Paper ZOO-402

Unit : B - Neuroendocrinology

Principles of Electrical Signaling: Action potential

The Nernst Potential.

The diffusion potential level across a membrane that exactly opposes the net diffusion of a particular ion through the membrane is called the *Nernst potential* for that ion. The magnitude of this Nernst potential is determined by the *ratio* of the concentrations of that specific ion on the two sides of the membrane. The greater this ratio, the greater the tendency for the ion to diffuse in one direction, and therefore the greater the Nernst potential required to prevent additional net diffusion. The following equation, called the *Nernst equation,* can be used to calculate the Nernst potential for any univalent ion at normal body temperature of 98.6°F (37°C):

$$\text{EMF (millivolts)} = \pm 61 \log \frac{\text{Concentration inside}}{\text{Concentration outside}}$$

where EMF is electromotive force.
When using this formula, it is usually assumed that the potential in the extracellular fluid outside the membrane remains at zero potential, and the Nernst potential is the potential inside the membrane. Also, the sign of the potential is positive (+) if the ion diffusing from inside to outside is a negative ion, and it is negative (–) if the ion is positive. Thus, when the concentration of positive potassium ions on the inside is 10 times that on the outside, the log of 10 is 1, so that the Nernst potential calculates to be –61 millivolts inside the membrane

Calculation of the Diffusion Potential When the Membrane Is Permeable to Several Different Ions
When a membrane is permeable to several different ions, the diffusion potential that develops depends on three factors:

(1) the polarity of the electrical charge of each ion,

(2) the permeability of the membrane (*P*) to each ion, and

(3) the concentrations (*C*) of the respective ions on the inside (*i*) and outside (*o*) of the membrane.

Thus, the following formula, called the *Goldman equation,* or the *Goldman-Hodgkin-Katz equation,* gives the calculated membrane potential on the *inside* of the membrane when two univalent positive ions, sodium (Na^+) and potassium (K^+), and one univalent negative ion, chloride (Cl^-), are involved.

$$\text{EMF (millivolts)} = -61 \cdot \log \frac{C_{Na^+_i} P_{Na^+} + C_{K^+_i} P_{K^+} + C_{Cl^-_o} P_{Cl^-}}{C_{Na^+_o} P_{Na^+} + C_{K^-_o} P_{K^+} + C_{Cl^-_i} P_{Cl^-}}$$

Let us study the importance and the meaning of this equation.

First, sodium, potassium, and chloride ions are the most important ions involved in the development of membrane potentials in nerve and muscle fibers, as well as in the neuronal cells in the nervous system. The concentration gradient of each of these ions across the membrane helps determine the voltage of the membrane potential.

Second, the degree of importance of each of the ions in determining the voltage is proportional to the membrane permeability for that particular ion. That is, if the membrane has zero permeability to both potassium and chloride ions, the membrane potential becomes entirely dominated by the concentration gradient of sodium ions alone, and the resulting potential will be equal to the Nernst potential for sodium. The same holds for each of the other two ions if the membrane should become selectively permeable for either one of them alone.

Third, a positive ion concentration gradient from *inside* the membrane to the *outside* causes electronegativity inside the membrane. The reason for this is that excess positive ions diffuse to the outside when their concentration is higher inside than outside. This carries positive charges to the outside but leaves the non-diffusible negative anions on the inside, thus creating electronegativity on the inside. The opposite effect occurs when there is a gradient for a negative ion. That is, a chloride ion gradient from the *outside to the inside* causes negativity inside the cell because excess negatively charged chloride ions diffuse to the inside, while leaving the non diffusible positive ions on the outside.

Fourth, as explained later, the permeability of the sodium and potassium channels undergoes rapid changes during transmission of a nerve impulse, whereas the permeability of the chloride channels does not change greatly during this process. Therefore, rapid changes in sodium and potassium permeability are primarily responsible for signal transmission in nerves.

Resting Membrane Potential of Nerves

The resting membrane potential of large nerve fibers when not transmitting nerve signals is about –90 millivolts. That is, the potential inside the fiber is 90 millivolts more negative than the potential in the extracellular fluid on the outside of the fiber.

