BIOCHEMICAL BASIS OF NEURONAL FUNCTION – An Overview

UNIVERSITY OF PNG
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
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VJ Temple

What is membrane potential?

- Membrane potential is due to electrical potential in cells;
- It involves separation of electrical charges across cell membrane;
- It is electrically negative inside the cell with respect to outside;
- K^+ conc. is higher inside cell compared to outside;
- Na^+ conc. is higher outside cell compared to inside;
- **Neuron is an excitable cell, because it is capable of generating and conducting electrical impulse by temporarily reversing its membrane potential**;
- Membrane potential is maintained by **Na^+, K^+-ion pump** with **ATPase** activity;
- It pumps Na^+ ions out of the cell and K^+ ions into the cell;

What are the two modes of impulse transmission?

- **Electrical mode of transmission**:
- Impulse transmission along axon – Membrane transmission:
  - Involves propagation of Action Potential along axonal membrane;
- **Chemical mode of transmission**:
- Impulse transmission across synapse – Synaptic transmission:
- Impulse transmission from neuron to muscle – Neuromuscular transmission
  - Involves chemical process by group of compounds called Chemical Neurotransmitters;

What factors induce ions to cross cell membrane?

- Three major factors that can induce ions to cross cell membrane are:
  - Difference in concentration of ions on both sides of the membrane;
  - Difference in electrical potential on both two sides of the membrane;
  - Action of an ion pump;

What are ion channels?

- Ion channels are special openings in cell membranes for passive movement of ions into cells;
- Ion movements based on concentration and electrical gradients are passive, but require the presence of **ion channels;**
- Most ion channels are selective for a particular ion, determined by size of central pore and electrical charges of chains of amino acids that are in the pore;

What are the different types of ion channels?

- **Leakage channels:**
  - They are open to the flow of ions all the time;
- **Gated channels:**
  - Can open or close through conformational change;
  - Conformational change may involve a change in diameter of the central pore, or movement of a part of the protein to obstruct the pore;

Major types of ion-gated channels are:

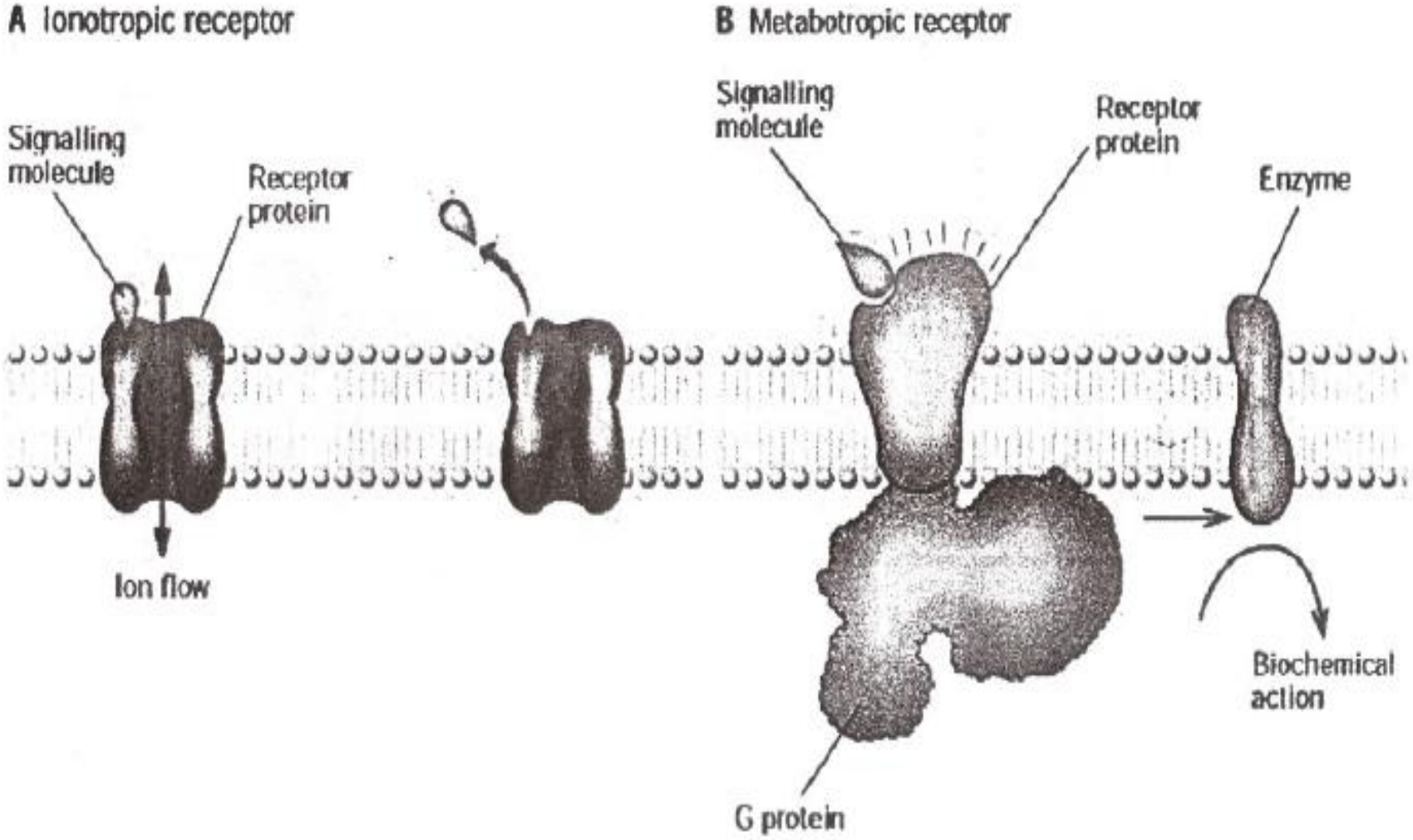
- Ligand gated ion channels;
- Voltage gated ion channel;
- Stretch sensitive channels;

