UNIT-III

PART - 1

PRECLINICAL SCREENING MODELS: FOR ANS ACTIVITY

Points to be covered in this topic

- ❑ Sympathomimetics
- ❑ Sympatholytics
- ❑ Para-sympathomimetics

❑ INTRODUCTION

- Sympathomimetics are the agents that mimic the activity of Adrenaline in the periphery.
- The **sympathetic receptors** are mainly found in **heart. Blood vessel, bronchi, uterus, GI tract and eye.**
- Stimulation of sympathetic nerves may trigger following responses: Heart rate **stimulation**, coronary arteries **dilatation, constriction** of pulmonary vessels, bronchial muscle **relaxation, inhibition** of gastric secretion, gastrointestinal sphincters, **contraction** and **stimulation** of uterus.

❑ ANIMAL MODELS USED FOR SCREENING SYMPATHOMIMETICS

S. NO.	SCREENING METHOD	DESCRIPTION
	A. IN- VIVO MODEL	
1	Bioassay of adrenaline by monitoring blood pressure in dogs	Intravenous (femoral vein) injection of adrenaline increases the blood pressure by activation of sympathetic receptors (α and b).
2	Rabbit eye model	Topical application of adrenaline (0.1%) or sympathomimetic solution dilates the pupil by activation of B2 receptor on the eye.
3	Cat spleen model	Sympathomimetics contract the spleen and trigger the noradrenaline release. Sympathomimetics are administered into the splenic artery by cannula thereafter the sample is collected for the estimation of the nor-adrenaline content.

4	Spinal cat model	Cats are also used to assay the adrenaline by measuring blood pressure. Posterior pituitary of cat is extracted out and followed the same procedure as dogs.
B. IN VITRO MODEL		
1	Bioassay of adrenaline using rabbit intestine	The intestinal duodenum is used in this model. Gl smooth muscle contains B_2 receptors and that is responsible for GI relaxation during sympathetic stimulation. Matching bioassay technique can be used to determine the potency of the test substance.
2	De Jalon's Method on Rat's Uterus	A virgin female rat is used to isolate uterine horn. Administration of sympathomimetics inhibits the contractile response of carbachol (0.75 pg/ml).ICs values (percent inhibition) of sympathomimetics are calculated.
3	De Jalon's Method on Rat's Uterus	This model is used to determine the heart rate on isolated frog's heart. Sympathomimetics like adrenaline and isoprenaline increases the heart rate and force of contraction by activation of B, receptor on the heart.

IN VIVO MODELS

I. A Bioassay of adrenaline by monitoring blood pressure in dogs:

- **Measuring blood pressure** is main parameter to evaluate **Adrenaline** and other **sympathomimetics.**
- When **Adrenaline is injected** by i.v. route to the animals, **blood pressures** (systolic and diastolic) **elevate rapidly** to a peak due to sympathetic stimulation

❖ Methodology:

- Healthy **dogs** are selected and anesthetized with Barbiturate
- Blood pressure is monitored by a **kymograph**.
- The **standard solution of adrenaline** (4% w/v in 0.1 HCl) is prepared and **different dilutions of adrenaline** are prepared by diluting it with saline.
- **Test sample** solution is also prepared in **saline.**
- Animals should be given Atropine Sulphate To eliminate vagal action.
- There will be **no fall in BP in Atropinised** animal after electrical stimulation or **Acetylcholine injection.**
- **Adrenaline** is injected into the **femoral vein** through the **venous cannula.**
- **two successive responses** of the same dose of Adrenaline are recorded.
- Then the test and the standard samples are given in **alternate** (intervals of 5min.) till both produce similar rise in blood pressure.

❖ Interpretation:-

Sympathomimetics raise the blood pressure.

The potency of the test sample is estimated by comparing the rise in BP of the test sample with the standard preparation of adrenaline.

II. Rabbit eye model:

- Administration of **sympathomimetic** drugs on **eye dilates the pupil.**
- They activate the **β_2 receptor** on the eye and cause the **ciliary muscle relaxation** and produce **mydriasis effect.**

❖ Methodology:

- Healthy young **rabbits** (2-3 kg) are used in this model.
- Animals are divided into different groups: control (vehicle treatment), standard and test group (test sympathomimetic treatment).

- 0.1% **Adrenaline Hydrochloride** solution is used as a **standard**.
- Eyes of rabbits are washed with distilled water and left for 5 min.
- Vehicle/standard drug/test drug is **applied topically** to their respective groups and **measured the pupil diameter**.
- **Calculate** the mean diameter in each group

Interpretation:- **Compare** mean pupil diameter of **standard** and **test groups** with the **control group.**

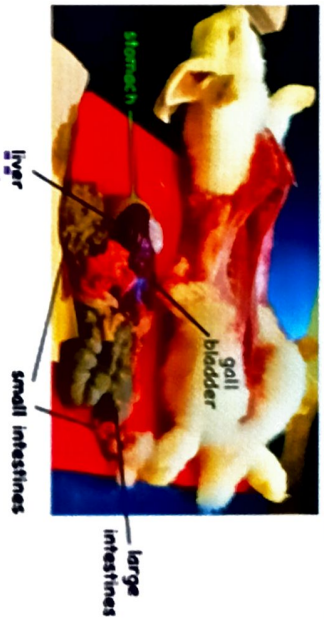
The topical administration of adrenaline or other **sympathomimetics increases the pupil diameter** and produce **mydriasis effect** in the eye.

