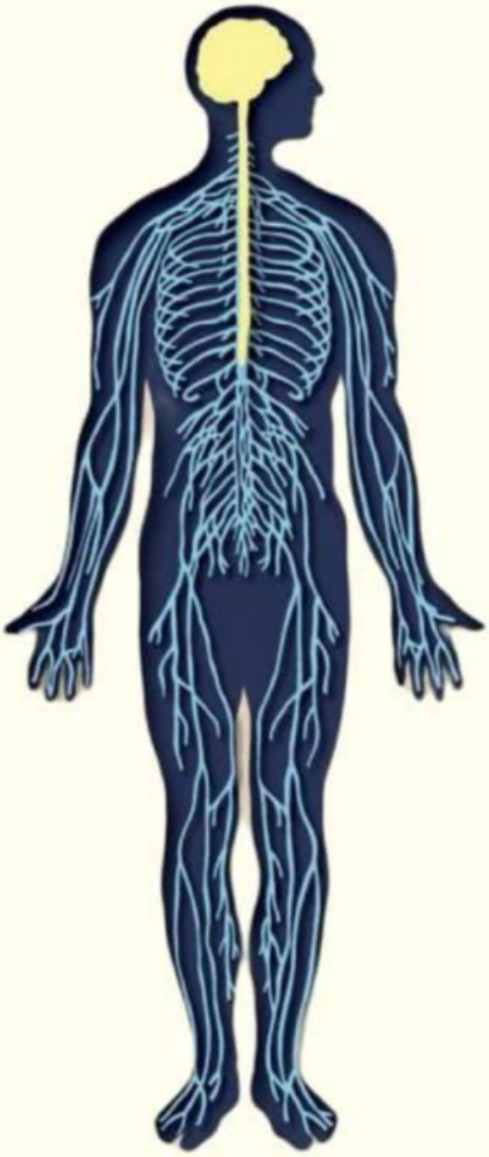
Wisdom
Dentistry 2023

SHEET NUMBER : 4,5



2023

PHYSIOLOGY

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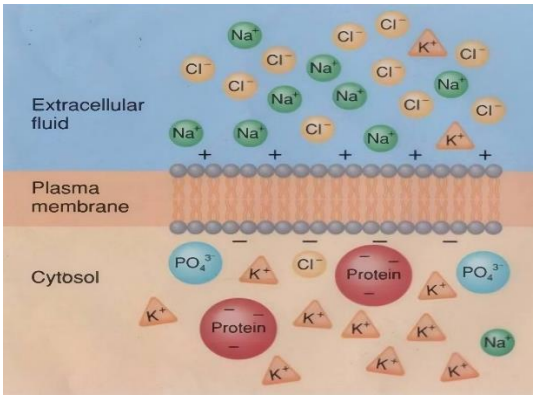
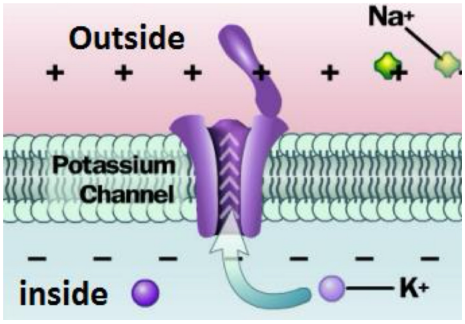
Plasma Membranes of Excitable tissues

In the past sheets, we've talked about transporting particles generally, in this sheet we'll talk about transporting again, but specifically about transporting charged particles through the plasma membrane such as K^+, Na^+, Ca^{+2}, Cl^- and so on, what will happen in this case?

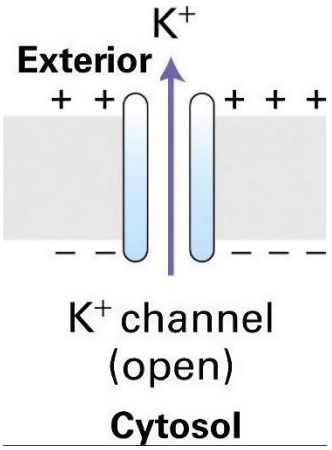
As you know, the membrane of our cells separates two compartments:
1- The Extracellular Matrix (ECM), outside the cells. (**High concentration of Na^+ ions**)
2- The Cytoplasm, inside the cells. (**High concentration of K^+ ions**)





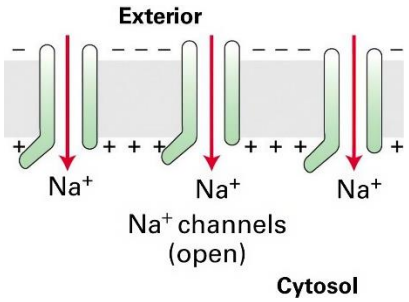
According to the concentration gradient, **K^+** ions have a high tendency to move from the inside toward the outside of the cells, meanwhile **Na^+** ions have a high tendency to move from the outside toward the inside of the cells.

Assuming a membrane that is permeable only for **K^+** ions, these ions will move from the inside toward the outside of the cell, creating a potential (electrical) across the membrane (**negative inside, positive outside**) and will reach the equilibrium, but what type of equilibrium? Chemical equilibrium (equal concentrations)? Actually, it won't get to this type of equilibrium, it will get **Electrochemical** Equilibrium (**Electro**: from the potential, **Chemical**: from the concentration).



In this case, reaching Electrochemical Equilibrium doesn't mean reaching Chemical equilibrium, we still have a concentration gradient of **K^+** ions, **high** inside and **low** outside, but the number of **K^+** ions that moving outside is **equal** to ones that moving inside, and that's due to the **Electrochemical Equilibrium** part (**electrical potential**).

Another example, assuming a membrane that is permeable only for **Na^+** ions, these ions will move from the outside toward the inside of the cell, creating a potential across the membrane (**positive inside, negative outside**).

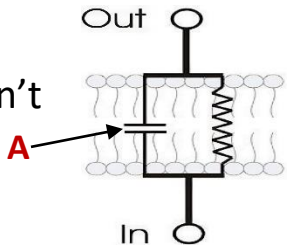


What if we have a membrane that is permeable only for **Cl^-** ions? These ions will move from the outside toward the inside of the cell, creating a potential across the membrane (**negative inside, positive outside**).