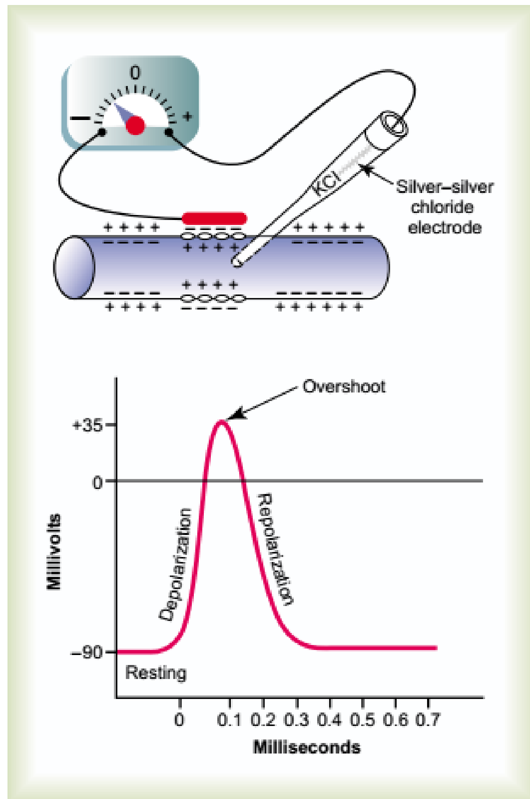


Figure 5–6

Typical action potential recorded by the method shown in the upper panel of the figure.

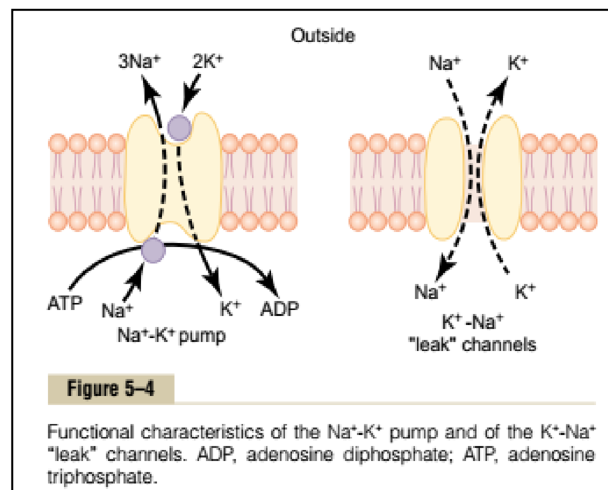


Figure 5–4

Functional characteristics of the Na^+-K^+ pump and of the K^+-Na^+ "leak" channels. ADP, adenosine diphosphate; ATP, adenosine triphosphate.

Nerve Action Potential

Nerve signals are transmitted by *action potentials*, which are rapid changes in the membrane potential that spread rapidly along the nerve fiber membrane. Each action potential begins with a sudden change from the normal resting negative membrane potential to a positive potential and then ends with an almost equally rapid change back to the negative potential. To conduct a nerve signal, the action potential moves along the nerve fiber until it comes to the fiber's end.

The upper panel of Figure 5–6 shows the changes that occur at the membrane during the action potential, with transfer of positive charges to the interior of the fiber at its onset and return of positive charges to the exterior at its end. The lower panel shows graphically the successive changes in membrane potential over a few 10,000ths of a second, illustrating the explosive onset of the action potential and the almost equally rapid recovery.

The successive stages of the action potential are as follows.

Resting Stage. This is the resting membrane potential before the action potential begins. The membrane is said to be "polarized" during this stage because of the –90 millivolts negative membrane potential that is present.

Depolarization Stage. At this time, the membrane suddenly becomes very permeable to sodium ions, allowing tremendous numbers of positively charged sodium ions to diffuse to the interior of the axon. The normal "polarized" state of –90 millivolts is immediately neutralized by the inflowing positively charged sodium ions, with the potential rising rapidly in the positive direction. This is called *depolarization*. In large nerve fibers, the great excess of positive sodium ions moving to the inside causes the membrane potential to actually "overshoot" beyond the zero level and to become somewhat positive. In some smaller fibers, as well as in

many central nervous system neurons, the potential merely approaches the zero level and does not overshoot to the positive state.

Repolarization Stage. Within a few 10,000ths of a second after the membrane becomes highly permeable to sodium ions, the sodium channels begin to close and the potassium channels open more than normal. Then, rapid diffusion of potassium ions to the exterior re-establishes the normal negative resting membrane potential. This is called *repolarization* of the membrane.

