◉Sheet ○Slides

Number

6

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In the previous lectures we have concluded that there's a higher concentration of K^+ inside nerve fibers, and a higher concentration of Na^+ outside.

We also compared the membrane to an electrical circuit; it works like a capacitor by separating charges across the plasma membrane.

The movement of any ion down its own electrochemical gradient will tend to move the membrane potential towards the equilibrium potential of that ion.

eg: If a membrane is permeable to Cl^-, electrical potential will be negative inside

If it's permeable to Ca^{2+}, electrical potential will be positive inside

The equilibrium potential that we calculate for ions is an <u>electrochemical</u> equilibrium since we are dealing with concentration parameters as well as parameters relating to electrical charges.

At equilibrium, energy created by concentration gradient (ΔG_{conc}) is equal to the energy created by electrical charges (ΔG_{volt})

$$\Delta G_{conc} + \Delta G_{volt} = 0$$

Sometimes we might find **the Nernst equation** in this form:

$$E_{ion} = -61.5 \log \frac{C_{in}}{C_{out}}$$

Note that we added a negative sign since we switched the logarithms.

The membrane potential is a result of the movement of Na^+, K^+ and Cl^- mainly. Since more than one ion channel is present in the plasma membrane, the membrane potential moves towards the "average" of the equilibrium potentials for the specific ions that can cross the membrane. The "average" depends on relative permeabilities for the different ions - the more permeable the membrane is for an ion, the more the equilibrium potential of that ion will influence the membrane potential. The membrane potential can be calculated using the Goldman-Hodgkin-Katz equation.

<u>Goldman-Hodgkin-Katz (GHK) equation</u>:

$$E_m = \frac{RT}{F} \ln \left(\frac{P_{Na+}[Na^+]_o + P_{K+}[K^+]_o + P_{Cl-}[Cl^-]_i}{P_{Na+}[Na^+]_i + P_{K+}[K^+]_i + P_{Cl-}[Cl^-]_o} \right)$$

where E_m: membrane potential

R: gas constant (8.314)

T: absolute temperature in Kelvin

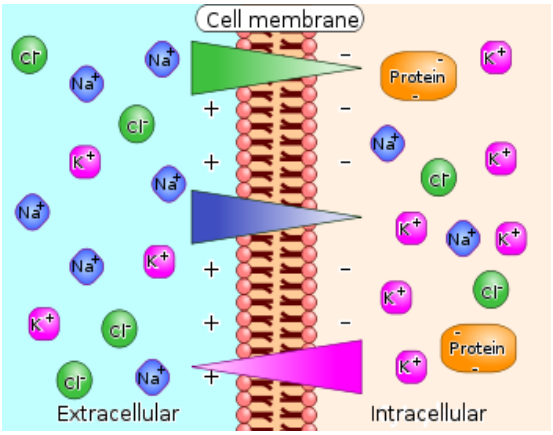
F: Faraday's constant (96485)

$[ion]_i$: ion's concentration inside

$[ion]_O$: ion's concentration outside

P_{ion}: permeability of membrane to ion

***** The effect of movement of Cl^- is a reversal of the movement of other positive ions like Na^+/K^+ since it has a valency of -1. This is why we reversed it in the equation (it becomes concentration inside ÷ concentration outside). This applies to all other negative ions.



Assume a membrane is highly permeable to K^+ and slightly permeable to Na^+ and is not permeable at all for Cl^-. In this case, the potential across it will be closer to the equilibrium potential for K^+ (E_{K+}) but not equal to it (around -80 mV) according to the GHK equation.

***** If the membrane has zero permeability for K^+ and Cl^-, its potential becomes equal to the equilibrium potential for Na^+ (E_{Na+}). If it had zero permeability for Na^+ and Cl^-, its potential equals the equilibrium potential for K^+ (E_{K+}) . Finally, if it had zero permeability for K^+ and Na^+, its potential equals the equilibrium potential for Cl^- (E_{Cl-}).