III. Cat Spleen model:

- **Sympathomimetics** **contract the spleen** and release the transmitter.
- Released **Nor-adrenaline** can be measured by collecting spleen, venous effluent and analyze the noradrenaline content.
- Healthy **cats** are used in this method.
- The sympathomimetics **increase** the **release of noradrenaline.**

IN VITRO MODEL

I. Bioassay of adrenaline using rabbit intestine:



- Adrenergic β_2 **receptors** are found in the **intestinal smooth muscles**.
- Activation of sympathetic β_2 receptors **relaxes the intestine via cAMP pathway.**

Methodology:-

- A healthy **rabbit** (2-3 kg) method is described.
- Rabbit is sacrificed and is dissected out to **isolate the duodenum** and small piece of duodenum is **suspended in the organ bath** containing **Tyrode solution** at 37.5°C.
- The **muscle** is allowed to **stabilize for 30 min**

- Rhythmic contractions are recorded by isotonic frontal writing lever on the kymograph.
- After recording baseline, adrenaline (1 µg/ml2 µg/ml, 4 µg/ml) is administered in an increasing order upto ceiling effect with proper washing interval.
- The test sample is administered and matched with the standard.
- Adrenaline relaxes the duodenum and the same property is taken upto consideration for finding out the potency of the test sample.
- Determine the dose of test solution producing the same response by a sequence of matching technique

II. De jolno's method on rat's uterus:

- Sympathomimetics relax the uterus by activation of β_2 receptor
- The **parasympathomimetic** like carbachol contracts the uterus, which is **inhibited by adrenaline.**

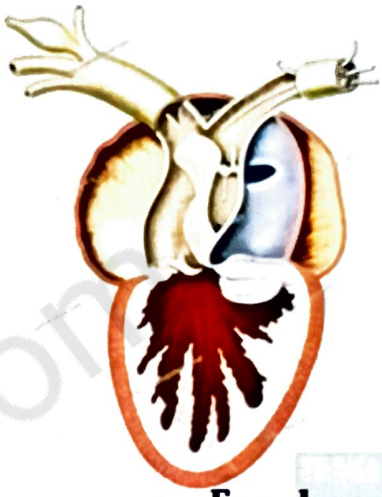
❖ Methodology:-

- The virgin **female rat** (100-150 g) is sacrificed to isolate the **uterine horns.**
- Uterine horns are ligated and suspended in a mammalian organ bath containing modified **Ringer solution** and maintained at 37°C with aeration.
- **Two identical contractions** for 30 sec. with Carbachol (0.75 ug/ml) are recorded.
- The **standard** solutions of Adrenaline and **test** solution are prepared.
- 3-5 different doses of standard adrenaline and test is selected for con. response curve.
- After washing the tissue preparation, adrenaline/test drug is **administered with carbachol** to record the response.

- Each dose-response is taken after 3 washings.
- **Percentage inhibition** is calculated against carbachol alone (100%).

❖ **Interpretation:-** The **ICs value** of **adrenaline** and **test drug** is calculated by concentration **response curve**. The potency of test drug is compared with adrenaline.

III. Isolated frog heart preparation:



Frog heart

➢ The **sympathomimetics** like adrenaline and isoprenaline increase the **heart rate** and **force of contraction** due to **activation of β_2 receptor** on heart, while noradrenaline administration has little effect on heart rate because Nor-adrenaline generally acts on α receptor.

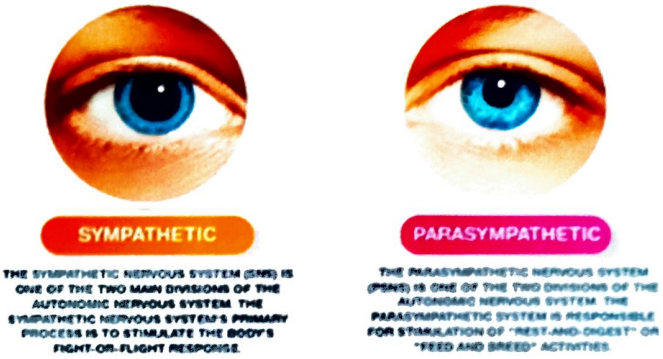
Methodology:-

- A healthy **frog** is sacrificed by pithing and dissected to expose the heart.
- **Sinus venous** is cannulated using a glass cannula and secured with thread.
- Then the heart is isolated along with the cannula and perfused with **Frog-Ringer** through the sinus venous.
- A **curved needle** is inserted in apex and attached to a startling heart lever for **recording** contractions on the kymograph.
- After recording baseline (basal heart rate), **Adrenaline** (2 pg/ml)/ **test** sympathomimetics are administered and **recorded the response**.
- Sympathomimetics **accelerate the heart rate.**

Interpretation:- **Observed heart rate** is **compared** with **basal heart rate** However, this method is now absolute due to constraints.

❑ INTRODUCTION

- Sympatholytics are agents that **inhibit** the action of the **sympathetic nervous system** by **blocking the sympathetic receptors** (α and β).



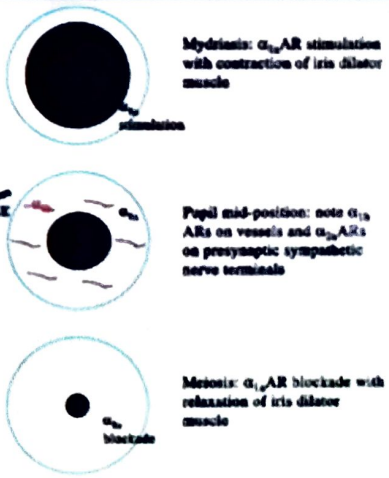
- They **decrease** the **blood pressure and heart rate, relax the vascular smooth muscle**, **inhibit** the **mydriasis effect** in the eye, and **inhibit** the **relaxing effects in tracheal** chain induced by sympathetic system activation.

