Introduction to Laboratory of Pharmacology
Routes Of Drug Administration

By

Assist. Lecturer

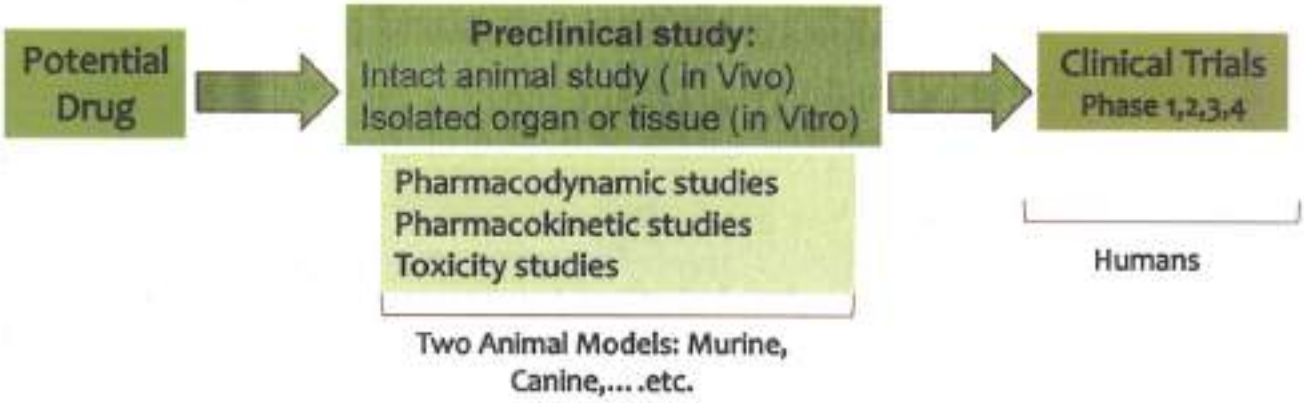
Nawar R. Hussein

Laboratory of Pharmacology

- Pharmacological experiments are designed to study the effects of drugs on tissues, organs, and other living subjects
- Find out new therapeutic agents
- Study the mechanism(s) by which the drug interact and affect the targets
- Study the possible toxic effects of the drug on humans
- Qualitative experiments: study the drug-body interaction
- Quantitive experiments: study the amount of active materials
- 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

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

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

Laboratory Animals

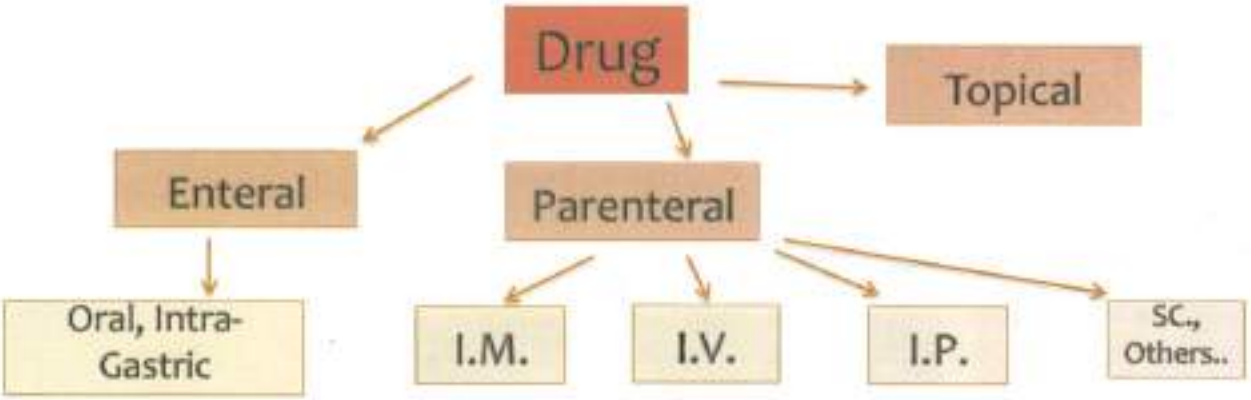
frog or toad: physiological studies

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

Rabbit: Expensive, the effect of some drug

Other: Cat, Dog, pig, and Monkey

Routes of Drug Administration



Route of administration

Placement of drug directly into any part of the GIT

It could be Oral, Sublingual ,Intragastric gavage, or Rectal.

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

Advantages:

- Most commonly used as it is safe, convenient, & painless procedure and some animals can be trained to cooperate voluntarily, depending on the compound being administered
- Economical as sterilization of drug products is not essential
- No need of any assistant

Disadvantages:

- Onset of action is slower
- Polar drugs cannot be given as they are not absorbed (e.g.: Streptomycin)
- Drugs are destroyed by the digestive juices (e.g.: Penicillin-G, Insulin, Oxytocin)
- 1st pass effect (those destroyed in liver before reaching systemic circulation) (e.g.: morphine, Isoprenaline)
- Bad test, bad smell and irritant drugs cannot be given
- Drugs cannot be given to unconscious and uncooperative patients
- Drugs cannot be given during emesis
- This route is not preferable since it inaccurate

Enteral Route of administration

Oral

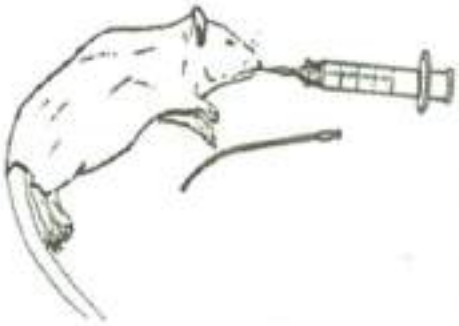
- **Additive to the drinking water:** the investigator must monitor the animals and assure that adequate fluid intake occurs
- **Additive to food:** investigator must assure adequate food intake

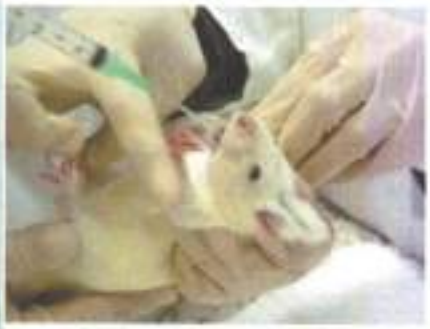
Intra-gastric gavage: is the administration of fluids directly into the lower esophageal or stomach using a ball ended feeding needle introduced into the mouth and thread down to esophagus.

- 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.
- Gavage is often used in research settings, instead of mixing substances in water or food, to ensure accurate dosing of animals.
- The investigator must be trained and well experienced with gavage

❑ Dose should not be exceed :

- 10ml/kg (1ml/100g) body weight for non-aqueous preparation
- 20ml/kg (2ml/100g) body weight for aqueous preparation





Enteral Route of administration

Sublingual / Buccal

- The drug is place beneath the tongue (sublingual) or crushed in mouth and spread over the buccal mucosa (buccal)
- **Advantages:**
  - Quick onset of action because of rapid absorption due to more blood supply in that region
  - Bypass the portal circulation, no 1st pass metabolism
  - Drug action can be terminated at any time when side effects are observed
- **Disadvantages :**
  - Distasteful, irritant drugs cannot be given
  - Higher molecular weight drugs cannot be absorbed (e.g.: insulin)
  - Examples: Isosorbide dinitrate tablets & Nitroglycerin tablets (for Angina), Isopranline sulfate tablets (for bronchial Asthma

Enteral Route of administration

- **Rectal**
- Thought rectum (suppositories, Enema)
- **Advantages :**
  - Useful in patient with nausea and vomiting
  - 1st pass metabolism is greatly bypassed as a major portion of the drug is absorbed from external haemorrhoidal veins.
  - Useful for gastric irritant drugs
- **Disadvantages :**
  - Chances of rectal inflammation
  - Absorption is irregular
  - Inconvenient and embarrassing to the patient
- **Examples**
  - Dulcolax & Glycerin suppositories, enemas, ointments for location
  - Aminophylline (Bronchodilator) & Indomethacin (Anti-inflammatory agent) Suppositories for systemic action

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.
- The choice of parenteral route of administration will depend on the volume and material to be injected, and the desired rate of absorption

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

Parenteral routes of Drug Administration

11. Intrathecal (intraspinal)

- Into subarachnoid space, cross BBB & blood CSF barrier
- Strict aseptic conditions & grater expertise is needed
- Painful & risky procedure
- Many radiopaque contrast media for myelography (to visualize spinal cord) are given through this route
- Xylocaine injection for providing Spinal Anesthesia

12. Intramedullary

- Injection into the tibial or sternal bone marrow

13. Intra-arterial

- Into the lumen of the desired artery

14. Retro-orbital

- In to the retro-bulbar space (the region behind the globe of the eye)

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.

