Ion Channels and Channelopathies



J Clin Invest. 2005;115(8) review series



Frances M. Ashcroft 2005

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Outline

Part I: Ion Channels

- Introduction
- Classification
- Structure
- Function

Part II: Channelopathies

- Long QT syndromes Type 1 and 2 : LQT1 and LQT2: delayed K⁺ channel
- Long QT syndrome type 3: LQT3: Na⁺ channel
- Epilepsy: Voltage-gated Ca²⁺ channel
- Diabetes Mellitus: ATP-sensitive K⁺ channel
- Cystic fibrosis: CFTR, Cl⁻ channel

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What are Ion Channels ?



Ion channels - structure

- are proteins that span (or traverse) the membrane
- have water-filled 'channel' that runs through the protein
- ions move through channel, and so through membrane

Ion channel - properties

- Selectivity: Each specific ion crosses through specific channels
- Gating: transition between states (closed ↔ open ↔ Inactivation)
 Voltage-gated ; Ligand-gated
- Channels mediate ion movement down electrochemical gradients.
- Activation of channel permeable to ion X shifts membrane potential towards to its Equilibrium Potential, E_X

Equilibrium Potential or Nernst Potential

The voltage at which there is zero net flux of a given ion (Electrical gradient = a chemical concentration gradient)

For K⁺ : ~ -90 mV

$$E_{K} = \frac{RT}{ZF} \ln \frac{[K^{+}]_{o}}{[K^{+}]_{i}}$$



Hermann (Walther) Nernst 1864-1941 1920 Nobel Prize for chemistry

- R = gas constant
- F = Faraday constant
- T = temperature (K)
- Z = valence (charge) of ion ~



K current (I_{K1}) is the major contributor for RMP

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Four Milestones in Ion Channel Research

1. Ionic conductance

Noble 1963 (Physiol/Medicine)





Alan L. Hodgkin

Andrew F. Huxley

3. Channel cloning sequencing

(Ach receptor, Na, Ca channels)



Japan Academy Prize 1985

Shosaku Numa (沼 正作)

2. Patch clamp methodology

Noble 1991 (Physiol/Medicine)





Erwin Neher

Bert Sakmann

4. K channel structure Noble 2003 (Chemistry)



Determining the structure of cell-membrane ion cells. be mission impossible. Also Al-blott meets the researcher who proved the doubters wrong, opening new windows on cellular function.

Rod MacKinnon

Hodgkin-Huxley Model Predicted the Existence of Ion Channels



Patch-Clamp Techniques



1991 Nobel Prize







Erwin Neher & Bert Sakmann Nobel prize for medicine in 1991 for the development of the patchclamp technique making possible the characterization of single ion channels





Channel cloning sequencing

NATURE VOL. 305 27 OCTOBER 1983

Cloning and sequence analysis of calf cDNA and human genomic DNA encoding α -subunit precursor of muscle acetylcholine receptor

Masaharu Noda, Yasuji Furutani, Hideo Takahashi, Mitsuyoshi Toyosato, Tsutomu Tanabe, Shin Shimizu, Sho Kikyotani, Toshiaki Kayano, Tadaaki Hirose*, Seiichi Inayama* & Shosaku Numa

Department of Medical Chemistry, Kyoto University Faculty of Medicine, Kyoto 606, Japan * Pharmaceutical Institute, Keio University School of Medicine, Tokyo 160, Japan



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NATURE VOL. 305 27 OCTOBER 1983

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Nobel Prize for Chemistry 2003

Protein x-ray crystallography

1) Purification



2) Crystallization

3) X-Ray Diffraction

Crystallized

X-ray beam







The Nobel Prize in Chemistry 2003 Peter Agre, Roderick MacKinnon

Crystal structure of ion channel

Classification of Ion Channels

1) Based on ion selectivity:

K⁺, Na⁺, Ca²⁺, Cl⁻ channels

2) Based on gating:

Voltage-gated : ions

Ligand-gated: Glutamate, GABA, ACh, ATP, cAMP



3) Based on rectification:

Inwardly or outwardly rectifying





Structure of K_{CSA} Channels: Selectivity Filter and Gating







Inner helices form "inverted teepee" structure







Doyle et al (1998) Science 280, 69

Open-Close Gating



Doyle et al. Science 1998;

Bacterial K channel selective filter: P-loop; Gating: intracellular side of the pore bundle crossing



Bacterial Na channel pore in the closed and "open" conformation

Ligand-Gated Channels



ATP-sensitive K channel



Also a weak inward rectifier

- Open when a signal molecule (ligand) binds to an extracellular receptor region of the channel protein.
- This binding changes the structural arrangements of the channel protein, which then causes the channels to open or close in response to the binding of a ligand such as a neurotransmitter.
- This ligand-gated ion channel, allows specific ions (Na+, K+, Ca2+, or Cl-) to flow in and out of the membrane.

