Antihistamines

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Lab.7

Antihistamines

Drugs that directly compete with histamine for specific receptor sites.

- Two histamine receptors:
  - H_1 histamine-1
  - H_2 histamine-2

Role in allergy and anaphylaxis

The symptoms resulting from intravenous injection of histamine are similar to those associated with anaphylactic shock and allergic reactions.

These include:-

1- contraction of airway smooth muscle.

2- stimulation of secretions.

3- dilation and increased permeability of the capillaries.

4- stimulation of sensory nerve endings.

Symptoms associated with allergy and anaphylactic shock result from the release of certain mediators from their storage sites. Such mediators include **histamine, serotonin, leukotrienes, and eosinophil** chemotactic factor of anaphylaxis. **In some cases**, these mediators cause a localized allergic reaction, producing actions on the skin or respiratory tract.

Antihistamines

H_2 Blockers or H_2 Antagonists

- Used to reduce gastric acid in PUD
- **Examples**:
- cimetidine (Tagamet),
- ranitidine (Zantac),
- Or famotidine (Pepcid)

Antihistamines

H_1 antagonists are commonly referred to as antihistamines

- Antihistamines have several effects:
  - Antihistaminic
  - Anticholinergic
  - Sedative

Mechanism of Action

BLOCK action of histamine at the receptor sites

- Compete with histamine for binding at unoccupied receptors.
- CANNOT push histamine off the receptor if already bound.

Mechanism of Action

- More effective in preventing the actions of histamine rather than reversing them.
- Should be given early in treatment, before all the histamine binds to the receptors.

Histamine vs. Antihistamine

Effects

Cardiovascular (small blood vessels)

- **Histamine effects:**
  - Dilation and increased permeability (allowing substances to leak into tissues)
- **Antihistamine effects:**
  - Prevent dilation of blood vessels
  - Prevent increased permeability

Histamine vs. Antihistamine Effects

Smooth Muscle (on exocrine glands)

- **Histamine effects:**
  - Stimulate salivary, gastric, lacrimal, and bronchial secretions
- **Antihistamine effects:**
  - Prevent salivary, gastric, lacrimal, and bronchial secretions

Histamine vs. Antihistamine Effects

Immune System

(Release of substances commonly associated with allergic reactions)

- **Histamine effects:**
  - Mast cells release histamine and other substances, resulting in allergic reactions.
- **Antihistamine effect:**
  - Binds to histamine receptors, thus preventing histamine from causing a response.

Antihistamines: Other Effects

Skin:

- Block capillary permeability, wheal-and-flare formation, itching

Anticholinergic:

- Drying effect that reduces nasal, salivary, and lacrimal gland secretions (runny nose, tearing, and itching eyes)

Sedative:

- Some antihistamines cause drowsiness.

Antihistamines:Therapeutic Uses

Management of:

- Nasal allergies
- Seasonal or perennial allergic rhinitis (hay fever)
- Allergic reactions
- Sleep disorders
- Motion sickness and nausea
- Somnifacients

Antihistamines: Therapeutic Uses

Also used to relieve symptoms associated with the common cold:

- Sneezing, runny nose
- Palliative treatment, not curative

Antihistamines: Side effects

- **Anticholinergic (drying) effects, most common:**
  - Dry mouth
  - Difficulty urinating
  - Constipation
  - Changes in vision
- **Drowsiness**
  - (Mild drowsiness to deep sleep)

Antihistamines: Two Types

- Traditional

  or

- Nonsedating/Peripherally Acting

Antihistamines:

Traditional

- Older
- Work both peripherally and centrally.
- Have anticholinergic effects, making them more effective than nonsedating agents in some cases.

Examples: diphenhydramine (Benadryl) chlorpheniramine (Chlor-Trimeton).

Antihistamines:

Nonsedating /Peripherally Acting

- Developed to eliminate unwanted side effects, mainly sedation.
- Work peripherally to block the actions of histamine; thus, fewer CNS side effects.
- Longer duration of action (increases compliance).

Examples: fexofenadine (Allegra)
loratadine (Claritin)

Important Implications: Antihistamines

- Instruct patients to report excessive sedation, confusion, or hypotension.
- Avoid driving or operating heavy machinery, and do not consume alcohol or other CNS depressants.
- Do not take these medications with other prescribed or **OTC** medications without checking with prescriber.

Important Implications: Antihistamines

- Best tolerated when taken with meals—reduces GI upset.
- If dry mouth occurs, teach patient to perform frequent mouth care, chew gum, or suck on hard candy (preferably sugarless) to ease discomfort.
- Monitor for intended therapeutic effects.

Mast cell stabilizers

1. Mast cell stabilizers inhibit the release of histamine and other autocoids from mast cells.

2. Specific agents include cromolyn and nedocromil.

3. Indications .

a. Cromolyn is available in several dosage forms:

- Oral solution for systemic mastocytosis
- Oral inhalation (nebulization) for asthma and prevention of bronchospasm.
- Nasal spray for allergic rhinitis.
- Ophthalmic solution for conjunctivitis and keratitis

b. Nedocromil is available as an ophthalmic solution for allergic conjunctivitis.

4. Adverse effects include headache, dry mouth, and dry eyes.

H2 receptor antagonists

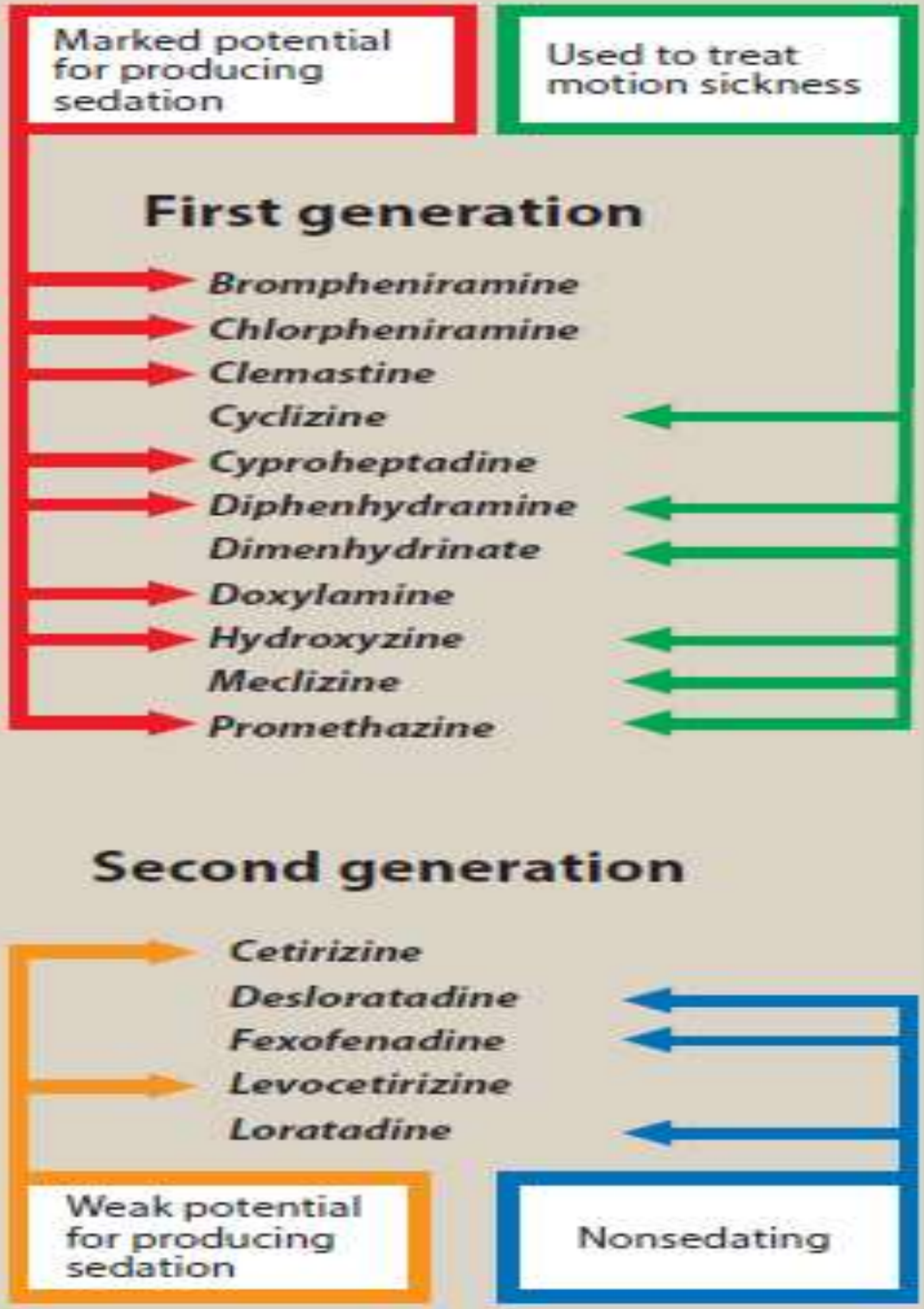
By competitively blocking the binding of histamine to H2 receptors, these agents reduce the secretion of gastric acid. Drugs such as cimetidine, ranitidine, famotidine, roxatidine, and nizatidine are used to inhibit basal, food-stimulated, and nocturnal secretion of gastric acid, reducing acid secretion by approximately 70%.

Therapeutic uses:

a. **Peptic ulcers:** All agents are equally effective in promoting the healing of duodenal and gastric ulcers. Patients with NSAID-induced ulcers should be treated with PPIs, because these agents heal and prevent future ulcers more effectively than do H2 receptor antagonists.

b. **Acute stress ulcers:** These drugs are given as an intravenous infusion to prevent and manage acute stress ulcers associated with high-risk patients in the intensive care setting. Because tolerance may occur with these agents, PPIs are used for this indication.

c. **Gastroesophageal reflux disease (GERD):** H2 receptor antagonists are effective for the treatment of heartburn or GERD. H2 receptor antagonists act by decreasing acid secretion; therefore, they may not relieve symptoms of heartburn for up to 45 minutes.



ANTIHISTAMINES

Sedating
Diphenhydramine
Dimenhydrinate
Chlorpheniramine
Brompheniramine
Doxylamine

Nonsedating
Fexofenadine
Cetirizine
Levocetirizine
Loratadine
Desloratadine

Mast cell stabilizers
Hydroxyzine
Ketotifen

ANTIHISTAMINES AND MAST CELL STABILIZERS

Azelastine
Olopatadine
Alcaftadine
Bepotastine
Clemastine
Cyproheptadine
Emedastine

Motion sickness and nausea
Cyclizine
Meclizine
Promethazine
Dimenhydrinate
Triprolidine

DRUGS ACTING ON EYE

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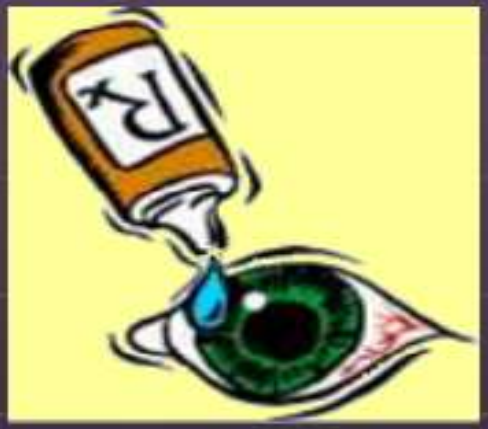
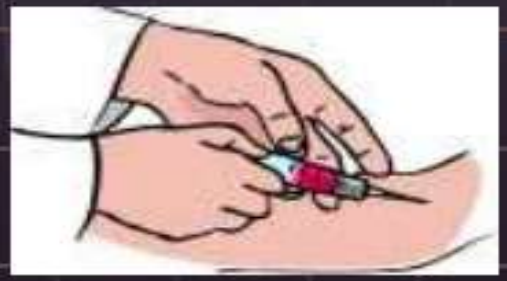
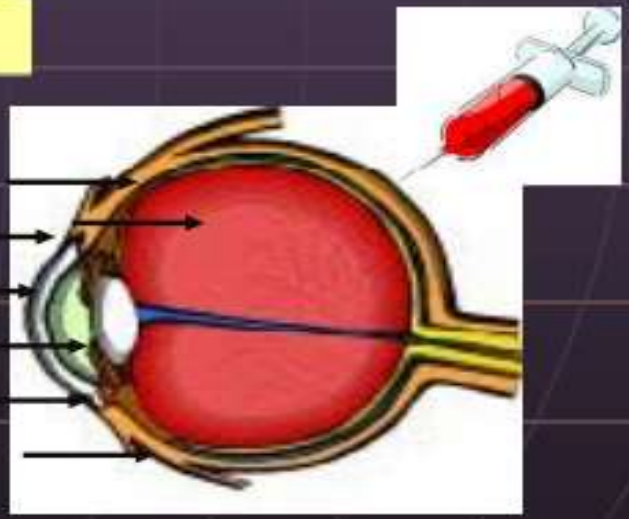
Drugs acting on eye

Route of Administration

- Local
  - Topical
  - Intravitreous
  - Subconjunctival
  - Subtenon
  - Retrobulbar
  - Intracameral
- Systemic







Blood aqueous barrier
Blood retinal barrier

Eyedrops

50 µl per drop

- Only 20 % of the administered drugs is retained
- One drop is enough.

Volume of fluid held by cul-de-sac 7 – 10 µl

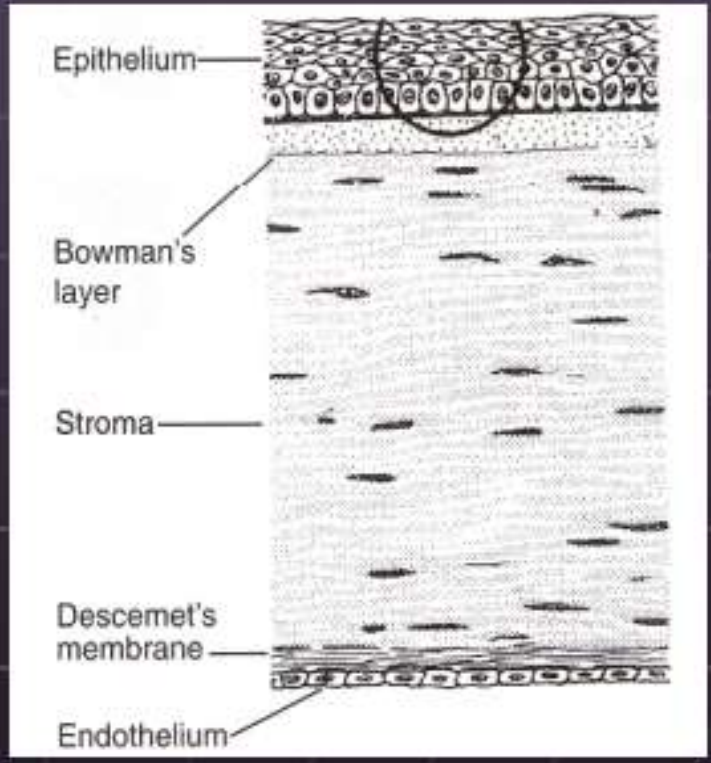
Improve ocular and decrease systemic absorption

- Digital pressure at medial canthal area
- Keep eyes closed for 5 min after taking drops
- Wait 10 minutes between drops
- Frequency ~ duration of action
- Order of using ~ pH

Drug Penetration

Factors that determine the amount of medication to penetrate the cornea

- Drug concentration
- Viscosity
- Lipid/water solubility
- pH
- Surfactants
- Reflex tearing
- Preservative



Form

- Solution and suspension
- Ointment
- Gel

Solution and Suspension

Most commonly used

Advantages

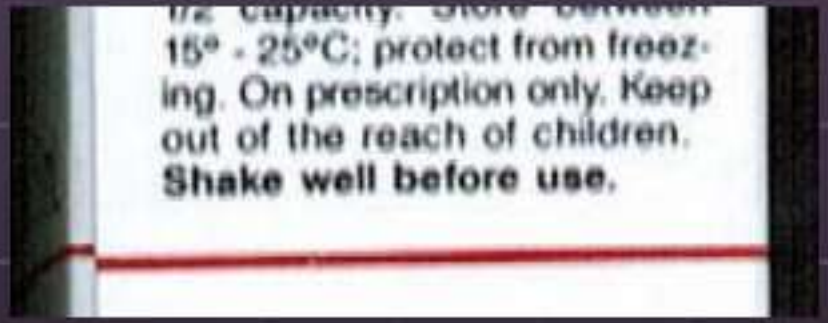
- Easily instilled
- Less interfere to vision
- Fewer potential complications

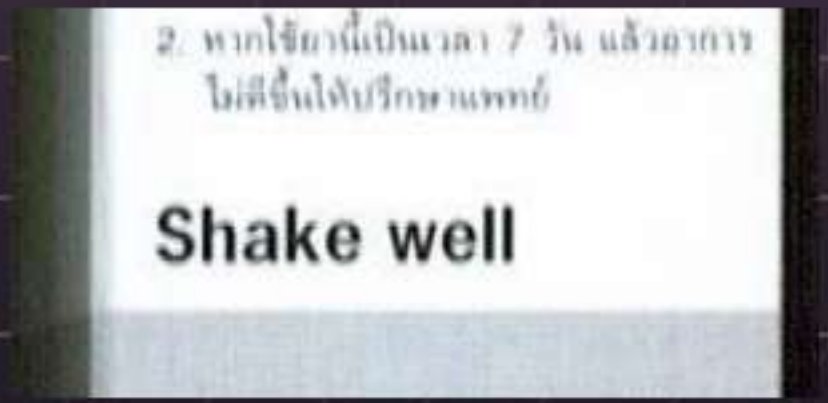
Disadvantages

- Short ocular contact time
- Imprecise and inconsistent delivery
- Frequent contamination
- Possibilities of ocular injury with the dropper tip

Suspensions

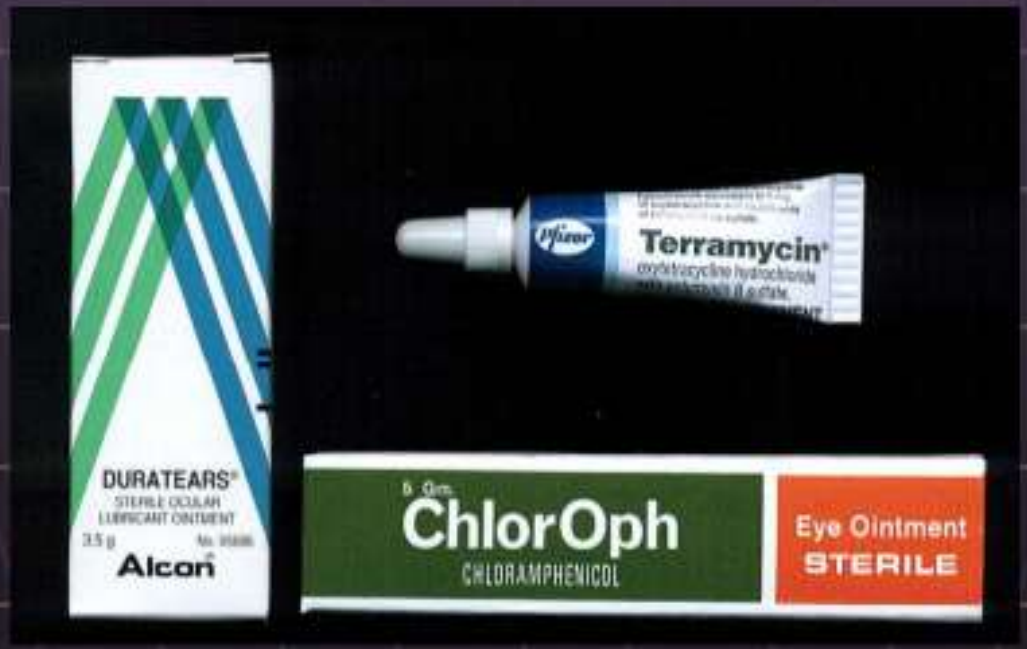
Must be re-suspended by shaking to provide an accurate dosage of drug





Ointment

- Consist of petrolatum and mineral oil
- Increase contact time
- Disturb vision



Gel

- Polymer-based aqueous gels
- Drug release occurs by diffusion and by erosion of the gel surface

Packaging

Standard colors for drug labeling and bottle cap

Yellow, Blue	B blocker
Red	Mydriatics and cycloplegics
Green	Miotics
Orange	Carbonic anhydrase inhibitors
Brown or tan	Anti-infective agents
Gray	NSAIDS
Pink	Steroids

Artificial tears

Anesthesia

Topical

- Cornea, conjunctiva
- Irritate 15 sec
- Duration 15 min
- For FB removal, IOP measurement, etc.
- Complications : toxic to epithelium

Tetracaine hydrochloride (Tetracaine) 0.5%

Benoxinate hydrochloride (Novesine) 0.4%

Mydriatics

I. Sympathomimetics

- Stimulate dilator muscle
- Complications: AACG, vasoconstriction
- For retinal examination, surgery

Phenylephrine hydrochloride (Neosynephrine)

10% 2-3 hr

Mydriatics

II. Parasympatholytics / Cycloplegics

- Inhibit constrictor m.and ciliary m. contraction
- Complications: AACG, flushing
- For ciliary spasm, refraction in children, retinal exam

Atropine sulfate 1% 2 wk

Scopolamine hydrobromide 0.25% 3-5 d

Hornatropine hydrobromide 1-2% 1-3 d

Cyclopentolate hydrochloride (Cyclogyl) 1% 24 hr

Tropicamide (Mydriacyl) 0.5-1% 4-5 hr

Antibiotics

Consider

- Severity and progression
- Suspected organism & Sensitivity
- Location & Penetration
- Toxicity
- Frequency and concentration

Antibiotics: Combination

Polymyxin B

Gramicidin

Neomycin

Antihistamine

Pheniramine maleate

Antazoline HCl

Vasoconstrictors

Phenylephrine HCl

Naphazoline HCl

Tetrahydrozoline HCl

Antazoline HCl
+ Tetrahydrozoline HCl

Pheniramine maleate
+ Naphazoline HCl

Mast-cell stabilizer

- Cromolyn sodium
- Lodoxamide
- Olopatadine HCl

Corticosteroid

- Anti-inflammation
- Suppress immune system and tissue reaction
- Precaution in treatment of infection especially viral and fungal infection

Dexamethazone 0.1%

Prednisolone acetate 0.5%, 1%

Fluorometholone 0.1%

Corticosteroids

Side effects

- Glaucoma
- Cataract
- Exacerbation of infection
- Ptosis
- Scleral melting

Artificial Tear

- Supplement
- Increase contact time
- For dry eye, CL wearer

- Frequency of usage ~ severity
- Preserve VS non preserve

Effect of Parasympathomimetic Drugs on Glandular Secretions in Rats

Lab- 6

ASIST. LECTURER

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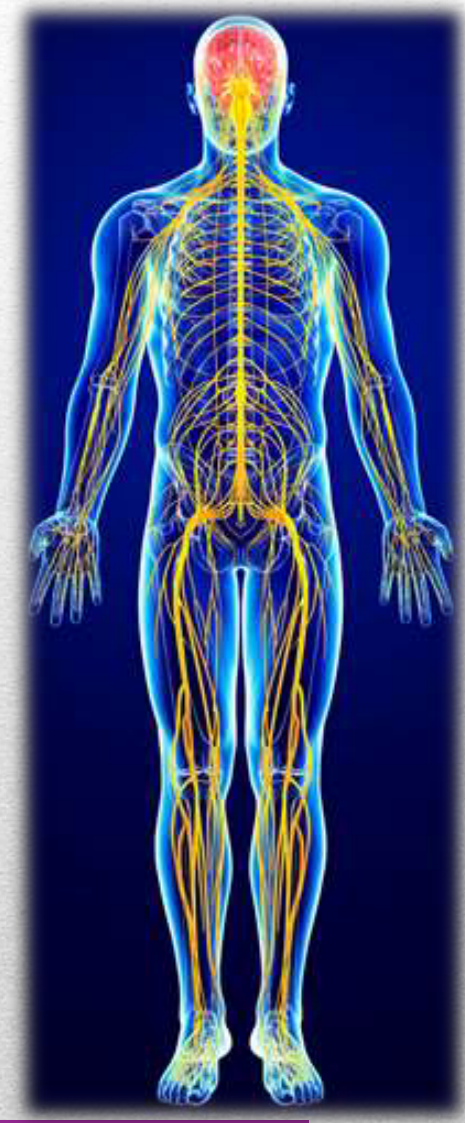
Nervous System

- The nervous system consists of the brain, spinal **cord, sensory organs,** and all of the nerves that connect these organs with the rest of the body.