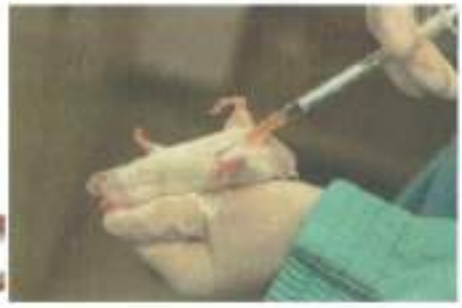
Intraperitoneal (IP) injections

- 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
- Suitable for irritating compound, such as ketamine or pentobarbital
- 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

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.**
- first 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.
- Second, the angle should be about 45 degree.
- Third, after the syringe needle has been in the abdomen, before injection, you should draw back the stylet to see if can draw out something, if not , you can go on. If draw out blood or urine, that shows you have fail



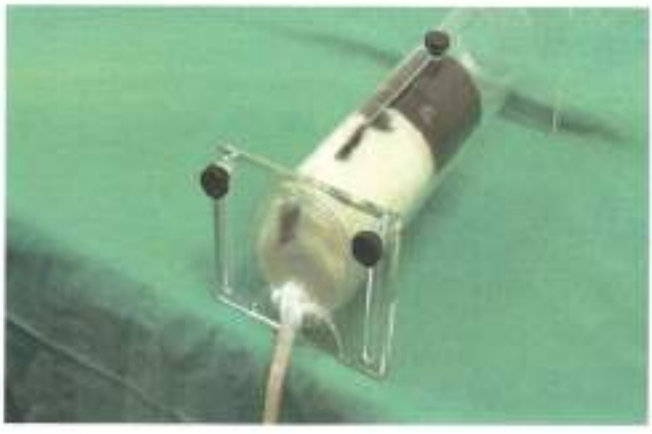


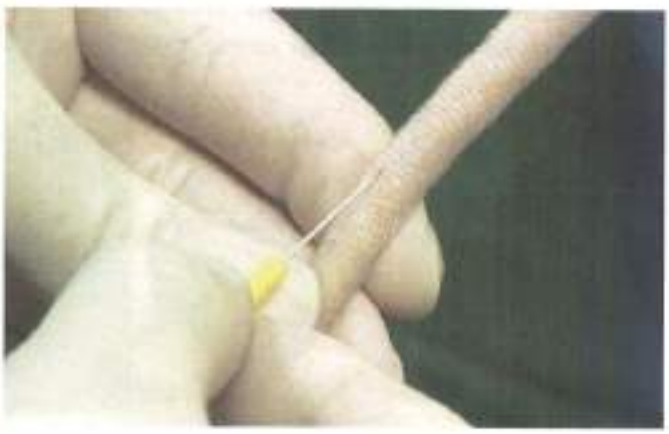

Intraperitoneal (IP) injections

- Complication of IP
- The potential for serious side effects such as peritonitis
- The risk of injecting the GI tract, kidney, loop of bowel, and bladder (may also cause its rupture or other vessels in the area)
- Some substances may cause mild-moderate irritation

Intravenous (IV) injections

- Also called Tail vein injection, is one of the commonly used route
- is 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. Not suitable for oily solutions or insoluble substances





Intravenous (IV) injections

Advantages

- Directly enters into the systemic circulation & no 1st pass effect & quicker onset of action
- Less dose is needed to achieve greater therapeutic effects
- Valuable in emergency
- Can be given evening unconscious, uncooperative patients those are having nausea, vomiting & diarrhea
- Hypertonic solution & GIT irritant drugs can be infused
- Large volume of fluids can be infused at a uniform rate
- Amount of the drug can be controlled with an accuracy

disadvantages

- Strict aseptic conditions are needed
- Needs specialized technician, painful, and risky because of the injected it cannot be recalled
- Introduction of any air or particulate matter produce embolism which is fatal
- Drugs in suspensions & oily drugs can not be given
- Venous thrombosis & Thrombophlebitis of the vein injected
- Necrosis around the site of action

Intramuscular (IM) injections

- intramuscular 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
- In rats ≤ 0.2 ml/site may be injected into gluteal muscles
- Deltoid muscle or gluteal mass of left or right buttock
- Vastus muscle underlying the lateral surface of the thigh

Intramuscular (IM) injections

Advantages:

- Absorption is more predictable, less variable & rapid compared to oral route
- Depot injections can be given

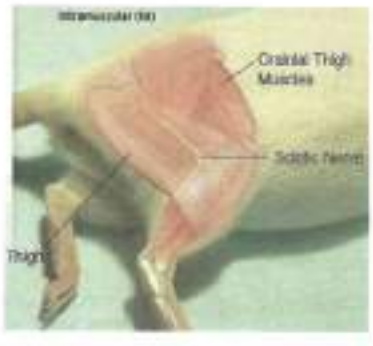
Disadvantages:

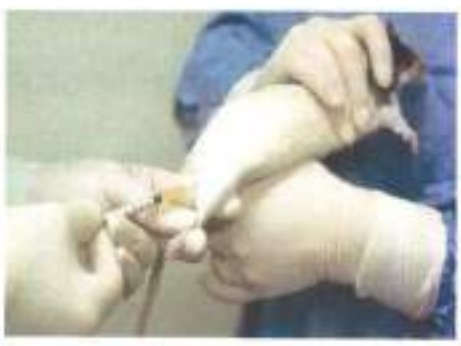
- Perfect aseptic conditions are needed
- Chances of abscess at the site of injection
- Chances of nerve damage leading to paresis of muscle supplied by it
- Large volumes cannot be given (maximum 5-10ml)

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





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 (inhalant anesthetic)
- The needle is being placed in the retro-bulbar space (the region behind the globe of the eye).

Routes of Drug Administration

❖Factors that should be considered before the administrating any substance (therapeutic or experimental) to an animal subject:

- PH
- Sterility
- Chemical nature (odor, taste, mucosal irritability, osmolarity, solubility, light sensitivity, and hazard status)
- The dose to be administered, frequency of administration, volume to be administered, and the solvent (if necessary)

❖**The route of administration is determined primarily by:**

- The properties of the drug (ex. Water or lipid solubility, ionization,...)
- Therapeutic objectives (ex. The need for a rapid onset of action, the need for long term administration, restriction to a local site,.....)

Characteristics of drugs and solvent (Vehicle)

❑PH of compound

- Knowing the pH of the compound and the vehicle is crucial for the administration of the drug.
- Aim for pH around 7, if the pH is higher or lower then
  - Buffer to pH 7 if possible
  - Dilute the solution using sterile normal saline
- Injecting a high or low pH preparation as IM or SC would be painful and cause tissue necrosis
- Examples:
  - Pentobarbital is a very basic pH (~11). It can only be administered as IV or IP injections
  - Ketamine has an acidic pH (~4). It is administered as IP . IM administration can cause tissue necrosis

Characteristics of drugs and solvent (Vehicle)

❑**Taste and odor**

- Drugs having bitter tastes cause low subject compliance
- Many taste-making techniques, such as physical barrier coatings, chemical modification and sensory masking
- Ex. Tetracycline, Doxycycline, and Metronidazole
- Adding 2.0-0.5 g sucrose/100ml water making the solution 2-5% sucrose enhances palatability

❑**Mucosal irritability**

- Check tissue compatibility when administering any compounds to mucosal surfaces, i.e. eyes, mouth and trachea

❑**Solubility**

- Insoluble compounds may require to be administered as a suspension
- Example is sulfamethoxazole-trimethoprim which administered as a suspension in the drinking water. This suspension must be shaken daily to assure proper dosage

Characteristics of drugs and solvent (Vehicle)

❑Light sensitivity

- Protect against light exposure either by dispensing in a colored glass or cover clear glass or plastic with foil

❑Solvents (vehicle) characteristics

- Wide range of vehicle types are available, ex. :
  - Water : for enteral administration
  - Corn oil : gavage administration
  - Sesame oil : gavage administration
  - Ethanol :

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

THANK YOU

Study of Absorption and Excretion of drugs in Human.

Lab-2 / Experiment on the human

By

Assist. Lecturer

Nawar R. Hussein

Absorption

- Defined as the passage of a drug from its site of administration into the plasma. Therefore, it is important for all routes of administration, **except** intravenous injection.
- Cell membranes form the barriers between aqueous compartments in the body.
- An epithelial barrier, such as the gastrointestinal mucosa or renal tubule, consists of a layer of cells tightly connected to each other so that molecules must traverse at least two cell membranes (inner and outer) to pass from one side to the other

Types of transport across cell Membrane

1. Diffusion through lipid layers
2. Transfer through aqueous pores
3. Transport by carrier proteins: which include two main subtypes:.
    a. Passive transport
    b. Active transport: Need energy to transport against concentration gradient.
4. Pinocytosis

Factors affect the absorption of drug from GIT

- **First: Biological factors**
- **Second: Physiochemical factors**
- **Third: Pharmaceutical factors**

Biological factors

1. surface area of GI absorption sites
2. pH of gastrointestinal fluids
3. Gastrointestinal motility
4. Influence of food and diet in GIT
5. Hepatic metabolism (first pass effect)
6. Gastrointestinal disorders and presence of disease states

Physiochemical factors

1. Drug dissociation constant
2. Lipid solubility
3. Dissolution rate of drugs
4. Drug stability and degradation condition in GIT
5. Drug interaction properties with other constituents

Pharmaceutical factors

1. Types of dosage forms
2. Influence of excipients
3. Polymorphisms

Excretion

- Is the elimination of drug molecules from the bloodstream outside the body.
- Drugs are excreted or eliminated from the body as parent compounds or metabolites.

Renal excretion

- The kidney is the most important organ for the excretion of drugs and/or their metabolites.
- Some compounds are also excreted via bile, sweat, saliva, exhaled air, tears, hairs or milk, the latter a possible source of unwanted exposure in nursing infants.
- Drugs need to be reasonably hydrophilic to be excreted by the kidney, so that they will remain in the fluid that becomes the urine.
- Patients with impaired kidney function usually have a reduced ability to eliminate hydrophilic drugs.

Factors affect the excretion of drug from the body

- **First: Biological factors**
- **Second: Physiochemical factors**
- **Third: Pharmaceutical factors**

Biological factors

1. surface area of excretion sites
2. pH
3. Hepatic metabolism
4. Renal and hepatic disorders and presence of disease states

Physiochemical factors

1. Lipid solubility
2. Dissolution rate of drugs
3. Drug interaction properties with other constituents
4. Molecular size
5. Protein and tissue binding
6. Doses administrated

Pharmaceutical factors

1. Types of dosage forms
2. Influence of excipients

Saliva

- In recent years, saliva has been utilized for TDM. The advantage is that collection is noninvasive and painless and so it has been used as a specimen of choice in pediatric TDM.
- Due to the low protein content of saliva, it is considered to represent the unbound or free fraction of drug in plasma. Since this is the fraction considered available for transfer across membranes and therefore responsible for pharmacological activity, its usefulness is easy to understand.
- Drugs excreted in saliva enter the mouth and may be reabsorbed and swallowed.

