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Molecular Structure of Ion Channels

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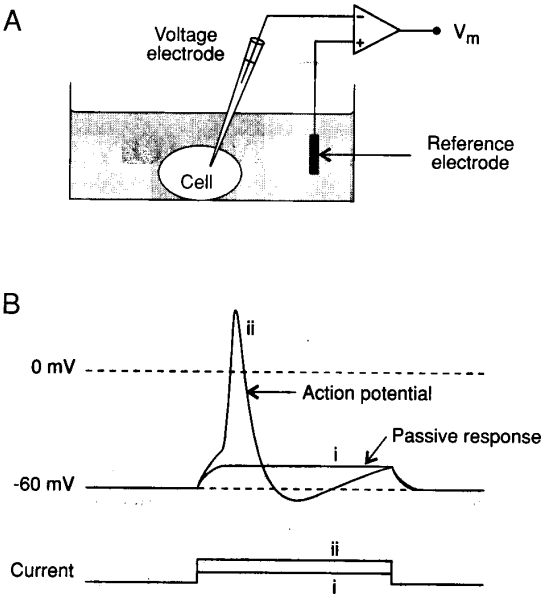
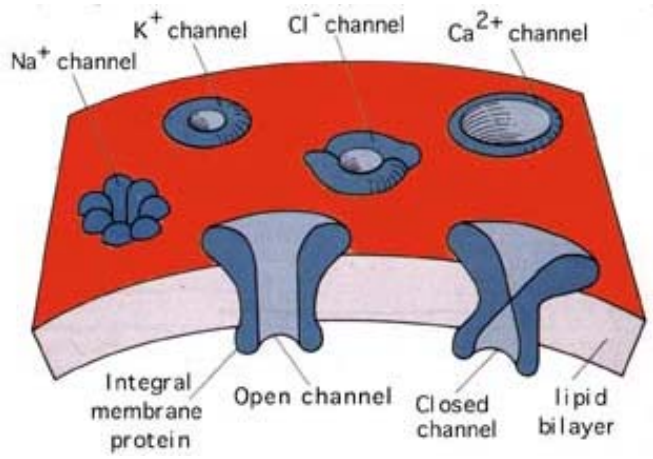
What are ion channels?

Ion channels are transmembrane proteins that allow for the flow of ions across the cell membrane. Biophysicists have focused on three specific properties of ion channels: **gating**, **selectivity** and **permeation**. Gating refers to the process of channels opening and closing, while selectivity is the property of restricting the flow of a particular ion through the channel. Permeation is the rate at which an ion can translocate through the channel. It should be noted a channel may be selective (K^+ channels are 10000X more selective for K^+ than Na^+) but still allow over 10^6 ions to flow per second. More recent studies have focused on the molecular structure of ion channels and determining key structural components involved in gating and selectivity, as well as assembly, protein-protein interaction, cellular regulation and modulation.

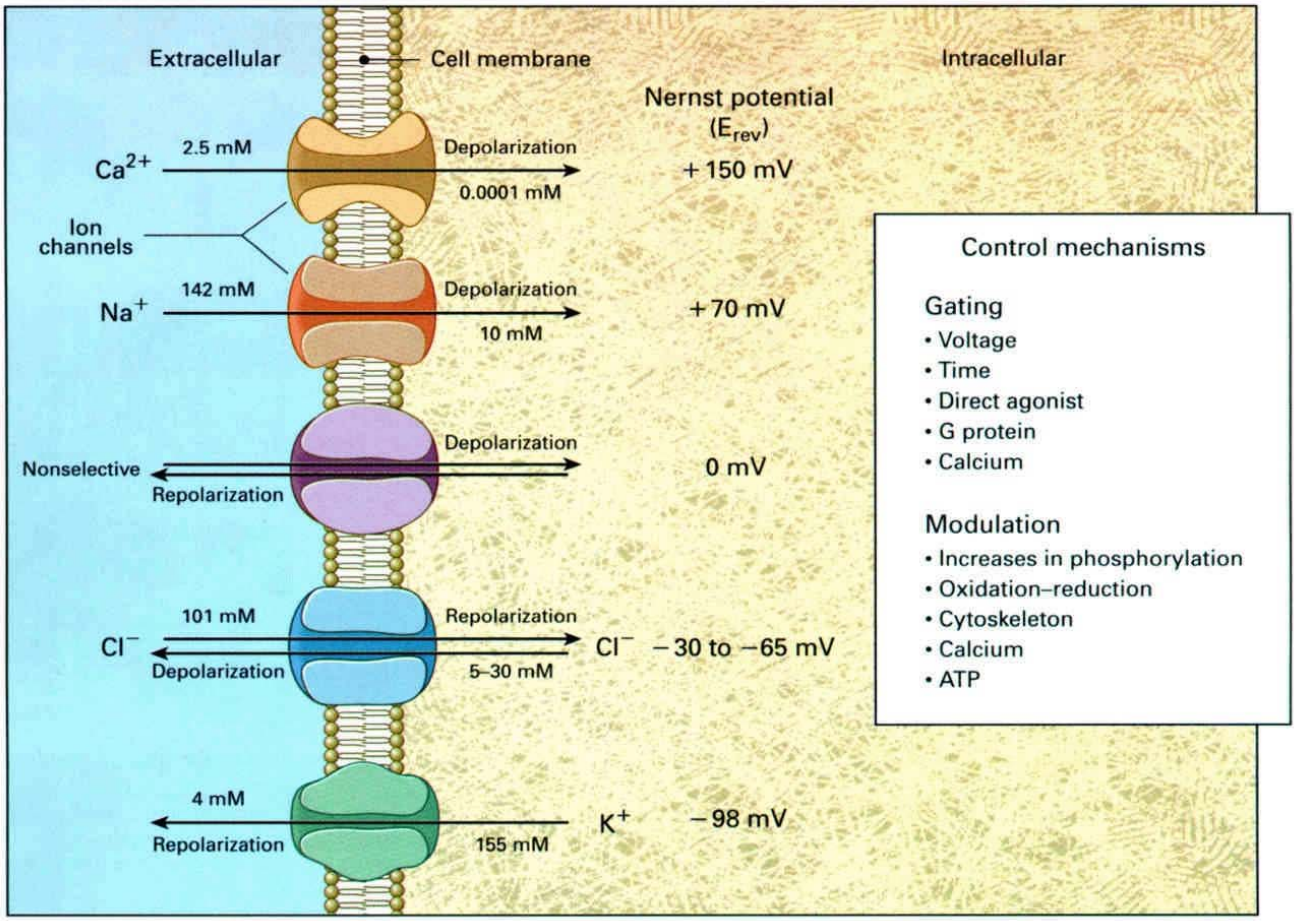
Ions due not easily diffuse across the lipid membrane due to the high energy barrier (~30 kT). Ion channels allow for the movement of ions across the membrane via a low energy water-filled conduction pathway.