To explain more fully the factors that cause both depolarization and repolarization, we need to describe the special characteristics of two other types of transport channels through the nerve membrane: the voltage-gated sodium and potassium channels.

Voltage-Gated Sodium and Potassium Channels

The necessary actor in causing both depolarization and repolarization of the nerve membrane during the action potential is the *voltage-gated sodium channel.* A *voltage-gated potassium channel* also plays an important role in increasing the rapidity of repolarization of the membrane. *These two voltage-gated channels are in addition to the Na^+-K^+ pump and the K^+-Na^+ leak channels.*

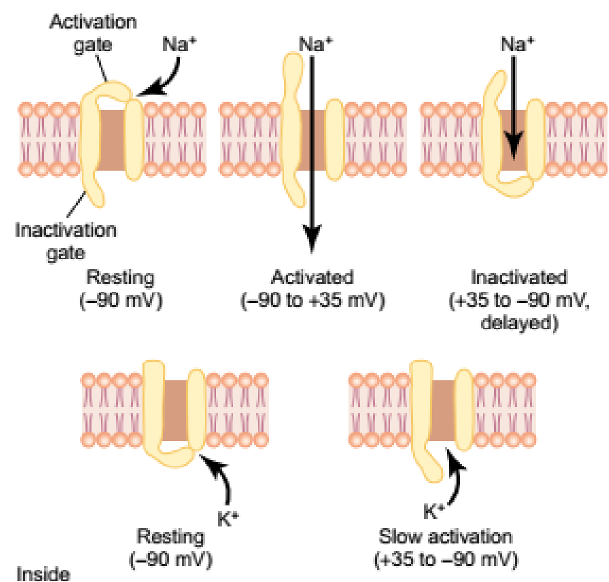


Figure 5–7

Characteristics of the voltage-gated sodium *(top)* and potassium *(bottom)* channels, showing successive activation and inactivation of the sodium channels and delayed activation of the potassium channels when the membrane potential is changed from the normal resting negative value to a positive value.

Voltage-Gated Sodium Channel—Activation and Inactivation of the Channel

The upper panel of Figure 5–7 shows the voltage-gated sodium channel in three separate states. This channel has two *gates*—one near the outside of the channel called the *activation gate,* and another near the inside called the *inactivation gate.* The upper left of the figure depicts the state of these two gates in the normal resting membrane when the membrane potential is –90 millivolts. In this state, the activation gate is closed, which prevents any entry of sodium ions to the interior of the fiber through these sodium channels.

Activation of the Sodium Channel. When the membrane potential becomes less negative than during the resting state, rising from –90 millivolts toward zero, it finally reaches a voltage—usually somewhere between –70 and –50 millivolts—that causes a sudden conformational change in the activation gate, flipping it all the way to the open position. This is called the *activated state;* during this state, sodium ions can pour inward through the channel, increasing the sodium permeability of the membrane as much as 500- to 5000-fold.

Inactivation of the Sodium Channel. The upper right panel of Figure 5–7 shows a third state of the sodium channel. The same increase in voltage that opens the activation gate also closes the inactivation gate. The inactivation gate, however, closes a few 10,000ths of a second after the activation gate opens. That is, the conformational change that flips the inactivation gate to the closed state is a slower process than the conformational change that opens the activation gate. Therefore, after the sodium channel has remained open for a few 10,000ths of a second, the inactivation gate closes, and sodium ions no longer can pour to the inside of the membrane. At this point, the membrane potential begins to recover back toward the resting membrane state, which is the repolarization process.

Another important characteristic of the sodium channel inactivation process is that the inactivation gate will not reopen until the membrane potential returns to or near the original resting membrane potential level. Therefore, it usually is not possible for the sodium channels to open again without the nerve fiber's first repolarizing.

Voltage-Gated Potassium Channel and Its Activation

The lower panel of Figure 5–7 shows the voltage-gated potassium channel in two states: during the resting state (left) and toward the end of the action potential (right). During the resting state, the gate of the potassium channel is closed, and potassium ions are prevented from passing through this channel to the exterior. When the membrane potential rises from –90 millivolts toward zero, this voltage change causes a conformational opening of the gate and allows increased potassium diffusion outward through the channel. However, because of the slight delay in opening of the potassium channels, for the most part,

they open just at the same time that the sodium channels are beginning to close because of inactivation. Thus, the decrease in sodium entry to the cell and the simultaneous increase in potassium exit from the cell combine to speed the repolarization process, leading to full recovery of the resting membrane potential within another few 10,000ths of a second.

