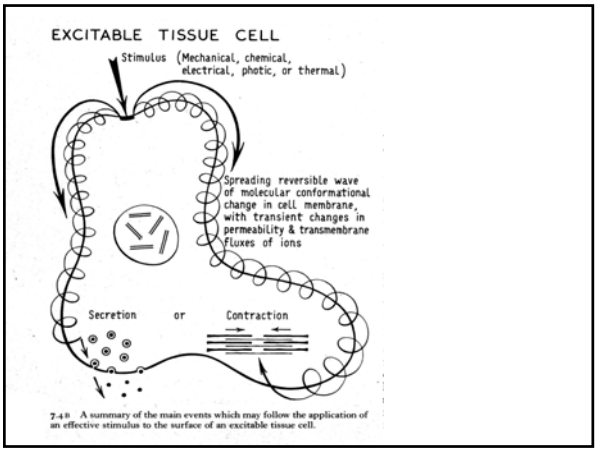
STNS 602 Cellular and Molecular neuroscience

Lecture : Molecular Structure of Sodium and Potassium Channels

Lecturer: Dr. Naiphinich Kotchabhakdi Ph.D.

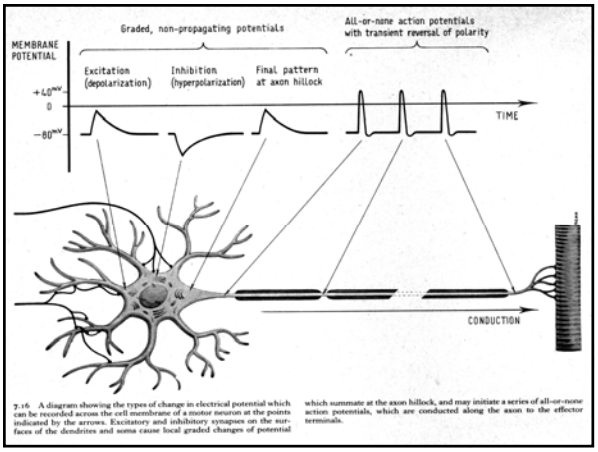
Neuro-Behavioural Biology Center,
Institute of Science and Technology,
Mahidol University, Salaya,
Nakorn pathom 73170 Thailand



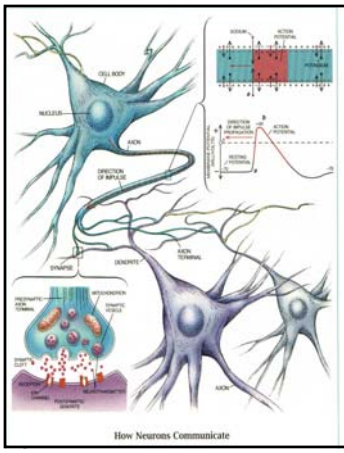
7.4b A summary of the main events which may follow the application of an effective stimulus to the surface of an excitable tissue cell.

Movements of water and electrolytes through plasma membrane of excitable cells and neurons

1. Passive channels
2. Water channels (Aquaporins)
3. Ion pumps (e.g. Na+/K+ ATPase)
4. Ligand-gated ionic channels (Receptors)
5. Voltage-gated ionic channels
6. Mechanical sensitive ionic channels
7. G-proteins-coupled receptors
8. Gap junctions (Nexus, Nexin channels)
9. Leak channels



7.16 A diagram showing the types of change in electrical potential which can be recorded across the cell membrane of a motor neuron at the points indicated by the arrows. Excitatory and inhibitory synapses on the surfaces of the dendrites and soma cause local graded changes of potential which summate at the axon hillock, and may initiate a series of all-or-none action potentials, which are conducted along the axon to the effector terminals.



Ionic Regulation of Neuronal excitability

Sodium ions
Potassium ions
Calcium ions
Chloride ions

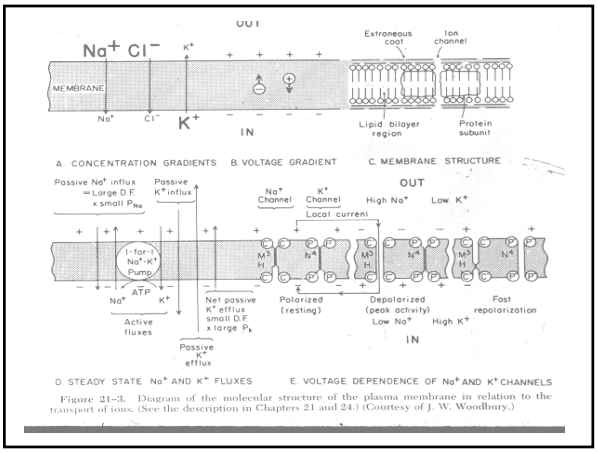
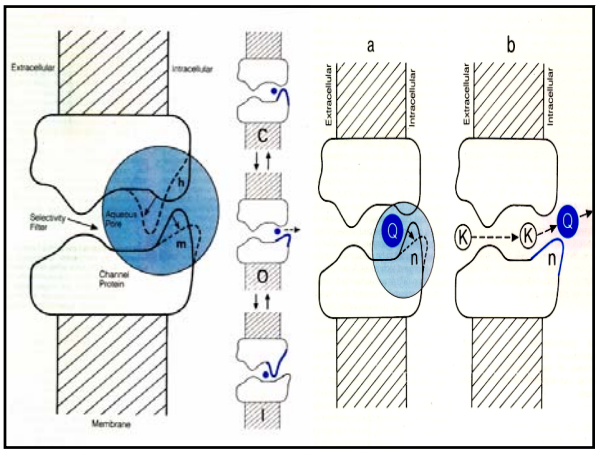


Figure 21–3. Diagram of the molecular structure of the plasma membrane in relation to the transport of ions. (See the description in Chapters 21 and 24.) (Courtesy of J. W. Woodbury.)



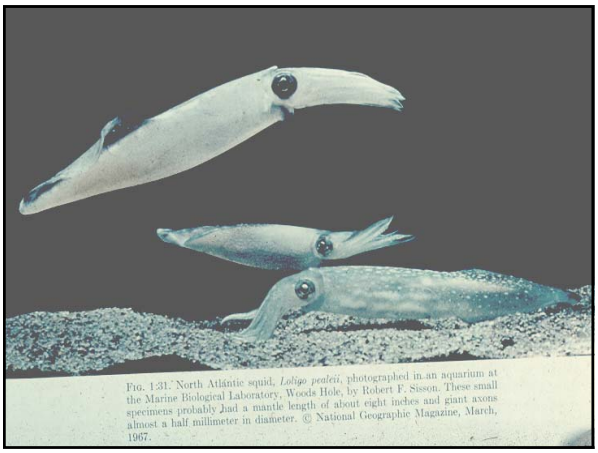
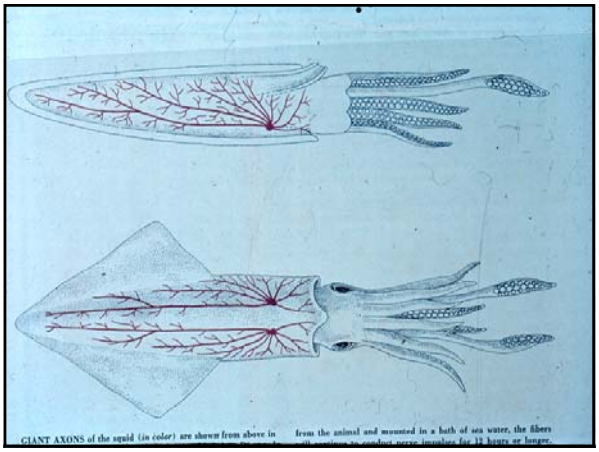
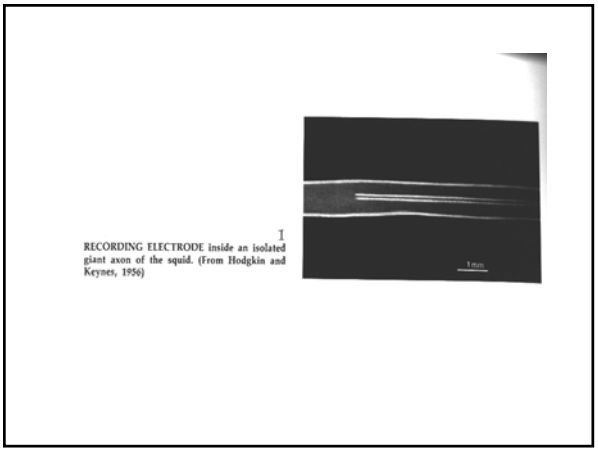


FIG. 1-31. North Atlantic squid, *Loligo pealeii*, photographed in an aquarium at the Marine Biological Laboratory, Woods Hole, by Robert F. Sisson. These small specimens probably had a mantle length of about eight inches and giant axons almost a half millimeter in diameter. © National Geographic Magazine, March, 1967.



GIANT AXONS of the squid (*in color*) are shown from above in from the animal and mounted in a bath of sea water, the fibers



RECORDING ELECTRODE inside an isolated giant axon of the squid. (From Hodgkin and Keynes, 1956)

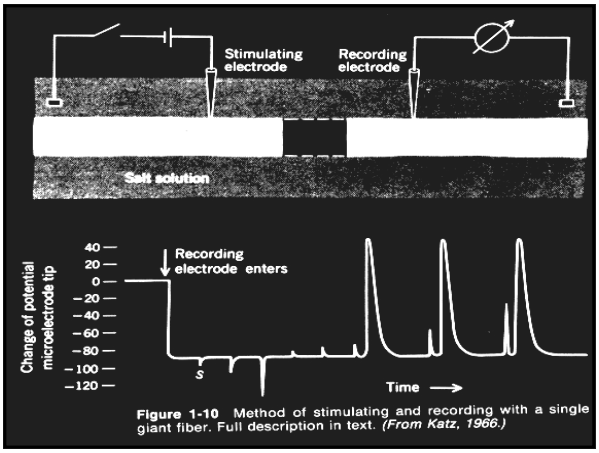
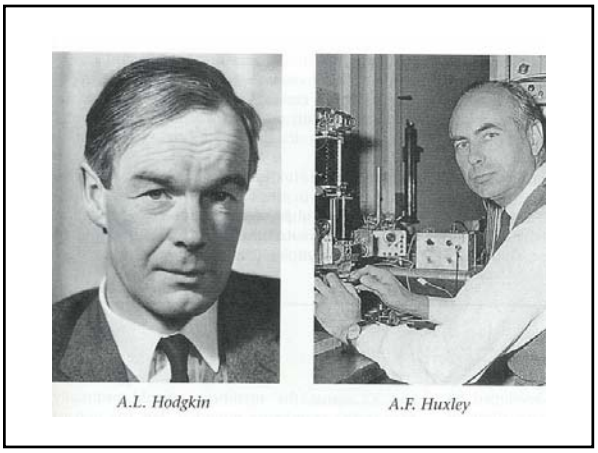


Figure 1-10 Method of stimulating and recording with a single giant fiber. Full description in text. *(From Katz, 1966.)*



A.L. Hodgkin *A.F. Huxley*

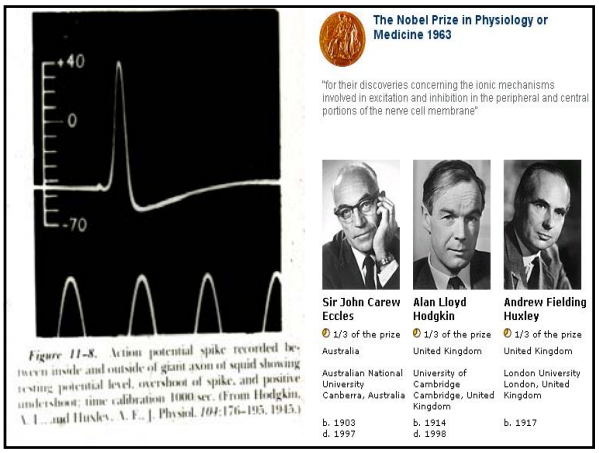
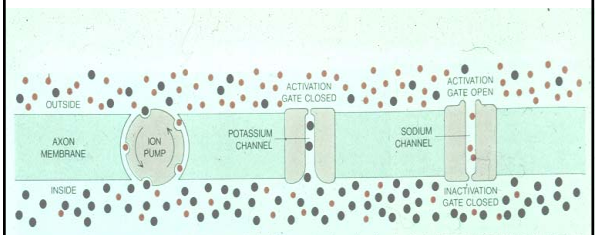


Figure 11–8. Action potential spike recorded between inside and outside of giant axon of squid showing resting potential level, overshoot of spike, and positive undershoot; time calibration 1000 sec. (From Hodgkin, A. L., and Huxley, A. F., J. Physiol. *104*:176–195, 1945.)

