UNIVERSITY OF PAPUA NEW GUINEA
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL SEMINAR
BIOCHEMISTRY OF THE MUSCLE – An Overview

(ALL DIAGRAMS IN HANDOUT AVAILABLE IN BMS OFFICE)

What are the three major types of muscles in the body?

- Major types of muscle tissues are Skeletal muscle, Cardiac muscle and Smooth muscle
- Under light microscope:
  - Skeletal and Cardiac muscles appear striated,
  - Smooth muscle is non-striated

What is the general structure of Striated muscles?

- Striated muscles are made up of bundles of highly specialized multinucleated cells that are capable of contraction
- Each multinucleated muscle cell is called a **Myofiber**
- Each Myofiber is made up of regularly arranged filaments called **Myofibrils**
- Myofibril consists of repeating units called **Sarcomere**
- **Sarcomere is the functional unit of the Myofibril**
- Parallel alignment of Myofibrils with their Sarcomere in register give muscle fibers a periodic pattern of alternating Light and Dark bands, (i.e., Striations) seen under the light microscope

Give a brief description of the components of skeletal muscle fiber:

- Skeletal muscle fiber is made up of:
  - Bundles of Myofibrils
  - **Sarcoplasmic Reticulum (SR)**:
    - A specialized organelle containing Calcium ions that plays key role in regulation of muscle contraction
    - It stores and release Ca^{2+} ions in excitation-contraction coupling
    - It maintains low intracellular concentration of Ca^{2+} ions
  - Transverse Tubules (T-tubules):
    - It passes from Sarcolemma across a Myofibril
    - It carries depolarization from the Sarcolemma into Myofibrils

- Sarcoplasmic Reticulum is connected to Sarcolemma via Transverse (T) Tubule
- Sarcoplasmic Reticulum is connected to T-Tubules via terminal Cisternae
- Muscle fiber is surrounded by Electrically Excitable plasma membrane called **Sarcolemma**
- Cytoplasm of muscle fiber is called **Sarcoplasm**
  - Sarcoplasm contains Glycogen, ATP, Creatine Phosphate and Glycolytic enzymes

What is a Dihydropyridine Receptor?

- Voltage-gated receptor located on the Sarcolemma
- It acts as Calcium ion channel

What is a Ryanodine Receptor?

- Voltage-gated receptor located on the Sarcoplasmic Reticulum
- It acts as Calcium ion channel

What are the components of the Sarcomere?

- **Fig 1** shows schematic diagram of myofibril in striated muscle as seen under Phase-Contrast Microscope
- Sarcomere is the functional unit of Myofibril
    - Alternating **Dark Bands** (**Aniso-tropic bands** – meaning Birefringent in polarized light) and
    - **Light Bands** (**Isotropic band** – meaning not altered by polarized light) can be observed
        - These bands are the **A-band** and **I-band**, respectively
- Central region of **A-band** appears less dense than the rest of the band and is referred to as **H-band** or **H-zone**
- Middle of **H-band** is a dark **M line**, made up of **M protein** and joins the fibers that make the A-band
- **I-band** is bisected by a very dense and narrow **Z-line**, that demarcates the ends of the **Sacromeres**,
    - **Sarcomere is a Z line – to – Z line repeat**
    - Sarcomere is the region between two Z-lines
    - Z-line is made up of proteins called **α-Actinin and Desmin**

What components in the Sarcomere represent the Thick and Thin Filaments?

- **A-band** represents **Thick filaments**
    - A-bands (Thick filaments) do not change their length or width during muscle contraction
- **Thin filaments** extend from Z-line, across I-band and through A-band up to H-band. Thin filaments do not change length or width during contraction
- Thick and Thin filaments overlap and interact in the A-band
- Cross-bridges (or links) from Thick filaments make contact with Thin filaments
- Darkest portion of A-band is the region in which Thick and Thin filaments overlap
- H-band is the portion of the A-band where there is no overlap between Thick and Thin filaments
- During muscle contraction the H-zone gets shorter in size

What is the component and structure of Thick filament?

- Primary component of Tick filament is **Myosin**
- Two types of Myosin:

- Type-1 Myosin is not found in muscle cells
- Type-2 Myosin is required for **muscle contraction**, and can also be found in non-muscle cells

- **Type-2 Myosin** is made up of:
  - Two Identical Heavy Polypeptide chains and
  - Two pairs of different Light Polypeptide chains
  - NH_2-terminal end of Heavy chains is a Globular structure to which the Light chains are bound
  - Two Heavy chains coil around each other forming a **Coiled Coil structure** (**Fig. 2a**)

What are the component parts of the Myosin in skeletal muscle?

- Limited digestion of Type-2 Myosin using the enzymes Trypsin and Papain gives the products shown in **Fig 2b**

- **Action of Trypsin on Myosin**:
  - Two Myosin Fragments (**Meromyosins**) are produced:
    - **Light Meromyosin (LMM**) and
    - **Heavy Meromyosin (HMM**)
  - **LMM**, is from the tail of myosin, is insoluble, has no ATPase activity and does not bind to F-Actin, but can still form filaments
  - **HMM** is soluble, exhibits ATPase activity and can bind to F-Actin

- **Action of Papain on HMM:**
  - **HMM** is split into Two Identical Globular Sub-fragments (**S-1**) and One Rod-shaped sub-fragments (**S-2**)
  - Each S-1 fragment contains:
    - **ATPase activity**,
    - **Actin binding site**, and
    - **Two light chain-binding sites**
  - S-2 fragment has no ATPase activity and cannot bind to F-Actin

- Type-2 myosin exhibits Three Separate biological activities that are important structurally and functionally. These are:
  - Self-assembly activity:– Myosin molecules spontaneously assemble into filaments in solutions of physiological ionic strength and pH
  - ATPase activity:– Globular ends of myosin can hydrolyze ATP
  - Myosin can bind to the Polymerize form of Actin (F-Actin)

What are the components of the Thin Filament?

- Primary components of Thin Filaments are **Actin, Tropomyosin**, and **Troponin**
- **Actin** is a polymer of globular-shaped subunits called G-Actin
  - G-Actin can be polymerized to form F-Actin (a helical structure of linked G-Actin molecules)
- **Tropomyosin** is a Regulator protein
  - Tropomyosin plays an integral role in muscle contraction by regulating the interaction of Actin and Myosin

- **Troponin** is a complex of 3 polypeptides chains each of which exhibits a different activity that is essential for overall function of the complex

What are the functions of each component in Troponin?

- Troponin components and functions are:
  - **Troponin C** (TnC): Binds Ca^{2+} and act just like Calmodulin, it allows the interaction of Actin and Myosin
  - **Troponin T** (TnT): Binds Troponin to Tropomyosin
  - **Troponin I** (TnI): Inhibits interaction of Actin and Myosin

- Functionally Troponin complex acts together with Tropomyosin to mediate the regulation of muscle contraction by Calcium ions

What changes occur in the Sarcomere of skeletal muscle during contraction?

- Length of Thick and Thin filaments do not change during contraction
- Lengths of **H-zones** and **I-bands** are reduced during contraction, because of overlap between Thick and Thin Filaments
- Lengths of each Sarcomere unit are reduced because Thin Filaments "Slide" across Thick Filaments during contraction
- Thin and Thick filaments exhibit polarity
- Thick filaments are bipolar
- **Cross-bridge** occurs between Thick and Thin Filaments
  - Oppositely oriented Thin filaments, surrounding each end of Thick filaments, moves across Thick filaments pulling the Z-discs of each sarcomere closer together (**Fig. 3**)

What is the sequence of events in Excitation-contraction coupling that causes the cross-bridge cycle for muscle contraction?

