Lecture 3: CHOLINERGIC SYSTEM  
(Parasympathetic Nervous System) AND DRUGS

1. *cholinoceptor activating drugs*
2. *cholinesterase-inhibiting drugs*

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**Acetylcholine (Ach)**
- is a major neurohormonal transmitter
- it is an ester of acetic acid and choline
- synthesis: Ach is synthesized in the cytoplasm of the cholinergic neuron, from choline and acetyl-coenzyme A, by the enzyme choline acetyltransferase (CAT). Choline is an essential amino-acid transported from extracellular fluid into the cytoplasm of cholinergic neuron by a carrier that actively cotransports sodium. Acetyl-coenzyme A is synthesized in mitochondria from nerve ending.
- storage: Ach is stored into vesicles from nerve ending.
- release: Ach is released when an action potential arrives at the nerve ending and determines an increase in the concentration of intracellular calcium. Elevated calcium levels promote the fusion of vesicles with the presynaptic membrane and release of Ach into the synapse. Release of Ach occurs in small cuanta (1 cuanta = 1000 – 50000 molecules of Ach from a vesicle)
- after release into synaptic space: Ach binds to either the cholinergic receptors or active site of acetylcholinesterase (AchE).
  - Binding to cholinergic receptors leads to a biological response.
  - Binding to active site of AchE terminates Ach action. Cholinesterase is enzyme that cleaves acetylcholine to acetate and choline to end its action. It’s located in both pre and postsynapse.
- AchE cleaves, in 2 steps, Ach to choline and acetate:
  - first step: complex Ach-AchE is hydrolysed to choline and acetylated enzyme;
  - second step: acetylated enzyme is hydrolysed to AchE and acetate.
  Choline is transported back into the nerve ending by an uptake system, where it is acetylated and stored until release by a subsequent action potential.
- T1/2 of Ach in synaptic space is very short (milliseconds).
Cholinoreceptors
Two classes of receptors for Ach are recognized: muscarinic and nicotinic receptors.

Muscarinic receptors
- are coupled with protein G.
- subtypes: M₁, M₂, M₃, M₄ and M₅
- are located:
  - presynaptic: on cholinergic nerve endings (activation inhibits further Ach release) and adrenergic nerve endings (activation inhibits NA release);
  - postsynaptic: primarily on autonomic effector cells in the CNS, autonomic ganglia, heart, smooth muscle, endothelial cells of blood vessels, eye, glands of gastrointestinal, respiratory and uro-genital tract, sweats glands etc.

### Muscarinic receptors

<table>
<thead>
<tr>
<th>Receptor Name</th>
<th>Typical Locations</th>
<th>Result of Ligand Binding</th>
<th>Biological effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>M₁</td>
<td>CNS; Most abundant in cerebral cortex, hippocampus and striatum; Autonomic ganglia; Glands (gastric and salivary) Enteric nerves</td>
<td>Formation of IP₃ and DAG, increased intracellular calcium</td>
<td>Increased cognitive function (learning and memory); Increased seizure activity; Increase in secretions; Increase in depolarization of autonomic ganglia</td>
</tr>
<tr>
<td>M₂</td>
<td>Myocardium, smooth muscle, some presynaptic sites; CNS neurons</td>
<td>Inhibition of adenylyl cyclase, opening of potassium channels</td>
<td>Heart: decrease heart rate, decrease conduction velocity; Smooth muscle: increase contraction; Peripheral nerves: neural inhibition; CNS: neural inhibition, increase tremors, hypothermia, analgesia</td>
</tr>
<tr>
<td>M₃</td>
<td>Exocrine glands, vessels (smooth muscle and endothelium); CNS neurons</td>
<td>Formation of IP₃ and DAG, increased intracellular calcium</td>
<td>Glands: increase secretion (predominant in salivary gland); Smooth muscle: increase contraction (predominant in some, e.g. bladder, bronchia)</td>
</tr>
<tr>
<td>M₄</td>
<td>CNS neurons; possibly vagal nerve endings</td>
<td>Opening of potassium channels, inhibition of adenylyl cyclase</td>
<td>Facilitation of dopamine release; Analgesia</td>
</tr>
<tr>
<td>M₅</td>
<td>Vascular endothelium, especially cerebral vessels; CNS neurons</td>
<td>Formation of IP₃ and DAG, increased intracellular calcium</td>
<td>Facilitation of dopamine release; Facilitation of cerebral vascular vessels</td>
</tr>
</tbody>
</table>