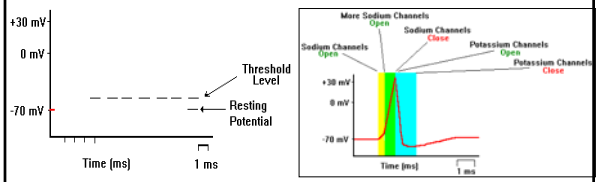
The Nobel Prize in Physiology or Medicine 1963

"for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane"

Sir John Carew Eccles	Alan Lloyd Hodgkin	Andrew Fielding Huxley
1/3 of the prize	1/3 of the prize	1/3 of the prize
Australia	United Kingdom	United Kingdom
Australian National University Canberra, Australia	University of Cambridge Cambridge, United Kingdom	London University London, United Kingdom
b. 1903 d. 1997	b. 1914 d. 1998	b. 1917

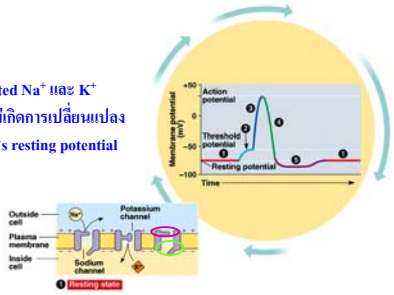


AXON MEMBRANE separates fluids that differ greatly in their content of sodium ions (*colored dots*) and potassium ions (*black dots*). The exterior fluid is about 10 times richer in sodium ions than in potassium ions; in the interior fluid the ratio is the reverse. The membrane is penetrated by proteins that act as selective channels for preferentially passing either sodium or potassium ions. In the resting state, when no nerve impulse is being transmitted, the two types of channel are closed and an ion pump maintains the ionic disequilibrium by pumping out sodium ions in exchange for potassium ions. The interior of the axon is normally about 70 millivolts negative with respect to the exterior. If this voltage difference is reduced by the arrival of a nerve impulse, the sodium channel opens, allowing sodium ions to flow into the axon. An instant later the sodium channel closes and the potassium channel opens, allowing an outflow of potassium ions. The sequential opening and closing of the two kinds of channel effects the propagation of the nerve impulse, which is illustrated below.



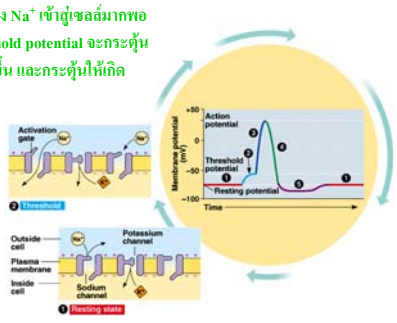
ระยะที่ 1: Resting State

-ทั้ง voltage-gated Na^+ และ K^+ channel ปิด ไม่เกิดการเปลี่ยนแปลงต่อ membrane's resting potential



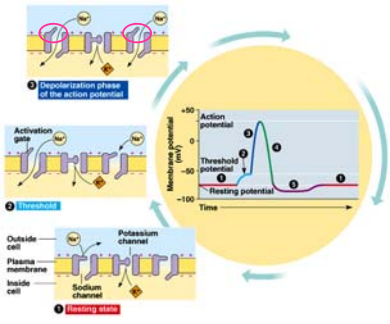
ระยะที่ 2: Threshold

-สิ่งเร้ามากระตุ้น ทำให้ Na^+ channel บางส่วนเปิด ถ้าการไหลของ Na^+ เข้าสู่เซลล์มากพอจนถึงระดับ threshold potential จะกระตุ้น Na^+ gate เปิดมากขึ้น และกระตุ้นให้เกิด action potential

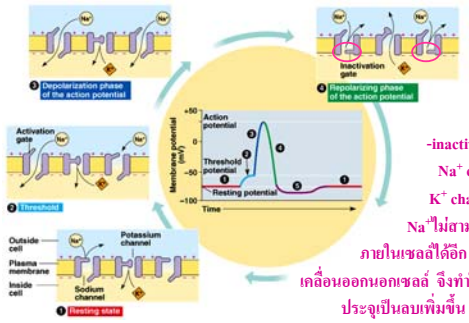


ระยะที่ 3: Depolarization

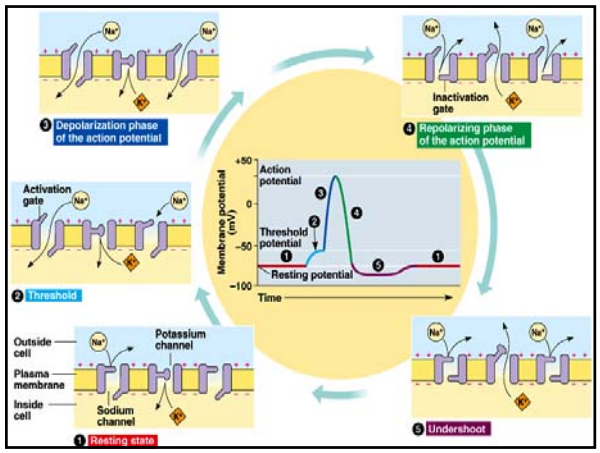
-activation gate ของ Na^+ channel เปิด แต่ K^+ channelยังคงปิดอยู่ ดังนั้นการเคลื่อนที่ของ Na^+เข้าภายในเซลล์จึงทำให้ภายในเซลล์มีประจุเป็นบวกมากขึ้น (หรือเป็นลบลดลง)

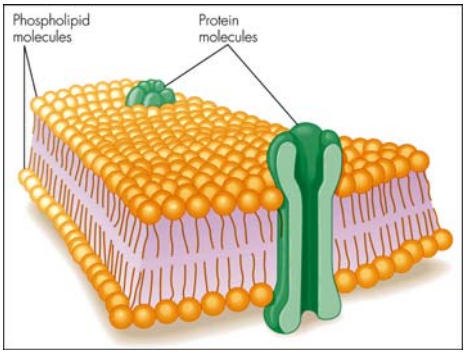


ระยะที่ 4: Repolarization

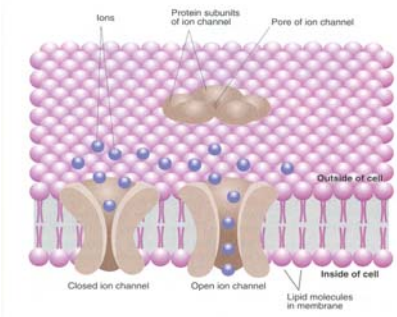


-inactivation gate ของ Na^+ channel ปิด และ K^+ channel เปิด ทำให้ Na^+ไม่สามารถเคลื่อนเข้าสู่ภายในเซลล์ได้อีก ในขณะที่ K^+จะเคลื่อนออกนอกเซลล์ จึงทำให้ภายในเซลล์มีประจุเป็นลบเพิ่มขึ้น กลับคืนสู่สภาวะ resting membrane potential

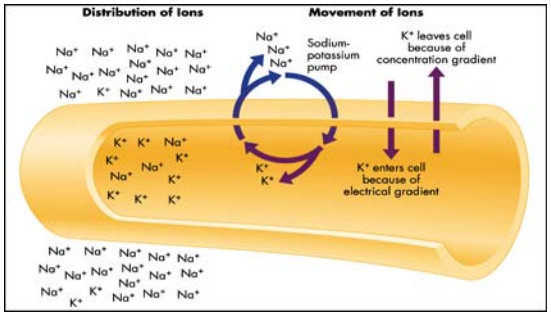




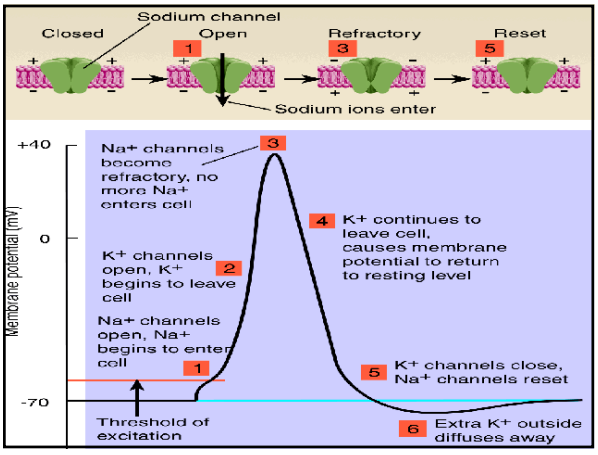
The membrane of a neuron
Embedded in the membrane are protein channels that permit certain ions to cross through the membrane at a controlled rate.

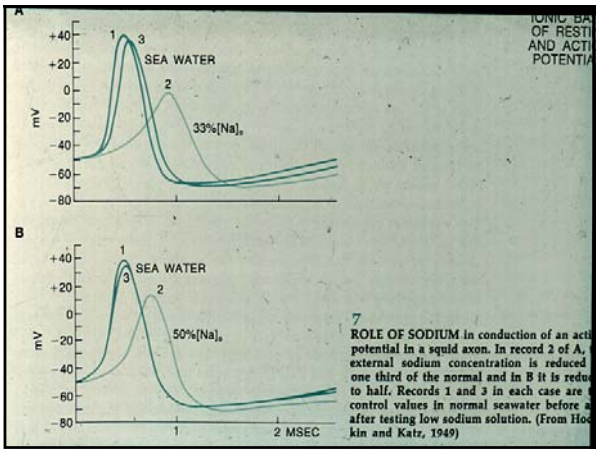


Ion channels. When they are open, ions can pass through them, entering or leaving the cell.



The sodium and potassium gradients for a resting membrane





7
ROLE OF SODIUM in conduction of an acti potential in a squid axon. In record 2 of A, external sodium concentration is reduced one third of the normal and in B it is redu to half. Records 1 and 3 in each case are control values in normal seawater before after testing low sodium solution. (From Hod kin and Katz, 1949)

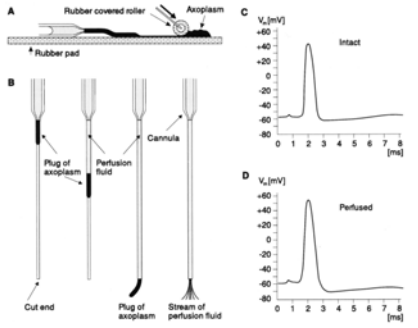
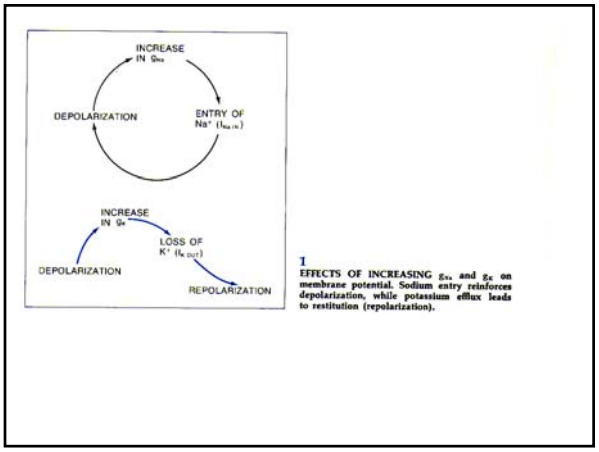
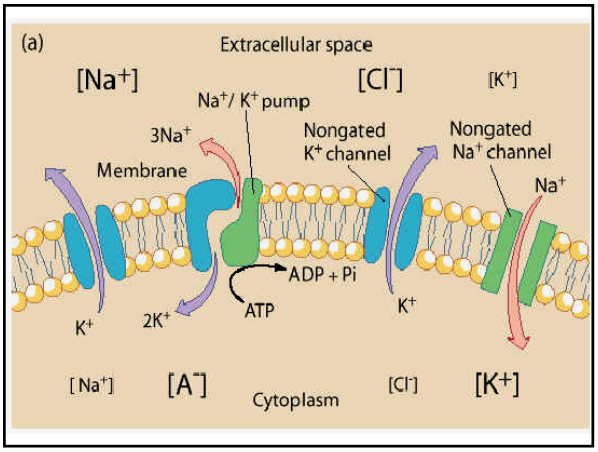
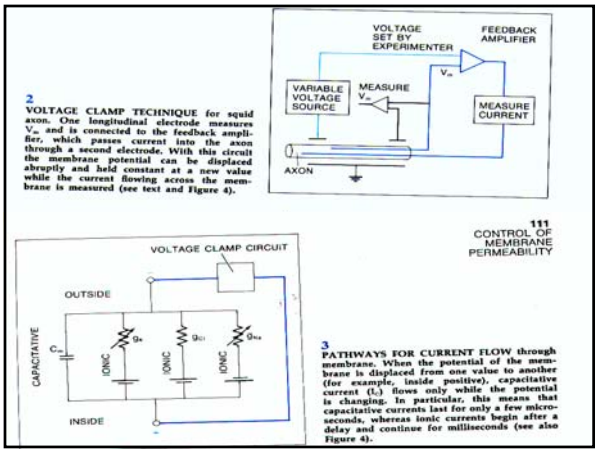


Fig. 4.8 Preparation of the squid axon for a voltage clamp experiment, where the internal ionic concentrations of the axon are changed. (A) The axoplasm is first squeezed out with a roller. (B) The axon is filled with perfusion fluid. (C) The axon impulse is measured before perfusion. (D) The axon impulse after perfusion.



1
EFFECTS OF INCREASING g_{Na} and g_K on membrane potential. Sodium entry reinforces depolarization, while potassium efflux leads to restitution (repolarization).





2
VOLTAGE CLAMP TECHNIQUE for squid axon. One longitudinal electrode measures V_m and is connected to the feedback amplifier, which passes current into the axon through a second electrode. With this circuit the membrane potential can be displaced abruptly and held constant at a new value while the current flowing across the membrane is measured (see text and Figure 4).

111
CONTROL OF MEMBRANE PERMEABILITY

3
PATHWAYS FOR CURRENT FLOW through membrane. When the potential of the membrane is displaced from one value to another (for example, inside positive), capacitative current (I_c) flows only while the potential is changing. In particular, this means that capacitative currents last for only a few microseconds, whereas ionic currents begin after a delay and continue for milliseconds (see also Figure 4).