Models for Voltage Gate



the S4 segment is responsible for detecting voltage changes.

The movement of positively-charged S4 segments within the membrane electric field

Transition between Close, Open, and Inactivation States



Closed At the resting potential, the channel is closed.





Open In response to a nerve impulse, the gate opens and Na⁺ enters the cell.





Inactivated For a brief period following activation, the channel does not open in response to a new signal.

Inactivation Gating of Voltage-Gated Channels -Ball and Chain



(Gulbis et al, Science 2000)

N-terminal inactivation gate

A positively charged inactivation particle (ball) has to pass through one of the lateral windows and bind in the hydrophobic binding pocket of the pore's central cavity. This blocks the flow of potassium ions through the pore. There are four balls and chains to each channel, but only one is needed for inactivation.

Structural Basis of Gating in a Voltage-gated Channel



A: a subunit containing six transmembrane-spanning motifs. S5 and S6 and the pore loop are responsible for ion conduction (channel pore). S4 is the the *voltage sensor, which* bears positively charged amino acids (Arg) that relocate upon changes in the membrane electric field. N-terminal ball-and-chain is responsible for inactivation

B: four such subunits assembled to form a potassium channel.

Channel Function: Single Channel and Whole-cell Current

- Ion channels are not open continuously but open and close in a stochastic or random fashion.
- Ion channel function may be decreased by
 - decreasing the open time (O),
 - increasing the closed time (C),
 - decreasing the single channel current amplitude (i)
 - or decreasing the number of channels (n).



Channel Function: Single Channel and Whole-cell Current



Depolarizing voltage pulses result in brief openings in the seven successive recordings of membrane current Close correlation between the time courses of microscopic and macroscopic Na+ currents

Physiological Function of Ion Channels

- Maintain cell resting membrane potential: inward rectifier K and CI channels.
- Action potential and Conduction of electrical signal: Na, K, and Ca channels of nerve axons and muscles
- Excitation-contraction (E-C) coupling: Ca channels of skeletal and heart muscles
- Synaptic transmission at nerve terminals: glutamate, Ach receptor channels
- Intracellular transfer of ion, metabolite, propagation: gap junctions
- Cell volume regulation: Cl channel, aquaporins
- Sensory perception: cyclic necleotide gated channels of rods, cones
- Oscillators: pacemaker channels of the heart and central neurons
- **Stimulation-secretion coupling:** release of insulin form pancreas (ATP sensitive K channel)

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- Instruction
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Part II: Channelopathies

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Channelopathies?

1. Definition: Disorders of ion channels or ion channel disease Diseases that result from defects in ion channel function. Mostly caused by mutations of ion channels.

2. Channelopathies can be inherited or acquired:

a. Inherited channelopathies result from mutations in genes encoding channel proteins (major)

b. Acquired channelopathies result from *de novo mutations,* actions of drugs/toxins, or autoimmune attack of ion channels

• Drug/Toxin - e.g. Drugs that cause long QT syndrome

- **3. Increasingly recognized as important cause of disease** (>30 diseases).
- **4. Numerous mutation sites may cause similar channelopathy** e.g. cystic fibrosis where >1000 different mutations of CFTR described

Molecular Mechanisms of Channel Disruption

IV. Gating



III. Conduction

II. Processing

Consequences of Ion Channel Mutations

- Mutation of ion channel can alter
 - -Activation
 - -Inactivation
 - -Ion selectivity/Conduction
- Abnormal gain of function
- Loss of function

Cardiac Channelopathies

- Long QT Syndrome (types 1-12, various genes)
 - Short QT Syndrome (Kir2.1, L-type Ca²⁺ channel)
 - Burgada Syndrome (I_{to}, Na⁺, Ca²⁺ channels)
 - Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) (RyR2, SR Ca release)

ECG and QT interval



FYI: ECG Recording 120 Years Ago





First recorded in 1887

In order to conduct the weak current of the heart's electrical activity, Einthoven used electrolyte (saline-filled) tubs ["E" in photo] as *electrode* contacts to each of three limbs, the right arm, the left arm, and the left foot, respectively.¹⁰ He chose two of these limb electrodes to monitor each *lead*, making one electrode positive and the other electrode negative to record each of his three classic *bipolar limb leads*. He named these bipolar limb leads *Lead I* (left arm positive, right arm negative), *Lead II* (left foot positive, right arm negative), and *Lead III* (left foot positive, left arm negative). Note that the original string galvanometer consisted of massive equipment that filled a room.