- The brain and spinal cord form the control center known as the **central nervous system (CNS),** where information is evaluated and decisions are made.

- The sensory nerves and sense organs of the **peripheral nervous system (PNS)** send information to CNS and regulate organs functions by efferent and afferent nerves.



Autonomic Nervous System

- The autonomic nervous system conveys all the outputs from the CNS to the rest of the body, except for the motor innervation of skeletal muscle
- Compose of two neurons; **the pre-ganglionic neuron** with cell body in the CNS and the **post-ganglionic neuron** with cell body in the autonomic ganglia

- **The main processes that it regulates are:**
  - Control circulation, respiration, digestion, and body temperature
  - Contraction and relaxation of vascular and visceral smooth muscle
  - All exocrine and certain endocrine secretions
  - The heartbeat
  - Energy metabolism, particularly in liver and skeletal muscle
- **Three main parts**
  - sympathetic
  - parasympathetic
  - The enteric nervous system

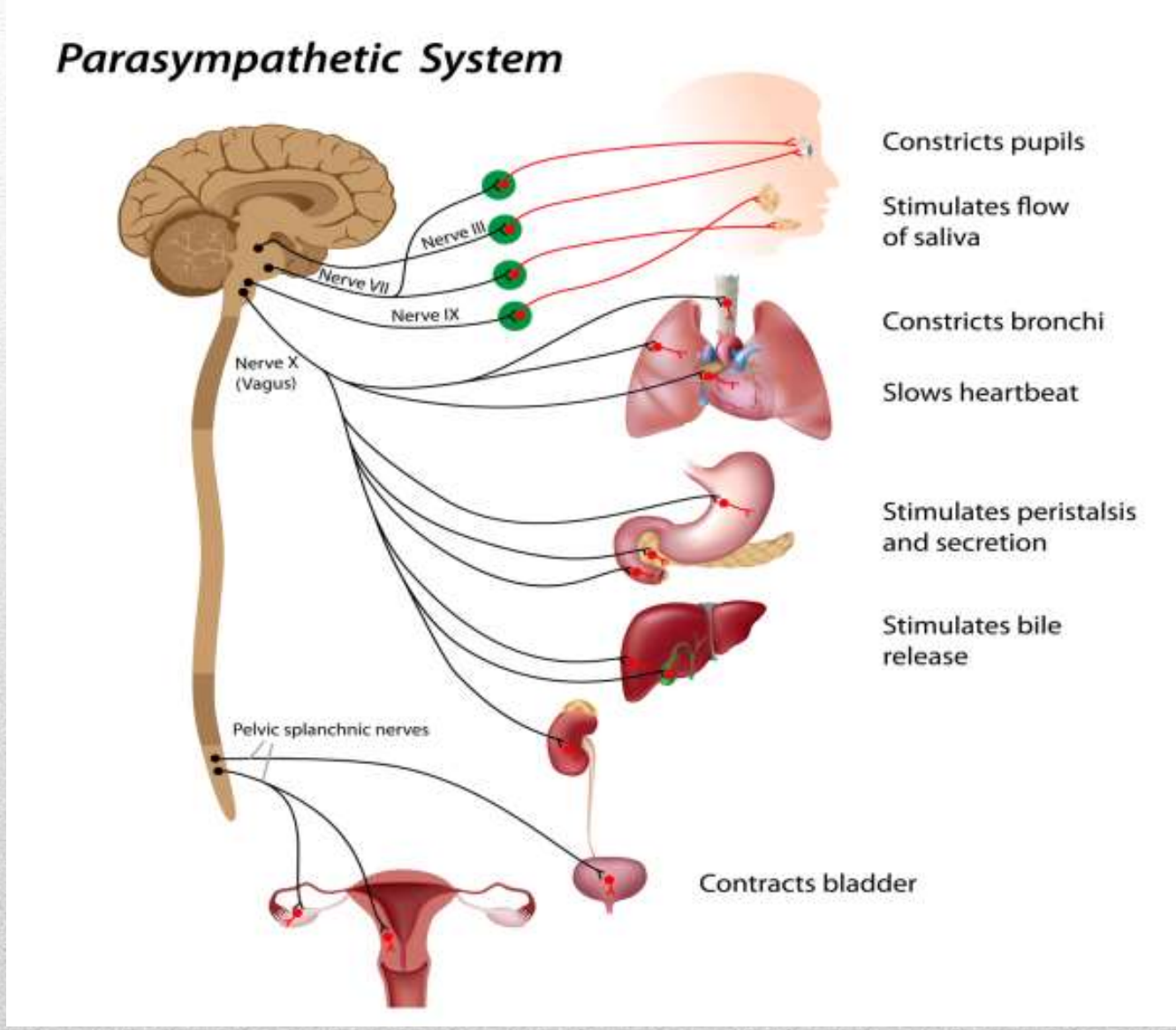
Autonomic Nervous System

- The Parasympathomimetic nervous system innervates a large number of organs.
- The neurotransmitter acetylcholine (**Ach**) mediates the transmission of impulses from the preganglionic neurons to postganglionic neurons as well as the transmission of impulses from postganglionic nerve terminals to the effector organs.
- The action of Ach at the effector organ can be mimicked by drugs like **Carbachol, methacholine, or muscarine.** The sites at which Ach and the Parasympathomimetics act are called Muscarinic receptors (M receptors) and they are sensitive to block by **atropine.**

Parasympathetic N.S.

- Ach is the only neurotransmitte r of this system that can act on two types of receptors:

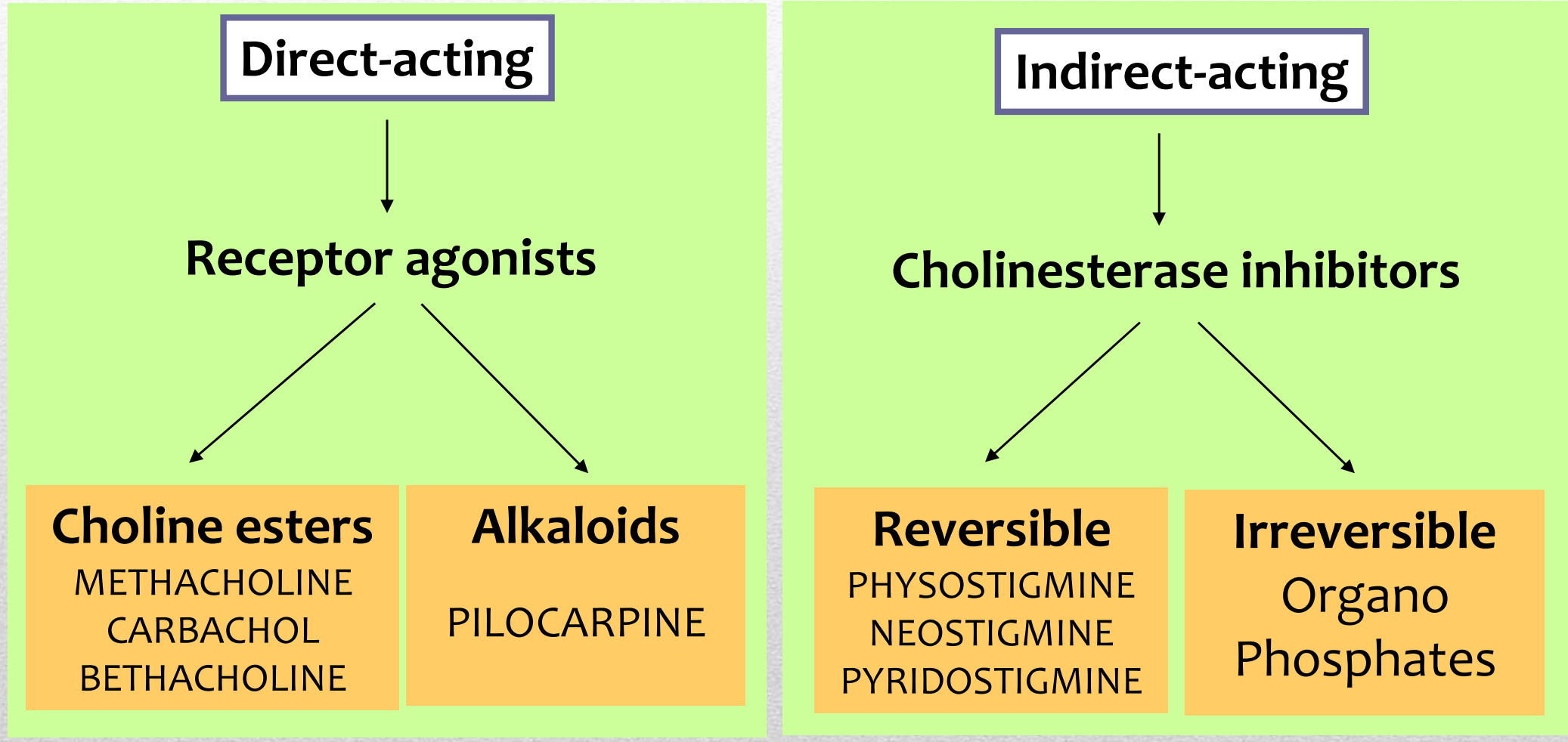
- **Nicotinic R.**
- **Muscarinic R.**



Muscarinic Receptors

- **G. protein coupled receptors**
- **Five types of these receptors (M1-M5):**
- **M1 receptors (neural): C.N.S**., gastric parietal cells, they mediate an **excitatory effect** result an increase of acid secretion
- **M2 receptors (cardiac);** present in the heart, they exert an **inhibitory effects** causing **bradycardia**.
- **M3 receptors (glandular);** present in the smooth muscle of **G.I.T**., bronchial, bladder and exocrine glands; like sweat gland, salivary and lacrimal gland. They mediate **excitatory effect** which increases the glandular secretion and contraction of visceral smooth muscle
- **M4 and M5 receptors**; present in C.N.S. but their functions are not fully identified.

parasympathomimetic Drugs



Antimuscarinic agents

- Block **M.R.** cause an inhibition of all M. function
- Have little or no action at skeletal **N.M.J.** or autonomic ganglia, they do not block **N.R** .
- Reverse excessive cholinergic effects (**reverse DUMBBELLS**)

(DUMBBELLS)

D – Defecation (diarrhea)
U - Urination (peeing)
M - Miosis (constriction of the pupil of the eye)
B - Bradycardia (slow heart beat)
B - Bronchospasm (difficulty breathing)
E – Emesis (excite skeletal muscle and CNS, vomiting)
L - Lacrimation (tearing)
L - Lethargy (fatigue)
S - Salivation (excessive drooling)

Antimuscarinic agents

- **Atropine (Hyoscyamine):**
  - Orally absorbed , cross **BBB**
  - Competitive antagonist to Ach at all muscarinic receptors

- **Scopolamine**: anti-motion sickness
- **Pirenzepine**: block **M.R.** of the gastric parietal glands; hence it is preferred in the treatment of gastric ulcer
- **Ipratropine**: useful in the treatment of asthma to decrease secretion & dilate obstructed air ways.

Principle

- Ach and parasympathomimetics stimulates the secretion of different glands in the body, like mucous, sweat, salivary and tear glands as well as secretory activity of the stomach, intestine and pancreas.

- In the rat, there is a special horseshoe-shaped gland is located within the bony orbit called **Harderian gland**.

- This gland Involves muscarinic receptors.

Aim of the experiment

1. This exp. was designed to show the stimulatory effect of cholinergic drug on glandular secretion

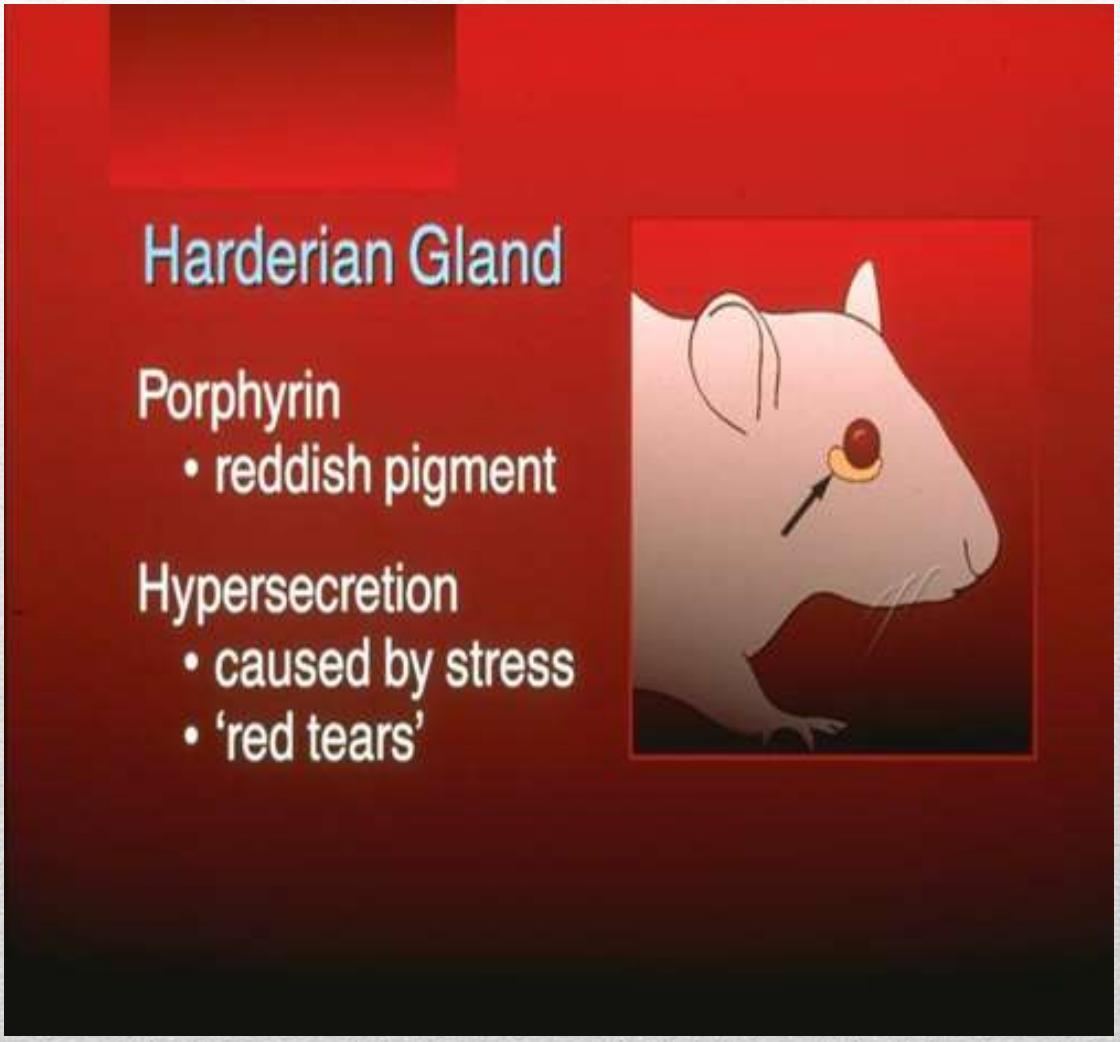
2. To prove that these glands are parasympathetically innervated and contain a muscarinic type of receptors.

Effect of Parasympathomimetic Drugs on Glandular Secretions in Rats

The Harderian gland

- The Harderian gland (or Harder's lacrimal gland) is an exocrine gland, horseshoe-shaped located within the bony orbit.

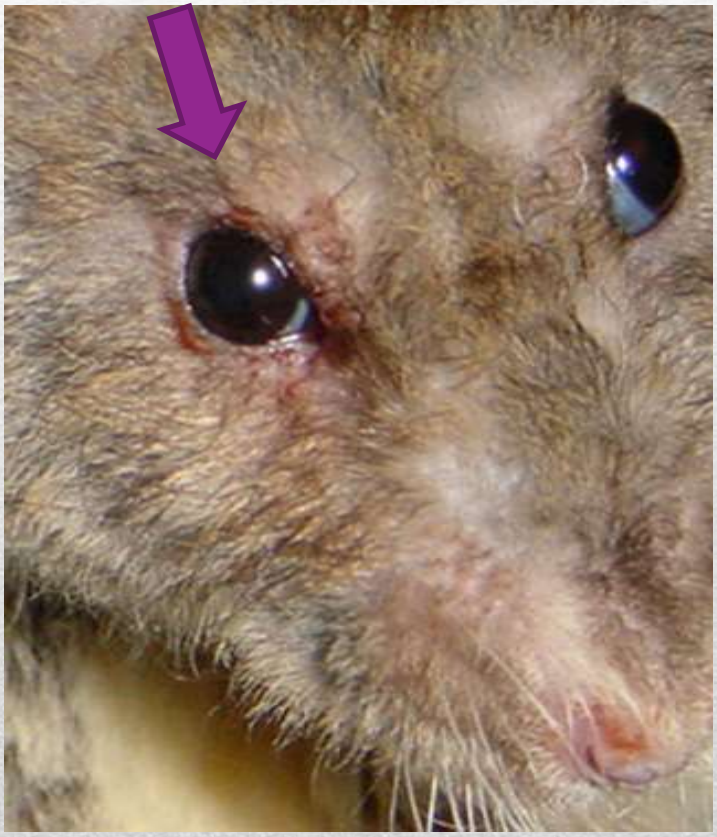
- Secretions from this gland include the **reddish pigment porphyrin**.



The Harderian gland

- **Harderian** secretions coat the eye, and drain down into the nose through the nasolacrimal duct. A rat may smear the secretions around his nose.
- Involves muscarinic receptors
- Hypersecretion in stressed rats is often referred to as "red tears" (chromodacryorrhea)
- **Rats overproduce porphyrin when they are:**
  - Stressed, ill, or poorly fed
  - Water deprivation
  - Joint pain , morphine withdrawal
  - Acetylcholine injections
- After injection with acetylcholine, **for example,** profuse amounts of porphyrin were secreted almost immediately and overflowed the eye to stain the eyelids within minutes.

The Harderian gland secretion



The Harderian gland

- Cholinergic agonists such as neostigmine, carbachol, and pilocarpine caused chromodacryorrhea.
- Nicotine does not !!
- Atropine blockes chromodacryorrhea induced by systemic administration of direct-acting cholinergic agonists !!
- Thus, chromodacryorrhea appears to be a muscarinic receptor-related event.

Experimental protocol

Procedure:

1. Inject 100 mg/kg of Pilocarpine I.P into a rat.

2. Examine the eyes for tears by wiping the eyelids with cotton to detect the bloody tears. Note salivation and nasal secretion…..etc.

3. Inject another rat with 1 mg/kg of atropine I.P, wait about 15-25 minutes, and inject the rat with Pilocarpine 100 mg/kg I.P.

