

Review Article

*Mechanisms of Disease*FRANKLIN H. EPSTEIN, M.D., *Editor***ION CHANNELS — BASIC SCIENCE
AND CLINICAL DISEASE**MICHAEL J. ACKERMAN, M.D., PH.D.,
AND DAVID E. CLAPHAM, M.D., PH.D.

ION channels constitute a class of proteins that is ultimately responsible for generating and orchestrating the electrical signals passing through the thinking brain, the beating heart, and the contracting muscle. Using the methods of molecular biology and patch-clamp electrophysiology, investigators have recently cloned, expressed, and characterized the genes encoding many of these proteins. Ion-channel proteins are under intense scrutiny in an effort to determine their roles in pathophysiology and as potential targets for drugs.

Defective ion-channel proteins are responsible for cystic fibrosis,¹ the long-QT syndrome,² heritable hypertension (Liddle's syndrome),^{3,4} familial persistent hyperinsulinemic hypoglycemia of infancy,^{5,6} hereditary nephrolithiasis (Dent's disease), and a variety of hereditary myopathies,⁷⁻⁹ including generalized myotonia (Becker's disease), myotonia congenita (Thomson's disease), periodic paralyses, malignant hyperthermia, and central core storage disease (Table 1).

Elucidating the mechanisms of these diseases will benefit medicine as a whole, not just patients with a particular disease. For instance, although the inherited long-QT syndrome is not common, identifying the underlying defects in the KVLQT1 and HERG potassium channels and the SCN5A sodium channels may benefit the study of ventricular arrhythmias, which are responsible for 50,000 sudden deaths each year in the United States. Likewise, al-

though a defect in the recently cloned epithelial sodium channel (ENaC) is the basis of a very rare form of inherited hypertension (Liddle's syndrome, or pseudoaldosteronism), normal ENaC may serve as an alternative target in attempts to correct the physiologic defects created by the cystic fibrosis transmembrane regulator (CFTR), which is mutated in patients with cystic fibrosis, and work with ENaC may provide insight into the mechanism of essential hypertension.

This review focuses on ion channels as functioning physiologic proteins, sources of disease, and targets for therapy. We will discuss two prominent diseases caused by defects in ion-channel proteins, as well as two specific ion channels whose recent molecular identification raises new prospects for pharmacologic manipulation.

PHYSIOLOGY OF ION CHANNELS

Ion channels are macromolecular protein tunnels that span the lipid bilayer of the cell membrane. Approximately 30 percent of the energy expended by cells is used to maintain the gradient of sodium and potassium ions across the cell membrane. Ion channels use this stored energy much as a switch releases the electrical energy of a battery. They are more efficient than enzymes; small conformational changes change (gate) a single channel from closed to open, allowing up to 10 million ions to flow into or out of the cell each second. A few picoamps (10^{-12} A) of current are generated by the flow of highly selected ions each time the channel opens. Since ion channels are efficient, their numbers per cell are relatively low; a few thousand of a given type are usually sufficient. Ion channels are usually classified according to the type of ion they allow to pass — sodium, potassium, calcium, or chloride — although some are less selective. They may be gated by extracellular ligands, changes in transmembrane voltage, or intracellular second messengers.

Conductance is a measure of the ease with which ions flow through a material and is expressed as the charge per second per volt. The conductance of a single channel, γ , as distinguished from the membrane conductance (G) of all the channels in the cell, is defined as the ratio of the amplitude of current in a single channel (i) to the electromotive force, or voltage (V):

$$\gamma = \frac{i}{V}.$$

The direction in which ions move through a channel is governed by electrical and chemical concentra-

From the Department of Pediatrics and Adolescent Medicine, Mayo Foundation, Rochester, Minn. (M.J.A.); and the Department of Cardiology, Children's Hospital Medical Center, Department of Neurobiology, Harvard Medical School, Boston (D.E.C.). Address reprint requests to Dr. Ackerman at the Department of Pediatrics and Adolescent Medicine, Mayo Eugenio Litta Children's Hospital, Mayo Foundation, Rochester, MN 55905.

©1997, Massachusetts Medical Society.

TABLE 1. HERITABLE DISEASES OF ION CHANNELS.

DISEASE	MODE OF INHERITANCE*	ION-CHANNEL GENE (TYPE)	CHROMOSOME LOCATION	NO. OF AMINO ACIDS	COMMON MUTATIONST
Cystic fibrosis	AR	<i>CFTR</i> (epithelial chloride channel)	7q	1480	Δ F508 (70 percent of cases) and >450 other defined mutations
Familial persistent hyperinsulinemic hypoglycemia of infancy	AR	<i>SUR1</i> (subunit of ATP-sensitive pancreatic potassium channel)	11p15.1	1582	Truncation of NBD2 (nucleotide-binding domain 2)
Hypercalciuric nephrolithiasis (Dent's disease)	X-linked	<i>CLCN5</i> (renal chloride channel)	Xp11.22	746	1 intragenic deletion, 3 nonsense, 4 missense, 2 donor splice, 1 microdeletion
Liddle's syndrome (hereditary hypertension; pseudoaldosteronism)	AR	<i>ENaC</i> (epithelial sodium channel) α subunit β subunit γ subunit	12p 16p 16p	1420 640 649	R564stop, P616L, Y618H (all in β subunit); premature stop codon in β and γ subunits; C-terminal truncation
Long-QT syndrome (cardiac arrhythmia)	AD				
LQT1		<i>KVLQT1</i> (cardiac potassium channel)	11p15.5	581	1 intragenic deletion, 10 missense
LQT2		<i>HERG</i> (cardiac potassium channel)	7q35–36	1159	2 intragenic deletions, 5 missense
LQT3		<i>SCN5A</i> (cardiac sodium channel)	3p21–24	2016	Δ KPQ1505–1507, N1325S, R1644H
Myopathies					
Becker's generalized myotonia	AR	<i>CLCN1</i> (skeletal-muscle chloride channel)	7q35	988	D136G, F413C, R496S
Central core storage disease	?	<i>RYR1</i> (ryanodine calcium channel)	19q13.1	5032	R163C, I403M, Y522S, R2434H
Congenital myasthenic syndrome	?	<i>nAChR</i> (nicotinic acetylcholine receptor) ϵ subunit	17p	473	T264P, L269F
		α subunit (slow channel)	2q	457	G153S
Hyperkalemic periodic paralysis	AD	<i>SCN4A</i> (skeletal-muscle sodium channel)	17q23–25	1836	T698M, T704M, M1585V, M1592V
Hypokalemic periodic paralysis	AD	<i>CACNLIA3</i> (dihydropyridine-sensitive calcium channel)	1q31–32	1873	R528H, R1239H
Malignant hyperthermia	AD	<i>RYR1</i>	19q13.1	5032	G341R, G2433R
Masseter-muscle rigidity (succinylcholine-induced)	?	<i>SCN4A</i>	17q23–25	1836	G1306A
Myotonia levior	AD	<i>CLCN1</i>	7q35	988	Q552R
Paramyotonia congenita	AD	<i>SCN4A</i>	17q23–25	1836	V1293I, G1306V, T1313M, L1433R, R1448C, R1448H, V1589M
Pure myotonias (fluctuations, permanins, acetazolamide-responsive)	AD	<i>SCN4A</i>	17q23–25	1836	S804F, G1306A, G1306E, I1160V
Thomsen's myotonia congenita	AD	<i>CLCN1</i>	7q35	988	D136G, G230E, I290M, P480L

*AR denotes autosomal recessive, and AD autosomal dominant.

†Missense mutations are represented by the standard nomenclature (AxxxB, meaning that at amino acid position xxx, amino acid A has been replaced by amino acid B).

tion gradients. Ions flow passively through ion channels down a chemical gradient. Electrically charged ions also move in an electrical field, just as ions in solution flow to one of the poles of a battery connected to the solution. The point at which the chemical driving force and the electrical driving force are exactly balanced is called the Nernst potential (or reversal potential [E_{rev}]). Above or below this point of equilibrium, a particular species of ion flows in the direction of the dominant force. The net flow of electricity across a cell membrane is predictable given the concentrations of ions and the number, conductances, selectivities, and gating properties of the various ion channels.

