Ion Channels: Structural Basis for Function and Disease

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Ion channels are ubiquitous proteins that mediate nervous and muscular function, rapid transmembrane signaling events, and ionic and fluid balance. The cloning of genes encoding ion channels has led to major strides in understanding the mechanistic basis for their function. These advances have shed light on the role of ion channels in normal physiology, clarified the molecular basis for an expanding number of diseases, and offered new direction to the development of rational therapeutic interventions.

Ion channels reside in the membranes of all cells and control their electrical activity.[1] These proteins underlie subtle biological events such as the response of a single rod cell to a beam of light, the activation of a T cell by its antigen, and the fast block to polyspermy of a fertilized ovum. They also mediate spectacular events like heart beats, systemic fluid and electrolyte homeostasis, and our memories. Over the past 40 years, increasingly sensitive electrophysiological methods have allowed characterization of channel function at remarkable resolution-single channel molecules can be observed in real time (Fig 1A). However, it is only in the last few years with the application of molecular genetic technology that we have begun to discern the structural basis for ion channel function. Cloning of genes for channels and their regulatory subunits has revealed amino acid sequences and made feasible studies of structure and function that were impossible with native tissues alone. Recent advances in our understanding of the mechanistic underpinnings for normal function of sodium (Na^+) and potassium (K^+) channels are discussed in this article. These insights have clarified the etiology for an expanding number of disease states and allow disorders mediated by ion channels to be divided into two broad mechanistic groups: those resulting from loss of channel function and those consequent to gain of channel function. Three exemplary pathophysiological correlates are examined, Long QT syndrome, Liddle's syndrome and pseudohypoaldosteronism type 1 (leading to life-threatening cardiac rhythm disturbances, systemic hypertension, and hypotension, respectively). Future challenges for ion channel research are considered.

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Ion Channel Function: Gating and Ion Selectivity

Ion channels perform two operations. First, they open and close in response to specific stimuli; this process is called "channel gating." Second, they catalyze the flux of specific ions across the membrane, an activity referred to as "ion selective conduction." Some channels are ligand-gated. Thus, binding of cGMP to a cyclic-nucleotide gated (CNG) channel in the retina leads to a conformational change in channel structure and a shift from a closed, nonconducting channel state to an open state that is maintained as long as cGMP is bound. Other ion channels are voltage-gated. The single Na^+ channel shown in Fig 1A undergoes a series of state transitions when the membrane is depolarized from its resting level (−80 mV) to a more positive potential (−10 mV). The channel moves first from a closed (or resting) state to an open state that allows Na^+ to flow into the cell; it proceeds to another nonconducting conformation, the inactive state, in which it sits silently despite maintained membrane depolarization (Fig 1B).

