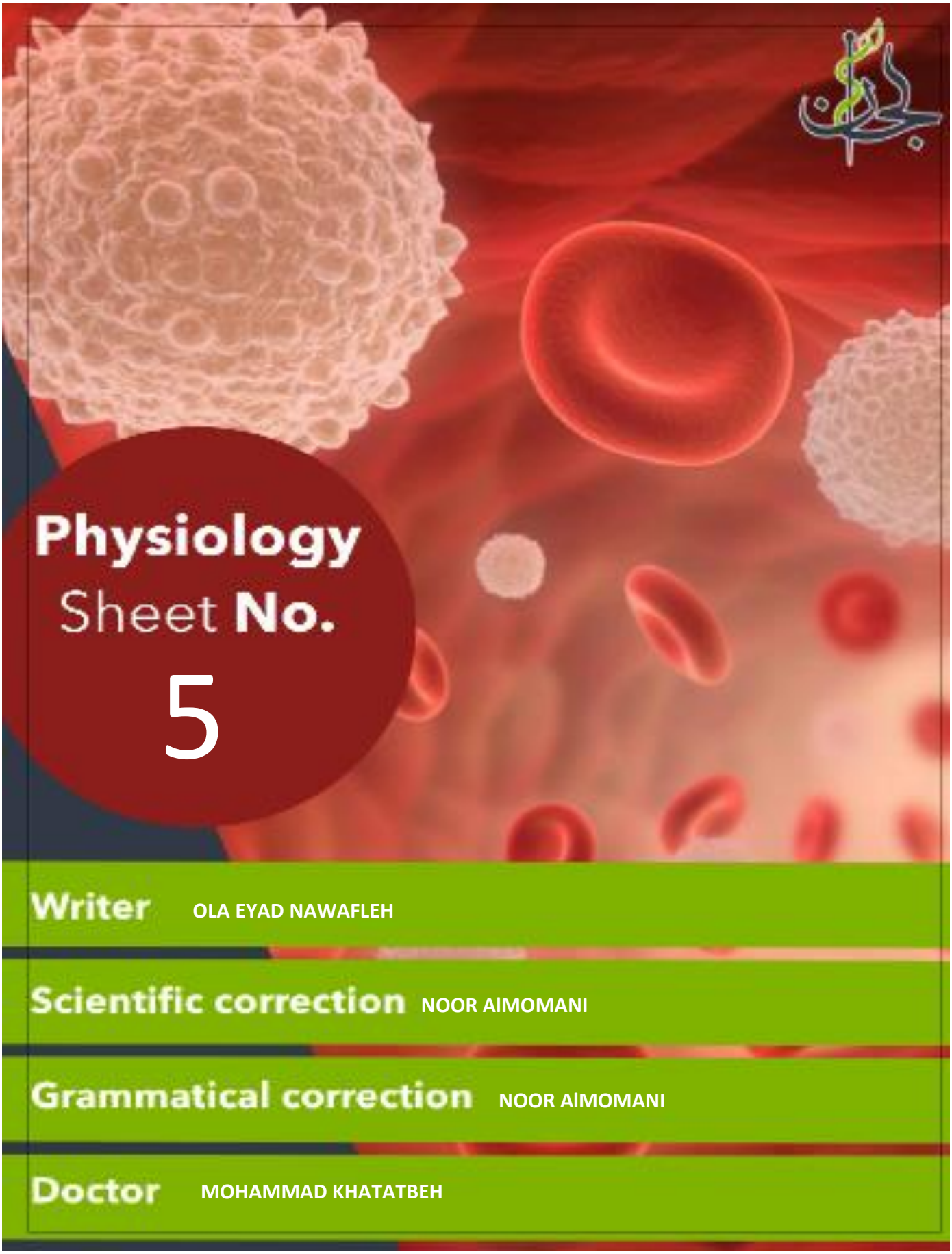

Physiology

Sheet No. 5

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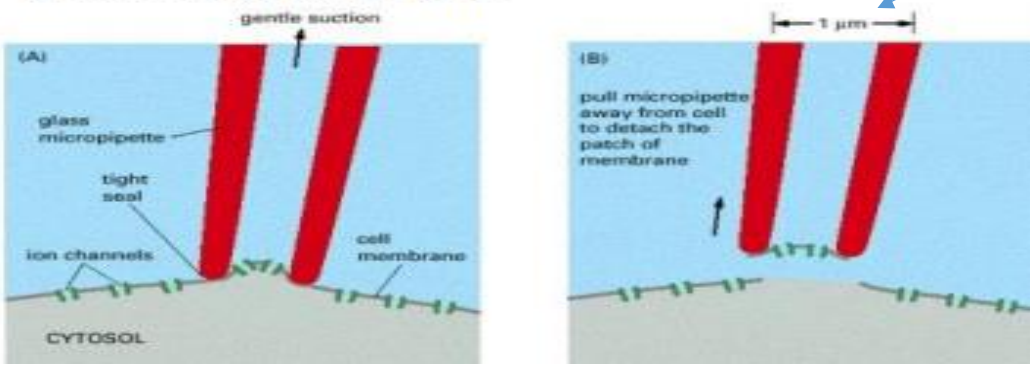
Measuring currents at specific membrane potential

- Once you have a potential gradient , we can have currents created by these ions ; you can have sodium , potassium or chloride currents according to the condition that we have across the membrane (the potential that we have across the membrane).
- Technique that is used for this issue is called PATCH CLAMP : It is a technique that is used to measure the current that can pass through the membrane at fixed voltage point.
- In this technique we are measuring the current across a part of the membrane , currents that depend on permeability or conductance , so if we have high number of sodium channels activated, we will have high sodium current . In this way we can study the current that can pass across the membrane .

Patch Clamp

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



Patch Clamp

- electronic device is employed to maintain, or "clamp," the membrane potential at a set value
- recording the ionic current through individual channels