Potassium iodide (KI)

- It's a salt of iodine added to Iodized table salt to keep most people healthy under normal conditions.
- KI is a safe and medically effective drug; Short-term use of KI at the proper dosage is safe for most people. KI is available without a prescription.
- The thyroid gland needs iodine to carry out its hormone production and iodine deficiency can cause hypothyroidism and most of the stable iodine in our bodies comes from the diet.

Objectives

- The aims of this experiment is to illustrate the considerable variation that exists in the rate of absorption and excretion of potassium iodide in two different dosage forms **(capsule, solution)** when administered orally.
- At the end of the practical class the student shall be able to:

1. Quantitatively estimate the levels of iodide in the saliva.
2. Understand the importance of timing sample collection in relation to drug intake when estimating drug levels.
3. Understand the importance of bioavailability and pharmacokinetics in clinical practice.

Materials

Drugs and solutions:

a) Potassium iodide 300mg capsules
b) Potassium iodide 300mg/5ml solution
c) Sulphuric acid 10% solution
d) Hydrogen peroxide 5%
e) Starch solution 1% in distilled water.

Apparatus: Droppers, containers and test tubes.

Procedure

Assigned students into 2 groups

1. A random sample of students was allocated to receive potassium iodide 300 mg in capsules and another receives potassium iodide 300 mg in solution.
2. Two samples of saliva are collected every 10 minutes for 1 hour.

These samples are tested as follow:

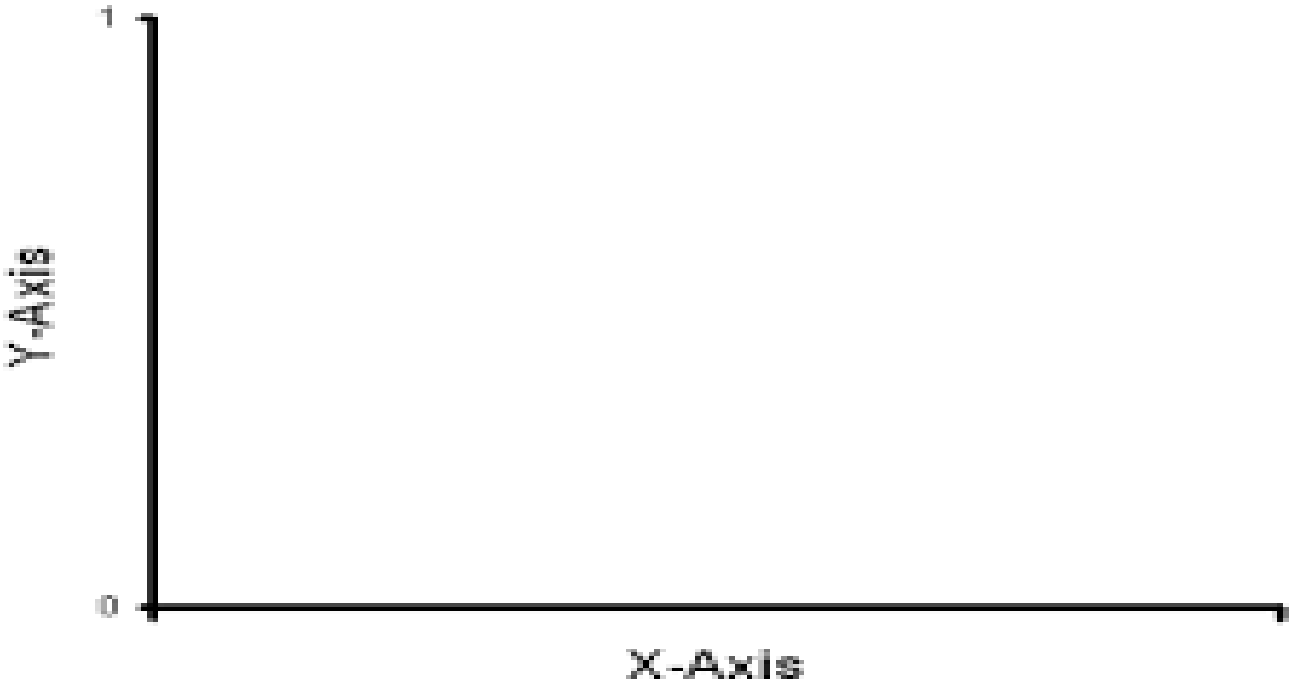
Testing the samples of saliva :

- 4 drops (saliva) + 5 drops (H_2O_2) + 4 drops (H_2SO_4) +1 ml starch solution
- Shaking for 3 seconds.
- Blue color indicate a positive test (presence of iodide) ,the intensity of which indicates the concentration of KI.
- The approximate values are obtained by color intensity (+ve,++ve,+++ve….etc.).
- Tabulate the results and plotted in a graph paper (X axis time, Y axis concentration) to show the rate of excretion consequent to absorption as below:

Result

Time	KI (capsule)	KI (solution)
	Presence of iodide in Saliva	Presence of iodide in Saliva
10 min		
20 min		
30 min		
40 min		
50 min		
60 min		

Plot the graph (X axis time, Y axis concentration (intensity of the color) to show the rate of excretion consequent to absorption of capsules vs. solution dosage forms



Practical Pharmacology

Barbiturates

thiopental and dose calculations

lab 3

By

Assist. Lecturer

Nawar R. Hussein

Overview:

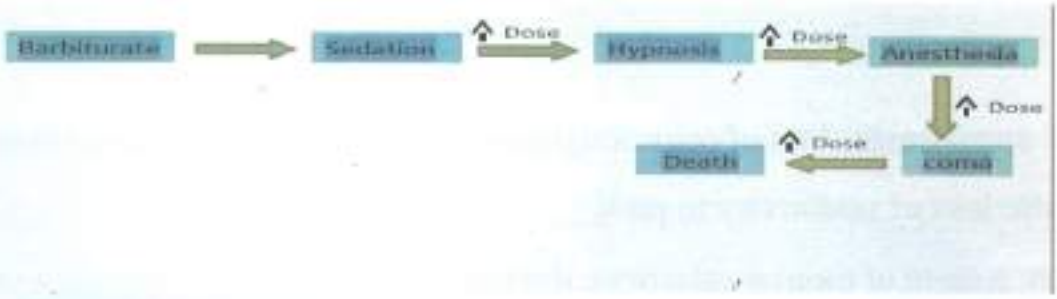
- **General anesthesia:** loss of consciousness in addition to loss of sensation
- **Analgesia:** loss of sensitivity to pain.
- **Sedation:** a state of mental calmness, decreased response to environmental stimuli,
- **Euthanasia:** is the practice of intentionally ending a life to relieve pain and suffering.

Barbiturate

- Class of drugs that act as central nervous system depressants, and can therefore produce a wide spectrum of effects, from mild sedation to total anesthesia
- Long acting barbiturates, ex. **Phenobarbitone which has 6 8 hr (duration of action), 4-5 days elimination half life**
- Short acting barbiturates, ex. **Butobarbitone** and **Pentobarbitone** 2 4 hrs. (duration of action)
- Ultra short acting barbiturates, ex. **Thiopentanl** .(Duration of action **20-25 min**, h1/2 = **8-10**)
- They are also effective as **anxiolytics, hypnotics, and anticonvulsants**
- Barbiturates also have analgesic effects, however these effects are somewhat weak preventing barbiturates from being used in surgery in the absence of other analgesics
- They have addiction potential, both physical and psychological

Barbiturate

- CNS DEPRESSANT



- Used in anesthesia and the treatment of epilepsy and seizure disorders
- Respiratory effects are dose dependent :
  - At hypnotic, little effects.
  - At high dose, depress respiratory center.
  - Death due to respiratory failure.

Barbiturate

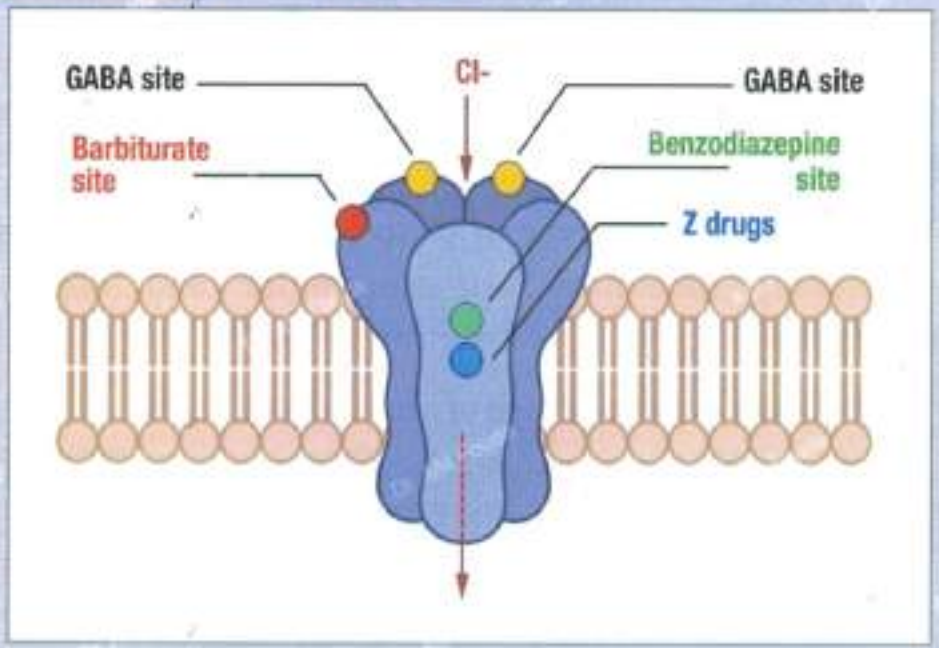
✓Barbiturates have now largely been replaced by benzodiazepines in routine medical practice, mainly because benzodiazepines are significantly less dangerous in overdose