- Excitation of nerves causes Action potential to reach muscle
- Wave of depolarization moves down T-tubules
- Calcium ion channels open on Sarcoplasmic Reticulum
  - *Dihydropyridine receptor (Voltage-gated Ca^{2+} channel receptor) on Sarcolemma activates Ryanodine receptor (Voltage-gated Ca^{2+} channel receptor) on Sarcoplasmic Reticulum*
- Intracellular concentration of Ca^{2+} ions Increases
  - *Ryanodine receptor releases Calcium ions*
- Troponin C binds released Ca^{2+} ions
- Troponin C-Calcium complex undergoes conformation change
- Complex formed interacts with Tropomyosin freeing the Myosin-binding Site on Actin
- Cross-bridge cycle occurs (Myosin forms Cross-bridge with Actin)

Outline the sequence of events in the Cross-bridge cycle that produces muscle contraction (Basic Contraction Reaction)

- Myosin forms cross-bridge with Actin
- Myosin is tightly attached to Actin in the absent of ATP
- Binding of ATP to Myosin results in conformation change and releases Actin
- Myosin hydrolyzes ATP to ADP and Pi (which are not released) and then binds to a new site on Actin

    - Pi is then released and Myosin binds tightly to Actin
- Binding induces conformational change in Myosin, resulting in pulling of Actin across the Thick Filament and the release of ADP;
    - Conformational change that occurs in Myosin generates the "Power stroke"
- If ATP is present, it binds to Myosin, thus causing the release of Myosin from Actin
    - ATP is required for the release of Myosin from Actin
- If ATP is depleted, as it is upon death, Myosin remains tightly bound to Actin and "Rigor Mortis" takes place (i.e., stiffening of the body that occurs after death)

In other words:

- Cyclic formation and dissociation of complexes between Actin filaments and S-1 heads of Myosin leads to contraction of muscle
- On binding to Actin, Myosin releases its bound ADP and Pi, causing a conformational change to occur in Myosin protein, which moves the Actin filament along the Thick filament
- ATP then binds to Myosin, displacing the Actin
- Hydrolysis of ATP returns the S-1 head of Myosin to its original confirmation

How is Striated muscle contracting regulated?

- Ca^{2+} ions are the major regulatory molecule of contraction in striated muscle
- When concentration of Ca^{2+} ions is very low in the Sarcoplasm (less than 1.0 μM), Tropomyosin lies in a groove of Actin protein in such a way that it prevents the S-1 head of Myosin to interact with Actin
- On muscle stimulation by a nerve impulse, Ca^{2+} ions are released from the Sarcoplasmic Reticulum raising the Cytosolic Ca^{2+} concentration from the resting contraction of less than 1.0 μM to about 10.0 μM
- Ca^{2+} ions bind to sites on TnC, which then interacts with TnI and TnT to alter their interaction with Tropomyosin
- Tropomyosin then moves out of the way, allowing the S-1 head of Myosin to interact with Actin and initiate a cycle of contraction

How is the Relaxation of the skeletal muscle regulated?

- For relaxation: Sarcoplasmic Reticulum has a membrane enzyme (Ca^{2+}-ATPase) that removes Ca^{2+} ions from the Sarcoplasm after an electrical impulse passed
- Removal of Ca^{2+} ions from the Sarcoplasm results in the removal of the conformation changes of the Troponin-Tropomyosin complex
- Tropomyosin then returns to the groove of the Actin, where it inhibits Myosin binding, causing relaxation of the muscle

What ion regulates the contraction of skeletal muscle?

- Ca^{2+} ions control muscle contraction by Allosteric mechanism involving Troponin, Tropomyosin, Actin and Myosin
- At rest the Ca^{2+} ions are sequestered in the Sarcoplasmic Reticulum, thus keeping the Ca^{2+} ions concentration in the Sarcoplasm very low

What are the components of Smooth Muscle?

- Smooth muscle contain:
    - Thick Filaments (Myosin)
    - Thin Filaments (Actin and Tropomyosin)
- Smooth muscle do not contain:

- Sarcomere units and Troponin
- Smooth muscles are not striated because Sarcomere units are not aligned

What mechanisms causes increase in Intracellular concentration of Ca^{2+} ions in Smooth muscle?

- Mechanisms that increase Intracellular concentration of Ca^{2+} ions in smooth muscle include:
  - Depolarization of Sarcolemma opens Voltage-gated Ca^{2+} ion channels and releases Calcium ions
  - Depolarization causes Sarcoplasmic Reticulum to release Ca^{2+} ions
  - Hormones and Neurotransmitters stimulate Sarcoplasmic Reticulum to release Ca^{2+} ions via IP_3-gated Calcium ion channels

Briefly describe contraction and relaxation of Smooth muscle: (**Fig. 4**)

- **Contraction of Smooth muscle**:
  - Action potential causes wave of depolarization
  - Sarcolemma of smooth muscle is depolarized
  - Voltage-gated Ca^{2+} channels are opened
  - Intracellular concentration of Ca^{2+} ions increases
  - Ca^{2+}ions binds Calmodulin
  - Calmodulin-Ca^{2+} ion complex activates Myosin Light-chain Kinase
  - Myosin-Light chain Kinase Phosphorylate Myosin
  - Phosphorylated Myosin bind to Actin forming Myosin-Actin cross-bridge
  - Cross-bridge leads to contraction (pulling of Actin across Myosin)

- **Relaxation of Smooth muscle**:
  - Myosin Light-chain Phosphatase de-phosphorylates (remove phosphate group) from the Myosin-Actin complex
  - De-phosphorylated Myosin then releases Actin resulting in relaxation of the smooth muscle

What is the role of Caldesmon in smooth muscle contraction?

- **Caldesmon** is a protein that plays a Ca^{2+}-dependent role in the regulation of smooth muscle contraction
  - Caldesmon binds to Tropomyosin and Actin at low concentration of Ca^{2+} ions, preventing Interaction of Myosin with Actin, and therefore keeps the muscle in a relaxed state
  - At high concentrations of Ca^{2+} ions, Calmodulin binds Caldesmon, releasing Actin, which then binds to Myosin and contraction can occur
  - Caldesmon can also be Phosphorylated
  - Phosphorylated Caldesmon cannot bind to Actin, thus allowing Myosin to bind causing Contraction

What are the different types of fibers in skeletal muscle?

- Fibers in skeletal muscle can be subdivided into two types based on their energy requirement and function:
- Type-1 is the Slow-Twitch-Oxidative fibers;
- Type-11 is the Fast-Twitch-Glycolytic fibers
  - Type-1 fibers are Red because they contain Myoglobin and Mitochondria

- Metabolism is aerobic and they maintain relatively sustained contractions
- Myosin ATPase activity is low and their energy utilization is low

- Type-11 fibers are White because they lack Myoglobin and contain few mitochondria
- Metabolism is anaerobic and exhibit relatively short durations of contraction
- Myosin ATPase activity is high and their energy utilization is high

Briefly explain the major cause of Muscle Fatigue

- Primary cause of muscle fatigue is due to accumulation of H^+ ions (not of Lactate) in muscle tissue. Increase of H^+ ions (decreased pH) can affect the function of muscle in a number of ways:
  - Reduces release of Ca^{2+} ions from Sarcoplasmic Reticulum;
  - Reduces activity of Actino-Myosin ATPase
  - Affects conformation of some muscle proteins involved in contraction

What are Myopathies? Give examples:

- Myopathies may be caused by several factors:
  - Congenital factors (muscular dystrophies): Duchenne's muscular dystrophy: Caused by defect in protein Dystrophin, which anchors the Cortical Cytoskeleton of muscle cell to Trans-membrane Glycoproteins
  - Viral infection, Acute damage due to Anoxia, Infections, Toxins or Drugs
  - Muscle denervation
  - Muscle weakness can occur due to lack of energy producing molecules or failure in the balance of electrolytes within and surrounding the muscle cell necessary for neuromuscular function
  - Normal muscle that is overused will end up weak or in spasm until rested
  - In severe cases of overuse, especially where movements are strong and erratic as might occur during convulsions, damage to muscle cells may result
  - Severely damaged muscle cells release Myoglobin, a condition known as Rhabdomyolysis
  - In all cases of muscle weakness:
    - Serum/Plasma electrolytes should be checked along with Creatine Kinase (CK) activity,
    - A full drug history should be taken to exclude pharmacological and toxicological causes,
    - History of alcohol abuse should be excluded
    - Neuromuscular electrophysiological studies should be performed to detect neuropathies
    - Where a genetic cause is suspected, a muscle biopsy should be taken for Histo-pathological studies and measurements of muscle enzymes
    - In contrast to Rhabdomyolysis, serum CK and Myoglobin are frequently normal in patients with Myopathy

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CNS METABOLISM - Review

- Brain or Cerebral energy metabolism is often considered to reflect predominantly, if not exclusively, Neuronal energy metabolism or CNS metabolism in general
- Other cell types, namely Glial and Vascular Endothelial cells not only consume energy but also play active role in the flux of energy substrates to Neurons

Why does the cerebral tissue need energy?

- Neuron is the functional unit of the CNS
- Neuron is an excitable cell, because it is capable of generating and conducting electrical impulse by temporarily reversing its membrane potential
- Major functions of neurons are excitation and conduction, which are reflected in the unceasing electrical activity of Cerebral tissue
- Electrical energy is derived from chemical processes
- Energy consumption is used for active transport of ions needed to sustain and restore the membrane potentials discharged during the process of excitation and conduction
- Thus, cerebral tissue requires constant supply of energy

What substrates are used for energy production in cerebral tissue?