Nicotinic receptors
- are ligand gated cation channel: their activation causes opening of the channel and rapid flow of cations resulting in the depolarisation and on action potential.
- binding of acetylcholine (the ligand) to the nicotinic receptor, changes the receptor shape, and let sodium enter the muscle cell → skeletal muscle contraction (Influx of sodium depolarizes the muscle sarcolemma near the motor endplate. This depolarisation triggers another type of sodium channel, the voltage-gated sodium channels which spread the excitation over the muscle cell and leads to the release of calcium ions from...
sarcoplasmic reticulum. Calcium ions initiate a series of biochemical events involving troponin, tropomyosin and myosin leading to muscle contraction.)

- there are 2 subtypes: $N_n$ and $N_m$.
- are located:
  - $N_n$: autonomic ganglia, adrenal medula, CNS;
  - $N_m$: neuromuscular junction.

### Nicotinic receptors

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<td>$N_n$</td>
<td>Postganglionic neurons, some presynaptic cholinergic terminals, adrenal medula, CNS</td>
<td>Opening of $Na^+$, $K^+$ channels, depolarization</td>
</tr>
<tr>
<td>$N_m$</td>
<td>Skeletal muscle neuromuscular endplates</td>
<td>Opening of $Na^+$, $K^+$, $Ca^{2+}$ channels, depolarization</td>
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### Acetylcholine mechanism of action

- molecule of Ach is flexible, this is why it may bind on:
  - muscarinic receptors: when methyl profile of Ach is evident
  - nicotinic receptors: when carbonilic profile is evident
- structure of molecule of Ach is $CH_3-CO-O-CH_2-CH_2-N^+(CH_3)_3$
- molecule of Ach has an “onium” group: -$N^+(R)_3$
- active points of molecule:
  - cationic group (onium group)
  - esteric oxygen
  - hydroxil attached to C3
  - methyl attached to C2
- muscarinic receptor has 4 active sites on its surface:
  - anionic group $\rightarrow$ affinity site
  - positive charged group which interact with esteric oxygen
  - positive charged group which interact with hydroxil attached to C3
  - hydrophobic region which interact with methyl attached to C2
- the bind of Ach on muscarinic receptors increase the permeability for $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$:
  - heart: increase permeability for $K^+$ and $Cl^-$ $\rightarrow$ membrane hyperpolarisation, decrease heart rate (bradicardia is possibly preceded by tachycardia),
  - autonomic ganglia, smooth and skeletal muscle: increase permeability for $Na^+$ $\rightarrow$ increase of peristalism and muscle tone
  - gland cells: increase permeability for $Ca^{2+}$ $\rightarrow$ hypersecretion.
- the bind of Ach on nicotinic receptors (ligand gated $Na^+/K^+$ channel):
  - mechanism in 2 stages:
    - the bind of few molecules of Ach $\rightarrow$ increase the number of open channels
    - the bind of a big number of molecules of Ach $\rightarrow$ block the channels in open position
- on $N_n$ $\rightarrow$ short period of transmition of impulses with membrane depolarising $\rightarrow$
  followed by blockage of repolarising of membrane
- on $N_m$ $\rightarrow$ skeletal muscle fasciculations $\rightarrow$ followed by skeletal muscle relaxation
Pharmacodynamic effects of Acetylcholine

Cardiovascular: Bradycardia (possibly preceded by tachycardia), vasodilation (all vascular beds including pulmonary and coronary – M3) and hypotension, reduction of the contraction strength (atrial and ventricular cells, \( \text{I}_{\text{K}^+}, \text{I}_{\text{Ca}^2+} \) diastolic depolarization, NO-inhibitable ATP?), negative chronotropic effect (inhibition of adrenergic activation).