- Narrow therapeutic index
- Suppress REM sleep,
  - **barbiturate shortened the time taken to sleep and lengthened the delay to rapid eye movement (R.E.M.) sleep**
- Quick tolerance
- High potential for dependence and abuse (addiction).
- Potent inducer for liver metabolizing enzymes

Mechanism of action

(γ-aminobutyric acid)

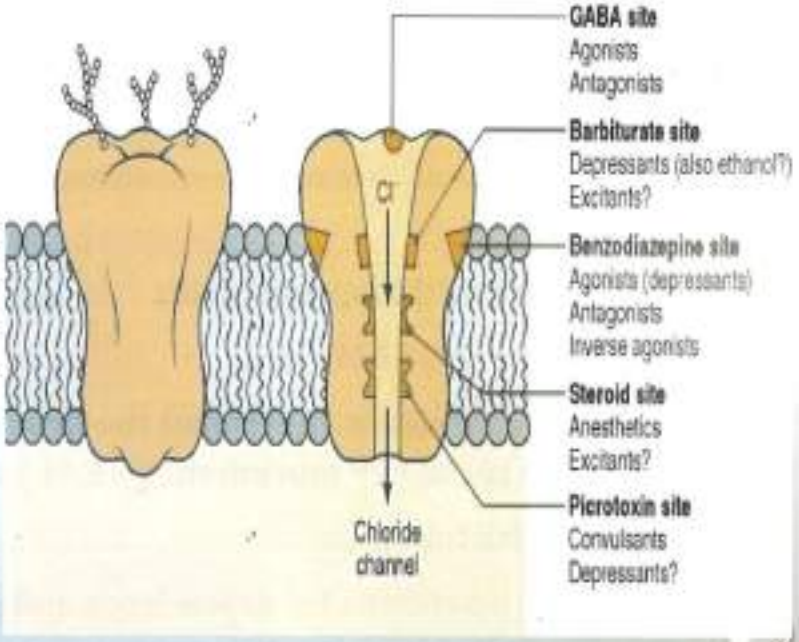
GABA-A Receptor



Mechanism of action

GABA Modulators:

Enhance binding of inhibitory GABA with its receptors GABA-A binding site, they cause allosteric modulation of GABA action on GABA-A receptor, prolonged the duration of the GABA-gated Cl^--channel opening (influx of Cl^-)→ hyperpolarization →**CNS depression.**



At higher concentration:

Barbiturate directly increase cl^- conductance (GABA mimetic action contrast BZDs which have only GABA facilitatory action).

Block the AMPA receptor (a subtype of glutamate receptor), leading to decrease the activity of excitatory glutamate neurotransmitter and depress the neuronal depolarization effect.

They inhibit the ca^{+2}- dependent release of neurotransmitters.

The action of barbiturates is non selective i.e. increase dose of barbiturates → generalized CNS inhibition.

Barbiturate

Pharmacokinetics

- High lipid solubility ⟹ cross blood brain barrier, rapid onset.
- Redistribution to other tissues outside the CNS ⟹ short duration of action.
- Liver: All metabolized into inactive metabolites except (**Phenobarbital excreted unchanged**).
- Renal excretion.
- Alkalization of urine in case of toxicity.

Adverse effects

- Drowsiness, disorientation, respiratory depression.
- Tolerance: decreased responsiveness to the drug upon repeated administration.
- Dependence: both psychological and physiological.
- Withdrawal is muscle more severe than that associated with opiates and can result in death.
- Peripherally hypotension due to myocardial depression and depression of vasomotor center (VMC).

Potent inducer for liver metabolizing enzymes

- Chronic use of barbiturates will cause upregulation, or induction, of the microsomal enzymes (CYPs), increasing the metabolism of endogenous substrates and other drugs metabolized by these enzymes this can lead to patients requiring larger dosages of medication to achieve therapeutic effect and/or increased clearance.
- This enzyme induction also causes barbiturate tolerance due to increased barbiturate metabolism.

Barbiturate

- **Advantages:**
  - Rapid anesthetic onset;
  - Provides a prolonged duration of surgical anesthesia.
  - Can be a sedative.
  - Anesthetic agent or euthanasia agent depending on the dose
- **Disadvantages:**
  - Prolonged recovery time.
  - Inadequate analgesic properties.
  - Extremely expensive.
  - Narrow margin of safety.
  - Produces respiratory depression at higher dosages.
  - Potent inducer for liver metabolizing enzymes.
  - They have addiction potential, both physical and psychological.

Calculate your dose

The dose is the amount of drug taken at any one time.

- Weight of drug (e.g. 250 mg).
- Volume of drug solution (e.g. 10 ml, 2 drops).
- The number of dosage forms (e.g. 1 capsule, 1 suppository).
- Other quantity (e.g. 2 puffs).

The dosage regimen is the frequency at which the drug doses are given. Ex. 2.5 ml twice a day, one tablet three times a day...

- Accurate dosing is critical for the proper utilization of all pharmaceuticals.
- First you need to know what volume you want to inject into the animal with each treatment being administered, then you need to know how much drug should be in that given volume.

Calculate your dose

- To calculate the correct dose of drug you need to know
  - **The concentration of the drug**
  - **The weight of the animal**
  - **The recommended dose rate of the drug for each specific animal model**
- **Concentration of the drug**
  - **Mg/ml:** manufacturers usually provide concentrations of their product in milligrams (mg) of drug per (ml) of solvent
  - **% : grams per 100 ml:** 10% solution of drug A is 10gm/100ml, a 2% solution of drug A is 2gm/100ml or (20mg/ml)
    - **IU/ml:** international units per ml of, like some of the fat soluble vitamins
  - **Powders:** the mg of active drug in the vial. For example, drug Telazol tiletamine and zolazepam) comes in powdered form with comes in powdered form with 500mg per vial:
    - If you add 5ml of sterile water for injection to the vial thus providing 5ml of 100mg/ml drug
    - If you add 2.5ml of sterile water for injection, will make 2.5ml of a 200mg/ml solution

Calculate your dose

- **Weight of the animal**
  - It is always best to use a scale and get an accurate weight
  - If you cannot weigh the animal prior to injection, you need to be experienced in estimating the weight
- **Dose rate of the drug**
  - Always look up the drug dose for the species you are working with - it often varies

Calculate your dose

Practice

- For most applications the following formula is applicable:

  $(C1)(V1) = (C2)(V2)$

- Ex. You have 20 ml of a 10 mg/ml solution and you want to make 15 ml of a 2.5 mg/ml solution. Set up the math as follows:

  C1 = 10 mg/ml C2 = 2.5 mg/ml V1 = unknown V2 = 15 ml (10 mg/ml) (V1) = (2.5 mg/ml) (15 ml)

  V1 = 3.75 ml

So you dilute 3.75 ml of C1 to a final volume of 15 ml therefore you need to add 15 - 3.75 =11.25 ml of diluent

- How to administer xylazine at a dose rate of 10mg/kg to a 300 g rat?

You are using 2% xylazine.

The proper dose for a 300g rat is: 10x0.3kg= 3mg of xylazine 2% xylazine is 20 mg/ml

3/20=0.15 ml of 2% xylazine

Calculate your dose

Practice

- Let's say you want to treat 15 rats with 75mg/kg of a compound at the rate of 0.1ml/20 gm of body weight and you want to prepare enough drug to dose 4 days, weight of each rat is 100gm.
  - Number of animals = 15
  - Dose to each animal = 75mg/100gm OR 7.5mg/100gm
  - Volume injected to each rat =0.1ml/20gm OR 0.5ml/100gm
  - Thus, each 7.5mg compound should be dissolved in 0.5ml vehicle (for one rat)
  - (7.5mg/0.5ml) x 15 = 112.5mg/7.5ml (for 15 rats in each day)
  - (112.5mg/7.5ml) x 4 = 450mg/30ml (to 15 rats for 4 days)
  - So, we weight 450mg compound and dissolve it in 30ml vehicle, and inject each rat with 0.5ml of solution for 4 days
- Steps may be done at random!!

Experiment protocol

- Thiopental 1 gm vial
- Thiopental , 40 mg/kg I.P in mice.
- 3 mice receive **I.P**

Measured Parameters

- ✓ General activity
- ✓ Characteristics of breathing
- ✓ **Onset of sleep (mins)**
- ✓ **Duration of sleep (mins)**

- Barbiturates are hypnotic drugs:
  - **Onset of action** is the time required to loss the righting reflex.
  - **Duration of action** in mice can be measured by the 'sleeping time' (i.e. The time from the loss of righting reflex to recovery of reflex).

Experiment protocol

- **The loss of righting reflex (LORR) assay** was used to evaluate sedative/hypnotic effects
- **Righting reflex** the ability to assume an optimal position when there has been a departure from it
- The onset time of sleep was noted for all animals. After induction of sleep, mice were placed in the inverted position and when sedation was over, the mice came to normal posture and time was noted
- Record:
  - LORR was recorded as the time at which the animal was unable to turn itself ***(onset of action)***
  - The time to regain the righting reflex ***(duration of action)***

Judgment of sleep and wake

← Loss of righting reflex (sleep)

Recovery of righting reflex (wake) →

Experiment protocol

Report

- Name & aim of experiment
  - ➢ You need to write the name of experiment and the aim of work, for ex.
    - **We aimed to investigate and evaluate the sedative/hypnotic effects of Thiopental administered IP in mice**
- Methods: species/drug/dose/ROA
  - ➢ Mention the animal used, the drug injected with the doses. Describe the work. For example:
    - **Three mice were injected with thiopental 40 mg/kg IP. The LORR and duration of sleep were recorded..... etc.**
- Results: onset of action/duration of action/analyze data (t-test)
- Discussion: interpretation of the results.