By allowing for changes in the ionic gradient across the cell membrane, changes in ion channel activity can lead to changes in the membrane potential which is critical in a number of cellular functions:

1) cardiac, skeletal and smooth muscle contraction
2) cellular excitability
3) secretion of neurotransmitters and hormones
4) regulation of cell volume
5) maintaining ionic gradients across the membrane

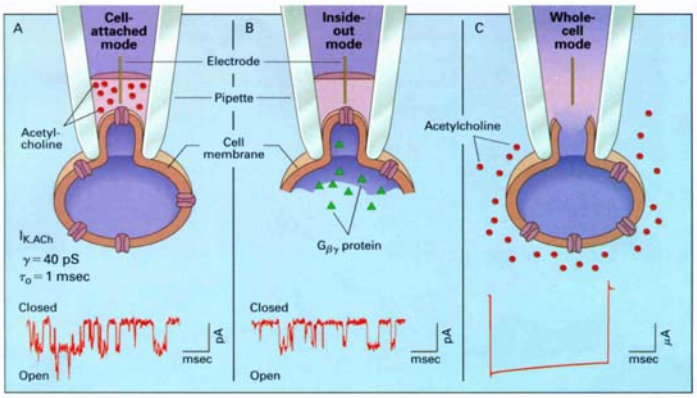


Ions normally flow down its electrochemical gradient which is governed by diffusional and electrical forces, and can be determined by calculating its Nernst potential. The activity of the ion channel (i.e. the frequency at which it opens and closes to allow ions to flow) is governed by intrinsic properties of the channel protein or accessory proteins and can be modulated by cellular factors.

How Do We Study Ion Channels?

Patch Clamp Technique

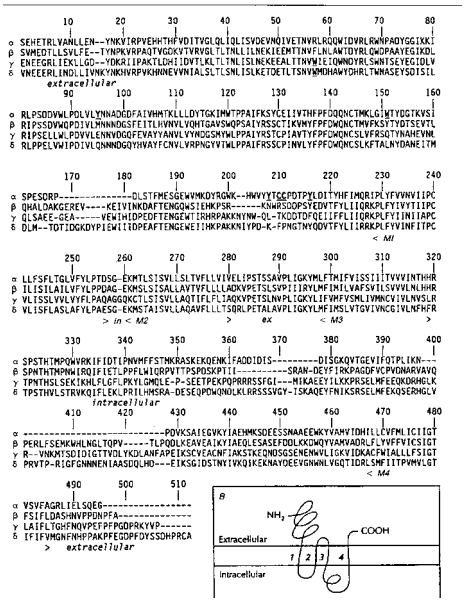
The patch clamp technique is a powerful tool since it can allow for the high temporal resolution (µsec) of the gating of a single ion channel protein. This technique developed by Ernst Neher and Bert Sakmann, uses a fine-tipped glass electrode pulled to a diameter of <1 µm to record the activity of ion channels on the surface membrane (plasmalemma) of the cell. A number of varying techniques can allow for the study of a single ion channel (e.g. cell-attached, inside-out) or all of the channels on the cell membrane (e.g. whole-cell). With the advent of the patch clamp technique, the biophysical properties (gating, permeation, selectivity), regulation, expression, identification of new channels and modulation of ion channels has been made possible.



Molecular Biology

The use of molecular biology techniques has provided "channelologists" the ability to study the molecular structure and function of ion channels. The cloning of the first ion channel, the α, β and γ subunits of the nicotinic acetylcholine receptor from the electric ray, *Torpedo californica*, by Numa and colleagues (Nature 299:793-7, 1982; Nature 301:251-5, 1983) has led to the cloning of hundreds of other ion channels. The development of site-directed mutagenesis by Michael Smith and the expression of foreign cDNA/cRNA in host cells (e.g. *Xenopus* oocytes, mammalian cultured cells) have provided the opportunity to alter the protein structure of ion channels to investigate the structure/function properties.

Amino acid sequence of the nicotinic acetylcholine receptor subunits and the putative structure



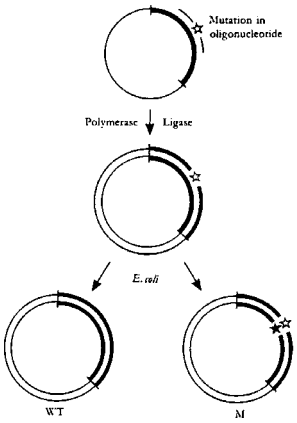
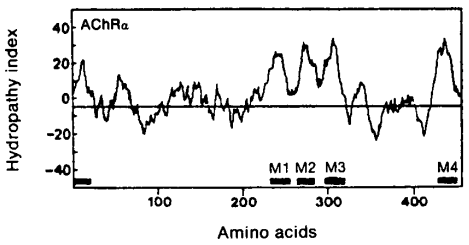


Fig. 4.3. Site-directed mutagenesis. The plasmid single-stranded DNA template contains a cloned gene (heavy line). A synthetic DNA oligonucleotide contains a mutant codon flanked by regions complementary to the cloned gene. After annealing to the template the rest of the plasmid is made double stranded, producing a hybrid with a mismatch in the mutated region. Replication in *E. coli* produces double-stranded copies of the wild-type (WT) and mutant (M) forms. The mutant carries the original mutant codon (white star) and its complementary base sequence (black star). (From Nicholl, 1994.)

Site-directed mutagenesis

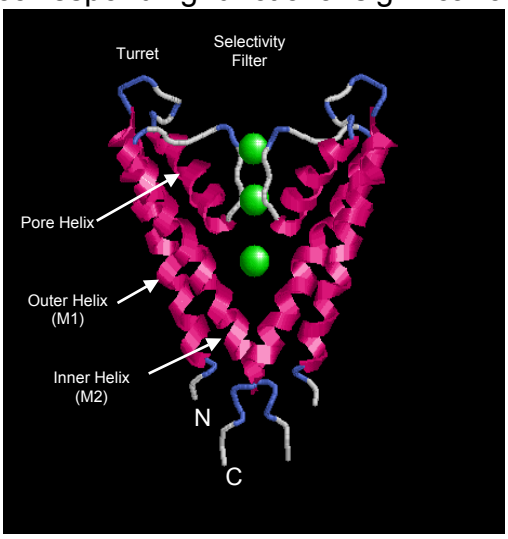
Based on the primary protein sequence of the nicotinic acetylcholine receptor channel, the putative secondary topology of the protein can be deduced from the hydropathy profile (Kyte-Doolittle plots). The hydropathy plot of the α subunit of the nAChR revealed a membrane spanning protein with 4 transmembrane domains comprised of α-helices, and extracellular amino and carboxyl termini.

Hydropathy plot of the nAChR α subunit. The horizontal axis shows the amino acid residue while the vertical axis shows the sum of the hydropathy indices. The black bars indicate the position of the hydrophobic regions. The first black bar is the signal sequence required for the N-terminus to be placed outside, and is removed by enzymatic cleavage on the mature protein. The other four hydrophobic segments are the transmembrane spanning regions (M1 – M4).