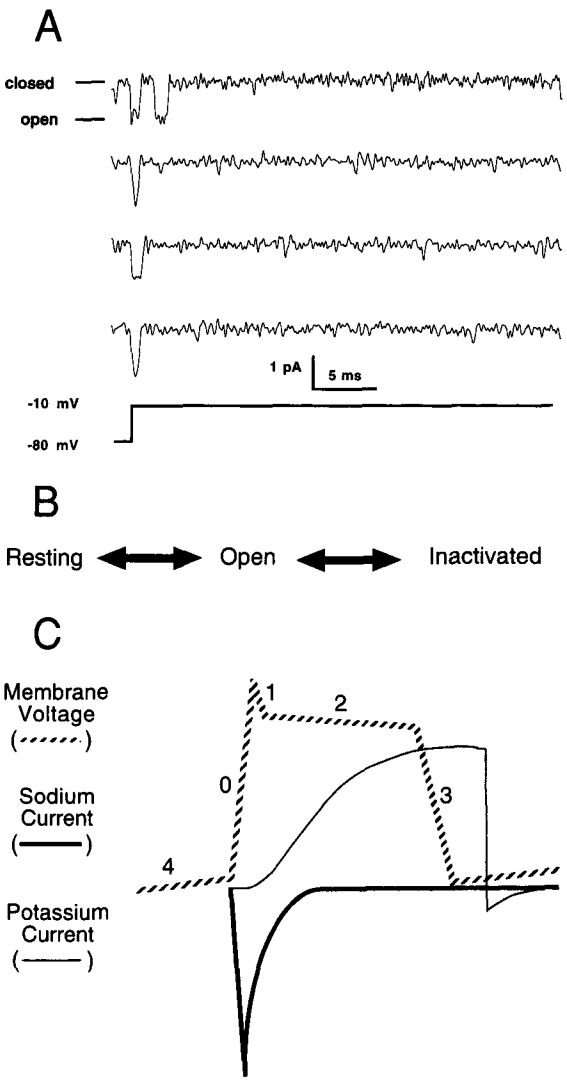

Figure 1. Single channel recording of a Na^+ channel with a gating scheme and a diagram of a cardiac action potential with two contributing channel currents. (A) Single channel recording from a cardiac voltage-gated Na^+ channels (courtesy of Ted Cummins and Fred Sigworth, Yale University). In response to a change in voltage from −80 mV to −10 mV, the channel moves from resting to open conformation and then to an inactive state. (B) Scheme for gating of an inactivating voltage-gated channel. (C) Diagram of the phases of a cardiac Purkinje cell action potential with currents through two contributing channels superimposed, based on Fozzard.[69] The channel currents associated with the five phases of the action potential are: phase 0, rapid depolarization-I_{Na} and I_{Ca}; phase 1, fast repolarization-I_{to}; phase 2, plateau-I_{Ca}; phase 3, delayed repolarization-I_{Ks}, I_{Kr}; phase 4, pacemaker depolarization-I_{IR}, I_f.

An open ion channel forms a water-filled conduction pore across the membrane bringing the external and intracellular solutions into continuity. Ions passively diffuse through the pore in a direction determined solely by differences in ion concentration and electrical potential across the membrane. This does not indicate that channel proteins are inert scaffolding; the number and type of ions that move through a channel reveal its influence. Thus, some K^+ channels catalyze the flow of 100,000,000 K^+ ions each second through a single channel complex, yet maintain a preference for K^+ over Na^+ of 10,000 to 1.[2] We will consider the dramatic progress in understanding of the molecular basis for opening and inactivation of voltage-gated channels as well as the channel regions that mediate ion conduction and selectivity.

Ion Channel Function: The Cardiac Action Potential

A cell's electrical behavior is determined by the gating and selectivity attributes of its ion channels.[3-5] In the heart, cells of the conduction pathway exhibit a pattern of cyclical excitability with five phases. A Purkinje cell action potential and its two primary channel currents are schematized in Fig 1C. Phase 0 is an explosive rise in membrane potential due to a rush of Na^+into the cell (down its concentration gradient) through voltage-gated Na^+ channels that open with membrane depolarization; these channels then rapidly inactivate (Fig 1A, B). Phase 1 is a brief repolarization step mediated by voltage-gated K^+ channels that open with depolarization and, like phase 0 Na^+ channels, rapidly inactivate. Because outward flow of K^+ (down its concentration gradient) makes the cell interior more negative, opening K^+ channels shifts the cell toward more negative potentials. Phase 2, the plateau in the action potential, is coincident with myocardial contraction and results from the cumulative activity of a number of channel and carrier-type transporters. The duration of phase 2 is determined by voltage-gated K^+ channels that open with a delay in response to membrane depolarization and remain open until the membrane is again hyperpolarized (Fig 1C). These delayed outward K^+ currents return the membrane to its resting potential during phase 3 and

allow the heart to relax. Phase 4, the pacemaker potential, is a slow rise in membrane potential attributed to closing of other K^+ channels and instigates the next cycle of excitation and contraction. In broad outline, cardiac excitation results from rapid, voltage-dependent gating of Na^+ channels, delayed, voltage-dependent gating of K^+ channels, and the ability of both channels to discriminate between Na^+ and K^+ ions.