Experiment protocol

- **Report:**
  - **Results:**
    - Compare Onset and duration for between IP and SC routes using t test **(two groups, use excel, $p<0.05$ is considered significant difference)**

Animals	I.P. LORR time	SC LORR time	I.P.sleep time	SC sleep time
Mouse 1				
Mouse 2				
Mouse 3				

- **Discussion**: mention and discuss your results, for example:

From the results obtained, we noted that onset of action was faster in IP than SC route. This is due to ..... etc.

Thank you

Study of the action of drugs on eyes

Lab. 4

By

Assist. Lecturer

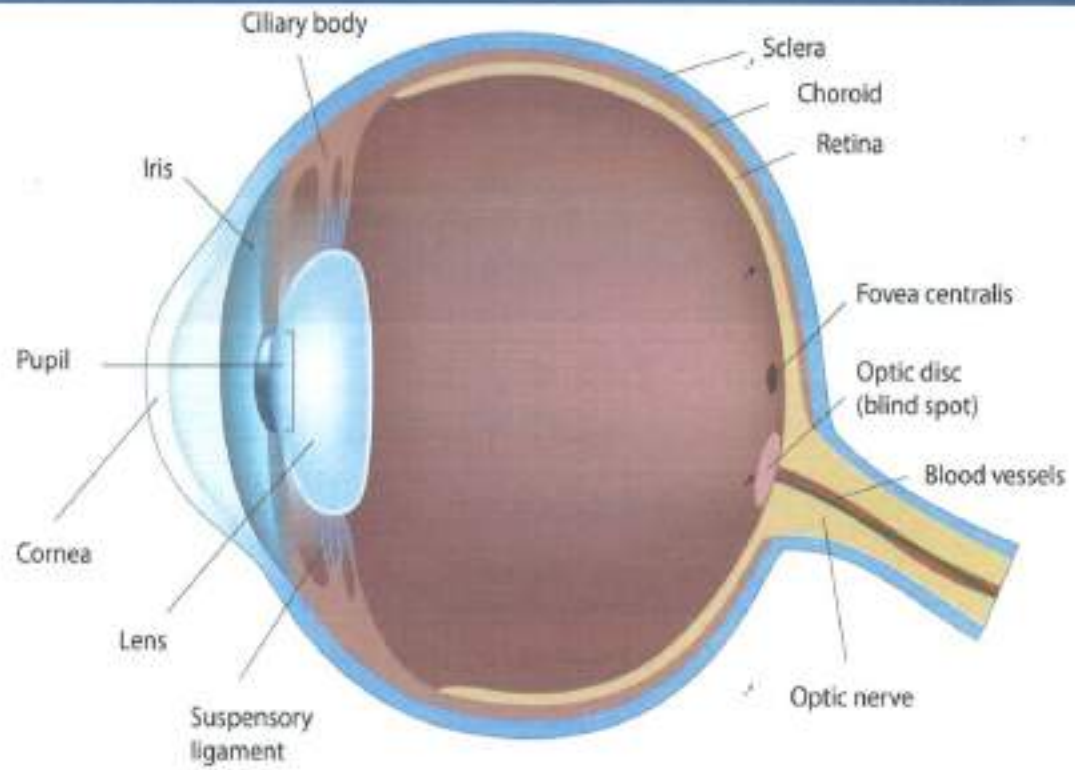
Nawar R. Hussein

Eye

- is specialized sensory organ that mediate vision. The eye focuses images from external environment onto retina & convert them into electrical signals, which then recognized by the brain.

Anatomy & Physiology of the Eye

The main compartments of the human eye are cornea, iris, lens, ciliary body and vitreous humour.

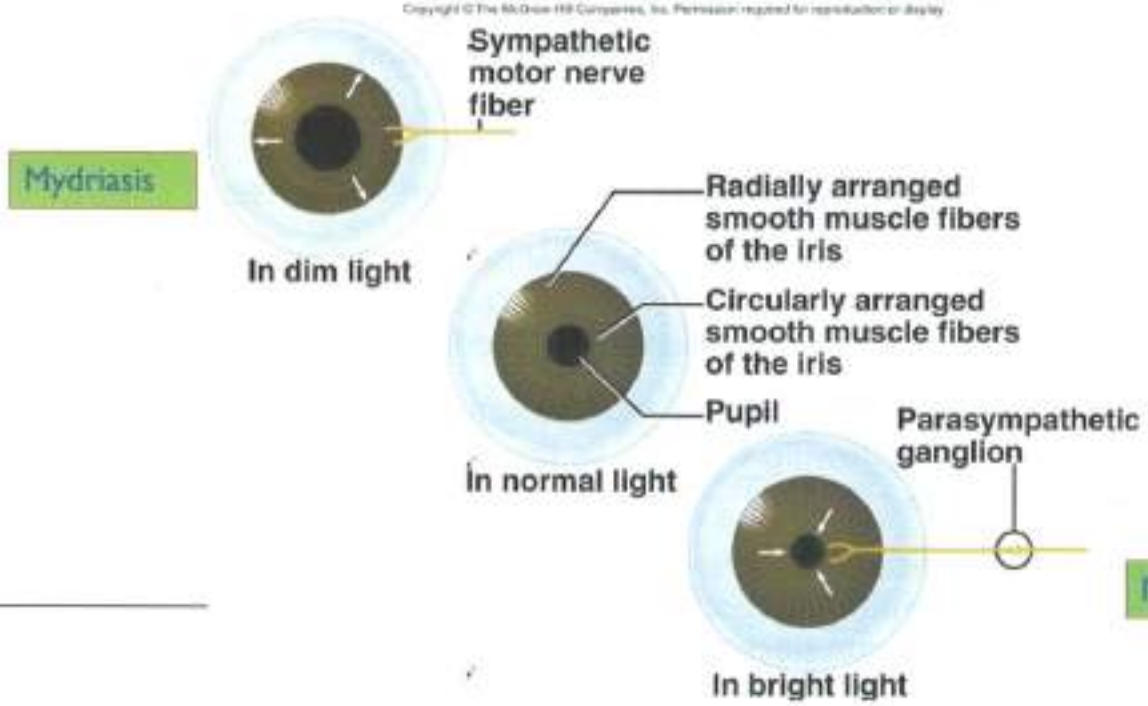


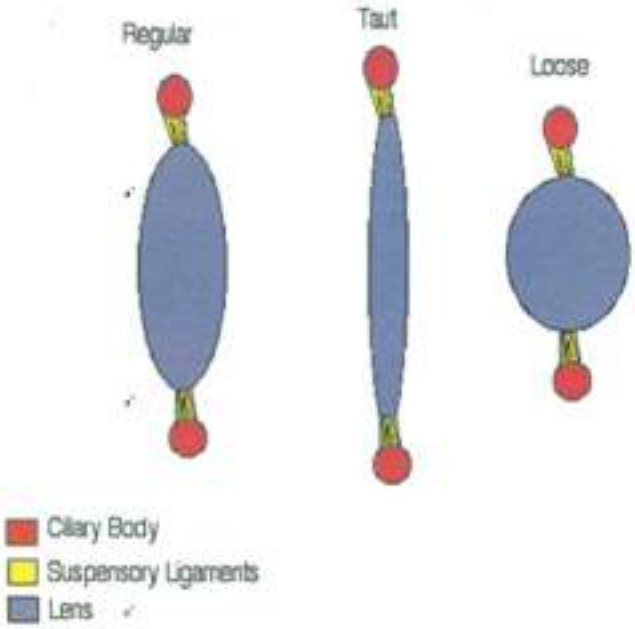
Pupil diameter

- The pupil is the hole in the center of iris. The diameter of the pupil (pupil size) & hence the amount of light entering the eye is regulated by two anatomically innervated set of smooth muscles:
- radial muscles which innervated by adrenergic fibers (containing alpha1- receptors).
- circular muscles which innervated by cholinergic fibers (containing M3-receptors).
- Notes: Miosis: is due to either contraction of circular muscle or relaxation of radial muscle. Mydriasis: is due to either contraction of radial muscle or relaxation of circular muscle

