Kv7 CHANNELS IN SMOOTH MUSCLE AS THERAPEUTIC TARGETS FOR VASCULAR AND AIRWAY DISEASES

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DISCLOSURE

- U.S. Patents issued:
  - Patent 8686017 “Methods of using proteinacious channels to identify pharmaceutical treatments and risks, and treatments resulting therefrom”
  - Patent 20,140,155,368 “Combination pharmaceuticals and methods thereof using proteinacious channels as treatments for medical conditions”
Arginine-Vasopressin (AVP): A pituitary hormone that regulates water balance and blood pressure.

↓ Blood Pressure

↑ Plasma Osmolality

Vascular Smooth Muscle

Pituitary

AVP

AVP

V1a

V2

Kidney

Blood Pressure

Plasma Osmolality
Plasma AVP Concentrations

Change in Osmolarity

Change in Blood Pressure

AVP Concentration-dependent Ca\(^{2+}\) Signaling in Vascular Smooth Muscle Cells (A7r5 cells)

**Physiological range**

- **EC\(_{50}\) = 150pM**
- **EC\(_{50}\) = 5nM**

**Spiking Frequency (s\(^{-1}\))**

**Peak Ca\(^{2+}\) (nM)**

- **Peaks** at various AVP concentrations:
  - 0-500pM
  - 500pM to 800nM
Novel AVP signaling pathway identified in A7r5 rat aortic smooth muscle cell line.
Isolated $K_v$ currents are non-inactivating.

Isolated $K_v$ currents activate at very negative voltages and are suppressed by AVP

$V_{0.5} = -38.0 \pm 1.6 \text{ mV}$
$s = 8.3 \pm 0.4 \text{ mV}$
$n=21$

AVP-sensitive \( K_v \) currents in A7r5 cells have characteristics of neuronal “M currents”

1. Non-inactivating delayed rectifier \( K^+ \) currents that activate over a relatively negative voltage range.

2. Inhibited in response to activation of G protein-coupled receptors, resulting in increased electrical excitability.

Are the vascular smooth muscle currents mediated by KCNQ (Kv7) channels?
Kv currents are inhibited by selective KCNQ channel blockers linopirdine & XE991 and reversibly activated by selective KCNQ channel activator flupirtine.

KCNQ5 is expressed in A7r5 cells.
Knocking down KCNQ5 expression or function abolishes the AVP-sensitive KCNQ currents.
Novel AVP signaling pathway identified in A7r5 rat aortic smooth muscle cell line

High (EC$_{50}$=5 nM) AVP Pathway

Low (EC$_{50}$=150pM) AVP Pathway
Vasoconstrictor responses measured in pressurized rat mesenteric arteries

Outer diameters ~ 300-400 µm
AVP concentration-dependent constriction of rat mesenteric arteries

Novel AVP signaling pathway

10 nM AVP Pathway

- AVP
- V1aR
- DAG + IP3 + PIP2
- Release of SR Ca2+ Store

PLC

30 pM AVP Pathway

- Ca2+ Influx
- PLC
- PLD
- PKC
- KCNQ
- CaL
- Ca2+ Influx
- K+
- EM Ca2+

Vasoconstriction

Release of SR Ca2+ Store
M-currents measured in freshly isolated mesenteric artery myocytes

Kv currents are enhanced by KCNQ channel activators (retigabine and flupirtine) and abolished by KCNQ blockers (XE991 and linopirdine).

Mackie et al., JPET 2008
AVP suppresses KCNQ currents, but not 4-AP-sensitive Kv currents

Mackie et al., JPET 2008
KCNQ channel activator flupirtine: concentration-dependent vasodilation

Mackie et al. *JPET* 2008
In Vivo Effects of KCNQ Channel Modulators

**Linopirdine (n=3)**

**Flupirtine (n=3)**

% of Basal MAP

% of Basal MVR

% of Basal HR

Dose (mg/kg i.v)

Mackie et al. *JPET* 2008
COX-2 Inhibitors

Celecoxib
(Celebrex®)

Rofecoxib
(Vioxx®)
Celebrex®, but not Vioxx®, activates native or overexpressed vascular KCNQ channels.
Celebrex® but not Vioxx® can relax rat mesenteric arteries pre-constricted with AVP

Brueggemann et al. *Mol Pharm* 2009
KCNQ channel expression in mesenteric artery myocytes: KCNQ1, KCNQ4, & KCNQ5 are detected by RT-PCR

Mackie et al., JPET 2008
Diclofenac distinguishes between overexpressed homomeric and heteromeric KCNQ4 & KCNQ5 channels

Diclofenac distinguishes between natively expressed homomeric and heteromeric KCNQ4 & KCNQ5 channels