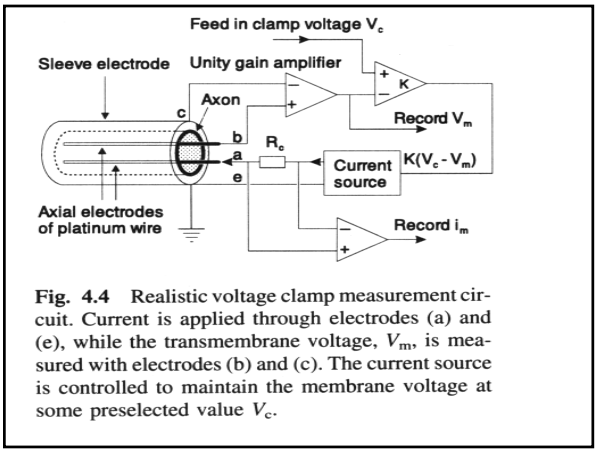


Fig. 4.4 Realistic voltage clamp measurement circuit. Current is applied through electrodes (a) and (e), while the transmembrane voltage, V_m, is measured with electrodes (b) and (c). The current source is controlled to maintain the membrane voltage at some preselected value V_c.

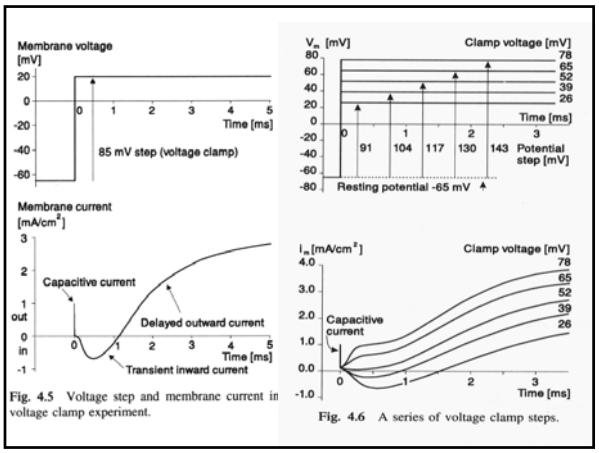
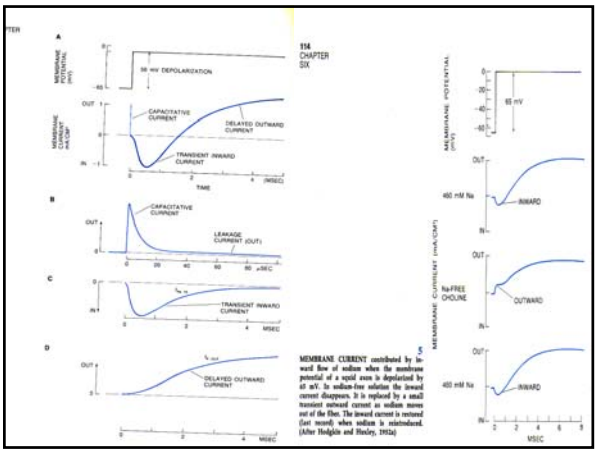
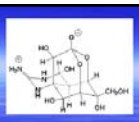


Fig. 4.5 Voltage step and membrane current in voltage clamp experiment.

Fig. 4.6 A series of voltage clamp steps.



Tetrodotoxin

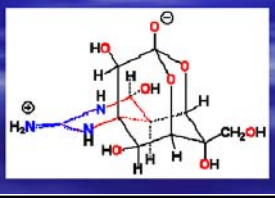


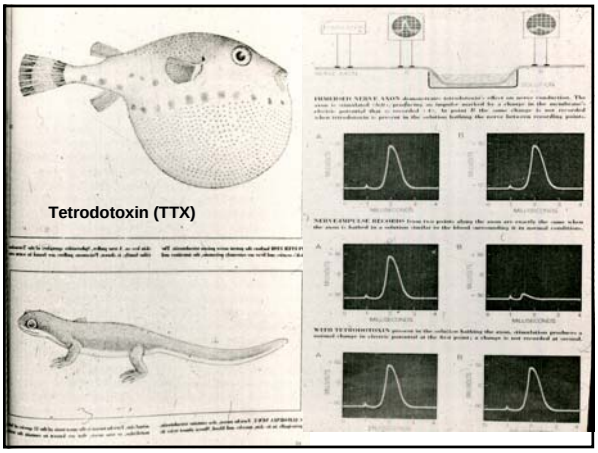
- Found in Pufferfish
- 1000 times deadlier than cyanide
- Tetrodotoxin is found in liver, muscles, skin, and ovaries.
- The victim will eventually die of respiratory paralysis.
- There is no known antidote for this poison.

- Puffer fish is eaten as a delicacy in Japan.
- Only licensed chefs are allowed to prepare Fugu.

- It acts by blocking the conduction of nerve impulses along nerve fibres and axons.
- Guanidinium ring is resonance stabilised
- The five hydroxyl groups give it additional stability

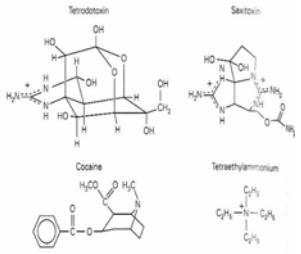
Chemical Name: Tetrodotoxin
Molecular Formula: $C_{11}H_{17}N_3O_8$

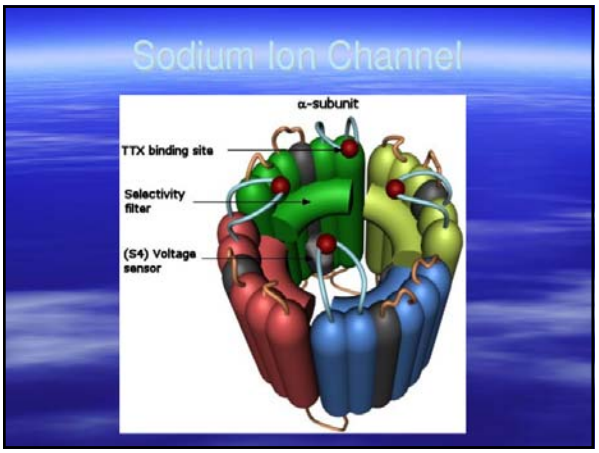




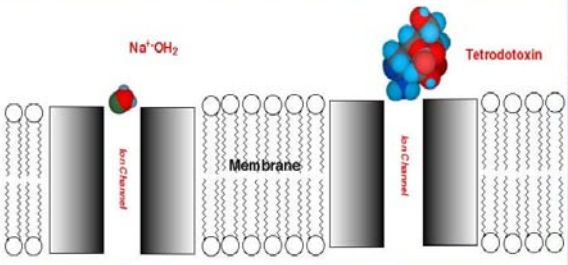
Tetrodotoxin (TTX)

Figure 9-4 Drugs that block voltage-gated Na^+ and K^+ channels. Tetrodotoxin and saxitoxin both bind to Na^+ channels with a very high affinity. **Tetrodotoxin** is produced by certain puffer fish, newts, and frogs. **Saxitoxin** is synthesized by the dinoflagellates *Gonyaulax* that are responsible for red tides. Consumption of clams or other shellfish that have fed on the dinoflagellates during a red tide causes paralytic shellfish poisoning. **Cocaine,** the active substance isolated from coca leaves, was the first substance to be used as a local anesthetic. It also blocks Na^+ channels but with a lower affinity and specificity than tetrodotoxin. **Tetraethylammonium** is a cation that blocks certain voltage-gated K^+ channels with a relatively low affinity. The red plus signs represent positive charge.





- Tetrodotoxin blocks the flow of sodium ions into nerve cells
- It contains positively charged guanidino group.
- This binds to negatively charged carboxylate groups of sodium channel



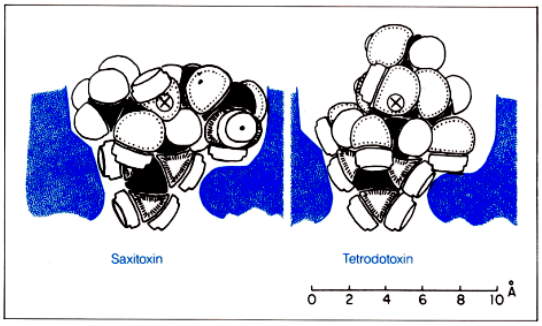
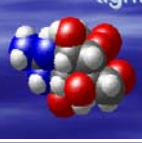
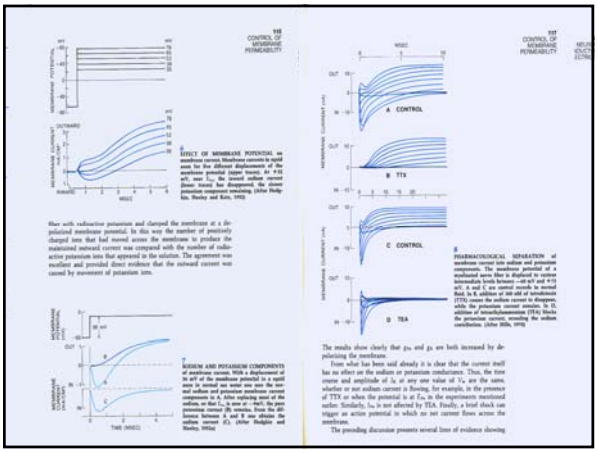


Figure 10 Saxitoxin and tetrodotoxin on their receptor. The shading on the atoms of toxin represent: Carbon, black; Hydrogen, white; Oxygen, dotted margin; Nitrogen, dash-margin. The stippled area represents the receptor in the sagittal section with a narrow selectivity filter below. Most of the receptor is hydrogen bond accepting, and there is a negative charge associated with the selectivity filter. A circled X has been drawn in the same position with respect to the receptor in both cases. The X falls on a hydroxyl group attached to an unusually electropositive carbon. (Taken from Hille, 1975).

Hydrated Na+ ion binds reversibly on a nanosecond time-scale; whereas TTX is bound for tens of seconds.

TTX is much larger than the Na+ ion and thus the TTX-Na channel-binding site is extremely tight ($Kd = 10^{-10}$ nM)





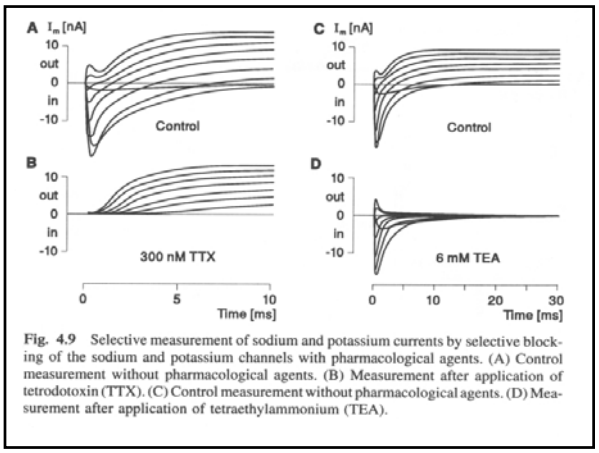
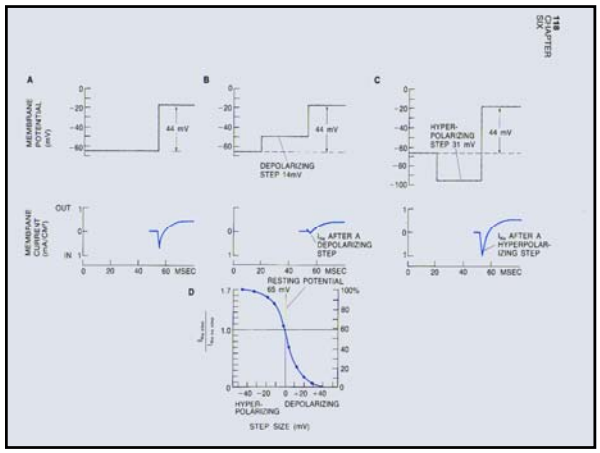


Fig. 4.9 Selective measurement of sodium and potassium currents by selective blocking of the sodium and potassium channels with pharmacological agents. (A) Control measurement without pharmacological agents. (B) Measurement after application of tetrodotoxin (TTX). (C) Control measurement without pharmacological agents. (D) Measurement after application of tetraethylammonium (TEA).



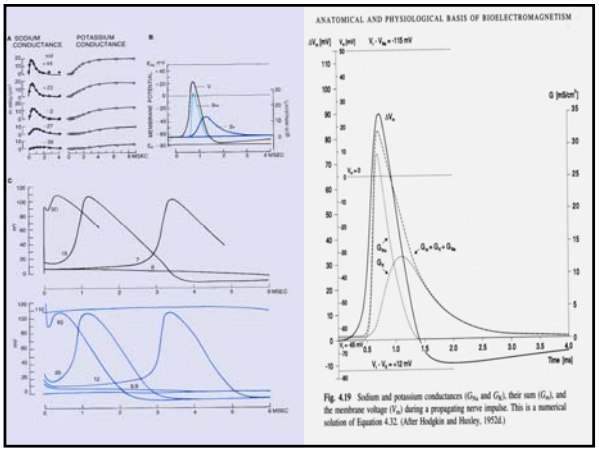


Fig. 4.19 Sodium and potassium conductances (G_{Na} and G_K), their sum (G_m), and the membrane voltage (V_m) during a propagating nerve impulse. This is a numerical solution of Equation 4.32. (After Hodgkin and Huxley, 1952d.)

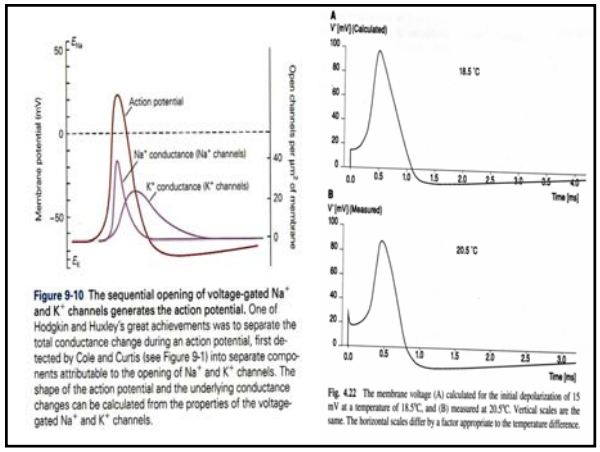


Figure 9-10 The sequential opening of voltage-gated Na^+ and K^+ channels generates the action potential. One of Hodgkin and Huxley's great achievements was to separate the total conductance change during an action potential, first detected by Cole and Curtis (see Figure 9-1) into separate components attributable to the opening of Na^+ and K^+ channels. The shape of the action potential and the underlying conductance changes can be calculated from the properties of the voltage-gated Na^+ and K^+ channels.