4. Examine for bloody tears, salivation and nasal secretion….etc

Results

Parameters	Pilocarpine	Atropine+ Pilocarpine	Effectors organ/gland	Receptor
Bloody tears				
Salivation				
Urination				
Defecation				
Tremor				
Sk.M. contraction				

LAB 2: BARBITURATES

Assist. Lecturer:

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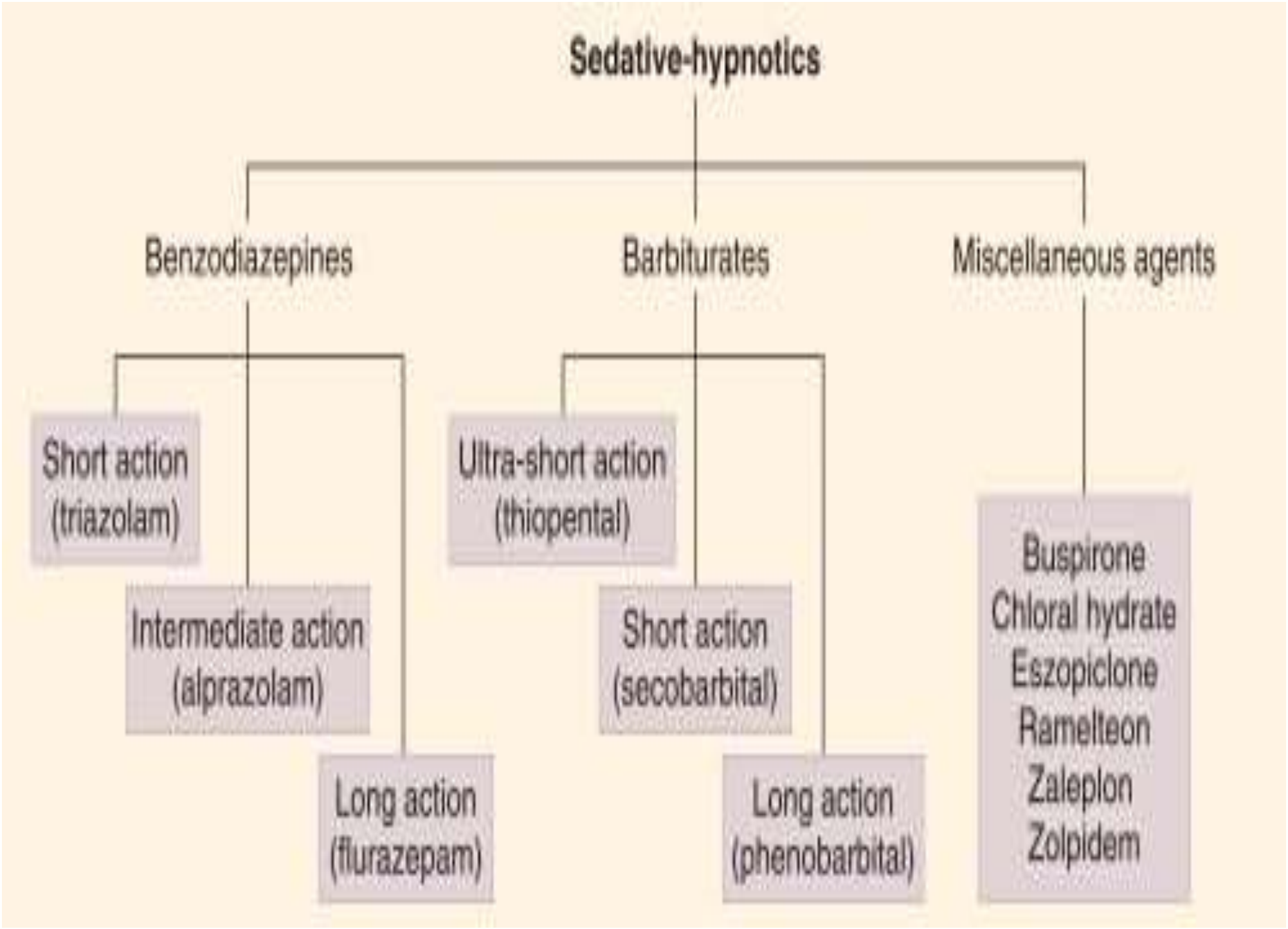
Sedative-Hypnotic Drugs

- **The sedative-hypnotics** belong to a chemically heterogeneous class of drugs, almost all of which produce dose-dependent CNS depressant effects.
- **Sedative** is a drug that reduce excitement & calm the person
- **Hypnotic** is a drug that produces sleep-resembling normal sleep
- **A major subgroup** is the **Benzodiazepines**, other including :

Barbiturates and **miscellaneous agents**


Sedative-hypnotics
Benzodiazepines
Barbiturates
Miscellaneous agents
Short action (triazolam)
Intermediate action (alprazolam)
Long action (flurazepam)
Ultra-short action (thiopental)
Short action (secobarbital)
Long action (phenobarbital)
Buspirone
Chloral hydrate
Eszopiclone
Ramelteon
Zaleplon
Zolpidem

Principle

- **Sedation**: Reduction of anxiety
- **Addiction**: the state of response to a drug whereby the drug taker feels compelled to use the drug and suffers anxiety when separated from it
- **Anesthesia** : Loss of consciousness associated with absence of response to pain
- **Anxiolytic** : A drug that reduces anxiety, a sedative agent

Terms to Learn

- **Dependence**: the state of response to a drug whereby removal of the drug evokes unpleasant, possibly life-threatening symptoms, often the opposite of the drug's effects.

- **REM sleep**: phase of sleep associated with rapid eye movements; most dreaming takes place during REM sleep.

- **Tolerance**: reduction in drug effect requiring an increase in dosage to maintain the same response

Types of Neurotransmitter

- **Excitatory N.T :**

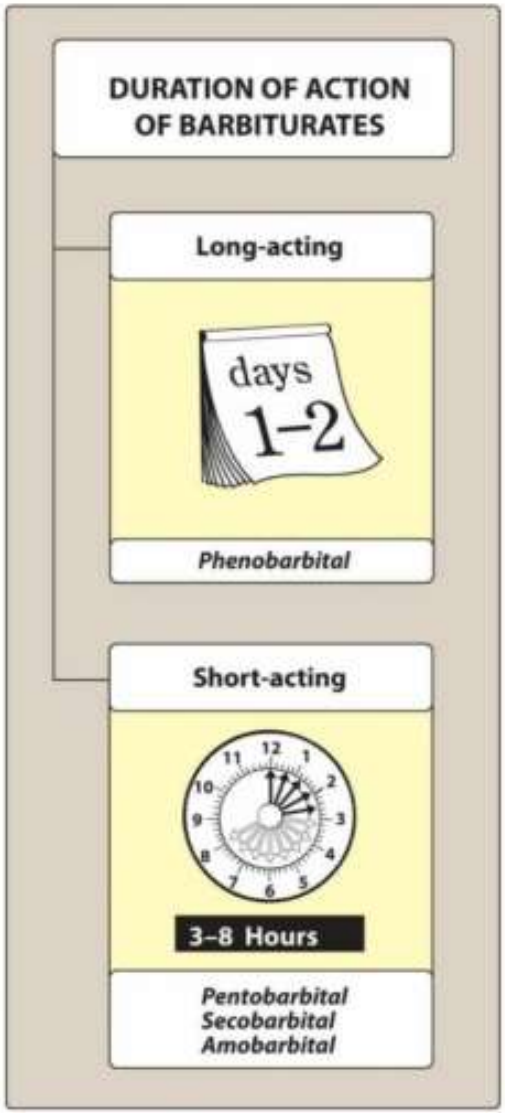
1. **Acetylcholine**
2. **Glutamate**

- **Inhibitory N.T :**

1. **GABA**
2. **Glycine**

Barbiturates

- **Phenobarbital: long acting**
- **Secobarbital: short action**
- **Thiopental: ultra short acting**



Mechanism of Action

- **Barbiturates** depress neuronal activity in the midbrain reticular formation, facilitating and prolonging the inhibitory effects of GABA receptor.
- **Barbiturates** also bind to multiple isoforms of the GABA A receptor
- **Barbiturates** increase the duration of GABA-mediated chloride (influx of Cl ion) channel opening (hyperpolarization)
- **Barbiturates** may also block the excitatory transmitter glutamic acid, and at high concentration, sodium channels.

Barbiturates + GABA receptor

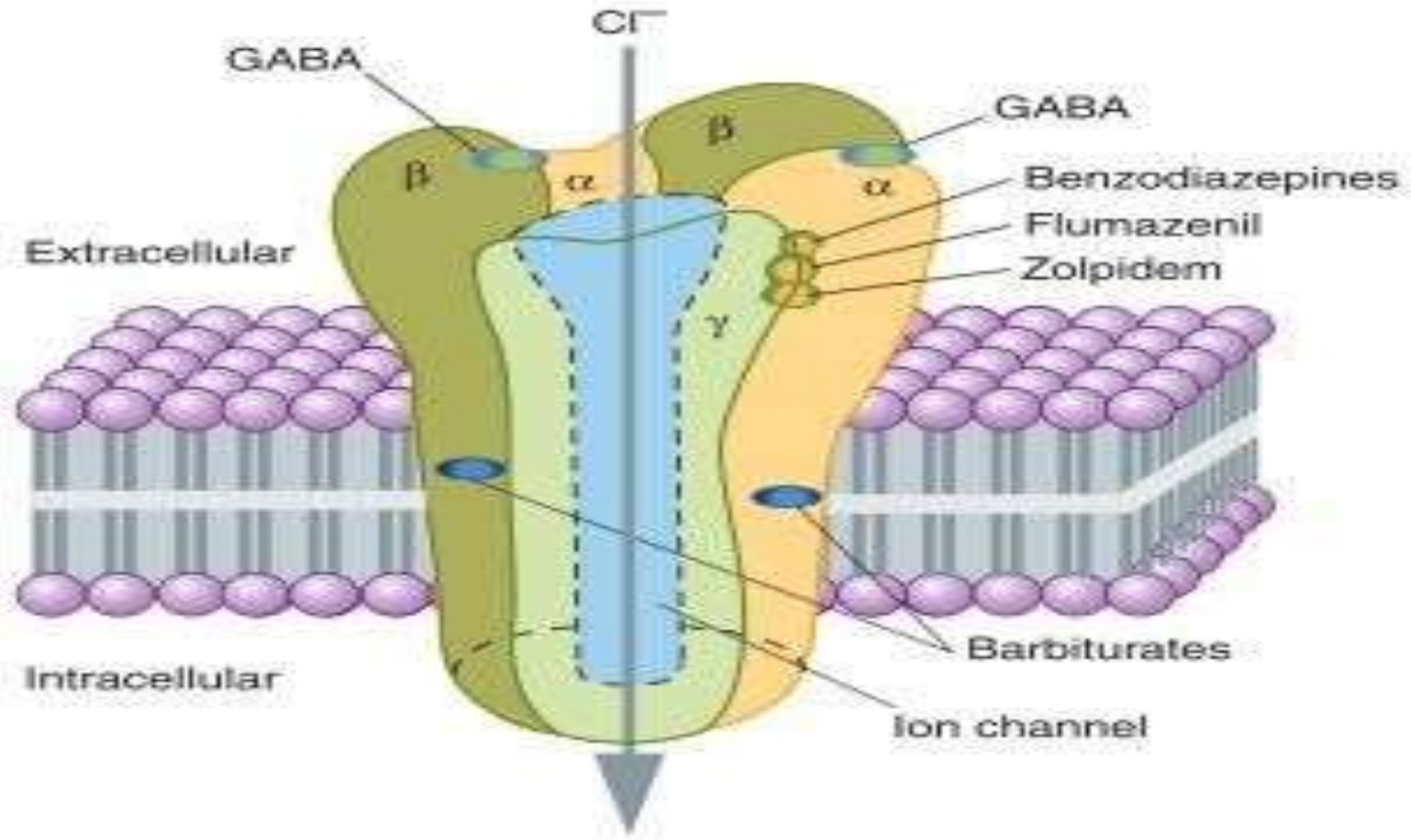
Activation of GABA receptor

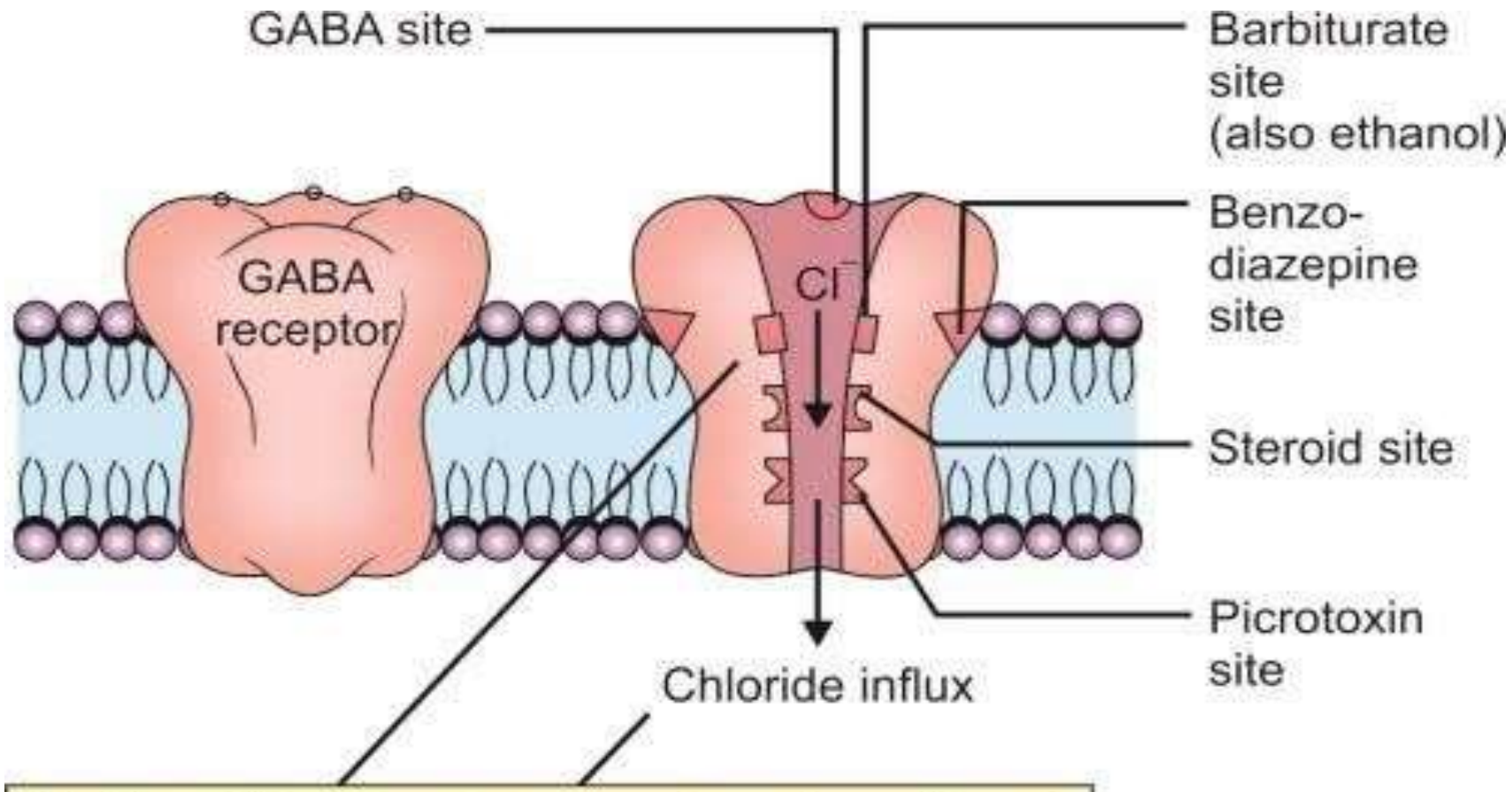
Opening of Chloride channel
Increase the duration of GABA gated channel opening

Hyperpolarization of cells

CNS depression

GABA - receptor



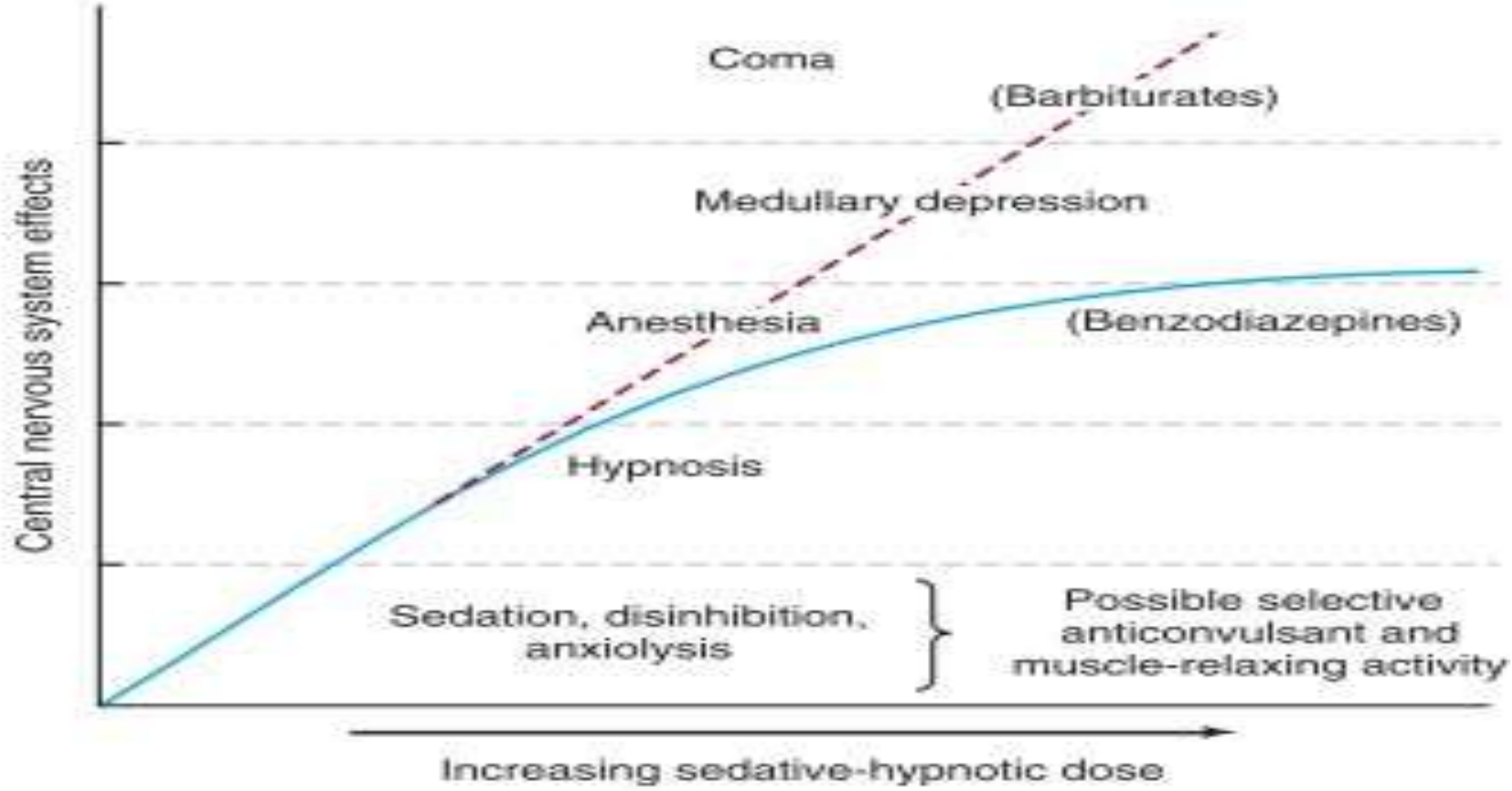


Binding of barbiturate, benzodiazepine, ethanol, steroid or picrotoxin to their allosteric sites on GABA receptor, modulates the effect of GABA binding to its site on the receptors; that is it enhances the hyperpolarizing chloride currents

Clinical Applications

- Anesthesia (**thiopental**)
- Insomnia and sedation (**secobarbital**)
- Seizure disorders (**phenobarbital**)

CNS depressant



Pharmacokinetics

- Most sedative-hypnotic drugs are lipid-soluble and are absorbed well from the gastrointestinal tract, with good distribution to the brain.

- The CNS effects of thiopental are terminated by rapid redistribution of the drug from brain to other highly perfused tissues.

- Renal excretion

Adverse effects

- Drowsiness, severe respiratory & cardiovascular depression.
- Tolerance
- Dependence liability> benzodiazepine
- Enzyme induction may lead to multiple drug interactions
- Withdrawal symptoms is much more sever than opioid and can results in death (no antidote).
- C.I. in pregnancy

Methods

- **1-** Check the weight of two mice / rats.

- **2-** Inject one animal with normal saline I.P and mark it as control, and another animal with Phenobarbital I.P or S.C in a dose of 50 mg/kg.

- **3-** Inject one animal with normal saline I.P and mark it as control, and another animal with Thiopental I.P or S.C in a dose of 30 mg/kg.

- **4-** Record the observations and time of occurrence in a table form.

Evaluation of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) (Lab 3)

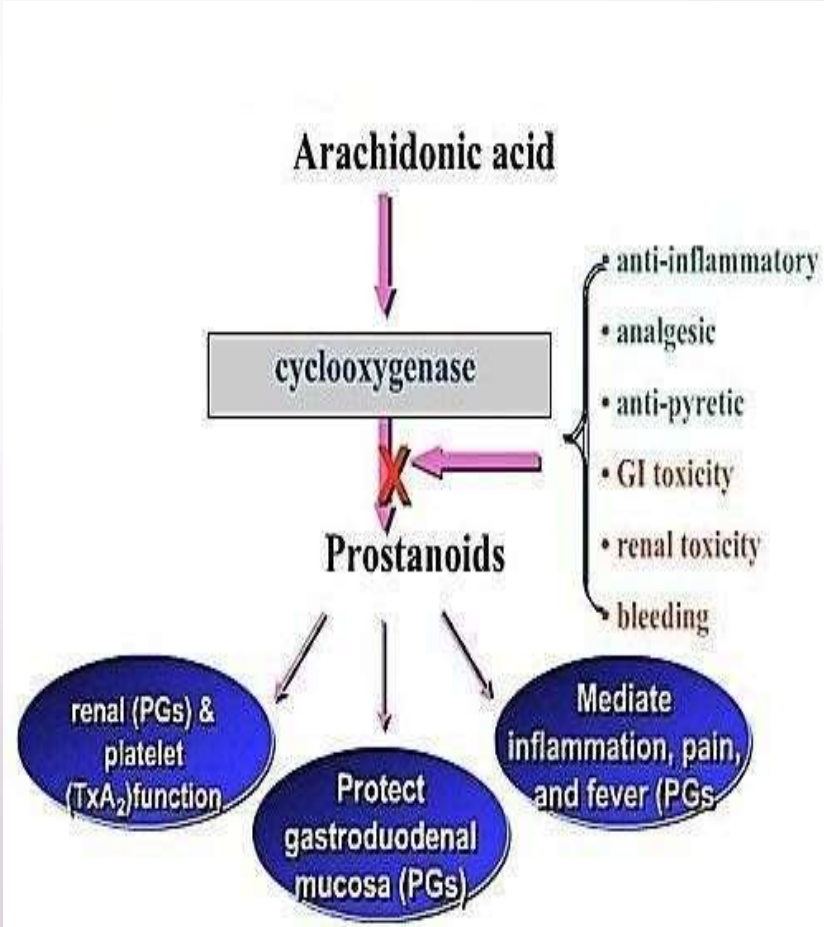
Asst. Lec. Nibras Hasaballah Jasem.