❑ Animal models used for screening sympatholytics

Sr. no.	SCREENING MODEL	DESCRIPTION
	A. IN VIVO MODELS	
1	Adrenergic antagonism in the rodent's eye	Sympatholytics block the action of sympathetic system (mydriasis effect) in the eye. Rodents (rats and mice) are used in this model
2	Blood pressure response in dogs	Anesthetized male. Mongrel dogs are used in this model. Administration of sympatholytics decreases the blood pressure by blocking a and ß receptors
3	Nictitating membrane prolapses in cats	Cats are used in this model. Sympatholytic administration exerts a relaxing effect on the nictitating membrane.

4	**Cardio-vascular response in rats**	**Administration of sympatholytics in albino rats decreases the blood pressure and heart rate. Sympatholytics also inhibit the cardiovascular response of sympathomimetic agents.**
	B. IN VITRO MODELS	
1	Isolated frog heart preparation	This model is used to determine the changes in heart rate on isolated frog heart. The sympatholytic like propranolol (200 µg/ml) reduces the heart rate by blocking of B, receptor on the heart or membrane stabilising effect.
2	Isolated guinea pig tracheal chain	This model is used to determine β_2 agonistic and antagonistic activity. Stimulation of β_2 receptor relaxes the tracheal muscle, which is inhibited by β_2 antagonist. Percent inhibition of test drug is recorded against isoprenaline-induced relaxation of guinea pig tracheal chain.
3	Isolated aortic muscle strips	Aortic muscles mainly contain α_1 receptor. sympathetic stimulation contracts the aorta while blocking of sympathetic a receptor relax the aortic muscle. Rabbits, cats, and rats are generally used to isolate the aortic muscle.

❑ IN VIVO MODELS



I. Adrenergic antagonism in rodent's eye:

- Sympathomimetics like noradrenaline, adrenaline, and isoprenaline induces the mydriasis.
- This effect is blocked by α and β blockers.

❖ **Methodology:-**

- **Albino rats** (120-150 g) are used in this model.
- Rats are divided into control (vehicle) and tests group having 4-6 animals in each.
- Vehicle/test drugs are **administered s.c.** to the respective groups.
- After 30 min of **vehicle/test drugs administration**, **Nor-adrenaline** (0.1 mg/kg, iv) **or Adrenaline** (0.05 mg/kg, iv) or **Isoprenaline** (20 mg/kg, i.v.) are injected.
- The pupil diameters of each animal is **measured** and the **mean value** in each group is reported.

❖ **Interpretation:-** Mean pupil diameter of test groups is compared with control group. The **sympatholytic** drugs antagonize effects of **sympathomimetics.** Therefore, the pupil diameter of test groups is **lesser** than the control group

II. Blood pressure response in dogs

➢ Sympatholytics **decreases** the blood pressure by **blocking α and β receptors.**

➢ **Rats and cats** are also used to monitor changes in blood pressure induced by drugs.

❖ **Methodology:-**

- Male **Mongrel dogs** (15-20 kg) are anesthetized with Chloralose (100 ng/kg, i.v.) and maintained with Nitrous Oxide.
- **Brachiocephalic veins** were cannulated one for dextrose (5% w/v) infusion and the other for the Succinylcholine infusion.
- Left carotid artery is cannulated and connected to a blood pressure recorder.
- The femoral vein is cannulated for **drug administrations.**
- **Atropine** is also injected (2 mg/kg) at the start of the experiment to avoid reflex vagal action.

- After recording the baseline, **vehicle/standerd/test** drug is administered via the femoral vein and blood pressure is recorded.
- Blood pressure of standard and test drugs are compared with vehicle treatment.

❖ **Interpretation:-** Sympatholytics **decrease the blood pressure** as compared to vehicle.

III. Nictitating membrane prolapse in cats:

➢ Sympatholytics exert **relaxing effect on nictitating membrane.**

❖ **Methodology:-**

- **Cats** (3-5 kg) are divided into **control and test group.**
- Vehicle/test sympatholytic is **administered orally** and after 30 min, degree and duration of the prolapse of the nictitating membrane are recorded.
- The relative affinity of the different compound is compared by mean duration of prolapse in hours, by the dose in mg/kg body weight.

❖ **Interpretation:-** Sympatholytic gents **relax the nictitating membrane** and the results are compared with the control group

IV. Cardiovascular response in rats

➢ Administration of sympatholytics in **albino rats** **decreases the blood pressure and heart rate.**

➢ Sympatholytics also **inhibit** the cardiovascular response of sympathomimetic agents.

❑ IN-VITRO MODELS

I. Isolated frog heart preparation:

➢ The sympatholytic like propranolol **reduces** the heart rate by **blocking of β receptor** or membrane stabilizing effect on the heart.

❖ **Methodology:-**

- In this model, **sympatholytics are injected** instead of sympathomimetics.
- After **recording basal heart rate**, Propranolol (200 µg/ml)/test Sympatholytics are administered and record the respornse by using force transducer on physiograph.
- **Observed heart rate** is compared with basal heart Similarly, effect of sympatholytics on sympothomimetics induced changes in the heart rate can also be evaluated.

❖ **Interpretation:-** **Sympatholytics reduce** sympathomimetics induce increase heart rate.

II. Isolated guinea pig tracheal chain:

➢ This test is used to determine β_2 **agonistic and antagonistic** activity.

➢ **Stimulation of β_2** relaxes tracheal muscle.

❖ **Methodology:-**

- Albino **guinea pig** (300-350gm)is sacrificed and the **tracheal chain** is isolated
- The trachea is then cut into 6-10 ring of the same width and connected in series.
- The ring are mounted on organ bath containing **Tyrode solution** and maintained at 37^0C.
- Tissue is washed again and repeated with **increased test dose**.
- **Percentage inhibition** response is calculated in respected of isoprenaline alone.

