
<th>Route</th>
<th>( ED_{50} ), mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal Electroshock Mouse</td>
<td>IP</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Maximal Electroshock Mouse</td>
<td>PO</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Maximal Electroshock Rat</td>
<td>PO</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Amygdala Kindling Rat</td>
<td>PO</td>
<td>3.0 to 9.0&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>6 Hertz Seizures Mouse</td>
<td>IP</td>
<td>10.0 to 25.0&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Pentylenetetrazol Rat</td>
<td>PO</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Pentylenetetrazol Mouse</td>
<td>PO</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Picrotoxin Mouse</td>
<td>IP</td>
<td>3.6 to 9.9&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Pilocarpine Mouse</td>
<td>PO</td>
<td>NE @ 50</td>
<td></td>
</tr>
<tr>
<td>Bicuculline Mouse</td>
<td>IP</td>
<td>NE @ 30</td>
<td></td>
</tr>
<tr>
<td>Strychnine Mouse</td>
<td>IP</td>
<td>NE @ 30</td>
<td></td>
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</tbody>
</table>

NE = not effective.

<sup>1</sup> = \( ED_{50} \) not calculated, range indicates doses where compound was active.

*KCNQ2/Q3 openers represent mechanistically novel, broad spectrum anti-epileptic agents.*
Conclusions: Epilepsy

- KCNQ activators exhibit broad spectrum activity in animal seizure models
  - Maximal electroshock
  - Pentylenetetrazol
  - Kindling
  - 6-Hz

- Molecules with KCNQ activating properties exhibit efficacy in open- and controlled-clinical trials of treatment-resistant epilepsy

- KCNQ2/Q3 activators represent a novel class of agents for the treatment of epilepsy.
Acknowledgments