- mechanism of PATCH CLAMP : the patch pipette contains a solution with an ionic composition similar to that of cytoplasm, whereas the solution exposed to the outer surface of the membrane has a composition similar to that of normal extracellular fluid .

If we assume that a cellular membrane is permeable **only** to K+, which is found in a very high concentration inside the cell. K+ will diffuse to the extracellular fluid because of the concentration gradient. The diffusion of K+ will result in a movement of positive charges outside the cell and leaving behind negative charges inside the cell. This will create an electrical potential difference across membrane (positive outside and negative inside). Creation of this potential difference will oppose diffusion of K+ to the outside at a certain concentration difference. When you reach a point at which diffusion of K+ is completely opposed by the potential difference created across membrane and the net diffusion for K+ is zero even though you still have a concentration gradient, you have reached the equilibrium potential for K+ (E_K). The equilibrium potential for any univalent ion at normal temperature can be calculated by Nernest equation:

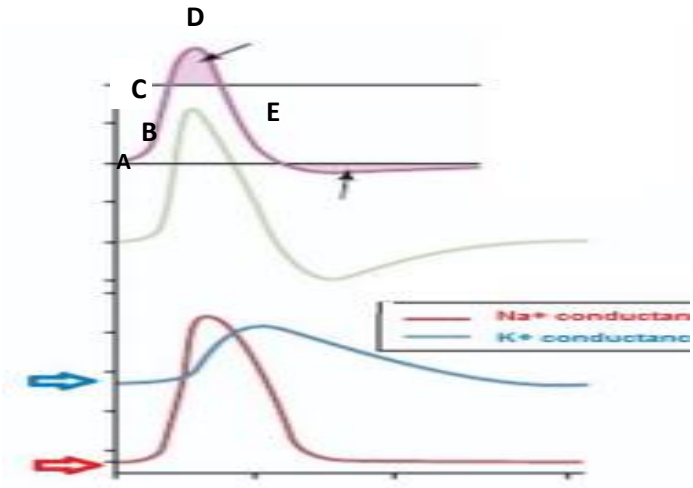
$$E\ (mV) = -\ 61.\log\ (Ci/Co)$$

E = equilibrium potential for a univalent ion
Ci = concentration inside the cell.
Co = concentration outside the cell.