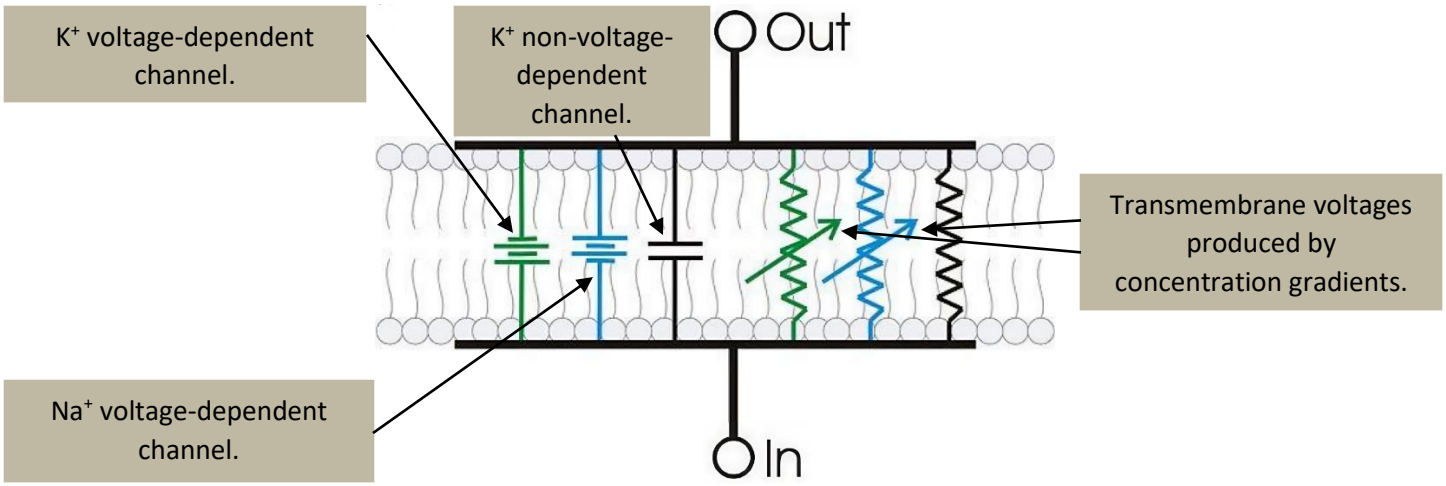
What if we have a membrane that is permeable only for **Ca^{+2}** ions? These ions will move from the outside toward the inside of the cell, creating a potential across the membrane (**positive inside, negative outside**).

As we are talking about charges, and a lipid bilayer (**membrane separating them**), we can think about this membrane as an electrical circuit, how is that? :) كمل دراسة بتعرف

The symbol **A** represents a Capaciter (**used to separate charges**), now you should know that the cell's membrane works as a capaciter, isn't it?



Here is more complicated one (Dr. Mohammad didn't say any details about it, but it's written in the slides):



Nernest Equation

We can calculate the potential across membrane using the **Nernest Equation** if the membrane is permeable for only one ion.

$$E = \frac{RT}{ZF} \ln \frac{[C]_{out}}{[C]_{in}}$$

E: Equlibrium, **R**: Gas constant, **T**: Absoulte temperaturem **Z**: Valence
F: Faraday's constant, **C**: Concentration, **out**: outside the cell, **in**: inside the cell.

Electrochemical Equilibrium

$$\Delta \boldsymbol{G}_{conc} + \Delta \boldsymbol{G}_{volt} = \mathbf{0}$$

ΔG_{conc}: The difference in energy generated by the concentration gradient.

ΔG_{volt}: The difference in energy generated by the voltage across the membrane.

$$= zFV - RT \ln \frac{[C]_{out}}{[C]_{in}} \rightarrow V = \frac{RT}{zF} \ln \frac{[C]_{out}}{[C]_{in}} \rightarrow V = 2.3 \frac{RT}{zF} \log_{10} \frac{[C]_{out}}{[C]_{in}}$$

R, **T** and **F** are **constants**, replacing them with their values and when **z=1** for K^+:

$$E_{K^+} = 61.54 \log \frac{[K^+]_{out}}{[K^+]_{in}}$$

R, **T** and **F** are **constants**, replacing them with their values and when **z=1** for Cl^-:

$$E_{Cl-} = 61.54 \log \frac{[Cl^-]_{in}}{[Cl^-]_{out}}$$

R, **T** and **F** are **constants**, replacing them with their values and when **z=2** for Ca^{+2}:

$$E_{Ca^{+2}} = \frac{61.54}{2} \log \frac{[Ca^{+2}]_{out}}{[Ca^{+2}]_{in}}$$

To avoid calculating these during the exam, memorize the next table.

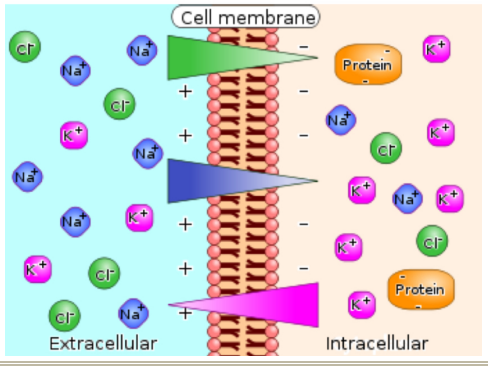
Ion	Extracellular (mM)	Intracellular (mM)	Nernst potential (mV) + → positive inside in comparison to the outside. - → negative inside in comparison to the outside.
Na^+	145	15	60
Cl^-	100	5	-80
K^+	4.5	160	-95
Ca^{+2}	1.8	10^{-4}	130

Our excitable cells have a very high permeability for K^+ ions and very low permeability for Na^+ ions, which results in creating potential, which is **negative inside and positive outside**, closer to the equilibrium potential for K^+ ions, but it will never reach it, because we have some permeability for Na^+ ions.

However, there are differences in permeabilities of membranes for ions which create differences in potentials over them, we have some membranes generate potentials equal to −70, −80, −90 and some aren't even excitable.