University of Tikrit \ College of Pharmacy
Department of Pharmacology & Toxicology

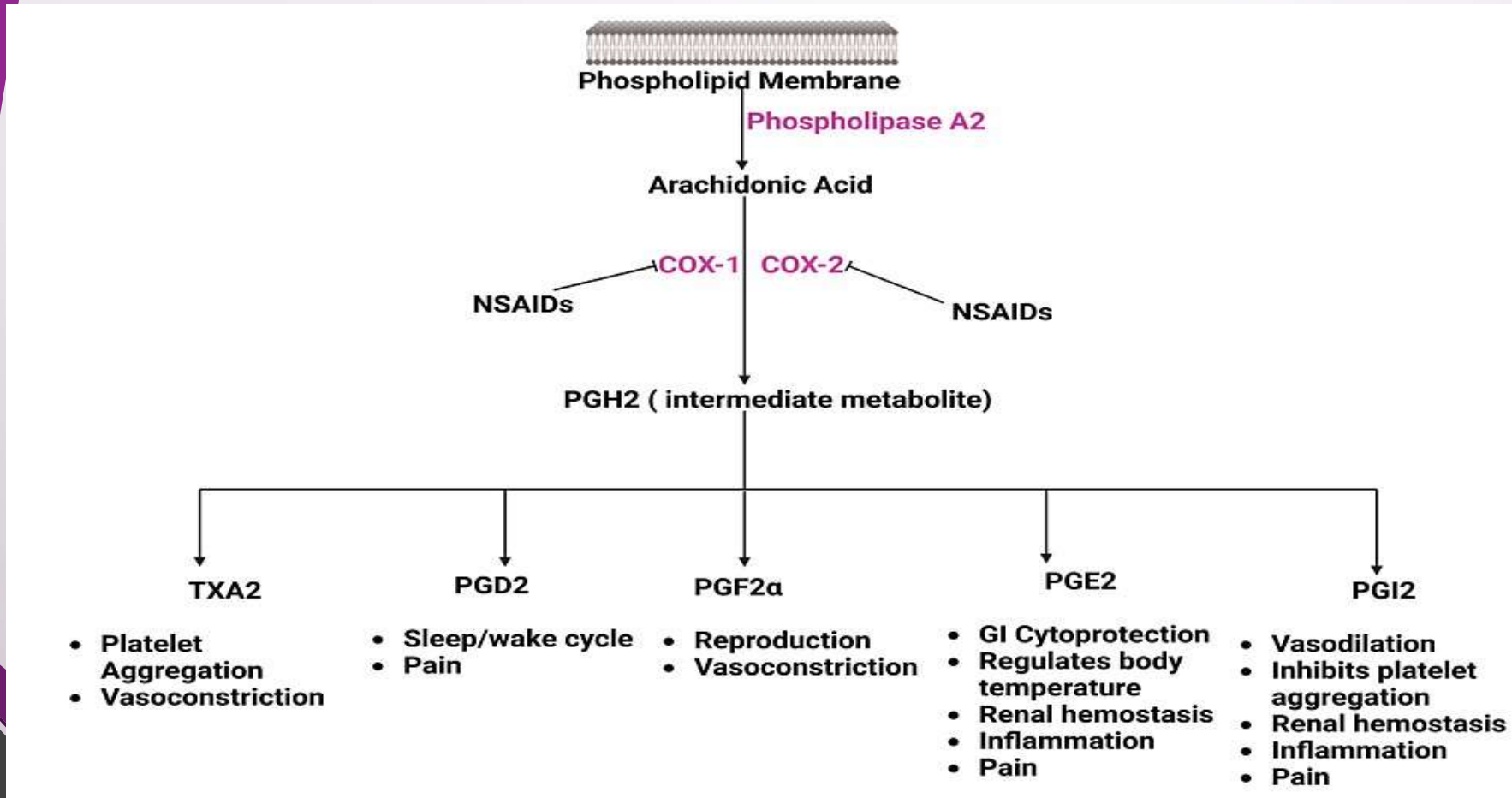
Nonsteroidal Anti-Inflammatory Drugs (NSAIDs):

•The **NSAIDs** are a group of drugs that differ in their **antipyretic**, **analgesic**, & **anti- inflammatory activities**.

•They act primarily by inhibiting the COX enzymes that catalyze the first step in prostanoid biosynthesis. This leads to decreased **prostaglandin** synthesis with both beneficial & unwanted effects.



Synthesis of prostaglandins



Clinical uses of NSAIDs

NSAIDs are used to relieve pain & reduce signs of inflammation.

NSAIDs are a common treatment for chronic health problems such as rheumatoid arthritis & osteoarthritis.

General Adverse Effects of NSAIDs:

•Dyspepsia, nausea & vomiting. Gastric damage may occur in chronic users, with risk of hemorrhage.

• Skin reactions.

•Reversible renal insufficiency seen mainly in individuals with compromised renal function.

•All NSAIDs (except COX-2 inhibitors) prevent platelet aggregation & therefore may prolong bleeding.

In vivo analgesic evaluation techniques:

❖ **Principle:**

Pain is induced in a suitable animal & the response of the animal to the painful stimuli is recorded with or without administration of the analgesic agent.

❖ **Classification of methods:**

1. Methods for central analgesic activity:

- Hot plate method
- Tail immersion method
- Tail clip method

2. Method for peripheral analgesic activity:

- Writhing method
- Formalin test in rats

Writhing method:

•The painful stimulus is induced by IP injection of an irritant substance (acetic acid)

•The animals create a characteristics stretching behavior, which is called writhing.

(writhing is constriction of abdomen, turning of trunk (twist) & extension of hind legs).

•The number of writhes for each animal is counted during certain time period (during 30 minutes), beginning 5 minutes after injection of acetic acid.

Experimental protocol:

Control group

•The control group is given acetic acid IP (10 ml/kg) & after 5 minutes the number of writhes is recorded for each animal during 20 minutes.

• The number of writhes is recorded

Treated group

•Treated animals are administered the drug (diclofenac sodium at dose 10 mg/kg) IP, 5 minutes prior to acetic acid administration. Then acetic acid is given IP.

•5 minutes are allowed to elapse, the mice are then observed for a period of 20 minutes & the number of writhes is recorded.

•If the drug possesses analgesic activity, the animal that received the drug will give lower number of writhes than the control, i.e. the drug having analgesic activity that inhibits writhing.

• **Calculate % inhibition:**

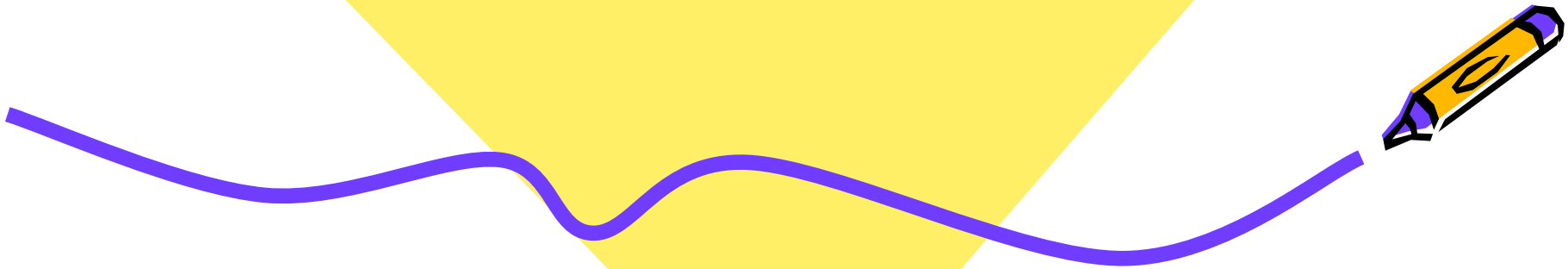
% inhibition = [No. of writhing in control group - No. of writhing in treated group] / No. of writhing in control group] × 100

Writhing test		
Group	**No. of writhing**	**% inhibition**
Control	**40**	**0**
Group I: Drug A	**20**	**50%**
Group II: Drug B	**30**	**25%**

THANK YOU FOR YOUR ATTENTION

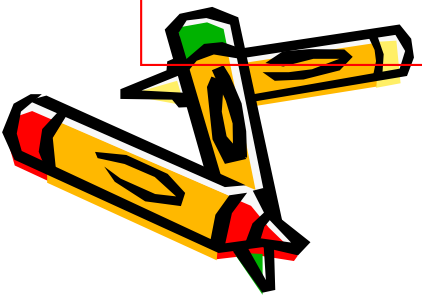
Acute toxicity study

Determination of LD50



HISTORICAL BACKGROUND

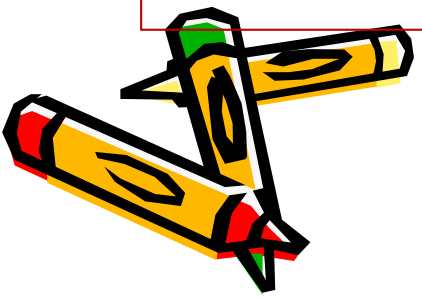
- Originally developed in 1927 by J.W. Trevan the LD50 test was used to determine the potency of **digitalis extracts**, **insulin**, and **diphtheria antitoxin**. Scientists soon developed other methods for determining potency but the LD50 catch on as a "scientific" measure of toxicity..??. The ease of performing an LD50, as well as the urgent need for getting concrete numbers quickly, has made results of the test a standard in toxicology studies. Governments also liked the numerical results that the LD50's provided and quickly mandated (authorized) the test for assessing the toxic effects of products ranging from drugs , pesticides to industrial solvents.



Definition

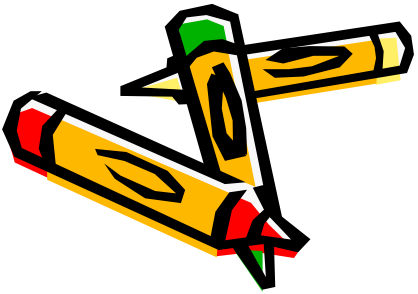
LD 50: IS STAND FOR MEDIAN LETHAL DOSE

- An LD50: represents the individual dose of drug (**Xenobiotic**) **usually per kg (or gm for small animals) of body weight** required to kill **50 percent** of a population of test animals. It is an index determination of drugs and poison's virulence. The lower(1mg/kg) the LD50 dose, the more toxic the drug (xenobiotic).



Toxicological Significance

- *It is the most common test of acute toxicity assessment ;* Before a product on new drug formulation is released to the market, its potential safety to the users is evaluated by a series of biological tests on lab. animals. Each animals given a single dose of the tested materials. usually three or more dose levels are tested.



❑ **Related terms**

Median effective dose or ED50: This is the dose (mg/kg), which produces a desired response in 50 per cent of test population.

Therapeutic index: It is an approximate assessment of the safety of the drug. It is the ratio of the median lethal dose and the median effective dose. Also called as therapeutic window or safety.

Therapeutic index (T. I) = LD50/ED50

The larger the therapeutic index, the safer is the drug. Penicillin has a very high therapeutic index, while it is much smaller for the digitalis preparation

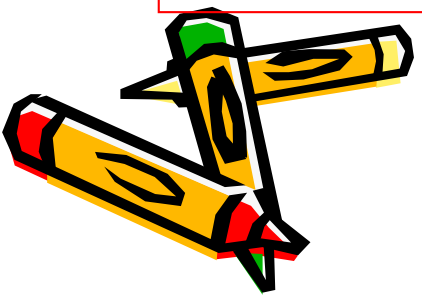
The types of exposures are

1-**Acute**, which is exposure to a chemical for **24 hours or less**.

2-**Chronic**, which is exposure to a chemical for **more than 3 months**.

3-**Sub-acute**, which is exposure to a chemical for **1 month or less**.

4-**Sub-chronic**, which is exposure to a chemical **between 1 to 3 months**.

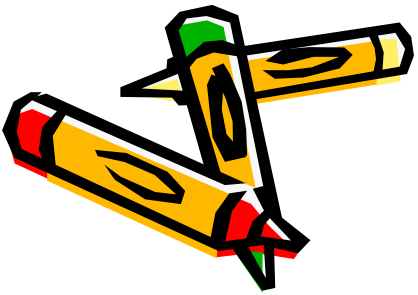


Differences between acute and chronic toxicity

- Acute toxicity is characterized by:
- Sudden, sever exposure, rapid absorptions of toxicant.
- Usually involve single large dose exposure.
- It is reversible.
- Interfere with essential physiological processes.

Chronic toxicity

- Prolong exposure lasting overs days, months , years.
- Symptoms may not be immediately apparent.
- Often irreversible.
- Affect on chains of biochemical events.



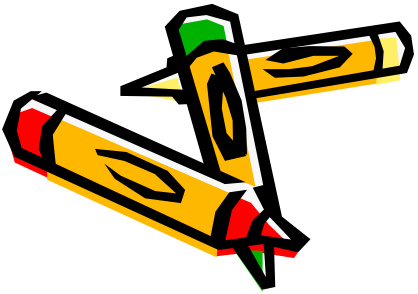
Objective (aim)

To determine the LD50 of a drug (Neostigmine) in a given species (mouse).

Experimental method

- **Thompson and Weil Method:**

There are many methods in determining LD50 such as Bliss, sequential method, grouping method ect. The method used in this experiment is the one described by Thompson and Weil.



Experimental animals

- mice

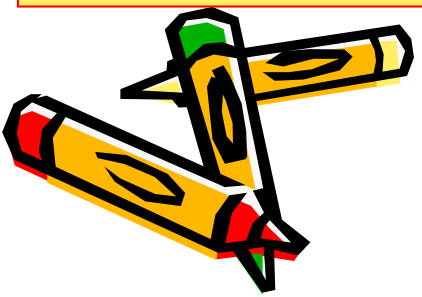
➢ Display the videos concerning mouse restraint technique ,then the i.p injection in mouse.

Toxicant (Drug): Physostigmine

- **Physostigmine**, is an alkaloid (tertiary amine) isolated from seeds of the plant Physostigma venenosum from West Africa. Physostigmine is used in ophthalmology, in eye drops (in form of physostigmine sulfate), in combination with pilocarpine, for the lowering of intraocular pressure, in glaucoma.
- Physostigmine is also used as an antidote at the poisoning by compounds with anticholinergic effect (e.g. atropine, scopolamine and imipramine) and tricyclic anti-depressants, as well as at the poisoning with anti-cholinergic organophosphates

- **Pharmacological mechanisms**

➢ Physostigmine is used as antidote in organophospate poisoning (e.g. poisoning with parathion, malathion and dichlorvos) because it binds to the enzyme acetylcholinesterase (AChE) reversibly and preserves the enzyme from irreversible phosphorylation by organophosphates. Physostigmine serves as alternate substrate for AChE.



Mechanism of intoxication

- Acetylcholine (ACh) plays an important role as a neurotransmitter in the CNS and in the parasympathetic nervous system (PNS). At the high concentrations of ACh, neuromuscular transmission may be blocked and the adverse effects can occur.

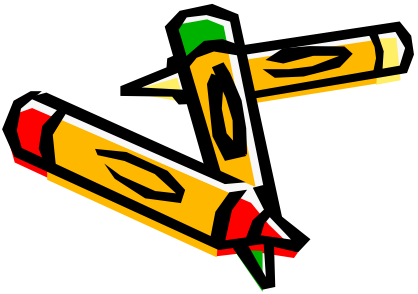
-In similarity with other anti-cholinergic agents, physostigmine is an inhibitor of the enzyme AChE, which catalyzes hydrolysis of ACh into choline and acetic acid.

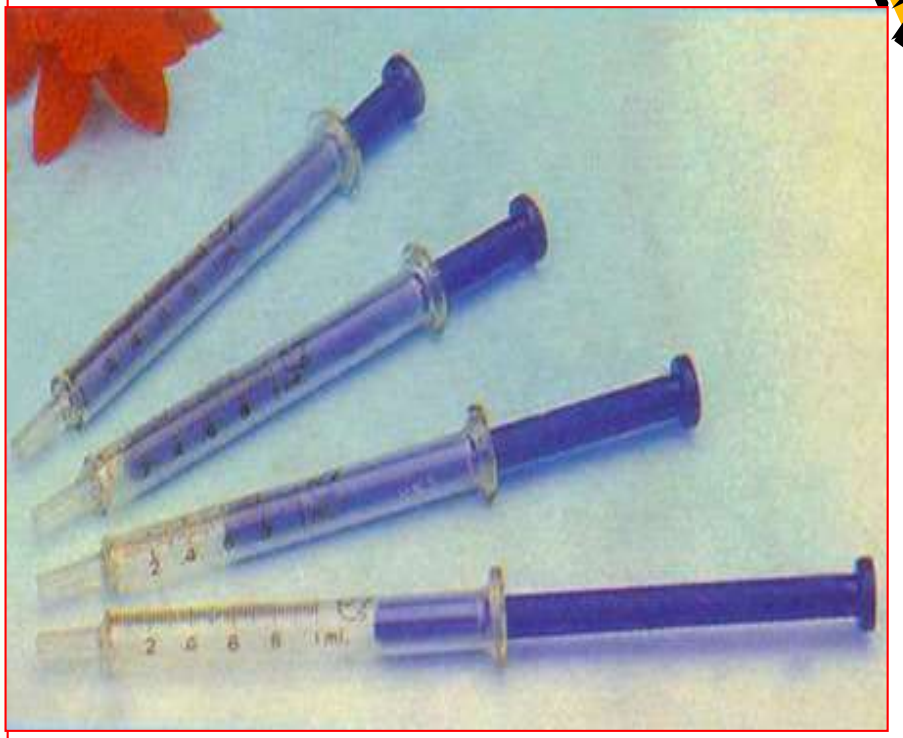
- The following acute toxic effects can be produced:
- a) stimulation of muscarinic receptor responses at autonomic organs; muscarinic effects include nausea, vomiting, abdominal pain, diarrhea, increased salivation, perspiration and tearing, blurred vision (miosis), respiratory tract secretions, bradycardia and atrio-ventricular block;
- b) nicotinic receptor stimulation, followed by muscle twitching, weakness and paralysis;
- c) stimulation of cholinergic receptor sites in the CNS, following in severe cases by CNS depression, convulsions, coma, and death.

Target organs: CNS and PNS.

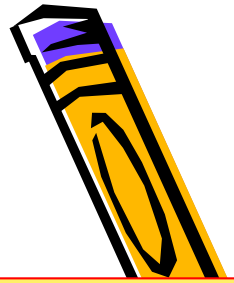
Experimental apparatus

- mice cage, animal's equi-armbalance, 1ml injection syringe, and electronic calculator





Experimental procedure & calculations



the method used in this experiment is the one described by Thompson and Weil. It involves the use of moving averages, and is calculated by the use of logarithms and antilog to the following formula:

Log M = Log Da + d (*f+1*) , K= 3& n= 6

Where*

M= estimated LD50

D= dosage

Da= lowest dose level

d= Log of constant ratio between the doses (i.e. log of geometric factor)

f= the function of (r) used in the calculation of an LD50.See the table listed in for calculation of LD50.

n= number of animals used for each dose level.

K= number of doses -1

r- Vales: the number of death for each dose levels.

6*f* = the function for the LD50,this is used in calculation the confidence limits.

Log confidence limits (95%)= Log M$\pm$2d (6*f*).

Confidence limits (95%) = it is the tang which when the experiment, is repeated 100 times, 95% time out of 100, the LD50 value will be within this range.

- Weils table for calculation of LD50

Where n= 6 k=3

r-values	*f*	*6f*	r-values	*f*	*6f*
0, 0, 3, 6	1.000 000	0.22361	1, 1, 4, 6	0.060000	0.3226
0, 0, 4, 6	0.83333	0.21082	1, 1, 5, 6	0.400000	0.30724
0, 0, 5, 6	0.66666	0.16667	1, 2, 5, 6	0.20000	0.36000
0, 0, 6, 6	0.50000	0.0000	1, 2, 6, 6	0.0000	0.26833
0, 1, 2, 6	1.00000	0.26874	1, 3, 3, 6	0.40000	0.39799
0, 1, 3, 6	0.83333	0.27889	1, 3, 4, 6	0.20000	0.42000
0, 1, 4, 6	0.66667	0.26874	1, 1, 4, 6	0.0000	0.36878
0, 3, 6, 6	*0.00000*	0.22361	2, 0, 4, 6	*0.75000*	*0.32566*
1, 1, 2, 6	*1.00000*	*0.32249*	2, 0, 5, 6	*0.50000*	*0.29580*
1, 1, 3, 6	*0.80000*	*0.33706*	2, 0, 6, 6	*0.25000*	*0.23717*
0, 2, 4, 6	*0.50000*	*0.29814*	2, 0, 3, 6	*1.0000*	*0.33541*

Example:

Group of six rats were administered single oral dose of drug X at **four** successive dose levels; namely **1.00 mg/kg, 1.2 mg/kg,1.44 mg/kg, and 1.77 mg/kg**. Death occurring in each group were **0, 2, 4, and 6**. Calculate the LD50 and confidence limits of the product X.