Electrophysiologic concepts are simplified by recalling the Nernst potentials of the four major ions

across the plasma membrane of cells. These are approximated as follows: sodium, +70 mV; potassium, –98 mV; calcium, +150 mV; and chloride, –30 to –65 mV (Fig. 1). The positive and negative signs reflect the intracellular potential relative to a ground reference electrode. When only one type of ion channel opens, it drives the membrane potential of the entire cell toward the Nernst potential of that channel. Thus, if a single sodium-selective channel opens in a cell in which all other types of channels are closed, the transmembrane potential of the cell will become E_{Na} (+70 mV). If a single potassium channel opens, the cell's transmembrane potential will become E_K (–98 mV). Because cells have an abundance of open potassium channels, most cells' transmembrane potentials (at rest) are approximately

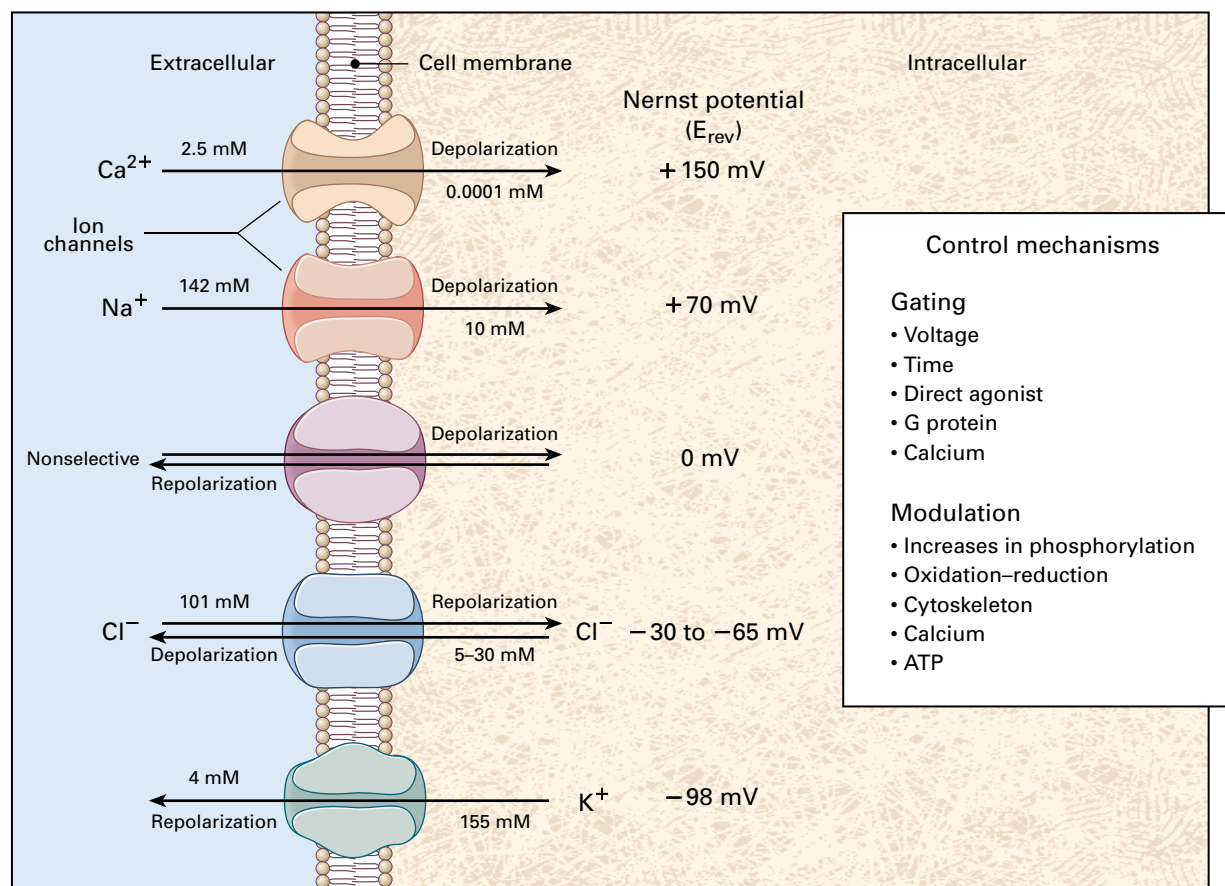


Figure 1. Physiology of Ion Channels.

Five major types of ion channels determine the transmembrane potential of a cell. The concentrations of the primary species of ions (sodium, calcium, chloride, and potassium) are millimolar. The ionic gradients across the membrane establish the Nernst potentials of the ion-selective channels (approximate values are shown). Under physiologic conditions, calcium and sodium ions flow into the cells and depolarize the membrane potential (that is, they drive the potential toward the values shown for E_{Ca} and E_{Na}), whereas potassium ions flow outward to repolarize the cell toward E_K . Nonselective channels and chloride channels drive the potential to intermediate voltages (0 mV and -30 to -65 mV, respectively).

-70 mV, near E_K . When more than one type of ion channel opens, each type “pulls” the transmembrane potential of the cell toward the Nernst potential of that channel. The overall transmembrane potential at a given moment is therefore determined by which channels are open and which are closed, and by the strength and numbers of the channels. A cell with one open sodium channel and one open potassium channel, each with the same conductance, will have a transmembrane potential halfway between E_{Na} (+70 mV) and E_K (-98 mV), or -14 mV. The result is the same when there are 1000 equal-conductance, open sodium and potassium channels. Ion channels are both potent and fast, and they are tightly controlled by the gating mechanisms of the cell (Fig. 1).

The modern way to see an ion channel in action is to use the patch-clamp technique. With this method,¹⁰ a pipette containing a small electrode is pressed against the cell membrane so that there is a tight seal between the pipette and the membrane (Fig. 2). In essence, the electrode isolates and captures all the ions flowing through the 1 to 3 μm^2 of membrane that is defined by the circular border of the pipette. In this fashion, the ionic current passing through a single ion channel can be collected and measured. Several geometric configurations can be used if a mechanically stable seal is formed. The current passing through the attached patch (cell-attached configuration), a detached patch (inside-out or outside-out configuration), or the whole cell can be measured, providing information about ion channels within the