❖ **Interpretation:-** **β_2 sympatholytic activity** is considered if the test drug **inhibit** the **relaxing response of Isoprenaline.**

III. Isolated aortic muscle strips

- This model is **described** both **sympathomimetics & sympatholytic** agent.
- Aorta muscle contain **α_1 receptor**. Sympathetic stimulation contracts the aorta.

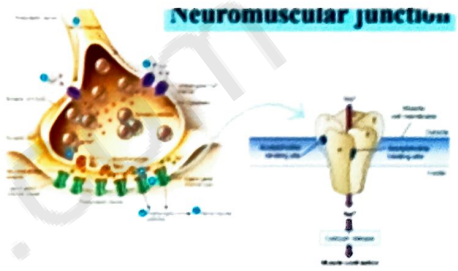
❖ **Methodology:-**

- **Cat**(3-5kg) are dissected to expose **thoracic aorta.**
- Two spiral aortic strips (20-30 mm) are cut from thoracic aorta.
- **One** is used as a **standard** and **other** is used for the test.
- The tissue preparation are allowed to **stabilize for 60-90 min.**
- During the equilibration period, the bathing solution is replaced with a fresh solution in every 15-20 min.
- The **strip were connected** to isotonic lever of the gravity type. The recording was taken on kymograph.
- Percentage inhibition of standard and test drug are recorded against noradrenaline- induced contraction.

❖ **Interpretation:-** the α1 receptor blockers **decrease** the **contractile response of noradrenaline**

❑ INTRODUCTION

- Parasympathomimetic drugs are also known as **cholinomimetic/cholinergic drugs**.
- **Acetylcholine** is an endogenous neurotransmitter.
- Parasympathomimetic drugs can act by cholinergic receptor (muscarinic and nicotinic receptor)
- **Muscarinic receptor** mainly present in the **gastric glands** (M_1), **heart** (M_2), and **smooth muscles** (M_3) and their activation Causes the gastric secretion, cardiac depression, and smooth muscle contraction.
- **Nicotinic receptors** are present in the **autonomic ganglion** (N_N) and **neuromuscular junction** (N_M) and their activation cause the stimulation of autonomic ganglion and skeletal



❑ Animal models used for screening parasympathomimetics

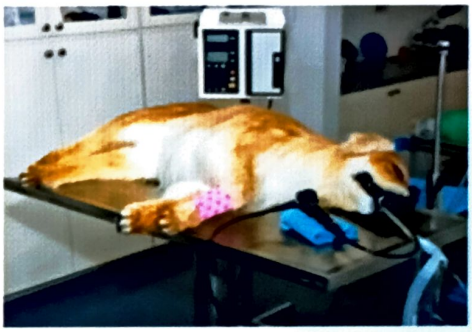
The models for screening parasympathomimetics are based on action of standard acetylcholine on the particular parasympathetic innervations.

SR. No.	Screening models	Description
	A. IN VIVO MODEL	
1	Blood pressure response in anesthetized animals	Dogs, cats, and rats are generally used for this model. Intravenous (femoral vein) injection of acetylcholine (2 µg) produces fall in blood pressure due to vasodilatation.
2	Miosis effect on rabbit eye	Parasympathomimetic agents produce miosis effects (pupil contraction) on the eye by contracting the ciliary muscle.

B. IN VITRO MODEL		
1	Isolated ileum preparation	The intestinal ileum of guinea pigs, rats, and rabbits are used for this model GI smooth muscle contains M_3 receptor and that is responsible for contractions during parasympathetic stimulation. Different bioassay techniques can be used to determine the potency of the test substance by comparing it with known standard of acetylcholine.
2	Frogs rectus abdominis muscle preparation	The frog's rectus abdominis muscle is generally used to assay of spasmolytic drugs. The stimulation of parasympathetic system preparations. contracts the muscle

❑ IN-VIVO MODELS

I. Blood pressure response in anesthetized animals:



➢ Parasympathetic stimulation produces **fall in blood pressure** due to **vasodilatation.**

❖ **Methodology**:-

- Dogs are used in this method.
- **Dogs** are anesthetized with a suitable anesthetic agent and prepared for blood pressure monitoring. Animals are kept on artificial respiration.
- The **carotid artery** is cannulated for **recording blood pressure**.
- The **femoral vein** is cannulated for **injecting acetylcholine** or test drugs. After stabilization, (standard drug) Acetylcholine /test drug is injected and blood pressure is recorded.

❖ **Interpretation:-** The **fall in blood pressure** after administration is **compared** with the **fall by the standard** acetylcholine preparation Blood pressure response **depends upon the dose** of **Acetylcholine**. Acetylcholine at 2 µg causes fall in blood pressure without any changes in heart rate

II. Miosis effect on rabbit eye:

- **Parasympathomimetics** like carbachol, pilocarpine, and physostigmine **contract the pupil** (miosis) and **decrease intraocular pressure** by muscarinic (**M_3 receptor**) action.
- The activation of M_3 muscarinic receptors causes **ciliary muscle contraction**, resulting in **decrease in diameter** of the ring of ciliary muscle

❖ **Methodology:-**

- Male **rabbits** (2-3 kg) are generally used in this model.
- Animals are divided into different groups **control** (vehicle treatment), **standard, and test group** (test sympathomimetic treatment).
- 0.5-2% **Pilocarpine** and/or 0.25-0.5% Physostigmine ophthalmic solution is used as a **standard.**
- Eyes of rabbits are **washed with distilled water** and left for 5 min.
- Vehicle/standard/test drug is **applied topically** to their respective groups and measured the pupil diameter.
- **Calculate** the **mean pupil diameter** in each group.
- Vehicle/test drug is injected intraperitoneally. After 30 min pupil diameter is monitored at every 10 min interval upto 120 min.