Fig. 4.22 The membrane voltage (A) calculated for the initial depolarization of 15 mV at a temperature of 18.5°C, and (B) measured at 20.5°C. Vertical scales are the same. The horizontal scales differ by a factor appropriate to the temperature difference.

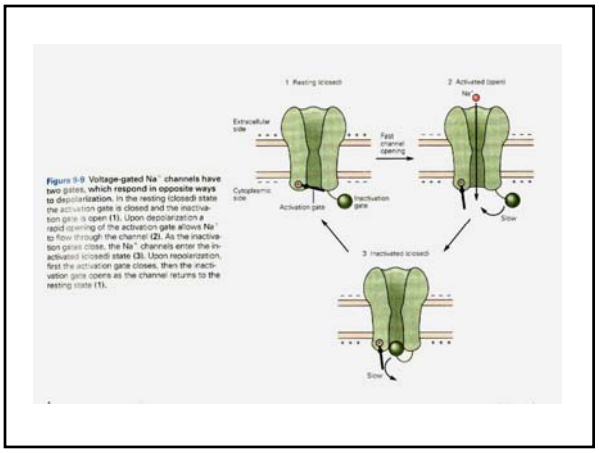


Figure 9-9 Voltage-gated Na^+ channels have two gates, which respond in opposite ways to depolarization. In the resting (closed) state the activation gate is closed and the inactivation gate is open (1). Upon depolarization a rapid opening of the activation gate allows Na^+ to flow through the channel (2). As the inactivation gate closes, the Na^+ channels enter the inactivated (closed) state (3). Upon repolarization, first the activation gate closes, then the inactivation gate opens as the channel returns to the resting state (1).

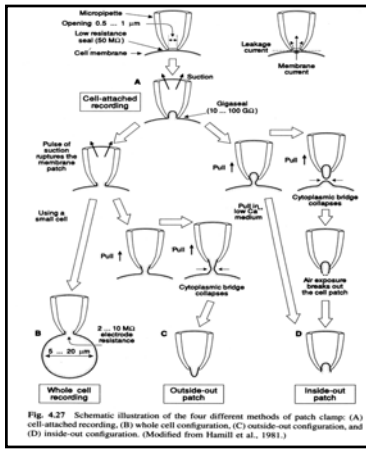
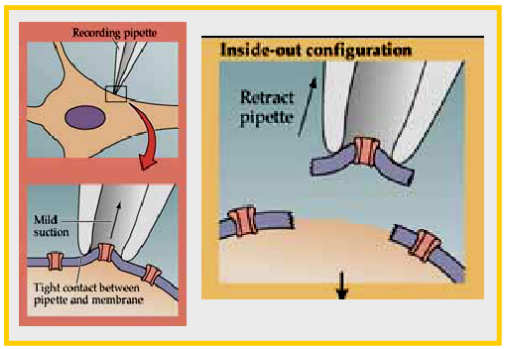


Fig. 4.27 Schematic illustration of the four different methods of patch clamp: (A) cell-attached recording, (B) whole cell configuration, (C) outside-out configuration, and (D) inside-out configuration. (Modified from Hamill et al., 1981.)

Membrane Patch Clamping Technique

Measuring individual ion channel opening



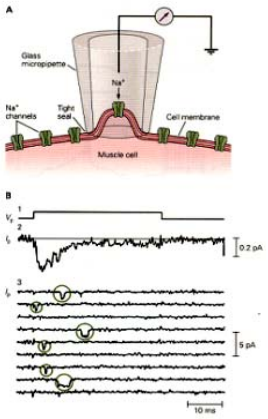


Figure 9-12 Individual voltage-gated channels open in an all-or-none fashion.

A. A small patch of membrane containing only a single voltage-gated Na^+ channel is electrically isolated from the rest of the cell by the patch electrode. The Na^+ current that enters the cell through these channels is recorded by a current monitor connected to the patch electrode.

B. Recordings of single Na^+ channels in cultured muscle cells of rats. **1.** Time course of a 10 mV depolarizing voltage step applied across the patch of membrane (V_p = potential difference across the patch). **2.** The sum of the inward current through the Na^+ channels in the patch during 300 trials (I_p = current through the patch of membrane). The trace was obtained by blocking the K^+ channels with tetraethylammonium and subtracting the capacitive current electronically. **3.** Nine individual trials from the set of 300, showing six individual Na^+ channel openings (**circles**). These data demonstrate that the total Na^+ current recorded in a conventional voltage-clamp record (see Figure 9-3C) can be accounted for by the all-or-none opening and closing of individual Na^+ channels. (From Sigworth and Neher 1980.)

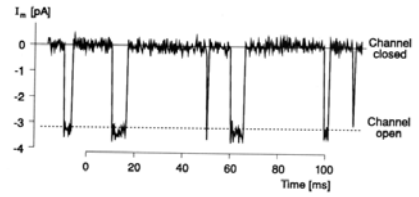
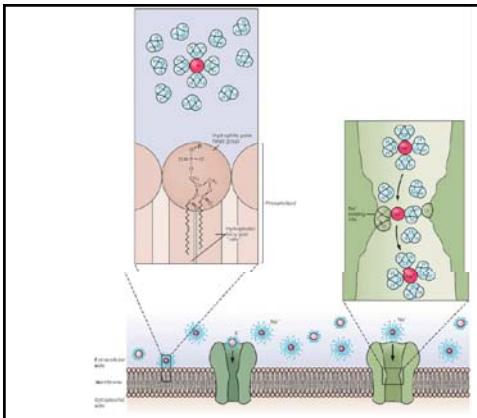
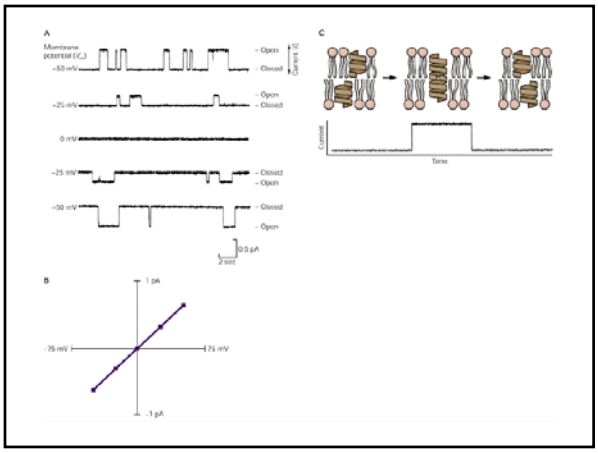
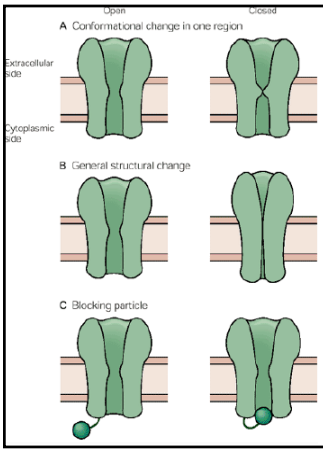


Fig. 4.28 Registration of the flow of current through a single ion channel at the neuromuscular endplate of frog muscle fiber with patch clamp method. (From Sakmann and Neher, 1984.)







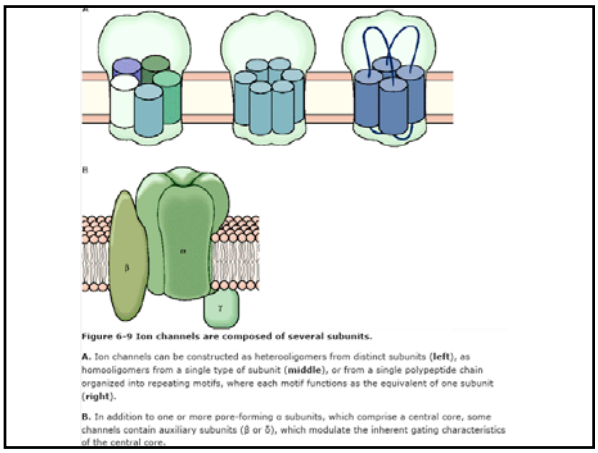
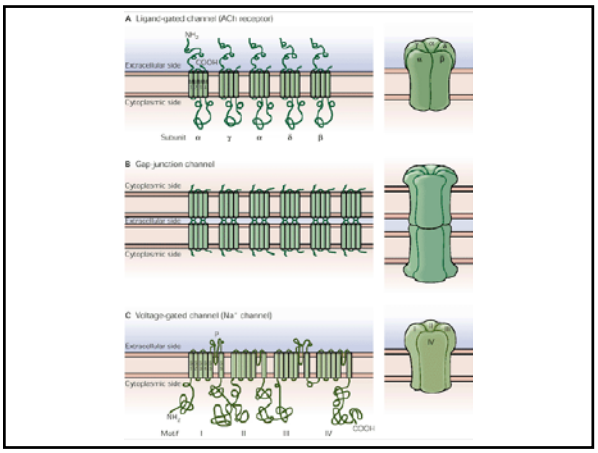


Figure 6-9 Ion channels are composed of several subunits.

A. Ion channels can be constructed as heterooligomers from distinct subunits (**left**), as homooligomers from a single type of subunit (**middle**), or from a single polypeptide chain organized into repeating motifs, where each motif functions as the equivalent of one subunit (**right**).

B. In addition to one or more pore-forming α subunits, which comprise a central core, some channels contain auxiliary subunits (β or δ), which modulate the inherent gating characteristics of the central core.



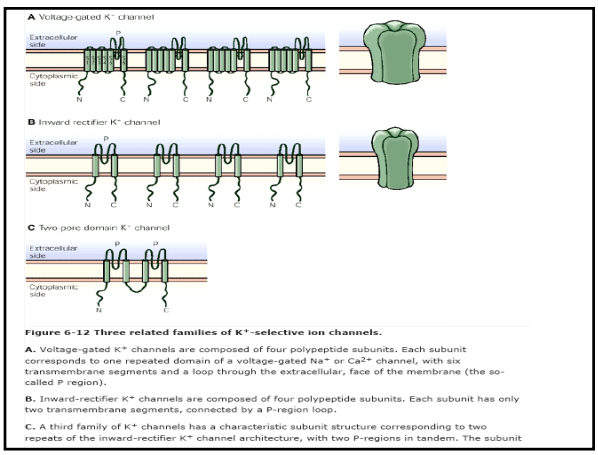


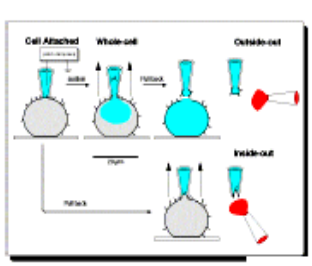
Figure 6-12 Three related families of K^+-selective ion channels.

A. Voltage-gated K^+ channels are composed of four polypeptide subunits. Each subunit corresponds to one repeated domain of a voltage-gated Na^+ or Ca^{2+} channel, with six transmembrane segments and a loop through the extracellular, face of the membrane (the so-called P region).

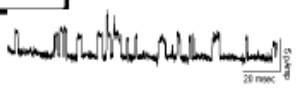
B. Inward-rectifier K^+ channels are composed of four polypeptide subunits. Each subunit has only two transmembrane segments, connected by a P-region loop.

C. A third family of K^+ channels has a characteristic subunit structure corresponding to two repeats of the inward-rectifier K^+ channel architecture, with two P-regions in tandem. The subunit

Patch clamp



Measures the current flowing through SINGLE channels.



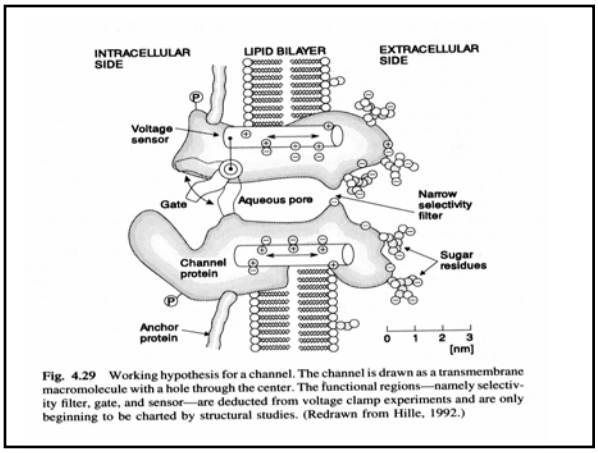
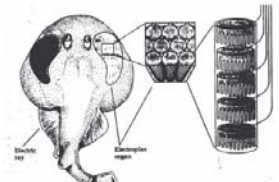


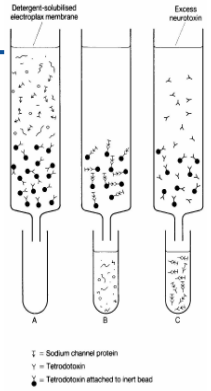
Fig. 4.29 Working hypothesis for a channel. The channel is drawn as a transmembrane macromolecule with a hole through the center. The functional regions—namely selectivity filter, gate, and sensor—are deducted from voltage clamp experiments and are only beginning to be charted by structural studies. (Redrawn from Hille, 1992.)