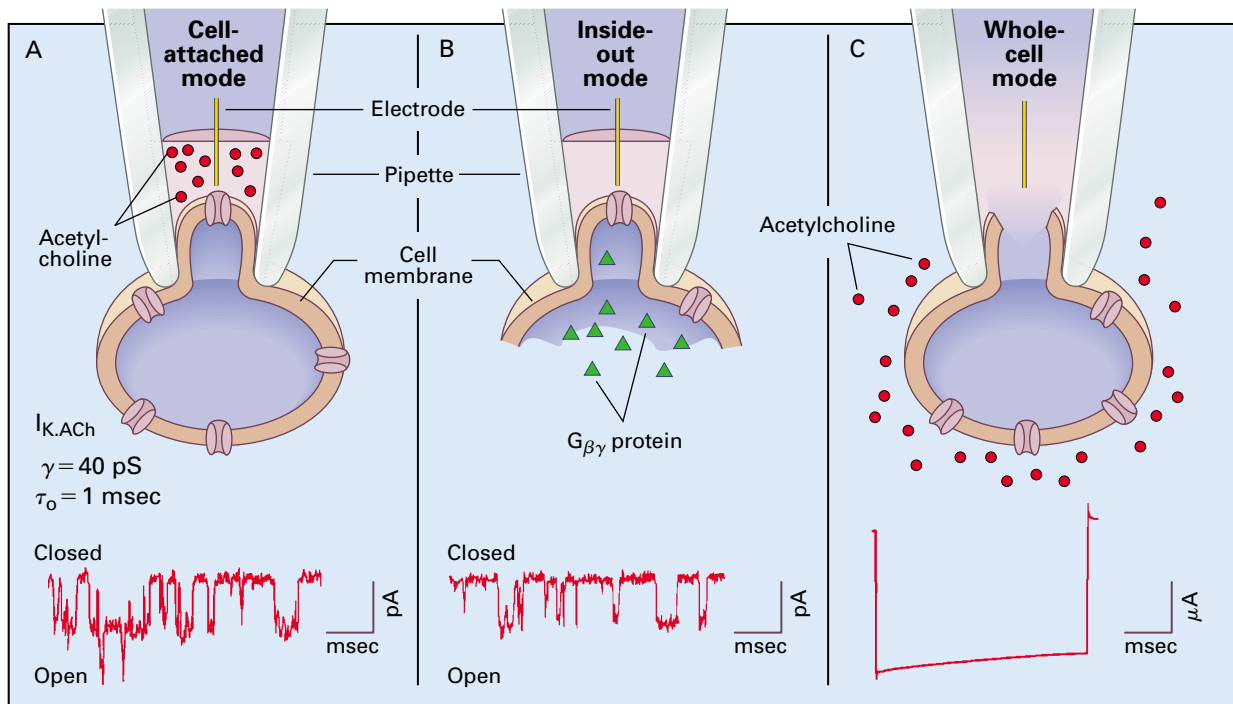


Figure 2. Patch-Clamp Measurement of Ion-Channel Activity, with the Acetylcholine-Sensitive Potassium Channel ($I_{K_{ACh}}$) Used as an Example.

In the “cell-attached” mode (Panel A), a pipette is pressed tightly against the cell membrane, suction is applied, and a tight seal is formed between the pipette and the membrane. The seal ensures that the pipette captures the current flowing through the channel. In the cell-attached membrane patch, the intracellular contents remain undisturbed. Here, acetylcholine in the pipette activates the $I_{K_{ACh}}$, which has a characteristic open time (τ_o) of 1 msec and a conductance (γ) of 40 pS.

In the inside-out mode (Panel B), after a cell-attached patch has been formed, the pipette is pulled away from the cell, ripping off a patch of membrane that forms an enclosed vesicle. The brief exposure to air disrupts only the free hemisphere of the membrane, leaving the formerly intracellular surface of the membrane exposed to the bath. Now the milieu of the intracellular surface of the channels can be altered. In this figure, adding purified $G_{\beta\gamma}$ protein to the exposed cytoplasmic surface activates the $I_{K_{ACh}}$.

In the whole-cell mode (Panel C), after a cell-attached patch has been formed, a pulse of suction disrupts the membrane circumscribed by the pipette, making the entire intracellular space accessible to the pipette. Instead of disrupting the patch by suction, a pore-forming molecule, such as amphotericin B or nystatin, can be incorporated into the intact patch, allowing ions access to the interior of the cell but maintaining a barrier to larger molecules. In this figure, the net current ($I_{K_{ACh}}$) after the application of acetylcholine is shown.

environment of the cell, in isolation from the rest of the cell, or over the entire cell, respectively.

MOLECULAR BLUEPRINTS OF ION CHANNELS

Many ion channels have been cloned by assaying their function directly with the use of oocytes from South African clawed toads (*Xenopus laevis*).¹¹ These oocytes are large enough to be injected with exogenous messenger RNA (mRNA) and are capable of synthesizing the resulting foreign proteins. In “expression cloning,” in vitro transcripts of mRNA from a complementary DNA (cDNA) library derived from a tissue known to be rich in a particular ion channel are injected into individual oocytes. Subsequently, the currents in the oocytes are meas-

ured by two-electrode voltage clamp techniques. The cDNA library is serially subdivided until injected mRNA from a single cDNA clone is isolated that confers the desired ion-channel activity. Moreover, mutant cDNA clones with engineered alterations in the primary structure of the protein can be expressed and the properties of the ion channel can be studied to determine which regions of the protein are critical for channel activation and inactivation, ion permeation, or drug interaction.

Most ion-channel proteins are composed of individual subunits or groups of subunits, with each subunit containing six hydrophobic transmembrane regions, S1 through S6 (Fig. 3A).¹³ The sodium and calcium channels comprise a single (α) subunit containing four repeats of the six transmembrane-span-

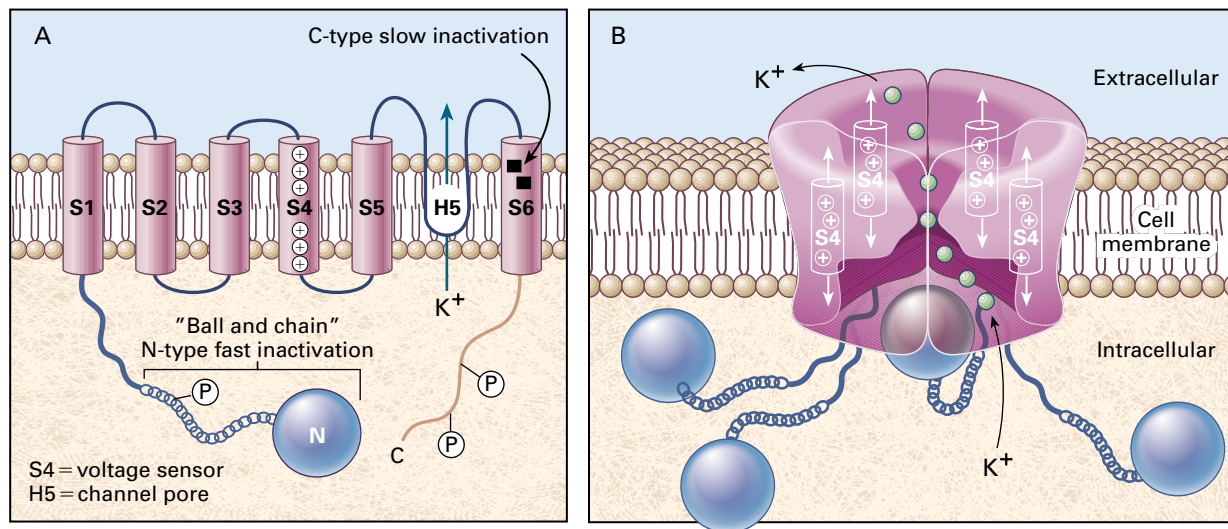


Figure 3. Structure of Ion Channels.

Panel A shows a subunit containing six transmembrane-spanning motifs, S1 through S6, that forms the core structure of sodium, calcium, and potassium channels. The “ball and chain” structure at the N-terminal of the protein is the region that participates in N-type “fast inactivation,” occluding the permeation pathway. The circles containing plus signs in S4, the voltage sensor, are positively charged lysine and arginine residues. Key residues lining the channel pore (H5) are found between S5 and S6. The genes for sodium and calcium channels encode a protein containing four repeats of this basic subunit, whereas the genes for voltage-activated potassium channels (Kv) encode a protein with only a single subunit. The genes for Kir channels encode a simple subunit structure containing only an H5 (pore) loop between two transmembrane-spanning segments. P denotes phosphorylation.