In excitable cells the membrane potential isn't constant. When the cell is stimulated, the membrane potential changes. These changes in membrane potential are due to changes in permeability of plasma membrane to different ions. The permeability of one cell to a certain ion can vary at different positions across the plasma membrane of that same cell; this occurs due to the control of transport mechanism.

Resting Membrane Potential:

The resting membrane potential is the membrane potential for a cell under resting conditions; when no stimulus is introduced.

In neurons, resting membrane is equal to **-90 mV**. This represents the potential difference between the inside and outside when the neuron is not active. We usually ignore Cl^- . Resting membrane values are different in different cell types; for example in

cardiac muscle cells it could be -60 mV, in smooth muscle cells -50, in skeletal muscle cells – 70, and in non-excitable cells it could be -10 or even +10 or +20mV.

The question is why do we have different resting membranes? It is according to the permeability to Na^+, K^+, and the presence of the Na^+ - K^+ pump.

Factors affecting resting membrane potential:

1. Activity of K^+ channels

Using the Nernst equation, we calculate that it corresponds to an equilibrium potential of -94 mV.

2. Activity of Na^+ channels

The cell membrane of the nerve is slightly permeable to Na^+ resulting in an equilibrium potential of +61 mV.

* Using the Goldman equation, we can calculate the potential inside the nerve cell as -86 mV. This potential is much closer to the equilibrium potential of K^+ (E_{K+}) since the membrane is much more permeable to K^+ than Na^+ (100 times more).

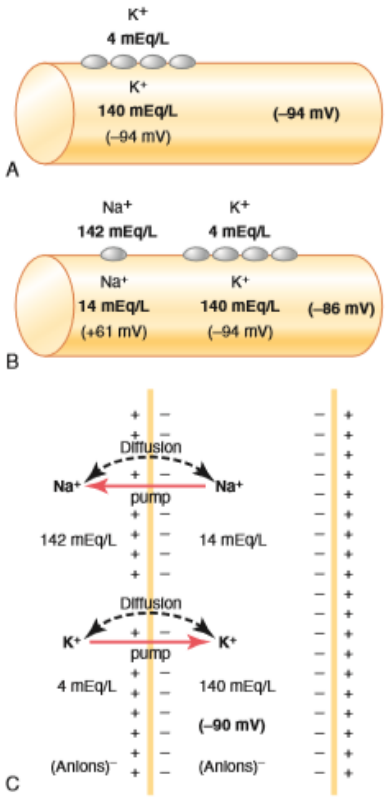


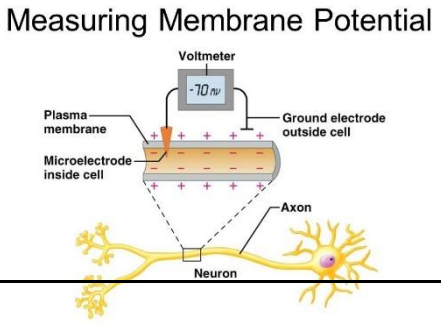
Figure 5-5. Establishment of resting membrane potentials in nerve fibers under three conditions: **A,** when the membrane potential is caused entirely by potassium diffusion alone; **B,** when the membrane potential is caused by diffusion of both sodium and potassium ions; and **C,** when the membrane potential is caused by diffusion of both sodium and potassium ions plus pumping of both these ions by the Na^+-K^+ pump.

3. Na^+/K^+ pump (electrogenic)

If it is working alone it can establish a resting potential of -4 mV; means negative inside and positive outside. This is because for every 3 Na^+ pumped outside 2 K^+ are pumped inside, causing a continual loss of positive charges from inside the membrane, adding an extra 4 mV of negativity.

* Taking the effect of the Na^+/K^+ pump into account, the net membrane potential when all these factors are operative at the same time is about **-90 mV**.