FYI: ECG Recording 120 Years Ago



AP Correlation to ECG Waveform

- P wave: Electrical activation (depolarization) of the atrial myocardium.
- PR segment: This is a time of electrical quiescence during which the wave of electrical excitation (depolarization) passes through mainly the AV node.
- QRS wave: Depolarization of the ventricular myocardium.
- T wave: Ending of ventricular myocardium repolarization
- ST segment: Ventricular repolarization



LQTS-facts

- Normal QT interval: 360-440 ms
- Delayed repolarization of the myocardium, QT prolongation (>450 in man; > 470 in women).
- Increased risk for syncope, seizures, and SCD in the setting of a structurally normal heart
- 1/2500 persons.
- Usually asymptomatic, certain triggers leads to potentially life-threatening arrhythmias, such as Torsades de Pointes (TdP)

FYI: QT Interval Ranges

QT interval ranges

	Age 1 to 15	Adult man	Adult woman
Normal	Less than 0.44 second	Less than 0.43 second	Less than 0.45 second
Borderline	0.44 to 0.46 second	0.43 to 0.45 second	0.45 to 0.47 second
Prolonged	Greater than 0.46 second	Greater than 0.45 second	Greater than 0.47 second

Source: Jacobson C. Long and short of it: What's up with the QT interval? http://hosted.mediasite. com/mediasite/Viewer/?peid=9ed8856fcdab4bc0bb066c25a148435b1d.

FYI: Genetic Basis for LQT syndromes

Туре	Locus	Gene	Protein	Function	Frequency
LQT1	11p15.5	KCNQ1	KV7.1 α	$I_{Ks} \downarrow$	30%-35%
LQT2	7q35	KCNH2	KV11.1 α	I _{Kr} ↓	25%-30%
LQT3	3p21	SCN5A	NaV1.5 α	I _{Na} ↑	5%-10%
LQT4	4q25	ANK2	Ankyrin-B	I _{Na,K} ↓	1%-2%
				$I_{NCX} \downarrow$	
LQT5	21q22.1	KCNE1	minK β	I _{Ks} ↓	1%
LQT6	21q22.1	KCNE2	MiRP1 β	$I_{kr}\downarrow$	Rare
LQT7*	17q23	KCNJ2	Kir2.1 α	$I_{\kappa_1} \downarrow$	Rare
LQT8†	12p13.3	CACNA1C	CaV 1.2 α 1c	I _{Ca.L} ↑	Rare
LQT9	3p25	CAV3	Caveolin-3	I _{Na} ↑	Rare
LQT10	11q23	SCN4B	NaV1.5 β4	I _{Na} ↑	Rare
LQT11	7q21	AKAP9	Yotiao	I _{Ks} ↓	Rare
LQT12	20q11.2	SNTA1	A1-syntrophin	I_{Na}^{\uparrow}	Rare

Cardiac action potential

- Phase 0. Influx of Na+ (INa). Induces membrane depolarization
- Phase 1. Efflux of K+ (Ito). Limits the Na+ spike
- Phase 2. Influx of Ca²⁺ (ICa). Activation of I_{K.} Balance between Ca²⁺ influx and K⁺ efflux. Ca²⁺ enters the cell to trigger the Ca²⁺-induced Ca²⁺ release.
- Phase 3. Efflux of K⁺ (I_K) increases. Repolarization starts
- Phase 4. Restoration of the resting potential: equilibrium potential of K via I_{K1}. and Na⁺ / K⁺ pump, Na⁺ / Ca²⁺ pump.
 B Ventricular myocyte action potential



Pathophysiology of LQT (1, 2, 3)



as harsh, sudden noises

• LQTS3: Slow heart rate while sleeping

Source: National Heart, Lung, and Blood Institute. What is long QT syndrome? http://www.nhlbi.nih.gov/health/dci/Diseases/ qt/qt_all.html.