- Glucose is the major substrate for energy production in cerebral tissue
- Cerebral tissue utilizes glucose directly from arterial blood
- Insulin is not required for uptake of glucose by cerebral tissue
- Brain can utilize Glycogen store (about 0.1%) to maintain cerebral metabolism for a very short time, when blood glucose is low
- Apart from Glucose, Mannose can be used to sustain normal cerebral metabolism
    - Mannose easily crosses the blood–brain barrier, is converted to Fructose-6-phosphate that enters the Glycolytic pathway
    - Mannose is not normally present in the blood and cannot therefore be considered as a substrate for cerebral energy metabolism

- Fructose, Galactose, Lactate and Pyruvate have limited permeability across the blood–brain barrier, therefore cannot directly serve as substrates for cerebral energy metabolism
- Lactate and Pyruvate when formed within the blood-brain barrier are useful metabolic substrates for cerebral metabolism

How significant is O_2 supply to brain energy metabolism?

- Brain represents about 2 to 3% of total body weight of an average adult, but it utilizes about **20 to 25% of the total O_2 consumed by the whole organism**
- In children up to 4 years of age, the brain utilizes about 50% of the total O_2 consumed by the whole organism
- Cerebral tissue utilizes O_2 more than other tissues;
    - Example: it utilizes about 20 times more O_2 than muscle tissue when at rest.
- Oxygen consumption varies throughout the brain:

- Grey matter utilizes about twice more O_2 than White matter (which contains fewer cells than the Grey matter)
- Cerebral O_2 consumption continues unabated day and night, (Sleep reduces cerebral O_2 uptake by only 3%)
- Oxygen stored in the brain is extremely small compared to the rate of utilization, thus the brain requires the continuous replenishment of its oxygen by the circulation
- Consciousness is loss if cerebral blood flow is completely interrupted

TAKE NOTE:

- Reduced cerebral O_2 uptake occurs under certain conditions that lead to depressed consciousness

    - Examples include: Insulin induced hypoglycemia, Diabetic coma, Cerebral tumors, Uremia, Gross liver damage that culminate in hepatic coma and exposure to depressant drugs used during surgery.

What are some of the uses of O_2 consumed by cerebral tissue?

Some uses of O_2 include:

- Energy metabolism via Oxidative Phosphorylation
- Maintenance of energy component in blood-brain barrier
- Functioning of specific enzyme systems:
    - Mixed Functional Oxygenases used in the biosynthesis of Neurotransmitters and other biologically active compounds

Briefly explain how carbohydrate is metabolized in cerebral tissue

- Aerobic and Anaerobic Glycolysis occurs in cerebral tissue
- HMP shunt (Pentose Phosphate Pathway) also occurs in cerebral tissue mainly for the production of NADPH, required for the biosynthesis of Fatty acids and Steroids

- Carbohydrates such as Maltose, Fructose, Galactose, Hexose-phosphates and Intermediate metabolites such as Lactate, Pyruvate and Glyceraldehydes are used only after their conversion to Glucose via Gluconeogenesis
    - Thus, these compounds act by raising the Blood Glucose Level

- Cerebral tissue can utilize Mannose directly and rapidly from the blood to restore or maintain normal metabolic functions
    - Mannose can directly enter the Glycolytic pathway of cerebral tissues, without raising blood glucose level
    - Mannose like Glucose can easily cross the blood-brain-barrier and can be converted to Mannose-6-phosphate by the enzyme Hexokinase

    - **Phospho-mannose Isomerase** is an active enzyme in cerebral tissue that converts Mannose-6-phosphate to Fructose-6-phosphate, which then enters the Glycolytic pathway.

The reaction is as follows:

$$\text{Mannose} + \text{ATP} \xrightarrow{1} \text{Mannose-6-P} \xrightarrow{2} \text{Fructose-6-Phosphate}$$

(NB: 1 = Hexokinase; 2 = Phosphomannose Isomerase)

{Mannose is not normally present in blood in any appreciable amount and is therefore of no Physiological significance}.

Briefly comment on the amino acid content in cerebral tissue

Cerebral tissue contains:

- Very high concentration of free amino acids compared to that in plasma
- Highest amount of free Glutamate, compared to any other mammalian tissue
- Some unusual amino acids such as: Gamma-Aminobutyrate (GABA), N-Acetyl-Aspartate and Cystathione.

TAKE NOTE:

- GABA is an inhibitory neurotransmitter that acts by increasing the passage of Chloride ions through the Post-synaptic membrane of Neurons
- Glutamate is involved in several metabolic processes such as: the biosynthesis of GABA, Detoxification of Ammonia and as Neurotransmitter

How is Ammonia formed in cerebral tissue?

Formation of Ammonia in cerebral tissue:

- In cerebral tissue ammonia is produced mainly via Adenylate Deaminase reaction

$$\text{AMP} \xrightarrow{\text{Adenylate Deaminase}} \text{IMP} + NH_4^+$$

- High concentration of Glutamate in blood causes ammonia toxicity
    - Glutamate Dehydrogenase (GDH) catalyzes the formation of Ammonia from Glutamate

$$\text{Glutamate} + \text{NAD} + H_2O \xleftrightarrow{\text{Glutamate DH}} \text{Alpha-oxoglutarate} + NH_3 + \text{NADH} + H^+$$

How is ammonia removed from cerebral tissue?

- Rate of urea formation in the cerebral tissue is too low to account for the removal of ammonia via the urea cycle, WHY???
    - Mitochondrial N-Acetyl-Glutamate activated Carbamoyl-Phosphate Synthetase that catalyzes the first reaction in Urea cycle, is low or absent in cerebral tissue

- Thus, removal of ammonia from Cerebral tissue involves **2 reactions**:
    - **First** is formation of Glutamate from Alpha-Oxoglutarate and Ammonia, by Glutamate Dehydrogenase (GDH)

Glutamate DH

Alpha-oxoglutarate + NH_3 + NADH + H^+ ⟵======⟶ Glutamate + NAD + H_2O

- **Second** is formation of Glutamine from Glutamate and Ammonia, by Glutamine Synthetase

Glutamine Synthetase

Glutamate + NH_3 + ATP ==========> Glutamine + ADP + P_i

- Concentration of Ammonia in Cerebral tissue will be kept low if there is adequate supply of Alpha-Oxoglutarate
- Extensive utilization of Alpha-Oxoglutarate (produced in the TCA cycle) within the cerebral tissue, would deplete Intermediates from the TCA cycle and thus affect energy supply to the Brain, unless a mechanism of replenishing the intermediates is available
- One of such mechanism is known as the **Anaplerotic (Filling-up**) reaction, which is the formation of TCA cycle intermediates in the cerebral tissue
- Anaplerotic reactions can increase the concentrations of TCA cycle intermediates, allowing an increased rate of oxidation of Acetyl-CoA

List some of the Anaplerotic reactions

Anaplerotic reactions include:

- Pyruvate Carboxylase reaction: formation of Pyruvate from Oxaloacetate using ATP and Biotin
- Some Transamination reactions
- Glutamate Dehydrogenase reaction to form Alpha-Oxoglutarate
- Succinyl-CoA formation from Isoleucine, Valine, Methionine, and Threonine

SOME FACTORS THAT CAN AFFECT CEREBRAL METABOLISM

- Oxygen and Glucose are two major substrates required for normal energy metabolism in cerebral tissue
- Hypoxia and Ischaemia can severely affect energy metabolism in cerebral tissue

How does hypoxia affect cerebral metabolism?

- After a brief period of hypoxia:
    - Drastic slowdown in Oxidative metabolism occurs in Cerebral tissue
    - Rate of Glycolysis is increased in Cerebral tissue
    - Lactic acid production is increased, which can consequently leads to intracellular acidosis

- These changes can be explained on the basis of **Pasteur effect**
    - **Inhibition of Glycolysis in the presence of oxygen**
    - Pasteur effect reflects the increased energy yield obtained via Aerobic metabolism of glucose as compared to Anaerobic metabolism

- Hypoxia causes an increase in Glucose utilization from cerebral blood stream, followed by a decrease in cerebral glucose concentration
    - Resulting is an increase in Lactic acid production in cerebral tissue
    - Gradual increase in Spinal fluid Lactate level occurs during hypoxia.