Research Method for Measuring the Effect of Voltage on Opening and Closing of the Voltage-Gated Channels—The "Voltage Clamp." The original research that led to quantitative understanding of the sodium and potassium channels was so ingenious that it led to Nobel Prizes for the scientists responsible, Hodgkin and Huxley. The essence of these studies is shown in Figures 5–8 and 5–9.

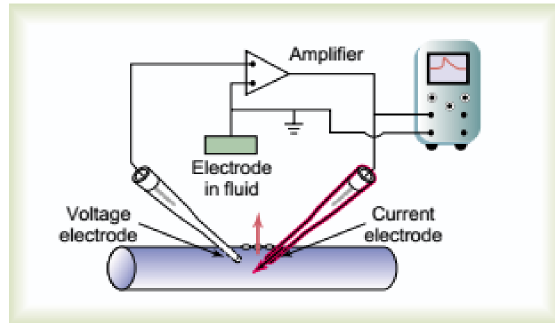


Figure 5–8

"Voltage clamp" method for studying flow of ions through specific channels.

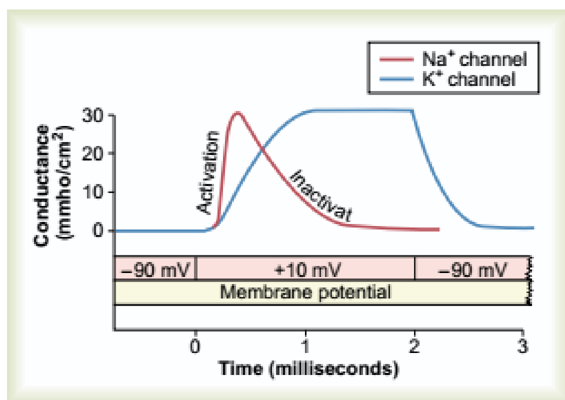


Figure 5–9

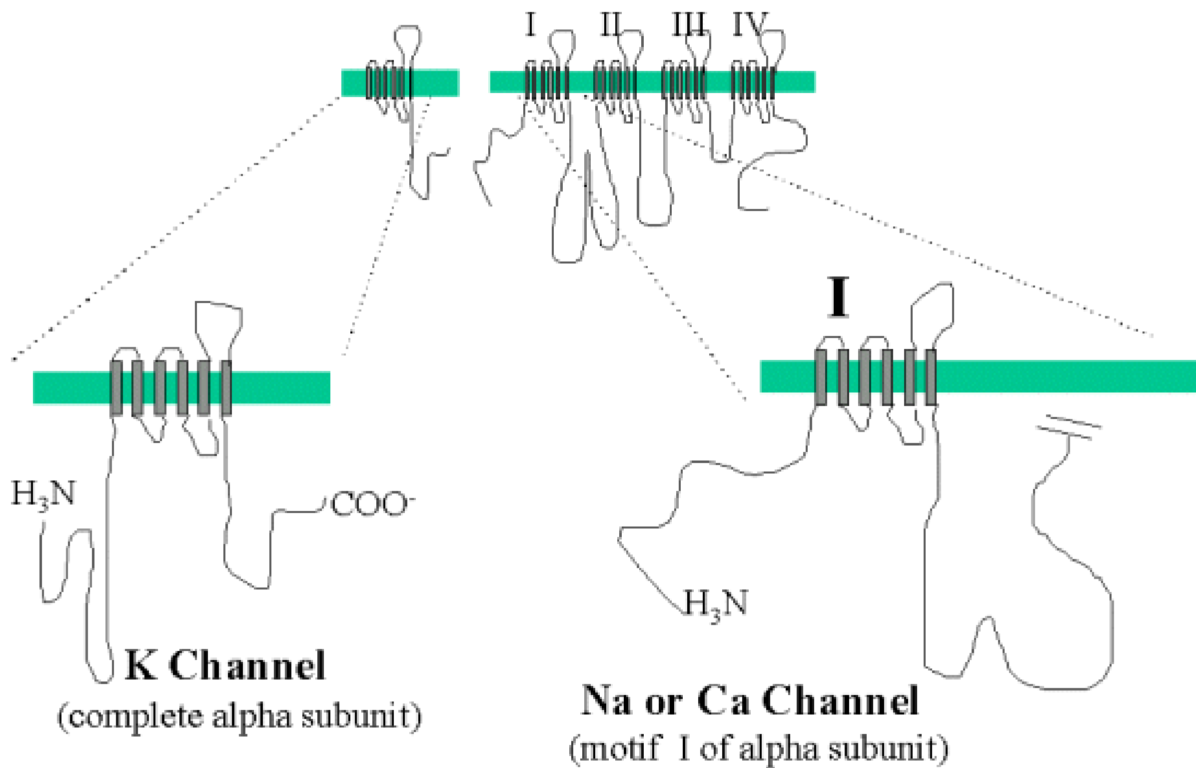
Typical changes in conductance of sodium and potassium ion channels when the membrane potential is suddenly increased from the normal resting value of –90 millivolts to a positive value of +10 millivolts for 2 milliseconds. This figure shows that the sodium channels open (activate) and then close (inactivate) before the end of the 2 milliseconds, whereas the potassium channels only open (activate), and the rate of opening is much slower than that of the sodium channels.