Let's say we have a membrane with a high permeability for K^+ ions and very low permeability of Na^+ ions, we will get a potential which will be very close to the equilibrium potential for potassium (-95mV), why close to it not equal to it?
- Again, because of Na^+ ions, they will make the potential less negative.

As we mentioned, **Nernest Equation** can calculate the potential for a membrane that is permbeable for only one ion, but **our cells' membranes** are permable for **multiple ions**, so we need another equation.



Goldman Hodgkin Katz Equation

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

P: Permeability of the membrane to that ion.

The movement of the **chloride ion** from outside to inside effect is a reversal of the movement of **sodium ion** from outside to inside effect, it also has the same effect of **potassium ion** that is moving from inside to outside.

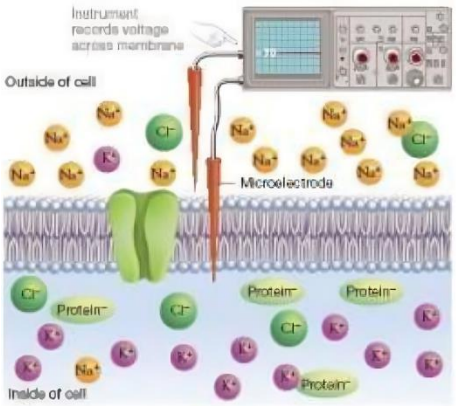
We should mention, if we used this equation in case of a membrane is permeable for only one ion, we'll get back to **Nernest equation**.

So far, we've seen two factors that play a rule in modulation of the potential across the membrane:

1- **High** permeability for **K^+** ions.

2- **Low** permeability for **Na^+** ions.

We can measure the potential across the membrane using the voltmeter as shown in the picture, we must place the electrodes just at the inside (**not deep**) and just at the outside (**not far**) of the membrane.



لَنْ أَبْرَحَ البَابَ حَتَّىٰ أَبْلِغَ القِمَمَ

وَأَرَىٰ بِعَيْنِيْ سَقْفَ الحُلْمِ مُنْتَظِمِ

Resting membrane potential

What determines the rest potential?

1. Activity of the K^+ channels (**most influential**):
The K^+ ions move from the inside toward the outside and cause a negative potential for the membrane.

- Contribution of **K^+ diffusion**:
As mentioned earlier, if the membrane is permeable only for K^+, the calculated E_{K^+} is about (-94mV):
$C_{out}K^+ = 4meq/l \rightarrow C_{in}K^+ = 140meq/l \rightarrow E_{K^+} = 61*\log(4/140) = -94mV$

Which is not far from the recorded membrane potential, but not exactly equal.

2. Activity of the Na^+ channels:
The membrane has less permeability for Na^+, so the rest potential will be closer to the equilibrium potential of K^+, but they aren't equal.

- Contribution of **Na^+ diffusion**:
The permeability of the membrane for Na^+ is much less than that of K^+, so if the membrane is permeable only to Na^+, the calculated $E_{Na^+} = +61mV$.

Because of the permeability of the membrane for these two ions, the **E** would be between (**-94mV and +61mV**), the calculated **E** for these two ions is **–86 mV**, which is not far from the E_{K^+} (because of the higher permeability of membrane for K^+ than for Na^+ → 200 times more for K^+ than Na^+)

3. **Activity of the Na+ /K+ pump**:
It pumps **3 Na^+** ions from the inside toward the outside and **2 K^+** ions from the outside toward the inside, it can alone create a membrane potential which will be negative inside.

- Contribution of **Na^+/K^+ pump**:
It produces **-4 mV**.

All these factors, during rest, will give a net membrane potential of **-90mV**, which is the resting membrane potential.

In this sheet we will talk about membrane at resting state and its properties, which means that the cell is not stimulated by any stimulus so it will have these specific resting properties.

NOTES:

1- membrane resting potential **can be changed** by a stimulus.

2-If we activated **more K^+ channels** the potential will be shifted to **more negative.**

(Because normally there are more K^+ ions inside the cell so channels will move these ions from **the higher** concentration to **the lower** one, reducing the positive charge inside the cell while increasing it outside in addition increasing the negative potential (charge of the cytoplasm compared to the ECM charge)) **because of the ion's positive charge**

3- if we activated **more Na^+ channels** the potential will be shifted to **less negative**.

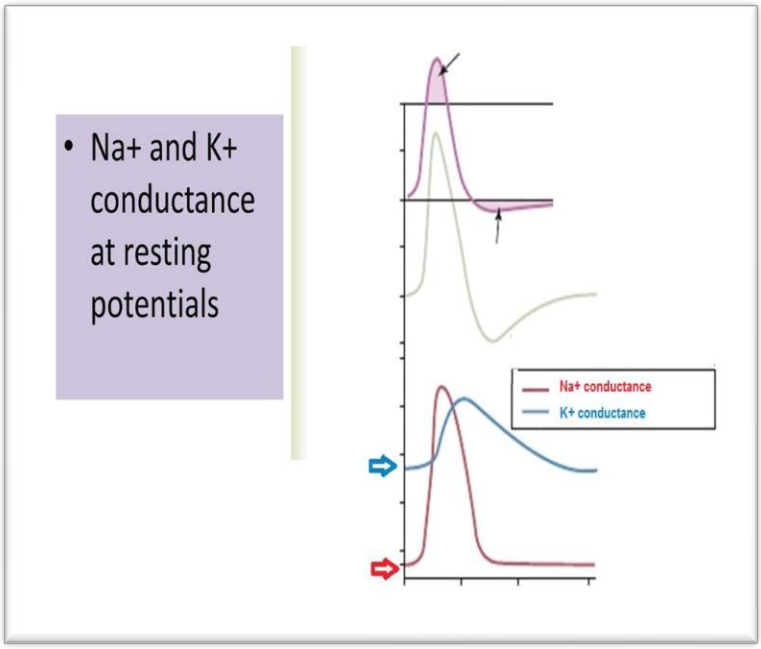
(Because normally there are more Na^+ ions outside the cell, so these ions will move into the cell making the cytoplasm more positive while increasing the negative charge outside of it) **because of the ion's positive charge**

4-From now on if we are at resting potential **the permeability of K^+ is higher than permeability of Na^+**.