Cloning of Genes Encoding Ion Channels

Identification of the structural determinants of gating and selectivity followed cloning of the genes encoding ion channels. The first Na^+ channel gene was isolated by a direct biochemical approach; channel protein was purified from the electric organ of the *Electrophorus electricus* eel in sufficient quantity to allow production of anti-channel antibodies, and these were subsequently used to screen and isolate the gene from an eel cDNA expression library.[6] K^+ channels have no high abundance tissue source; the first K^+ channel gene was isolated after its identification as the cause of a motion disorder in mutant fruit flies.[7-9] Scores of related Na^+ and K^+ channel genes and their human homologues were then identified based on their homology to the sequences of these first two genes.[10] More recently, novel ion channel genes have been isolated by screens for expression of channel function rather than DNA sequence homology[11-14] and by computer analysis of the rapidly expanding database of genomic and expressed nucleotide sequences for channel-like motifs.[15,16]

Classification of Ion Channels by Structure

Once genes for voltage-gated Na^+, Ca^{2+}, and K^+ channels and ligand-gated CNG channels were isolated, their membership in an extended molecular superfamily was revealed. Marked by similarities in primary sequence and predicted membrane topology, voltage-gated Na^+ and Ca^{2+} channels contain four homologous domains in tandem, each with six predicted membrane-spanning segments (Fig 2A). Voltage-gated K^+ channel subunits and CNG channel subunits are similar in size and topology to a single Na^+ channel domain (Fig 2B). Inward rectifier K^+ channels and epithelial Na^+ channels share a proposed two transmembrane topology (Fig 2C), whereas the recently identified outward rectifier (Fig 2D) and open rectifier K^+ channel subunits (Fig 2E) are built with two domains similar to those observed in previously identified K^+ channels.

Assigning Structure to Function: Voltage-Dependent Gating

Channel regions that participate in gating and ion selectivity were first suggested by comparison of the amino acid sequences predicted from each newly isolated gene.[6,17,18] Candidate regions were then evaluated and influential residues identified by studying the functional effects of site-directed mutations.

Resting to Open Transition

Hodgkin and Huxely[3] first proposed that changes in voltage might cause the movement of charged "gating particles" within nerve membranes to turn Na^+ and K^+ conductances on and off. Thirty years later, cloning revealed that voltage-gated channels carry a novel charge-containing motif not observed before in membrane proteins: at every third or fourth position in the fourth predicted transmembrane segments (S4), a positive arginine or lysine residue is present. That movement of these S4 charges might mediate the effects of voltage on channel activation gained early support[19,20] and has now been firmly established.[21,22]

Yang et al[21,23] investigated the S4 segment in the fourth homologous domain (D4) of a human skeletal muscle voltage-gated Na^+ channel. They started with a channel mutant, first identified in patients with paramyotonia congenita,[24] in which the initial arginine in S4/D4 is mutated to cysteine. When they added a cysteine-specific reagent to the external solution, the channel became covalently modified and its gating was changed. Modification was speeded by membrane depolarization as if voltage caused outward movement of the S4/D4 segment and greater exposure of the cysteine to reagent in the bath. Study of each of the seven remaining positively charged S4/D4 residues supported the hypothesis that this region held the long-sought