Torpedo electroplax



Cloning the sodium channel

- Start with tissue with loads of target: eel electroplax
- Purify protein by affinity to TTX
- Microsequence N-terminal
- Design degenerate probe
- Screen electroplax cDNA library



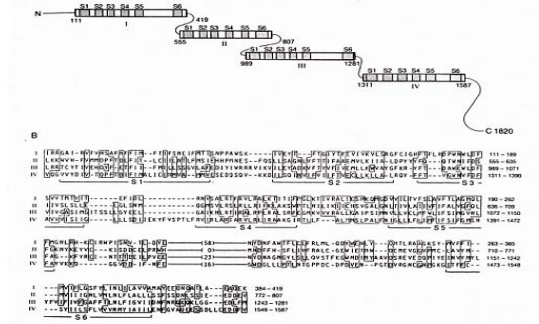


Figure 10.3 Domain structure and amino acid sequences of the four regions of internal homology of the sodium channel protein. (A) The 1820 amino acid sequence contains four homologous domains: I, II, III and IV. The first domain stretches from residue 111 to residue 419, the second from residue 555 to residue 807, the third from residue 989 to residue 1281 and the fourth from residue 1311 to residue 1587. Each homologous domain consists of six transmembrane segments, S1, S2, S3, S4, S5 and S6. (B) Amino acid sequence of the four homologous domains. The six transmembrane segments in each domain are labelled; S4 can be seen to contain a number of positively charged residues (R=arginine, K=lysine) and has been proposed as the voltage sensor. The number of residues in each line of the figure are given on the right; gaps (–) have been inserted to achieve maximum homology; the non-homologous regions of I, III and IV are shown by lines and the number of residues in each indicated in brackets; identical residues and conservative substitutions in all four domains are boxed. Reprinted by permission from Noda *et al.* (1984), *Nature*, **312**, 121–127. Copyright ©1984, Macmillan Magazines Ltd.

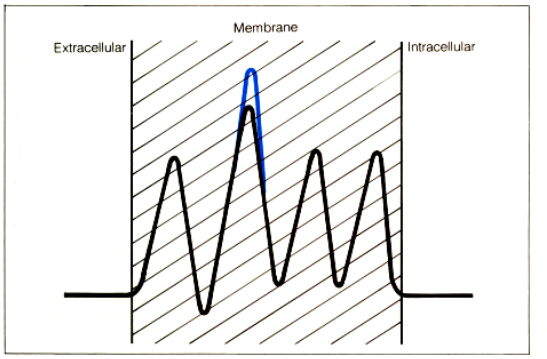
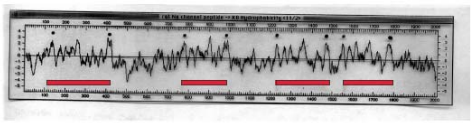


Figure 5 Energy profile along the diffusion path of a sodium ion in a sodium channel. The representation shows four energy peaks or barriers between which are three energy wells or binding sites. (The energy levels are usually given in RT units, the

DNA Strider shows the repeat structure



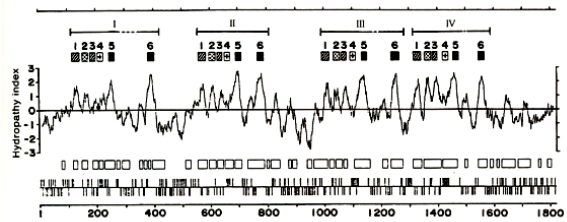


Fig. 4.17. Hydropathy profile of the electric eel voltage-gated sodium channel. The vertical axis shows the mean hydropathy index of 19 adjacent amino acid residues from / = -9 to / = 9. Positive peaks represent segments with a high proportion of hydrophobic residues that are interpreted as α-helices that cross the plasma membrane lipid bilayer. Homologous domains I to IV are shown, and the S1 to S6 segments within each one of them. The line of white boxes shows all sections of predicted α-helix or β-strand structure. The bottom line shows positions of the positively charged arginine and lysine residues as upward lines and the negatively charged aspartate and glutamate residues as downward lines. (From Noda *et al.*, 1984. Reprinted with permission from *Nature* **312**, p. 126, Copyright 1984 Macmillan Magazines Limited.)

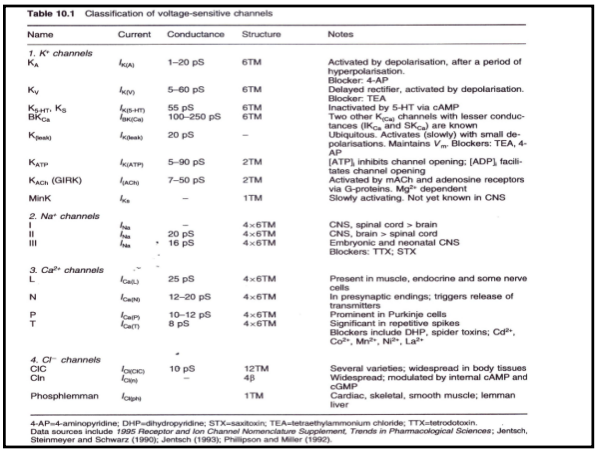


Table 10.1 Classification of voltage-sensitive channels

Name	Current	Conductance	Structure	Notes
1, K^+ channels				
K_A	$I_{K(A)}$	1–20 pS	6TM	Activated by depolarisation, after a period of hyperpolarisation. Blocker: 4-AP
K_V	$I_{K(V)}$	5–60 pS	6TM	Delayed rectifier, activated by depolarisation. Blocker: TEA
K_{5HT}, K_S	$I_{K(5\text{-}HT)}$	55 pS	6TM	Inactivated by 5-HT via cAMP
BK_{Ca}	$I_{BK(Ca)}$	100–250 pS	6TM	Two other $K_{(Ca)}$ channels with lesser conductances (IK_{Ca} and SK_{Ca}) are known
$K_{(leak)}$	$I_{K(leak)}$	20 pS	–	Ubiquitous. Activates (slowly) with small depolarisations. Maintains V_m. Blockers: TEA, 4-AP
K_{ATP}	$I_{K(ATP)}$	5–90 pS	2TM	[ATP]$_i$ inhibits channel opening; [ADP]$_i$ facilitates channel opening
K_{ACh} (GIRK)	$I_{K(ACh)}$	7–50 pS	2TM	Activated by mACh and adenosine receptors via G-proteins. Mg^{2+} dependent
MinK	I_{Ks}	–	1TM	Slowly activating. Not yet known in CNS
2. Na^+ channels				
I	I_{Na}	–	4×6TM	CNS, spinal cord > brain
II	I_{Na}	20 pS	4×6TM	CNS, brain > spinal cord
III	I_{Na}	16 pS	4×6TM	Embryonic and neonatal CNS
				Blockers: TTX; STX
3. Ca^{2+} channels				
L	$I_{Ca(L)}$	25 pS	4×6TM	Present in muscle, endocrine and some nerve cells
N	$I_{Ca(N)}$	12–20 pS	4×6TM	In presynaptic endings; triggers release of transmitters
P	$I_{Ca(P)}$	10–12 pS	4×6TM	Prominent in Purkinje cells
T	$I_{Ca(T)}$	8 pS	4×6TM	Significant in repetitive spikes
				Blockers include DHP, spider toxins; Cd^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, La^{2+}
4, Cl^- channels				
ClC	$I_{Cl(ClC)}$	10 pS	12TM	Several varieties; widespread in body tissues
Cln	$I_{Cl(n)}$	–	4β	Widespread; modulated by internal cAMP and cGMP
Phosphlemman	$I_{Cl(ph)}$		1TM	Cardiac, skeletal, smooth muscle; lemman liver

4-AP=4-aminopyridine; DHP=dihydropyridine; STX=saxitoxin; TEA=tetraethylammonium chloride; TTX=tetrodotoxin.
Data sources include *1995 Receptor and Ion Channel Nomenclature Supplement, Trends in Pharmacological Sciences*; Jentsch, Steinmeyer and Schwarz (1990); Jentsch (1993); Phillipson and Miller (1992).

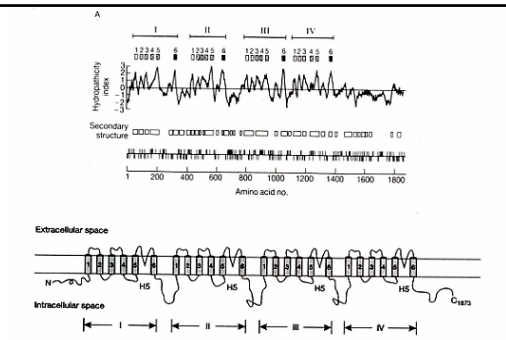


Figure 10.16 Structure of the calcium channel. (A) Hydropathy plot of the polypeptide chain. The further above the horizontal line the greater the hydrophobicity. Roman I, II, III and IV indicate the position of the homologous domains, while the boxes 1–6 denote the homologous segments within each domain. The white rectangles beneath the hydropathicity plot indicate where the α-helix or β-sheet is predicted and the vertical bars on the line beneath show the position of positively charged residues (Lys, Arg) (upward) and negatively charged residues (Asp, Glu) (downward). (B) Putative disposition in the membrane. In reality (as with the other channel proteins) the four homologous domains make use of the third dimension to cluster together around (in this case) a calcium-selective pore. Reprinted by permission from *Nature*, **328**, 313–318. Copyright © 1987 Macmillan Magazines Ltd.

Topology of the sodium channel

There are 24 transmembrane domains, arranged in 4 quarters

There is internal similarity between each set of 6.

This suggests that the channel has evolved from intragenic duplication of an ancestral, 6-pass channel that presumably formed a tetramer

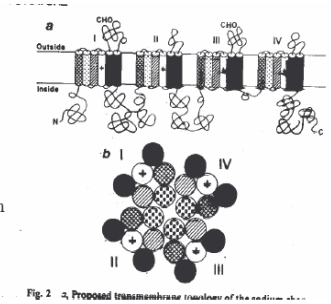


Fig. 2 a, Proposed transmembrane [illegible]

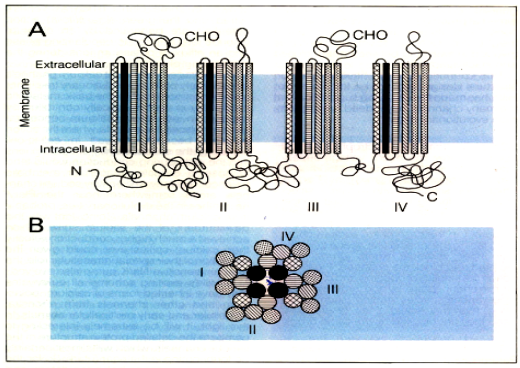
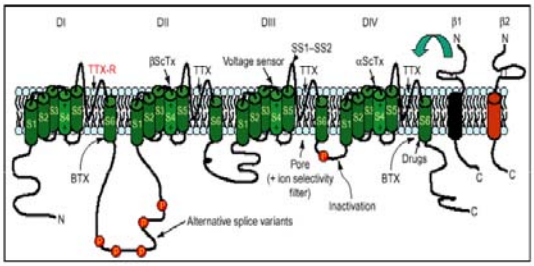


Figure 2 (A) Transmembrane topology of sodium channel proposed by Noda *et al.*, 1986. (B) Proposed arrangement of the transmembrane segments viewed in the direction perpendicular to the membrane. The six segments (S1-S6, numbered from left to right) in each of the four repeating subunits (I-IV) are indicated by the shading. The S4 segment is highly positively charged and may be involved in gating. (Based on Noda *et al.*, 1986).

Mammalian voltage-gated sodium channels



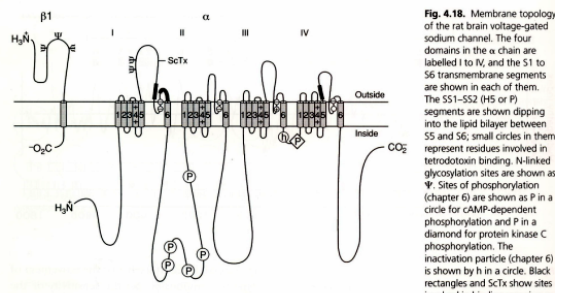


Fig. 4.18. Membrane topology of the rat brain voltage-gated sodium channel. The four domains in the α chain are labelled I to IV, and the S1 to S6 transmembrane segments are shown in each of them. The SS1–SS2 (H5 or P) segments are shown dipping into the lipid bilayer between S5 and S6; small circles in them represent residues involved in tetrodotoxin binding. N-linked glycosylation sites are shown as Ψ. Sites of phosphorylation (chapter 6) are shown as P in a circle for cAMP-dependent phosphorylation and P in a diamond for protein kinase C phosphorylation. The inactivation particle (chapter 6) is shown by h in a circle. Black rectangles and ScTx show sites involved in binding scorpion α-toxins. The principal α subunit contains the transmembrane pore; two auxiliary β subunits are present. The β2 chain (not shown in this diagram) is similar to the β1 chain in its topology. (From Catterall, 1992.)

topological arrangement shown in fig. 4.18. Evidence in agreement with this comes from the positions of phosphorylation sites (which must be cytoplasmic), and of glycosylation sites and binding sites for scorpion toxins (which must both be on the extracellular side of the membrane), and also from antibody binding studies (see Catterall, 1988, 1992; Trimmer & Agnew, 1989).