Panel B shows four such subunits assembled to form a potassium channel. Although no mammalian voltage-dependent ion-channel structure has been revealed at high resolution by x-ray crystallography, the dimensions of the pore region shown here were derived by using high-affinity scorpion toxins and their structures (as determined by nuclear magnetic resonance imaging) as molecular calipers.¹² The pore region appears to have wide intracellular and extracellular vestibules (approximately 2.8 to 3.4 nm wide and 0.4 to 0.8 nm deep) that lead to a constricted pore 0.9 to 1.4 nm in diameter at its entrance, tapering to a diameter of 0.4 to 0.5 nm at a depth of 0.5 to 0.7 nm from the vestibule.

ning motifs. Voltage-gated potassium channels (Kv; this nomenclature refers to **K** channel, voltage-dependent) are composed of four separate subunits, each containing a single six-transmembrane-spanning motif (Fig. 3B).¹⁴ The subunits are assembled to form the central pore in a process that also determines the basic properties of gating and permeation characteristic of the channel type. The peptide chain (H5 or P loop) between the membrane-spanning segments S5 and S6 projects into and lines the water-filled channel pore. Mutations in this region alter the permeation properties of the channel. S4 contains a cluster of positively charged amino acids (lysines and arginines) and is the major voltage sensor of the ion channel. Voltage-dependent “fast inactivation” of the channel is mediated by a tethered amino-terminal-blocking particle (the “ball and chain”) that swings in to occlude the permeation pathway.¹⁵

The most recently discovered family of ion-channel proteins is that containing the inwardly rectifying potassium-selective channels (Kir, for **K** channel, inward rectifier). These channels determine the transmembrane potential of most cells at rest, because

they are open in the steady state. Kir channels are known as inward rectifiers because they conduct current much more effectively into the cell than out of it. Despite this biophysical property of the Kir channels, the physiologically important current is the outward one that accompanies the efflux of potassium ions. The topography of Kir channels resembles that of Kv channels, but the subunits in Kir channels lack the S1 to S4 segments present in Kv channels.¹⁶ With only two transmembrane-spanning segments, Kir channels have a deceptively simple domain surrounding the conserved H5 pore. However, pore formation by different combinations of subunits, direct gating of G proteins, and interactions with other proteins adds considerable complexity to the behavior of the Kir channels.

HERITABLE DISEASES ASSOCIATED WITH ION-CHANNEL MUTATIONS

Cystic Fibrosis

One in 27 white persons carries a mutant *CFTR* gene, and 1 in 2500 to 3000 is born with cystic fi-

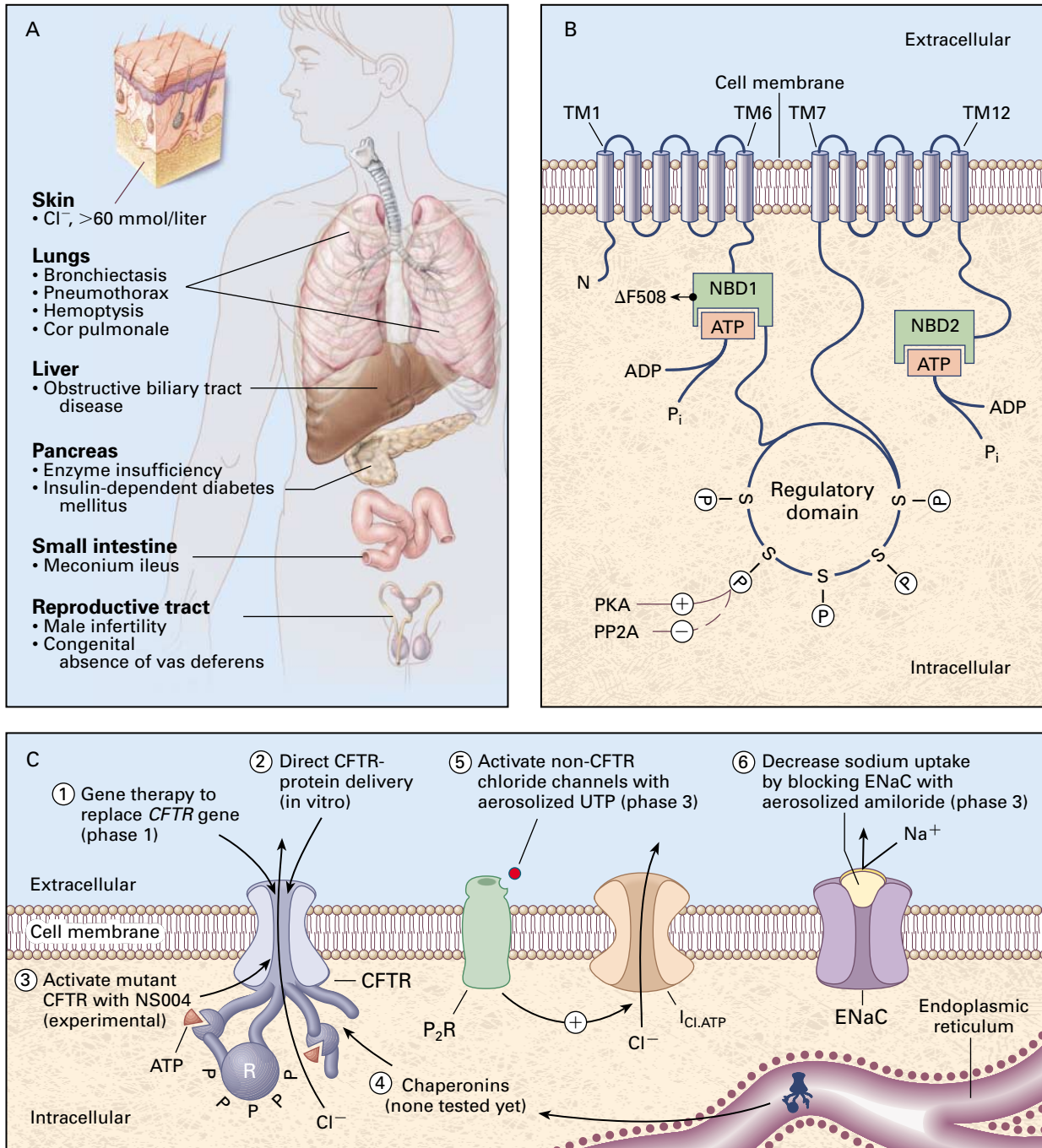


Figure 4. Cystic Fibrosis and CFTR.

In cystic fibrosis, defective apically located membrane chloride channels (CFTR) in a variety of epithelial cells do not allow the egress of chloride ions into the lumen. Control over epithelial sodium channels is also lost, increasing the reabsorption of sodium from the lumen. Thick, desiccated mucus results, which accounts for the primary clinical manifestations of the disease (Panel A).¹⁷ CFTR contains 12 transmembrane segments (TM1 through TM12, Panel B), several of which (TM1, TM6, and TM12) contribute to the chloride-channel pore. There are also two nucleotide-binding domains (NBD1 and NBD2) and a regulatory domain. The chloride channel is regulated by ATP binding and hydrolysis at the nucleotide-binding domains and by the phosphorylation (P) of serine residues (S) in the regulatory domain. The most common mutation in cystic fibrosis, found in more than 70 percent of cases, involves a deletion of a single amino acid (phenylalanine) in NBD1 ($\Delta F508$). PKA denotes protein kinase A, PP2A protein phosphatase 2A, and P_i inorganic phosphorus.

Molecular strategies to treat cystic fibrosis (Panel C) include replacing the mutant chloride channel by gene therapy (1) or protein delivery (2); improving the secretion from the existing mutant CFTR protein with CFTR-channel openers, such as NS004 (3) or "chaperonins" for $\Delta F508$ in the endoplasmic reticulum (4); bypassing the CFTR defect by activating other chloride channels with aerosolized uridine triphosphate (UTP) (5); and blocking the increased reabsorption of sodium through epithelial sodium channels (ENaC) with aerosolized amiloride (6). The investigational stages of these strategies are given in parentheses. P_2R denotes type-2 purinergic receptor, and R regulatory domain.

brosis (among blacks the incidence is 1 in 14,000, and among Asians it is 1 in 90,000). The manifestations of cystic fibrosis stem from a defect in a chloride-channel protein, CFTR, that does not allow chloride to cross the cell membrane (Fig. 4A).¹⁷ The *CFTR* gene encodes a chloride channel that is activated by the binding of ATP to its nucleotide-binding domains and by the phosphorylation of key serine residues in its regulatory domain; the phosphorylation is mediated by cyclic AMP and protein kinase A (Fig. 4B).¹⁸⁻²¹ *CFTR* also appears to regulate the absorption of sodium through ENaC, the epithelial sodium channel, and to activate other "outwardly rectifying" chloride channels.