A7r5 cells

Mesenteric Artery myocytes

# LIST OF US PATENTS ISSUED PRIOR TO 2011 THAT CLAIM COMPOUNDS ACTIVATING KCNQ CHANNELS

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Company</th>
<th>Target channel</th>
<th>Chemical class</th>
<th>Representative compound</th>
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<tbody>
<tr>
<td>6,326,385</td>
<td>Icagen</td>
<td>KCNQ2/Q3</td>
<td>N-aryl benzamide</td>
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<td>6,372,767</td>
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<td>ICA-27243 analogs</td>
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<td>Bisarylamines</td>
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<td>7,741,332</td>
<td>Icagen</td>
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<td>Fused ring heterocycles</td>
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<td>Fused ring heterocycles</td>
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<td>6,469,042</td>
<td>BMS</td>
<td>KCNQ2, KCNQ2/Q3</td>
<td>Fluoro oxindole derivatives</td>
<td>BMS-204352 analogs</td>
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<tr>
<td>6,855,829</td>
<td>BMS</td>
<td>KCNQ2, KCNQ5</td>
<td>3-fluoro-2-oxindole and 2,4-disubstituted pyrimidine-5-carboxamide</td>
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<td>7,632,866</td>
<td>Tel Aviv U.</td>
<td>KCNQ2/3, Q1, Q1/E1</td>
<td>Derivatives of N-phenylantranilic acid and 2-benzimidazolone as potassium channel and/or neuron activity modulators</td>
<td></td>
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</table>
A small molecule activator of KCNQ2 and KCNQ4 channels
Haibo Yu, Meng Wu, Corey Hopkins, Julie Engers, Steve Townsend, Craig Lindsley, Owen B McManus, and Min Li.

“ML213 was identified following a high throughput fluorescent screen of the Molecular Libraries Small Molecule Repository (MLSMR) library and structure activity relationship (SAR) studies using fluorescent and electrophysiological assays to determine potency and selectivity of test compounds. ML213 is a potent activator of potassium voltage-gated channel, KQT-like subfamily, member 2 (KCNQ2) (Kv7.2, EC50 = 230 nM) and KCNQ4 (Kv7.4, EC50 = 510 nM) and selective against the other members of the KCNQ family of ion channels (KCNQ1, KCNQ3 and KCNQ5).”
hKv7.4

control

10 µM ML213

hKv7.5

control

10 µM ML213

hKv7.4/7.5

control

10 µM ML213

I, nA

Time (s)
leftward shift of activation curve ($\Delta V_{0.5}$), mV

\begin{align*}
\text{hKv7.4} & \quad \text{EC}_{50} = 1.5 \pm 0.1 \mu\text{M} \\
\text{hKv7.5} & \quad \text{EC}_{50} = 3.4 \pm 0.4 \mu\text{M} \\
\text{hKv7.4/7.5} & \quad \text{EC}_{50} = 3.2 \pm 0.4 \mu\text{M}
\end{align*}
Cerebral Aneurysm and Subarachnoid hemorrhage (SAH).
Hypothetical model of the role of KCNQ channels in cerebral vasospasm.

Bharath Mani’s project
KCNQ channels are expressed and functional in basilar artery myocytes.

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**Graph:**

- **KCNQ Subtype**
  - 1 2 3 4 5
  - 500 bp

- **Current (I) vs. Voltage (V) Graph:**
  - Controls
  - Flupirtine 10µM
  - XE991 10µM

- **V, mV:**
  - -80 -60 -40 -20 0 20

- **I, pA/pF:**
  - 0.3 0.2 0.1 0.0
Celebrex® prevents or reverses vasoconstriction in basilar arteries

A

B

C

Serotonin 75nM
Celecoxib

Vessel outer diameter (µm)

Celecoxib

1µM
10µM
20µM
30µM

Serotonin 75nM
AVP 100pM
Endothelin 100pM

[celecoxib] µM
0 5 10 15 20 25 30

% relaxation
0 20 40 60 80 100

Celecoxib 30µM
Wash out
Vehicle

Serotonin 75nM

Vessel outer diameter (µm)

20 min

275 300 325 350 375 400

20 min

200 250 300 350
Retigabine or Celecoxib prevents basilar artery vasospasm in a rat model of SAH

KCNQ CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS AS PHYSIOLOGICAL AND THERAPEUTIC TARGETS

High (EC$_{50}$=5 nM) AVP Pathway

Low (EC$_{50}$=150pM) AVP Pathway

Release of SR Ca$^{2+}$ Store

Ca$^{2+}$ Influx

Vasoconstriction
KCNQ CHANNELS IN AIRWAY SMOOTH MUSCLE CELLS AS PHYSIOLOGICAL AND THERAPEUTIC TARGETS?
Kv7 CURRENTS IN AIRWAY SMOOTH MUSCLE CELLS ARE ENHANCED BY FLUPIRTINE AND CELECOXIB.
SUPPRESSION OF Kv7 CURRENTS IN AIRWAY SMOOTH MUSCLE CELLS BY METHACHOLINE AND HISTAMINE IS REVERSED BY FLUPIRTINE.