❖ **Interpretation:-** Topical application parasympathomimetics **decrease pupil diameter** and produce **mitotic effect**. Mean pupil diameter of standard and test groups are compared with the control group.

❑ IN VITRO MODEL

I. Isolated ileum preparation:

- GI smooth muscles contain **muscarinic M_3 receptors**, which are **activated** by **parasympathetic stimulation**.
- Activation of M_3 receptor causes **contraction of GI smooth muscle**.

❖ Methodology:-

- The animal is sacrificed and intestinal part is removed and kept in organ bath containing Tyrod solution. (35-37 °C)
- Ileum (2-3 cm in length) is then isolated and extra fatty tissue, nerve and blood vessels are removed.
- The writing lever is tied up **with tissue** and extra 0.5 load tension is applied over the lever for proper tissue relaxation.
- After stabilization of tissue preparation, **baseline is recorded** on **kymograph**, then dose-response curve of standard acetylcholine solution (1 µg onward) is recorded upto ceiling effects.
- The **tissue is washed** with **fresh Tyrode solution** 3-4 times at 1 min interval of each response.
- Then a test preparation (agonist) is added and **contraction response is recorded.**
- The response of test is **matched with the standard** by adding Acetylcholine.

❖ **Interpretation:-** If the contraction is observed after adding test substance, similar to standard substance, it is considered as a **parasympathomimetic activity** (agonist).

II. Frog's rectus abdominis muscle preparation:

- Rectus abdomínís muscle is a **voluntary muscle preparation,** which produces **slow contraction** in response to acetylcholine.

❖ Methodology:-

- **Frog** is sacrificed and **rectus muscle** is isolated.
- Isolated rectus muscle is then ligated and mounted in an organ bath containing **Frog Ringer solution**.
- lever is used to **record** the contraction **response** on a **kymograph**.
- After stabilization, Acetylcholine standard preparation is **added** to record the response upto ceiling response.
- Tissue preparation is washed out after each response.
- Then a test preparation (agonist) is **added** and contraction response is recorded. The response of test is matched with the standard by adding Acetylcholine to find out the potency of the test.
- For **evaluation of parasympatholytic and spasmolytic agents**, **dose response curve** of Acetylcholine is recorded.
- Then tissue is washed out and the test substance is added in a bath solution and after 90 sec. without washing known standard Acetylcholine solution is added and **record the response**.

❖ Interpretation:-

Parasympatholytic and spasmolytic test drugs decrease the contraction response of Acetylcholine.

UNIT-III

PART - 2

PRECLINICAL SCREENING MODELS: FOR ANS ACTIVITY

Points to be covered in this topic

- ❑ Skeletal muscle relaxants
- ❑ Drug acting on eye
- ❑ Local anethetics

❑ SKELETAL MUSCLE RELAXANTS

- Skeletal muscle relaxants are used to **reduce** muscle spasticity or muscle tension.
- muscle relaxants are categorised into two therapeutic groups: neuromuscular blockers and spasmolytics.
- Neuromuscular blockers act on periphery by blocking the neurotransmission (ACh) at neuromuscular end plate (Nm receptors).
- The spasmolytics, also known as "**centrally-acting**" muscle relaxants and are used to **relieve** **musculoskeletal pain** and **spasms.**
- In the screening of neuromuscular blocking agents, following parameters considered: **potency of the drug, the time required for maximal effect, duration of action, cumulative effects, and reversibility** by antagonism.

❑ Animal models used for screening skeletal muscle relaxants

Sr. No.	Screening models	Description
	IN VIVO MODELS	
1	Skeletal muscle relaxant activity by using rotarod	Rodents are used in this model. Administration of muscle relaxant like diazepam or d-tubocurarine decrease the muscular tone and reduce the fall time on rotator drum (20-25 rpm).
2	Skeletal muscle relaxant activity using actophotometer	Skeletal muscle relaxants relax the muscle and lead to decrease the locomotion. Albino mice or rats are generally used in this test.

IN VITRO MODELS		
1	Frog's rectus abdominis muscle preparation	The frog's rectus abdominis muscle is also used to assay of spasmolytic drugs. The skeletal muscle relaxant drugs inhibit acetylcholine induced contraction.
2	Phrenic nerve diaphragm preparation	Rats and guinea pig are used to isolate respiratory muscles in phrenic nerve-diaphragm preparations Electrodes are used to induce stimulation. Neuromuscular blocking agents oppose the effect of to neuromuscular transmission

❑ IN VIVO MODELS

I. Skeletal muscle relaxant activity by using rotarod:

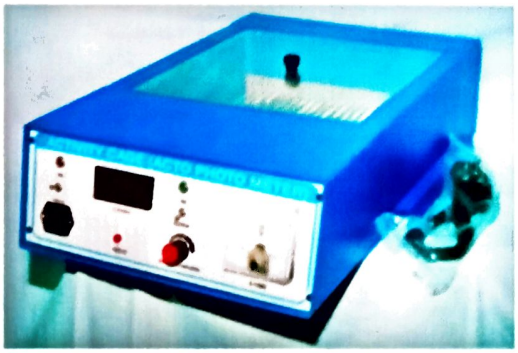
- The rotarod assembly is useful for screening of drugs that **affecting motor coordination.**
- Skeletal muscle relaxant drugs **decrease** the **muscle gripping strength** of animals and decreases fall down time on the rotarod.

❖ Methodology:-

- **Albino mice** (25-30 g) are used in this model.
- Animals are divided into three groups, each group contains 5-6 animals.
- Control group is treated with **vehicle**, the standard group treated with **Diazepam** and test group received **test drug.**
- 30 min after oral administration of drugs, animal is placed on **rotating drum** (speed: 25 rpm) and **recorded the fall time** of each animal.
- The **mean fall time** of each group is **calculated and compared** with the control group.