What are the different types of Ion-Gated channels?

- **Ligand Gated Ion Channels** or Ligand Sensitive Channels (**Figs. 1 & 2**)
  - Open or close in the presence of a signaling molecule,
  - Involve in conduction across neuromuscular junctions and synapses
  - Ligand gated channels are usually highly distributed at Postsynaptic sites and have specific type of receptors

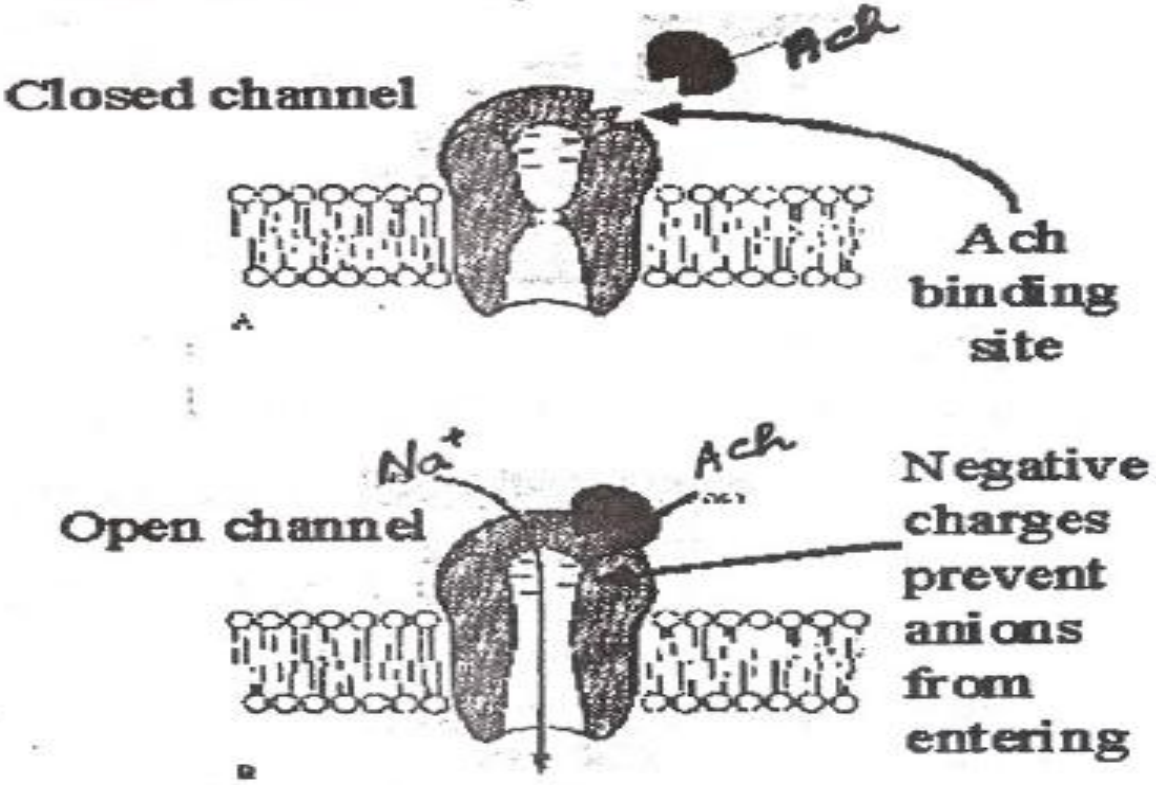
Ligand-gated channels

Fig 1



Excitability: Rapid Changes in Membrane Potential

The Chemically Gated Channel



A chemical, such as the neurotransmitter Acetylcholine (Ach), triggers a conformational change in the channel protein which allows Na+ ions to enter the cell, causing depolarization.