Answer:

Log M = Log Da + d (*f+1*) , K= 3& n= 6

= 0.00 + Da+ 0.0792(0.50000+1)

= 0.0792(1.5000)

Log M = 0.1188

Anti log or (LD50) = 1.314 mg/kg

Log confidence limits (95%) = log M ± 2d (6*f*)

= 0.1188+ 2×0.792×0.29814

= 0.1188 + 0.047

The log of the upper limit = 0.1658

Antilog of the upper limit = 1.465

The log of the lower limit =0.1188-0.047

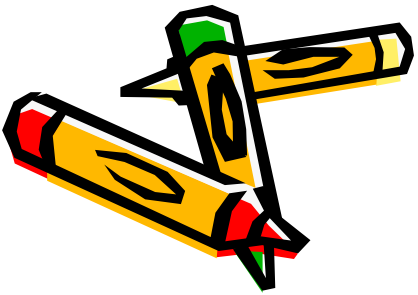
= 0.0718

Antilog of the lower limit = 1.18

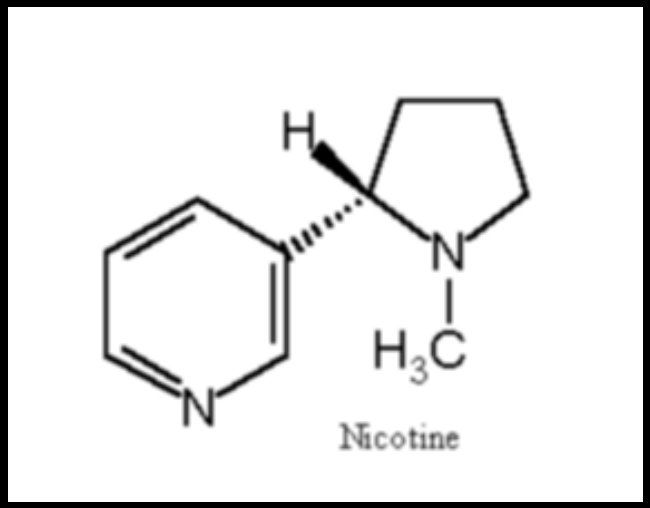
There for the 95% confidence limits are = 1.18 - 1.465 mg/kg

HOMEWORK

- Q1) Define the LETHAL DOSE50(LD50) and give example.




OK!



Nicotine

Lab: 2

NICOTINE

- Nicotine –potent poison and bitter flavor
- Nicotine is a colorless, toxic alkaloid made up of carbon, hydrogen and nitrogen.
- The primary reason why nicotine use has turned into worldwide concern is because of its ability to induce a state of euphoria in the brain of the smoker thus leading to addiction.
- Tobacco leaves contain up to 10% nicotine

Tobacco Overview

- Naturally found in leaves of *Nicotiana tabacum* are prepared in different ways, depending on the intended use of the tobacco

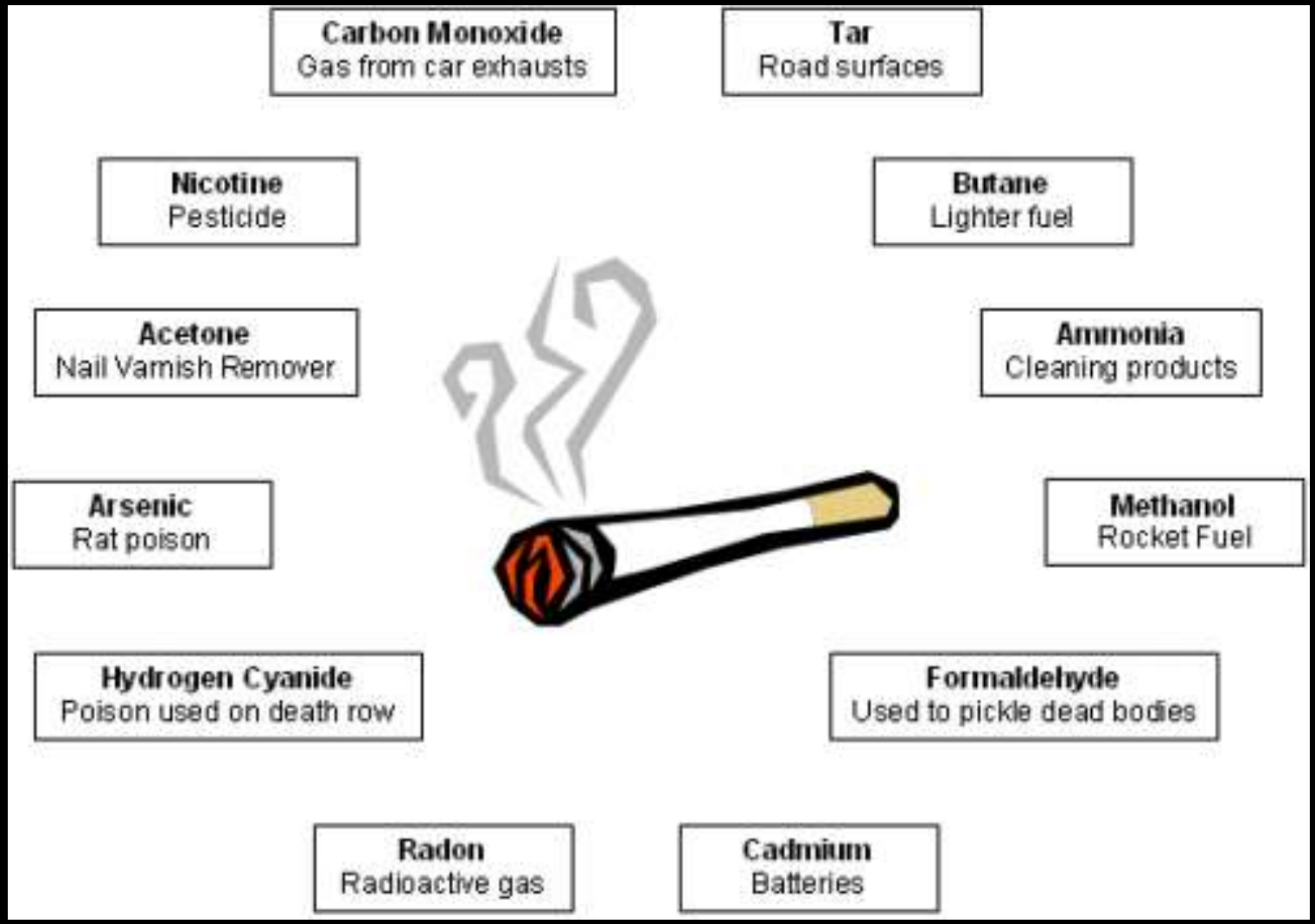
- Leaves dried, then grounded

- Indigenous to North America)

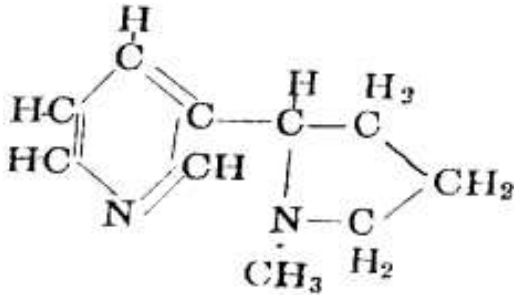
Addiction

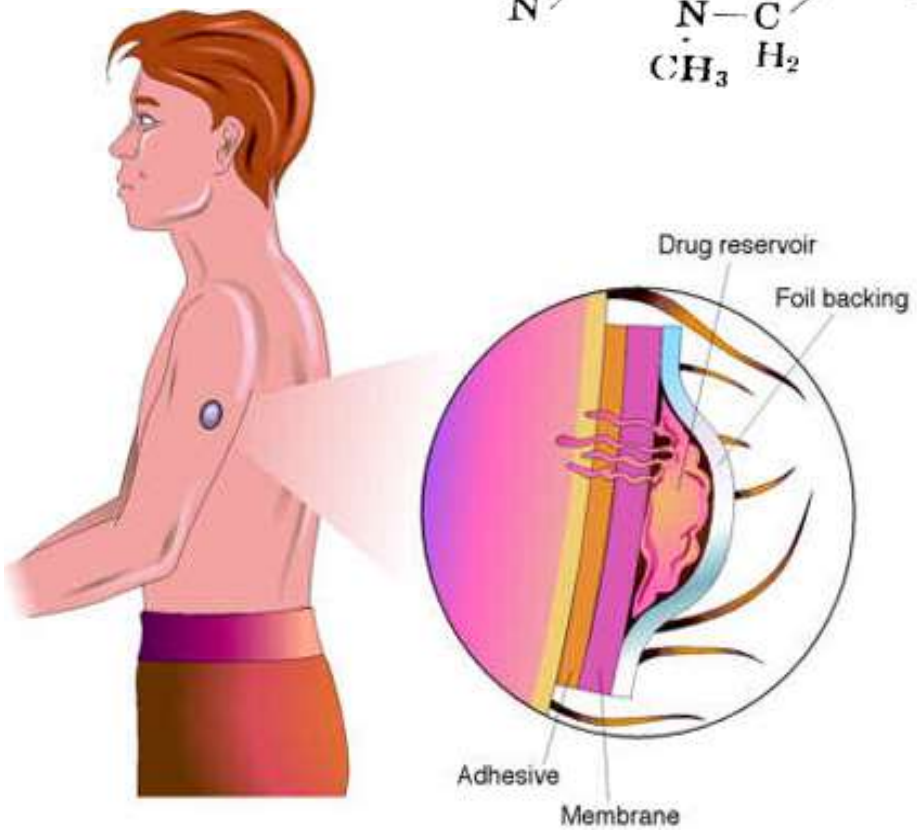
- Nicotine meets both the psychological and physiological measures of addiction
  - **Psychological** - People who are addicted to something will use it spontaneously, without regard for its negative effects on their health or their life
  - **Physiological** - anything that turns on the reward pathway in the brain is addictive. Because stimulating this neural circuitry makes you feel so good, you will continue to do it again and again to get those feelings back

Nicotine and other agents in tobacco smoke



Pharmacokinetics





- Readily absorbed from all over the body, including
  - Lungs (smoked)
  - Mucosa (cigar, chewing tobacco, gum, nasal spray)
  - Skin (patch)
  - Gastrointestinal tract (uncommon)

Pharmacokinetics

- **Absorption**
  - The most common way to get nicotine into your bloodstream is through inhalation
  - Your lungs are lined by millions of alveoli, which are the tiny air sacs where gas exchange occurs.

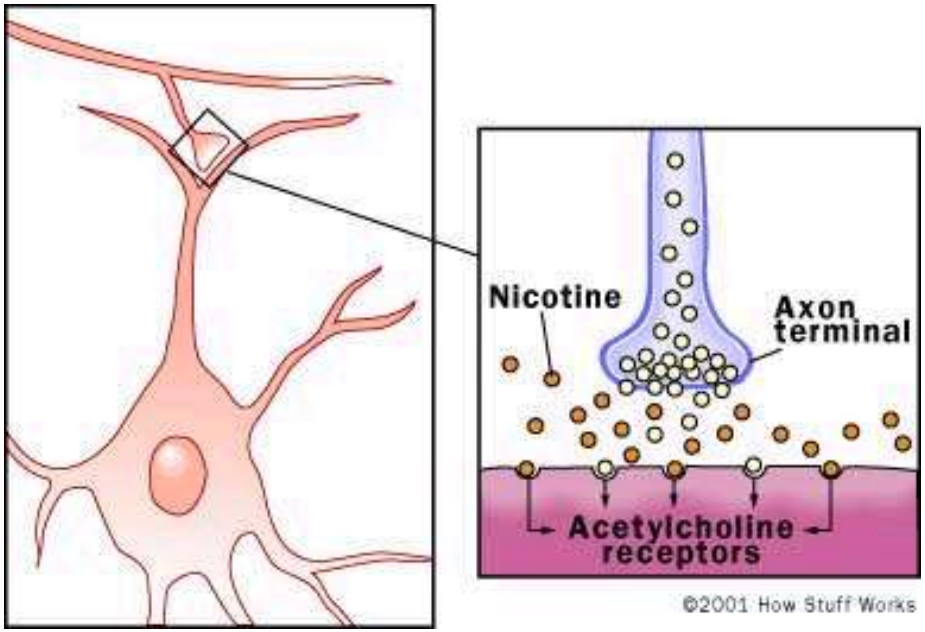
  - **Nicotine** taken in by cigarette or cigar smoking takes only 10-15 seconds to reach the brain but has a direct effect on the body for only ~30 minutes

Pharmacokinetics

- **Metabolism & Elimination**
  - About 80 percent of nicotine is broken down to cotinine by enzymes in your liver (e.g., CYP2A6)
  - Nicotine is also metabolized in your lungs to cotinine and nicotine-N-oxide
  - Cotinine and the remaining nicotine is filtered from the blood by your kidneys and excreted in the urine

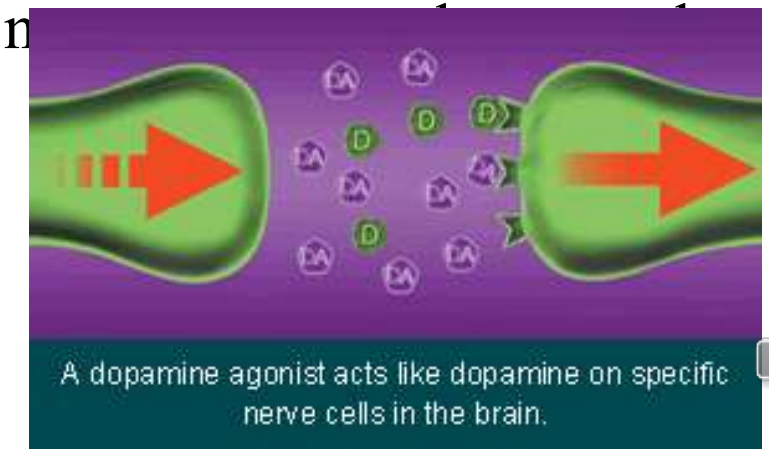
Pharmacodynamics

- **Nicotine** is a direct agonist for nicotinic ACh receptors
- **Nicotine** initially causes a rapid release of adrenaline, the "fight -or- flight" hormone

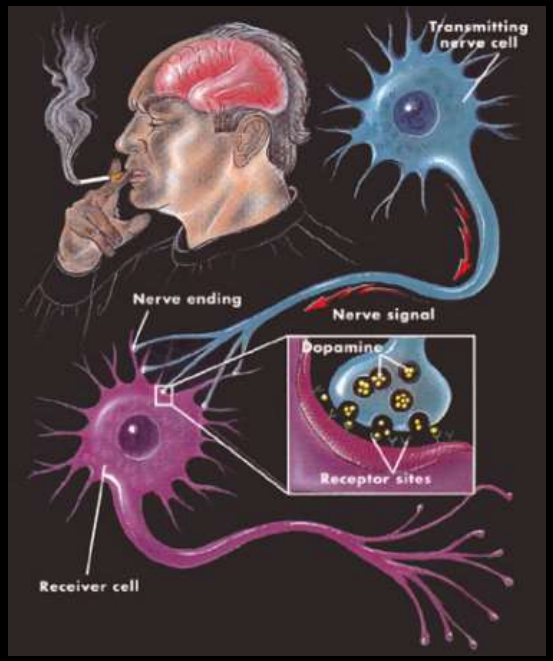


Pharmacodynamics

- **nAChRs** found in limbic system (e.g. striatum, hippocampus), midbrain (e.g. substantia nigra), various cortical areas (frontal lobe) and associated with mood enhancement, appetite suppression and anxiety reduction.
- **nAChRs** both postsynaptic and presynaptic, facilitating ACh, DA, 5-HT and Glutamate action
- Nicotine also increases release of various neurohormones
- Has powerful effects on peripheral n
  other organs



Nicotine affects nicotinic Acetylcholine receptors at the autonomic ganglia and the NMJ to produce peripheral effects



Peripheral Effects

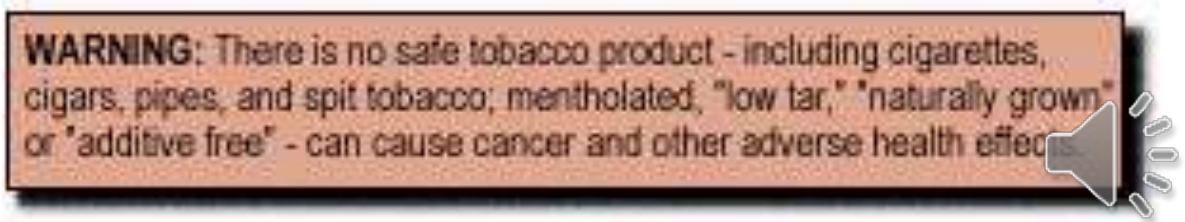
- **A sympathomimetic-** Adrenal glands release epinephrine and norepinephrine
  - Increases heart rate, blood pressure, respiration
- **A parasympathomimetic**
  - Increases smooth muscle (GI tract) activity
  - Increases HCL production in stomach/nausea
  - Chronic diarrhea, Colitis
- Body weight- appetite suppression, increased metabolic rates

Nicotine Forms

- Tobacco-Nicotiana tabacum
  - Leaf (Chewing)
  - Snuff (powdered)
  - Nicotine lozenges
  - Transdermal Patch…others

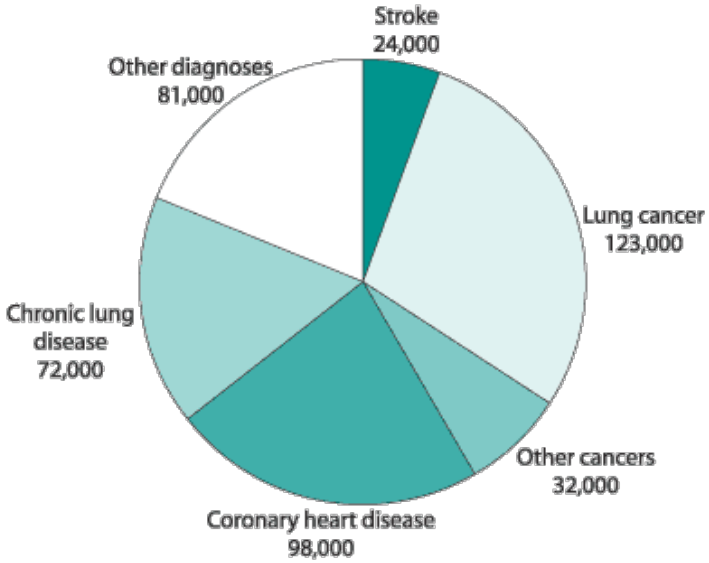
Acute toxicity effects of nicotine

- Classic stimulant effects of arousal (e.g. increased heart rate and blood pressure, alertness, appetite suppression)
- Carbon monoxide (in smoked form) reduces oxygen transport to heart and other organs
- Vasoconstriction
- Can have calming (anxiolytic) effects in some individuals
- Mild euphoria
- Cognitive enhancements
- Antidepressant effects



Chronic effects: CANCER

- Tobacco use accounts for one-third of all cancers
  - **Cancers** relating to tobacco include:
    - Mouth
    - Pharynx
    - Larynx
    - Esophagus
    - Stomach
    - Lung
    - Cervix
    - Kidney
    - Bladder
    - Throat
    - Pancreas



- Cigarette smoking has been linked to about 90 percent of all lung cancer cases
- 430,000 annual deaths are attributed to cigarette smoking

??Physical Withdrawal Symptoms of Nicotine

- irritability
- impatience
- hostility
- anxiety
- depressed mood
- difficulty in concentrating
- restlessness
- decreased heart rate
- increased appetite or weight gain

Nicotine Is a Toxic Substance

- 60 mg

- Nicotine poisoning
  - Accidental swallowing
  - Excessive absorption
  - Exposure to pure nicotine

Homework

- Q1) Discuss the cause of death and the mode of action of nicotine toxicity on animals studies.

PRACTICAL TOXICOLOGY

LAB: 1

REVIEW

- **Toxicology: is the study of the adverse effects of chemicals on living organism.**
- **It is study of symptoms, mechanisms , treatments and detection of poisoning.**
- **Toxicologist is trained person to examine the nature of those effects (including their cellular, biochemical, and molecular mechanism of action) and assess the probability of their occurrence.**

INTRODUCTIONS

- **Toxicity: is the degree to which a substance can harm humans or animals can be acute, subacute, chronic, and subchronic.**
- **Poison: It is any substance which when introduce to or absorbed by living organism destroys or injures health. It can cause death even in vary small amounts.**
- **Toxin:- generally refers to toxic substances that are produced by biological systems such as plants, animals, fungi or bacteria.**
- **Venom:- It is biological toxin that is injected by bite or sting to cause its effect.**

The types of exposures are

1-**Acute**, which is exposure to a chemical for **24 hours or less**.

2-**Chronic**, which is exposure to a chemical for **more than 3 months**.

3-**Sub-acute**, which is exposure to a chemical for **1 month or less**.

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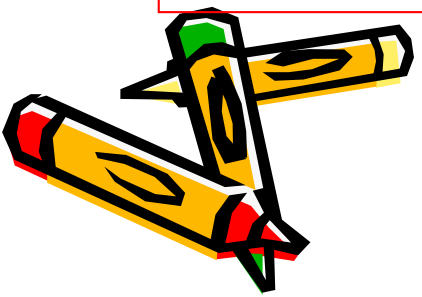


FACTORS AFFECTING TOXICITY

1. pathway of administrations (toxin is applied to skin, ingested, inhaled or injected).
2. The time of exposure
3. The number of exposure (single or multiple).
4. The physical form of toxin (solid, liquid, gas).
5. The genetic makeup of individual.
6. An individual's overall health

CLASSIFICATION OF TOXICANTS

1. Target organ (hepatotoxin, neurotoxin).
2. Intended use (pesticide, solvent).
3. Source (natural, synthetic).
4. Special effect (carcinogen, endocrine disrupter).
5. Physical state (gas, solid, liquid).
6. Toxicity (extremely, slightly).
7. Chemical composition (heavy metal, organophosphate).

TYPES OF TOXIC RESPONSES

1. Immediate:- minute to hours after single exposure.

2. Delayed: days to years after exposure.

3. Effect at site of contact, GIT, Lung and Skin.

4. Effect distant from exposure site, CNS, and Kidney

REVERSIBLE VS IRREVERSIBLE

Largely determine by:

1. Tissue involved

2. Length of exposure

3. Magnitude of tissue insult

Reversible: rapidly regenerating tissue like liver, intestinal mucosa and blood cells.