More than 450 mutations have been identified in *CFTR*, which contains 1480 amino acids. A deletion of phenylalanine at position 508 ($\Delta F508$) accounts for more than 70 percent of cases of cystic fibrosis and is associated with severe pancreatic insufficiency and pulmonary disease. The $\Delta F508$ CFTR channel conducts chloride reasonably well when it is incorporated into a cell membrane, but because of improper folding the mutant protein becomes stuck in intracellular organelles and is not inserted into the cell membrane.²² The majority of mutant CFTR proteins are processed abnormally, like the $\Delta F508$ mutant, but some mutations cause either defects in regulation or defective conduction through the CFTR channel.²³

Different *CFTR* genotypes may provide opportunities to develop unique therapeutic strategies. For instance, misfolded mutants could be escorted to the membrane by yet-to-be-invented "chaperonins," whereas the action of poorly conducting mutant proteins may be enhanced by CFTR-specific channel openers. Molecular genotypes are correlated with the severity of pancreatic insufficiency, but not with the severity of pulmonary disease.²⁴ An exception is the A455E *CFTR* mutant (in which alanine is changed to glutamic acid at position 455), which has been associated with mild lung disease and accounts for 3 per-

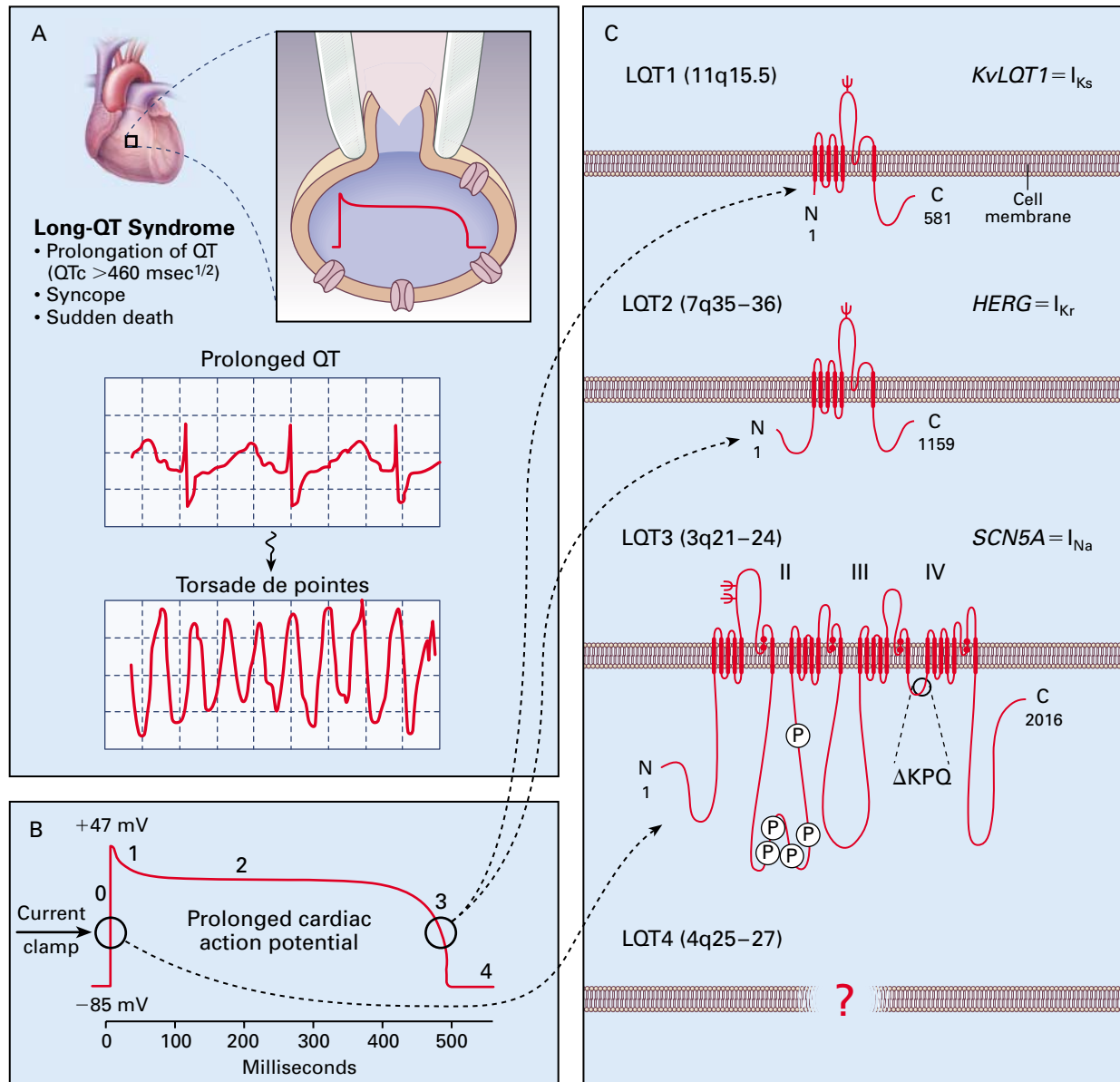
cent of cases of cystic fibrosis in the Netherlands.²⁵ In addition, a primarily genital phenotype of cystic fibrosis that involves the congenital bilateral absence of the vas deferens has been described in otherwise healthy males who are heterozygous for the $\Delta F508$ *CFTR* mutation.²⁶

Pulmonary disease accounts for over 90 percent of mortality from cystic fibrosis, and therefore treatment is mostly directed at ameliorating lung disease. Therapy includes antibiotics to eliminate common respiratory pathogens (*Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus*), recombinant human DNase to decrease the viscosity of secretions, and antiinflammatory drugs to reduce the inflammatory response.²⁷ The recognition of the ion-channel defect in cystic fibrosis has led to novel approaches, such as replacing the defective channel gene by gene transfer with either viral carriers such as adeno-associated virus or nonviral carriers such as cationic liposomes (now in phase I trials)²⁸; stimulating the activation of reduced numbers of functional ion channels with a CFTR-channel opener (NS004, a substituted benzimidazolone)²⁹; mobilizing mutant CFTR proteins to the cell surface^{30,31}; counteracting the defect in chloride efflux by blocking the influx of sodium with amiloride^{32,33}; and bypassing CFTR-mediated conductance of chloride by activating other chloride channels, such as $I_{Cl,Swell}$, $I_{Cl,Ca}$, and $I_{Cl,ATP}$ ³⁴ (Fig. 4C).

Long-QT Syndrome

A more detailed understanding of cardiac arrhythmogenesis is emerging as the workings of most of the types of ion channels underlying cardiac action potentials are elucidated.^{35,36} The various long-QT syndromes are the first genetically determined arrhythmias known to be caused at the molecular level by defects in myocardial ion channels (Fig. 5).

The congenital long-QT syndrome has an estimated incidence of 1 in 10,000 to 1 in 15,000. It is characterized by prolongation of the QT interval



corrected for heart rate (QTc) to more than 460 $\text{msec}^{1/2}$, and it is an important but relatively rare cause of sudden death in children and young adults (Fig. 5A). The majority (two thirds) of persons with the long-QT syndrome are identified during routine electrocardiographic screening or after the evaluation of a primary relative who is affected. Approximately one third of subjects are identified during a clinical evaluation for unexplained syncope or cardiac or respiratory arrest. These subjects are at an annual risk of 5 percent for an abrupt syncopal episode. Without treatment, symptomatic subjects have

a 10-year mortality rate approaching 50 percent. Often the arrhythmia is a torsade de pointes polymorphic ventricular tachycardia, typically triggered by adrenergic arousal.³⁷ Genetic origins were suggested for this syndrome by descriptions both of the autosomal recessive form associated with congenital deafness (Jervell and Lange-Nielsen syndrome)³⁸ and of an isolated autosomal dominant form (Romano-Ward syndrome).^{39,40}

Substantial progress has been made toward elucidating the molecular basis of the most common inherited subtypes of the long-QT syndrome (Fig.

Figure 5. The Long-QT Syndrome.