Fig 2.

- **Voltage gated ion channels** or voltage sensitive channels (**Fig. 3**)
  - Open or close in response to electrical potential differences across the cell membrane,
  - Forms the basis for conduction of nerve signals along axons

- **Stretch sensitive channels**
  - Opens or closes when mechanical force is applied,
  - Mediates mechanical sensitivity

Voltage-gated Channel

In voltage-gated ion channels a change in the membrane potential toward depolarization triggers opening of the ion gates.

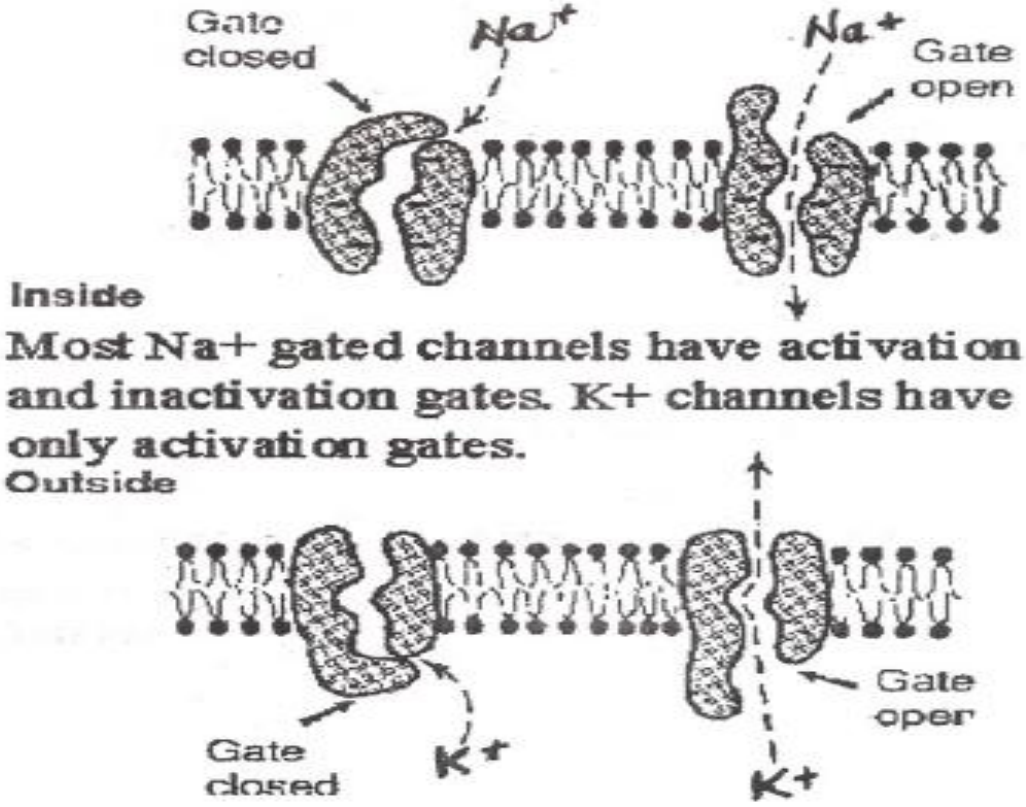


Fig 3

What are receptors?

- Receptor is a membrane bound protein with high affinity for specific molecule;
- It responds to the presence of signaling molecule by initiating a chain of events that brings about a response by the neuron;
- Each receptor is specific for a particular signaling molecule and for close chemical analogs of the signaling molecule

What type of receptors are in Ion-Gated channels?

- **Ionotropic Receptors**: (**Fig 1a**)
  - Cause the opening or closing of ion channels, and a quick, short lasting electrical response;
  - Receptor protein sometimes itself an ion channel, which opens or closes in presence of signaling molecule;
  - Receptor may be coupled to adjacent channel;