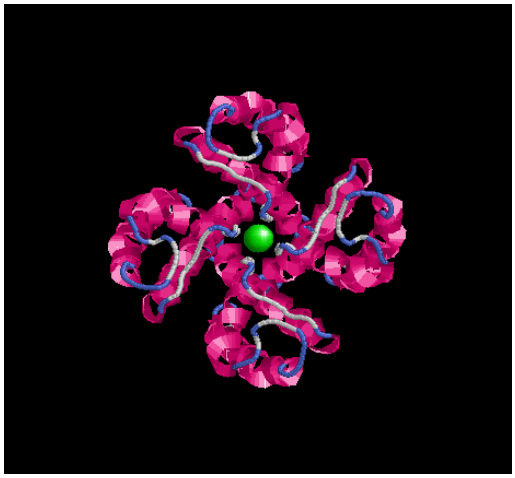


Crystallization of Ion Channels

The recent crystallization of the bacterial K^+ channel from *Streptomyces lividans* (KcsA) by Mackinnon and coworkers (Doyle *et al.,* Science 280:69-77, 1998), and more recently of a voltage-gated potassium channel isolated from the thermophilic archaebacteria *Aeropyrum pernix* (Jiang *et al.,* Nature 423:33-41, 2003; Jiang *et al.,* Nature 423:42-48, 2003) has provided for the first time detailed structural information of an ion channel and the corresponding functional significance.

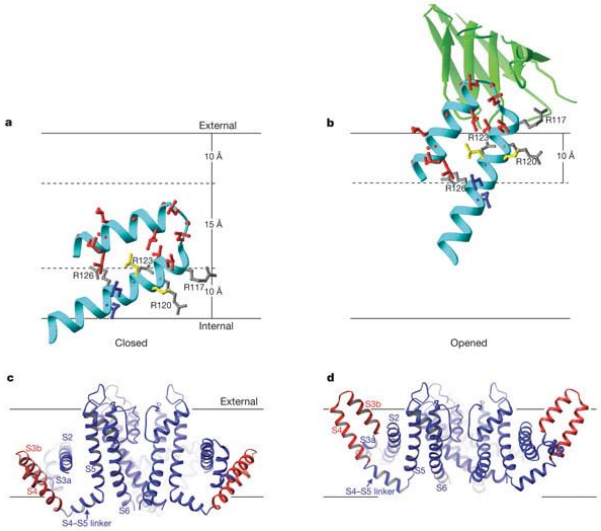


Side view of the KcsA channel. The channel is comprised to two transmembrane spanning domains (M1 and M2) separated by the pore region. This channel, like, other potassium channels, can accommodate up to 3 ions within the pore at one time. For that reason, it is referred to as having a single-file multi-ion pore. The permeating ions do not interact with the amino acid side chains within the selectivity filter, but with the carbonyl oxygens of the protein backbone.



View of the KcsA channel from the top. The channel displays four-fold symmetry with the conduction pathway in the middle.

Crystal structure of a voltage-gated K^+ channel



Electromechanical Model of Voltage-Gated Ion Channels
A simplistic model of voltage-gated ion channels is one where the channel is in 3 different states; **open** (activated), **closed** (rested), and **inactivated**.

1) Closed (rested) state. No ions can flow through the channel
2) Open (active) state. Upon membrane depolarization (change in the electrical field across the membrane), there is a voltage-dependent confirmational change in the protein which opens the pore or moves the activation gate and allow for the flow of ions. The channel can re-enter the closed state from which it can re-open again.
3) Inactivated state. Even in the presence of the stimulus (i.e. membrane depolarization), ion flow ceases due to a second confirmational change which prevents ion flow. This protein confirmation is distinct from the closed state. From this state, the channel does not normally open. Only upon membrane repolarization, the channels recover from inactivation, return to the closed state and can subsequently reopen.

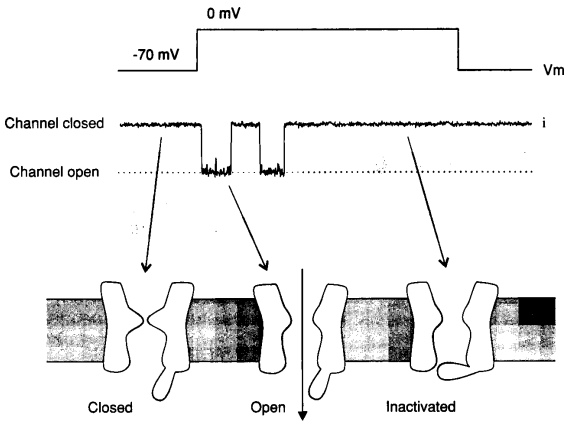


FIGURE 3.5 VOLTAGE-DEPENDENT GATING
Voltage-gated channels open in response to a change in the membrane potential. At negative membrane potentials, most voltage-gated Na^+ channels are closed. Depolarization produces a voltage-dependent conformational change in the channel protein that leads to opening of the channel pore. This is followed by a further conformational change that causes the channel to enter an inactivated state in which it no longer conducts ions. On repolarization the channel returns once more to the closed state (not shown).

Ligand-gated channels open upon binding of a specific ligand(s) or agonist resulting in a conformational change in the protein. The channel will stay open as long as the ligand is bound to the channel. During prolonged exposure to the ligand, the channel may undergo desensitization in which the channel will enter a closed state even with agonist bound. Desensitization can result also in the internalization of the channel. In some cases, binding of the ligand can close the channel such as with ATP binding to the ATP-sensitive K^+ channel.

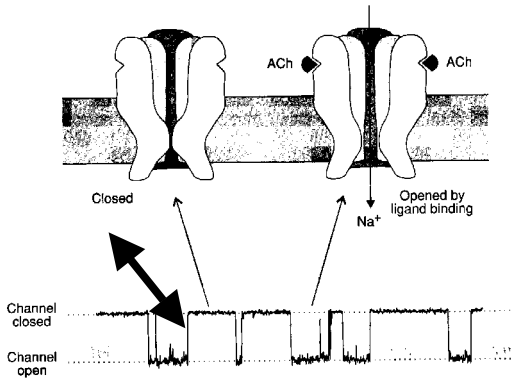


FIGURE 3.4 LIGAND-GATED CHANNEL ACTIVATION
Ligand-gated channels open in response to binding of a ligand. The pore of the acetylcholine receptor channel is closed in the absence of acetylcholine (left). Binding of acetylcholine (ACh) to its receptor site on the channel protein produces a conformational change that results in opening of the channel pore (right). Ions can then move through the pore, resulting in single-channel currents such as those shown in the recording below.