LQT syndromes: proarrhythmic mechanisms

- Upregulation of inward currents
 Or
- Downregulation of outward currents
- EADs→ triggers
- Dispersion of APDs
 → substrates
 → reentry



Example 1:

LQT1 and LQT2

Downregualtion of delayed K⁺ channel, I_{Ks} and I_{Kr}

LQT1: KCNQ1 (KvLQT1) mutations



LQT2: KCNH2(HERG) MUTATIONS



LQT 1 and 2: I_{Ks} and I_{Kr} downregulation



Example 2:

LQT3

Inactivation of Na⁺ channel

LQT3: Increased persistent Na Current



Functional mechanisms in LQT3



Example 3: Epilepsy - a CNS Channelopathies



Epilepsy is a disorder marked by disturbed electrical rhythms in the central nervous system

FYI: Ion Channels Implicated in Epilepsy

Voltage-gated Sodium channelType I α_1 subunitSCN1AGeneralized epilepsy with febrile seizures plus syndrome (GEFS+)Type I β_1 subunitSCN1BGeneralized epilepsy with febrile seizures plus syndrome (GEFS+)Type I α_1 subunitSCN1ASevere myocolonic epilepsy of infancy (SMEI) Intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTCS)Type I α_1 subunitSCN1AInfantile spasms (IS)Type I α_1 subunitSCN1AInfantile spasms (IS)Type I α_1 subunitSCN2ABenign familial neonatal-infantile seizures (BFNIS)Calcium channelP/Q-type α_1 subunitCACNA1AEpisodic tatxia type 2 (EA2) Familial hemiplegic migraine (FHM) Spinocerebellar atxia type 4 (SCA 6) CACNB4Potassium channelKy1.1KCNA1Episodic tatxia type 1 (EA1) M-channelKCNQ3BK channelKCNQ3Beneralized epilepsy with paroxysmal dyskinesia (GEPD)Chloride channelCLC-2CLCN2Juvenile absence epilepsy (JAE) Epilepsy with grand mal seizures on awakening (EGMA) CAELigand-gated Acetylcholine receptor β_2 subunitCHRN82 α_4 subunitAutosomal dominant frontal lobe epilepsy (ADNFLE) α_4 subunitGABA receptor γ_2 subunitGABRG2GEFS+, CAE, SMEI M_1	Channel	Protein	Gene	Syndrome
Sodium channelType I α_1 subunitSCN1AGeneralized epilepsy with febrile seizures plus syndrome (GEFS+)Type I β_1 subunitSCN1BGeneralized epilepsy with febrile seizures plus syndrome (GEFS+)Type I α_1 subunitSCN1ASevere mycolonic epilepsy of infancy (SMEI) Intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTCS)Type I α_1 subunitSCN1AInfantile spasms (IS)Type I α_1 subunitSCN1AInfantile spasms (IS)Type I α_1 subunitSCN2ABenign familial neonatal-infantile seizures (BFNIS)Calcium channelP/Q-type α_1 subunitCACNA1AEpisodic tatxia type 2 (EA2) Familial hemiplegic migraine (FHM) Spinocerebellar atxia type 6 (SCA 6) CACNB4Potassium channelKv1.1KCNA1Episodic tatxia type 1 (EA1) McchannelKv1.1KCNQ3Benign familial neonatal convulsions (BFNC) KCNQ3BK channelKCNQ4Benign familial neonatal convulsions (BFNC) KCNQ3Chloride channelCLC-2CLCN2Juvenile absence epilepsy (JAE) Epilepsy with grand mal seizures on awakening (EGMA) CAELigand-gatedAcetylcholine receptor α_4 subunitGABRA2GEFS+, CAE, SMEI GABRA2GABA receptor γ_2 subunitGABRA2GEFS+, CAE, SMEI Juveni	Voltage-gated			
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Type I α_1 subunit Type I α_1 subunitSCN1A SCN1ASevere myoclonic epilepsy of infancy (SMEI) Intractable childhood epilepsy with 		Type I β_1 subunit	SCN1B	Generalized epilepsy with febrile seizures plus syndrome (GEFS+)
Type I α_1 subunitSCN1AIntractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTCS)Type I α_1 subunitSCN1AInfantile spasms (IS)Calcium channelP/Q-type α_1 subunitSCN2ABenign familial neonatal-infantile seizures (BFNIS)Calcium channelP/Q-type α_1 subunitCACNA1AEpisodic ataxia type 2 (EA2) Familial hemiplegic migraine (FHM) 		Type I α_1 subunit	SCN1A	Severe myoclonic epilepsy of infancy (SMEI)