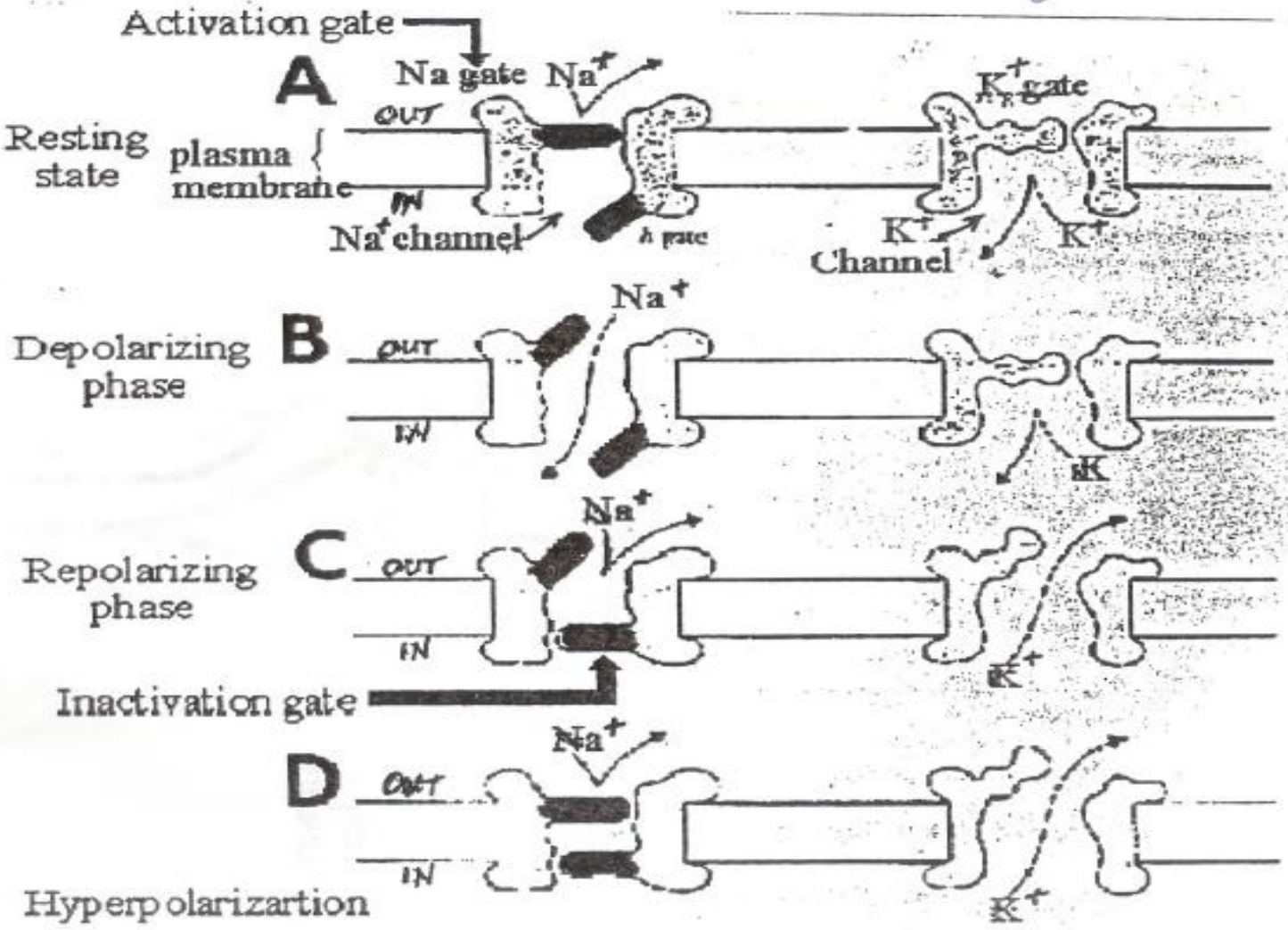
- **Metabotrophic Receptors: (Fig 1b)**
  - Exerts its effect via a biochemical cascade that takes longer to develop than with Ionotropic receptor, but lasts longer
  - Exerts its effect via G protein (GTP binding proteins)

What are the types of Voltage Gated Ion channels?

- **Two types** of voltage gated ion channels are present in the membrane (**Fig. 4**)
- Sodium voltage gated channels: Permeable to Na^+
- Potassium voltage gated channels: Permeable to K^+
- They are sensitive to changes in Membrane Potential, conformational changes occurs as the Potential alters;
- Na^+ voltage gated channel has two gates:
  - Activation Gate,
  - Inactivation Gate
- K^+ channel has only one gate;

The Function of Voltage Gated Channels

Fig 4



What are Ion Pumps, give example?

- Require a source of energy (ATP) to pump ions up a concentration gradient,
- Ions enter one side and are actively moved to the other side and released,
- Movement through Ion pump is a multi-step process, and may be as much as 100 times slower than an ion channel
- Besides Na^+/K^+ exchange pump, pumps for Ca^{2+} and Cl^- exist as well

Na+ / K+ ion exchange pump:

- In every cycle 3 Na^+ ions are moved out of cell, and 2 K^+ ions are moved in,
- Energy is obtained from ATP;
- Allows neuron to generate differences in ion concentration across membrane that is essential to function;

MEMBRANE TRANSMISSION

What is the mechanism for the resting potential in neruon?