A

100 nM methacholine
10 µM F

B

control, n=4
- 100 nM MC, n=4
- 10 µM XE-991, n=3

C

control, n=3
- 30 µM His, n=3
- 10 µM XE-991, n=3

D

I/Ic at -20mV

C C H Hist Hist+ F XE-991 C MC MC+ F XE-991

0.0 0.5 1.0

* # * #
PRECISION-CUT LUNG SLICES: FUNCTIONAL EFFECTS OF KCNQ CHANNEL MODULATION ON AIRWAY DIAMETER

A

B

1 µM methacholine

10 µM retigabine

area µm²

0 10000 20000 30000 40000 50000 60000

time, min

0 20 40 60 80 100
KCNQ POTASSIUM CHANNEL ACTIVATORS ATTENUATE METHACHOLINE-INDUCED CONSTRICTION OF RAT AIRWAYS.

PROFOUND CONSTRICTION OF HUMAN AIRWAYS INDUCED BY Kv7 CHANNEL BLOCKER XE991; ATTENUATION OF HISTAMINE-INDUCED AIRWAY CONSTRICTION BY FLUPIRTINE.
FORMOTEROL

**Drug class:** Long-acting $\beta_2$-adrenergic agonist (LABA)

**Use:** Bronchospasm, Exercise-induced asthma

**DESENSITIZATION TO LABAs**

Regular treatment with both long- and short-acting $\beta_2$-agonists results in tolerance to their bronchoprotective effects. Twice daily administration of long-acting $\beta_2$-agonists results in blunted responses to repeated doses of short-acting $\beta_2$-agonists, as with rescue inhalers used in the setting of an acute asthma attack.
FORMOTEROL ALONE TRANSIENTLY RELAXES RAT AIRWAYS, WHEREAS IN COMBINATION WITH RETIGABINE IT INDUCES GREATER AND MORE SUSTAINED BRONCHODILATION

COMBINING RETIGABINE WITH FORMOTEROL IMPROVES RELAXATION OF ASTHMATIC HUMAN AIRWAYS

H = 25 pM histamine
FF = 30 pM formoterol fumarate
R = 10 µM retigabine
CONCLUSIONS

- KCNQ (Kv7) K⁺ channels contribute to stabilization of resting membrane potential and are essential intermediates in vasoconstrictor and bronchoconstrictor signal transduction in vascular and airway smooth muscle, respectively.

- In intact arteries, drugs that activate vascular KCNQ currents induce vasodilation. Drugs that inhibit KCNQ currents are vasoconstrictors. These effects contribute to changes in arterial blood flow and systemic blood pressure.

- Drugs currently in clinical use may have unexpected cardiovascular side effects due to previously unrecognized effects on vascular KCNQ channels. To predict these cardiovascular side effects, screening of drugs for actions on vascular KCNQ currents will be required. Some drugs may distinguish among KCNQ channels formed from different subunit combinations.

- Kv7 channels in airway smooth muscle may be important new targets for treatment of airway diseases such as COPD or asthma. Combination therapy with other bronchorelaxants may improve outcomes.
Acknowledgments:

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Matthias Majetschak & Abhi Tripathi

Robert Love, Chris Wigfield, Jeffrey Schwartz

National Heart, Lung, & Blood Institute
Low [AVP] but not high [AVP] constrictor effects depend on PKC activation and L-type Ca^{2+} channels.
KCNQ currents? Isolated $K_v$ currents are not affected by blockers of other types of $K^+$ channels.

Mackie et al., JPET 2008
Celebrex® dilates mesenteric arterioles and enhances blood flow in vivo

A

control

AVP

AVP + celecoxib

20 μm

13.9±0.5μm

9.7±0.4μm

16.4±0.3μm

B

blood flow, 10⁻³mm³/sec

control AVP AVP + celecoxib

n=11 n=5 n=11
Heteromeric KCNQ4/5 channels in mesenteric artery myocytes.

Brueggemann et al. *JBC* 2014

**Phase**

<table>
<thead>
<tr>
<th>KCNQ4-KCNQ5</th>
<th>PLA</th>
<th>DAPI</th>
</tr>
</thead>
</table>

**KCNQ4-TRPC6**

**Ab ctrl.**

**Graph:**

![Graph showing PLA signal/cell comparison between KCNQ4-KCNQ5 and KCNQ4-TRPC6](image)

Brueggemann et al. *JBC* 2014
Adv-GFPControl

50 µm

50 µm

*dnKCNQ5, n=6
control non-fluorescent, n=9
control GFP, n=6
dnKCNQ4, n=5

**

V, mV -80 -60 -40 -20 0
I, pA/pF 0.4 0.6

Brueggemann et al. JBC 2014