The establishment of the resting membrane potential is due to high permeability to K^+, some permeability to Na^+ and the presence of the Na^+/K^+ pump; all our body's cells have high amounts of proteins inside but it don't play a major



role in making a difference in cell membrane potential among different cell types.

We measure the resting membrane potential just across the plasma membrane using a galvanometer; that means we put an electrode just above the cell membrane and one just below the cell membrane to obtain the correct membrane potential. If we measure it far outside and far inside the cell, it would be electroneutral (no electrical difference). This is due to the Donnan effect; the negatively charged proteins in the cell are neutralized by cations (positive ions) so it's electroneutral both on the outside and the inside.

***** The maximum resting membrane potential is -94 mV and occurs at maximum permeability to K^+. Even if the membrane has high permeability for Cl^-, we can't go below -94 mV.

If we look at the movement of charged particles from an electrical view, we can replace the idea of permeability by the "conductance of ions". Conductance is the reversal of resistance. Any increase in the conductance of that membrane means decreasing resistance, so the ions can move across it. This is similar to Fick's law of diffusion (the rate of diffusion (flux or # of particles moving) = permeability * ΔC). In Fick's law, ΔC is the driving force, while in conductance, ΔV is the driving force. Therefore increasing permeability of an ion increases its conductance.

Conductance (G) of plasma membrane (Ohm's Law)

- $I = \Delta V/R$ current=potential difference/resistance
- $G = 1/R$ conductance=1/resistance
- $I = G\Delta V$ current=conductance*potential difference

The membrane potential created can be measured using the chord conductance equation based on the conductance values as well as equilibrium potential values of the ions contributing to the membrane potential. This equation is similar to the Goldman equation but here we rely mostly on conductance, while in the GHK equation we rely on relative permeabilities.

The cord Conductance equation describes the contributions of permeant ions to the resting membrane potential

$$V_m = \frac{g_K}{g_{tot}} E_K + \frac{g_{Na}}{g_{tot}} E_{Na} + \frac{g_{Cl}}{g_{tot}} E_{Cl}$$

V_m: membrane potential

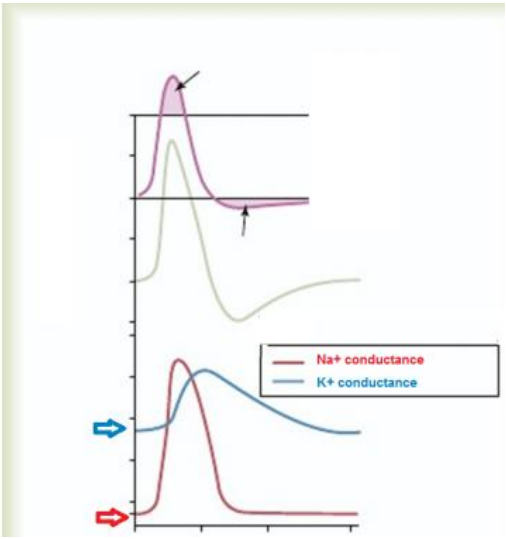
g_{ion}: conductance of ion

g_{tot}: total conductance of membrane

E_{ion}: equilibrium potential of ion

Activation of Na^+ channel will increase Na^+ current, and activation of K^+ channels will increase the K^+ current.

- Na+ and K+ conductance at resting potentials



Can we measure the current across the cell membrane? Yes. One of the methods is patch clamp technique