Classification of Ion Channels

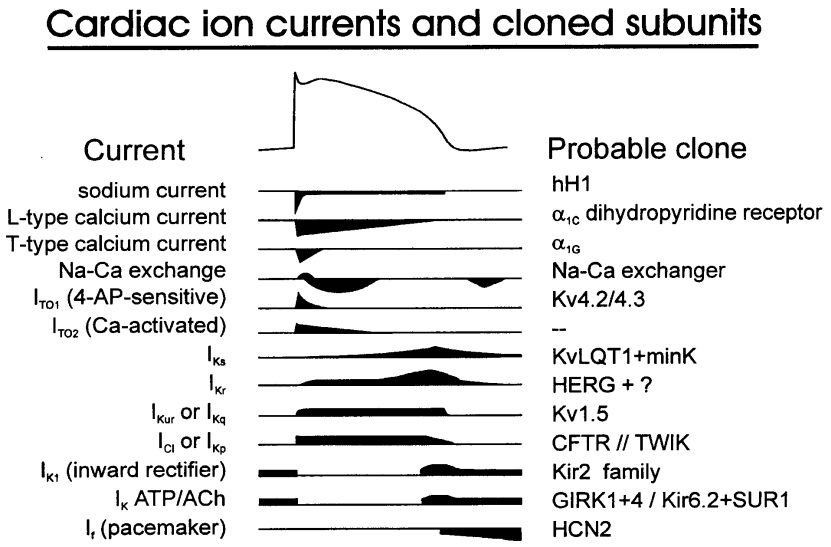
1. Ionic selectivity, e.g. K^+, Na^+, Ca^{2+}, Cl^-, nonselective
2. Functional properties
   voltage-gated channels
   ligand-gated channels (e.g. nicotinic acetylcholine receptor, ACh-sensitive K^+ channel)
   mechanosensitive channels (e.g. swell-activated Cl^- channels)
3. Molecular properties

Cardiac Action Potential

The cardiac action potential is a composite of the integrative activity of numerous ion channels. It can be divided into 5 distinct phases:

1) phase 0 – upstroke of the action potential
   initiated primarily by the activation of voltage-gated sodium channels (I_{Na})
2) phase 1 – initial repolarization
   due to the opening of transient outward potassium channels (I_{to1})and possibly Ca^{2+}-activated Cl^- channels (I_{to2})
3) phase 2 – plateau phase
   balance between depolarizing Ca^{2+} currents through L-type (or dihydropyridine-sensitive) Ca^{2+} channels (I_{Ca-L})and repolarizing potassium currents through a number of different voltage-gated potassium channels (ultrarapidly activating delayed potassium channel I_{Kur}; rapdily- and slowly-activating delayed rectifying potassium channels, I_{Kr} and I_{Ks};
1) phase 3 – repolarization
   due to the reduction in I_{Ca-L}, and an increase in I_{Kr}, I_{Ks} and the inwardly-rectifying potassium channel (I_{K1})
2) phase 4 – resting potential
   controlled primarily by I_{K1}, however in other cells types the pacemaker current (I_f) also contributes to the resting membrane potential

Other ion channels, exchange and pump currents can also modify the action potential waveform.

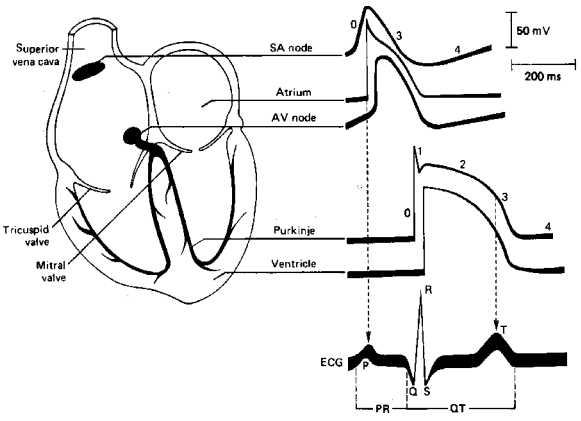


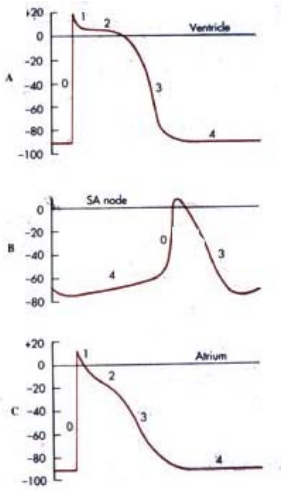
Synders DJ, *Cardiavasc Res* 42:377-390 (1999)

Fig. 1. Ionic and molecular basis of the cardiac action potential. Schematic indication of the time course of depolarizing inward currents (downward) and repolarizing outward currents (upward). The established or most probable corresponding clones are indicated. Abbreviations: see text.

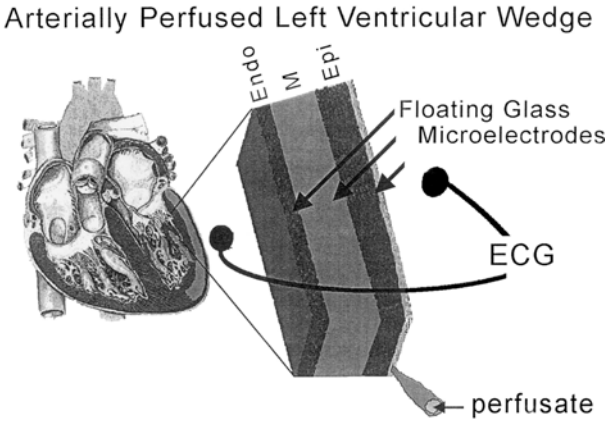
Regional andTransmural Differences in Action Potential Waveforms

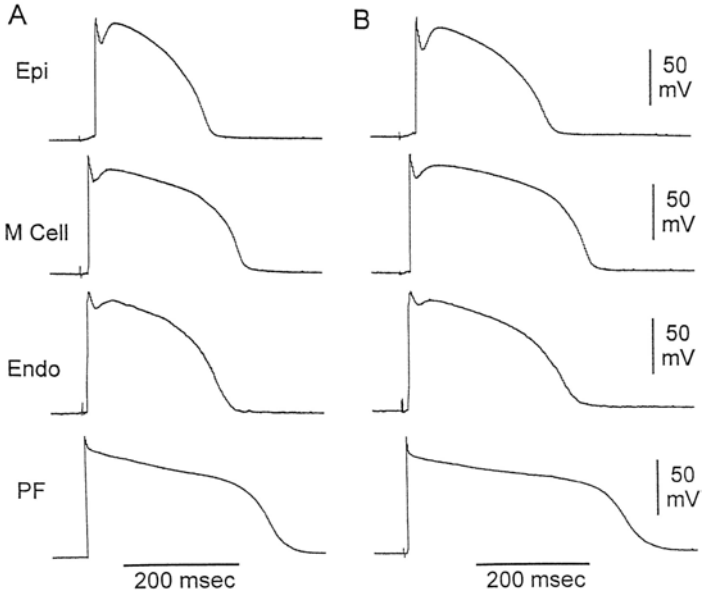
Differences in action potential waveforms is observed in nodal (sinoatrial, atrioventricular), atrial and ventricular tissue.