5-we are talking here about **ions** moving; so instead of saying permeability we say that we are changing the **conductance** of that membrane to that ion.

For example, we can increase sodium conductivity; HOW?

- Simply by activating more Na^+ channels.



In the adjacent pic (red square) at **resting potential** we have about 200 times conductance for potassium than sodium, and because of that we are establishing a resting membrane potential(**-ve**), because of the high conductivity of K^+.
We can change the conductance by changing the activity of these channels.

Cord Conductance eqn of plasma membrane

Ohm's law

- $I = \Delta V/R$
- G (conductance)= 1/R
- $I = G.\ \Delta V$

When we talked about the permeability of particles, we used Fick's law but here we're talking about ions, so we'll use electrical terms.

I: Current.

V: The voltage difference across the plasma membrane (**the driving force that moves ions**).

R: Resistance across the plasma membrane.

G: Conductance; how that membrane conducts or lets a specific ion move through it.

(Conductance is inversely proportional with the resistance; so, if we have a **high conductance** for an ion that's mean we have **low resistance** and vice versa).

- Also, we can measure the whole membrane voltage according to its conductivity for different ions.

It can be calculated by this equation:

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

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

G: Conductance for the ion

G_{tot}: Total conductances

E: Equilibrium potential.

Measuring Currents at Specific Membrane Potential

Patch Clamp Technique

We can measure the activity of different channels by measuring their currents like Na^+, K^+ or Cl^- depending on the ion that is moving.

Patch clamp technique: The technique by which we can measure the currency of different ions; to study which voltage-gated channels are active at a specific membrane potential (voltage).

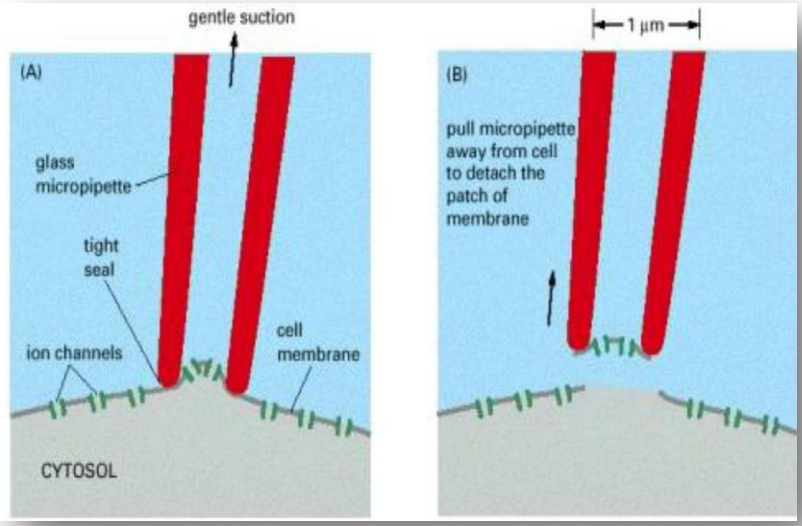
1-We have a tip (**tip of a pipette**) that is very small (**smaller than the cell**).

2- By sealing a part of the membrane that has the channels we are studying.

3- At the tip of the pipette we will have 1 or more sodium channels for ex (**that are voltage gated**) we found that at (**-80**) they are inactive, so we changed the potential to (**-60**) under the microscope to track their behavior and observe whether they become active or not.

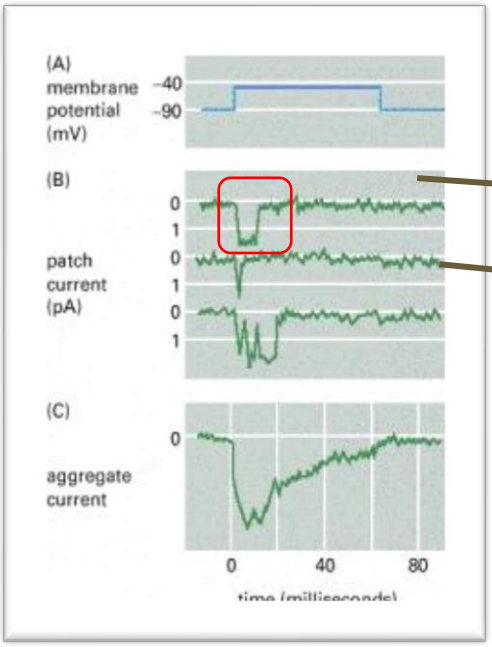
How we're shifting? By clamping the potential of that membrane (by the two electrodes which clamp the potential to (**-60**) so I study the current at the chosen potential).

In the below picture (**B**) there is a part of a membrane that is sealed off, we can first place the pipette inside a solution that is similar to the extracellular fluid (**high concentration of Na^+**)and then placing the pipette in a solution that is similar to intracellular fluid (**high concentration of K^+**) now by clamping the potential at (**-60 or -40**) -for example- and by studying the behavior of these channels we will figure out what happens to these channels: get activated or inactivated.



Watch this short video for more understanding.

https://youtu.be/mVbkSD5FHOw



This picture shows the recording of currents in patch clamp.

Clamping the potential to (**-40**).

We will start to see currents.

Here we consider that the current is from outside to inside.

(يعني حركة الايونات من الخارج للداخل)

للتوضيح أكثر المخطط في هذا المثال ينزل للأسفل (المربع الأحمر) بالتالي حركة الأيونات من الخارج إلى الداخل فالأيونات التي تتحرك هي ايونات الصوديوم الموجبة وحركتها تقلل الشحنة الموجبة في الخارج وتزيدها من الداخل، من الممكن بمثال اخر أن تكون الحركة بالعكس إذا كانت لأيونات البوتاسيوم فالمخطط سوف يكون للأعلى بعكس هذا المخطط.

- If it is the opposite, it will be designed to the reversal direction.