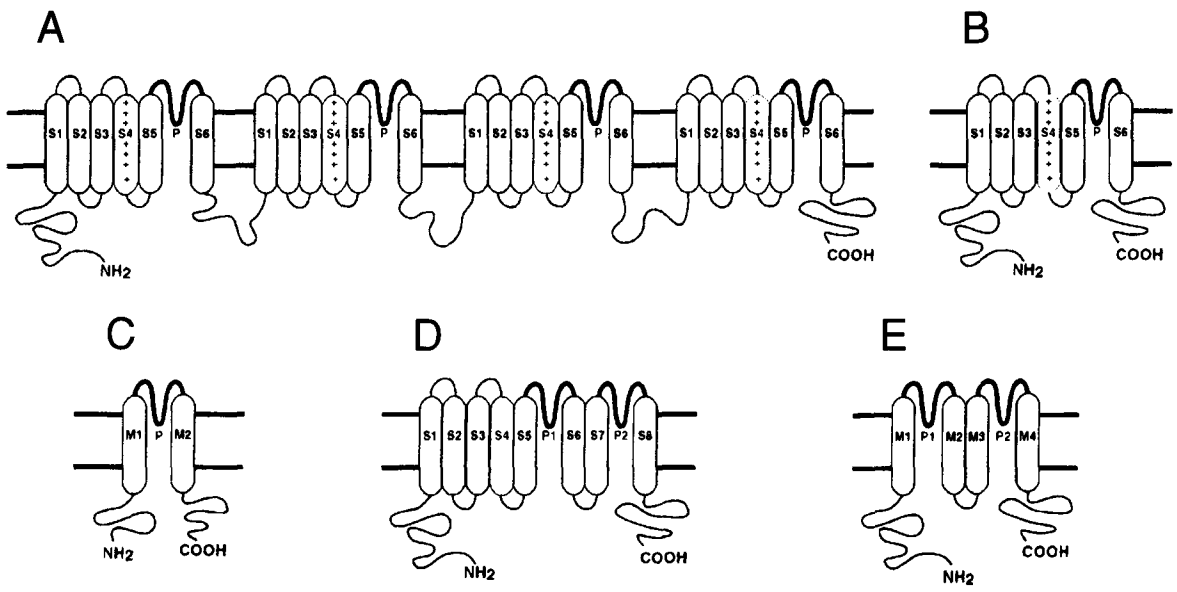

Figure 2. Probable membrane topologies of ion channel α-subunits. (A) Voltage-gated Na^+ and Ca^{2+} channels; (B) voltage-gated K^+ channels and CNG-gated channels; (C) inward rectifier K^+ channels and epithelial Na^+ channels; (D) two P domain outward rectifier K^+ channel[15,16]; and (E) two P domain open rectifier K^+ channel.[14]

gating particles[21]; whereas wild-type channels were unaffected by cysteine-specific probes, channels with cysteine at the 1st, 2nd, or 3rd charged site altered gating. Notably, the second and third sites were modified only under some circumstances: *external* reagent had no effect if the membrane was hyperpolarized (and channels were at rest), but acted on depolarization; moreover, *internal* reagent had no effect if the membrane was depolarized, but acted on hyperpolarization. These sites seemed to move completely across the membrane; exposed inside the membrane at rest, they gained exposure to the external solution with depolarization.

How gating charge movements are translated into channel opening is not yet known. However, a hypothesis for the channel's structure near S4 suggests that the first three charged sites move into continuity with the external solution (or into a vestibule large enough to accommodate the cysteine probes which are ~6°A in diameter), whereas the fourth charged site remains exposed to the internal compartment. In α-helical conformation, the third and fourth sites could be separated by as little as 4.5°A (if extended, by no more than 10 to 11°A). Thus, gating charges seem to travel through short canals (canaliculi) in the protein to reach vestibules in contact with cellular or external solution and, thereby, limit their unfavorable interaction with membrane lipids (Fig 3A).[25] This suggests how gating charges move completely across 40°A of hydrophobic cell membrane in just a few 10 millionths of a second: only some S4 charges traverse the membrane, and their movement is over a limited distance. A similar model for the role of the S4 in voltage-gated K^+ channel function has been cogently argued.[26]

Open to Inactive Transition

The inrush of Na^+ in phase 0 of the cardiac action potential is explosive and regenerative because Na^+ influx drives the membrane to more positive potentials, leading even more voltage-gated Na^+ channels to open. The process is restrained by transition of the channels to an inactive state a few milliseconds after they open (Fig 1A). Armstrong and Bezanilla first proposed a "ball and chain" model for fast inactivation based on their observation that internal protease treatment produced channels that did not inactivate.[27] They suggested that protease removed a portion of the channel (the ball) that moved into the channel pore after it was open to physi-

cally block ion conduction (Fig 3B). In fact, this simple mechanism seems to be operative in both K^+ and Na^+ channels. In Shaker K^+ channels, the intracellular amino-terminus of the protein functions as a tethered open channel blocker.[28,29] When these residues are removed by protease treatment (or by genetic deletion), the channels no longer show fast inactivation. Remarkably, application of the deleted "inactivation ball" as a soluble peptide to the intracellular face of the channel restores inactivation. Moreover, varying the length of the "chain" of residues between the "ball" and the rest of the channel varies the speed of inactivation. In Na^+ channels, it is the cytoplasmic residues linking homologous domains III and IV that are critical for fast inactivation and function to block open channels.[19,30,31] This was shown first by inhibition of inactivation by internally applied peptide-specific antibodies and, subsequently, by site-directed mutation. Deletion of residues mediating fast inactivation often reveals a slow inactivation process that proceeds over 100's of milliseconds and is mediated by the S5, P, and S6 domains in K^+ channels.[32]