TAKE NOTE:

- The earliest detectable Neuro-chemical change in brain resulting from Hypoxia is not elevation of cerebral lactate concentration, but a reduction in Acetylcholine Synthesis
- Major effect of Hypoxia on the Nervous system is reduction in the rate of conversion of Pyruvate to Acetyl-CoA with a resultant decrease in both the biosynthesis of Acetylcholine and the activity of the TCA cycle
- In situation of low Acetyl-CoA availability the brain may use the available Acetyl-CoA for energy production so as to maintain membrane potentials in preference to its use in the biosynthesis of any compound

How does Ischaemia affect cerebral metabolism?

- During Ischaemia:
    - Glucose and O_2 supply are deficient
    - Cerebral glucose concentration and Glycogen store are depleted
    - Coma can occur leading to cerebral tissue damage

- Hypoglycemia can severely affect cerebral energy metabolism because glucose is almost exclusively used by the brain as the substrate for energy metabolism

TAKE NOTE:

- During starvation cerebral tissue can use Ketone bodies (especially Beta-hydroxybutyrate, and Acetone) as substrate for energy metabolism
- Concentration of Ketone bodies are usually very high in the blood during starvation, thus they are able to cross the blood-brain barrier without much restriction
- Vitamin deficiency can lead to abnormality in cerebral metabolism and function
- Effect of vitamin deficiency can be either direct or indirect, because of the role of vitamins on biochemical processes.

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BIOCHEMICAL BASIS OF NEURONAL FUNCTION – An Overview
(ALL DIAGRAMS IN HANDOUT AVAILABLE IN BMS OFFICE)

- Neuron is functional unit of Nervous system
- Neuron is an excitable cell, because it is capable of generating and conducting electrical impulse by temporarily reversing its membrane potential
- Synapse is contact between two neurons
- Neuromuscular junction is contact between neuron and muscle fiber

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 (Neurotransmitters) 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

Membrane Potential and Electrical potential:

- Membrane potential involves separation of electrical charges across membrane
- Leads to difference in electrical potential across cell membrane,
- Electrically negative inside the cell with respect to outside
- Indeed an electrical potential (the **membrane potential**) exists across the plasma membrane of all cells.
- Most cells however, are electrically inactive, as this membrane potential does not vary with time.
- In all cells the membrane potential is maintained by the action of the **Na^+, K^+-ion pump** with **ATPase** activity, with a high concentration of K^+ inside the cell and a high concentration of Na^+ outside.
- The energy for this process is derived from the hydrolysis of ATP. This system pumps Na^+ ions out of the cell, whereas K^+ is moved into the cell.

What are the factors that can induce ion to cross the cell membrane of a cell?

A number of factors can induce ion to cross the cell membrane, three of such factors include:

- The difference in concentration of the ion on the two sides of the membrane.
- The difference in electrical potential on the two sides of the membrane.
- The action of an ion pump.

It is important to not that ion movements based on concentration and electrical gradients are passive, but require the presence of ion channels. Ion pump requires energy expenditure (ATP).

ION CHANNELS AND ION PUMPS:

General structure of Ion Channel:

- Hollow cylinder with central core, typically 0.3 - 0.6 nm diameter, through which ions can flow.
- Ion channel usually consists of several chemically similar subunits (4 in case of sodium channel).
- Each subunit consists of several transmembrane domains (6 in case of sodium channel) each domain is a helical coil of protein that crosses the membrane.
- Trans-membrane domains connected by amino acid chains.
- Amino acid chains confer specific properties on channel.

Selectivity of Ion channel:

- Most ion channels are selective for a particular ion, determined by size of central pore and electrical charges of chains of amino acids that dip into pore.

Types of Channels:

Leakage channels: This type of channel is 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

What are the different types of gated channels?

- **Ligand gated channels** or ligand sensitive channels **(Figs. 1 & 2)** - open or close in the presence of a signaling molecule, involved in receiving information from other neurons, modulation of neuron's response over time, and some forms of sensory reception.
- **Voltage gated channels** or voltage sensitive channels **(Fig. 3**) - open or close in response to electrical potential differences across the cell membrane, form the basis for conduction of nerve signals along axons.
- **Stretch sensitive channel** - opens or closes when mechanical force is applied to it, mediates mechanical sensitivity.

What types of receptors are linked to gated channels?

- Ligand gated channels are usually highly distributed at postsynaptic sites and have specific type of receptors.
- Receptor is a membrane bound protein that has a high affinity for some other specific molecule, and responds to the presence of the signaling molecule by initiating a chain of events that brings about a response by the neuron.
- Vast majority of neurotransmitter receptors belong to a class of proteins known as the serpentine receptors.
    - Exhibits a characteristic transmembrane structure: that is, it spans the cell membrane, not once but seven times.
- Each receptor is specific for a particular signaling molecule and for close chemical analogs of the signalling molecule.

Types of Receptors in Gated Ion channels:

Ionotropic receptors:

- Cause opening or closing of ion channels and 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:

- 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 (guanine nucleotide binding proteins).

What are the types of voltage gated ion channels?

- Two types of voltage gated ion channels are present in the membrane: One is selectively permeable to Na^+ ions, the other to K^+ ions **(Fig. 4)**.
- These integral membrane proteins are sensitive to the membrane potential, undergoing conformational changes as the potential alters.
- Each Na^+ channel has an activation gate and an inactivation gate, while K^+ channels have only one gate.

Ion pumps:

- 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 the pump is a multi-step process, and may be as much as 100 times slower than an ion channel.
- Ionic currents too small to have a direct effect on electrical signalling, but by working continuously, hour after hour, ion pumps have significant role in maintaining concentration gradients.
- Besides Na^+/K^+ exchange pump, pumps for calcium ion (Ca^{2+}) and chloride ion (Cl^-) exist as well.

Na^+/K^+ exchange pump:

- In every cycle three sodium ions (Na^+) are moved out of cell, and two potassium (K^+) ions are moved in, one ATP hydrolysed to ADP.
- Allows neuron to generate differences in ion concentration across membrane that is essential to function.

Operation of the Na^+/K^+ exchange pump:

- Pump open to interior, 3 Na^+ ions enter.
- ATP hydrolyzed causing reconfiguration of pump molecule, interior opening closes, and exterior opening opens.
- Na^+ ions leave into extracellular fluid.
- Two K^+ ions enter from extracellular fluid.
- K^+ ions destabilize pump molecule and cause it to return to original configuration.
- K^+ ions leave into intracellular fluid.

What do you understand by the term excitable cells?
Why are neurons and muscle cells said to be excitable cells?

- Neurons and muscle cells are unusual in having large membrane potentials that can change rapidly under certain circumstances, thus they are said to be excitable cells.
- Electrical potential and its changes are basis for signalling ability of neurons and contractile function of muscle.

IMPULSE TRANSMISSION ALONG THE AXON (MEMBRANE TRANSMISSION):

- Exact chemical mechanisms involved in this process is not fully understood, however it is generally agreed that the electrical polarity of the neuron and of excitable cells in general, is due to the unequal distribution of Sodium ions and Potassium ions on both sides of the cell membrane.

It is important to note that:

- Neuron has low internal concentrations of Na^+ and Cl^-;
- High internal concentration of K^+, and abundance of small electrically charged organic molecules;
- In addition the extracellular fluid contains few charged molecules and little K^+, but large amounts of Na^+ and Cl^-;

- **In the resting state, the neurons are electrically negative in the inside compared to the outside. One explanation for this 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?
How does Tetrodotoxin affect action potential?

Action Potential:

- Action potential is a temporary change in the membrane potential that is transmitted along the axon. It is usually initiated in the cell body, travels in one direction normally.

Figure 4: Diagrammatic representation of the role played by the voltage gated ion channels in propagation of the action potential.

- At **the resting state** the 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**: The action potential begins with the activation gates of sodium channels opening, allowing Na^+ ions to enter the cell and causing a sudden depolarization, which leads 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.

- **Repolarization phase**: The sodium inactivation gates close and the potassium gates open. This causes Na^+ ions to stop entering the cell and K^+ ions to leave the cell, causing repolarization. Until the membrane is repolarized it cannot be stimulated, called the **absolute refractory period**.
- Excess potassium leaves the cell causing a brief **hyper-polarization**. Sodium activation gates close and potassium gates begin closing. The sodium-potassium pump begins to re-establish the resting membrane potential. **This process, which is energy dependent, requires a constant supply of ATP. Thus inhibition of energy supply to the 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**.

- **Action potentials 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.

(Note that signals that make the cytoplasm more positive are said to depolarize the membrane, while those that make it more negative are said to hyperpolarize the membrane.)