- Exact chemical mechanisms not fully understood,
- Generally agreed that the electrical polarity of the neuron and of excitable cells in general, is due to the unequal distribution of Na^+ ions and K^+ ions on both sides of neuronal membrane;
- Neuron has:
  - Low internal concentrations of Na^+ and Cl^- ions
  - High internal concentration of K^+ ions
  - Abundance of small electrically charged organic molecules;

- At rest, neurons are electrically negative in the inside compared to the outside
- One explanation is that at rest the permeability of the neuronal membrane for K^+ ions is much higher than that for Na^+ ions;
- Therefore, K^+ ions freely diffuse out creating a potential to balance the concentration gradient of K^+ ions inside the cell;
- This process on completion gives the so-called "**Resting Potential**" of the neuron;

How are voltage-gated ion channels related to Action Potential?

- **Action Potential: (Fig 4)**
- Action potential is a temporary change in membrane potential that is transmitted along the axon
- It is usually initiated in the cell body, travels in one direction normally;
- It can be separated into Phases:

Resting phase:

- Sodium activation gates are closed,
- Sodium inactivation gates are open, and potassium gate is closed;
- Resting membrane potential is at around – 70 mv inside the neuron;

Depolarization phase:

- Action potential begins with activation gates of Na^+ channels opening,
- Na^+ ions enter the cell causing a sudden depolarization, leading to the Spike of the Action Potential;
- Excess Na^+ ions enter the cell causing **Reversal of Potential,** becoming briefly more Positive on the inside of the cell membrane;

Re-polarization phase:

- Sodium inactivation gates close and Potassium gate open;
- Causes Na^+ ions to stop entering the cell and K^+ ions to leave the cell, leading to Re-polarization;
- Until the membrane is Re-polarized it cannot be stimulated, called the **Absolute Refractory Period;**

Hyper-polarization:

- Excess potassium leaves the cell causing a brief **hyper-polarization**
- Sodium activation gates close and Potassium gates begin closing
- Na^+, K^+ - ion pump begins to re-establish the resting membrane potential (pumping Na^+ ions out and K^+ ions into the cell);
- **An energy process requiring constant supply of ATP;**
- **Inhibition of energy supply to nervous system can lead to inhibition of impulse transmission along the axon;**
- During hyper-polarization the membrane can be stimulated but only with a greater than normal depolarization, the **Relative Refractory Period;**

IMPORTANT TO NOTE!!

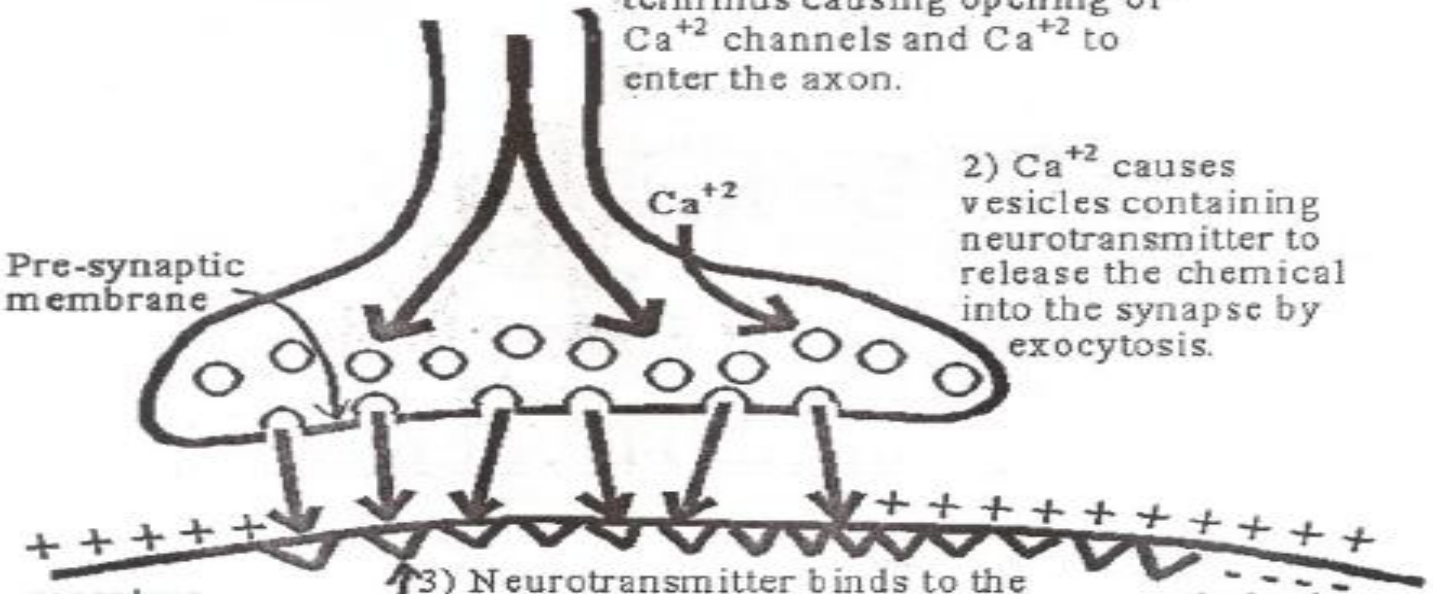
- **Action potential are self-propagated and once started the action potential progresses along the axon membrane;**
- **It is all-or-none, that is there are no different degrees of action potentials: You either have one or you do not;**
- **Action potential** arises from large**, transient changes in the permeability of the plasma membrane of the neuron to Na^+ ions and K^+ ions;**
- **Neurotoxin, Tetrodotoxin**, a highly **potent poison from the Puffer fish**, blocks the conduction of nerve impulses along **Axons** and so leads to respiratory paralysis by binding very tightly to the Na^+ channel and blocking its action;
- (Signals that make the cytoplasm more Positive are said to Depolarize the membrane, those that make it more Negative are said to Hyperpolarize the membrane)