Irreversible: CNS damage, carcinogenesis and mutagenesis

ROUTE AND SITE OF EXPOURE

1. Exposure phase: the major routes of exposure are

a. Gastrointestinal tract (ingestion)

b. Lungs (inhalation)

c. Skin (topical, percutaneous or dermal)

d. parenteral (I.V , I.M)

2.TOXICOKINETICS PHASE

1. Absorption
2. Distribution
3. metabolism and
4. excretion.

3.TOXICODYNAMIC PHASE

The science of toxicology is based on the principle that there is a relationship between a toxic reaction (response) and the amount of poison received (dose).

MECHANICAL APPROACHES AND TREATING UNWANTED TOXICITY:

The first line of defenses is to remove the:

a. Ingested poison are frequently treated by:-

1. oral administering of activated charcoal which adsorbs the poison.

2. Ipecac syrup which causes vomiting in order to empty the stomach.

3. Cathartics which remove the poison from gut.

4. Gastric lavage where the stomach is washed out and drained using tube.

TREATING UNWANTED TOXICITY

B. poison which are injected into the body (from bites and strings from venomous animals) are usually treated by the use of constriction band which limits the flow of lymph or blood to the area.

The second line of defense involves the removal of poison (drugs) from blood stream by:

1. changing the urine pH.

2. Forced diuresis.

3. Hemodialysis.

4. Exchange transfusion.

The third line is administered of antidote which either remove the harmful substance from the blood or counteract its effect.

Pharmacology lab

Lab 4

Drugs acting on the eye

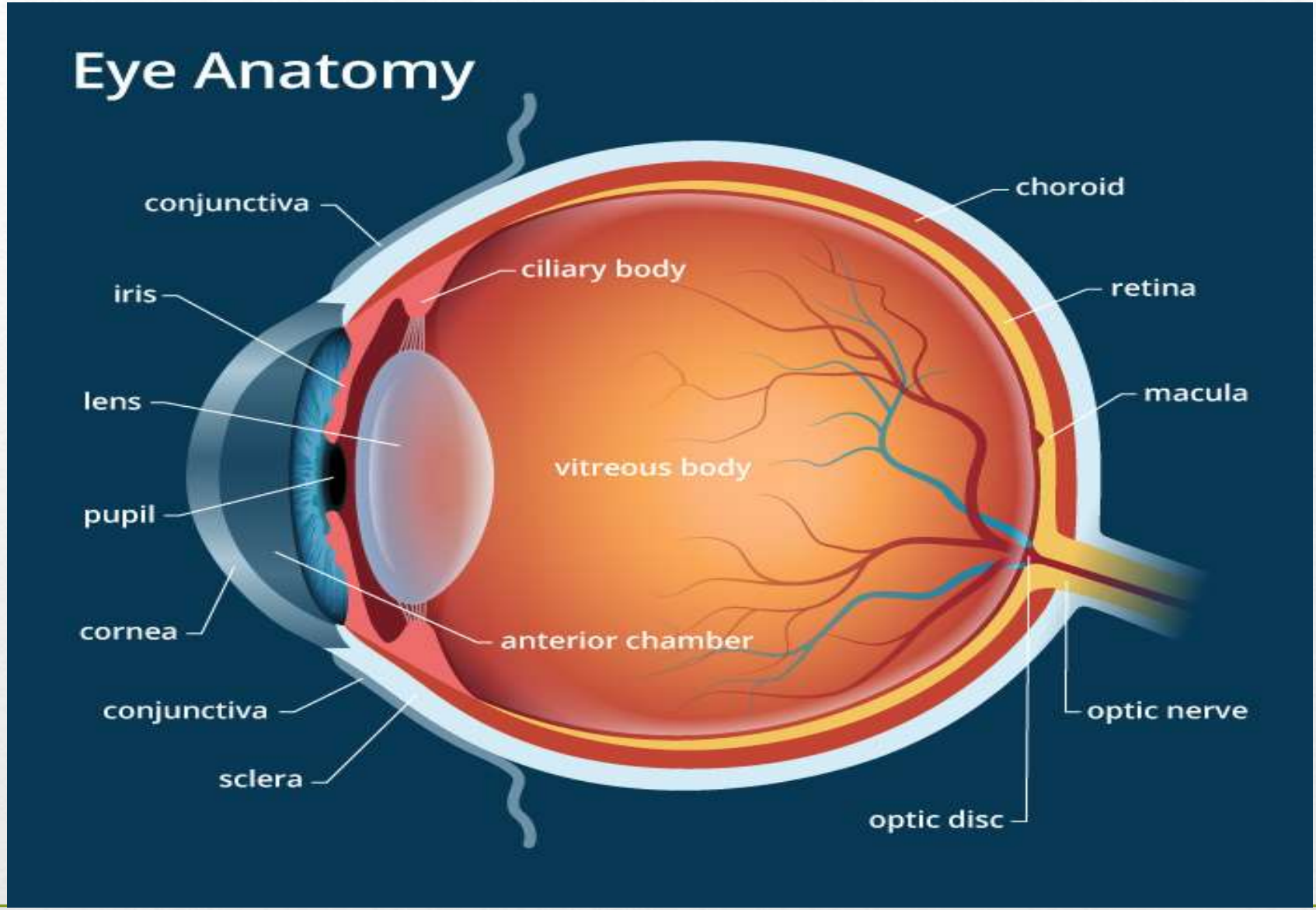
Asisst. Lecturer:
Nibras Hasaballah

The composition of the human eye

The main compartments of the human eye are :

- Cornea
- Iris
- Lens
- Ciliary body and vitreous humor.

The composition of the human eye



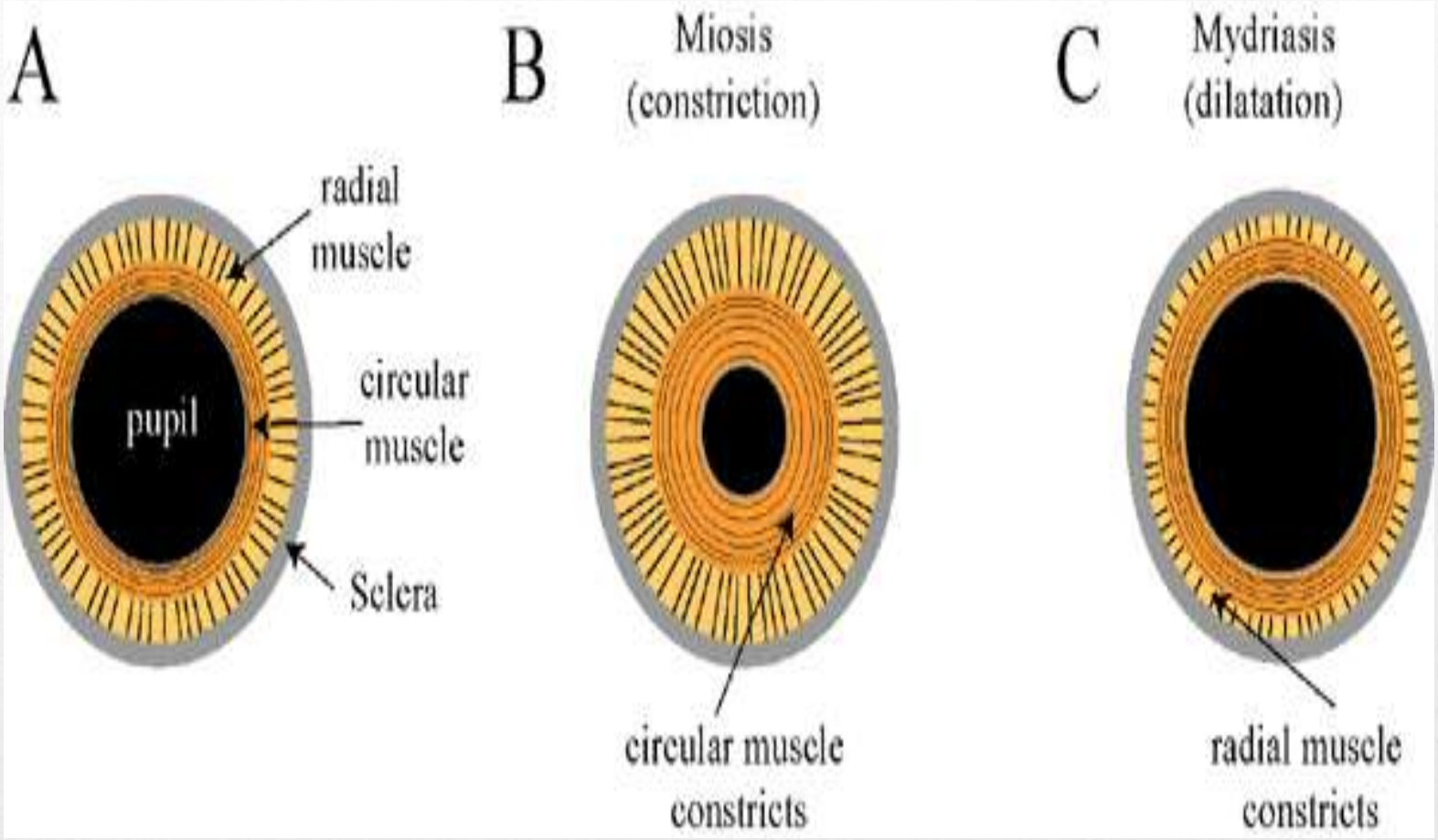
Iris

Iris involve;

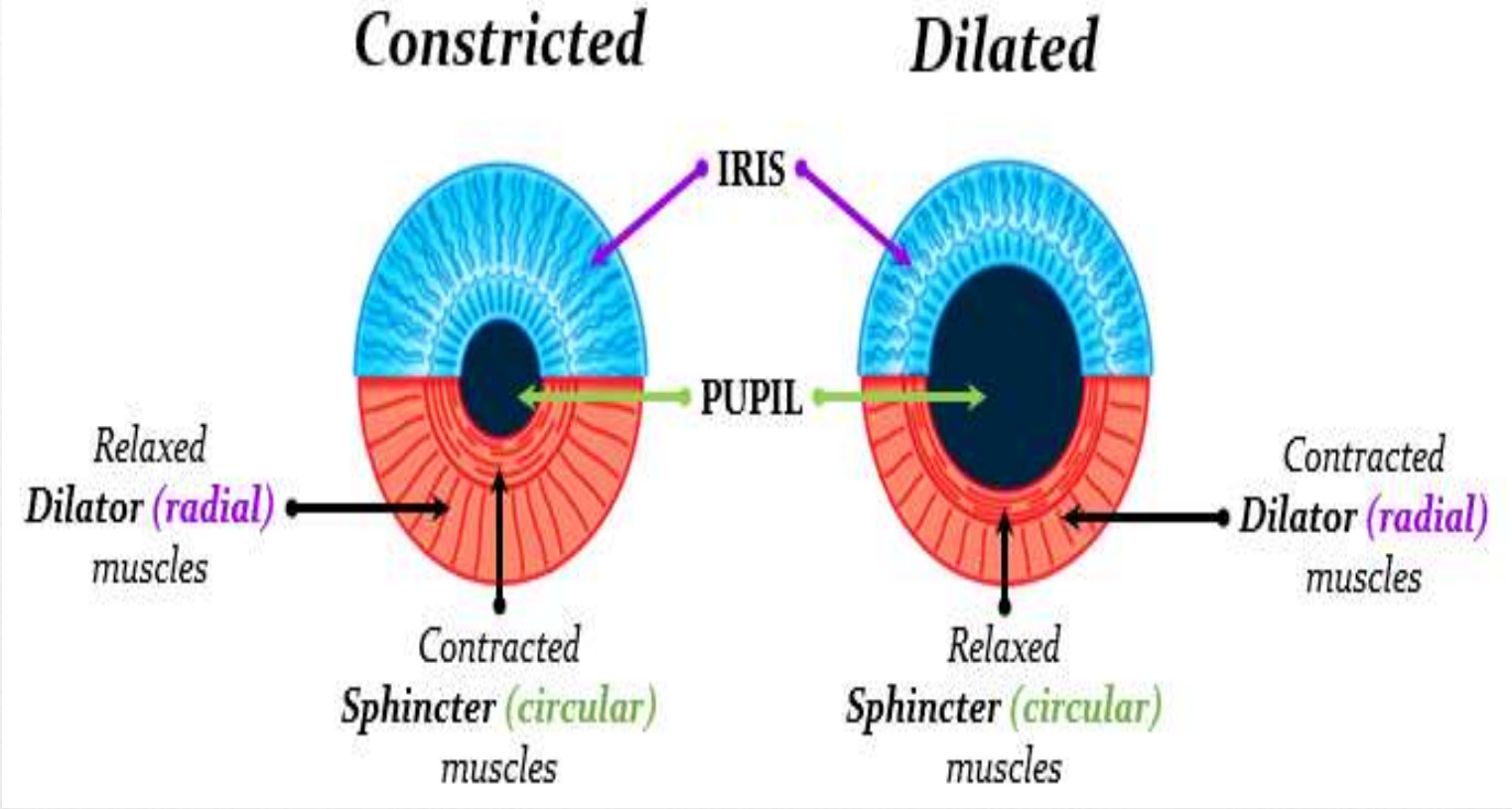
- **Circular muscle** (Muscarinic receptors).
- **Radial muscle** (Alpha-receptors).

- **Miosis**: smallness of the pupils of the eyes, is due to either contraction of circular muscle or relaxation of radial muscle.

- **Mydriasis**: dilatation of the pupils of the eyes , is due to either contraction of radial muscle or relaxation of circular muscle.


A
radial muscle
pupil
circular muscle
Sclera
B
Miosis (constriction)
circular muscle constricts
C
Mydriasis (dilatation)
radial muscle constricts

Working of Iris



Autonomic Nervous System

ANS Drugs & Pupil

	Miosis	Mydriasis
Cholinergic System	⊕ Cholinergics	⊖ Anticholinergics
Adrenergic System	⊖ Antiadrenergics	⊕ Adrenergics

Eye muscle	Receptor	Effect (muscle)	Effect (pupil)
Radial muscle (iris)	α_1 (SNS)	Contraction	Mydriasis
Circular muscle (iris)	M_1 (PNS)	Contraction	Miosis
Ciliary muscle	β_2 (SNS) M_2 (PNS)	Relaxation Contraction	– Accommodation

Alpha-receptors in iris

- **Alpha-agonist** → Contraction of radial muscle of iris (**Mydriasis**).
- **Alpha 1 agonist** such as **phenylephrine** → mydriasis, also increase the out flow of aqueous humor from the eye and reduce IOP.
- **Alpha-blocker** → Relaxation of radial muscles of iris (**Miosis**)

Alpha agonist

- **Alpha agonist** eye drops help reduce redness & irritation of the eyes by vasoconstriction of swollen blood vessels in the eye.
- Some ophthalmic alpha agonist used by ophthalmologists target the contraction of radial muscle to dilate the pupils (mydriasis) for examination or surgery. **Example**:
- **Naphazoline hydrochloride**
- **Phenylephrine hydrochloride**
- **Oxymetazoline hydrochloride**

Ciliary body

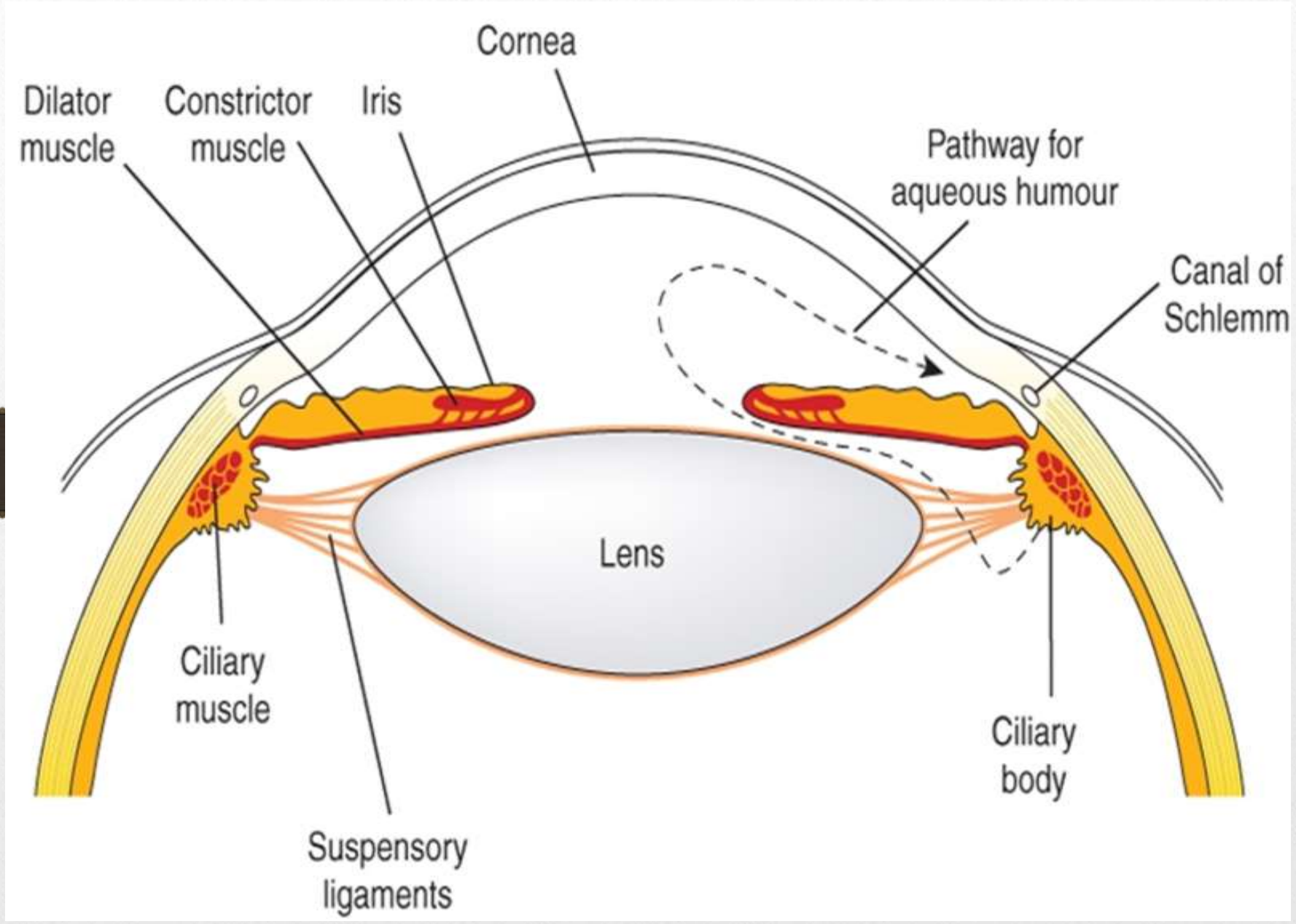
Ciliary body involve:

- **- Ciliary epithelium (B2 receptors):**

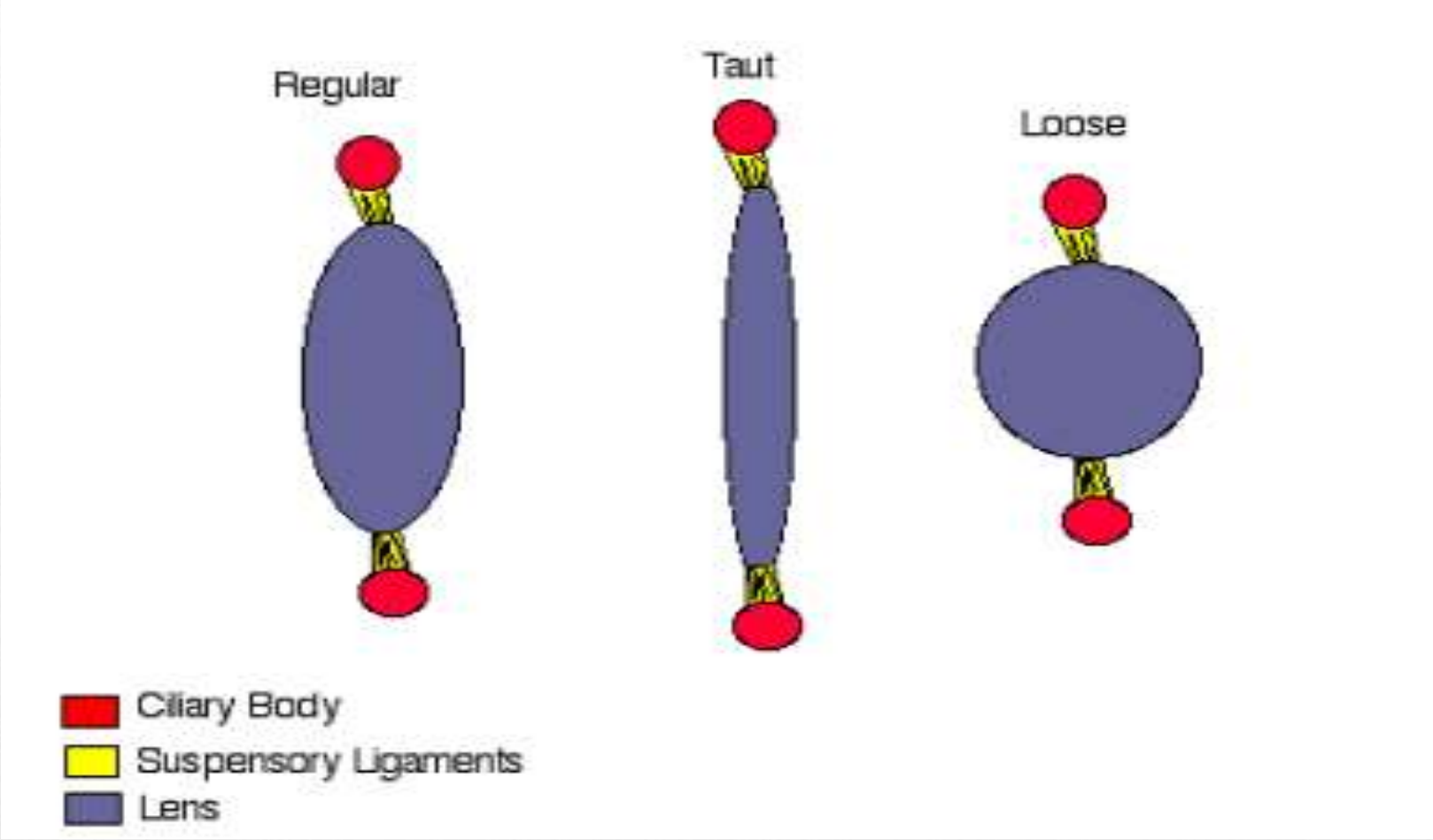
responsible for secretion of aqueous humor.