SYNAPTIC TRANSMISSION: (See Fig. 5)

- Synaptic transmission refers to the propagation of nerve impulses from one nerve cell to another. This occurs at a specialized cellular structure known as the Synapse – a junction at which the axon of the presynaptic neuron terminates at some location upon the postsynaptic neuron.
- The end of a presynaptic axon, where it is juxtaposed to the postsynaptic neuron, is enlarged and forms a structure known as the terminal button.
- An axon can make contact anywhere along the second neuron: on the dendrites (an axo-dendritic synapse), the cell body (an axo-somatic synapse) or the axons (an axo-axonal synapse).
- Nerve impulses are transmitted at synapses by the release of chemicals called neurotransmitters.
- 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.
- When the neurotransmitter binds to the receptor ion channels are opened (ligand-gated channels) allowing Na^+ ions and K^+ ions to flow in and out of the postsynaptic cell, respectively.
- **If the ions depolarize the postsynaptic cell they produce an excitatory postsynaptic potential (EPSP).** Examples of neurotransmitters that can produce EPSPs include Acetylcholine, Epinephrine, and Norepinephrine.

- **If the ions make the postsynaptic membrane more negative (hyper-polarization) they produce an inhibitory postsynaptic potential (IPSP).** The major transmitters producing IPSPs are glycine and GABA (gamma amino-butyric acid).
- The resulting depolarization of the postsynaptic membrane initiates a new action potential in that cell.
- The neurotransmitter action on the receptors on the postsynaptic membrane may be terminated either by specific enzymes, reuptake, or diffusion into glea cells.

NEUROMUSCULAR TRANSMISSION:

- A different type of nerve transmission occurs when an axon terminates on a skeletal muscle fiber, at a specialized structure called the neuromuscular junction.
- An action potential occurring at the site is known as neuromuscular transmission.
- At a neuromuscular junction, the axon subdivides into numerous terminal buttons that reside within depressions formed in the motor end-plate.

Neurotransmission at neuromuscular junction involves several steps (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 the cell.
- The terminal region of the axon is loaded with vesicles containing the neurotransmitter called 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.
- The flow of ions depolarise the membrane, producing an EPSP. In muscle a single impulse usually causes enough depolarisation to reach threshold.
- An action potential is generated in the muscle membrane.
- The 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, and organophosphate insecticides).
- The choline is recycled. A choline pump transports it back into the nerve terminal and there it is converted back into Ach.

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 in addition to the Na^{+} ion channels open up.
- This allows Ca^{2+} ions to enter the cell, thus increasing the concentration of Ca^{2+} ions in the cell.
- The Ca^{2+} ions then activate a **calcium-Calmodulin-dependent protein kinase**. This enzyme is responsible for the phosphorylation of a specific protein that is attached to the surface of the presynaptic membrane. The protein is called **SYNAPSIN-1**.

- When in the de-phosphorylated form, this protein (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. Some of these receptors form gated channels through the membrane.
- 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 in that cell.
- Once the signal has been delivered the neurotransmitter must be removed so that new signals may be received. In some cases an enzyme breaks down the neurotransmitter. In other cases the neurotransmitter is recycled - it is transported back into the presynaptic nerve. In still other cases these 2 methods are combined. Some drugs inhibit the enzymes that break down transmitters (examples include nerve gases, Physostigmine.) Other drugs act by inhibiting recycling of neurotransmitters (example Prozac, Cocaine).

What are the effects of chemical neurotransmitters on membrane receptors?
Effects of neurotransmitters on receptors on the post-synaptic membrane vary:

- 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 central nervous system (CNS) is inhibited by tetanus toxin.
- Black widow spider toxin, alpha-latrotoxin, stimulates fusion and depletion of neurotransmitter vesicles.

- The plant poison, physostigmine, nerve gases and organophosphorus pesticides inhibit acetylcholinesterase, the enzyme that splits ACh into acetate and choline.
- The muscle ACh receptor is blocked by the South American arrow poison, curare.
- The plant drug, 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 neurotransmitters in the brain. This has an excitatory effect.
- Ca will inhibit transmitter release.

Diseases affecting Synapses and Neuromuscular junctions:

- Eaton-Lambert syndrome: patient produces antibodies that attack his own Ca channels. This results in low Ca in the synapse and transmitter release is inhibited.
- Myasthenia gravis: 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 the neurotransmitter, serotonin, in parts of the brain.

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NEUROMUSCULAR AND SYNAPTIC TRANSMITTERS – An Overview

(ALL DIAGRAMS IN HANDOUT AVAILABLE IN BMS OFFICE)

Table 1: Names of some common neurotransmitters

Transmitter molecule	Derived From	Sites of Synthesis
Acetylcholine	Choline	CNS, Parasympathetic nerves
Serotonin {5-Hydroxytryptamine (5-HT)}	Tryptophan	CNS, Chromaffin cells of Gut, Enteric cells
GABA	Glutamate	CNS
Glutamate		CNS
Aspartate		CNS
Glycine		CNS
Histamine	Histidine	Hypothalamus
Epinephrine	Tyrosine	Adrenal medulla, some CNS cells
Norepinephrine	Tyrosine	CNS, Sympathetic nerves
Dopamine	Tyrosine	CNS
Adenosine	ATP	CNS, Peripheral nerves
ATP		Sympathetic, Sensory and Enteric nerves
Nitric oxide (NO)	Arginine	CNS, GIT

What are neurotransmitters?

- Neurotransmitters are a diverse group of chemical compounds ranging from simple amines like Dopamine and amino acids like Gamma-Amino-Butyric Acid (GABA), to polypeptides like Enkephalins
- Other neurotransmitters like Peptide neurotransmitters (Neuro-peptides) are derived from large protein called Pre-opiomelanocortin (POMC)
- Mechanisms by which neurotransmitters elicit responses in both presynaptic and postsynaptic neurons are as diverse as the mechanisms employed by growth factor and cytokine receptors

Synaptic Transmission:

- Propagation of nerve impulses across the Synapse by release of neurotransmitters

Neuromuscular Transmission:

- Axon terminates on skeletal muscle fiber at neuromuscular junction (Motor end-plate)
- Acetylcholine is the neurotransmitter used at neuromuscular junction

Neurotransmitter Receptors:

- Neurotransmitters released from Presynaptic terminal binds to specific receptors on surface of Postsynaptic cell
- Some neurotransmitter receptors are found on synaptic membrane of Presynaptic neurons
    - Receptors on Presynaptic neuron usually act to Inhibit further release of neurotransmitters
- Major types of receptors: **Ionotropic** and **Metabotropic**
- Link between neurotransmitters and intracellular signaling is by either
    - G-proteins (small GTP-binding and hydrolyzing proteins) or
    - Protein Kinases, or the receptor itself in the form of a Ligand-gated ion channel

- One major characteristic of neurotransmitter receptors is that they are subjected to **Ligand-Induced Desensitization**: i.e., they can become unresponsive upon prolonged exposure to their neurotransmitter

ACETYLCHOLINE (Ach):

Outline the biosynthesis of Ach:

- Acetylcholine (Ach) is an excitatory neurotransmitter
- Neurons that synthesize and release ACh are termed Cholinergic neurons (**Fig. 1**)
- Ach is synthesized from Choline and Acetyl-CoA in reaction catalyzed by Acetylcholine Transferase (Choline Acetyl-Transferase) [**Fig. 2a**]

How is Ach removed from receptors? (**Fig. 2b**)

- Removal of Ach is by hydrolysis catalyzed by Acetylcholinesterase
- Acetylcholinesterase found at nerve endings is anchored to the plasma membrane through Glycolipids

What types of receptors are used by Ach?