❖ **Interpretation:-** Skeletal muscle relaxant drug **decreases** the **mean fall time** as compared to the control group

II. Skeletal muscle relaxant activity using actophotometer:



- Skeletal muscle relaxants **relax the muscle** and lead to decrease the locomotion.
- The locomotor activity is assessed with the help of **actophotometer**, which consists of photocells in the outer wall.
- **Interruptions of photocell beams** (locomotor activity) are **recorded** by means of a six digit counter.

❖ **Methodology:**

- **Albino mice** (25-30 g) or rats (150-200 g) are selected and divided into control and test group.
- **Control rats** receive vehicle and **test groups** receive test drugs.
- **After 30 min of oral administration**, animals are subjected to **locomotion count**.
- The animal is placed in the **actophotometer** and locomotion count for 10-15 min is **recorded**.
- The mean locomotion count is **calculated and compared** with the control group.

❖ **Interpretation:-** If the **test drug** decreases the locomotion count as compared to control, the test drug is considered as a muscle relaxant.

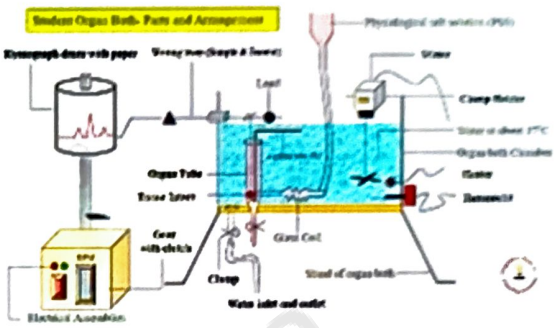
❑ IN VITRO MODELS

I. Frog's rectus abdominis muscle preparation:

- This model is used to assay the skeletal muscle relaxant drugs.
- Test drugs inhibit the acetylcholine-induced muscle contraction.

❖ Methodology:

- **Frog** is sacrificed and **rectus muscle** is isolated.
- Isolated rectus muscle is then **ligated and mounted** in an organ bath containing **Frog Ringer solution.**
- lever is used to record the contraction response on a **kymograph**
- After stabilization of tissue preparation, cumulative **dose response curve of Acetylcholine is recorded** with and without the presence of skeletal muscle relaxant.

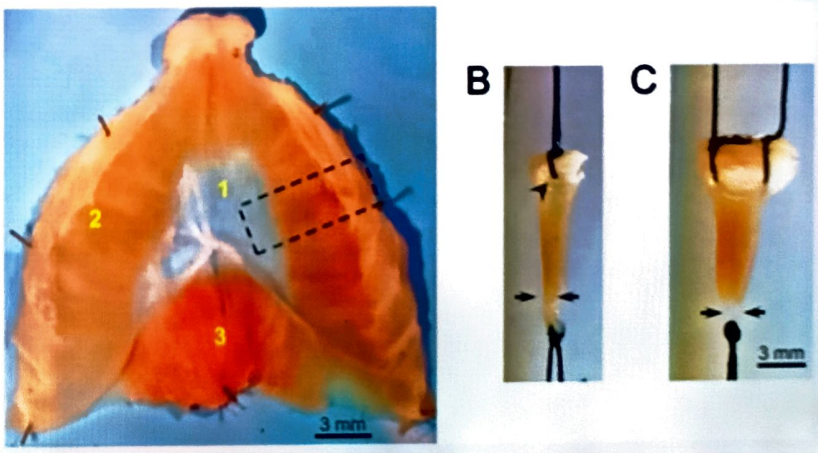


- After getting the **cumulative dose response curve** of Acetylcholine, the tissue is washed out (2-3 times) and then test drug is added in organ bath solution.
- After 1-2 min **without washing,** again **cumulative dose response curve** of Acetylcholine is **recorded.**

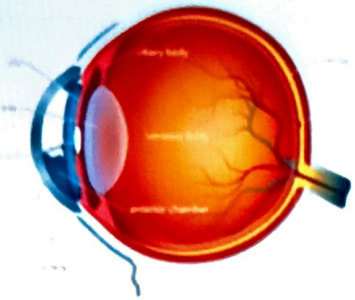
❖ **Interpretation: Percentage inhibitory response** is observed **after adding the test drug**. it is considered as a skeletal muscle relaxant.

II. Phrenic nerve diaphragm preparation

- **Rats and guinea pig** are used to isolate respiratory
- Muscles in **phrenic nerve-diaphragm** preparations **Electrodes** are used to **induce stimulation.**
- Neuromuscular blocking agents oppose the effect of to neuromuscular transmission.



PRECLINICAL SCREENING MODELS: FOR ANS ACTIVITY



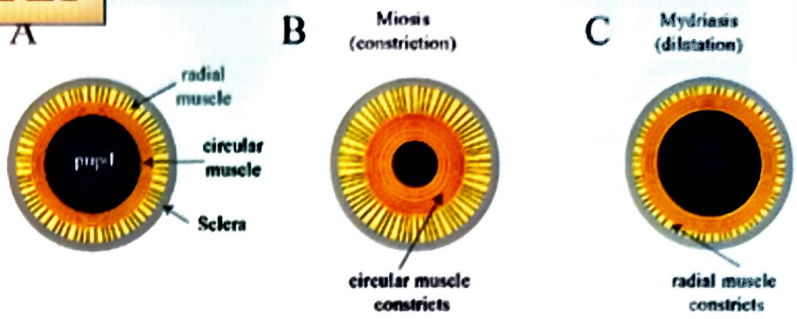
Unit-3 (part - 6)