$ \begin{array}{c c} Type I \alpha_1 \mbox{ subunit} \\ Type II \alpha_1 \mbox{ subunit}$		Type I α_1 subunit	SCN1A	Intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTCS)
Type II α_1 subunitSCN2ABenign familial neonatal-infantile seizures (BFNIS)Calcium channelP/Q-type α_1 subunitCACNA1AEpisodic ataxia type 2 (EA2) Familial hemiplegic migraine (FHM) Spinocerebellar ataxia type 6 (SCA 6) CACNB4Calcium channelKv1.1KCNA1HChildhood absence epilepsy (CAE) ^a Potassium channelKv1.1KCNA1Episodic ataxia type 1 (EA1) Benign familial neonatal convulsions (BFNC) KCNQ3Potassium channelKv1.1KCNA2 KCNQ3Benign familial neonatal convulsions (BFNC) KCNQ3Chloride channelCLC-2CLCN2Juvenile myoclonic epilepsy (JME) Juvenile absence epilepsy (JME) Livenile absence epilepsy (JAE) Epilepsy with grand mal seizures on awakening (EGMA) CAELigand-gated Acetylcholine receptor β_2 subunitCHRNB2 GABA receptorAutosomal dominant frontal lobe epilepsy (ADNFLE) α_4 subunitGABA receptor γ_2 subunitGABRA1 GABRA1JME JME		Type I α_1 subunit	SCN1A	Infantile spasms (IS)
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$ \begin{array}{c cccc} Familial hemiplegic migraine (FHM) \\ Spinocerebellar ataxia type 6 (SCA 6) \\ CACNB4 & Episodic ataxia type 2 (EA2) \\ \hline T-type \alpha_1 subunit & CACNA1H & Childhood absence epilepsy (CAE)^a \\ \hline Potassium channel & K_V1.1 & KCNA1 & Episodic ataxia type 1 (EA1) \\ M-channel & KCNQ2 & Benign familial neonatal convulsions (BFNC) \\ KCNQ3 & & & & & & & & & & & & & & & & & & &$	Calcium channel	P/Q-type α_1 subunit	CACNA1A	Episodic ataxia type 2 (EA2)
$\begin{array}{c c} & & & & & & & \\ & & & & & & \\ & & & & $				Familial hemiplegic migraine (FHM)
$\begin{array}{c cccc} CACNB4 & Episodic ataxia type 2 (EA2) \\ \hline T-type \alpha_1 subunit & CACNA1H & Childhood absence epilepsy (CAE)^a \\ \hline Potassium channel & K_V1.1 & KCNA1 & Episodic ataxia type 1 (EA1) \\ \hline M-channel & KCNQ2 & Benign familial neonatal convulsions (BFNC) \\ \hline KCNQ3 & \\ BK channel & CLC-2 & CLCN2 & Juvenile myoclonic epilepsy (JME) \\ \hline Juvenile absence epilepsy (JAE) & Epilepsy with grand mal seizures on awakening (EGMA) \\ \hline CACNA1H & CHRNB2 & Autosomal dominant frontal lobe epilepsy (ADNFLE) \\ \hline \alpha_4 subunit & CHRNA4 & \\ \hline GABA receptor & \gamma_2 subunit & GABRG2 & GEFS+, CAE, SMEI \\ \hline \alpha_1 subunit & GABRG1 & JME \\ \hline \end{array}$				Spinocerebellar ataxia type 6 (SCA 6)
I-type α_1 subunitCACNATHChildhood absence epilepsy (CAE)aPotassium channel $K_V 1.1$ $KCNA1$ Episodic ataxia type 1 (EA1)M-channel $KCNQ2$ $KCNQ3$ Benign familial neonatal convulsions (BFNC)BK channel $KCNQ3$ Generalized epilepsy with paroxysmal dyskinesia (GEPD)Chloride channelCLC-2 $CLCN2$ Juvenile myoclonic epilepsy (JME) Juvenile absence epilepsy (JAE) Epilepsy with grand mal seizures on awakening (EGMA) CAELigand-gatedAcetylcholine receptor β_2 subunit $CHRNB2$ α_4 subunitAutosomal dominant frontal lobe epilepsy (ADNFLE) α_4 subunitGABA receptor γ_2 subunit $GABRG2$ GEFS+, CAE, SMEI ME			CACNB4	Episodic ataxia type 2 (EA2)