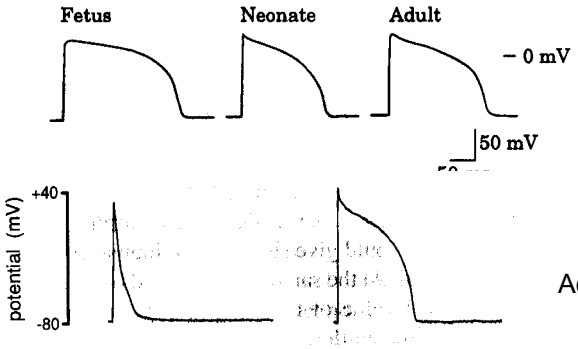


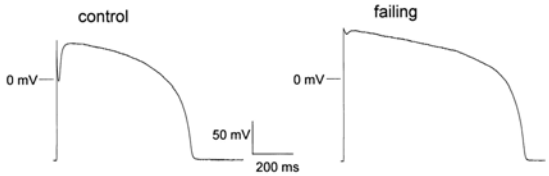
Across the ventricular wall, there are distinct differences in action potential waveform; more prominent phase 1 in epicardial cells and prolonged durations in midmyocardial cells.





Changes in the action potential waveform occur during cardiac development and disease, and differences are seen amongst different species.





Action potentials of rat and guinea pig

K^+ Channels

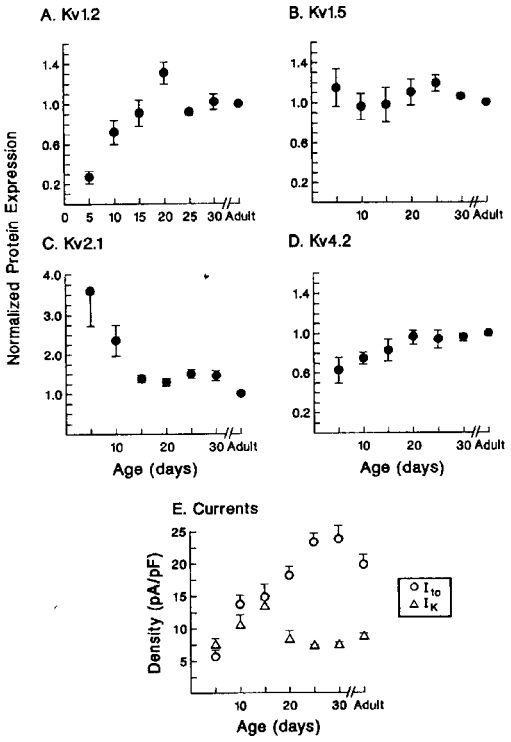
1) Voltage-gated (Shaker-like)
   - high diversity of channel subtypes
   - categorized as Kv1.X to Kv11 (pore-forming α subunit); however only Kv1 – Kv4 form functional channels
   - many different permeations/combinations of K^+ channel assembly which the cell can use to modulate excitability
   - current activated by voltage (depolarization) above –40 mV\

Developmental and species differences are found between the different types of voltage-gated K^+ channels

Table 3 Developmental Variations in Voltage-Gated K^+ Currents in Mammalian Ventricular Myocytes

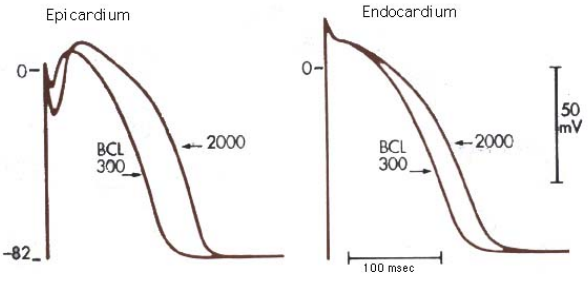
Current	Fetal	Neonatal	Adult	Species
I_{to}	ND	−	++++	Dog
		+	++++	Human
	+	+++	++++	Mouse
	ND	++	+++	Rabbit*
	+	++	++++	Rat
I_{Kr}	++	++	−	Mouse
	ND	+	+	Rat
I_{Ks}	−	++	−	Mouse
	ND	−	−	Rat
I_K	+	++	++	Mouse
	+	++	++	Rat
I_{Kur}	ND	+	−	Rat

* Properties of rabbit I_{to} also change with age (Sánchez-Ghapula et al., 1994). + = detectable; ++ = moderate density; ++++ = high density; − = not detected; ND = not determined.

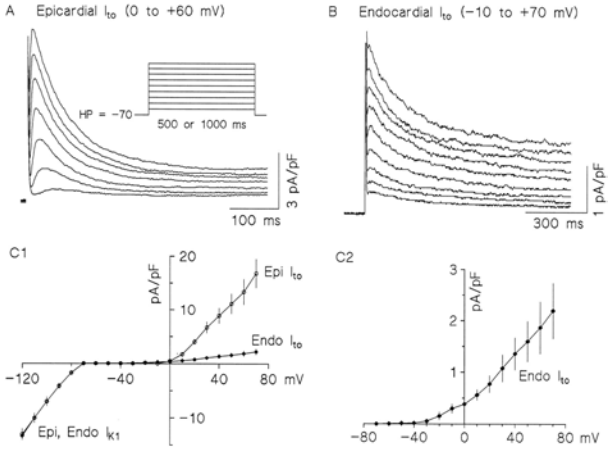


Examples

I_{to} (transient outward K^+ channel)
- contributes to the initial repolarization of the action potential during phase 1
- molecular candidates Kv1.4, Kv4.2, Kv4.3
- Kv4.2/Kv4.3 down-regulated during human heart failure

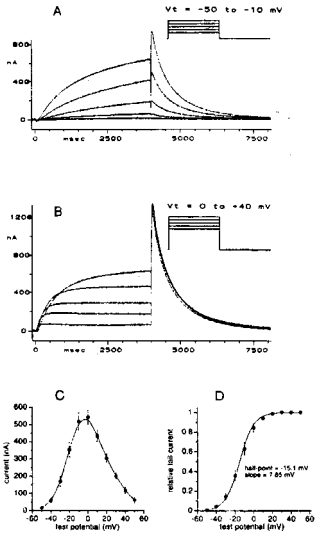


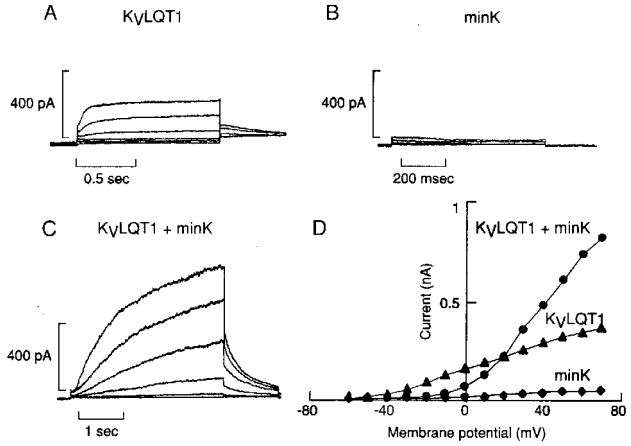
Effect of cycle lengths of 300 and 2000 ms on action potentials recorded from the epicardium and endocardium. (B&L Fig. 23-9, pg. 371)



