Initial testing of an ion channel panel for early *in vitro* detection of seizure liability

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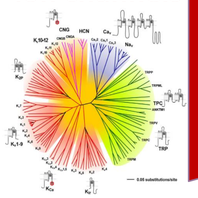
Seizure liability remains a significant cause of attrition throughout drug development. The resulting loss of competitiveness, delays, increased costs, and considerable safety risk all emphasise the need for improved methodologies to accurately detect seizure liability earlier in drug development. Current methods rely on nonclinical rodent and non-rodent studies. Ideally, novel, high-throughput *in vitro* methods would provide an earlier prediction of seizurogenic risk that could eliminate liabilities early in discovery while there are still options in chemistry and reduce the reliance on costly animal studies with questionable translation. The involvement of numerous ion channels in seizure provides an opportunity for a new paradigm in screening. Akin to the success of screening against a panel of ion channels to reduce cardiovascular safety liability (CiPA), the involvement of ion channels in seizure suggests that a similar approach to early seizure detection is valid.

1. ION CHANNEL SELECTION

15 ion channels were selected based on weight of evidence

100s of ion channels



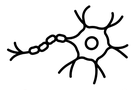
Expression profile

Function

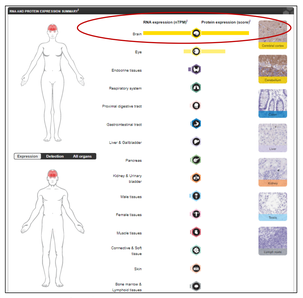
Human mutations

Pharmacology

15 seizure related ion channels



Example evidence for selection of Kv3.1 *(KCNC1)*



www.proteinatlas.org

www.omim.org

HGNC Approved Gene Symbol: KCNC1

Cytogenetic location: 11p15.1 *Genomic coordina*

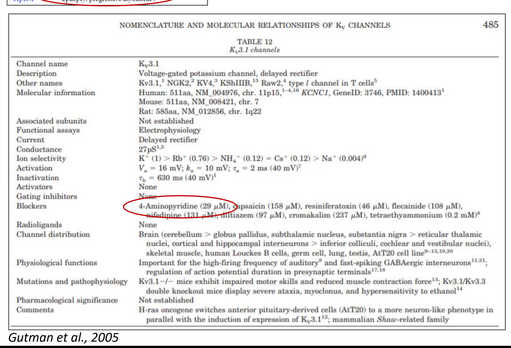
Gene-Phenotype Relationships

Location	Phenotype
11p15.1	Epilepsy, progressive myoclonic 7

UniProtKB/Swiss-Prot Summary for KCNC1 Gene

› Voltage-gated potassium channel that plays an important role in the rapid repolarization of fast-firing brain neurons.

www.genecards.org



NOMENCLATURE AND MOLECULAR RELATIONSHIPS OF K_V CHANNELS 485

TABLE 12
$K_V3.1$ channels

Channel name	$K_V3.1$
Description	Voltage-gated potassium channel, delayed rectifier
Other names	Kv3.1,[1] NGK2,[2] $KV4$,[3] KShIIIB,[35] Raw2,[4] type *f* channel in T cells[5]
Molecular information	Human: 511aa, NM_004976, chr. 11p15.[1–4,35] *KCNC1*, GeneID: 3746, PMID: 1400413[1] Mouse: 511aa, NM_008421, chr. 7 Rat: 585aa, NM_012856, chr. 1q22
Associated subunits	Not established
Functional assays	Electrophysiology
Current	Delayed rectifier
Conductance	27pS[1,3]
Ion selectivity	K^+ (1) > Rb^+ (0.76) > NH_4^+ (0.12) = Cs^+ (0.12) > Na^+ (0.004)[6]
Activation	V_a = 16 mV; k_a = 10 mV; τ_a = 2 ms (40 mV)[2]
Inactivation	τ_h = 630 ms (40 mV)[3]
Activators	None
Gating inhibitors	None
Blockers	4-Aminopyridine (29 μM); tityustoxin (158 μM), resiniferatoxin (46 μM), fleicainide (108 μM), nifedipine (131 μM), diltiazem (97 μM), cromakalim (237 μM), tetraethyammonium (0.2 mM)[6]
Radioligands	None
Channel distribution	Brain (cerebellum > globus pallidus, subthalamic nucleus, substantia nigra > reticular thalamic nuclei, cortical and hippocampal interneurons > inferior colliculi, cochlear and vestibular nuclei), skeletal muscle, human Louckes B cells, germ cell, lung, testis, AtT20 cell line[6–13,19,20]
Physiological functions	Important for the high-firing frequency of auditory[7] and fast-spiking GABAergic interneurons[11,21]; regulation of action potential duration in presynaptic terminals[17,18]
Mutations and pathophysiology	Kv3.1−/− mice exhibit impaired motor skills and reduced muscle contraction force[35]; Kv3.1/Kv3.3 double knockout mice display severe ataxia, myoclonus, and hypersensitivity to ethanol[14]
Pharmacological significance	Not established
Comments	H-ras oncogene switches anterior pituitary-derived cells (AtT20) to a more neuron-like phenotype in parallel with the induction of expression of $K_V3.1$[19]; mammalian *Shaw*-related family