- To understand and analyze what ions are producing this current due to the raise in their conductivity, and what is the direction of this current? to answer these questions we bring two or more solutions with different concentrations

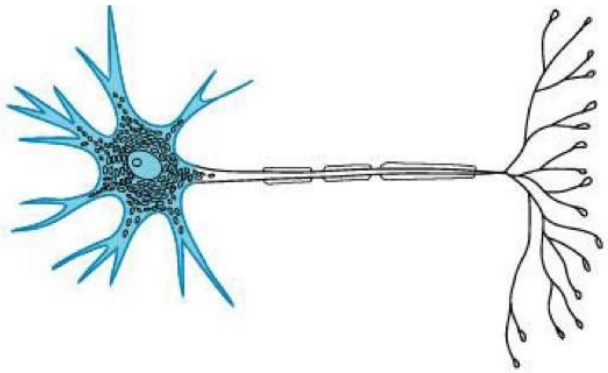
For example: we put solution with high concentration of NaCl (sodium chloride), we will notice a current production at this specific voltage then we change the solution to $CaCl_2$ (calcium chloride), no current will be observed, so this indicates that the current is produced by the movement of Na ions. (This example is for the movement of ions from outside to inside)

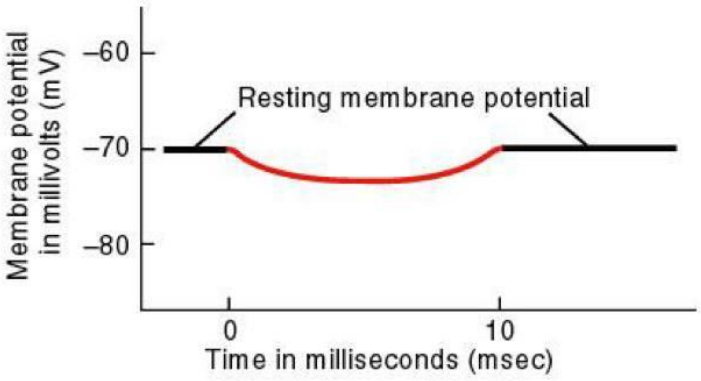
مثال اخر:

If I have KCL solution and I change the current by changing the solution to make sure that it is potassium current or if it's produced by the movement of Cl^- from the outside to the inside not the movement of K^+ from inside to outside.

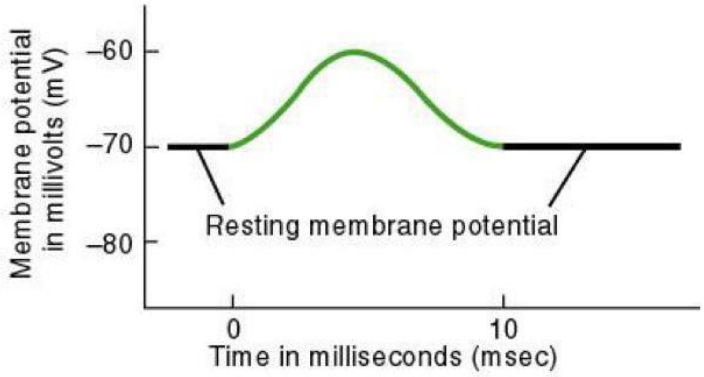
يعني بغير المحلول حتى اتأكد مثلا اذا التيار الناتج من حركة الكلور مش من حركة البوتاسيوم.

I





(a) Hyperpolarizing graded potential



(b) Depolarizing graded potential

12.10

Changes in Resting membrane potential.

We said that we can shift this potential to more or less than negative.

More negative potential:

Called "**Hyper polarization**"

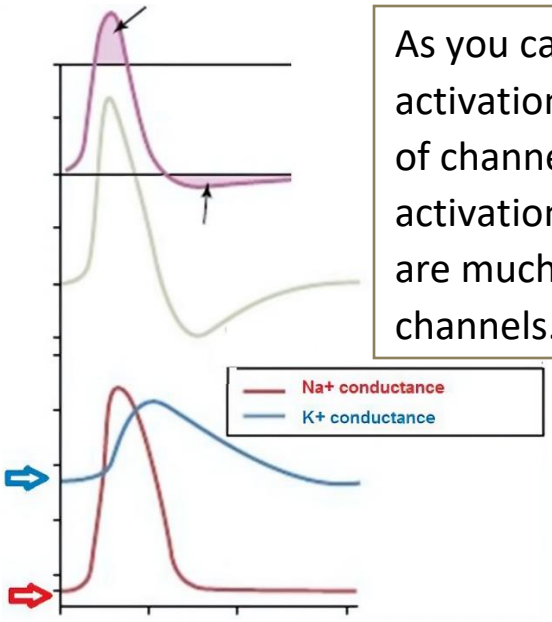
By activating more potassium channels (voltage gated) by changing the membrane potential to a specific number, so it's activated by the new voltage.

Less negative potential:

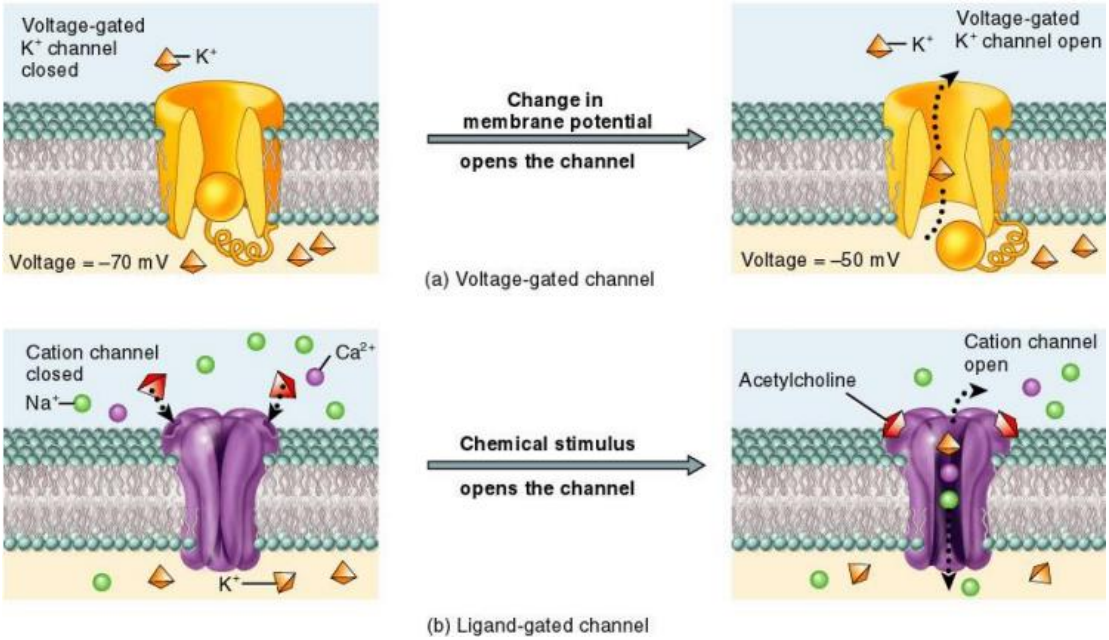
Called "**Depolarization**"