DRUGS ACTING ON EYES

Contents to be covered in this topic

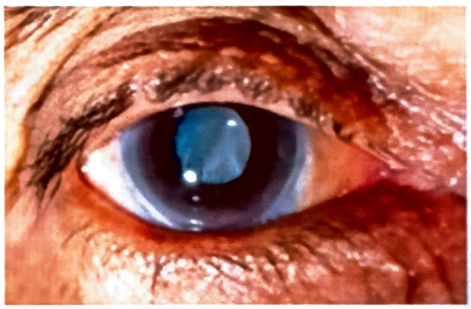
- INTRODUCTION
- ANIMAL MODELS FOR SCREENING ANTI-GLAUCOMA DRUGS
- IN-VIVO MODELS
  I. Steroid-induced glaucoma
  II. Methyl cellulose-induced glaucoma
- ANIMAL MODEL USED FOR CATARACT
- IN-VIVO MODELS
  I. Selenite induced cataract
  II. UV radiation induced cataract
- IN-VITRO MODELS
  I. Isolated lens model

❑ DRUGS ACTING ON EYES



- The eye is a complex **sensory organ** responsible for **vision.**
- The **pupil dilation and contraction are controlled** by the **iris** muscle fibers (circular and radial) that are **triggered** by parasympathetic and **sympathetic system.**
- The **stimulation** of **sympathetic and parasympathetic** nerves produces **mydriasis** and **miosis** respectively and their **blockading produces opposite effects.**
- Topical administration of local anesthesia on eye **reduces the corneal reflexes** and produces miosis.

❑ Animal models for screening anti-glaucoma drugs



- Glaucoma is an **ocular disease** that is characterized by **high intraocular pressure** (IOP) and further **leads to ocular blindness.**
- The rise in IOP is caused by **increasing aqueous formation** and **reducing outflow.**
- The **normal IOP in human** being ranges from **20-30 mmHg** and can rise upto 60-70 mmHg in glaucoma patients.
- A wide variety of animal models has been used to study glaucoma.
- These include monkeys, dogs, cats, rodents, and several other species.

Sr. no.	Type of models	Screening model
1	In vivo models	Steroid-induced glaucoma
		Methyl cellulose-induced glaucoma

❑ IN-VIVO MODELS

I. Steroid-induced glaucoma:

- Administration of **glucocorticosteroids** can lead to the **development of ocular hypertension** and **glaucoma** through a **reduction in aqueous humor outflow.**
- Models using steroid-induced ocular hypertension have been developed in many animals such as **rabbits bovine, and sheep.**

❖ Methodology:

- **Adult rabbits** or cats are used in this test.
- Animals are divided into three groups each containing 5-6 animals.
- The groups are divided into normal (vehicle), control (Dexamethasone), and test group (Dexamethasone + test drug).
- The normal group is treated with **sterile saline topically**
- In control group and test group, glaucoma is induced by 10 pl topical administration of examethasone in the eye for 2-3 weeks.
- The test group is treated with test preparation along with Dexamethasone.
- The IOP is monitored weekly by using **tonometer.**

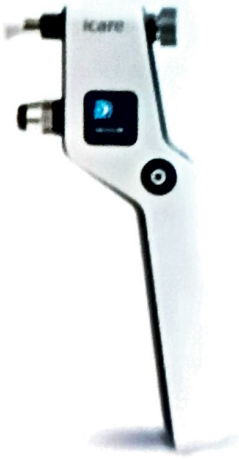
❖ **Interpretation:** Anti-glaucoma drug **reduces the mean IOP** as compared to control group.

II. Methyl cellulose-induced glaucoma:

- Local inj. of **Methyl cellulose** (0.5-4% w/v) in the anterior chamber of eye can **increase IOP** and **lead to glaucoma.**

❖ Methodology:-

- **Rabbits** are divided into three groups **each containing 5-6 animals.**
- The groups are divided into normal **(saline)**, control **(Methyl cellulose)**, and test group **(Methyl cellulose + test drug).**



- The **normal group** is treated with sterile saline locally.
- In **control group** and **test group**, glaucoma is **induced** by a **series of four intra-anterior chamber inj.** of Methyl cellulose in the eye.
- The intraocular hypertension is produced within **6-8 weeks**.
- The **test group** is treated with **test preparation** along with Methyl cellulose.
- The IOP is monitored weekly by using **tonometer** (tonopen).

❖ **Interpretation:-** The results of the **test group** are compared with **control group**.

❑ ANIMAL MODEL USED FOR CATARACT

- Cataractous is one of the **leading causes** of **visual dysfunction**.
- Cataract is characterized by the **clouding or opacity of the eye lens.**
- Progression of cataract depends on the several risk factors such as
- **aging, UV-radiation, smoking, diabetes and systemic hypertension**.

Several animal models are available for assessment of anticataract agent.

Sr.no.	Type of model	Screening models
1	In vivo models	Selenite induced cataract
		UV radiation induced cataract
2	In vitro models	Isolated lens model

❑ IN VIVO MODELS

I. Selenite-induced cataract:


- **Sodium Selenite**-induced cataract .
- Sodium Selenite causes oxidative damages of the **lenticular cell** and further leads to cataract formation

❖ Methodology:-

- **Albino rats** are used. Animals are divided into **three groups** each containing **5-6 animals**.
- The groups are divided into normal, control, and test group.

- A **normal group** is treated with **saline** (10 ml/kg, p.o.).
- **Control group** is treated with single dose Sodium Selenite.
- Sodium selenite gave to the suckling rats of **10-12 days of age**, The test group is treated for 4-6 days after treatment of sodium selenite.
- **After 16th days** the **opacity of the lens observed** by using ophthalmoscope

❖ **Interpretation:-** Anticataract test drug **reduces lens opacity and oxidative damages**

II. UV radiation-induced cataract:

- The cornea is a **major absorber** of the UV radiation.
- UV radiation reaches the lens and generates ROS.
- These ROS **increases** the **oxidative stress** and **damages the lens proteins.**

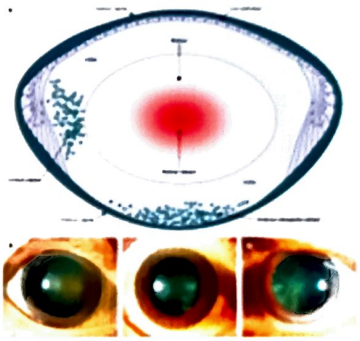
❖ Methodology:

- **Albino rats** are generally used for this study.
- The groups are divided into **normal, control, and test group**.