Eyes: contraction of ciliary muscle and smooth muscle of the iris sphincter (miosis) – aqueous humor outflow, drainage of the anterior chamber.

- GI - increases in tone, amplitude of contractions, and peristaltic activity of the stomach and intestines, enhances secretory activity of the gastrointestinal tract.

- Urinary bladder - increase ureteral peristalsis, contract the detrusor muscle of the urinary bladder, increase the maximal voluntary voiding pressure, and decrease the capacity of the bladder.

- Other effects – increased secretion from all glands that receive parasymptatetic enervation (salivary, lacrimal, tracheobronchial, digestive and exocrine sweat glands)

- IMPORTANT - BROCHOCONSTRICTION

Muscarinic actions

1) Eye: constriction of pupil of eye (miosis) occurs due to contraction of circular fibres of iris

2) Fall in I/O pressure occurs due to better drainage through canal of Schlemm which occurs due to opening up of trabecular meshwork at the base of ciliary muscle

3) CVS: Bradycardia and vasodilation occur which leads to fall in blood pressure. Then reflex tachycardia occurs.

4) Negative inotropic effect

5) Negative chronotropic effect

6) Negative dromotropic effect

- Produces dilatations of all vascular beds

- LUNGS: smooth muscle of bronchi contracts.

- Increased secretions of glands of tracheobronchial mucosa.

- Dangerous in asthmatics patients

- GIT: increase in secretory and motor activity of gut

- Increased in salivary and gastric secretions

- URINARY BLADDER: detrusor muscle contracts, sphincter relaxed thus promoting micturition

- Exocrine glands: increased secretions of salivary, lacrimal and sweat glands

- NMJ: Causes depolarizations

- CNS: in large doses causes central stimulation with tremors and convulsions.

Therapeutic status of Acetylcholine:

Acetylcholine is a quaternary ammonium compound that cannot penetrate membranes. Due to its very brief duration of action, acetylcholine cannot be used clinically. Compounds with better selectivity and longer duration of action are generally used.
Classification of cholinomimetic drugs

1. **Cholinoreceptor activating drugs (Direct acting cholinomimetic drugs or Receptor agonists)**

**Classification**
- Nonselective agonists:
  - esters: - Acetylcholine
    - esters of acetic acid: Methacholine
    - esters of carbamic acid: Carbachol, Betanechol
  - alcaloids: - on muscarinic receptors (M>N): - natural: Pilocarpine
    - synthetic: Muscarine, Arecholine
    - on nicotinic receptors (N>M): - natural: Lobeline, Nicotine
    - synthetic: Difenilmethylpiperidine
- Selective agonists: - M₁: Cevimeline, Xanomeline
  - M₂: Oxotremorine
  - M₃: Aceclidine
  - on nicotinic receptor: Varenicline.

**Mechanisms of action:**
Agonists on muscarinic receptors
- Stimulation of muscarinic receptors M1 and M3 determine excitatory effects (because of formation of IP3 and DAG and of increased intracellular calcium) \(\rightarrow\) smooth muscle contraction, hypersecretion of exocrine glands.
These drugs bind to muscarinic receptor $\rightarrow$ activates receptor binds G-protein $\rightarrow$ removal of GDP and addition of GTP to G-protein $\rightarrow$ dissociation of G-protein from muscarinic receptor $\rightarrow$ separation of G-protein into alpha and beta-gamma subunits $\rightarrow$ Alpha subunit interacts with and activates Phospholipase C $\rightarrow$ Phosphatidyl inositol biphosphate (PIP) complex $\rightarrow$ Phospholipase breaks down PIP into inositol 1,4,5-triphosphate ($IP_3$) and diacylglycerol $\rightarrow$ $IP_3$ interacts with endoplasmic reticulum membrane which releases $Ca^{2+}$ $\rightarrow$ $Ca^{2+}$ binds to calmodulin forming a complex $\rightarrow$ this complex binds to caldesmon $\rightarrow$ when caldesmon is bound by $Ca^{2+}$/calmodulin complex this allows myosin-actin interactions to occur $\rightarrow$ muscle contracts, increase secretion.