By activating more sodium channels (chemical)**.**

When activating these channels we will reach a point –threshold– at that point all voltage gated sodium channels are opened, so the membrane potential will be less negative and after the sodium channels reach potential equilibrium for sodium which is (**+61**) these channels will be inactivated (**the conductance for Na decreased**) and this potential motivate K^+ channels to open.



As you can see, the rate of activation for these two types of channels are different, the activation rate of Na^+ channels are much higher than the K^+ channels.



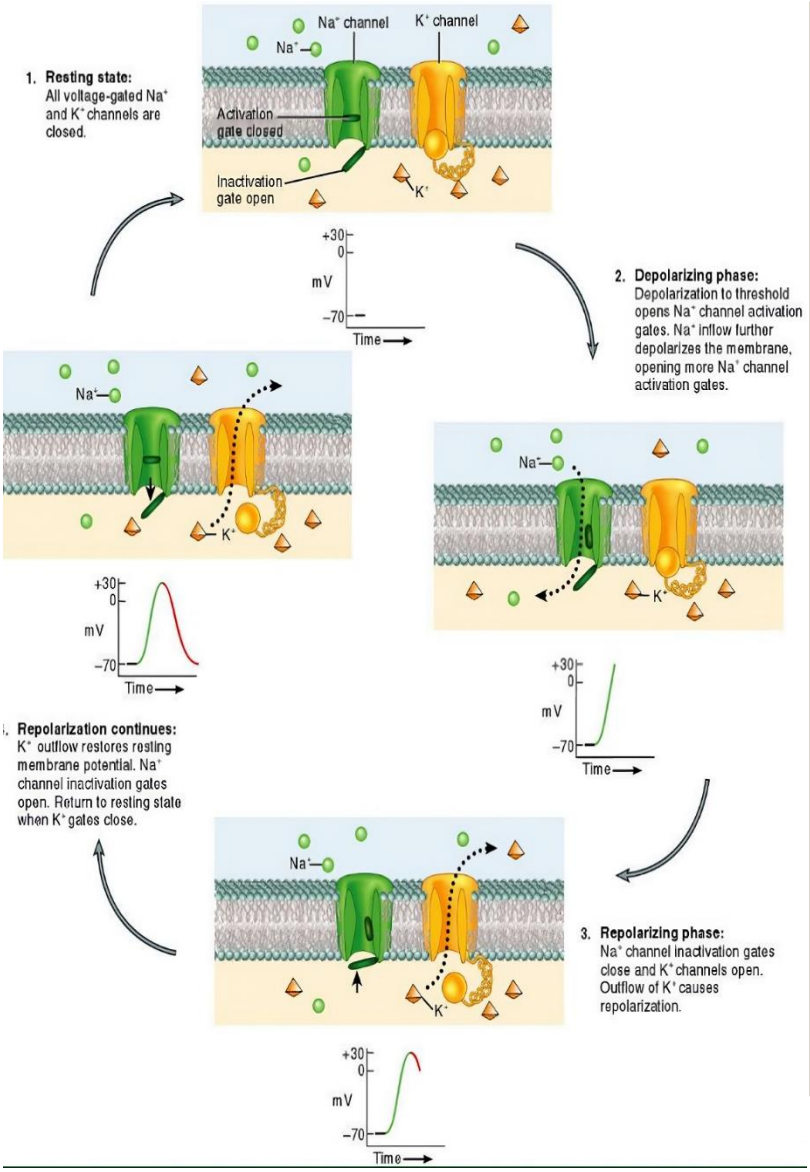
There are two types of channels:

1-**Voltage-gated channel**:

Changes its behavior according to the change in voltage across the plasma membrane.

2-**Ligand-gated channel**:

Changes its behavior if a ligand is bound to the receptor on it.



1-Resting: Both Na^+ and K^+ channels are closed.

2-Depolarizing: Na^+ channels are opened, Na^+ ions' flow toward inside increases, and this leads to more activation of Na^+ channels.

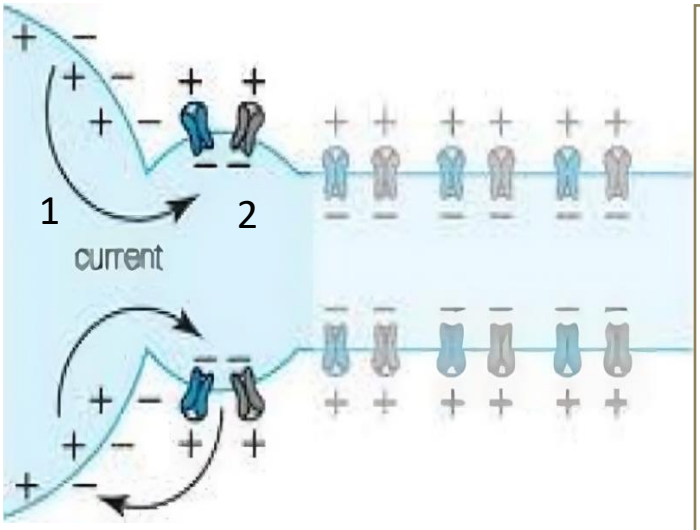
3-Repolarizing: Na^+ channels get inactivated, K^+ channels are opened, K^+ ions' flow toward outside increases, that leads the membrane to get back to the resting state, after that K^+ channel will be inactivated.

Note: Resting state is called polarized state too, so "Repolarizing" means regenerate the polarized state, which is resting state.