A person with the long-QT syndrome may have unexplained syncope, seizures, or sudden death (Panel A). More likely, the person will be asymptomatic and identified by electrocardiographic screening during a routine evaluation or the screening of a primary relative who is symptomatic. The strict electrocardiographic definition of a prolonged QT interval varies according to age and sex, but generally a QT interval corrected for heart rate (QTc) greater than 460 msec^{1/2} is considered abnormal. According to Bazett's formula, the QTc is calculated by dividing the QT interval by the square root of the R-R interval. In patients with the long-QT syndrome, the T-wave morphology is often abnormal. This base-line rhythm can degenerate into a polymorphic ventricular tachycardia, classically a torsade de pointes, as shown here, after a stimulus that is not precisely understood but that often takes the form of adrenergic arousal.

The prolonged QT interval as measured on the electrocardiogram results from an increased duration of the cardiac action potential (Panel B). The ventricular action potential is maintained at a resting membrane potential (approximately -85 mV) by inwardly rectifying potassium currents (I_{K1} , phase 4). Once an excitatory stimulus depolarizes the cell beyond a threshold voltage (for example, -60 mV), sodium currents are activated that quickly depolarize the cell (I_{Na} , phase 0). These sodium channels are rapidly inactivated, allowing transient potassium currents to return the action potential to the plateau voltage (phase 1). The plateau lasts about 300 msec and provides time for the heart to contract. The plateau is maintained by the competition between outward-moving potassium currents and inward-moving calcium currents (phase 2). Progressive inactivation of calcium currents and increasing activation of potassium currents repolarize the cell to the resting membrane potential (phase 3).

On a molecular basis, the autosomal dominant LQT1 and LQT2 are caused by defects in potassium-channel genes (*KvLQT1* and *HERG*) involved in phase 3 repolarization (Panel C). LQT3 is caused by a defective sodium-channel gene, *SCN5A*. A common *SCN5A* mutation in families with LQT3 involves a deletion of three amino acids (Δ KPQ) in the III-IV cytoplasmic linker loop, which is known to regulate inactivation. The mutant sodium channel fails to become completely inactivated, resulting in sustained depolarization and prolonging the cardiac action potential. The linear topology of the proteins responsible for LQT1, LQT2, and LQT3 is shown, with the amino acids numbered beginning with the N-terminal — a total of 581, 1159, and 2016 amino acids, respectively. The chromosomal locations for these genes are shown in parentheses.

5C).^{2,36} Recent studies of 16 families with chromosome-II-linked long-QT syndrome type 1 (LQT1) implicated KvLQT1, a 581-amino-acid protein with sequence homology to voltage-activated potassium channels.⁴¹ One intragenic deletion and 10 missense mutations were identified. The combination of the KvLQT1 and I_{Ks} subunits (the latter of which contains 130 amino acids, also known as minK) appears to reconstitute the cardiac I_{Ks} current.^{42,43} I_{Ks} ("s" denotes "slow") is one of the principal delayed-rectifying potassium currents responsible for phase 3 repolarization in the heart (Fig. 5B). LQT1 may account for half the incidence of the long-QT syndrome in its autosomal dominant forms.

Mutations in a second potassium channel, the human ether-a-go-go-related gene (*HERG*), have been identified in subjects with the long-QT syndrome type 2 (LQT2), which has been linked^{44,45} to chromosome 7q35-36. *HERG* is responsible for the other major potassium current (I_{Kr} ["r" denotes "rapid"]) that participates in phase 3 repolarization. It is a unique voltage-gated potassium channel; its secondary structure is that of a typical voltage-activated (Kv) potassium channel (Fig. 3A), but it behaves more like an inwardly rectifying (Kir) potassium channel.⁴⁶ The role of *HERG* in normal cardiac physiology appears to be to suppress depolarizations that lead to premature firing. Subjects with LQT2 may therefore be prone to sudden cardiac death, because they lack protection from arrhythmogenic afterbeats. Class III antiarrhythmic drugs block *HERG* channels. In addition, antihistamines such as terfenadine and antifungal drugs such as ketoconazole have been implicated in acquired cases of the long-QT syn-

drome because of their ability to block I_{Kr} (*HERG*-mediated) current.

The third subtype of the long-QT syndrome (LQT3) has been linked to the gene for the cardiac sodium channel (*SCN5A*) on chromosome 3p21-24.⁴⁷ This channel is responsible for the fast upstroke of the cardiac action potential (phase 0, Fig. 5B), which ensures contractile synchrony by causing the potential to spread rapidly throughout the heart muscle. A deletion of three amino acids, Δ KPQ1505-1507, in a region thought to control rapid inactivation has been demonstrated in LQT3-linked families. The mutant sodium channel fails to inactivate completely, resulting in reopenings of the channel and long-lasting bursts of channel activity.^{48,49} The resulting prolonged inward current lengthens the action potential (and thus the QT interval). Finally, a fourth heritable type of long-QT syndrome (LQT4) has been linked to chromosome 4q25-27. Its causative gene has not been identified, although a gene encoding a calcium-calmodulin kinase has been proposed.⁵⁰

Current therapies for the long-QT syndrome include β -adrenergic-antagonist drugs, cardiac pacing, and left cervicothoracic sympathectomy. The majority of families with heritable long-QT syndrome have type 1, 2, or 3, offering the prospect of genetic screening and directed antiarrhythmic therapy. Theoretically, therapies that augment potassium-channel activity may be used in subjects with potassium-channel defects (LQT1 and LQT2),⁵¹ and those with sodium channel-linked defects (LQT3) may benefit from drugs that decrease sodium-channel activation (such as mexiletine).⁵²

TABLE 2. ION CHANNELS AND DRUGS THAT AFFECT THEM.

Calcium channels
Antianginal drugs (amlodipine, diltiazem, felodipine, nifedipine, verapamil)
Antihypertensive drugs (amlodipine, diltiazem, felodipine, isradipine, nifedipine, verapamil)
Class IV antiarrhythmic drugs (diltiazem, verapamil)
Sodium channels
Anticonvulsant drugs (carbamazepine, phenytoin, valproic acid)
Class I antiarrhythmic drugs
IA (disopyramide, procainamide, quinidine)
IB (lidocaine, mexiletine, phenytoin, tocainide)
IC (encainide, flecainide, propafenone)
Diuretic drugs (amiloride)
Local anesthetic drugs (bupivacaine, cocaine, lidocaine, mepivacaine, tetracaine)
Chloride channels
Anticonvulsant drugs (clonazepam, phenobarbital)
Hypnotic or anxiolytic drugs (clonazepam, diazepam, lorazepam)
Muscle-relaxant drugs (diazepam)
Potassium channels
Antidiabetic drugs (glipizide, glyburide, tolazamide)
Antihypertensive drugs (diazoxide, minoxidil)
Class III antiarrhythmic drugs (amiodarone, clofilium, dofetilide, N-acetylprocainamide, sotalol)
Drugs that open potassium channels (adenosine, aprikalim, levcromakalim, nicorandil, pinacidil)

TARGETING ION CHANNELS

Drugs that target ion channels include calcium-channel blockers (used in patients with hypertension), potassium-channel blockers (used in patients with non-insulin-dependent diabetes mellitus), some diuretics and antiseizure medications, and essentially all antiarrhythmic drugs (Table 2). Recent progress in the basic understanding of the ATP-sensitive potassium channel ($I_{K,ATP}$) and the G-protein-activated potassium channel ($I_{K,ACh}$) shows the opportunities for drug design.