SYNAPTIC TRANSMISSION: Impulse transmission across the synapse (Fig.5)

- Synaptic transmission refers to propagation of nerve impulses across the synapse
- An axon can make contact anywhere along the second neuron: on the Dendrites (Axo–Dendritic Synapse), the cell body (Axo–Somatic Synapse) or the axons (Axo– Axonal Synapse);
- Nerve impulses are transmitted at synapses by release of neurotransmitters;
- Neurotransmitters that are released diffuse across the synaptic cleft to the Post-synaptic cell where they bind to specific receptors;
- Neurotransmitter binds to the receptor and opens ion channels (ligand-gated channels) allowing Na^+ ions and K^+ ions to flow in and out of the Postsynaptic cell, respectively;

Synaptic Transmission

Fig 5



4) Graded potentials spread and can summate to produce a threshold depolarization and an action potential in the neuron's trigger region.

5) The neurotransmitter is broken down or removed from the synapse in order for the receptors toreceive another stimulus.

- **If the ions depolarize the Postsynaptic cell they produce Excitatory Postsynaptic Potential (EPSP);**
  - Examples of neurotransmitters that can produce EPSPs: Acetylcholine, Epinephrine, and Norepinephrine;
- **If the ions make the Postsynaptic membrane more Negative (Hyper-polarization) they produce Inhibitory Postsynaptic Potential (IPSP):**
  - Examples of neurotransmitters producing IPSPs: Glycine and GABA (Gamma Amino-Butyric Acid);
- Resulting depolarization of the postsynaptic membrane initiates a new action potential in the postsynaptic cell;
- Neurotransmitter action on the receptors on the postsynaptic membrane may be terminated either by:
  - Specific enzymes,
  - Reuptake, or
  - Diffusion into Glea cells