Another means for studying the flow of ions through an individual type of channel is to block one type of channel at a time. For instance, the sodium channels can be blocked by a toxin called *tetrodotoxin* by applying it to the outside of the cell membrane where the sodium activation gates are located. Conversely, *tetraethylammonium ion* blocks the potassium channels when it is applied to the interior of the nerve fiber.

Figure 5–9 shows typical changes in conductance of the voltage-gated sodium and potassium channels when the membrane potential is suddenly changed by use of the voltage clamp from –90 millivolts to +10 millivolts and then, 2 milliseconds later, back to –90 millivolts. Note the sudden opening of the sodium channels (the activation stage) within a small fraction of a millisecond after the membrane potential is increased to the positive value. However, during the next millisecond or so, the sodium channels automatically close (the inactivation stage).

Note the opening (activation) of the potassium channels. These open slowly and reach their full open state only after the sodium channels have almost completely closed. Further, once the potassium channels open, they remain open for the entire duration of the positive membrane potential and do not close again until after the membrane potential is decreased back to a negative value.

Voltage-gated ion channels are a class of **transmembrane** ion channels that are activated by changes in electrical **potential difference** near the channel; these types of ion channels are especially critical in neurons, but are common in many types of cells.



Gating of Protein Channels:

- A means of controlling ion **permeability of the channels** (Figure 4–4) for selective gating of sodium and potassium ions.

It is believed that some of the gates are **actual gate-li**ke extensions of the transport protein molecule, which can close the opening of the channel or can be lifted away from the opening by a **conformational change** in the shape of the protein molecule itself.

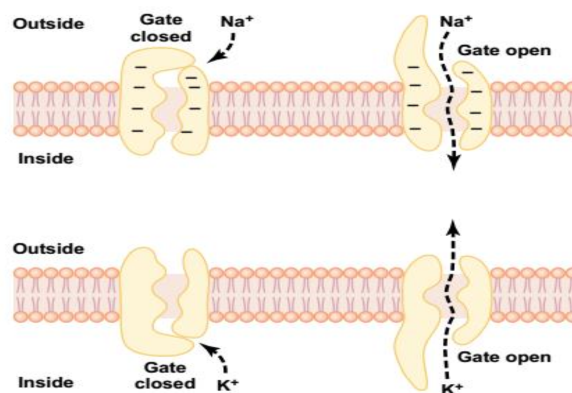


Figure 4–4

Transport of sodium and potassium ions through protein channels. Also shown are conformational changes in the protein molecules to open or close "gates" guarding the channels.

The opening and closing of gates are controlled in 2 principal ways:

1. ***Voltage gating***- here, the molecular conformation of the gate or of its chemical bonds responds to the electrical potential across the cell membrane. This is the **basic mechanism** for eliciting **action potentials in nerves** that are responsible for nerve signals.