It seems very reasonable to assume that the four domains of the protein chain are clustered together to form a cylindrical rosette with an aqueous pore

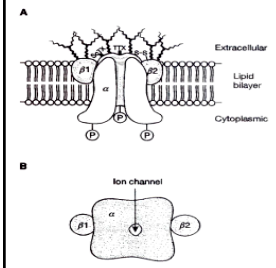


Figure 10.2 Subunit structure of brain Na^+ channel. (A) Schematic cross-section of channel inserted in membrane. The channel complex consists of the 260 kDa α-subunit and the two smaller β_1- and β_2-subunits. All three subunits are heavily glycosylated on their extracellular surfaces and the α-subunit has binding sites for α-scorpion toxins (ScTX) and tetrodotoxin (TTX). The intracellular surface of the α-subunit has numerous phosphorylation sites. The β_1-subunit is associated non-covalently with α while the β_2-subunit is linked via disulphide bonds. (B) Surface view from the extracellular side showing the ion channel in the centre of the α-subunit. From Catterall (1992), *Physiological Reviews*, **72**, S15–S48; with permission.

Internal repeats are emphasised by a matrix plot

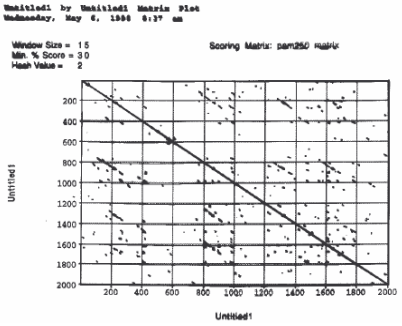


Table 10.2 Amino acid residues in TM4

K channels																				
Shaker	R	V	I	R	L	V	R	V	F	R	I	F	K	L	S	R	H	S	K	G	L
Shab	Q	V	F	R	I	M	R	I	L	R	V	L	K	L	A	R	H	S	T	G	L
Shaw	E	F	F	S	I	I	R	I	M	R	L	F	K	V	T	R	H	S	S	G	L
Shal	F	V	T	R	V	F	R	V	F	R	I	F	K	F	S	R	H	S	Q	G	L
Na channels	S	A	L	R	T	F	R	V	L	R	A	L	K	T	I	S	V	I	P	G	L
Ca channels	S	V	L	R	C	I	R	L	L	R	L	F	K	I	T	K	Y	W	T	S	L

Conventional one-letter code for amino acids; those with side chains bearing positive charges emphasised: H=histidine; K=lysine; R=arginine.
After Catterall (1992), *Physiological Reviews*, **72**, S15–S48.

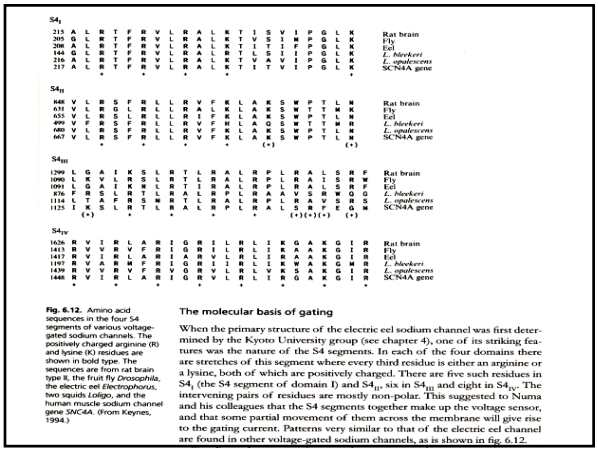
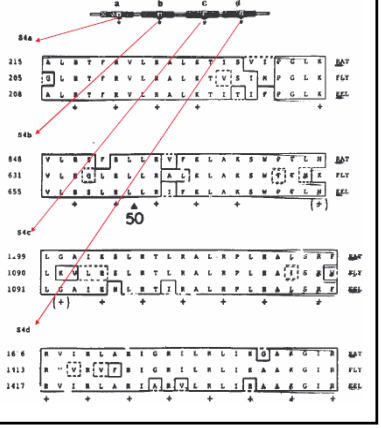


Fig. 6.12. Amino acid sequences in the four S4 segments of various voltage-gated sodium channels. The positively charged arginine (R) and lysine (K) residues are shown in bold type. The sequences are from rat brain type II, the fruit fly *Drosophila*, the electric eel *Electrophorus*, two squids *Loligo*, and the human muscle sodium channel gene *SNC4A*. (From Keynes, 1994.)

The molecular basis of gating

When the primary structure of the electric eel sodium channel was first determined by the Kyoto University group (see chapter 4), one of its striking features was the nature of the S4 segments. In each of the four domains there are stretches of this segment where every third residue is either an arginine or a lysine, both of which are positively charged. There are five such residues in $S4_I$ (the S4 segment of domain I) and $S4_{II}$, six in $S4_{III}$ and eight in $S4_{IV}$. The intervening pairs of residues are mostly non-polar. This suggested to Numa and his colleagues that the S4 segments together make up the voltage sensor, and that some partial movement of them across the membrane will give rise to the gating current. Patterns very similar to that of the electric eel channel are found in other voltage-gated sodium channels, as is shown in fig. 6.12.

Conserved charges in the S4 helices



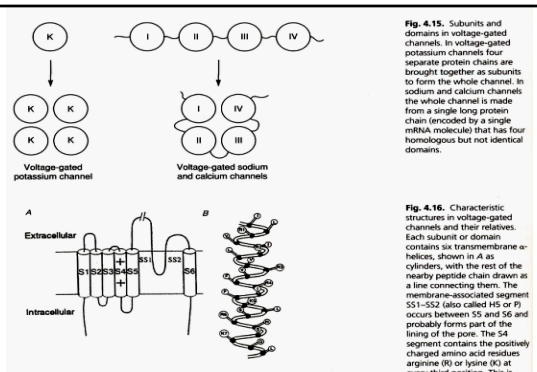
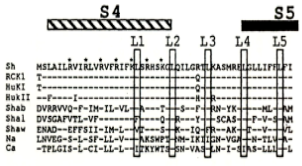


Fig. 4.15. Subunits and domains in voltage-gated channels. In voltage-gated potassium channels four separate protein chains are brought together as subunits to form the whole channel. In sodium and calcium channels the whole channel is made from a single long protein chain (encoded by a single mRNA molecule) that has four homologous but not identical domains.

Fig. 4.16. Characteristic structures in voltage-gated channels and their relatives. Each subunit or domain contains six transmembrane α-helices, shown in A as cylinders, with the rest of the nearby peptide chain drawn as a line connecting them. The membrane-associated segment S5–S52 (also called H5 or P) occurs between S5 and S6 and probably forms part of the lining of the pore. The S4 segment contains the positively charged amino acid residues arginine (R) or lysine (K) at every third position. This is shown for the *Shaker* potassium channel in *B*. (From Catterall, 1993. Reproduced from *Trends in Neurosciences*, with permission from Elsevier Trends Journals.)

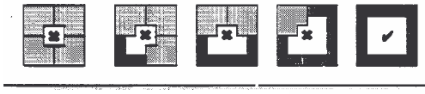
Molecular studies in recent years have shown that they and similar channels form a superfamily of related proteins with a number of structural features in common. Not all members of the superfamily are gated solely by changes in membrane potential, and so partly for this reason the terms 'voltage-dependent' or 'voltage-sensitive' are sometimes used instead of 'voltage-gated' to describe them.



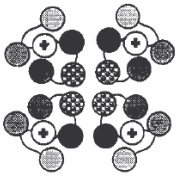
sodium channels. They injected mRNAs made from the altered cDNAs into *Xenopus* oocytes, so that the mutant sodium channels would be expressed in the oocyte membrane and could be investigated by voltage clamping large patches of membrane. They found that the steepness of the relation between channel opening and the membrane potential was progressively reduced as the positively charged residues of the S4, segment were replaced by neutral or

Fig. 6.13. Amino acid sequences in the S4 and leucine-heptad regions of various voltage-gated channels. The top seven sequences are from potassium channels, the sodium channel sequence (Na) is the second domain of the rat brain IIa sodium channel, and the calcium channel sequence (Ca) is from that of the skeletal muscle dihydropyridine receptor. Amino acids identical to *Shaker* (Sh) are shown by dashes. Asterisks show positively charged R and K residues in S4. Boxes show leucine residues in the heptad repeat. (From McCormack *et al.*, 1991.)

A 4-state gating model



The 4 S4 helices suggest an obvious mechanism for channel gating.



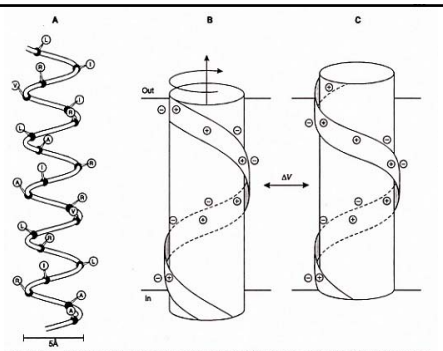
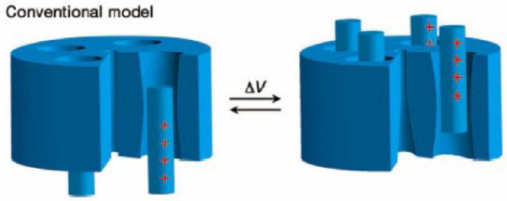


Figure 10.18 S4 'sliding helix' voltage sensor. (A) 'Ball and stick' representation of the S4 helix of domain 4 of the sodium channel. Dark circles represent the α-carbon atoms of each amino acid residue; open circles are labelled with the amino acid single-letter code and show the direction in which the side chain projects. (B and C) Movement of the helix in response to voltage change (Δ*V*) across the membrane. At resting potential (B) all positively charged side chains are paired with fixed negative charges on other transmembrane domains of the channel protein. The helix is held in this position by the negative internal potential of the resting membrane (V_m). On depolarisation the helix 'screws' outwards by 5 Å and through 60°. As indicated in C this leaves an unpaired negative charge on the P-face of the membrane and an unpaired positive charge on the E-face. Further explanation in text. From Catterall (1992) *Physiological Reviews*, **72**, S15–S48; with permission.

The 'sliding helix' model for channel gating

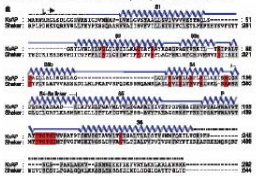


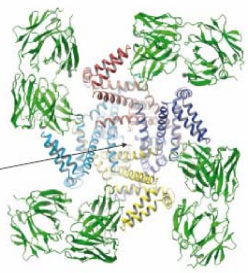
Problems with structure

- Voltage gated channels were thought to be impossible to crystallise
- McKinnon's group tried many organisms, but could not get stable crystals
- Thought that this meant that part of the protein was very mobile
- So used sequence from thermophilic bacterium
- AND raised monoclonals to S4 domain
- WERE able to crystallise

The structure

Comparing Shaker with KvAP, shows that it's a true voltage-gated channel



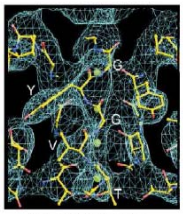


Fab fragments (attached to S4 helix) in green

The pore

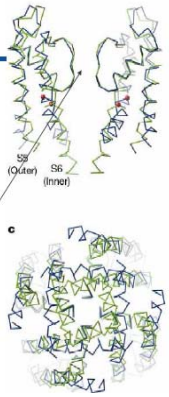
The S4 helix is in a very unexpected place : a 'paddle'

The channel



4 green K+ ions line the channel

S5 and S6 line the channel



Selectivity filter

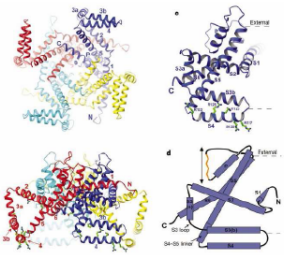
S3 and S4 contribute to the voltage-sensing 'paddle'

S3 can be split in two : S3a that sits wit the main protein, and S3b, that forms an antiparallel helix against S4.

S3a and S3b are joined by a loose loop.

S4 is linked to S5 by a much tighter linker.

So while the paddle is free to move, changes in position of S4 could be expected to move S5, and hence change the shape of the pore.



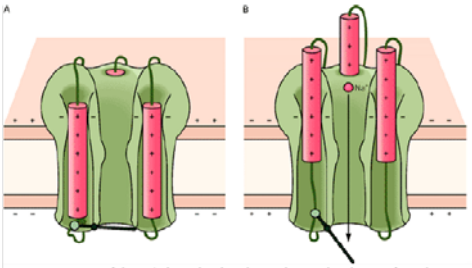


Figure 9-16 Gating of the Na^+ channel is thought to rely on redistribution of net charge in the S4 region.

A. At rest, the inside-negative electric field across the membrane biases the positively charged S4 helix toward the inside of the membrane. One of the positive charges is stabilized by interaction with a negative charge in another part of the channel. The remainder of the charged region lies in a water-filled cavity in the channel wall that is continuous with the cytoplasm.

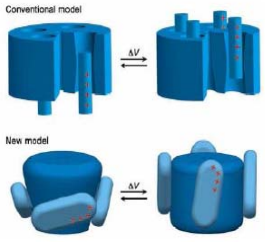
B. When the cell is depolarized the change in electrical field across the membrane drives the S4 region toward the extracellular face of the membrane. This change in configuration opens the activation gate by a mechanism that is not well understood. (Adapted from Yang et al. 1996.)

A new model for gating

In the traditional sliding helix model, 4 S4 helices were buried within the protein and moved in response to the field change as the membrane depolarised

In the new model, the 4 paddles make large movements through the membrane, and pull the base of the S5 helix open.

In both models, all 4 quarters have to decide to open before the pore is big enough to pass ions.



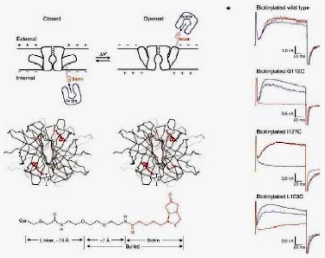
Cysteine scanning mutagenesis

Principle: mutate the only C in the protein, then introduce C's around the S4 domain.

Bind biotin.

Then ask:

Does avidin bind from outside, inside or both?