Human eye anatomy

Regulation of the amount of the light

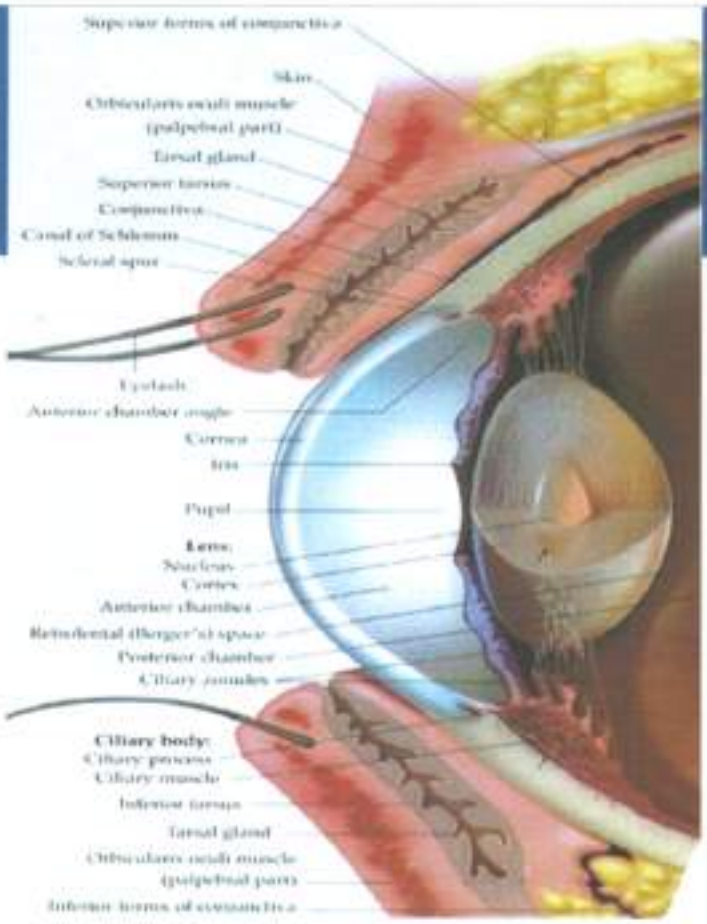




· Figure : The contraction and relaxation of the lens

Accommodation for near & far vision

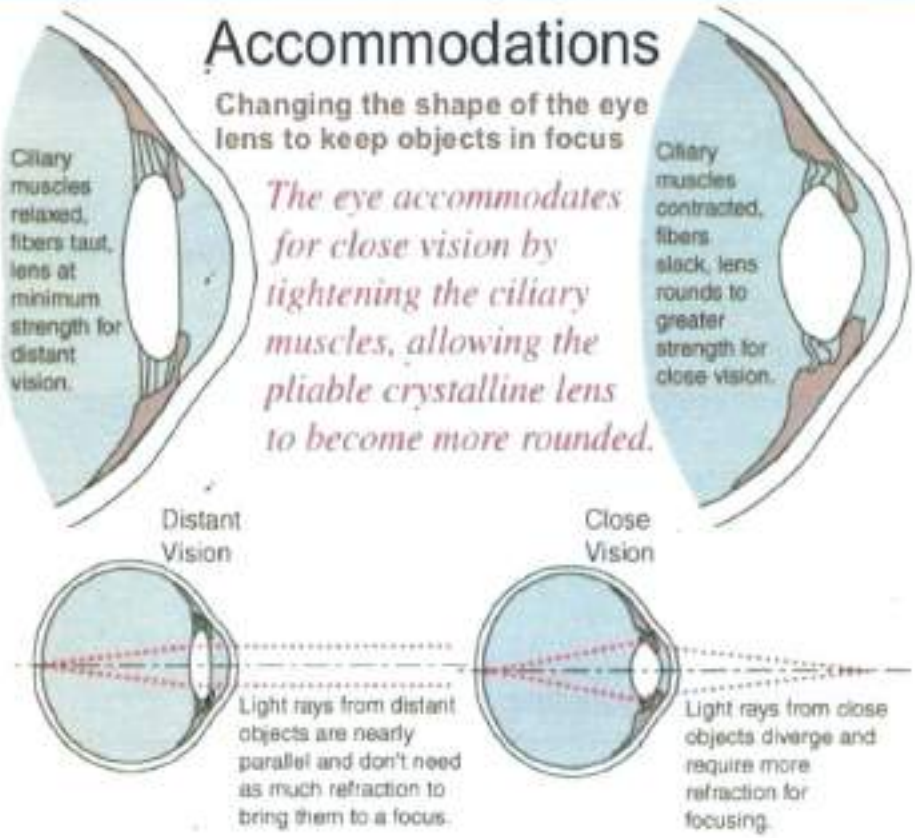
- Ciliary body involves ciliary muscle and ciliary epithelium
- Ciliary muscle (M receptors): responsible for near or far vision.
- M-agonist → Ciliary M. Contraction → Lens contraction → near vision
- Anti-Muscarinic → Ciliary M. Relaxation → Lens relaxation → far vision
- Contraction of ciliary muscle in response to cholinergic activation (M_3 receptors) causes these suspensory ligaments to relax allowing the lens to become more convex & thus reducing its focusing to near objects only.
- relaxation of the ciliary muscle (e.g. by antimuscarinic agents) the suspensory ligaments will be stretch allowing the lens to become more flat so that the lens focused for distant objects.
- Drugs that antagonized accommodation for near vision termed cycloplegics, they are exclusively muscarinic antagonists. sympatholytic agents do not alter accommodation for near vision since there are no adrenergic receptors in the ciliary muscle.



- Figure : Sagittal section in the eye showing the lens and ciliary body

Human eyes accommodation

Light focusing



Drugs and human eye

Topical administration

- Eye drops
  - Principally absorbed through the cornea
  - Short drug-eye contact time
- Eye ointments
  - Allow a prolonged contact time
- Eye lotions
  - Used for irrigation

Local injections and systemic treatment

- Physiological barriers limit systemically administered drug penetration to the eye
- Ex. acetazolamide for severely raised intraocular pressure

Drugs and human eye

Ophthalmic anesthetics

•Ophthalmic anesthetics are agents that act locally to block pain signals at the nerve endings in the eyes

•Anaesthetic drops:

- Initial assessment of minor trauma
- Removal of conjunctival and corneal foreign bodies
- In surgery

•Example:

- Propracaine Hydrochloride 0.5% (Alcaine)
- Tetracaine 0.5%

•Side effects:

- Allergy: local or systemic

Alcaine eye drop

Drugs and human eye

- **Dilating Drops (mydriatic medications)**
- Mydriatics are used to enlarge the pupil for eye examinations
- Used in diagnosis and surgery
- **Parasympathetic antagonists (parasympatholytics)**
  - Paralyzing the iris sphincter muscle
  - Make the pupil larger and paralyze the muscle involved in focusing of the lens (accommodation)
  - Blurry eyes especially for up close (reading, near play)
  - ❑ Examples :
  - Tropicamide: (Mydriacyl) 0.5% and 1%. Action up to 6 hours
  - Cyclopentolate: (Cyclogyl) 0.5%, 1% and 2%. Action up to 24 hours
  - Homatropine: 2% and 5%. Action: 2-3 days.
  - Atropine: (Atropisol) Drops 0.5% or 1%, ointment 1%. Action: 1-2 weeks
- **Sympathetic agonists (sympathomimetics)**
  - Stimulate the iris dilator muscle.
  - Ex: Phenylephrine: 2.5% and 10%. Action 3-6 hours.

Drugs and human eye

Miotic agents

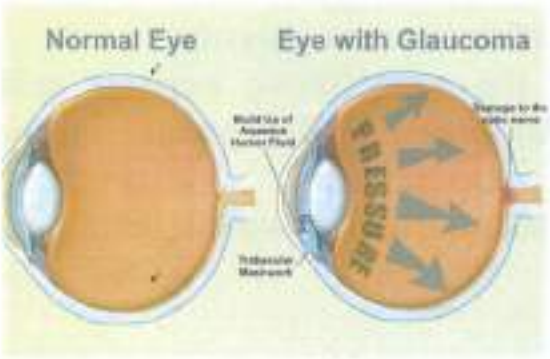
- Dapiprazole (α1-antagonist)
- Pilocarpine (M3 agonist)
- Isoproterenol, thromboxane A2, yohimbine, Tolazoline, prostaglandin growth factor 2α (PGF2α), ionomycin, thapsigargin,.. etc.

Glaucoma

- Disease of the eye in which fluid pressure within the eye rises
- May lead to vision lose
- Affects both eyes
- Symptoms :Loss of peripheral vision Sensitivity to light and glare

Problems with night vision, and Blurred vision

- Characterized chiefly by an increase in IOP above 21 mmHg & may be as high as 70 or 80 mmHg during the attack



Pathophysiology of glaucoma

- The aqueous humor is a transparent, gelatinous fluid. It is secreted from the ciliary epithelium.
- In glaucoma, aqueous humor builds up and increases pressure within the eye
- Ciliary Epithelium (B2-Receptors) Responsible for secretion of aqueous humor.
- Ciliary muscle contraction → Increases flow → Decreases IOP.
- Ciliary muscle Relaxation → Decreases flow → Increases IOP (Glaucoma).

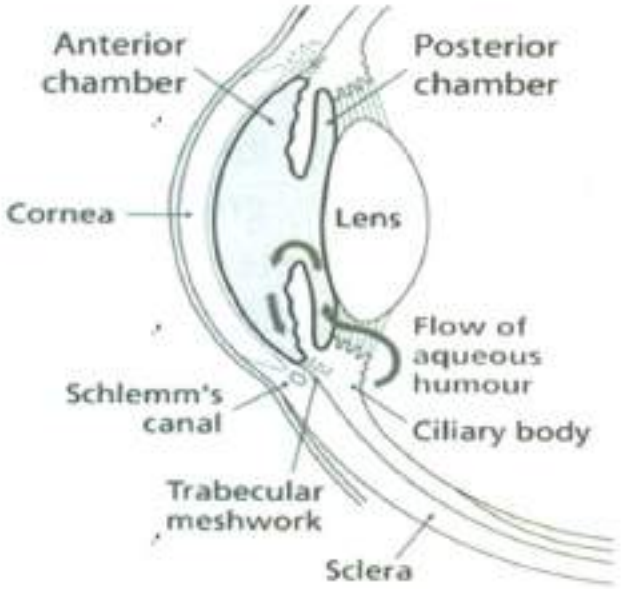


Figure 1: Production and flow of aqueous humour in the eye. © 2013 British Journal of Anaesthesia

Glaucoma

Prostaglandins

- Treat open-angle glaucoma
- Prostanoid selective FP receptor agonist
- Increase the outflow of the aqueous humor
- Ex. latanoprost (Xalatan) and bimatoprost (Lumigan)

Beta blockers

- Reduce the production of intraocular pressure
- Blocks the action of the sympathetic nervous system, Causing reduction of intraocular pressure. The precise mechanism of this effect is not known
- Ex. timolol (Betimol, Timoptic) and betaxolol (Betoptic)

Glaucoma

- **Alpha-adrenergic agonists**
- Reduce the production of aqueous humor and increase its outflow
- Ex. apraclonidine (Iopidine) and brimonidine (Alphagan)
- **Carbonic anhydrase inhibitors**
- Systemic administration (oral)
- Carbonic anhydrase is an enzyme founded in the biochemical production of aqueous humor
- Reduce the production of aqueous humor
- Ex. dorzolamide (Trusopt) and brinzolamide (Azopt)
- **Miotic or cholinergic agents**
- Increase the outflow of fluid within eyes
- Ex. pilocarpine (Isopto Carpine) and carbachol (Isopto Carbachol)

Objectives

- At the end of the practical class the student shall be able to:

1. Instill drugs carefully into the Rabbits eye by the pouch method without injuring the cornea.
2. Study the effects of drugs on the eyes

Methods

Place one drop of the agents in the following table into on eye of the rabbits and check for the parameters mentioned in the following table:

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**

❖ 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. This is also true for procaine (local anesthetic) as the cornea does not absorb it, so it cannot cause loss of corneal reflex.