- **- Ciliary muscle (M2 receptors):**

responsible for near or far vision.


Cornea
Dilator muscle
Constrictor muscle
Iris
Pathway for aqueous humour
Canal of Schlemm
Lens
Ciliary muscle
Ciliary body
Suspensory ligaments

The contraction and relaxation of the lens

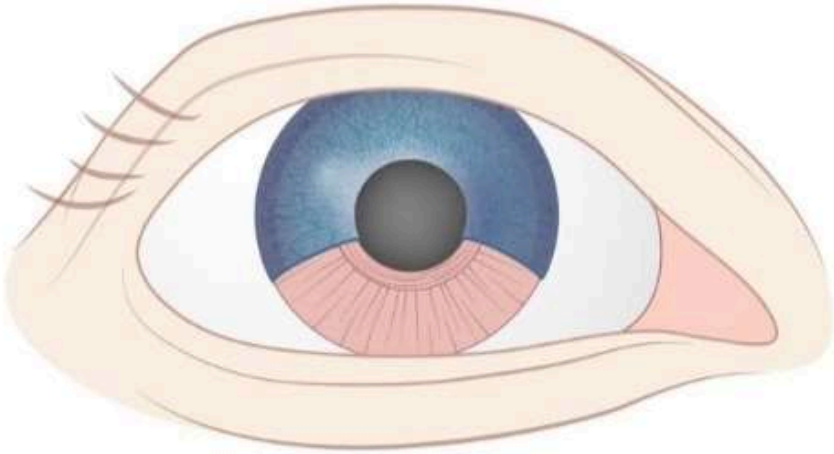


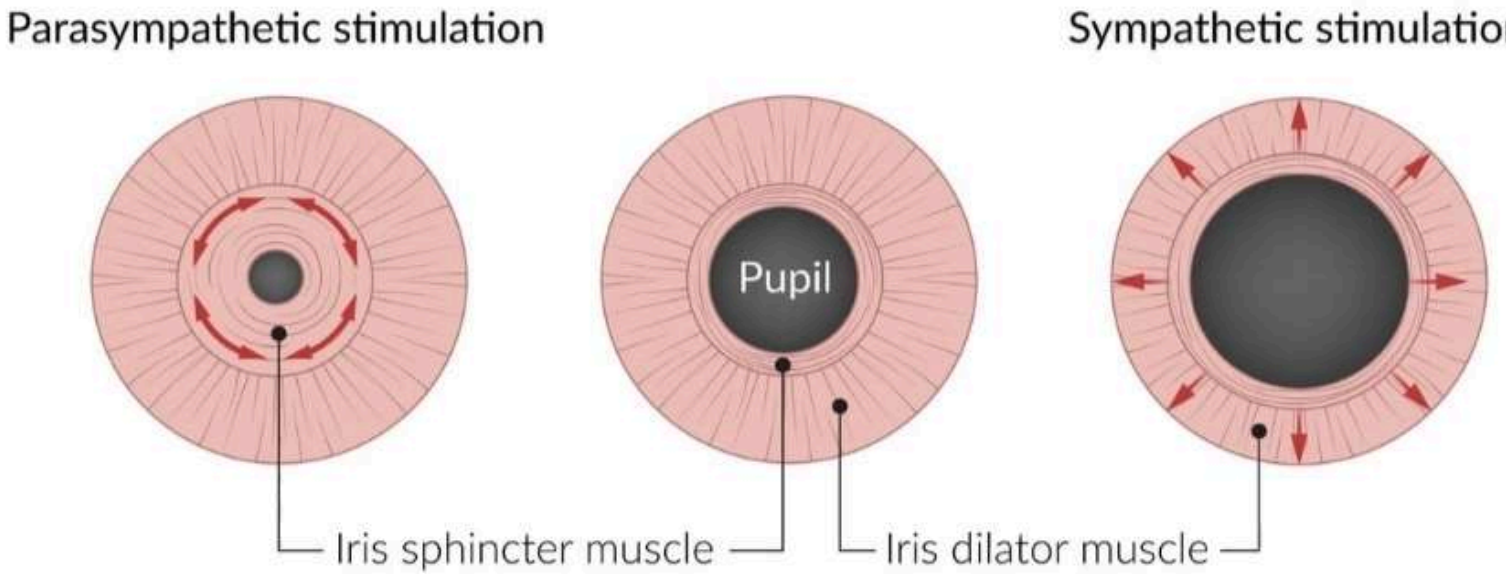
Ciliary Muscle
(Muscarinic receptors)

- Ciliary muscle contraction → Increases flow → Decreases IOP.
- Ciliary muscle Relaxation → Decreases flow → Increases IOP (Glaucoma).

Also

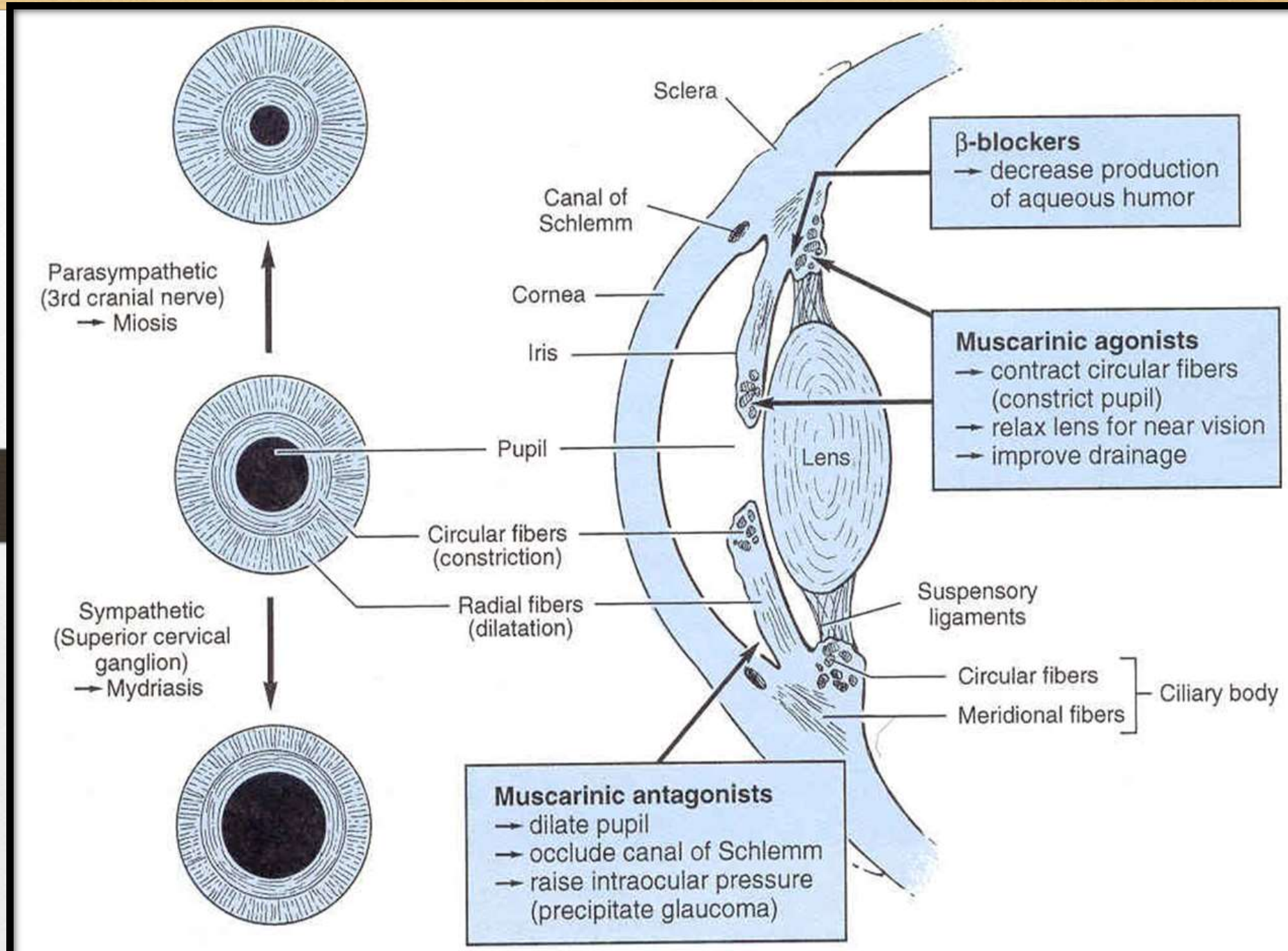
- **Muscarinic agonist** → Ciliary Muscle Contraction → Lens contraction → near vision
- **Anti-Muscarinic agent** → Ciliary Muscle Relaxation → Lens relaxation → far vision




Parasympathetic stimulation
Sympathetic stimulation
Pupil
Iris sphincter muscle
Iris dilator muscle

Ciliary Epithelium
(B2-Receptors)

- Responsible for secretion of aqueous humor.
- Contraction of ciliary muscle presses trabecular meshwork → enhancing the flow of aqueous humor through canal of Schlemm.


Parasympathetic
(3rd cranial nerve)
→ Miosis
Sympathetic
(Superior cervical
ganglion)
→ Mydriasis
Sclera
Canal of
Schlemm
Cornea
Iris
Pupil
Circular fibers
(constriction)
Radial fibers
(dilatation)
Lens
Suspensory
ligaments
Circular fibers
Meridional fibers
Ciliary body
β-blockers
→ decrease production
of aqueous humor
Muscarinic agonists
→ contract circular fibers
(constrict pupil)
→ relax lens for near vision
→ improve drainage
Muscarinic antagonists
→ dilate pupil
→ occlude canal of Schlemm
→ raise intraocular pressure
(precipitate glaucoma)

CILIARY BODY AND AUTONOMIC NERVOUS SYSTEM

- Sympathetic system increases aqueous production
  - Through stimulation of ß receptors
  - ß blockade decreases aqueous production
- Sympathetic system decrease aqueous production
  - Through activation of α2 receptors
  - α2 agonists decrease aqueous production

Drug induced miosis vs mydriasis

CONSTRICTED PUPILS (MIOSIS)

Sympatholytic agents
- Clonidine
- Opioids
- Phenothiazines
- Tetrahydrozoline and oxymetazoline
- Valproic acid

Cholinergic agents
- Carbamate insecticides
- Nicotine[b]
- Organophosphates
- Physostigmine
- Pilocarpine

Others
- Heatstroke
- Pontine infarct
- Subarachnoid hemorrhage

DILATED PUPILS (MYDRIASIS)

Sympathomimetic agents
- Amphetamines and derivatives
- Cocaine
- Dopamine
- LSD (lysergic acid diethylamide)
- Monoamine oxidase inhibitors
- Nicotine[b]

Anticholinergic agents
- Antihistamines
- Atropine and other anticholinergics
- Carbamazepine
- Glutethimide
- Tricyclic antidepressants

Methods

- Place few drops of the agents in the following table into the eyes of rabbits and check for the parameters mentioned in the same table, and the results are as follows:

Table of eye results

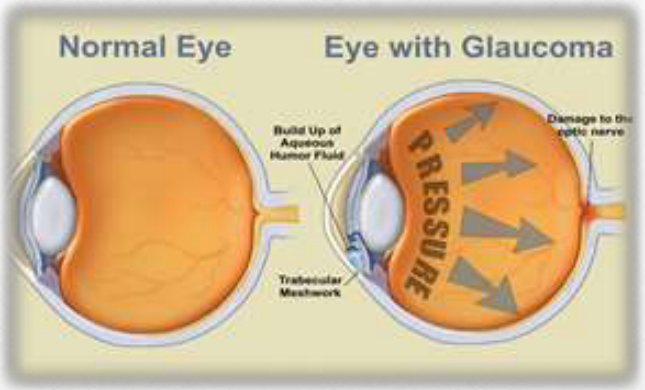
parameter / Agent	Pupil Size	Light Reflex	Accommodation	Conjunctival Blood vessels	Corneal sensation	IOP
Adrenaline	↔	+ve	↔	Pale	+ve	↔
Phenylphrine	Mydriasis	+ve	↔	Pale	+ve	Inc.
Pilocarpine	Miosis	+ve	Near Vision	Congestion	+ve	Dec.
Atropin	Mydriasis	-ve	Far Vision	Pale (Congested in High Dose)	+ve	Inc.
Xylocaine	↔	+ve	↔	↔	-ve	↔
procaine	↔	+ve	↔	↔	+ve	↔

(+ve) indicates the presence of the reflex
(-ve) indicates the absence of the reflex
(↔) indicates that there is no change

Results

- **Adrenaline** acts on alpha-receptors causing vasoconstriction of the epithelium of conjunctiva, but it does not cause mydriasis as it cannot be absorbed by the iris.
- **Phenylephrine** is alpha-receptor agonist → mydriasis
- **Atropine** is antimuscarinic agent, **scopolamine** & **tropicamide** → mydriasis
- **pilocarpine** → Muscarinic agent → miosis
- **Procaine, Xylocaine** (local anesthetic) as the cornea does not absorb it, so it cannot cause loss of corneal reflex.

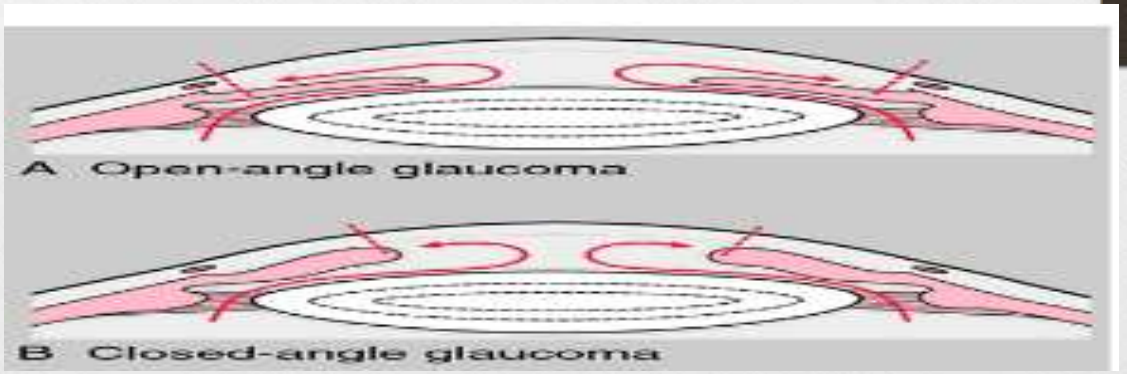
Glaucoma



- A group of eye conditions that damage the optic nerve, often due to high intraocular pressure (**IOP**).
- Leading cause of irreversible blindness.
- Characterized chiefly by **an increase in IOP above 21 mmHg & may be as high as 70 or 80 mmHg during the attack**

Types of Glaucoma

- **1. Open-Angle Glaucoma (POAG):** Gradual increase in IOP, asymptomatic until advanced.



- **2. Angle-Closure Glaucoma:** Sudden increase in IOP, emergency condition.

Phathophysiology

- **1. Increased Intraocular Pressure (IOP):** Imbalance between aqueous humor production and drainage.
- **2. Damage to the Optic Nerve:** Reduced blood flow and nutrient supply to the optic nerve.

Symptoms and signs

- **1. Open-Angle Glaucoma:** No early symptoms, slow peripheral vision loss.
- **2. Angle-Closure Glaucoma:** Severe eye pain, headache, blurred vision, nausea.
- **3. Advanced Signs:** Tunnel vision, optic disc cupping.

Treatment Options

- **1. Medications:**
- **- Prostaglandin analogs (increase fluid drainage).**
- **- Beta-blockers (reduce fluid production).**
- **- Carbonic anhydrase inhibitors.**
- **2. Laser Therapy: Trabeculoplasty (enhances drainage).**
- **3. Surgical Interventions: Trabeculectomy or drainage implants.**

CLASS OF DRUG	DRUG NAMES	MECHANISM OF ACTION	SIDE EFFECTS
β-Adrenergic antagonists (topical)	*Betaxolol, carteolol, levobunolol, metipranolol, timolol*	Decrease of aqueous humor production	Ocular irritation; contraindicated in patients with asthma, obstructive airway disease, bradycardia, and congestive heart failure.
α-Adrenergic agonists (topical)	*Apraclonidine, brimonidine*	Decrease of aqueous humor production and increase of aqueous outflow	Red eye and ocular irritation, allergic reactions, malaise, and headache.
Cholinergic agonists (topical)	*Pilocarpine, carbachol*	Increase of aqueous outflow	Eye or brow pain, increased myopia, and decreased vision.
Prostaglandin-like analogues (topical)	*Latanoprost, travoprost, bimatoprost*	Increase of aqueous humor outflow	Red eye and ocular irritation, increased iris pigmentation, and excessive hair growth of eye lashes.
Carbonic anhydrase inhibitors (topical and systemic)	*Dorzolamide* and *brinzolamide* (topical), *acetazolamide*, and *methazolamide* (oral)	Decrease of aqueous humor production	Transient myopia, nausea, diarrhea, loss of appetite and taste, and renal stones (oral drugs).


Thank
you!!

Introduction to Laboratory of Pharmacology

Lab1

Routes Of Drug Administration

*Tikrit University /College of Pharmacy /Department of Pharmacology &Toxicology

*Assist lecturer:

Nibras Hasaballah Jasem

2024-2025

Laboratory of Pharmacology

- **Pharmacological experiments** are designed to:

1 study the effects of drugs on tissues, organs, and other living subjects

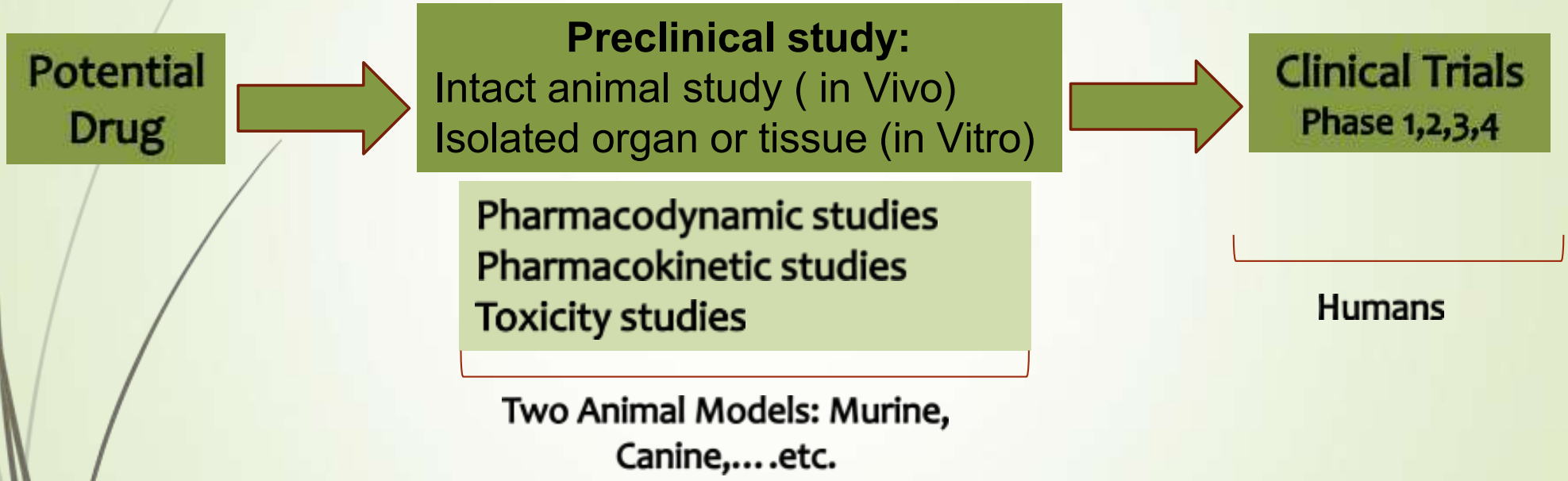
2 Find out new therapeutic agents

3 Study the mechanism(s) by which the drug interact and affect the targets

- **Clinical Trials:** Study the effect (s)/side effect (s) of drugs on humans

Significance of Pharmacological studies

- **Drug Development:**



- **Evaluating and Exploring doses, mechanisms, side effects,… etc.**

Laboratory Animals

Rats were first used for experimental purposes in the mid 1800s

Carefully bred rats are used in animal testing for a number of reasons, including their frequent reproduction, genetic purity and similarities to human biology

Lifespan	2.5-3.5 years
Adult weight	M 300-500g, F 250-300g
Birth weight	5-6g
Heart rate	330-480 beats/minute
Respiratory rate	85 breaths/minute
Body temp.	35.9-37.5°C

Rats

Rats are generally fed a diet containing low fiber, protein and fat

Rat rooms are usually maintained at 30-70% relative humidity and a temperature of 18-26°C

Rats should be adapted to handling to reduce stress

Blood can be collected from several sites in the rat including tail vein, retro-orbital sinus, vena cava or cardiac puncture

" Can receive oral, IP, IM, and IV"

Laboratory Animals

Mice

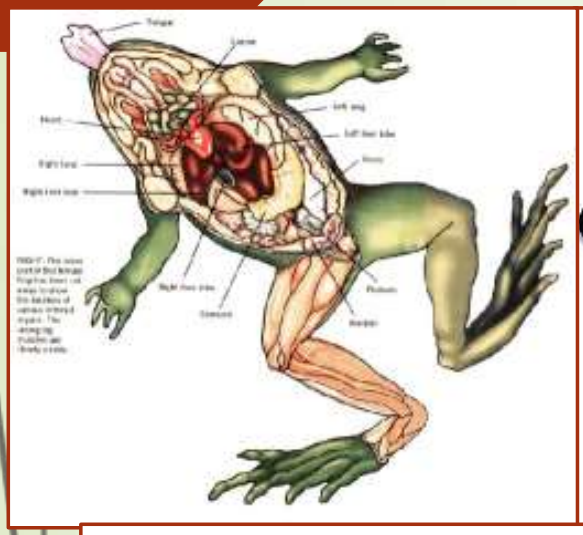
The mouse and human genomes are about **85** percent the same, and those similarities have made the mouse a powerful model for studying human biology and disease