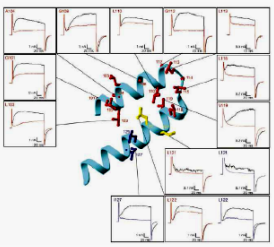


Some residues must move across the membrane

Red: residues accessible from outside

Blue: residues accessible from the inside

Yellow: residues accessible from both sides

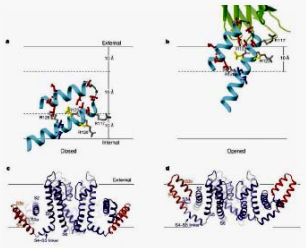


So how does the shape change?

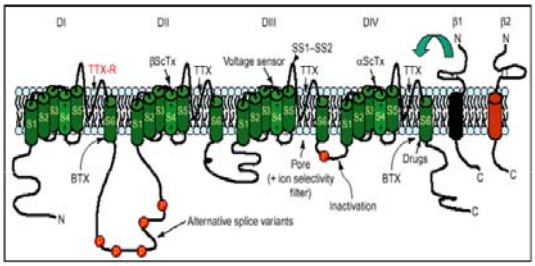
Fab binding mimics depolarisation

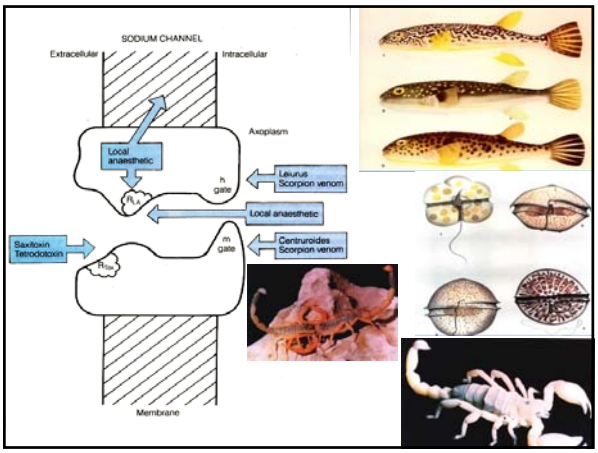
pulls paddle to surface

Opens channel by pulling at base of S5



Mammalian voltage-gated sodium channels





Mammalian voltage-gated sodium channel α subunits

Type	Gene Symbol	Name	Primary Tissue	Present in DRG	TTX sensitivity
$Na_v1.1$	SCN1a	type I	CNS, heart	+	+
$Na_v1.2$	SCN2a	type II	CNS	+ (embryonic)	+
$Na_v1.3$	SCN3a	type III	foetal brain	+ (embryonic)	+
$Na_v1.4$	SCN4a	SkM1 (μ1)	skeletal muscle	-	+
$Na_v1.5$	SCN5a	SkM2 (H1)	Heart	+ (embryonic, rat) (adult, mouse)	-
$Na_v1.6$	SCN8a	NaCh6	CNS, glial cells	+	+
$Na_v1.7$	SCN9a	PN1	SCG, CNS	+	+
$Na_v1.8$	SCN10a	SNS (PN3)	DRG	+ (small & medium cells)	-
$Na_v1.9$	SCN11a	NaN (SNS2)	DRG	+ (most small cells)	-
Na_X	SCN7a	NaG	sciatic nerve, lung	+	+(?)

Mutations in Voltage-Gated Channels Cause Specific Neurological Diseases

Several inherited neurological disorders are now known to be caused by mutations in voltage-gated ion channels. Patients with hyperkalemic periodic paralysis have episodes of muscle stiffness (myotonia) and muscle weakness (paralysis) in response to the elevation of K^+ levels in serum after vigorous exercise. Genetic studies have shown that the disease is caused by a point mutation in the α-subunit of the gene for the voltage-gated Na^+ channel found in skeletal muscle. Voltage-clamp

studies of cultured skeletal muscle cells obtained from biopsies of patients with this disorder demonstrate that the voltage-gated Na^+ channels fail to completely inactivate. This defect is exacerbated by elevation of external K^+. The prolonged opening of the Na^+ channels is thought to cause muscles to fire repetitive trains of action potentials, thus producing the muscle stiffness. As the fraction of channels with altered inactivation increases (as a result of continued K^+ elevation), the muscle resting potential eventually reaches a new stable depolarized level (around -40 mV), at which point most Na^+ channels become inactivated so that the membrane fails to generate further action potentials (paralysis).

Hyperkalemic periodic paralysis and SCN4a

SCN4A

Official Symbol SCN4A and **Name:** sodium channel, voltage-gated, type IV, alpha subunit [*Homo sapiens*]

Other Aliases: HYKPP, HYPP, NAC1A, Na(V)1.4, Nav1.4, SkM1

Other Designations: skeletal muscle voltage-dependent sodium channel type IV alpha subunit; voltage-gated sodium channel type 4 alpha

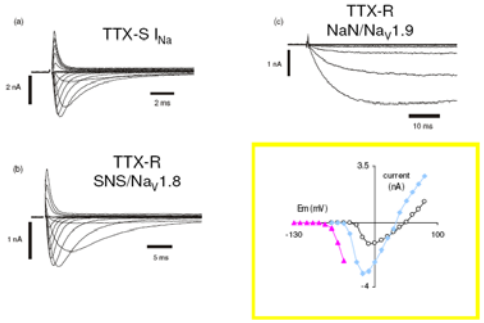
Chromosome: 17; **Location:** 17q23-q25.3

Annotation: Chromosome 17, NC_000017.9 (59369646..59404010, complement)

MIM: 603967

GeneID: 6329

TTX-s and TTX-r Na^+ currents in small diameter DRG neurons



Role of sodium channels in DRG

- $Na_V1.3$ and neuropathic pain

Not expressed in the adult PNS. Down-regulated by GDNF. Re-expression after nerve damage was thought to be responsible for ectopic discharges but KO animals are still neuropathic and still display ectopic discharges…

- $Na_V1.7$ and inflammation

Located at sensory neurons' terminals. Up-regulated by inflammatory mediators such as NGF. Mutations in $Na_V1.7$ are involved in human dominant inflammatory pathologies (Paroxysmal Extreme Pain Disorder and Primary Erythermalgia) and congenital inability to experience pain.

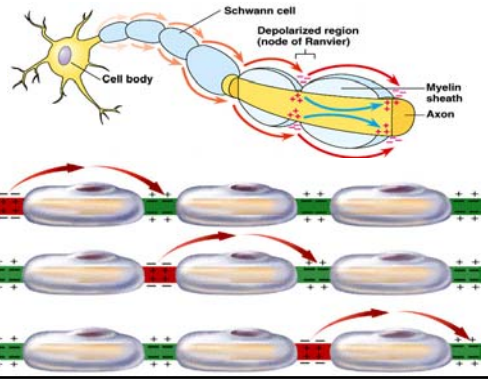
- $Na_V1.8$ and nociception

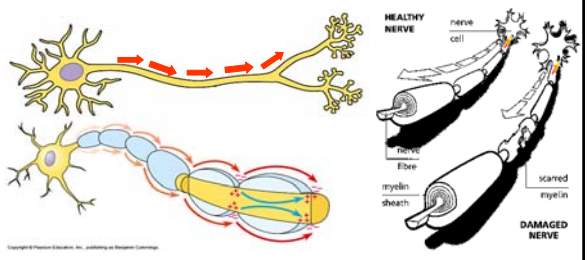
TTX-R sensory neuron-specific channel. Contributes the majority of the sodium current underlying action potentials in nociceptors. Activation facilitated by inflammatory mediators (PKA).

- $Na_V1.9$

Expressed in some nociceptors. Too slow to contribute to action potentials. Sets firing threshold. Up-regulated by G-protein pathways. Down-regulated after axotomy.

Saltatory propagation (Conduction)





ไมอิลินมีผลต่อการส่งกระแสประสาทอย่างไร?

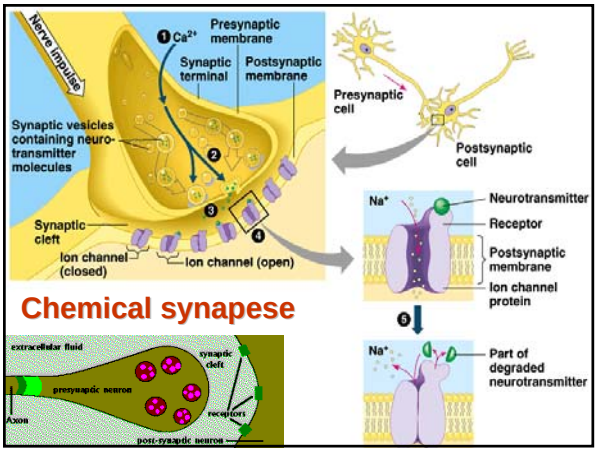
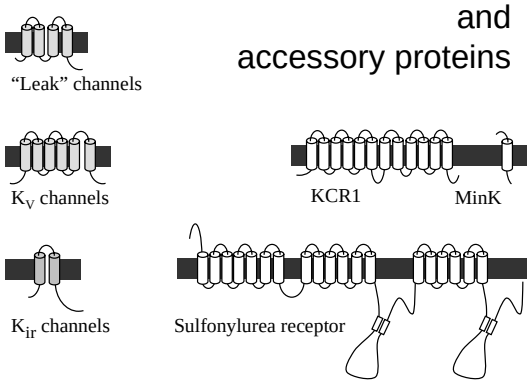


Table 4.5. *Homologies and nomenclature of some* Drosophila *and mammalian voltage-gated potassium channel genes*

	Mammal			
Drosophila		Mouse	Rat	Human
Shaker	Kv1.1	MBK1 MK1	RCK1, RBK1 RMK1, RK1	HBK1, HK1
	Kv1.2	MK2	RCK5, BK2 RK2, NGK1	HBK4
	Kv1.3	MK3	RCK3, RGK5 KV3	HGK5, HPCN
	Kv1.4		RCK4, RHK1 RK3	HBK4, HK2 hPCN2
	Kv1.5		RCK7, KV1 RK4	hPCN1
	Kv1.6		RCK2, KV2	HBK2
	Kv1.7	MK4		
	Kv1.8		RCK9	
Shab	Kv2.1	*MShab*	DRK1	DHK1
	Kv2.2		cdrk	
Shaw	Kv3.1	NGK2	KV4, Raw2 Raw2a	
	Kv3.2		*RkShIIIA*, Raw1	
	Kv3.3	MK5		KCNC3
	Kv3.4	MK6	Raw3	KCNC4
Shal	Kv4.1	*MShal*	*RShal*	
	Kv4.2		RK5	

Different laboratories have used different ways of naming the various mammalian channel genes; some of these are listed in the table. The Kv1.1 etc. notation was introduced by Chandy *et al.* (1991), and is now generally used. The other names can be converted to this system: DHK1, for example, becomes human Kv2.1 or hKv2.1. Partly after Pongs (1992), with information from Strong *et al.* (1993).

Structural types of K^+ channels and accessory proteins



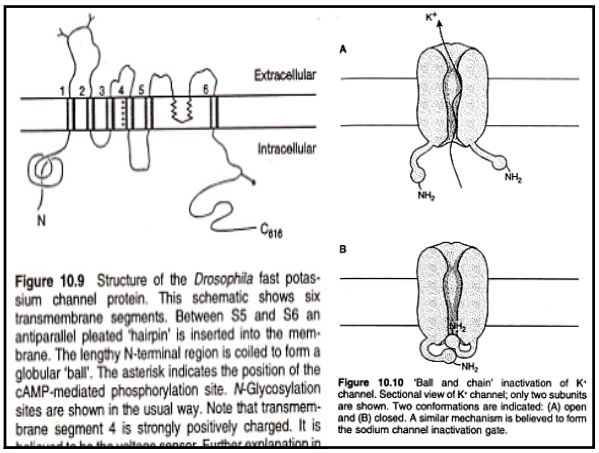


Figure 10.9 Structure of the *Drosophila* fast potassium channel protein. This schematic shows six transmembrane segments. Between S5 and S6 an antiparallel pleated 'hairpin' is inserted into the membrane. The lengthy N-terminal region is coiled to form a globular 'ball'. The asterisk indicates the position of the cAMP-mediated phosphorylation site. *N*-Glycosylation sites are shown in the usual way. Note that transmembrane segment 4 is strongly positively charged. It is

Figure 10.10 'Ball and chain' inactivation of K^+ channel. Sectional view of K^+ channel; only two subunits are shown. Two conformations are indicated: (A) open and (B) closed. A similar mechanism is believed to form the sodium channel inactivation gate.

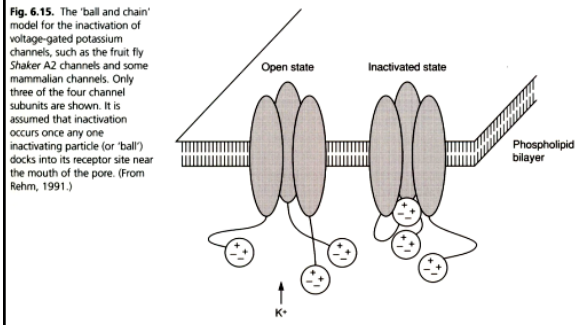
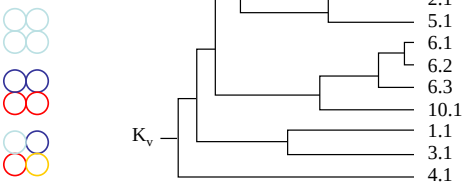


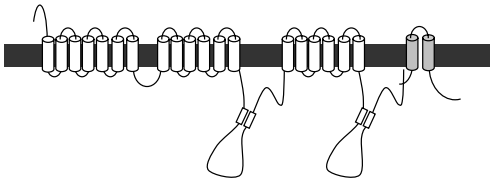
Fig. 6.15. The 'ball and chain' model for the inactivation of voltage-gated potassium channels, such as the fruit fly *Shaker* A2 channels and some mammalian channels. Only three of the four channel subunits are shown. It is assumed that inactivation occurs once any one inactivating particle (or 'ball') docks into its receptor site near the mouth of the pore. (From Rehm, 1991.)