But, before the action potential takes place, we must overcome the threshold, how is that happening? 1-**Ligands**. 2-**Ionic Currents**.

At this point, we should mention the non or all principle, that means if these Ligand or Ionic Current couldn't reach the threshold, nothing will happen because it couldn't activate the voltage-gated sodium channels, but if they could reach the threshold, the depolarization will start (by activating voltage-gated sodium channels) leading to the action potential.

We've already talked about Ligands, what about Ionic Currents?

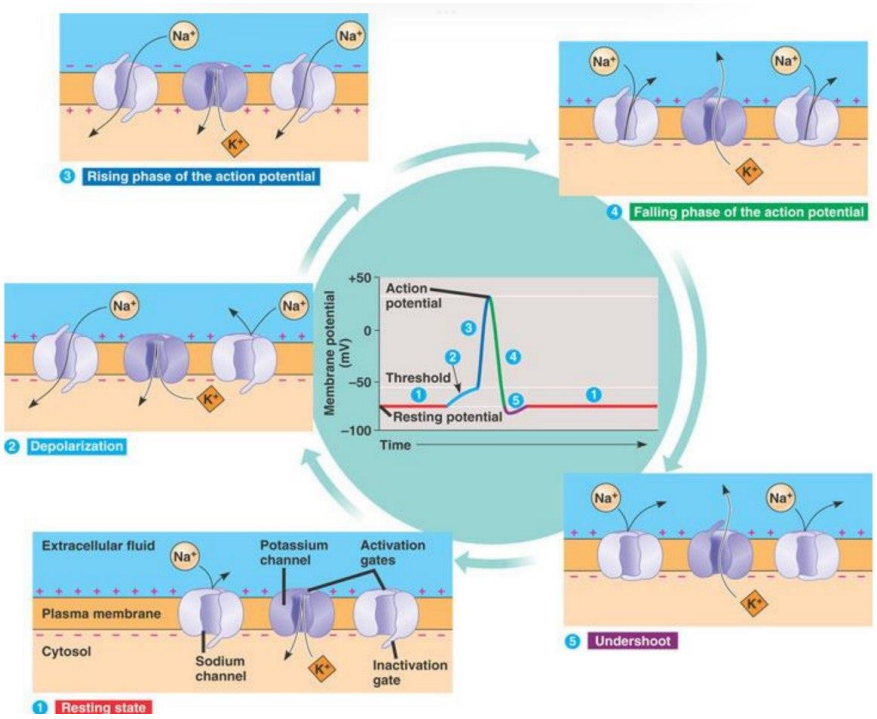


Assuming part 1 is in action potential, it becomes positive inside, and part 2 is still in the polarized state, so it's negative inside, if some positive ions (ionic current) moved from 1 toward 2, and more positive ions moved from outside toward inside part 2, it will overcome the threshold, leading to start the depolarization.

In conclusion, you don't have to activate all channels over membrane manually (by Ligands), it's enough to activate one part and the action potential can move along the membrane by ionic currents.

Action potential

- As we talked in the previous sheet, sodium channels must be activated by chemical, electrical or mechanical stimuli to reach the threshold, which is called the depolarization state (in which the membrane becomes less negative inside because of the sodium ions movement toward inside).



At 1) sodium channels start to open but at two they are having the highest rate and there's a big difference between the two

1)Resting state: all channels are closed (Na^+ and K^+ voltage gated channels).

2)Threshold: activation of some fast Na^+ chemical gated channels by stimuli and potassium gated ones at slower rate, if the stimuli were efficient enough the region will reach a specific potential "threshold" that triggers the action potential (the sodium rate is higher than the potassium).

3)Firing phase, also called Depolarization (rising phase of the action potential): more sodium voltage gated channels will open motivated by threshold (while K^+ channels are still closed) causing the fast entrance of sodium ions into the cytoplasm (increasing the Na^+ permeability), that causes a reduction in the negative potential of the membrane (becomes less -ve) even more because of the sodium ions positive charges (causing overshoot where Na+ ions try to reach equilibrium and

at this point the membrane has reached maximal exchanges). It's given a name to differentiate between this fast depolarization and the threshold one.

4)Repolarization (falling phase of the action potential):after the firing phase, caused by the Na+ ions, the membrane potential reaches a specific positive value ,this new potential inactivate the sodium channels and motivate the opening of more k+ voltage gated channels that transport these ions from the cytoplasm the the ECM (the cytoplasmic side is loosing the positive potassium ions to the outer surface of the membrane) making the inside more negative than the outside (increasing the membrane potential making it more -ve).

5)Undershoot (Hyperpolarization/Positive after Potential -old name): the potassium channels will close relatively slowly causing this "undershoot" hyperpolarization potential that is more negative than the resting one, this new potential inactive the potassium channels and the region will be back to it's resting state after a refactoring period

Important note: if the depolarized membrane didn't reach the threshold potential that triggers the action potential the membrane potential will return to the resting state.

Why the hyperpolarization has this old name?

Recording the action potential means recording the inside compared to the outside and because the first recordings of the membrane potential was according to the outside compared to inside it was named like that (reversal direction of diagram)—> you return down to reach the threshold then go further down for the depolarization then move back up for the repolarization so it's a mirror image upside down.

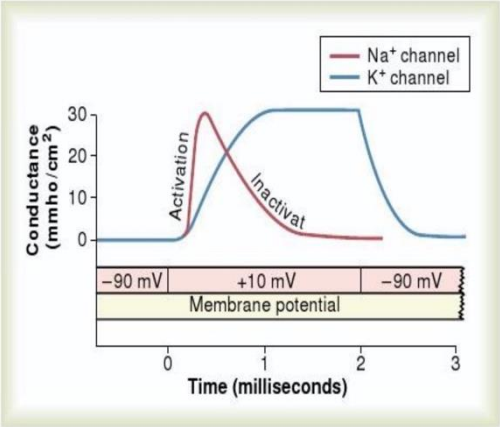
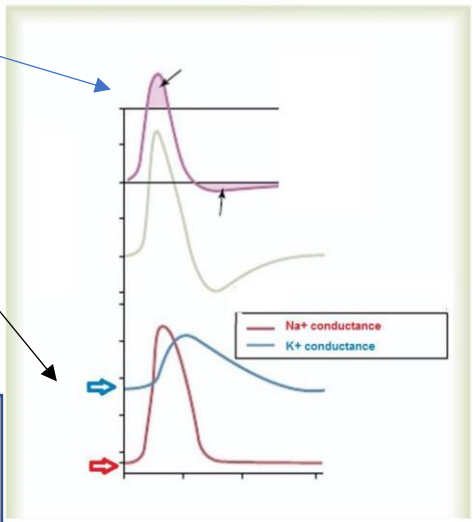


Figure 5–9

لاحظ ان قنوات البوتاسيوم تأخرت في الإغلاق

Overshot: is the peak of the action potential where's the membrane potential is positive it may be the period in black or all the red curve.