The ATP-Sensitive Potassium Channel

The ATP-sensitive potassium channel $I_{K,ATP}$ is a multimeric complex of inwardly rectifying potassium-channel subunits (Kir 6.2 = $K_{ATP-\alpha}$) and the sulfonylurea receptor ($SUR1 = K_{ATP-\beta}$).^{53,54} The genes for both are located on chromosome 11p15.1. *SUR1* binds sulfonylurea drugs. Mutations in the *SUR1* gene are responsible for persistent hyperinsulinemic hypoglycemia of infancy.^{5,6} Kir 6.2 is an inwardly rectifying potassium channel. Like other such channels, it has two transmembrane-spanning segments surrounding a pore domain. Expression of both *SUR1* and Kir 6.2 results in a potassium channel that is sensitive to intracellular ATP, inhibited by sulfonylurea drugs, and activated by diazoxide, as is consistent with the known properties of $I_{K,ATP}$ channels in pancreatic beta cells. The cardiac sulfonylurea receptor, *SUR2*, has a lower affinity for sulfonylurea drugs than does *SUR1*, and it may form the cardiac $I_{K,ATP}$ channel by combining with a homologue in the Kir 6 family.

The $I_{K,ATP}$ current has been characterized in heart, skeletal muscle, pituitary, brain, smooth muscle, and pancreas.⁵⁵ In the pancreas, it plays a major part in regulating glucose homeostasis and the secretion of insulin.⁵⁶ Rising plasma glucose concentrations increase intracellular concentrations of ATP in islet beta cells, which in turn inhibit $I_{K,ATP}$ channels. As these potassium channels close, the cell's membrane potential depolarizes away from E_K and enters the range in which voltage-dependent calcium channels are activated. The resulting influx of calcium triggers insulin secretion. As plasma glucose concentrations decline, intracellular concentrations of ATP decrease and $I_{K,ATP}$ channels become more active, hyperpolarizing the cell, closing the calcium channels, and terminating the secretion of insulin. Oral hypoglycemic drugs (such as glyburide) bind to the sulfonylurea receptor to inhibit the activity of $I_{K,ATP}$ and promote the secretion of insulin.⁵⁷

Drugs that open potassium channels include nicorandil, pinacidil, aprikalim, levcromakalim, and diazoxide. In vascular smooth muscle these drugs open $I_{K,ATP}$ channels, hyperpolarize cell membranes, and reduce calcium-channel activity, thus decreasing vascular tone. The drugs are therefore potentially cardioprotective and may provide novel therapeutic approaches in patients with cardiac disease or hypertension.⁵⁸⁻⁶⁰ The subtype specificity of sulfonylurea receptors (*SUR1* in the pancreas and *SUR2* in the heart) may be exploited to develop more specific drugs.

The G-Protein-Activated Potassium Channel

Vagally secreted acetylcholine binds to cardiac muscarinic type 2 receptors. Activating these G-protein-linked receptors slows the heart rate by opening a potassium-selective ion channel ($I_{K,ACh}$) composed of G-protein-activated inwardly rectifying Kir subunits. In turn, $I_{K,ACh}$ decreases spontaneous depolarization (pacemaker activity) in the sinus node and slows the velocity of conduction in the atrioventricular node.^{61,62} Muscarinic stimulation of $I_{K,ACh}$ can terminate arrhythmias, particularly supraventricular tachycardias, providing the basis for carotid massage and other vagotonic maneuvers.³⁵ Another G-protein-linked receptor agonist, adenosine, activates the same cascade in atria and pacemaking cells through type 1 purinergic receptors. Because muscarinic stimulation has many systemic effects, adenosine has become a favored treatment for supraventricular tachycardia; it is also useful in determining the underlying arrhythmic mechanism (usually a reentrant one).⁶³

The molecular mechanism of the activation of $I_{K,ACh}$ ($I_{K,G}$) is known.⁶⁴ Cardiac $I_{K,ACh}$ is a heteromultimer of two inwardly rectifying potassium-channel subunits, GIRK1 (Kir 3.1) and GIRK4 (Kir 3.4),⁶⁵ and it is activated after the direct binding of the $\beta\gamma$ subunits of G protein ($G_{\beta\gamma}$).⁶⁶ Similar $I_{K,ACh}$ currents and GIRK proteins are present in the brain.

Neuronal GIRK channel proteins are formed by heteromultimers of GIRK1 and GIRK2 in the cerebellum, midbrain, and cortex. In homozygous *weaver* mice that have profound ataxia due to the loss of granule-cell neurons during cerebellar development, a single point mutation in the highly conserved pore region of GIRK2 results in granule-cell death and failure of migration. The mutated *weaver*-mouse channel loses its potassium-ion selectivity and sensitivity to $G_{\beta\gamma}$, converting a regulated repolarizing potassium channel into a constitutively active, non-selective depolarizing channel and resulting in increased excitotoxic cell death.⁶⁷

CONCLUSIONS

A growing number of heritable diseases are known to be caused by ion-channel mutations. Chloride-channel defects underlie cystic fibrosis, certain myotonias, and heritable nephrolithiasis. Mutant sodium channels give rise to the long-QT syndrome and other myotonias, potassium-channel malfunction increases susceptibility to arrhythmias, and calcium-channel mutations can result in hypokalemic periodic paralysis, malignant hyperthermia, and central core storage disease. Identifying the structural framework of the major ion-channel proteins and resolving the precise relations between structure and function should make it possible to develop new therapies for patients with these disorders.

We are indebted to David A. Factor for his assistance with the figures.