- Ach receptors are Ligand-Gated Cation Channels composed of four different polypeptide subunits arranged as [$(\alpha 2)(\beta)(\gamma)(\delta)$]
- Ach receptors are of 2 types identified on the basis of their responsiveness to Toadstool Alkaloids: Muscarine and Nicotine
- Receptors are therefore called Muscarinic receptors and Nicotinic receptors
- Both types of receptors are abundant in human brain
- Nicotinic receptors are further divided into those found at neuromuscular junctions and those found at neuronal synapses
- Activation of Ach receptors by the binding of Ach leads to Influx of Na^+ ions into the cell and Efflux of K^+, causing depolarization of the Postsynaptic cell

Cholinergic Agonists and Antagonists (Table 2):

- Numerous compounds act as either Agonists or Antagonists of Cholinergic neurons (**Table 2**)
- Principal actions of Cholinergic Agonists / Antagonists are Excitation or Inhibition of Autonomic Effector cells that are innervated by Post-ganglionic Parasympathetic neurons and as such are referred to as Para-sympathomimetic Agents
- Cholinergic agonists include Choline esters (e.g., Ach), some protein- or alkaloid-based compounds
- Responses of Cholinergic neurons can be enhanced by administration of Cholinesterase (ChE) inhibitors
- ChE inhibitors have been used as components of Nerve Gases but also have significant medical application in the treatment of disorders such as Glaucoma, Myasthenia Gravis as well as in terminating the effects of neuromuscular blocking agents such as Atropine

Mode of action of some Cholinergic Antagonists:
See Figs. 3 & 4

CATECHOLAMINES:

How are the Catecholamines biosynthesized? (**Fig. 5**)

- Major Catecholamines: Dopamine, Nor-epinephrine (Nor-Adrenalin), Epinephrine (Adrenalin)
- Catecholamines formed from Phenylalanine and Tyrosine
- Phenylalanine Hydroxylase (mixed functional Oxygenase that utilizes molecular Oxygen) catalyzes conversion of Phenylalanine to Tyrosine
- Tetra-hydro-biopterine is required for reaction to occur
- Tyrosine is transported to appropriate Catecholamine–secreting neurons for conversion to either
    - Dopamine (Dopaminergic neurons) or
    - Nor-epinephrine (Noradrenergic neurons) or
    - Epinephrine (Epinephrinegic neurons)

What receptors are used by Catecholamines?

- Catecholamines bind to two classes of receptors α-Adrenergic and β-Adrenergic receptors
- Catecholamines therefore are also known as Adrenergic neurotransmitters
- Neurons that secrete them are called Adrenergic neurons
- Neurons that secrete Norepinephrine are called Noradrenergic neurons
- Adrenergic receptors are classical serpentine receptors that couple to intracellular G-proteins

Table 2: Natural Cholinergic Agonists and Antagonists

	Source of compound	Mode of Action
Agonists		
Nicotine	Alkaloid prevalent in tobacco plant	Activates Nicotinic class of Ach receptors
Muscarine	Alkaloid produced by Amanita muscaria mushrooms	Activates Muscarinic class of Ach receptors
α-Latrotoxin	Protein produced by the black widow spider	Induces massive Ach release, possibly by acting as Ca^{2+} ionophore
Antagonists		
Atropine (and related compound Scopolamine)	Alkaloid produced by deadly nightshade, Atropa belladonna	Blocks Ach actions only at Muscarinic receptors
Botulinus Toxin	Eight proteins produced by Clostridium botulinum	Inhibits release of Ach
α-Bungarotoxin	Protein produced by Bungarus genus of snakes	Prevents opening of Ach receptor channel
d-Tubocurarine	Active ingredient of Curare	Prevents opening of Ach receptor channels at Motor end-plate

How are Catecholamines removed from the receptors?

- Removal of Catecholamines from receptor sites is by active re-uptake mechanism
- They are pull back across the Presynaptic membrane into the Presynaptic knob, re-packaged and re-used

Catecholamine catabolism (Fig. 6a & 6b):

- Catabolism of Dopamine, Nor-epinephrine and Epinephrine to inactive compounds occur via sequential actions of the enzymes:
    - Catecholamine-O-Methyl-Transferase (COMT) and
    - Monoamine Oxidase (MAO)
- Compounds that inhibit the action of MAO have been shown to have beneficial effects in the treatment of clinical depression, even when tricyclic antidepressants are ineffective. Isoniazid is known to inhibit MAO.

What are some of the actions of Catecholamines?

- Catecholamines can exhibit both Excitatory and Inhibitory effects
- Excitatory effects include action:
    - Exerted upon Smooth muscle cells of vessels that supply blood to the skin and mucous membranes

  - On cardiac function that leads to an increase in heart rate and in force of contraction
  - Such as respiratory stimulation and increase in Psychomotor activity

- Inhibitory effects can be exerted upon:
  - Smooth muscle cells in the wall of the gut,
  - Bronchial tree of the lungs,
  - Vessels that supply blood to skeletal muscle

- Nor-epinephrine and Epinephrine also influence rate of metabolism by:
  - Modulating endocrine function such as: Insulin secretion
  - Increasing rate of Glycogenolysis and Fatty acid mobilization

How is Serotonin biosynthesized? (Fig. 7)

- Serotonin (5-Hydroxy-Tryptamine, 5-HT) is formed by Hydroxylation and Decarboxylation of Tryptophan

How is Serotonin degraded? (**Fig. 8**)

- Neurons that secrete Serotonin are termed Serotonergic
- After the release of Serotonin, a portion is taken back up by the presynaptic Serotonergic neuron in a manner similar to that of the re-uptake of Nor-epinephrine

Functions of Serotonin:

- Functions of Serotonin are exerted by interaction with specific receptors, some are Presynaptic and others are Postsynaptic
- Serotonin receptors are: $5HT_1$, $5HT_2$, $5HT_3$, $5HT_4$, $5HT_5$, $5HT_6$, and $5HT_7$
- Serotonin receptors are coupled to G-proteins that interacts with either Adenylate Cyclase or Phospholipase C

Gamma-Amino-Butyric Acid (**GABA)**:

- Several Amino Acids have distinct Excitatory or Inhibitory effects upon the nervous system
- Amino Acid derivative Gamma-Amino-Butyrate (GABA), also called 4-aminobutyrate, is a well-known inhibitor of presynaptic transmission in the CNS and Retina
- Formation of GABA occurs by Decarboxylation of Glutamate catalyzed by Glutamate Decarboxylase (GAD)
- GAD is present in many nerve endings in brain and in beta-cells of the Pancreas
- Neurons that secrete GABA are called GABAergic neurons
- GABA exerts its effects by binding to two distinct receptors, GABA-A and GABA-B
- **GABA-A receptors form a Chloride ion channel**
- Binding of GABA to GABA-A receptors increases Chloride ion conductance of presynaptic neurons
- GABA-B receptors are coupled to Intracellular G-protein and act by increasing conductance of associated K^+ channel

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PBL SEMINAR MBBS III
SYNOVIAL FLUID: Overview

- Normal joints in humans are surrounded by membrane called the Synovial membrane (or Synovium), which forms a capsule around the ends of the bones involved.
- Synovial membrane secretes a liquid: Synovial fluid (the meaning of Synovial is – "like egg white").

What are the functions of Synovial Fluid?

- Synovial fluid has many important functions: It serves as a
  - Lubricant, Shock absorber and Nutrient carrier.
- **As a lubricant:**
  - In healthy joins (healthy cartilage tissue) **Synovial fluid** makes the joint slicker than wet ice
  - Synovial fluid cannot function as a lubricant in joints with poor cartilage caused by inadequate production of **Glucosamine and Chondroitin sulfate which are the building blocks of cartilage**)
    - The normally thick synovial fluid becomes thin and watery Thus it cannot do the job it was intended to do as a lubricant

- **As a shock absorber or hydraulic fluid**:
  - Synovial fluid in contact with the cartilage in the joints protects the bones from the tremendous impact they would receive when we
    - Walk Run, Jump, Skip, etc.
  - Synovial fluid belongs to a group of liquids known as dilatent liquids.

Dilatent properties of Synovial fluid:

- Dilatent liquids are characterized by the rare quality of becoming thicker, that is, more viscous, when shear (force) is applied to them.
- Synovial fluid in the Knees and Hips assume a very viscous nature at the moment of shear in order to protect the joints, and then it thins out again to its normal viscosity instantaneously to resume its lubricating function between shocks.
- This change of state of Synovial fluid occurs over and over again, very rapidly, during the course of vigorous exercise, such as during an engagement in sports, dancing, walking, jumping, skipping, etc.
- When the body cannot produce enough Glucosamine and Chondroitin (building blocks of cartilage), this whole mechanism breaks down.
  - The viscosity is dramatically reduced, giving thin, watery synovial fluid, which then fails to function as a shock absorber and as a lubricant.
  - This results in pain, stiffness and decreased mobility that characterize Osteoarthritis (a condition that is primarily due to an imbalance between the rate of destruction and rate of production of cartilage).

Basic functions of Synovial fluid are:

- Lubrication to reduce frictional resistance to joint movement.
- Provide nutrition to articular cartilage
- Protect the joint structures when subjected to large compressive forces
- Provide a liquid environment within a narrow pH range.
- Remove various products of metabolism.

What is the general composition of Synovial fluid?