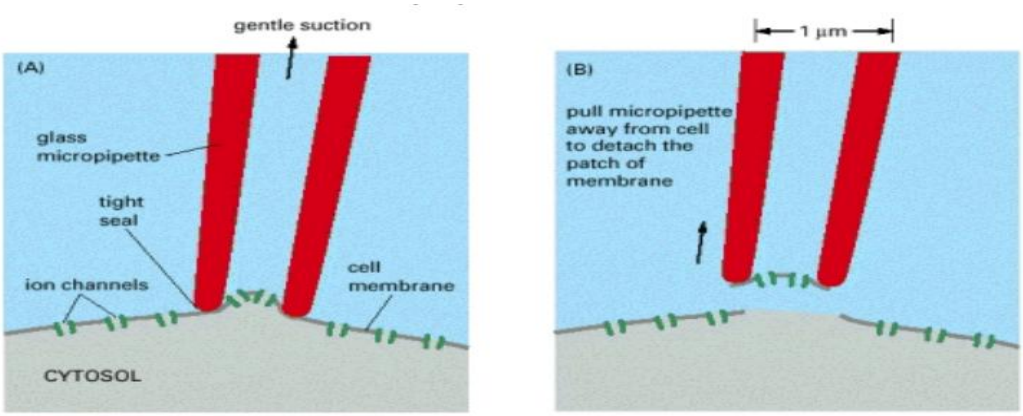
Patch Clamp Technique:

"The **patch clamp technique** is a laboratory technique in electrophysiology used to study ionic currents in individual isolated living cells, tissue sections, or patches of cell membrane. Patch clamping can be performed using the voltage clamp technique. In this case, the voltage across the cell membrane is controlled by the experimenter and the resulting currents are recorded. Alternatively, the current clamp technique can be used. In this case the current passing across the membrane is controlled by the experimenter and the resulting changes in voltage are recorded, generally in the form of action potentials". (from Wikipedia)

The patch clamp is electronic device that employed to maintain, or "clamp" the membrane potential at a set value, recording the ionic current through individual channels. (from slides)

We use the patch clamp technique to measure current across membranes. We seal part of the membrane with a micropipette and either keep it attached to cell membrane or detach it. This sealed part held by the pipette is a solution that is like extracellular fluid. We then place the micropipette in a free solution similar to intracellular fluid and measure the current created between intracellular and extracellular parts. We can alter the concentration of the solutions, as well as recording current through individual channels.

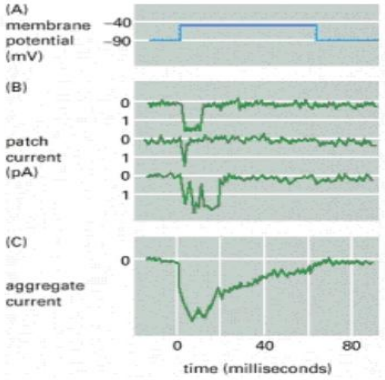
• Patch still attached to the rest of the cell, as in (A), or detached, as in (B).



Details of the setup of patch-clamping aren't required; we should only know that clamping at different voltages (different concentrations of solutions) produces different currents and thus different conductance.

In the figure below, there are some recorded currents using the patch clamp technique.

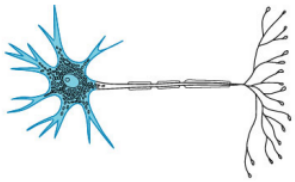
Recording of currents in Patch Clamp

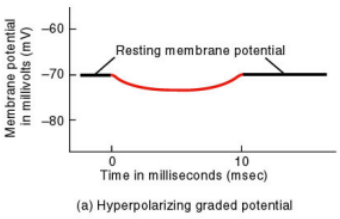


At resting potential we have high conductance for K^+ and very low conductance for Na^+. If we activate Na^+ channels, Na^+ enter the cell and their conductance increases so potential becomes less negative inside. This change in membrane potential is called **depolarization**. In **hyperpolarization**, the membrane becomes more permeable to K^+ for a short while, so K^+ flow out, making the membrane potential more negative inside. (details of the action potential will be discussed in the next sheet)

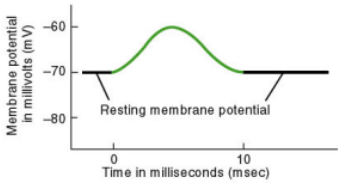
Therefore hyperpolarization means: movement towards the **more negative** potential, and depolarization means: movement towards the **less negative** potential.

- Changes in Resting membrane potential





(a) Hyperpolarizing graded potential



(b) Depolarizing graded potential