Potassium channel $K_V 1.1$ $KCNA1$ Episodic ataxia type 1 (EA1)M-channel $KCNQ2$ Benign familial neonatal convulsions (BFNC)KCNQ3BK channel $KCNMA1$ Generalized epilepsy with paroxysmal dyskinesia (GEPD)Chloride channelCLC-2 $CLCN2$ Juvenile myoclonic epilepsy (JME) Juvenile absence epilepsy (JAE) Epilepsy with grand mal seizures on awakening (EGMA) CAELigand-gated $Acetylcholine receptor$ β_2 subunit $CHRNB2$ α_4 subunitAutosomal dominant frontal lobe epilepsy (ADNFLE) α_1 subunitGABA receptor γ_2 subunit $GABRA1$ JMEJMEJME		T-type α_1 subunit	CACNA1H	Childhood absence epilepsy (CAE) ^a
M-channelKCNQ2 KCNQ3Benign familial neonatal convulsions (BFNC)BK channelKCNQ3Generalized epilepsy with paroxysmal dyskinesia (GEPD)Chloride channelCLC-2CLCN2Juvenile myoclonic epilepsy (JME) Juvenile absence epilepsy (JAE) Epilepsy with grand mal seizures on awakening (EGMA) CAELigand-gatedAcetylcholine receptor β_2 subunitCHRNB2 CHRNA4Autosomal dominant frontal lobe epilepsy (ADNFLE) GABA receptorM-channel γ_2 subunitGABRG2 GABRA1GEFS+, CAE, SMEI JME	Potassium channel	K _V 1.1	KCNA1	Episodic ataxia type 1 (EA1)
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Chloride channelCLC-2 $CLCN2$ Juvenile myoclonic epilepsy (JME) Juvenile absence epilepsy (JAE) Epilepsy with grand mal seizures on awakening (EGMA) 		BK channel	KCNMA1	Generalized epilepsy with paroxysmal dyskinesia (GEPD)
$\begin{array}{c} \label{eq:constraint} Juvenile absence epilepsy (JAE) \\ Epilepsy with grand mal seizures on \\ awakening (EGMA) \\ CAE \\ \\ Ligand-gated \\ Acetylcholine receptor & \beta_2 subunit & CHRNB2 \\ Acetylcholine receptor & \beta_2 subunit & CHRNB2 \\ a_4 subunit & CHRNA4 \\ \\ GABA receptor & \gamma_2 subunit & GABRG2 & GEFS+, CAE, SMEI \\ a_1 subunit & GABRA1 & JME \\ \end{array}$	Chloride channel	CLC-2	CLCN2	Juvenile myoclonic epilepsy (JME)
$\begin{array}{c} \mbox{Epilepsy with grand mal seizures on awakening (EGMA) \\ CAE \\ \mbox{Ligand-gated} \\ \mbox{Acetylcholine receptor} & \beta_2 \mbox{ subunit} & CHRNB2 \\ \mbox{GABA receptor} & \gamma_2 \mbox{ subunit} & CHRNA4 \\ \mbox{GABA receptor} & \gamma_2 \mbox{ subunit} & GABRG2 & GEFS+, CAE, SMEI \\ \mbox{ α_1 subunit} & GABRA1 $ JME \\ \end{array}$				Juvenile absence epilepsy (JAE)
$\begin{array}{c} \label{eq:Ligand-gated} \\ \mbox{Acetylcholine receptor} & \beta_2 \mbox{ subunit} & CHRNB2 & Autosomal dominant frontal lobe epilepsy (ADNFLE) \\ \mbox{α_4 subunit} & CHRNA4 \\ \\ \mbox{GABA receptor} & \gamma_2 \mbox{ subunit} & GABRG2 & GEFS+, CAE, SMEI \\ \mbox{α_1 subunit} & GABRA1 & JME \\ \mbox{α_1 subunit} & GABRA1 & JME \\ \end{array}$				Epilepsy with grand mal seizures on awakening (EGMA)
Ligand-gatedAcetylcholine receptor β_2 subunitCHRNB2Autosomal dominant frontal lobe epilepsy (ADNFLE) α_4 subunitCHRNA4GABA receptor γ_2 subunitGABRG2GEFS+, CAE, SMEI α_1 subunitGABRA1JME	Ligand gated			CAE
Acception p_2 subunitCHRND2Actosomal dominant noncer operations (ADINIEL) α_4 subunitCHRNA4GABA receptor γ_2 subunitGABRG2 α_1 subunitGABRA1JME α_1 subunitGABRA1	Acetylcholine receptor	B- subunit	CHRNR2	Autosomal dominant frontal John enilepsy (ADNELE)
GABA receptor γ_2 subunit $GABRG2$ GEFS+, CAE, SMEI α_1 subunit $GABRA1$ JME	Acetylcholine leceptor	p_2 subunit	CHRNA4	Autosomai dominant nontai iobe epilepsy (ADINFLE)
α_1 subunit $GABRA1$ JME	CARA receptor	w ₄ subunit	CARRC2	CEES + CAE SMEL
	UNDAT Teceptor	γ_2 subunit	CABRA1	IME
B subunit GABRD IME ^a		β subunit	GABRD	IME ^a