Constriction of the pupil pulls open the trabecular meshwork in the eye and this in turn facilitates drainage of aqueous humour and reduction of intra-ocular pressure.

- Stimulation of muscarinic receptors M2 on cardiac muscle determines inhibitory effects (because of inhibition of adenyl cyclase and of opening of potassium channels) $\rightarrow$ decrease heart rate, decrease conduction velocity.

Carbachol has substantial nicotinic activity because also stimulates the release of Acetylcholine from nerve ending (stimulate nicotinic receptors).

Pilocarpine has a more selective action in stimulating exocrine glands (sweat, salivary, lacrimal, bronchial) and has less effect on other receptors (smooth muscles, cardiovascular system).

**Pharmacokinetics particularities**

Acetylcholine is rapidly hydrolysed in the body by cholinesterases (see above).

Methacholine action is more prolonged because the added methyl group increases its resistance to hydrolysis by cholinesterases.

Carbachol and Betanechol are not inactivated by cholinesterases so their actions are more prolonged than those of acetylcholine.

Varenicline is a selective partial agonist on nicotinic receptors.

**Indications:**

- **Topic administration:**
  - **glaucoma** $\rightarrow$ Pilocarpine $\rightarrow$ it is standard cholinergic agent in the treatment of open-angle glaucoma
    $\rightarrow$ Carbachol $\rightarrow$ is alternative to Pilocarpine in the management of glaucoma when resistance or intolerance to Pilocarpine develops
    $\rightarrow$ Aceclidine (no longer used)
  - **ophthalmic surgical** $\rightarrow$ Acetylcholine (it is aid for the rapid production of miosis, so it is instilled directly into the anterior chamber of the eye)
  - **inhalatory** $\rightarrow$ Methacholine is used to provoke bronchoconstriction in the diagnosis of bronchial airway hypersensitivity.

- **Systemic administration:**
  - **postoperative** in abdominal distention, in gastric atony or gastroparesis, postoperative or postpartum urinary retention: Betanechol (Carbachol also has been used for this indication)
  - **dry mouth** (xerostomia from Sjogren syndrome or following radiotherapy for malignant neoplasms of the head and neck): Pilocarpine, Cevimeline,
  - **antidote in Atropine overdosage:** Pilocarpine,
  - **smoking cessation:** Varenicline.
Contraindications of cholinomimetic drugs:
- bronchial asthma and obstructive airways disease (because bronchoconstrictor effect could precipitate an asthmatic attack);
- coronary insufficiency (because of reduced coronary blood flow, especially if it is already compromised), bradycardia or heart block, hypotension;
- hyperthiroidia (because patients may develop atrial fibrillation due to excess of adrenaline);
- peptic ulcerations (because can aggravate the symptoms of acid-peptic disease);
- pregnancy.

Adverse effects of cholinomimetic drugs:
- abdominal pain, diarrhoea, increased urinary frequency, nausea and vomiting,
- sweating, salivation, lachrymation, rhinorrhoea,
- bradycardia, peripheral vasodilatation leading to hypotension and headache,
- bronchoconstriction.