Gutman et al., 2005

2. SELECTED SEIZURE RELATED ION CHANNELS

Sodium ion channels

Nav1.1 *(SCN1A)*
Nav1.2 *(SCN2A)*
Nav1.6 *(SCN8A)*

Ligand gated ion channels

GABA $\alpha_1\beta_2\gamma_3$ *(GABRA1/B2/G3)*
Nicotinic $\alpha_4\beta_2$ *(CHRNA4/B2)*
NMDA 1/2A (GRIN1/2A)

Calcium ion channel

Cav2.1 *(CACNA1A)*

Potassium ion channels

Kv1.1 *(KCNA1)*
Kv2.1 *(KCNB1)*
Kv3.1 *(KCNC1)*
Kv4.2 *(KCND2)*
Kv7.2/7.3 *(KCNQ2/KCNQ3)*
Kv7.3/7.5 *(KCNQ3/KCNQ5)*
KCa1.1 *(KCNMA1)*
KCa4.1 (KCNT1)

3. VALIDATION COMPOUNDS

	1	2	3	4	5	6	7	8
4-AP	✓						✓	
Amoxapine					✓			✓
Amoxicillin				✓		✓		✓
Bupropion					✓			✓
Chlorpromazine					✓			✓
Clozapine					✓			✓
Diphenhydramine					✓			✓
Enoxacin				✓		✓		✓
Linopirdine	✓						✓	
Paroxetine					✓			✓
Picrotoxin						✓	✓	
Pilocarpine			✓				✓	
Pentylenetetrazole						✓	✓	
Seroquel					✓			✓
Strychnine		✓					✓	✓

Seizurogenic compounds with various MOAs were used to validate the chosen ion channels

1 – Potassium channel antagonist
2 – Glycine receptor antagonist
3 – Muscarinic agonist
4 – Broad spectrum antibiotic
5 – CNS acting drug
6 – GABA-A receptor antagonist
7 – Used *in vivo* to induce seizures
8 – Causes seizures in humans

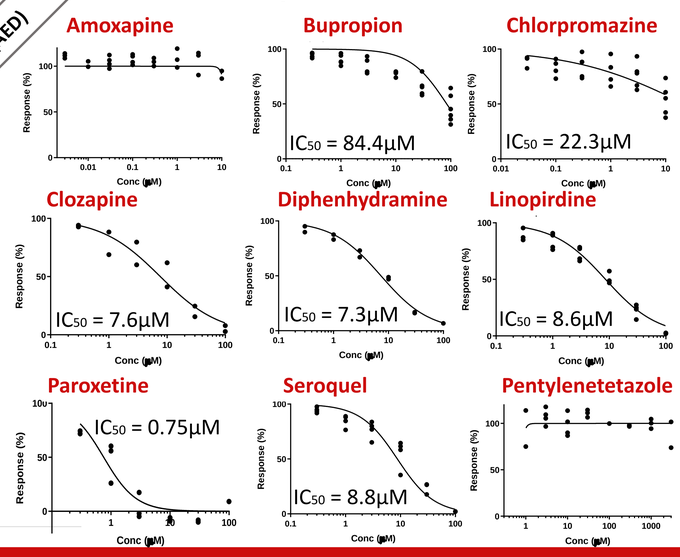
4. INITIAL SCREENING DATA

- The activity of the validation compounds was assessed in the seizure related ion channels which were stably expressed in recombinant CHO or HEK cell lines
- Ion currents were measured by automated patch-clamp (Q patch, Sophion/Ion Works, Molecular devices) at ambient temperature and 6 or 8-point curves were generated
- A compound that showed activity in our assay was considered a positive hit. An appropriate positive control was included for each ion channel

Ion channel	4-AP	Amoxapine	Bupropion	Chlorpromazine	Clozapine	Diphenhydramine	Linopirdine	Paroxetine	Pilocarpine	Seroquel	Strychnine	Amoxicillin	Enoxacin	Pentylenetetrazole	Picrotoxin	Phenytoin (AED)
	Seizure causing compounds										GABA antagonists					
Nav1.1		✓		✓	✓	✓										✓*
Nav1.2		✓		✓	✓	✓				✓						✓*
Nav1.6		✓		✓	✓	✓				✓						✓*
Kv1.1		✓		✓	✓	✓		✓		✓						
Kv2.1			✓	✓	✓	✓	✓	✓		✓						
Kv3.1	✓				✓		✓			✓						
Kv4.2 (4.3)		✓	✓	✓	✓	✓	✓	✓		✓						✓
Kv7.2/7.3		✓	✓	✓	✓	✓	✓	✓								
Kv7.3/7.5	IN HOUSE TESTING IN PROGRESS					✓										
KCa1.1																
KCa4.1		✓			✓											
Cav2.1																
GABA $\alpha_1\beta_2\gamma_3$											✓*	✓*	✓*	✓*		
Nicotinic $\alpha_4\beta_2$																
NMDA 1/2A				✓*		✓*				✓*						

= In house testing in progress; ✓* = validated from literature; AED = anti-epileptic drug

Example data: Kv2.1



5. DISCUSSION AND CONCLUSIONS

- Typically, the potassium channels were sensitive to more compounds than the sodium channels
- The 3 sodium ion channels tested have a similar inhibition profile, therefore inclusion of only one sodium channel may be sufficient
- The GABA-A receptor antagonists and pilocarpine show strong specificity for their targets
- The CNS acting drugs exhibit activity on many potassium and sodium ion channels, outside of their accepted MOAs
- These initial studies highlight the potential utility of a seizure ion channel screening panel to provide mechanistic information and support optimal drug design