Accessory Subunits

The core (or α) subunits depicted in Fig 2 can function by themselves; however, in most cells they are expressed in association with one or more accessory subunits. These additional subunits are essential to integrated cellular function. They regulate channel expression levels, modify functional activity, and influence pharmacological sensitivity. Some accessory subunits carry an inactivation ball and produce rapidly inactivating channels when assembled with K^+ channel α-subunits that normally remain open with maintained depolarization. For an excellent recent review, see Catterall.[33]

Assigning Structure to Function: Ion Selectivity

Voltage-gated Na^+, Ca^{2+}, and K^+ channels and CNG channels show fourfold symmetry. In each, a single conduction pore is formed, either through folding of four homologous domains, or aggregation of four independent subunits. The residues linking every fifth and sixth mem-

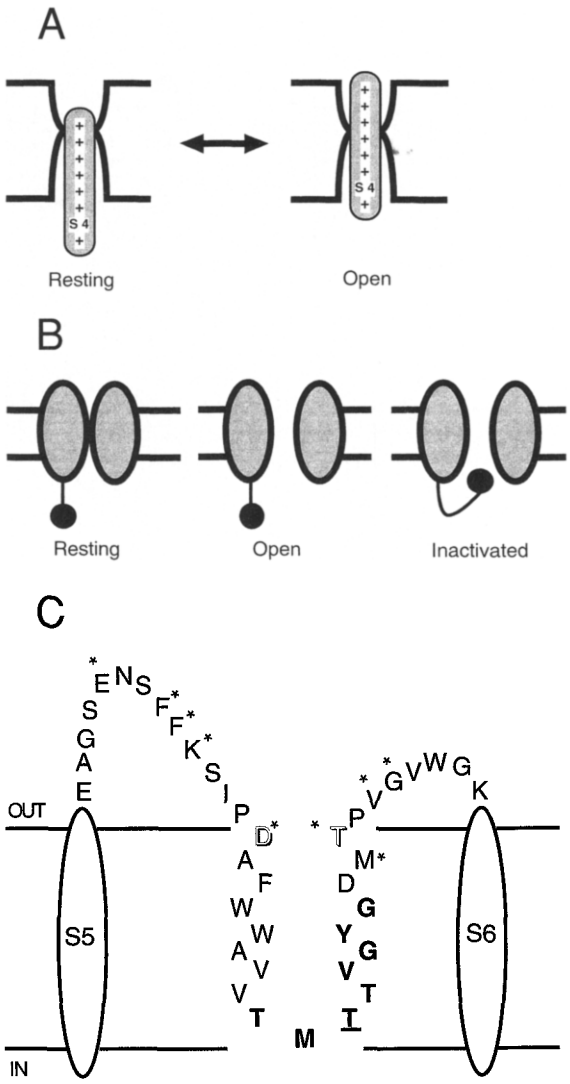

Figure 3. Schematic representations of channel structures involved in channel gating and pore formation. (A) The S4 segment of the Na^+ channel fourth homologous domain is diagrammed indicating the proposed relationship of the gating charges to the S4 canaliculus in the resting and open conformations, based on reference 21; (B) the "ball and chain" model of K^+ channel inactivation suggests the amino-terminal residues act as a tethered blocker that enters and occludes the internal channel vestibule after the channel is open, based on references 28 and 29. (C) The amino acids in the S5-S6 linker region of the Shaker K^+ channel that are pore-forming are indicated by their single letter codes; residues that are critical to charybdotoxin binding have an asterisk (*), the sites critical to external tetraethylammonium ion (TEA) binding are outlined, a site critical to internal TEA binding is underlined; the P domain "signature sequence" residues are bolded.

brane-spanning segment contribute to pore formation (P domains; Fig 2) and are arrayed centrally as four "pore loops".[34] Pores are the catalytic "active sites" of ion channels, and identification of P domains has been a major recent step in understanding the structural basis for channel function.[18,35-38]