NEUROMUSCULAR TRANSMISSION

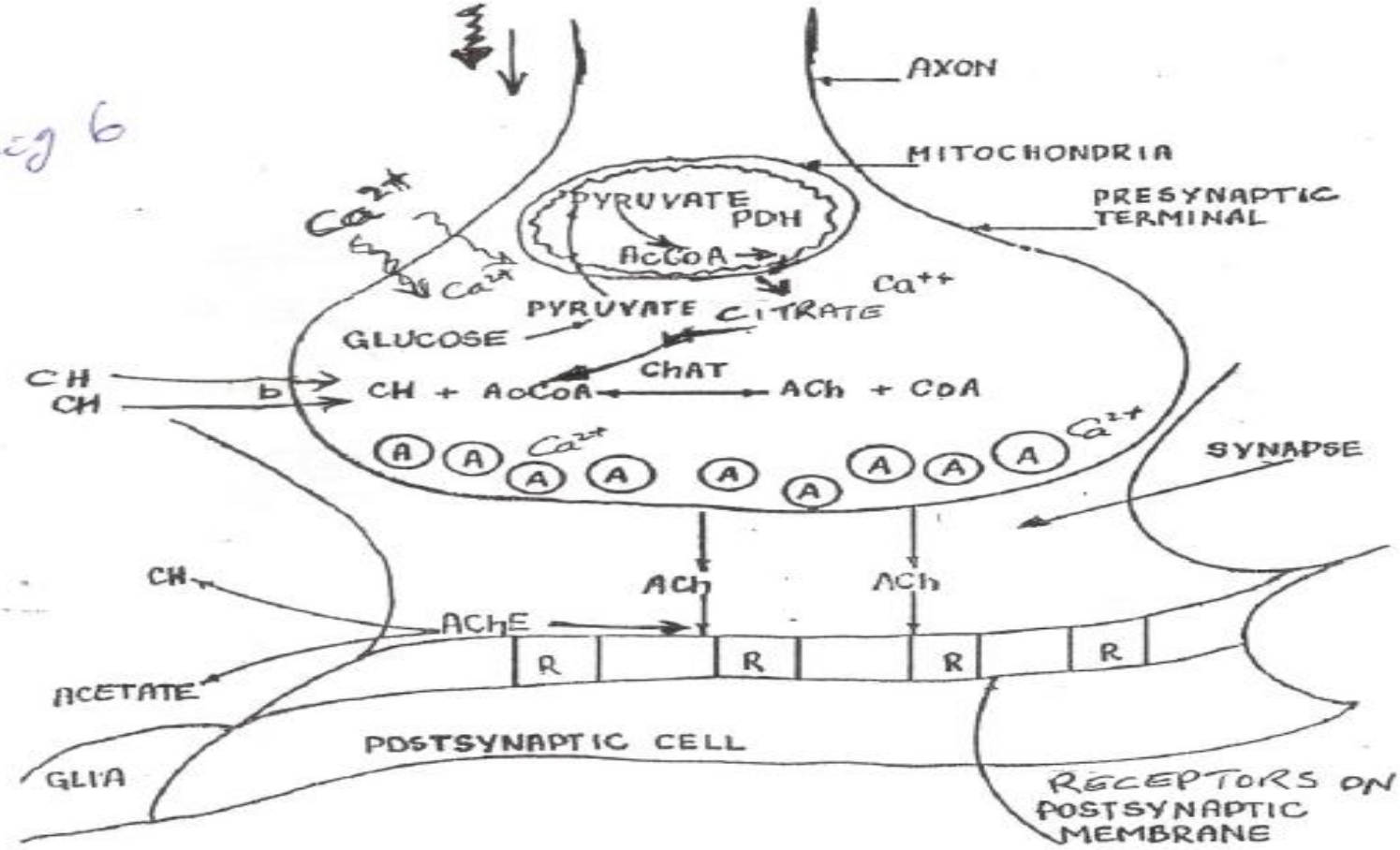
- Impulse transmission from neuron to skeletal muscle fiber, (neuromuscular junction)
- Action potential occurring at the site a neuromuscular transmission;
- At a neuromuscular junction, the axon subdivides into numerous terminal buttons that reside within depressions formed in the motor end-plate;

Outline the steps involved when Neurotransmission occurs at the neuromuscular junction (Fig. 6)

- When an action potential (inhibited by Tetrodotoxin) reaches the axon terminal it causes Ca channels to open, Ca^{2+} ions rushes into the cell because Ca^{2+} outside is much higher than Ca^{2+} inside;
- Terminal region of the axon is loaded with vesicles containing the neurotransmitter, Acetylcholine (ACh);
- Ca^{2+} causes some of the vesicles to fuse with the membrane and release their ACh (inhibited by Botulinum toxin);
- ACh diffuses across the neuromuscular junction and binds to the ACh receptor protein (inhibited by Curare) in the postsynaptic membrane;
- Binding causes an ion channel to open;

- Flow of ions depolarize the membrane, producing an EPSP;
- In muscle a single impulse usually causes enough depolarization to reach threshold;
- Action potential is generated in the muscle membrane;
- Muscle action potential causes release of Ca^{2+} from the Sarcoplasmic Reticulum of the muscle and this triggers muscle contraction;
- At the receptor site in the neuromuscular junction the ACh is broken down to acetate and Choline by the enzyme Acetylcholinesterase (inhibited by Physostigmine, Nerve gases, Organophosphate insecticides);
- Choline is recycled;
- Choline pump transports it back into the nerve terminal and there it is converted back into Ach;

Fig 6



PDH = PYRUVATE DEHYDROGENASE COMPLEX
R = CHOLINERGIC RECEPTORS
CH = CHOLINE
ChAT = CHOLINE ACETYL TRANSFERASE
AChE = ACETYLCHOLINESTERASE
b = SITES OF HIGH AFFINITY CHOLINE TRANSPORT SYSTEM
A = VESICLES CONTAINING ACETYLCHOLINE
ACh = ACETYLCHOLINE