When more ions are involved in creating the potential, we can calculate the potential according to Goldman-Hodgkin-Katz equation.

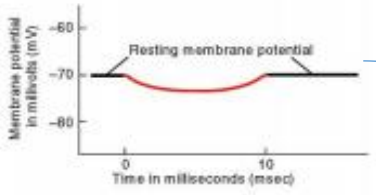
$$E_m = \frac{RT}{F} \ln \left(\frac{P_{Na+}[Na^+]_o + P_{K+}[K^+]_o + P_{Cl-}[Cl^-]_i}{P_{Na+}[Na^+]_i + P_{K+}[K^+]_i + P_{Cl-}[Cl^-]_o} \right)$$

P = permeability of the membrane to that ion.

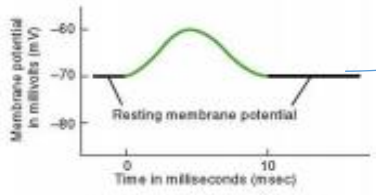


These channels are called voltage gated channels because they change their behavior according to the voltage that we have across the membrane

- A : This is the resting point(which called resting potential)where there is a very low conductance for sodium which means we have very low sodium ions and low active sodium channels, but at the same time we have a very high conductance for potassium which means there are specific number of potassium that are active .(much more active K+ channels than Na+ channels)
- By stimulation, the potential is changed to be less negative potential , here the number of activated sodium gated channel is increased and this represent B.
- C : There is a very fast shift in memrbrane potential; it becomes less negative and above zero. Here the potassium channels start to be activated but in a low rate.
  - Here it looks like it is trying to reach the the equilibrium potential for sodium which is(+61mV) but it will not reach (because potassium will prevent this to happen).
- D : Once we reach the top, there will be a decrease in conductance for sodium by closing of sodium gated channels.
- E : More potassium gated channels are activated and the potential return back toward the rest very fast .
  - The channels over membrane(K+ and Na+ gated channels) in these excitable cells can change there behaviour according to the voltage over that membrane.



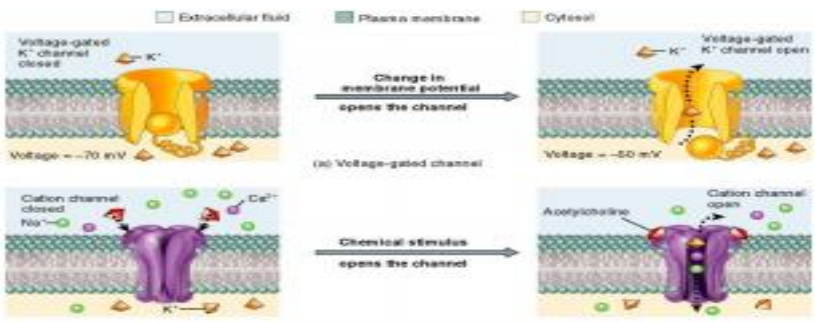
(a) Hyperpolarizing graded potential



(b) Depolarizing graded potential

A : If we have a resting membrane potential at (-70 mV) and we have activated more potassium channels, we will have more negative potential and this called (HYPERPOLARIZATION) . If the stimulus is over we return back to the resting state.

B:If we have a resting membrane potential and we have activated more sodium channels , we will have less negative potential (DEPOLERIZATION)



We can also activate channels by ionic current at the surface inside or outside the membrane

Here we have two types of channels:

1) Voltage gated channel that changes its behavior according to the change in voltage across the membrane (like Na+ and K+ gated channels).
2) Chemical gated channel , here we have ligand and receptor (IONS FLOW FROM HIGHER CONCENTRATION TO LOWER CONCENTRATION).