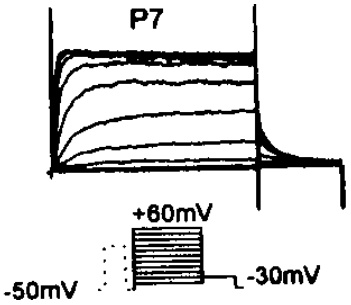
I_{Kr} (rapidly activating delayed rectifying K^+ channel)
- involved in the early and late repolarization during phase 3
- molecular clone – HERG (Human Ether-à-go-go Related Gene)

I_{Ks} (slowly activating delayed rectifying K^+ channel)
- involved in the late period of repolarization of phase 3
- formed by the coassembly of KvLQT1 and minK
- mutations in HERG, KvLQT1 and minK are associated with long QT syndrome





I_{Kur} (ultrarapid delayed rectifying K+ channel)
- contributes to repolarization during phase 3
- molecular clone – Kv1.5



2) Inward Rectifiers

high diversity of channel subtypes; Kir1.X to Kir8.X

voltage-independent channels

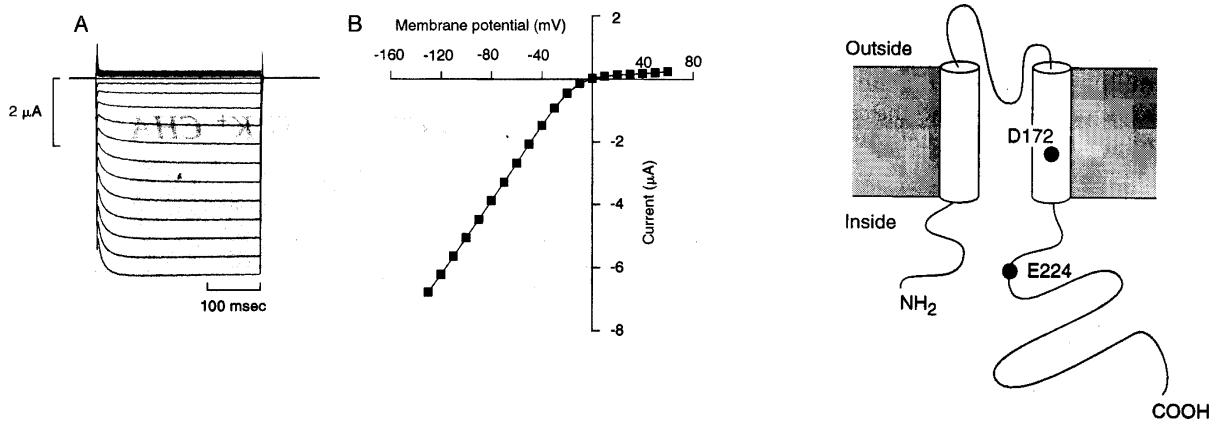
referred to as inward rectifiers since more current is conducted in the inward direction (K^+ flowing from outside the cell to inside) than outward

rectification due to internal block by polyamines (spermine, spermidine, putrescine) and Mg^{2+}

Examples

I_{K1} (inward rectifying K^+ channel)
- important for controlling the resting membrane potential and the late phase of the action potential repolarization
- absent in SA and AV nodal cells
- molecular candidates – Kir2.1, Kir2.2

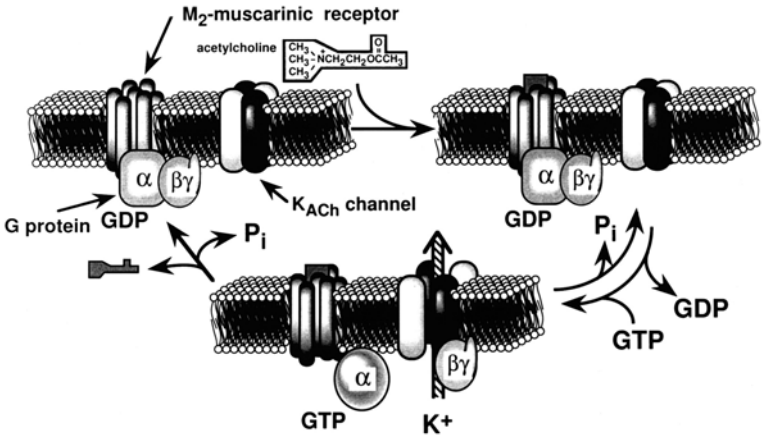
- strong inward rectifier, very little outward current



$I_{K\text{-}ACh}$ (acetylcholine-sensitive K^+ channel)

- activated indirectly by acetylcholine (ACh)
  - ACh binding to muscarinic receptors (M2) activate G proteins leading to the dissociation of Gα from Gβγ; Gβγ activates $I_{K\text{-}Ach}$

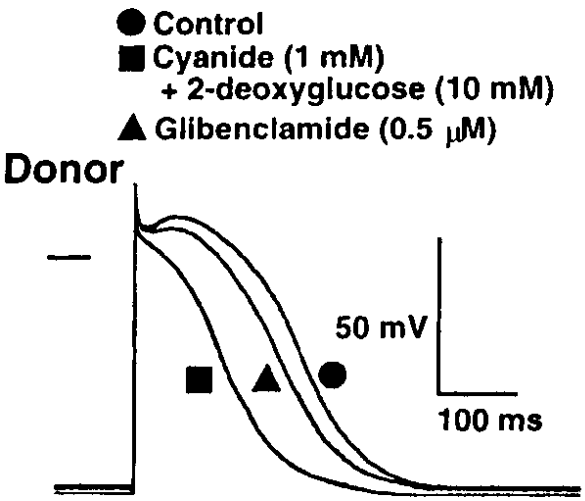
- comprised of the heterotetrameric assembly of Kir3.1 (GIRK1) and Kir3.4 (GIRK4)
- weak inward rectifier, i.e. more outward current component than I_{K1}
  - due to weaker block by polyamines and Mg^{2+}; two acidic residues implicated in rectification, aspartate (D172) and glutamate (E224) are asparagine (N) and glycine (G) in weak rectifiers
- localized mainly in SA and AV nodal cells, and atrial cells

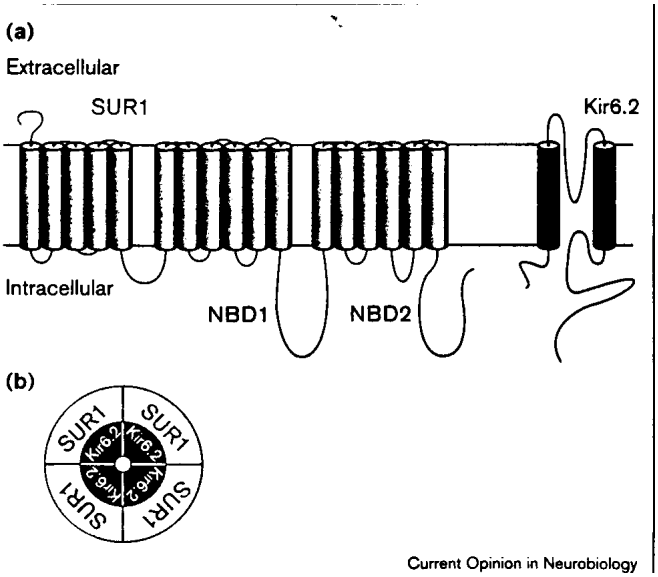


$I_{K\text{-}ATP}$ (ATP-sensitive K^+ channel)