Voltage-gated Ca Channels: Subunit Assembly and Subtypes

Ancillary subunits



Epilepsy: Voltage-gated Ca²⁺ Channel



Enhancement of T-type Ca current in thalamocortical networks produces spike wave absence epilepsy



gain-of-function

Epilepsy: Pathology and Symptom



Example 4:

ATP-Sensitive K⁺ Channel and Diabetes

Discovery of K_{ATP} Channel

Nature. 1983 Sep 8-14;305(5930):147-8.

ATP-regulated K+ channels in cardiac muscle.

<u>Noma A</u>.

Abstract

An outward current of unknown nature increases significantly when cardiac cells are treated with cyanide or subjected to hypoxia, and decreases on intracellular injection of ATP. We report here that application of the patch-clamp technique to CN-treated mammalian heart cells reveals specific K+ channels which are depressed by intracellular ATP (ATPi) at levels greater than 1 mM. For these channels, conductance in the outward direction is much larger than the inward rectifier K+ channel which is insensitive to ATP. AMP had no effect on the ATP-sensitive K+ channel, and ADP was less effective than ATP. Thus, the ATP-sensitive K+ channel seems to be important for regulation of cellular energy metabolism in the control of membrane excitability.

Nature. 1983 May 19-25;303(5914):250-3.

Acetylcholine activation of single muscarinic K+ channels in isolated pacemaker cells of the mammalian heart.

<u>Sakmann B, Noma A, Trautwein W</u>.

Abstract

Acetylcholine (ACh) released on vagal stimulation reduces the heart rate by increasing K+

conductance of pacemaker cells in the sinoatrial (S-A) node. Fluctuation analysis of ACh-act^{Saalland大学三人組と三内三人組} currents in pacemaker tissue showed this to be due to opening of a separate class of K+ channe

Development of Biosimulators and Analysis Tools

Computer simulations of Cell and Tissue functions herald a new age for the world of medical diagnosis and treatment





ATP-Sensitive Potassium Channel



Is composed of Kir6.x and sulfonylurea receptors (SURs)

- Inhibited by ATP
- Inhibited by sulfonylurea via SURs

ATP-Sensitive K channel Inhibited by ATP



Role of the K_{ATP} Channel in Insulin Secretion in Pancreatic β Cell



- Glucose enters the cell via the GLUT2 transporter
- Glycolytic and mitochondrial metabolism leads to an increase in ATP
- This results in K_{ATP} channel closure, membrane depolarization,
- Opening of voltage-gated Ca²⁺ channels, Ca²⁺influx,
- Exocytosis of insulin granules (insulin secretion).

Gloyn AL et al. N Engl J Med 2004;350:1838-1849.

K_{ATP} Channel Mutations Causing Lower ATP Sensitivity and Diabetes



The K_{ATP} Channel Couples Glucose Metabolism to Insulin Secretion



Example 5:

Cystic Fibrosis: Cl⁻ Channel Disease

Cystic Fibrosis: Facts

- Cystic fibrosis (CF) is autosomal recessive disease

- CF is a chronic, progressive, life threatening genetic disorder of pediatrics.

- It affect white population (1 in 3200 live births) but is uncommon among Asian and African population

- It affects exocrine glands (mainly sweat glands) and mucus gland present on the epithelial lining of lungs, pancreas, intestine, and reproductive system.

- CF is a defect in epithelial chloride channel protein, causes membrane to become impermeable to Chloride ion.