- Synovial fluid is a highly viscous fluid, which is thought to be a transudate of plasma (i.e., a dialysate of blood plasma that has filtered through semi-permeable walls of blood vessels) with the addition of Hyaluronic acid
    - Hyaluronic acid is one of the high molecular weight compounds called Glycosaminoglycan (GAG) that is produced by synovial cells
    - Hyaluronic acid is the compound that makes Synovial fluid viscous

- Synovial fluid is clear, almost colorless or straw-colored
- Synovial fluid has about one-third of the protein concentration of blood plasma and contains only low molecular weight proteins such as albumin
- Synovial fluid does not contain high molecular weight proteins such as fibrinogen therefore it does form a fibrin clot when aspirated
- Synovial fluid has low glucose content

What is Hyaluronic Acid (functions and properties)?

- Hyaluronic acid is a Glycosaminoglycan found in synovial fluid and cartilage.
- In synovial fluid, Hyaluronic acid is synthesized in synovial membrane.
- It consists solely of repeating disaccharide units of N-acetylglucosamine and glucuronic acid.
- The large molecular weight, poly-electrolyte-character, and large volume of water it occupies in solution all contribute to the properties of Hyaluronic acid as lubricant and shock absorbant.
- It acts as lubricant and shock absorber.
- It acts as barrier permitting metabolites to pass through it by diffusion but resist penetration by bacteria and other infectious agents.
- The amount of Hyaluronic acid in cartilage is variable but usually represents less than 1% of total Glycosaminoglycans.
- It can be present in a free state, but it is usually found as a part of Proteoglycan aggregates in cartilage.

What are GLYCOSAMINOGLYCANS (GAG) (also called **MUCOPOLYSACCHARIDES**) **and PROTEOGLYCANS?**

- These are un-branched Heteropolysaccharides made up of repeating disaccharide units in which **one component** is always:
    - An **Amino sugar** (either D-glucosamine or D-galactosamine).

    - The other component is usually uronic acid.
- When the Glycosaminoglycans are covalently linked to proteins the complex structure formed are called PROTEOGLYCANS.
- In other words the Polysaccharide portions of Proteoglycans are called Glycosaminoglycans.

What are the different types of GAGS?

There are seven types of Glycosaminoglycans; these are (See Table):

- Hyaluronic acid
- Chondroitin sulfate (made up of Chondroitin 4-sulfate and Chondroitin 6-sulfate)
- Keratan sulfate I & II
- Heparin
- Heparan sulfate
- Dermatan sulfate

General structure of the Proteoglycans in Cartilage:

- Proteoglycan is a macromolecule constructed of a protein core to which many Glycosaminoglycan chains are attached.
- Hyaluronic acid is non-covalently bound to Proteoglycan aggregate.
- Usually small Glycoproteins serve to stabilize non-covalent association of the Proteoglycan subunits with Hyaluronic acid in aggregate.
- Proteoglycan molecule is made up of 10% protein and 90% Glycosaminoglycans.
- In Osteoarthritis there is a characteristic reduction in aggregating Proteoglycans.

What is the general Composition of Articular Cartilage?

- Articular cartilage is elastic, fluid-filled, and backed by a relatively impervious layer of calcified cartilage and bone.
- About 80% of this specialized Hyaline cartilage is liquid (two-thirds of which is in the matrix).
- Collagen forms about half to two-third of the dry weight of cartilage.
- Chondroitin Sulfate (GAG) is in the matrix and comprises one-sixth to one-fourth of the dry weight of articular cartilage.
- Cartilage must remain resilient to act as a shock absorber;
- To retain minimal friction cartilage must maintain a smooth and unbroken surface.
- Once surface changes occur, friction increases and a vicious cycle of wear and cartilage destruction ensures.
- Diffusion of nutrients from synovial fluid into articular cartilage is greatly enhanced by the cyclic “kneading” of the cartilage in normal activity.
- Interference with the supply of nutrients may contribute to degenerative joint disease.

Osteoarthritic knees:

- Usually contains Synovial fluid with:
    - Increased cell numbers,
    - Increased levels of enzymes, and
    - Greater number of particles than normal knee

Arthritis may lead to the some changes in the composition of Synovial fluid:

- Increased plasma concentration.
- Increased protein content
- Increased number of cells.
- Possible changes in Hyaluronic acid structure.
- In various types of arthritis, Proteoglycans may act as auto-antigens, thus contributing to the pathologic features of these conditions.
- The amount of Chondroitin sulfate in cartilage diminishes with age, whereas the amounts of Hyaluronic acid and Keratan sulfate increase.
- These changes may contribute to the development of Osteoarthritis.

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GENERAL REVIEW OF NEUROCHEMISTRY – MBBS III

(ALL DIAGRAMS IN HANDOUT AVAILABLE IN BMS OFFICE)

CEREBRAL METABOLISM:

What are some major uses of energy in nervous system?

- Neuron is an excitable cell: capable of changing membrane potential
- Excitation and Conduction are two major functions of nervous tissues
  - Reflected in unceasing electrical activity of cerebral tissue
  - Electrical activity requires energy derived from metabolic processes
  - Energy required for Active Transport of ions needed to Sustain and Restore Membrane Potentials discharged during Excitation and Conduction
- Energy required for maintenance of blood-brain barrier
- Energy required for biosynthesis of neurotransmitters and other components
- Oxygen and Glucose are major substrates for energy production in cerebral tissue

How significant is O_2 supply for normal brain function?

- Brain represents 2 to 3% of total body weight of an average adult, yet it utilizes about **20 to 25% of total O_2 consumed by the whole organism**
- Brain tissue utilizes O_2 more than other tissues: e.g., it utilizes about 20 times more O_2 than muscle tissue when at rest
- O_2 consumption throughout the whole brain is not constant
  - Gray Matter utilizes about twice more O_2 than White Matter
    - Grey matter: energy is via Aerobic Glycolysis
    - White matter: energy is via Anaerobic Glycolysis

- Cerebral tissue contains mixed functional Oxygenases (Oxidases and Hydroxylases) that require molecular O_2 as substrates for biosynthesis of biologically active compounds
- Brain requires continuous supply of O_2 via circulation because O_2 stored in the brain is extremely small compared to its rate of utilization
- If cerebral blood flow is completely interrupted (Ischaemia), consciousness is loss within a few seconds, or the amount of time required for consuming the O_2 contained within the brain and its blood content
- Reduced cerebral O_2 uptake has been shown to occur under certain conditions that lead to depressed consciousness: Examples include:
  - Insulin Hypoglycemia, Diabetic Coma, Cerebral Tumors, Uremia, Gross Liver damage leading to hepatic coma, Anesthetic used during surgery

What are the sources of Glucose for cerebral metabolism?

- Cerebral tissue utilizes glucose directly from arterial blood
- Insulin does not mediate the uptake of blood Glucose by cerebral tissue

- Uptake of blood Glucose by cerebral tissue is via an High Affinity, High Capacity uptake system

- In conditions of low blood glucose, Cerebral tissue can utilize the small store of Glycogen (about 0.1%) to maintain cerebral metabolism for a very short time
- **During Hypoglycemia few Carbohydrates, such as Mannose, can act as substrates for cerebral energy metabolism**
- Not that compounds such as Maltose, Fructose, Galactose, Hexosephosphates, Lactate, Pyruvate and Glyceraldehyde cannot directly act as substrates for cerebral energy metabolism
  - They can act as substrate only after their conversion to glucose via Gluconeogenesis in the liver
    - Thus, these compounds act by raising blood glucose level

- **In the absence of glucose the brain can utilize Mannose directly and rapidly to restore or maintain normal metabolic function**
- **Mannose can enter directly into the Glycolytic pathway of brain tissues, without raising blood glucose level**
- **Mannose like glucose can easily cross the blood-brain-barrier and can be converted to Mannose-6-phosphate by Hexokinase**
- **Phosphomannose Isomerase, which** is an active enzyme in brain tissue then converts Mannose-6-phosphate to Fructose-6-phosphate, which then enters the Glycolytic pathway

How is ammonia metabolized in cerebral tissue?