- regulated by intracellular ATP and ADP levels; ATP maintains channel in the closed state, K_i ~ 25 μM. ADP weak blocker K_i ~275 μM, however MgADP activates the channel due to competitive interaction with ATP
- normally not open in the heart; becomes active under pathological conditions such as hypoxia or ischemia when cellular ATP levels decrease
  - activation leads to a shortening of the action potential duration
    - this will reduce Ca^{2+} entry and myocardial contractility

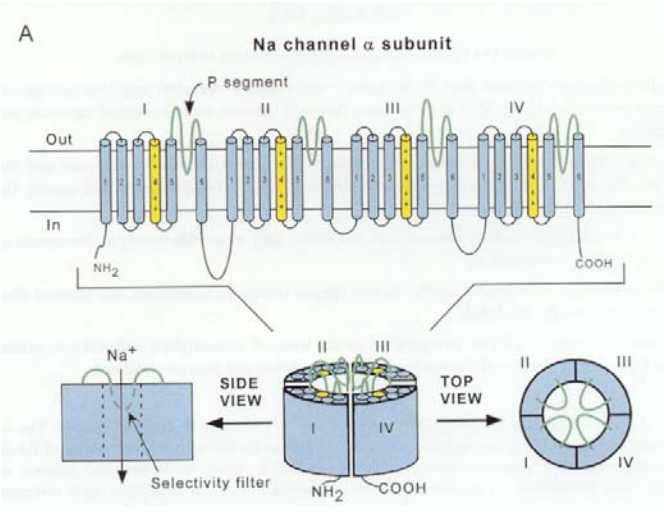
- suggested to be important in maintaining cellular ATP levels and therefore prevent further injury to the cardiac myocyte
- octameric channel comprised of 4 Kir6.2 subunits and and 4 sulfonylurea receptor (SUR2)

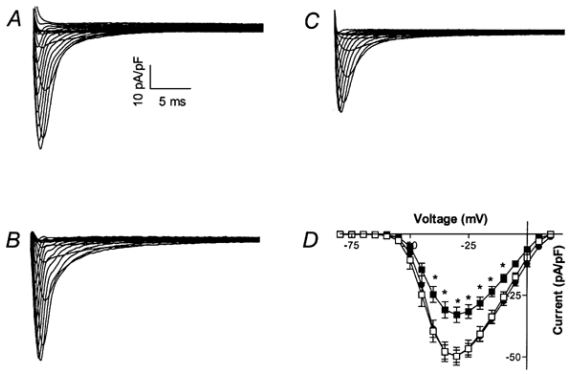




Voltage-Gated Sodium Channels

highest density in His-Purkinje cells compared to atrial or ventricular myocytes; absent in nodal cells
important for rapid propagation of the action potential
transmural difference in Na^+ channel expression – lowest in epicardium





Ashamalla et al., *J Physiol* 536: 439-443, 2001
A) right ventricle
B) left ventricle – endocardium
C) left ventricle – epicardium

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Voltage-Gated Calcium Channels

L-type Ca^{2+} Channels (long lasting)

These channels are important for initiating cardiac muscle contraction. Influx of Ca^{2+} through these channels leads to the activation of intracellular channels (ryanodine receptors; RyR) which result in a larger efflux of Ca^{2+} from the sarcoplasmic reticulum (SR). It is this Ca^{2+} that then binds to the myocardial contractile apparatus to induce muscle contraction.

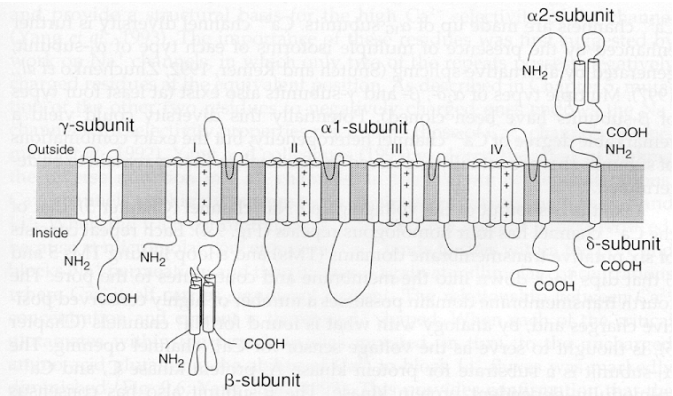
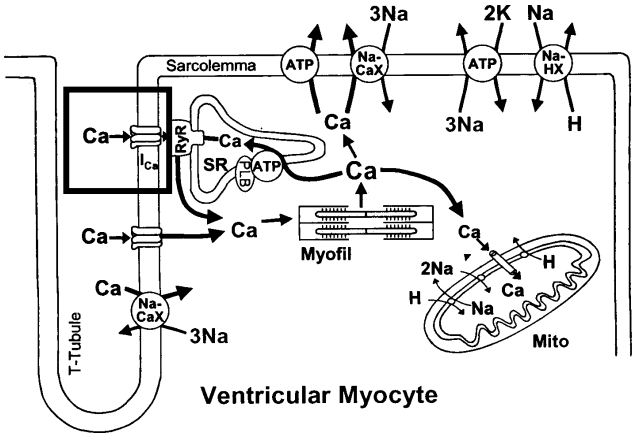


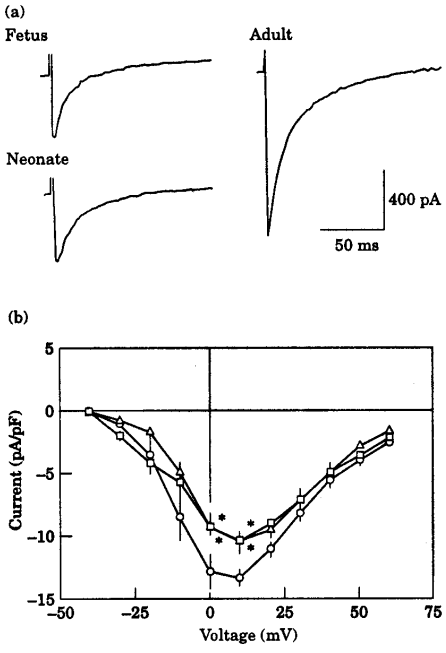
FIGURE 9.3 THE SKELETAL MUSCLE Ca^{2+} CHANNEL CONSISTS OF FIVE SUBUNITS

Putative membrane topology of the α_1-, α_2/δ-, β-, and γ-subunits of the voltage-gated Ca^{2+} channel. After Hockerman *et al.* (1997).