Handling, blood collection, and drug administration: same as rats

Lifespan	1-3 years
Adult weight	M 20-30 g, F 18-35g
Birth weight	1-2 g
Heart rate	310-840 beats/minute
Respiratory rate	80-230 breaths/minute
Body temp.	36.5-38°C

Easy to make disease models

Laboratory Animals



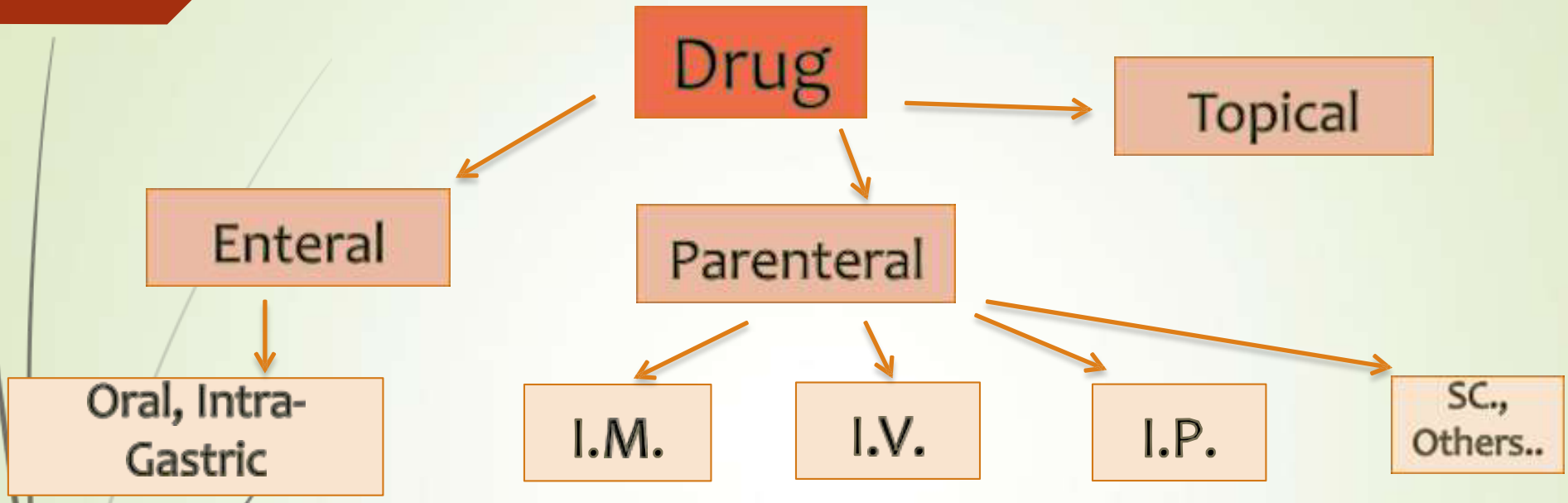
frog or toad: physiological studies

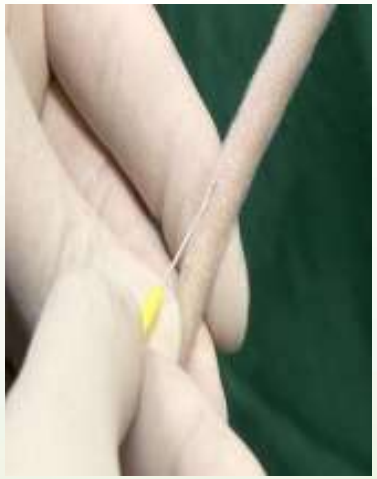
guinea pig: hypersensitive test (allergic reaction) or the screening of anti-asthmatic drug

Rabbit: Expensive, Used for studying the effect of some drug

Other: Cat, Dog, pig, and Monkey

Routes of Drug Administration





Enteral Route of administration

Placement of drug directly into any part of the GIT

It could be Oral, Sublingual ,Intra-gastric gavage, or Rectal.

1- Oral : Swallowing a drug through mouth, It may be done by adding desired drug To the drinking water or to the food

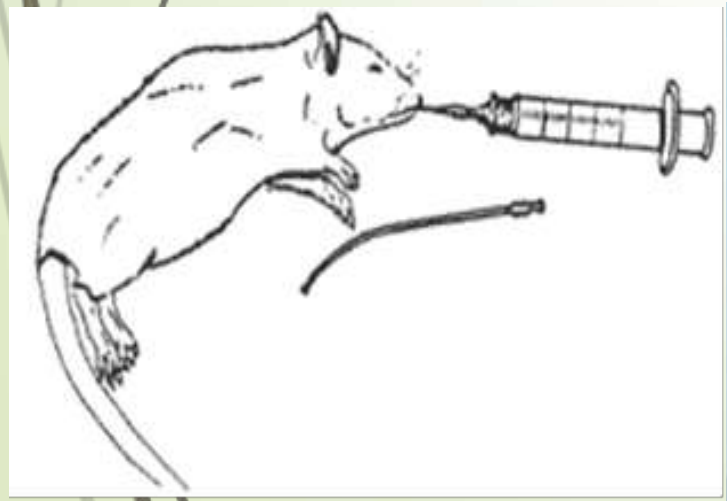
- The oral route is economical, convenient, relatively safe, and easy , some animals can be trained to cooperate voluntarily, depending on the compound being administered
- This route is not preferable since it inaccurate

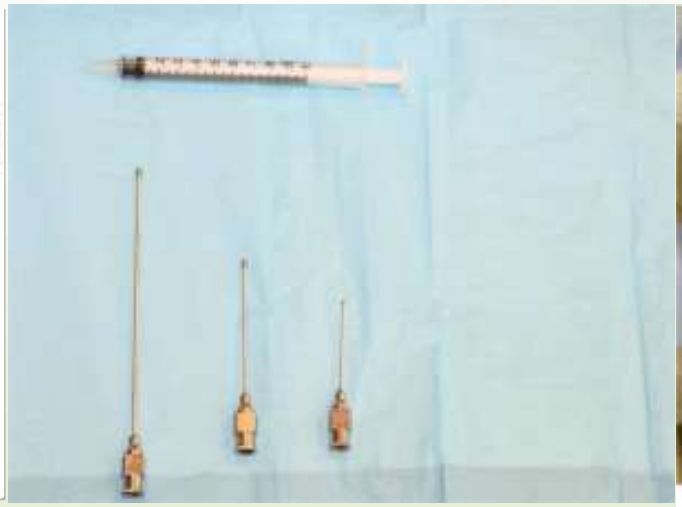
.

Enteral Route of administration

2- Intra-gastric gavage: is the administration of fluids directly into the lower esophageal or stomach.

- Gavage is often used in research settings, instead of mixing substances in water or food, to ensure accurate dosing of animals.
- A small, curved, metal tube, usually with a ball on the end (feeding needle) is often used with small rodents. Entrance may normally be obtained without anesthesia using ordinary hand restraint and the ball prevents trauma to the esophagus and oral cavity.





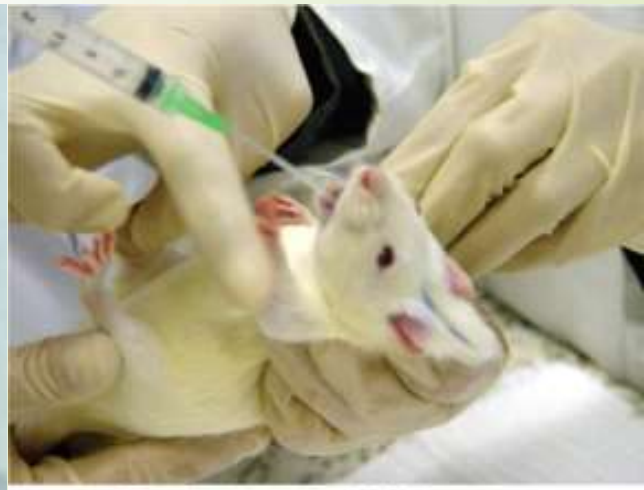


Figure 1 - Administration of DMBA by gavage.

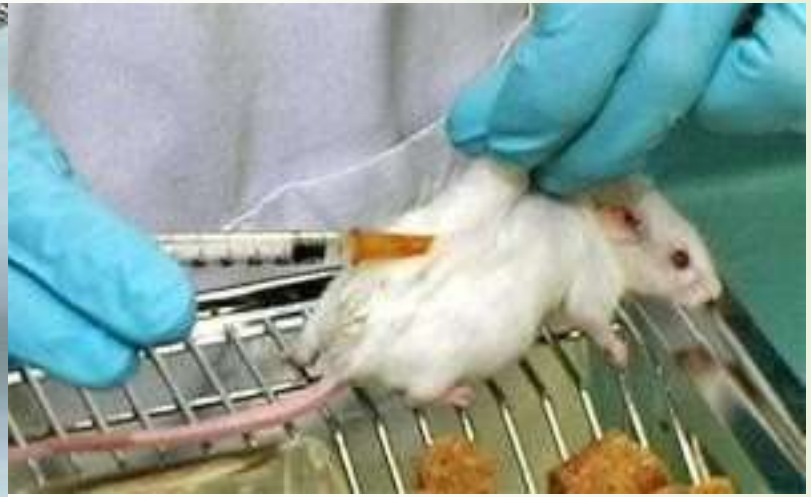
Parenteral routes of administration

- Routes other than Enteral are called Parenteral routes of administration
- Parenteral administration methods typically produce the highest bioavailability of substances because these methods avoid the first-pass effect of hepatic metabolism.

1. **Intravenous (IV)** directly in the vascular system through a vein
2. **Intraperitoneal (IP)** - injected into the abdominal cavity
3. **Intramuscular (IM)** injected into a muscle
4. **Subcutaneous (SC)** injected under the skin
5. **Intradermal (ID)** - injected between the layers of the skin
6. **Intracerebral (IC)-** injected into the brain
7. **Epidural :** injected into the epidural space of the spinal cord
8. **Intranasal:** sprayed into the nose for absorption across the nasal mucous
9. **Inhalation:** Inspiration through nose or mouth
10. **Intra-articular:** injection directly into the joint space

1-Subcutaneous (SC) injections

- The best spot to inject Subcutaneously is the loose skin on the back of the neck
- A mouse may easily be injected by one person, whereas a rat may require restraint by one person and injection by the other
- Not suitable for large volumes. Suitable for some insoluble suspensions



Procedure

- Lift the skin over the back to form a tent.
- place the mouse on the wire lid so it can hang on . Scruff the skin over the back and tent it up.
- Insert the needle at the tent base, Hold the needle parallel to the animal's body to also avoid puncturing underlying structures.
- Aspirate to ensure that the needle has not entered a blood vessel.
- Withdraw the needle and then press the skin to seal the needle's exit hole in the skin and to prevent the fluid from leaking out.

2-Intraperitoneal (IP) injections

- Commonly used in rats and mice since muscle mass is so small and veins are difficult to find
- Rapid absorption (almost as fast as IV) due to large peritoneal surface
- IP administration results in a faster absorption into the vasculature than SC administration l
- A mouse may easily be injected by one person, whereas a rat might require restraint by one person and injection by the other
- Volume of vehicle ranging between 2 ml/kg to 10 ml/kg

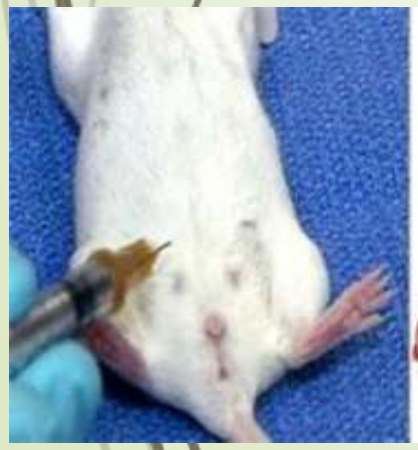
2- Intraperitoneal (IP) injections

- The injection site is usually on the animal's right lower abdominal quadrant
- Insert the needle at approximately 45 degree angle
- There are three points that you need to pay attention: **position/ angel / draw back.**

1 The position of injection is in the abdomen, not too high, not too low, if too high, liver may be hurt, if too low, bladder may be hurt.

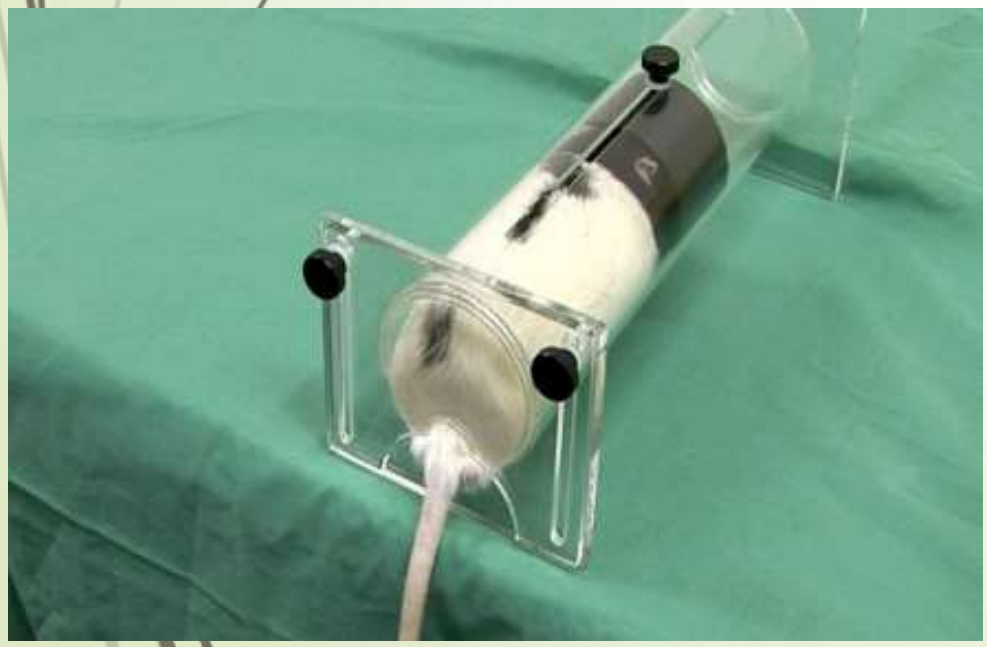
2 The angle should be **about 45 degree**.

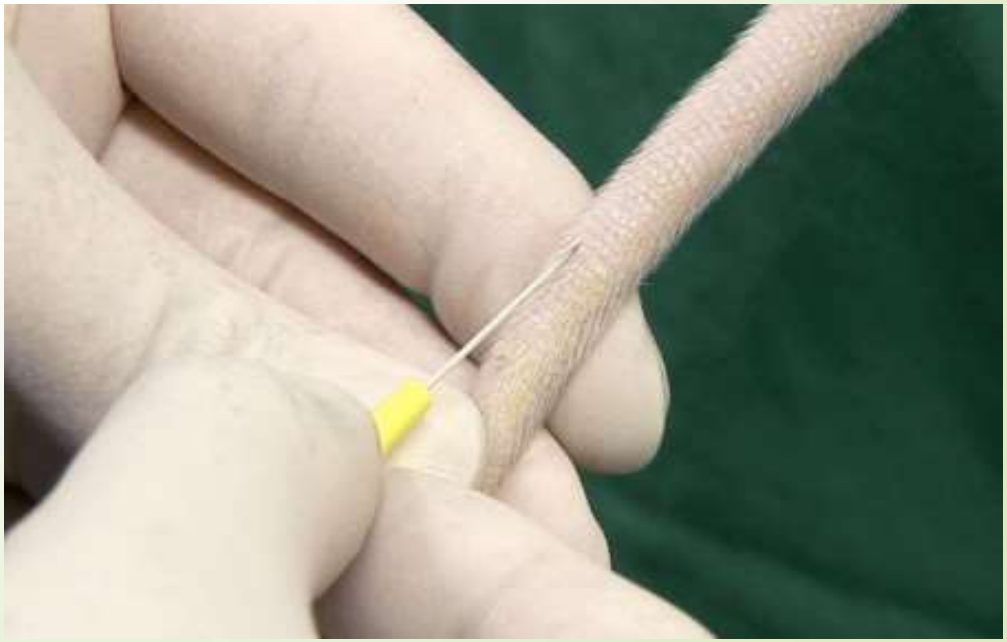
3 After the syringe needle has been in the abdomen, before injection, you should **draw back** : to see if can draw out something, if not , you can go on. If draw out blood or urine, that shows you have fail



3- Intravenous (IV) injections

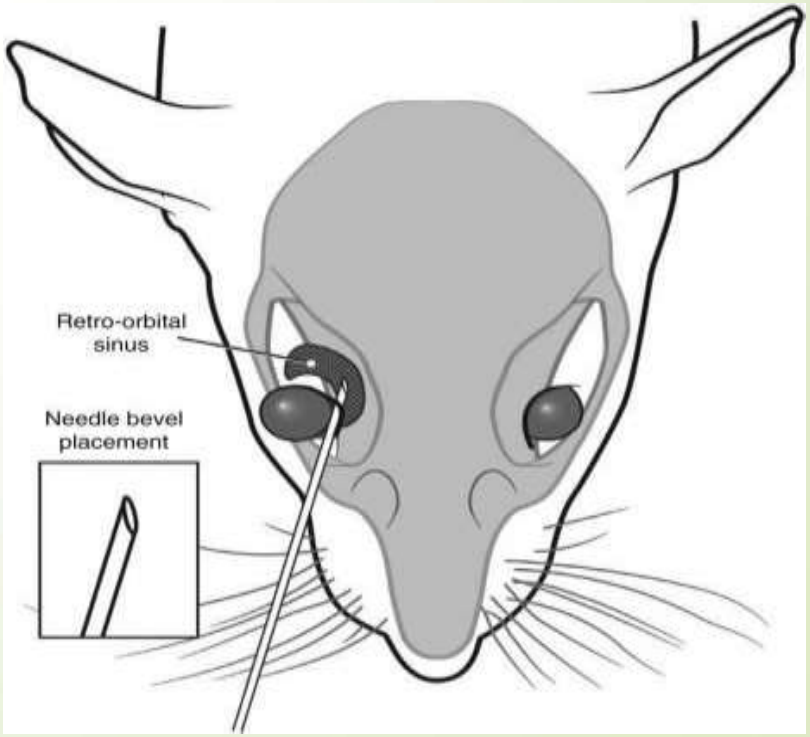
- The most efficient means of delivering substances to animals because it bypasses the need for solute absorption
- Technically difficult, and the use of a restraining device with appropriate size for the animal to be injected, is often required
- Performed in mice and rats, use the lateral tail vein located on either side of the tail
- The tail vein is difficult to find that's why mouse is often placed under a heat lamp to promote peripheral vasodilation
- Suitable for large volumes. Must inject slowly.





Retro-orbital injections in mice

- This technique is a useful alternative to tail vein injection.
- The mouse should be anesthetized so that it remains still during the procedure (using inhalant anesthetic)
- The needle is being placed in the retro-bulbar space (the region behind the globe of the eye).



4-Intramuscular (IM) injections

- IM injections result in uniform and rapid absorption of substances, because of the rich vascular supply
- Not recommended in mice and small species due to their small muscle mass
- Smaller volumes are administered intramuscularly than for subcutaneous delivery
- Suitable for aqueous or specialized depot preparations

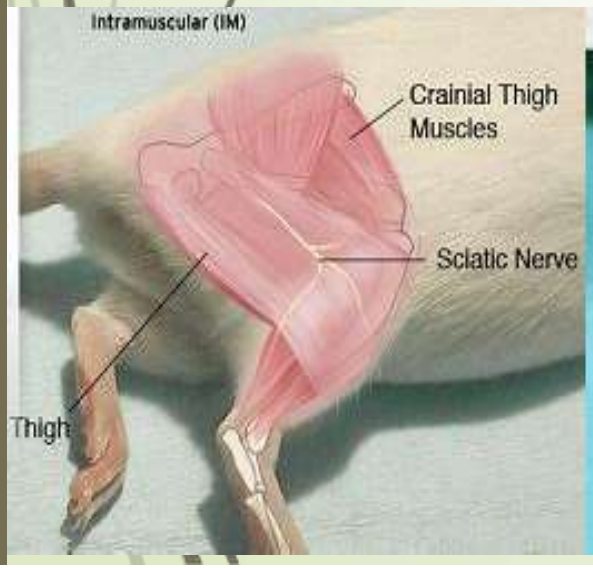
Intramuscular (IM) injections

Procedure

1 One person restrains the mouse by the scruff method with one hand and steadies the leg to be injected with the other.

2 The second person , aspirates and , bevel up. Direct the needle caudally (toward tail) if using the caudal thigh muscles or cranially (toward head) if using the quadriceps It is very important to avoid injuring the sciatic nerve.

3 Aspirate to ensure that you have not entered a blood vessel.
4 If no blood is seen, slowly inject the material



Injection site and volume in Rodents

Route	Maximum needle size	Optimal volume	Site
Gavage	Mice: 20 Gauge, (3.8cm) length Rat: 16 Gauge, (7.6cm) length	5 mL/kg (to 20 mL/kg)	intragastric
IV	25	Up to 5 mL/kg	tail or Retro-orbital vein
Sc.	22	Maximum of 5 mL/kg per site	Intrascapular (Scruff), neck, Flank
IM	25	Maximum of 0.05 mL/kg per site	caudal thigh , quadriceps muscles
IP	25	Maximum of 10 mL/kg	Lower ventral quadrants

Aim of the Experiment

1. Learn how to handling, treating and preparing animals for the experiments and the ethical guidelines during treatments with animal being .

2. Measure the required volume of a drug in a syringe using aseptic techniques.

3. Learn how to give different types of routes of administration in this lab which includes: Intraperitonial, intramuscular and subcutaneous.

Handling and restraint

- Good handling and restraint is the most important technique for correct administration.
- There are two styles of manual restraint:

a- Double handed manual restraint

b- Single handed restraint


BE KIND TO
ANIMALS