The first clue to which residues formed the pore in K^+ channels came from studies of blockade of Shaker K^+ channels by the scorpion venom neurotoxin, charybdotoxin. Previously, functional studies had indicated that charybdotoxin interacted with negatively charged sites on K^+ channels and inhibited by physically occluding the external pore entryway.[39-41] Now, systematic point mutation of negative residues in Shaker revealed that a unique glutamate residue in the channel region linking transmembrane stretches S5 and S6 was one such interaction site and was probably near the channel pore[35]; residues across this S5-S6 linker region were subsequently shown to be critical to toxin binding (Fig 3C).[42-44]

That the S5-S6 linker crossed the membrane to form the ion conduction pathway was shown by identification of two binding sites in this region for tetraethylammonium ion (TEA), one extracellular and one intracellular.[36,37] This was a surprising result because hydropathy analysis had suggested the region was likely to form a completely extracellular protein loop. In dramatic support for this model, a chimeric channel with 24 of its S5-S6 linker residues replaced by linker sites from another channel showed pore characteristics of the donor channel; the chimera showed high single channel conductance (unlike the native channel), high sensitivity to internal TEA (rather than low), and low sensitivity to external TEA (rather than high).[38] Notably, 2 years in advance of any of these experimental findings, evaluation of the Na^+ channel sequence had led Guy and Seetharamulu to propose from first principles that the residues linking S5 and S6 would dip into the membrane to form the pore.[18] Comparison of sequences of cloned K^+ channels and detailed studies of channels with point mutations in the P domain allowed Heginbotham et al to identify a "signature sequence" of eight highly conserved residues in the P domains of all K^+-selective ion channels (Fig 3C).[45,46]

Although membrane proteins like channels are not yet readily amenable to crystallization and direct structural determination, peptide toxins have provided an indirect means to assess spatial locations of pore residues. This has been achieved by combining the known three-dimensional structure of the toxins and identified sites of toxin-channel interaction.[47,48] From a first crude image of the Shaker K^+ channel vestibule using charybdotoxin and a first weak contact pair,[44] a sixth coupling pair and the largest interaction energy yet observed was recently reported.[49] The significance of that report lies in the fact that the contact was predicted from a pair-driven model of the pore and, so, validated its conclusions.

Channel Heterogeneity

The large variety of channel currents observed in vivo results first from the many separate genes encoding pore-forming channel subunits. This diversity is increased by alternative RNA splicing[50] and RNA editing,[51,52] which modify the encoded primary structure of the core subunits after transcription. Diversity is even more pronounced in channels that form as multimeric complexes because subunits can associate in varied combinations. Thus, voltage-gated K^+ channels assemble either as homotetramers or as heteromeric mixtures of two or more subunit types. Heteromeric channels exhibit new characteristics in gating, conduction, and pharmacology[53]; some, like cardiac K_{Ach} channels, require heteromeric association to form functional channel complexes.[54] Another degree of functional diversity is provided by modulatory accessory subunits that associate with the pore-forming core subunits.

Regulation of Channel Function

Channels are regulated in a cell-by-cell fashion; thus, adjacent cells in a tissue can express different channels or channel subtypes; for an excellent review see Levitan and Kaczmarck.[55] Specificity is achieved at multiple levels: which core and accessory subunits are expressed is controlled at the level of gene transcription, RNA editing, and RNA translation. Variation in channel levels and subtypes is observed with develop-

Table 1. Diseases Mediated by Ion Channels

Mechanism	*Disease*	*Channel*	*Reference*
Loss of function			
Low levels of surface expression			
Unstable protein	Cystic fibrosis	CFTR	70, 71
Autoimmune degradation	Myasthenia gravis	AchR	72
	Lambert-Eaton syndrome	VGCa	73
	Acquired myotonia	VGK	74
Open poorly, inactivate quickly	PHHI	K_{ATP}	75
	Thomsen's myotonia	CLC-1	76
	Becker's myotonia		
Decreased single channel conductance	Cystic fibrosis	CFTR	77
Channel down-regulation	Cystic fibrosis	CFTR	78
Decreased agonist affinity	Myasthenic syndrome-LAFC	AChR	79
Blockade by drugs or poisons	Long QT syndrome	HERG	60
Altered channel selectivity	*Weaver* mice	VGK	58
No channel function-undetermined	Dent's disease, XRN, XLRH	CLC-5	80
	PHA-1	α-ENaC	65
Gain of function			
Open too readily	HYPP	SCN4a	81
Inactivate slowly	Paramyotonia congenita	SCN4a	81
	Long QT syndrome	SCN5a	82
High open probability	Liddle's syndrome	ENaC	62, 63
	Malignant hyperthermia	RYR	83, 84
Activated by drugs or poisons	Cholinesterase inhibitors	AChR	85
Channel up-regulation	Neuronal cell death in stroke	NMDA	86