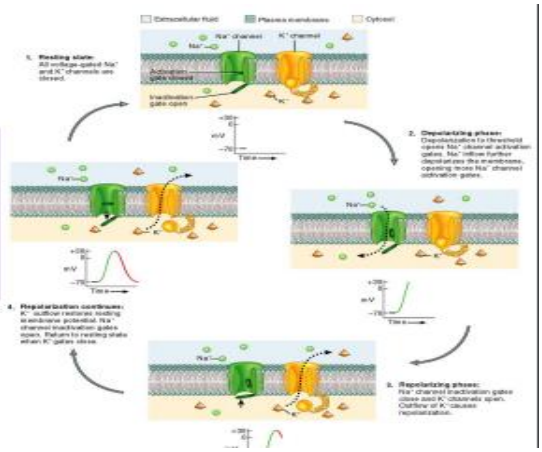
ACTION POTENTIAL:

As we have seen, the plasma membrane is **polarized** (has ability to separate opposite charges) during resting state. When the membrane potential decreases (becomes less negative), the membrane is in **depolarization** stage. While the change in membrane potential in opposite direction (becomes more negative than resting potential) is known as **hyperpolarization**.

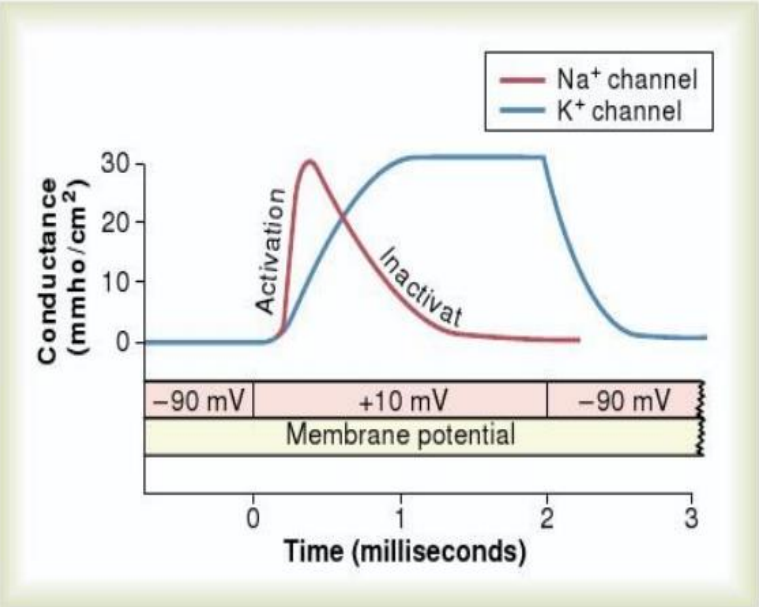
When a cell is depolarizing, it reaches a maximum according to stimulus, then the membrane potential returns to its resting state. The phase of returning from depolarized state to resting state is known as **repolarization**. These changes in membrane potential can be recorded by placing one electrode inside the cell and the other out side the cell. By recording of whole action potential in this way, we will obtain a **monophasic action potential**.

Let us consider the changes in membrane potential of an excitable cell to understand the events that appear during changes of membrane potential. To induce a change, a stimulus must be applied to change activity of channels at the membrane. Any increase in permeability of membrane to Na+ will result in diffusion of (+) charges inward. This event will decrease the membrane potential (becomes less negative). And conversely any increase in K+ diffusion (movement outward) will result in an increase in membrane potential (becomes more negative). The diffusion of these ions depends on the activity of Na+ and K+ channels that are found on the membrane. Activation of Na+ channels will induce depolarization, while activation of K+ channels will increase the potential difference across membrane.