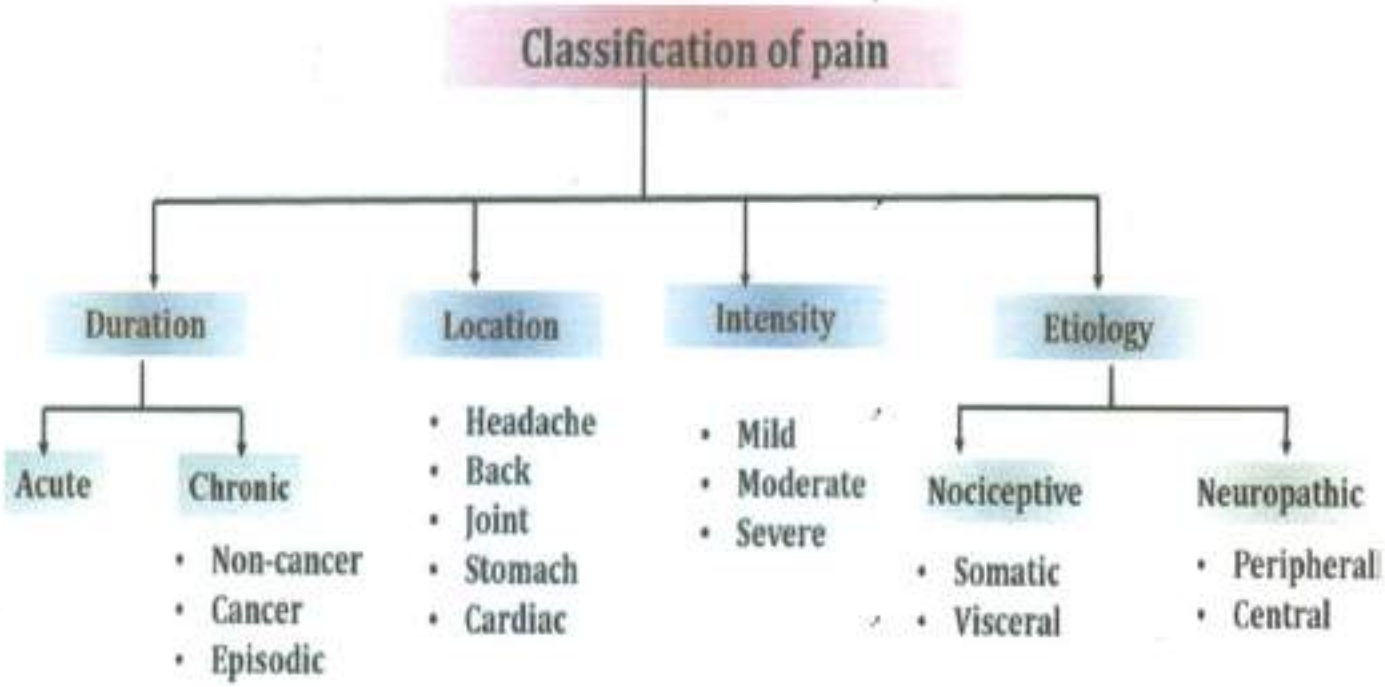
Thank you

Evaluation of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Lab. 5
By
Assist. Lecturer
Nawar R. Hussein

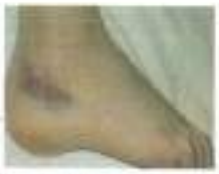
Pain

- Pain is an unpleasant sensory and emotional experience.
- Is one way the body tells you something wrong and needs attention.
- Acute pain typically comes on suddenly and has a limited duration (less than 3 months)
- It's frequently caused by damage to tissue such as bone, muscle, or organs, and the onset is often accompanied by anxiety or emotional distress.
- Chronic pain: Defined as a Disease of pain
- lasts 3 months or longer than acute pain and is generally somewhat resistant to medical treatment. It's usually associated with a long-term illness, such as osteoarthritis
- Pain may be
  - **Nociceptive pain**
  - **Neuropathic pain**
  - **Mixed category pain**



Pain

nociceptive pain (Acute pain)



- Nociceptors
  - Nerves which sense and respond to parts of the body that suffer from damage.
  - They signal tissue irritation, impending injury, or actual injury. When activated, they transmit pain signals (via the peripheral nerves as well as the spinal cord) to brain.
- Time limited, meaning when the tissue damage heals, the pain typically resolves. (Arthritis is a notable exception in that it is not time limited).
- Tends to respond well to treatment with conventional analgesics.
- Examples include sprains, bone fractures, burns, inflammation.

Pain

Neuropathic pain

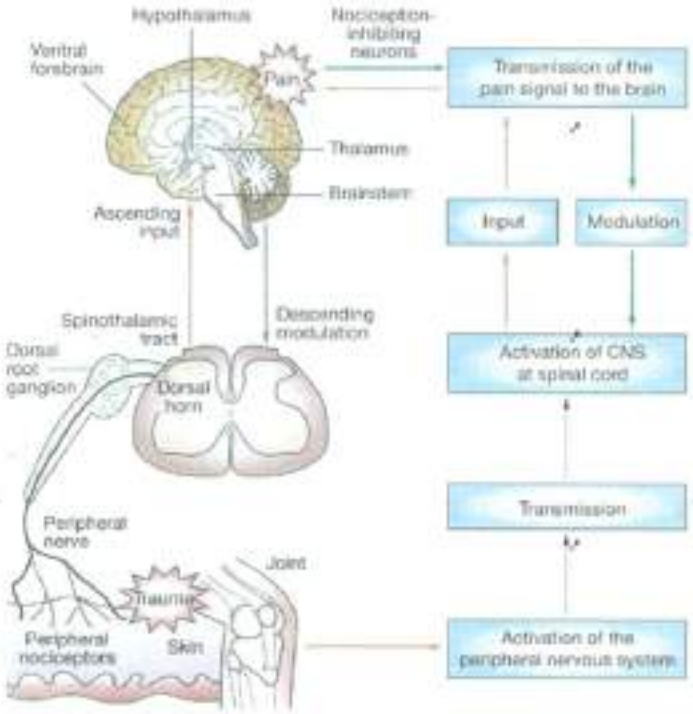
- result of an injury or malfunction in the peripheral or central nervous system. The pain is often triggered by an injury, but this injury may or may not involve actual damage to the nervous system
- The pain may persist for months or years beyond the apparent healing (chronic).
- Less response to treatment with conventional analgesics, but may respond well to other drugs such as anti-seizure and antidepressant medications.
- Examples : reflex sympathetic dystrophy / causalgia (nerve trauma), components of cancer pain, phantom limb pain, and peripheral neuropathy.

Pain

Mixed category pain

- Caused by a complex mixture of nociceptive and neuropathic factors.
- An initial nervous system dysfunction or injury may trigger the neural release of inflammatory mediators and subsequent neurogenic inflammation.
- For example, migraine headaches probably represent a mixture of neuropathic and nociceptive pain.
- Myofascial pain is probably secondary to nociceptive input from the muscles, but the abnormal muscle activity may be the result of neuropathic conditions.

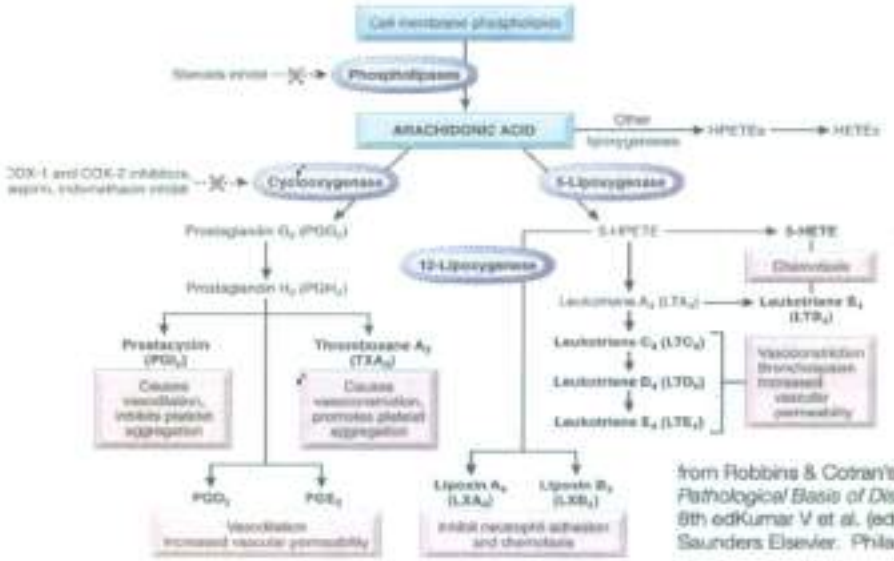
Pain stimulus formation



Inflammation

- Associated with injuries, Infections, antibodies, physical injuries
- Can be an exaggerated response with no apparent benefit
- Classic symptoms include warmth, pain, redness, and swelling
- Phases
  - Acute (Injury):
    - Transient local vasodilation
    - Increased capillary permeability
  - Delayed, subacute (infection):
    - Infiltration of leukocytes and phagocytic cells
  - Chronic proliferative phase (cancer):
    - Tissue degeneration and fibrosis