This drawing refers to the ratio of the conductance for both ions.



- Na+ and K+ conductance at resting potentials

You are rating the conductances with what's happening in the action potential at the start we have a higher conductance for potassium but opening the sodium channels changes the case.

At resting potential, the conductance of K+ is more than Na+

If stimuli affect the cell in other periods, it will not respond cause the channels are still open during action potential.

Please check on the professor's handout,
Very Important ..

University of Jordan
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Introduction Med, 2023/2024
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Membrane physiology and the basis of excitability

Ref: Guyton, 14th ed. 63-76, Jordan and 13th ed. pp: 61-71. 12th ed. pp: **57-69**,

MEMBRANE POTENTIALS AND ACTION POTENTIALS:

MEMBRANE POTENTIAL:

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 the 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 the 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.

In this equation, Goldman and his colleagues considered that these ions are mostly involved in the development of membrane potential.

According to this equation, the permeability of the membrane to an ion is very important in determining the membrane potential. If the membrane is permeable only to K+ and not permeable to Cl- and Na+, the membrane potential will be equal to E_{K+}.

Resting membrane potential:

In excitable cells the membrane potential is not constant. When the cell is stimulated, the membrane potential is changing. These changes in membrane potential are due to changes in permeability of plasma membrane to different ions. For example, when a neuron is stimulated, this will result in increased permeability to Na+. This will bring the membrane potential closely to E_{Na}. The recorded membrane potential for a cell under resting conditions when no stimulus is involved is known as **resting membrane potential**. For neurons, the recorded resting membrane potential is about (– 90 mV). This represents a potential difference between the inside to the outside when the neuron is not active.

Origin of resting membrane potential:

Contribution of K+ diffusion:

As mentioned earlier, if the membrane is permeable only for K+ the calculated E_{K+} is about (-94mV).

Co_{K+} = 4meq/l , Ci_{K+} = 140meq/l

E_{K+} = -61. log 140/4 = -94mV

Which is not far from the recorded membrane potential but not exactly.

The contribution of Na+ diffusion:

Membrane is also permeable to Na+. The permeability of the plasma membrane for Na+ is much less than that of K+. If the membrane is permeable only to Na+, the calculated E_{Na+} = + 61mV.

…….. (Co_{Na+} = 142meq/l , Ci_{Na+} = 14meq/l).

Because of the permeability of the membrane for the two ions, the E would be between (-94mV and +61mV). The calculated E for the two ions is –86mV, which is not far from the E_{K+} because of the higher permeability of membrane for K+ than for Na+ (100 times more).

So the Na+ contribution in resting potential is by bringing the membrane potential to a lower value than the calculated E_{K+}.

Contribution of Na+ - K+ pump:

As mentioned earlier, this pump is electrogenic. It moves more positive charges outside the cell (3 for 2). This will induce loss of positive charges from the cell and bring the membrane potential to a higher negativity (about –4mV additional negativity).

Therefore all these factors, during **rest**, will give a net membrane potential of –90mV (called **Resting Membrane Potential**).

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 outside 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.

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, the 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 the **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 full recovery in the 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.

Action potential and Ca++:

As discussed before, the raising phase of an action potential results by fast activation of Na+ channels. These are called *fast channels*. In some excitable cells, like cardiac muscle and uterine muscle, cells are equipped with another type of channels known as *slow* Na+ – Ca++ *channels*. These channels are activated at slower rate than Na+ channels. The slow and prolonged opening of slow channels will cause mainly Ca++ to enter the cell and prevents the rapid fall induced by activation of K+ channels, and the membrane potential is maintained for a while then the potential falls to its resting level. This is known as a **plateau** in action potential. The presence of plateau in this type of cell is important in prolonging the time of an action potential, giving more time for the cell to be able to respond to another stimulus, because the cell remains longer time in **refractory period**.

Refractory periods of an action potential:

During an action potential, the cell is not able to respond to another stimulus. From the firing stage to the end of the first third of falling phase the cell will not respond at all even by a stronger stimulus. In this stage the cell is said to be in **absolute refractory period**. From the beginning of the second phase until the resting membrane potential is achieved, the cell cannot respond to the usual stimulus, but a stronger stimulus can

change the membrane potential. In this period, the cell is in **relative refractory period**.

The periods depend on the activity of Na+ channels. These channels pass three states during action potential. During resting potential, Na+ channels are **closed but capable for opening** when stimulated. During the raising phase (firing), almost all Na+ channels are **opened**. And any other stimulus (even stronger one) will not cause activation of more Na+ channels. During this period, the membrane is in absolute refractory period.

In the third state, when voltage dependent Na+ channels become closed after the membrane potential has reached positive values. At this state, Na+ channels are not capable for opening. During all the falling phase of an action potential, these channels remain **closed and not capable for opening**. They can pass to the first state (closed and capable for opening) when the membrane potential returns to its normal level or to a more negative potential than resting potential. During this period, the membrane is in relative refractory period. This means that a stronger (suprathreshold) stimulus may activate the closed channels that are not capable for opening by normal stimulation. In addition to the role of voltage gated Na+ channels in establishing the relative refractory period, the presence of widely opened K+ channels during falling phase, which cause excess flow of positive charges to the outside, may also play a role by opposing stimulating signals.

Na+ -K+ pump and action potential:

This pump has **no** role in the electrical activity that are taking place during action potential. But it plays an important role in restoring ionic composition that has been altered during action potential. This role is important in maintaining the ionic composition of the intra- and the extra-cellular fluids.