- Changes in Channels activity results in action potential



Notice that at the resting membrane potential we have certain number of potassium channels that still active



A certain depolarization can cause fast activation of sodium channels but once we reach the peak (positive inside and negative outside) these soduim channels will close, and we will get the highest activation for potassium channels.

Then when we go back to the resting potential,the potassium gated channels will be inactivated . (But still have a certain number of active potassium channels)

The sodium channels are activated very fast but potassium channels are activated slowly.

Action potential and the role of Na+ channels:

On the membrane, most Na+ channels during resting state are inactive (closed). According to channel type, these channels can be activated by a chemical stimulus (in case of chemical gated channels), electrical stimulus (in case of voltage gated channels), or mechanical stimulus. In the case of chemical gated channels, binding of ligand to its receptor will induce activation of chemical gated Na+ channels. Once activated, the membrane potential will decrease (becomes less negative). Which means that the membrane depolarizes. The voltage changes in the membrane will cause the other type of channels (Na+ voltage gated channels) to be activated. Activation of these channels will cause more changes in membrane potential (more depolarization). More and more depolarization will occur in the membrane by a positive feed back mechanism. If we reach a point at which most voltage gated Na+ channels are activated, this will cause a sudden increase in Na+ permeability. This increase in Na+ permeability will even reverse the membrane potential (becomes positive inside and negative outside) (this is known as the **overshot** in the action potential), because Na+ is trying to approach its equilibrium potential (E_{Na}). At this point membrane has reached maximal changes in membrane potential (a peak of an action potential).

As we have seen, during depolarization there is a point at which a sudden increase in Na+ influx which induces rapid and maximal change in membrane potential. This point is known as **threshold** of an action potential. The rapid change in membrane potential during the raising phase of an action potential is known as **firing stage**. When a stimulus causes a depolarization that brings the membrane potential to the threshold, the membrane will respond by the firing stage of an action potential. If depolarization in the membrane has not reached threshold, the membrane will not enter firing stage, and instead, the potential returns to its resting level. Therefore the response in the membrane will be either by an action potential when threshold is achieved or no appearance of an action potential when the membrane potential has not reached threshold. For that reason induction of an action potential in excitable cells follows the **NONE OR ALL PRINCIPLE**.

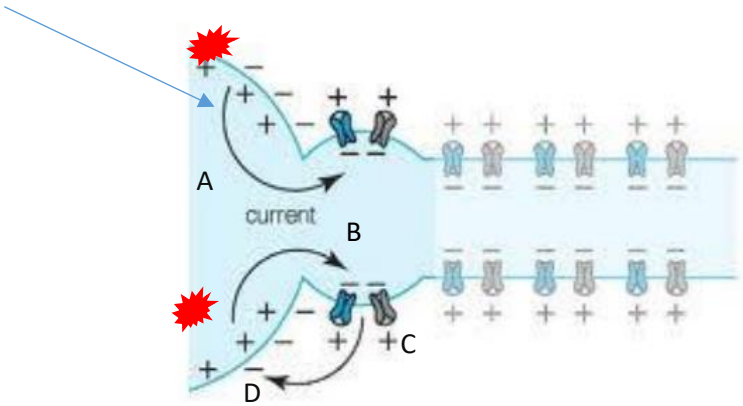
The voltage changes in membrane potential not only activate voltage dependent Na+ channels, but also inactivate these channels at certain potential difference. This inactivation appears because channels have changed their state from opened channels to closed channels due to voltage changes. The closing event of Na+ channels does not make these channels as the only responsible for bringing membrane potential to its resting level. But also, activation of voltage dependent K+ channels is the main player in returning the membrane potential to its resting level.

Action potential and K+ channels:

Although there is some leakage of K+ during resting state, which maintains the resting membrane potential close to E_{K+}, depolarization causes activation of voltage gated K+ channels. The activation of these channels is much slower than activation of Na+ channels. This results in a delay in the maximal activation of K+ channels.