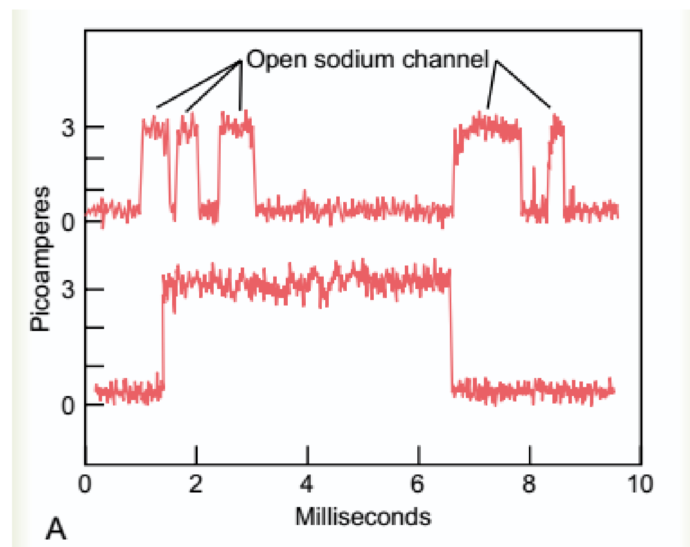
2. ***Chemical (ligand) gating***. Some protein channel gates are opened by the binding of a chemical substance (a ligand) with the protein; this causes a conformational or **chemical bonding change** in the protein molecule that opens or closes the gate.

    1. One of the most important instances is the effect of acetylcholine on the so-called **acetylcholine channel**. This gate is exceedingly important for the transmission of nerve signals from **one nerve cell to another** and from nerve cells to muscle cells to cause muscle contraction.

Selective Permeability of Protein Channels.

1. Many of the protein channels are highly selective for transport of one or more specific ions or molecules.
2. This results from the characteristics of the channel itself, such as its diameter, its shape, and the nature of the electrical charges and chemical bonds along its inside surfaces. For example, one of the most important –*sodium channel,* is only 0.3 by 0.5 nm in diameter, but more important, the inner surfaces of this channel are *strongly negatively charged.* Sodium ion attracts far more water molecules than does potassium.

Conversely, another set of protein channels is selective for potassium transport, These channels are slightly smaller than the sodium channels, only 0.3 by 0.3 nm but they are not negatively charged, and their chemical bonds are different.



Potassium, sodium and calcium channels all exist as protein complexes.

The alpha subunit contains the pore of the channel and the voltage-sensor that allows the channel to detect and gate in response to changes in the transmembrane voltage.

The accessory subunits (variously named as alpha2, beta, gamma etc.) might alter the behavior of the alpha subunit in a number of ways and may, in addition, provide a way of anchoring the channel to the cytoskeleton, even helping to target protein kinases to particular residues. **It is strange but true that the amino acids that line the pore of the voltage-gated channels are not those that are found within the transmembrane domains**

Sodium channels:

Sodium channels can often be isolated from cells as a complex of two types of protein subunits, α and β. The α –subunit has four repeat domains, labeled I through IV, each containing six membrane-spanning regions, labeled S1 through S6. The highly conserved S4 region acts as the channel's voltage sensor. The voltage sensitivity of this channel is due to positive amino acids located at every third position. When stimulated by a change in transmembrane voltage, this region moves toward the extracellular side of the cell membrane, allowing the channel to become permeable to ions.

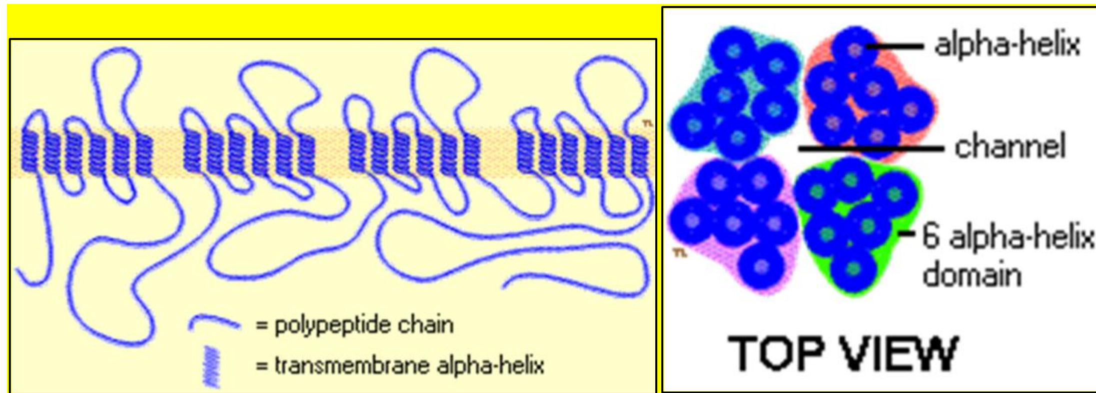
The ions are conducted through a pore, which can be broken into two regions. The more external (i.e., more extracellular) portion of the pore is formed by the "P-loops" (the region between S5 and S6) of the four domains. This region is the most narrow part of the pore and is responsible for its ion selectivity. The inner portion (i.e., more cytoplasmic) of the pore is formed by the combined S5 and S6 regions of the four domains.