- Urea cycle is not the major pathway for removal of Ammonia in Cerebral tissue
- Activity of Carbamoyl-phosphate synthetase, which is the first enzyme in Urea cycle, is extremely low or absent in Cerebral tissue
- **Removal of Ammonia from Cerebral tissue involves two reactions:**
  - **First is: Glutamate Dehydrogenase (GDH) reaction**

$$\text{Alpha-oxoglutarate} + NH_3 + NADH + H^+ \xleftarrow{\text{GDH}} \text{Glutamate} + NAD$$

  - **Second is: Glutamine Synthetase reaction**

$$\text{Glutamate} + NH_3 + ATP \xrightarrow{\text{Glutamine Synthetase}} \text{Glutamine} + ADP + P_i$$

- **Alpha-Oxoglutarate is taken form the TCA cycle, thus provided there is an adequate supply of Alpha-oxoglutarate the concentration of Ammonia in Cerebral tissue will be kept low**

- Extensive utilization of Alpha-oxoglutarate produced in TCA cycle within Cerebral Tissue, would deplete intermediates in the TCA cycle and thus affect energy supply to the Brain, unless a mechanism (Anaplerotic or Filling up reaction) of replenishing the intermediates is available
  - Anaplerotic reactions increase concentrations of TCA cycle intermediates, allowing increased formation Acetyl-CoA for maintenance of TCA cycle

Anaplerotic reactions include:

- Pyruvate Carboxylase reaction: catalyzes formation of Pyruvate from Oxaloacetate using ATP and Biotin
- Transamination reactions: forms Alpha-oxoglutarate, a TCA cycle intermediate
- Glutamate Dehydrogenase reaction: forms Alpha-oxoglutarate
- Succinyl-CoA formation from Isoleucine, Valine, Methionine, and Threonine

SOME FACTORS THAT CAN AFFECT CEREBRAL METABOLISM:

How does Hypoxia affect Cerebral Metabolism?

- Hypoxia causes drastic slowdown in rate of Oxidative metabolism
  - Resulting in increased Anaerobic Glycolytic activity and relative increase in conversion of Pyruvate to Lactate, which consequently leads to intracellular acidosis, in most cases
  - **Pasteur Effect**: **Inhibition of rate of Glycolysis in the presence of oxygen**
- **Hypoxia brings about Increase Glucose utilization from Cerebral blood stream, together with a Decrease in Cerebral glucose concentration**

- **Major effects of hypoxia on nervous system include:**
  - **Reduction in Rate of Conversion of Pyruvate to Acetyl-CoA with a resultant decrease in both biosynthesis of Acetylcholine and Activity of the TCA cycle**
- In situation of low Acetyl-CoA availability the Cerebral tissue may use the available acetyl-CoA for energy production so as to maintain membrane potentials in preference to its use in the biosynthesis of compounds

How does Ischaemia affect Cerebral Metabolism?

- Ischaemia: Glucose and Oxygen supply to Cerebral tissue are deficient
  - Causes rapid depletion of small store of Glycogen in Cerebral tissue
  - If condition persists then coma ensures leading to cerebral tissue damage

How does hypoglycemia affect cerebral metabolism?

- Hypoglycemia severely affect cerebral energy metabolism because, the brain uses glucose almost exclusively as substrate for energy metabolism
  - Unlike other tissues such as muscle, nervous system does not depend on Insulin for uptake of Glucose from blood
- During starvation Cerebral Tissue can use Ketone bodies (Beta-hydroxybutyrate, and Acetone) as substrate for energy metabolism
- Ketone Bodies are usually very high in the blood during starvation, thus they are able to cross the blood-brain barrier without much restriction

NEUROTRANSMISSION:

- Neuron is an excitable cell: it is capable of generating and conducting electrical impulse by temporarily reversing its membrane potential
- Synapse: contact between two neurons
- Neuromuscular Junction: contact between neuron and muscle fiber

What are the two ways of impulse transmission by neurons?

- **Impulse transmission along Axon:**
  - **Membrane Transmission**:
    - Mode of Transmission is Electrical
    - Propagation of Action Potential along membrane of Axon

- **Impulse transmission Across Synapse:**

  - **Synaptic Transmission (and also impulse transmission from Neuron to Muscle i.e., across the Neuromuscular Junction):**
    - Mode of Transmission is Chemical
    - Process carried out Chemical Compounds (Neurotransmitters)

Give a brief outline of the neurotransmitter Acetylcholine:

- Acetylcholine (ACh) is a simple molecule synthesized from Choline and Acetyl-CoA through the action of Acetylcholine Transferase (also called Choline Acetyltransferase)
- Neurons that synthesize and release ACh are termed Cholinergic neurons
- Acetylcholine is an excitatory neurotransmitter
- Removal of ACh from receptors at the Postsynaptic membrane is by hydrolysis catalyzed by True Acetyl-Cholinesterase located at nerve endings
- ACh receptors are Ligand-Gated Cation channels composed of four different polypeptide subunits arranged in the form [(α2)(β)(γ)(δ)]
- Two main classes of ACh receptors **Muscarinic receptors and Nicotinic receptors**
- Nicotinic receptors are further divided into those found at neuromuscular junctions and those found at neuronal synapses

CATECHOLAMINES:

- Major Catecholamines are **Norepinephrine, Epinephrine and Dopamine**
- Catecholamines are the neurotransmitters for the sympathetic nervous system effect
- Phenylalanine or Tyrosine can serve a precursor for their biosynthesis
- **Catecholamines bind to two different classes of receptors termed α- and β-Adrenergic receptors, which are** classical serpentine receptors that couple to intracellular G-proteins
- **Catecholamines are also known as Adrenergic neurotransmitters**
- Catecholamines are degraded to inactive compounds through sequential actions of the enzymes: Catecholamine-O-Methyl-Transferase (COMT), Monoamine Oxidase (MAO) and Aldehyde Dehydrogenase

Briefly comment on Gamma Amino Butyric Acid (GABA):

- Several amino acids have distinct excitatory or inhibitory effects on Nervous System
- Gamma-Amino-Butyrate, also called 4-Aminobutyrate, (GABA) is an inhibitor of Presynaptic Transmission in CNS, and Retina

- Biosynthesis of GABA occurs via Decarboxylation of Glutamate catalyzed by Glutamate Decarboxylase (GAD), which is found in many nerve endings in brain and in beta cells of the Pancreas
- Neurons that secrete GABA are called GABAergic neurons
- GABA exerts its effects by binding to two distinct receptors: GABA-A and GABA-B
- GABA-A receptors form Chloride ion channel:
    - Binding of GABA to GABA-A receptors increases Chloride ion conductance of Presynaptic neurons
- GABA-B receptors are coupled to Intracellular G-protein and act by increasing conductance of associated K^+ channel

What are some of the characteristics of Neurotransmitter Receptors?

- Neurotransmitter receptors are located on the surface of Postsynaptic neurons and also on some Presynaptic neurons
- Receptors on Presynaptic neurons act to inhibit further release of neurotransmitter
- Vast majority of neurotransmitter receptors belong to a class of proteins known as S**erpentine receptors**, because they exhibits a characteristic transmembrane structure that spans the cell membrane seven times
- **Link between neurotransmitters and Intracellular signaling is carried out by association either with G-proteins (small GTP-binding and hydrolyzing proteins) or with Protein Kinase, or by the Receptor itself in the form of a Ligand-gated ion channel (for example, the Acetylcholine receptor)**
- Neurotransmitter receptors are subjected to **ligand-Induced Desensitization**: That is, they can become unresponsive upon prolonged exposure to their neurotransmitter

What are the effects of Neurotransmitters on Post-synaptic receptors?

- Effects of neurotransmitters on Post-synaptic receptors varies:
- Some Neurotransmitters (such as, **Acetylcholine, Glycine, Glutamate, GABA)** have an "Inherent" biological activity.
    - Act directly to cause 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 Post-synaptic response
- Second Messenger systems involve compound (such as, cAMP, cGMP, ITP, PGs, Epoxides and Ca^{2+} ions) that 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
- Excitatory neurotransmitters bind to the receptors to cause depolarization of the membrane of the Post-synaptic cell
- Inhibitory neurotransmitters 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 & Diseases That Affect Neuromuscular Junction & Synaptic Transmission:

- ACh release in the NMJ is inhibited by Botulinum Toxin causing flaccid paralysis
- Glycine release in the CNS is inhibited by Tetanus Toxin causing spastic paralysis
- Black widow spider toxin, Alpha-Latrotoxin, stimulates fusion and depletion of neurotransmitter vesicles
- Plant poison, Physostigmine, Nerve Gases and Organo-phosphorus pesticides inhibit Acetyl-cholinesterase, the enzyme that degrades ACh into Acetate and Choline
- Muscle ACh receptor is blocked by the South American arrow poison, Curare
- Plant drug, 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 recycling of Dopamine and Norepinephrine neurotransmitters in the brain causing an excitatory effect