The delayed activation of K+ channels combined with inactivation of Na+ channels will result in a rapid returning of the membrane potential to its resting level causing the **falling phase** in the action potential. The membrane potential may go for a while to more negative potential than during resting potential, which is known as **positive afterpotential (after hyperpolarization)**. Followed by a full recovery in membrane potential (returns completely to its resting level). The positive after potential is probably due to an excess in K+ efflux, which causes more deficit of positive ions inside the cell.

Ionic currents cause depolarization



Once you have a situation like this one and you have the membrane potential changed at certain part for example becoming positive inside and negative out side as the tip of action potential

and the nearby membrane still at the resting state so we have two nearby regions one is positive inside and negative outside,and the other is positive outside and negative inside , this creates electrical current called (IONIC CURRENT) between two nearby regions it is like charges that are moving, the positively charged particles are moving similar between nearby region as shown.

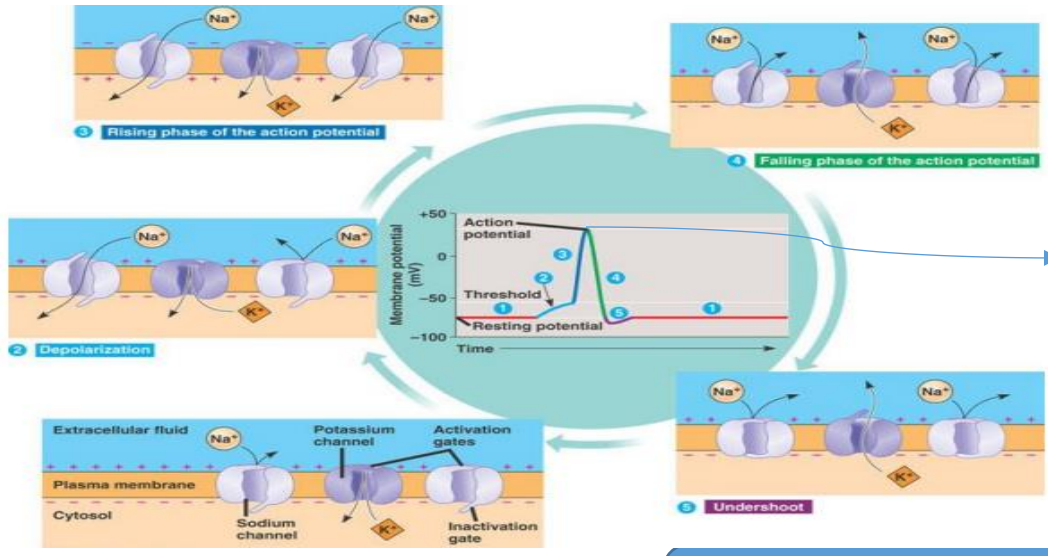
What happens if we have some positively charged particles have moved from region A to B ? the potential at that region becomes less negative (DEPOLARIZATION)because we have some positive charges have moved from A TO B and from C to D

We have some resistance to ionic currents (THE MOVEMENT OF IONS) so the channels have to be opened in order to reduce the resistance for the current , but across the membrane at the resting state we have very high resistance for the current because we have closed sodium channels and some potassium channels and the ions can pass through the membrane only according to the conductance

BY DEPOLARIZATION , more sodium channels are activated so there will be increase in the conductance.

The participation of negatively charged ions (chloride ions) is almost NEGLIGIBLE.

Once we have small depolarization (threshold) ,we are activating more voltage gated sodium channels; this happens by either ligand or by lateral current (IONIC CURRENT)



At this point , the activation of K+ gated channels is **started** and the activation of Na+ gated channels is **over** .

At which phase we have the highest activity for sodium gated channels ? **3**

At which phase we have the highest activity for potassium gated channels ? **4**

The threshold is a point where almost all sodium gated channels are activated.