Arachidonic acid metabolites and inflammation



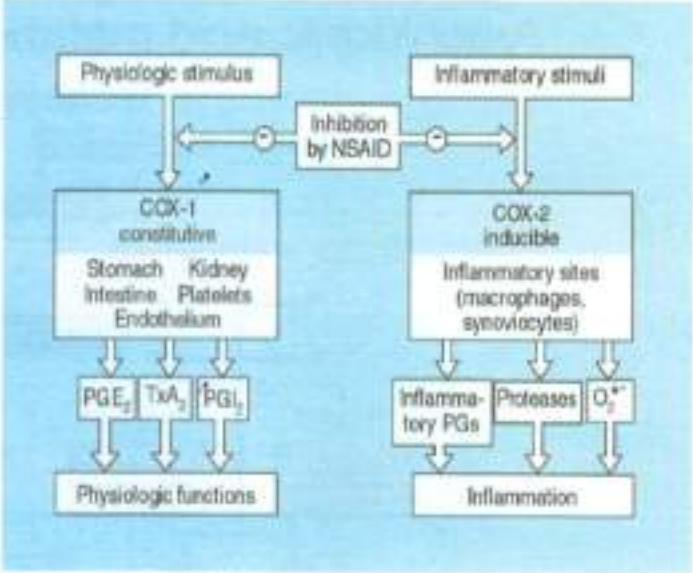
from Robbins & Cotran's
Pathological Basis of Disease
8th edKumar V et al. (eds).
Saunders Elsevier. Philadelphia (2010)

Prostaglandins

- There are currently nine known prostaglandin receptors on various cell types
- Prostaglandins act on a variety of cells, and have a wide variety of actions:
  - PGI2 (also called Prostacyclin) : inhibits platelet activation, and vasodilator
  - PGE2: decreases gastric acid secretion, increases gastric mucus secretion, labor (softens the cervix and causes uterine contraction), stimulates osteoblasts to release factors that stimulate bone resorption by osteoclasts, direct vasodilator, and induces fever (hyperpyrexia).
  - PGD2: contraction of the bronchial airways, involved in the regulation of reducing body temperature in sleep (opposite to prostaglandin E2), vasodilation, and male sexual development

COX1 Vs COX2

- COX-1 and COX-2 convert arachidonic acid to prostaglandin, resulting in pain and inflammation
- Cyclooxygenase-1 (COX-1) is known to be present in most tissues. In the gastrointestinal tract, COX-1 maintains the normal lining of the stomach. The enzyme is also involved in kidney and platelet function
- Cyclooxygenase-2 (COX-2) is primarily present at sites of inflammation
- Inhibition of COX-1 is undesirable while inhibition of COX-2 is considered desirable.
- COX-2 inhibitors potentially more selective for anti-inflammatory effects
- Less intestinal bleeding than with nonselective COX inhibitors



COX1 Vs COX2

- Inhibition of COX 1 is undesirable while inhibition of COX 2 is considered desirable
- COX 2 inhibitors are potentially more selective for anti-inflammatory effects
- Less intestinal bleeding than with nonselective COX inhibitors

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs):

•NSAIDs are available OTC

NSAIDs are a group of chemically dissimilar agents that differ in their antipyretic, analgesic, & anti-inflammatory activities.

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

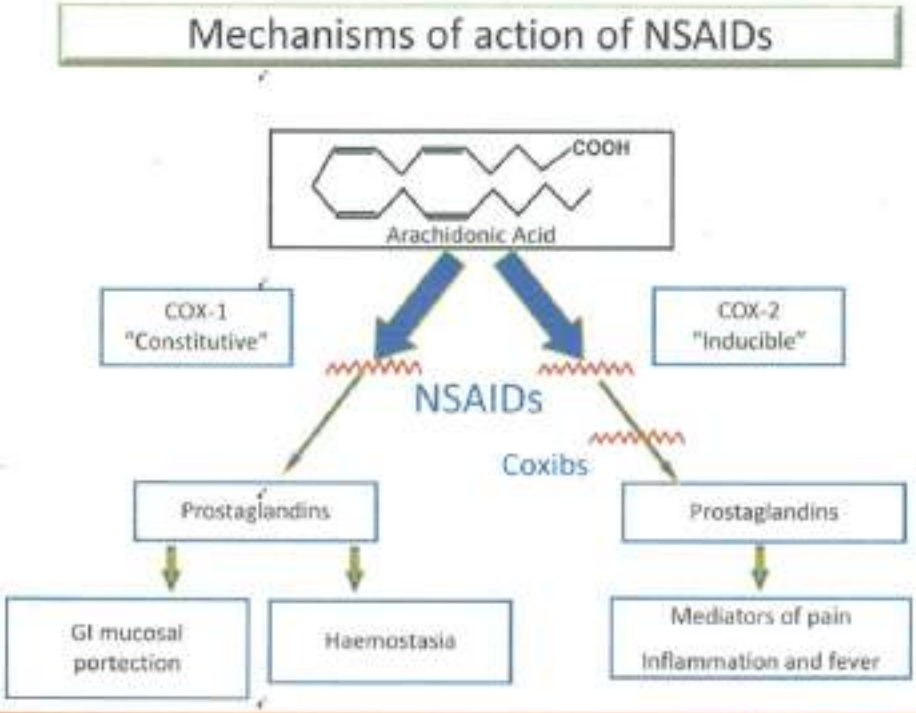
• Traditional NSAIDs include aspirin, ibuprofen, naproxen & many other generic & brand name drugs.

•A new NSAID like celecoxib, COX-2 inhibitor or a COX-2 selective NSAID

•Used to relieve pain and reduce signs of inflammation: fever, swelling, and redness

•Aspirin reduces fever by enhancing cutaneous blood flow and induces sweating and irreversibly inhibitor of inflammatory Cox 2 and PG.

•NSAIDs are a common treatment for chronic (long-term) health problems such as arthritis (rheumatoid arthritis, osteoarthritis, and others).



Aspirin as antiplatelet

Aspirin works by irreversibly inhibiting the enzyme cyclooxygenase (COX 1))(which is required to make the precursors of thromboxane within platelets), this reduces thromboxane synthesis Thromboxane is required to facilitate platelet aggregation and to stimulate further platelet activation

General unwanted effects of NSAIDs:

- Gastrointestinal (GI) and renal effects
- Dyspepsia, nausea & vomiting. Gastric damage may occur in chronic users, with a risk of hemorrhage.
- Reduce renal blood flow and decrease glomerular filtration by reducing prostaglandin synthesis, thus resulting in salt and water retention through stimulation of RAS.
- and thereby decrease the efficacy of diuretics, and inhibit the elimination of lithium and methotrexate
- On chronic use cause analgesic nephropathy
- All NSAIDs (except COX-2 inhibitors) prevent platelet aggregation & therefore may prolong bleeding.
- Hypocoagulability, which may be serious when combined with other drugs that also decrease blood clotting, such as warfarin
- Antagonize the effect of antihypertensives, such as ACE inhibitors, β-Blocker, and Loop diuretics, because part of the mechanism of action of these antihypertensive drugs is PG dependent pathway and inhibition of prostaglandin (synthesis(E 1, E 2 I 2 by NSAIDs leads to reduce the antihypertensive effect

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 rats(The noxious stimulus is an injection of dilute formalin (1% in saline) under the skin of the dorsal surface of the right hind paw).

Writhing method:

- **Principle**
- IP injection of the analgesic agent
- The painful stimulus is induced by IP injection of an irritant substance (e.g. acetic acid).
- Evolution of the analgesic effect by comparison to a control
- **Writhing**
- 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 (e.g., during 30 minutes), beginning 5 minutes after injection of acetic acid.

Acetic acid induced writhing method

- Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like acetic acid in mice.
- Analgesic activity of the test compound is inferred from a decrease in the frequency of writing
- the acetic acid-induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice
- Sensitive method for screening peripherally acting analgesjc and response is thought to **involve local peritoneal cells and mediated through the prostaglandin pathway.**

❖the test consists of :

- ✓ Injecting acetic solution intraperitoneally and then observing the animal for specific concentration of body referred as writhing
- ✓ A comparison of writhing is made between (Diclofenac –Na or meloxicam) and control sample given 30 minutes prior to acetic acid injection
- ✓ If the drug possesses analgesic activity, the animal that received the drug will give lower number of writhing than control, i.e the drug having analgesic activity will inhibit writhing.

Experimental protocol:

- Groups of animals are used for control & the treated mice.
- The control group is given acetic acid IP & after 5 minutes the number of writhes is recorded for each animal during 20 minutes.
- Treated animals are administered the drug (diclofenac or piroxicam) IP, 30 minutes prior to acetic acid administration. Then acetic acid is given IP.

Acetic acid in concentration of 0.7% is injected IP to all animals

- Five minutes are allowed to elapse, the mice are then observed for a period of 20 minutes & the number of writhes is recorded for each animal.

Results

Treatment	Dose (mg/kg)	Number of writhing	% inhibition
Control group (DW+ glacial acetic acid)	1ml/kg D.W + 0.7% i.p. (v/v) acetic acid (0.1ml/10g)		N/A
Group I : drug A (Diclofenac Na + glacial acetic acid)	10mg/kg i.p. Diclofenac + 0.7% i.p. (v/v) acetic acid (0.1ml/10g)		
Group II : drug B (Meloxicam + glacial acetic acid)	10mg/kg i.p. Meloxicam + 0.7% i.p. (v/v) acetic acid (0.1ml/10g)		

- **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