CFTR gene encode for the CFTR protein channel



CF occurs due to the deletion of 3 nucleotides which code for the phenylalanine from the CFTR (cystic fibrosis transmembrane conductance regulator) gene located on chromosome no.7 at position 508. This mutation is known as Δ F 508

Structure of the CFTR protein

CFTR protein is a cAMP induced Channel made up of five domains:

Two membrane-spanning domain (MSD1 & MSD2) that form Cl⁻ ion channel.

Two nucleotide binding domains (NBD1 & NBD2) that bind and hydrolyze ATP.

A regulatory R domain.



CFTR mutation: Loss of CI⁻ Channel Function



Pathology of Cystic Fibrosis - 1

In sweat glands:

CFTR is responsible for re-absorption of CI⁻ along with Na⁺ through epithelial Na channel (ENaC).

Impaired function of CFTR cause the production of hypertonic salty sweat, and ultimately dehydration.



SWEAT GLANDS

Pathology of Cystic Fibrosis - 2

In lung mucus glands:

- \bullet Loss of CFTR function to secrete chloride ion \rightarrow
- Loss or reduction of CI⁻ ion in luminal secretion \rightarrow
- Followed by active luminal Na⁺ absorption through ENaC \rightarrow
- \bullet Increases passive water absorption from the lumen arrow
- Impaired mucociliary action, accumulation of thick, viscous, dehydrated mucus
- Obstruction of air passage and recurrent pulmonary infections



Channelopathies: Summary

- Channel mutations are an increasingly recognized cause of disease.
- Many channelopathies are episodic despite persistently abnormal channel.
- Abnormalities in same channel may present with different disease states
- Mutations/ abnormalities in different channels may lead to same disease e.g. periodic paralysis or epilepsy
- Disease mechanism often unclear despite identification of mutation.

Thank you!

FYI: Human Channelopathies

Protein	Gene	Disease	Functional defect
Na _v 1.1	SCN1A	Generalized epilepsy with febrile seizures plus (GEFS+)	Hyperexcitability
Na,1.2	SCN2A	Generalized epilepsy with febrile and afebrile seizures	Hyperexcitability
Na _v 1.4	SCN4A	Paramyotonia congenita, potassium-aggravated myotonia, hyperkalemic periodic paralysis	Hyperexcitability
Na _v 1.5	SCN5A	LQTS/Brugada syndrome	Heart action potential
SCN1B	SCN1B	Generalized epilepsy with febrile seizures plus (GEFS+)	Hyperexcitability
KCNQ1	KCNQ1	Autosomal-dominant LQTS with deafness	Heart action potential/inner ear K* secretion
		Autosomal-recessive LQTS	Heart action potential
KCNH2	KCNH2	LQTS	Heart action potential
Kir2.1	KCNJ2	LQTS with dysmorphic features	Heart action potential
HERG	KCNH2	Congenital and acquired LQTS	Heart action potential and excessive
Anlwrin-R	ANKR	1015	Heart action notantial
Co 1 2	CACNA2	Timothy syndrome	Multicyctam dieordare
Vir6 2	KCN 111	Pareletant hyparineulinamic hyponhycamia of infancy	Inculin hyperescration
NI 0.2	NONDTT	Diabetes mellitus	Insulin hyposecretion
SUR1	SUR1	Persistent hyperinsulinemic hypoglycemia of infancy	Insulin hyposecretion
SUR2	SUR2	Dilated cardiomyopathy	Metabolic signaling
KCNE1	KCNE1	Autosomal-dominant LQTS with deafness	Heart action potential
		Autosomal-dominant LQTS	Heart action potential
KCNE2	KCNE2	LQTS	Heart action potential
CFTR	ABCC7	Cystic fibrosis	Epithelial transport defect
CIC-1	CLCN1	Myotonia (autosomal-recessive or -dominant)	Defective muscle repolarization
CIC-5	CLCN5	Dent disease	Defective endosome acidification
CIC-7	CLCN7	Osteopetrosis (recessive or dominant)	Defective bone resorption
CIC-Kb	CLCNKB	Bartter syndrome type III	Renal salt loss
RyR1	RyR1	Central core disease, malignant hyperthermia	Abnormal muscle activity
RyR2	RyR2	Catecholaminergic polymorphic tachycardia	Exercise-related cardiac arrhythmias

J Clin Invest. 2005;115(8)

and MORE...