The region linking domains III and IV is also important for channel function. This region plugs the channel after prolonged activation, inactivating it.

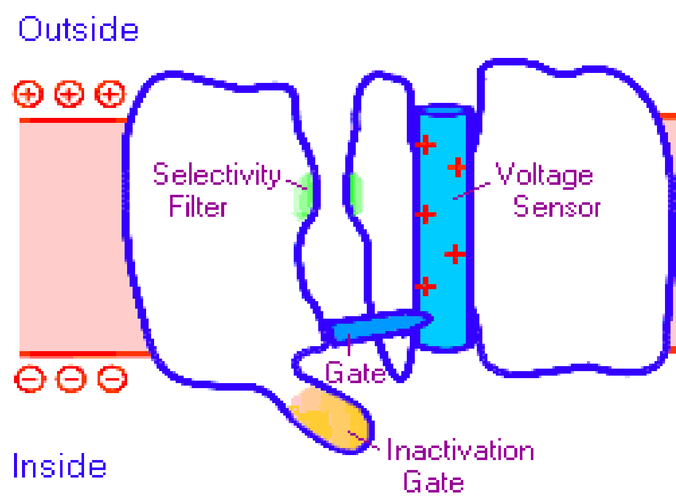
- **Length of the α helix ≡ width of the membrane.**
- **Impermeability to other ions**

The pore of sodium channels contains negatively charged amino acid residues, which attract the positive Na+ ion and keep out negatively charged ions such as chloride. The cations flow into a more constricted part of the pore that is 0.3 by 0.5 nm wide, which is just large enough to allow a single Na+ ion with a water molecule associated to pass through. The larger K+ ion cannot fit through this area. Differently sized ions also cannot interact as well with the negatively charged glutamic acid residues that line the pore.

- VGIC have 24 such transmembrane alpha-helices in the polypeptide chain.
- alpha-helices are shown spread out and in a row & divided into four groups. Each of these is an **homologous domain** with a similar sequence of amino acids.
- In an actual membrane, the alpha-helices are not in a line, but clustered. At the center of the four domains is the channel through which the sodium ions move.

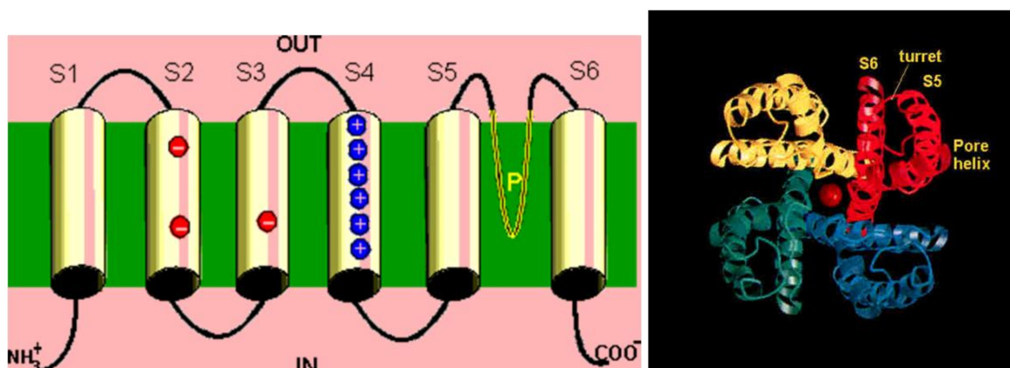


Opening of Channel by Voltage Sensor



Voltage-gated K+ channels:

- Tetramers of 4 identical subunits arranged as a ring, each contributing to the wall of the trans-membrane K+ pore.
- Each subunit is comprised of six membrane spanning hydrophobic α-helical sequences.



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Suggested References:

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2. Text Book of Medical Physiology - Arthur C. Guyton & John Edward Hall. 13th Ed.