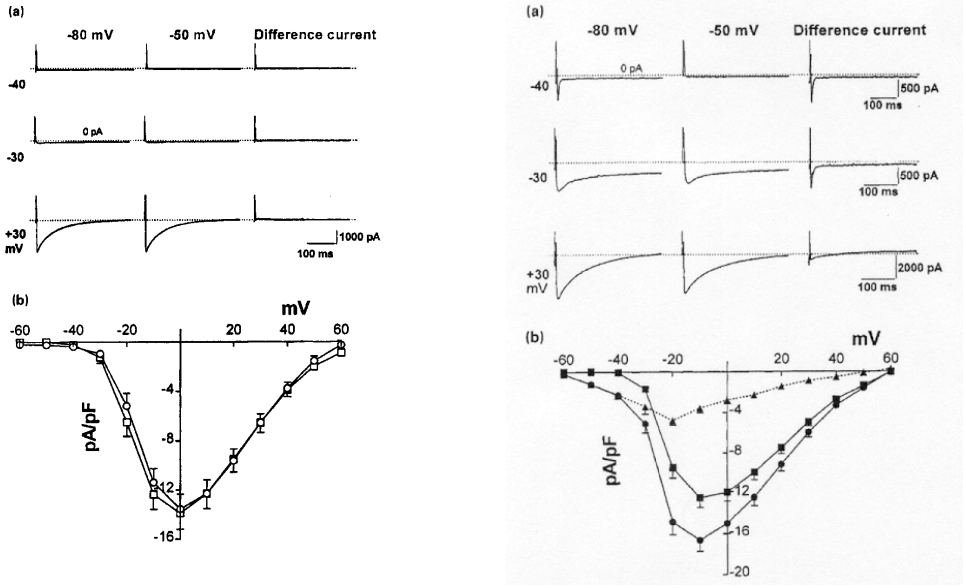
There is a high density of Ca^{2+} channels located in t-tubular system, in close proximity to intracellular ryanodine receptor.

Fetal and neonatal cardiac cells express slightly lower current density of Ca^{2+} channels compared to adult myocytes (Kato et al., *J Mol Cell Cardiol* 28:1515-22, 1996).



T-type Ca^{2+} Channel (transient)

1) high expression in nodal and Purkinje cells – may be important pacemaker activity
2) high expression in fetal and neonatal cells
3) very scarce in adult ventricular cells (rat, calf, rabbit) with the exception of guinea pig; yet to be measured in human cardiac myocytes
4) re-expression of channels during pathological conditions such as hypertrophy
5) potentially due to increase in growth factors during the disease which stimulates the expression of these channels



No difference in L-type Ca^{2+} channel density measured in control rat ventricular myocytes at a holding potential of –80 and –50 mV (left figure). Appearance of T-type Ca2+ channels in hypertrophic cardiac myocytes when held at –80 mV but not –50 mV (right figure) (Martinez et al., *J Mol Cell Cardiol* 31:1617-25, 1999).

Chloride Channels

- anion-selective channels
- 3 major types identified in cardiac cells
  - cAMP- and PKA-dependent Cl^- channels (CFTR)
  - Ca^{2+}-activated Cl^- channels
  - voltage-gated Cl^- channels
    - swell-activated (ClC-2?, ClC-3)
- influence membrane potential and action potential duration, regulates cell volume

CFTR Cl^- Channels

-present in ventricular myocytes, and less dense in atrial cells; not found in rabbit SA node, canine or rat ventricle

- larger outward currents (Cl^- flowing into the cell) will result in greater repolarizing current which will shorten the AP- the inward Cl^- currents will tend to depolarize the membrane potential to the Cl^- equilibrium potential (-46 mV) but this is relative minor due to the large I_{K1} currents.

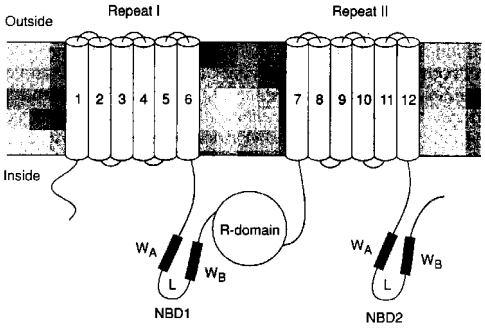
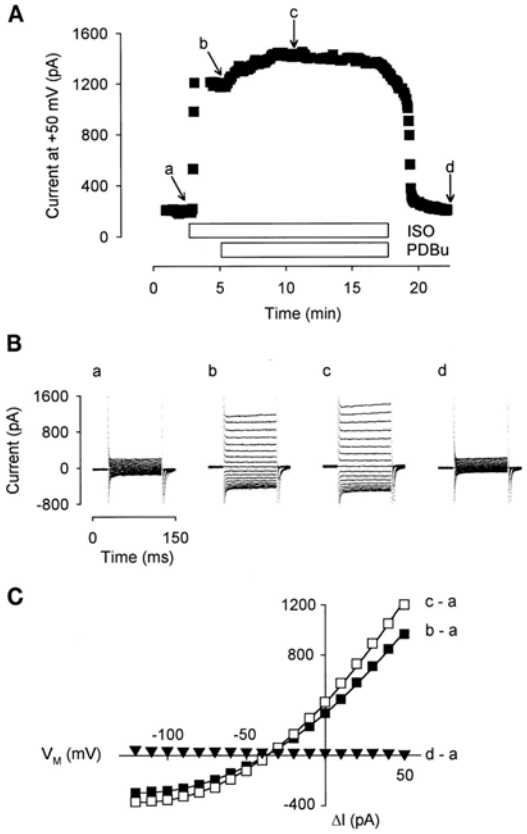


FIGURE 12.2 PUTATIVE TOPOLOGY OF CFTR
Putative membrane topology deduced from hydropathy analysis of CFTR. R, regulatory domain; NBD, nucleotide binding domains; W_A, Walker A motif; W_B, Walker B motif; L, linker motif.



Voltage-gated (swell-activated) Cl^- channels

- present both atria and ventricle, but more prominent in atria

Putative and crystal structure of a voltage-gated Cl^- channel

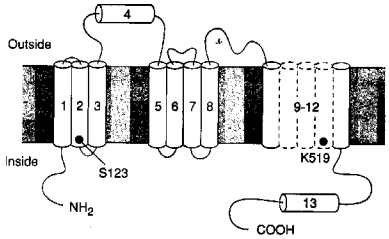


FIGURE 10.2 PREDICTED MEMBRANE TOPOLOGY OF VOLTAGE-GATED Cl^- CHANNELS
Putative membrane topology, based on hydropathy analysis. Two regions of intermediate hydrophobicity (D4 and D13) were originally supposed to span the membrane but are now thought not to do so. The region D9 through D12 is a hydrophobic region which is hypothesised to span the membrane but the precise number of transmembrane spanning domains is not established (between 3 and 5 is the favoured number). The positions of residues mentioned in the text are indicated.