Abbreviations: CFTR, cystic fibrosis transmembrane regulator; AChR, acetylcholine receptor channel; VGCa, voltage-gated calcium channel; VGK, voltage-gated potassium channel; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; K_{ATP}, ATP senstive potassium channel; CLC1, voltage-gated chloride channel type 1; HERG, human eag-related gene, a voltage-gated K^+ channel; XRN, X-linked recessive nephrolithiasis; XLRH, X-linked recessive hypophosphatemic rickets; PHA-1, pseudohypoaldosteronism type 1; LAFC, low-affinity, fast channel congenital myasthenic syndrome; HYPP, hyperkalemic periodic paralysis; SCN4a, a human skeletal muscle voltage-gated sodium channel; SCN5a, a human cardiac voltage-gated Na^+ channel; ENaC, epithelial Na^+ channel; RYR, Ryanodine receptor, a calcium release channel; NMDA, N-methyl-D-aspartate receptors, calcium channels.

mental stage, cell cycle, and external stimulation. Moreover, some channels are accurately localized to defined subcellular sites (such as motor end plates or dendrites), whereas others are sorted to apical or basolateral surfaces in polarized cells to achieve vectorial transport. Many channels are also subject to strict modulation by second messenger levels or by direct phosphorylation.[56,57] These mechanisms underlie a diverse repertoire of ion channel functions observed in normal cellular physiology.

Channel Dysfunction and Disease

As the molecular basis for ion channel function has been revealed, so has the basis for an increasing number of ion channel-mediated diseases. Table 1 categorizes disorders of known etiology into two groups: those resulting from loss of channel function and those due to gain of channel function. Loss of function can be a result of low levels of channel protein in the membrane. Thus, the most common variant of cystic fibrosis is marked by inherent instability of the CFTRΔ508 chloride channel protein leading to its rapid intracellular degradation. Autoantibodies mediate channel degradation in myasthenia gravis, Lambert-Eaton syndrome, and one form of acquired myotonia (Table 1). Loss of function disorders are also observed when channels reach the membrane but show decreased activity. Examples in humans include persistent hyperinsulinemic hypoglycemia of infancy (PPHI) and the inherited congenital myotonias (Thomsen's, Becker's) in which channels open poorly or for too short a time. Other loss of function disorders are the result of decreased single channel con-

ductance, inappropriate down-regulation, decreased agonist affinity, and blockade by drugs or poisons (Table 1). *Weaver* mice have a neurodegenerative disorder characterized by K^+ channels that open but are no longer selective.[58] Not yet reported for ion channels are diseases resulting from diminished transcription and translation or rapid degradation owing to improper secondary modification.

Disorders resulting from a gain of channel function include hyperkalemic periodic paralysis (HYPP) in which channels open too readily, and paramyotonia congenitia (PC) in which they inactivate too slowly. Other gain of function diseases are due to channels that have an abnormally high open probability as a result of mutations or exogenous activation by drugs or poisons (Table 1). The pathophysiology of neuronal cell death in stroke seems to involve dysfunctional up-regulation of NMDA Ca^{2+} channels (Table 1). No disorders are yet known to result from protein overproduction, diminished degradation, or increased single channel conductance. An excellent review of hereditary diseases of skeletal muscle ion channels is published.[59]