2. Cholinesterase-inhibiting drugs (Indirect acting cholinomimetic drugs or Anticholinesterases)

Classification
- reversible action:
  - natural (tertiary amine): Physostigmine (Eserine)
  - semisynthetic and synthetic (with a tertiary or quaternary amonium group):
    - alcohols: Edrophonium
    - esters of alcohols or carbamic acid: Neostigmine, Pyridostigmine, Ambenonium, Demecarium
  - other structures: Tacrine, Donepezil, Rivastigmine, Galantamine
- irreversible action → organophosphates: - drugs: Echotiophate, Metriphonate,
  - insecticides: Parathion, Malathion, Propoxur
  - nerve warfare gases: Sarin, Tabun, Soman

Mechanism of action
These drugs inhibit the enzyme acetylcholinesterase (AchE) in the synapse → this has as result the decrease of the inactivation of acetylcholine → accumulation of acetylcholine enhances the activation of the nicotinic and muscarinic receptors. Cholinesterase inhibitors indirectly provide a cholinergic action by prolonging the life time of acetylcholine.

Anticholinesterase drugs are either reversible or irreversible inhibitors of AchE, depending on the time of AchE inhibition. In general, reversible anticholinesterases are of more clinical value, whereas the irreversible ones are used as insecticides and war gases.

- Edrophonium (a quaternary alcohol) reversibly bind electrostatically and by hydrogen bonds to the active site of AchE, thus preventing access of acetylcholine. The enzyme-inhibitor complex does not involve a covalent bond and is correspondingly short-lived (on the order of 2–10 minutes).
- The carbamate esters (neostigmine, physostigmine etc) and other structures (Tacrine, Donepezil…) undergo a two-step hydrolysis sequence. The covalent bond of the
carbamoylated enzyme is considerably more resistant to the second (hydration) process, and this step is correspondingly prolonged (on the order of 30 minutes to 6 hours).

- Organophosphates undergo initial binding and hydrolysis by the enzyme, resulting in a phosphorylated active site. The covalent phosphorus-enzyme bond is extremely stable and hydrolyzes in water at a very slow rate (hundreds of hours). After the initial binding-hydrolysis step, the phosphorylated enzyme complex may undergo a process called aging. This process apparently involves the breaking of one of the oxygen-phosphorus bonds of the inhibitor and further strengthens the phosphorus-enzyme bond. The rate of aging varies with the particular organophosphate compound. If given before aging has occurred, strong nucleophiles like pralidoxime are able to split the phosphorus-enzyme bond and can be used as "cholinesterase regenerator" drugs for organophosphate insecticide poisoning. Once aging has occurred, the enzyme-inhibitor complex is even more stable and is more difficult to split, even with oxime regenerator compounds.

Actually pralidoxime competes with the organophosphates and is itself phosphorylated, making cholinesterase free. Pralidoxime combines with phospharyl group to form soluble complex setting esteratic site free and thus reactivating the enzyme.

**Pharmacokinetics particularities**

Indirect acting cholinomimetic drugs with reversible action (except Physostigmine) are poorly absorbed from gastro-intestinal tract, this is why much larger doses are required for oral administration than for parenteral injection. Only Tacrine, Donepezil, Rivastigmine, Physostigmine passes blood-brain barrier because are liposoluble.

Neostigmine and Pyridostigmine are poorly absorbed orally so requires larger doses than when given parenterally.

The organophosphate cholinesterase inhibitors (except for echothiophate) are well absorbed from the skin, lung, gut, and conjunctiva.

**Indications:**
- *myastenia gravis*:
  - for diagnosis: Edrofonium, Neostigmine,
  - for treatment: Neostigmine, Piridostigmine is the drug of choice, Ambenonium;
- *for the reversal of neuromuscular blockage in anaesthesia (decurarization)*: Edrofonium, Neostigmine, Piridostigmine, Ambenonium;
- *therapy of paralytic ileus and postoperative urinary retention*: Neostigmine, Piridostigmine;
- *intoxication with Atropine*: Neostigmine, Physostigmine;
- *glaucoma*: Ecotiophate, Demecarium, Neostigmine, Physostigmine.
- *infections with Schistosoma haematobium*: Metriphonate (alternative)

**Contraindications:** see above.

**Adverse effects:** see above.