Humans have multiple channel variants for each major ion involved in membrane excitation

Variants of human K_v channel subunits



the K^+-channels is the ATP-sensitive 'inward rectifier' channel



Cation channels as drug targets

Sodium channels:

Cardiac excitation	→	arrhythmia
Neural conduction	→	local anaesthetics
Cerebral excitation	→	epilepsy

Potassium channels:

Cardiac excitation	→	arrhythmia
Vascular smooth muscle tone	→	blood pressure
Pancreatic β-cells	→	insulin secretion

Calcium channels:

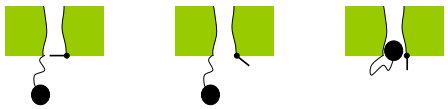
Cardiac excitation	→	arrhythmia
Vascular smooth muscle tone	→	blood pressure

Kinetics of sodium channel blocking by local anaesthetics

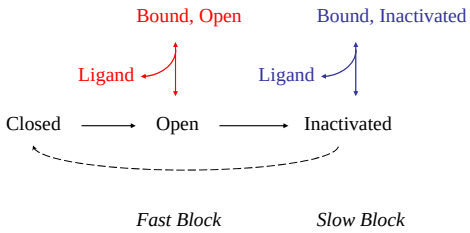
Depolarization *Spontaneous inactivation*

Closed ⟶ Open ⟶ Inactivated

Slow reactivation after membrane repolarization

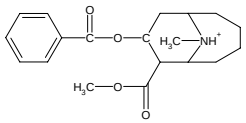


Kinetics of sodium channel blocking by local anaesthetics

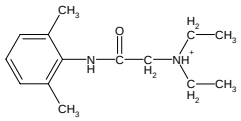


Local anaesthetics derive from cocaine

Cocaine

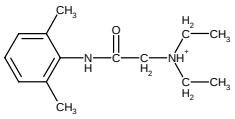


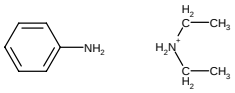
Lidocaine



Lidocaine and two partial analogues

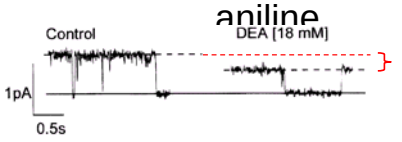
Lidocaine



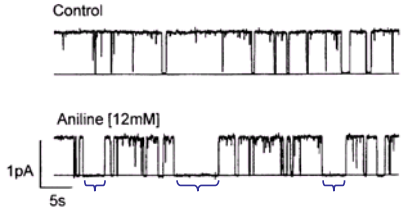


Aniline Diethylamine

Inhibition of a single Na_V channel by diethylamine and aniline



Incomplete block, immediate, all the time

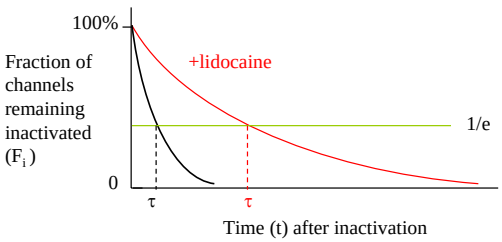


Complete block, delayed, part of the time

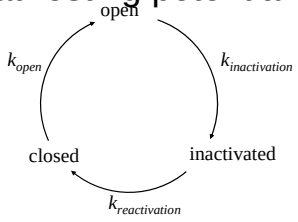
Kinetics of channel reactivation

$dF_i/dt = -F_i/\tau = -kF_i$

$F_i = e^{-t/\tau} = e^{-kt}$ $\tau > \tau$ $k < k$

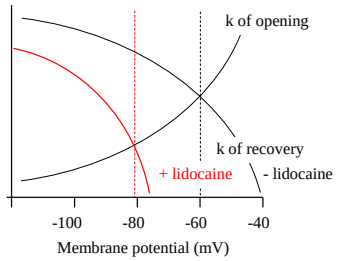


Dynamic equilibrium of channel states at resting potential

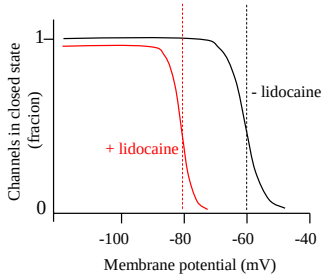


k_{open} [closed] = $k_{inactivation}$ [open] = $k_{reactivation}$ [inactivated]

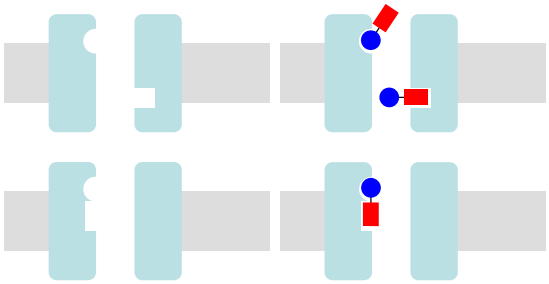
The effect of lidocaine on sodium channels varies with the resting potential



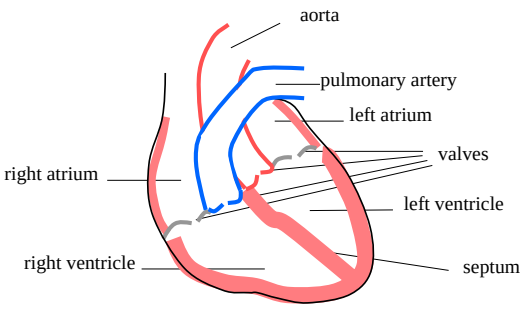
The effect of lidocaine on sodium channels varies with the resting potential



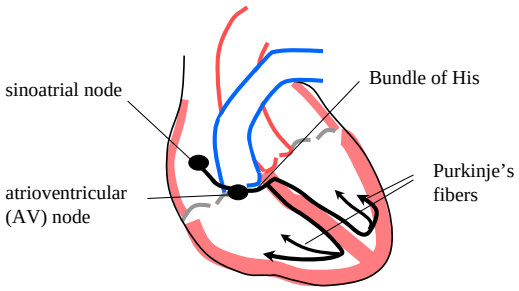
One or two binding sites for lidocaine?



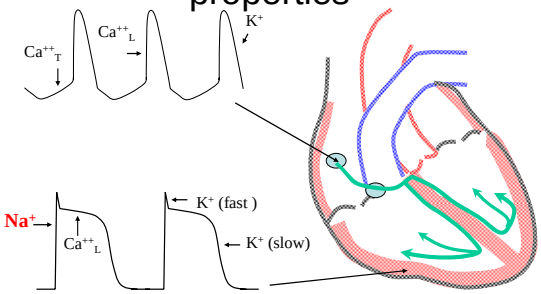
The heart



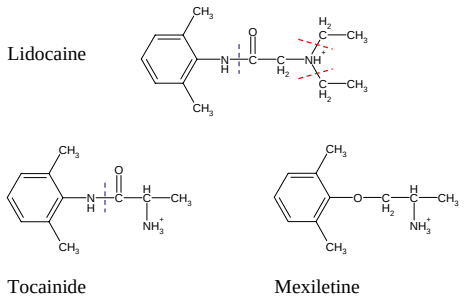
The excitation / conducting system of the heart



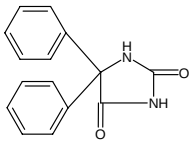
The excitation and the 'worker' cells have different ion flow properties



Lidocaine and two metabolism-resistant congeners



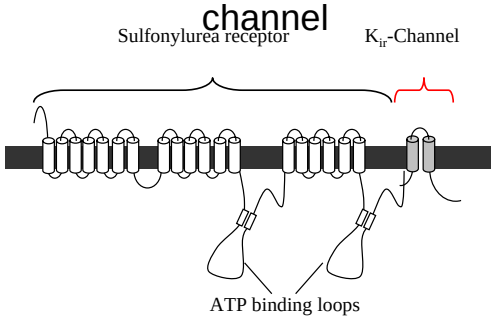
Phenytoin blocks sodium channels in the brain and is used in epilepsia



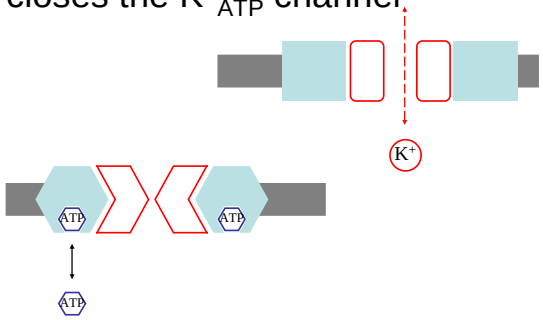
Properties of phenytoin:

- Good penetration of blood brain barrier
- Acts on several cation channels besides sodium (contribution to therapeutic effect unsettled)
- Strong enzyme inducer (hepatic metabolism, CYP3A4) → Multiple drug interactions

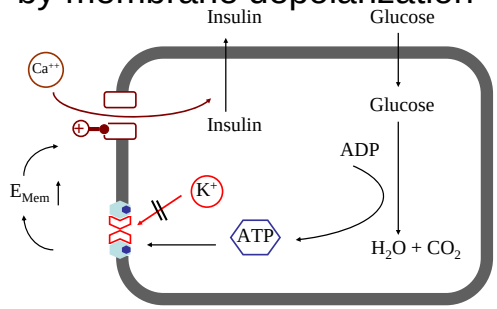
the K^+-channels is the ATP-sensitive 'inward rectifier' channel



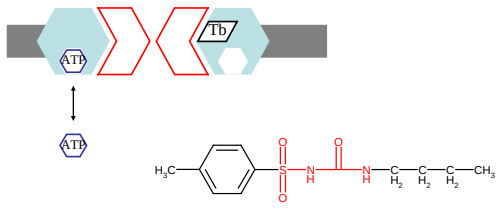
ATP binding to the sulfonurea receptor closes the K^+_{ATP} channel



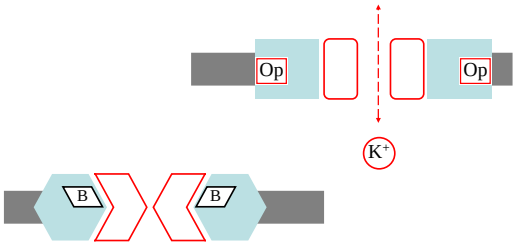
Insulin secretion in the pancreatic β-cell is triggered by membrane depolarization



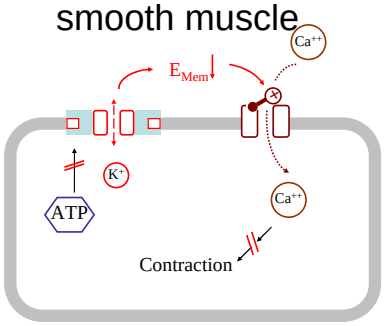
Sulfonylurea derivatives such as tolbutamide promote closing of the K_{ATP} channel

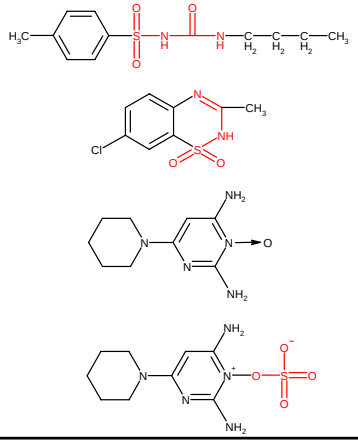


Both blockers and openers of K^+ channels bind to the sulfonurea receptor



K^+ Channel openers reduce cell contraction in the vascular smooth muscle



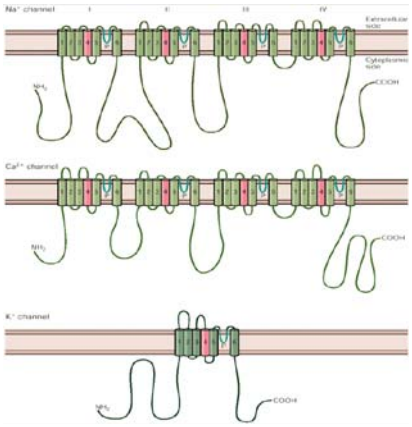


Tolbutamide

Diazoxide

Minoxidil

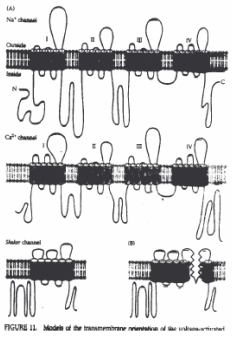
Minoxidil-4-sulfate



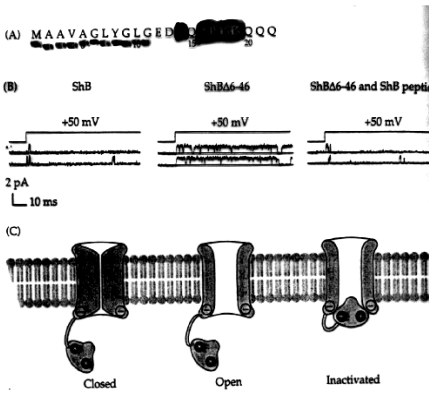
Shaker

Shaker is a quarter-sized, 'ancestral' K^+ channel found in *Drosophila*

Rather than being primitive, it is now known that there are multiple shaker-like channels in humans.



The 'ball and chain' model



Even smaller channels…