CHOLINERGIC NERVE TERMINAL

(ADAPTED & MODIFIED FROM BRAIN RESEARCH REVIEW, VOL. 1, No. 3, 1979)

Stages in chemical neurotransmission process and the role of Ca^{2+} ions in the release of neurotransmitters

- When the wave of depolarization (electrical impulse) reaches the presynaptic terminal of a neuron, voltage-gated Ca^{2+} ion channels and Na^{+} ion channels open up;
- Ca^{2+} ions enter the cell, increasing the concentration;
- Ca^{2+} ions then activate a **Calcium-Calmodulin-dependent protein kinase**;
- An enzyme responsible for Phosphorylation of a specific protein (**SYNAPSIN-1)** attached to the surface of the presynaptic membrane;
- In the de-phosphorylated form Synapsin-1 prevents the synaptic vesicles from making contact with the Presynaptic membrane;

- When synapsin-1 is phosphorylated it dissociates from the membrane, allowing the synaptic vesicles to attach to the presynaptic membrane;
- This enables the vesicles to release their store of neurotransmitter molecules into the synaptic cleft via the process of exocytosis;
- After the release Synapsin-1 is de-phosphorylated and displaces the empty synaptic vesicles;
- The displaced vesicles can then take up more neurotransmitter molecules and start the cycle over again;
- The neurotransmitter molecules that are released then diffuse across the synaptic cleft to the plasma membrane of the post-synaptic cell where they bind to specific receptors;

- Binding of the neurotransmitter molecules to the receptors may cause channels to open, allowing Na^+ ions and K^+ ions to flow in and out of the cell, respectively;
- The resulting depolarization of the post-synaptic membrane initiates a new action potential;
- Once the signal has been delivered the neurotransmitter must be removed from the receptors;
- An enzyme can breakdown the neurotransmitter
- In other cases the neurotransmitter is recycled - it is transported back into the presynaptic nerve;
- Some chemical or drugs may inhibit the enzymes that break down neurotransmitters (examples Nerve gases, Physostigmine);
- Other drugs act by inhibiting recycling of neurotransmitters (examples Prozac, Cocaine);

What are the effects of chemical neurotransmitters on membrane receptors?

- Some neurotransmitters such as **Acetylcholine, Glycine, Glutamate, GABA** have an “inherent” biological activity such that the neurotransmitter act directly to cause an increase in conductance to certain ions by binding to “Ligand-activated” ion channels at the post-synaptic membrane.;

- Other neurotransmitters such as **Norepinephrine, Dopamine** and **Serotonin**, have no direct activity but act indirectly via Second Messenger systems to bring about the post-synaptic response;
- These second messenger systems involve compound such as, cAMP, cGMP, ITP, PGs, Epoxides and Ca^{2+} ions;
- These messengers act in the cytosol to activate target proteins, including protein kinases, which in turn act on substances such as, ion channels, to produce the neurotransmission effect.

- Furthermore, if it is an excitatory neurotransmitter, then it causes depolarization of the membrane of the post-synaptic cell;

- If it is an inhibitory neurotransmitter, it will cause hyper-polarization by increasing the Chloride ion conductance of the post-synaptic membrane, thus making it more difficult for the cell to become depolarized;

Toxins and diseases that affect neuromuscular junction & synaptic transmission

- ACh release in the NMJ is inhibited by Botulinum toxin;
- Glycine release in the CNS is inhibited by Tetanus toxin;
- Black Widow Spider toxin, Alpha-Latrotoxin, stimulates fusion and depletion of neurotransmitter vesicles;
- Physostigmine, Nerve gases and Organophosphorus pesticides inhibit Acetylcholinesterase,
- Muscle ACh receptor is blocked by the South American arrow poison, Curare;
- Atropine, inhibits ACh receptors of the autonomic nervous system (but not the NMJ);
- Strychnine binds to Glycine receptor protein and inhibits IPSPs in the spinal cord;
- Cocaine blocks the recycling of Dopamine and Norepinephrine in the brain; This has an excitatory effect;

Diseases affecting Synapses and Neuromuscular junctions

- Eaton-Lambert syndrome: patient produces antibodies that attack his own Ca channels, which results in low Ca in the synapse inhibiting the release of neurotransmitter
- Myasthenia gravis: an autoimmune disease that damages the receptor proteins for Ach;
- Parkinson's disease: cells in the substantia nigra of the brain are deficient in the neurotransmitter, dopamine;
- Clinical depression: associated with low amounts of serotonin, in parts of the brain;