Correlation of Channel Function and Disease: Three Examples

Long QT syndrome (LQTS) is a cardiac disorder that predisposes the heart to torsade de pointes, a ventricular arrhythmia that can degenerate into ventricular fibrillation and cause sudden death. It can be acquired, most commonly as a side effect of treatment with class IA or III antiarrhythmic medications, or inherited. The electrocardiograms of affected individuals show an abnormally prolonged repolarization interval (QT_c). Recent molecular studies have established that two forms of inherited LQTS result from mutations in the genes encoding cardiac ion channels. SCN5a on chromosome 3 encodes the cardiac voltage-gated Na^+ channel that underlies the rapid rising phase of the cardiac action potential (Fig 1B, phase 0). Mutations in SCN5a produce channels with destabilized inactivation gates. Mutant channels open normally, but are not stable in the inactivated state; by reopening repetitively during depolarization, the channels prolong the cardiac action potential. A different mechanism underlies LQTS resulting from mutation in the human eag-related gene (HERG) locus on human chromosome 7. HERG encodes the core subunit of a K^+ channel and mediates a cardiac delayed-rectifier K^+ current, I_{Kr} critical to myocardial action potential repolarization (Fig 1B, phase 3). A number of mutations in HERG channels have now been identified in individuals with LQTS: all act to reduce channel function and prolong action potential duration. Drug-induced LQTS also results from decreased HERG channel function but is due to channel blockade.[60] These mechanistic insights have facilitated new therapeutic approaches. LQTS resulting from mutations in SCN5a (gain of function) has proven amenable to treatment with Na^+ channel blockers, whereas individuals with mutations in HERG (loss of function) have corrected their repolarization abnormalities in response to modest increases in extracellular K^+ concentration.[61]

Liddle's syndrome (pseudoaldosteronism) is an autosomal dominant disorder characterized by early onset of moderate to severe hypertension. Lifton and colleagues recently showed that this disorder results from mutations in subunits of the amiloride-sensitive epithelial Na^+ channel (ENaC) of the kidney and that mutations lead to constitutive channel activation (gain of function).[62-64] These channels are formed by co-assembly of α, β, and γ subunits. Affected patients have been found to carry mutations that lead to deletion of the cytoplasmic carboxyl termini of their β or γ ENaC subunits. Biophysical analysis of one β subunit mutation revealed channels with normal single channel conductance, ion selectivity and sensitivity to amiloride blockade, but excess channel openings in membrane patches. Because reabsorption of Na^+ through ENaC is regulated by aldosterone, a process that is a major determinant of net Na^+ reabsorption by the kidney, increased channel activity leads to increased Na^+ reabsorption and hypertension in affected individuals.

Autosomal recessive pseudohypoaldosteronism type 1 (PHA-1) is characterized by life-threatening dehydration in the neonatal period. It is characterized by marked hypotension, salt wasting, elevated K^+ levels, metabolic acidosis, and marked elevation in plasma renin and aldosterone levels. In this case, Lifton and colleagues

showed the disorder to result from mutations in α or β ENaC subunits leading to loss of channel function.[65] ENaC dysfunction seems to cause the disorder by facilitating renal tubular Na^+ loss. This leads to a secondary defect in secretion of K^+ and H^+ ions and stimulation of the renin-angiotensin system. However, salt reabsorption is not increased because the ENaC target is unresponsive.

Future Research Directions

An array of medical conditions are potentially amenable to treatment by agents that act on ion channels.[66] Abnormal ion channels are directly responsible for the pathophysiology of some diseases. In other cases, normal ion channels are inappropriately regulated. To develop rational therapeutic interventions targeted at ion channels, a series of unanswered queries lies ahead. Recognized currents that are key to tissue function must be identified at the molecular level (for example, the I_f current that mediates cardiac pacemaker function[67,68]). The biochemical rules that govern subunit association must be defined. The constituent subunits of heteromeric complexes that mediate function in vivo need to be delineated. The pattern of functional expression of channel subtypes through development needs to be described. Regulation and modulation of ion channel function in vivo remain poorly elucidated. To understand the structural basis for channel function, accurate three-dimensional information about the structure of ion channels is required. It seems likely that the mechanisms of most channel-mediated disorders are yet to be discerned, and that many diseases are yet to be appropriately attributed to ion channel dysfunction. To achieve therapies based on mechanistic insight, we have many barriers to surmount. The rapid progress made in the last few years makes such a goal seem feasible.

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