REFERENCES

- Davis PB, Drumm M, Konstan MW. Cystic fibrosis. *Am J Respir Crit Care Med* 1996;154:1229-56.
- Keating MT. The long QT syndrome: a review of recent molecular genetic and physiologic discoveries. *Medicine (Baltimore)* 1996;75:1-5.
- Shimkets RA, Warnock DG, Bositis CM, et al. Liddle's syndrome: heritable human hypertension caused by mutations in the β subunit of the epithelial sodium channel. *Cell* 1994;79:407-14.
- Snyder PM, Price MP, McDonald FJ, et al. Mechanism by which Liddle's syndrome mutations increase activity of a human epithelial Na^+ channel. *Cell* 1995;83:969-78.
- Thomas PM, Cote GJ, Wohlk N, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 1995;268:426-9.
- Dunne MJ, Kane C, Shepherd RM, et al. Familial persistent hyperinsulinemic hypoglycemia of infancy and mutations in the sulfonylurea receptor. *N Engl J Med* 1997;336:703-6.
- Hudson AJ, Ebers GC, Bulman DE. The skeletal muscle sodium and chloride channel diseases. *Brain* 1995;118:547-63.
- Koch MC, Steinmeyer K, Lorenz C, et al. The skeletal muscle chloride channel in dominant and recessive human myotonia. *Science* 1992;257:797-800.
- Ogawa Y. Role of ryanodine receptors. *Crit Rev Biochem Mol Biol* 1994;29:229-74.
- Hamill OR, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 1981;391:85-100.
- Soreq H. The biosynthesis of biologically active proteins in mRNA-microinjected *Xenopus* oocytes. *CRC Crit Rev Biochem* 1985;18:199-238.
- Aiyar J, Withka JM, Rizzi JP, et al. Topology of the pore-region of a K^+ channel revealed by the NMR-derived structures of scorpion toxins. *Neuron* 1995;15:1169-81.
- Catterall WA. Structure and function of voltage-sensitive ion channels. *Science* 1988;242:50-61.
- Jan LY, Jan YN. Tracing the roots of ion channels. *Cell* 1992;69:715-8.
- Hoshi T, Zagotta WN, Aldrich RW. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science* 1990;250:533-8.
- Jan LY, Jan YN. Potassium channels and their evolving gates. *Nature* 1994;371:119-22.
- Netter FH. Respiratory system. Ciba Collection Med Illustrations 1980;7:154.
- Rommens JM, Iannuzzi MC, Kerem B-S, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989;245:1059-65.
- Riordan JR, Rommens JM, Kerem BS, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245:1066-73. [Erratum, *Science* 1989;245:1437.]
- Kerem B-S, Rommens JM, Buchanan JA, et al. Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989;245:1073-80.
- Collins FS. Cystic fibrosis: molecular biology and therapeutic implications. *Science* 1992;256:774-9.
- Cheng SH, Gregory RJ, Marshall J, et al. Defective intracellular transport and processing of CFTR is the molecular basis for most cystic fibrosis. *Cell* 1990;63:827-34.
- Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993;73:1251-4.
- Dean M, Santis G. Heterogeneity in the severity of cystic fibrosis and the role of CFTR gene mutations. *Hum Genet* 1994;93:364-8.
- Gan K-H, Veeze HJ, van den Ouweland AMW, et al. A cystic fibrosis mutation associated with mild lung disease. *N Engl J Med* 1995;333:95-9.
- Anguiano A, Oates RD, Amos JA, et al. Congenital bilateral absence of the vas deferens: a primarily genital form of cystic fibrosis. *JAMA* 1992;267:1794-7.
- Ramsey BW. Management of pulmonary disease in patients with cystic fibrosis. *N Engl J Med* 1996;335:179-88.
- Alton EW, Geddes DM. Gene therapy for cystic fibrosis: a clinical perspective. *Gene Ther* 1995;2:88-95.
- Gribkoff VK, Champigny G, Barby P, Dworetzky SI, Meanwell NA, Lazdunski M. The substituted benzimidazolone NS004 is an opener of the cystic fibrosis chloride channel. *J Biol Chem* 1994;269:10983-6.
- Howard M, Frizzell RA, Bedwell DM. Aminoglycoside antibiotics restore CFTR function by overcoming premature stop mutations. *Nat Med* 1996;2:467-9.
- Sato S, Ward CL, Krouse ME, Wine JJ, Kopito RR. Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation. *J Biol Chem* 1996;271:635-8.
- Voilley N, Lingueglia E, Champigny G, et al. The lung amiloride-sensitive Na^+ channel: biophysical properties, pharmacology, ontogenesis, and molecular cloning. *Proc Natl Acad Sci U S A* 1994;91:247-51.
- Knowles MR, Olivier KN, Hohnke KW, Robinson J, Bennett WD, Boucher RC. Pharmacologic treatment of abnormal ion transport in the airway epithelium in cystic fibrosis. *Chest* 1995;107:Suppl:71S-76S.
- Knowles MR, Clarke LL, Boucher RC. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *N Engl J Med* 1991;325:533-8.
- Ackerman MJ, Clapham DE. Normal cardiac electrophysiology: understanding the action potential in the human heart. In: Chien K, ed. Molecular basis of heart disease: a companion to Braunwald's heart disease (in press).
- Roden DM, Lazzara R, Rosen M, Schwartz PJ, Towbin J, Vincent GM. Multiple mechanisms in the long-QT syndrome: current knowledge, gaps, and future directions. *Circulation* 1996;94:1996-2012.
- Moss AJ, Robinson JL. Long QT syndrome. *Heart Dis Stroke* 1992;1:309-14.
- Jervell A, Lange-Nielsen F. Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval, and sudden death. *Am Heart J* 1957;54:59-68.
- Romano C, Gemme G, Pongiglione R. Aritmie cardiache rare dell'età pediatrica. *Clin Pediatr* 1963;45:656-83.
- Ward OC. A new familial cardiac syndrome in children. *J Ir Med Assoc* 1964;54:103-6.
- Wang Q, Curran ME, Splawski I, et al. Positional cloning of a novel potassium channel gene: *KVLQT1* mutations cause cardiac arrhythmias. *Nat Genet* 1996;12:17-23.
- Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. K_vLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature* 1996;384:78-80.
- Sanguinetti MC, Curran ME, Zou A, et al. Coassembly of K_vLQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature* 1996;384:80-3.

44. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 1995;80:795-803.
45. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: *HERG* encodes the I_{Kr} potassium channel. *Cell* 1995;81:299-307.
46. Smith PL, Baukrowitz T, Yellen G. The inward rectification mechanism of the HERG cardiac potassium channel. *Nature* 1996;379:833-6.
47. Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 1995;80:805-11.
48. Bennett PB, Yazawa K, Makita N, George AL Jr. Molecular mechanism for an inherited cardiac arrhythmia. *Nature* 1995;376:683-5.
49. Dumaine R, Wang Q, Keating MT, et al. Multiple mechanisms of Na^+ channel-linked long-QT syndrome. *Circ Res* 1996;78:916-24.
50. Schott J-J, Charpentier F, Peltier S, et al. Mapping of a gene for long QT syndrome to chromosome 4q25-27. *Am J Hum Genet* 1995;57:1114-22.
51. Compton SJ, Lux RL, Ramsey MR, et al. Genetically defined therapy of inherited long-QT syndrome: correction of abnormal repolarization by potassium. *Circulation* 1996;94:1018-22.
52. Schwartz PJ, Priori SG, Locati EH, et al. Long QT syndrome patients with mutations of the SCN5A and HERG genes have differential responses to Na^+ channel blockade and to increases in heart rate: implications for gene-specific therapy. *Circulation* 1995;92:3381-6.
53. Inagaki N, Gonoi T, Clement JP IV, et al. Reconstitution of I_{KATP} : an inward rectifier subunit plus the sulfonylurea receptor. *Science* 1995;270:1166-70.
54. Aguilar-Bryan L, Nichols CG, Wechsler SW, et al. Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 1995;268:423-6.
55. Terzic A, Jahangir A, Kurachi Y. Cardiac ATP-sensitive K^+ channels: regulation by intracellular nucleotides and K^+ channel-opening drugs. *Am J Physiol* 1995;269:C525-C545.
56. Ashcroft FM. Adenosine 5'-triphosphate-sensitive potassium channels. *Annu Rev Neurosci* 1988;11:97-118.
57. Ashcroft SJ, Ashcroft FM. The sulfonylurea receptor. *Biochim Biophys Acta* 1992;1175:45-59.
58. Lopez JR, Jahangir R, Jahangir A, Shen WK, Terzic A. Potassium channel openers prevent potassium-induced calcium loading of cardiac cells: possible implications in cardioplegia. *J Thorac Cardiovasc Surg* 1996;112:820-31.
59. Challinor-Rogers JL, McPherson GA. Potassium channel openers and other regulators of $KATP$ channels. *Clin Exp Pharmacol Physiol* 1994;21:583-97.
60. Haeusler G, Lues I. Therapeutic potential of potassium channel activators in coronary heart disease. *Eur Heart J* 1994;15:Suppl C:82-8.
61. Shen W-K, Kurachi Y. Mechanisms of adenosine-mediated actions on cellular and clinical cardiac electrophysiology. *Mayo Clin Proc* 1995;70:274-91.
62. Pelleg A, Belardinelli L. Cardiac electrophysiology and pharmacology of adenosine: basic and clinical aspects. *Cardiovasc Res* 1993;27:54-61.
63. Malcolm AD, Garratt CJ, Camm AJ. The therapeutic and diagnostic cardiac electrophysiological uses of adenosine. *Cardiovasc Drugs Ther* 1993;7:139-47.
64. Wickman K, Clapham DE. Ion channel regulation by G proteins. *Physiol Rev* 1995;75:865-85.
65. Krapivinsky G, Gordon EA, Wickman K, Velimirović B, Krapivinsky L, Clapham DE. The G-protein-gated atrial K^+ channel I_{KACH} is a heteromultimer of two inwardly rectifying K^+ -channel proteins. *Nature* 1995;374:135-41.
66. Krapivinsky G, Krapivinsky L, Wickman K, Clapham DE. $G_{\beta\gamma}$ binds directly to the G protein-gated K^+ channel, I_{KACH} . *J Biol Chem* 1995;270:29059-62.
67. Navarro B, Kennedy ME, Velimirović B, Bhat D, Peterson AS, Clapham DE. Nonselective and $G_{\beta\gamma}$ -insensitive *weaver* K^+ channels. *Science* 1996;272:1950-3.



Lake District, Northern Italy

KEITH J. QUINTON, M.D.

CORRECTION

Ion Channels — Basic Science and Clinical Disease

Ion Channels — Basic Science and Clinical Disease . On page 1582, in Figure 5C, the label for the first gene defect should have read “LQT1 (11p15.5)” and for the third gene defect “LQT3 (3p21–24),” not “LQT1 (11q15.5)” and “LQT3 (3q21–24),” as printed. We regret the errors.