- A **normal group** is treated with **saline**.
- **Control group** is **unilaterally exposed to UV-radiation** (8 kJ/m^2) in the **300 nm wavelength** region for **1-2 weeks**.
- The **test group** is treated with test drug **along with UV exposure**.
- Lens opacity is observed by using ophthalmoscope/slit-lamp microscopy weekly during the protocol.
- **Antioxidants, total protein, ionic contents, ATPase enzymes and lipid peroxidation** were **measured** after the completion of the protocol.

❖ **Interpretation :-** Anticataract test drug reduces the lens opacity and oxidative damages

❑ In Vitro Models



II. Isolated lens model:

➢ Hydrogen peroxide induces cataract by **oxidative damage of lens protein,**

❖ **Methodology:**

- **Eye lenses** are isolated from **rats** and transferred into incubating media (Tyrode solution).
- The fresh lenses divided into normal, control, and test group.
- The **normal lenses** are placed in **incubation media** only.
- The **control lenses** is placed in **incubation media** containing inducing agent, **hydrogen peroxide**.
- **test lenses** are placed in **incubation media containing inducing agent with** test drug.
- The time required to complete opacity and lens biochemical parameter is **observed**.

❖ **Interpretation:-** If the test drug **increases,** time required for **complete opacification** it is considered as an **anticataract agent**.

❑ LOCAL ANESTHETICS

Local anesthetics are the drugs that cause the **reversible loss of sensory perception.**

Inhibiting block voltage-sensitive sodium channels and **inhibit** the **generation of the action potential.**

Clinically it is used to **relief pain** and in minor surgical procedure.

Several experimental animals like **rats, guinea pigs, rabbit, and frogs** are employed to screen local anesthetics.

- **Local anesthetics** are either applied topically on the desired surface area or **injected subcutaneously** to achieve

❑ Animal models for screening local anesthetics

The models are developed for screening of local anesthetic based on its effects, surface anesthesia, infiltration anesthesia, and conduction anesthesia. Some important models are discussed in this chapter.

Sr. no.	Screening model	Description
	IN VIVO MODEL	
1	Conduction anesthesia in rats sciatic nerve	Albino rats are used in this model. The sciatic nerves (located on legs) are blocked by administration of local anesthetic solution. Application of local anesthetics depresses the walking behavior.
2	Conduction anesthesia on the mouse tail	The tail flick or radiant heat method is used in this test. Administration of local anesthetics on mousetail root increases the reaction time

IN VITRO MODEL		
	Voltage clamp experiments	Single myelinated nerve fibers from the sciatic nerve of the frog are used in this study. Local anesthetics decrease the sodium and potassium ion current.
	Isolated sciatic nerve preparation	The isolated sciatic nerves from guinea pig or frog are used in this model. The administration of local anesthetics retards the action potential generation. Voltage clamp experiments are used to determine conductance. sodium and potassium

❑ IN-VIVO MODELS

I. Conduction anesthesia in rat's sciatic nerve:

- The local anesthetics **block the nerve conduction** and **modulate duration of cellular function**.
- **Vasoconstrictor** can also be added with a local anesthetic to enhance the **anesthetic effects**.
- Guinea pigs **resemble** peridural and paravertebral anesthesia in man.

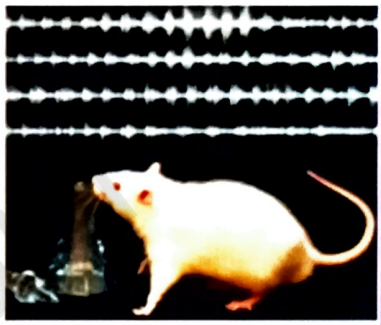
❖ Methodology:-

- **Male albino rats** (125-175 g) are used in this model.
- Animal are poised in prone position, **hind limbs are extended**, and the **depression for needle insertion** is located by palpation with the left index finger.
- 0.2 ml of 1% (w/v in saline) test local anesthetic or standard drug (e.g., procaine or lidocaine) is **injected to block the sciatic nerve** in the **junction of the biceps femoris** and the gluteus maximus muscles of legs.
- Tuberculin syringe with 24-25-gauge needle is used to administration of the drug.

- **One leg** is used for test drugs administration and **other leg** is used for **vehicle administration.**
- After the injection, the **walking behavior are monitored** in every 5 to 10 min time interval to **determine** the **recovery process**.

❖ **Interpretation:-** The local anesthetics increase the duration of block time as **compared to vehicle treated** animals.

II. Conduction anesthesia on the mouse-tail:



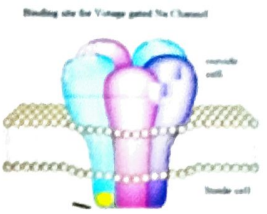
➢ This method used for determination of **anesthesia property** by **injecting** the local anesthetic into the **root of the tail.**

❖ **Methodology:-**

- **Albino mice** (22-25 g) are used in this method.
- **Before injecting** vehicle/ test/standard drug, the normal **reaction time is noted** against radiant heat (pretest values).
- The animals which show **reaction time** of **more than 6 sec** are not used for the test.
- animals are divided into **three groups** control (vehicle), **test** and **standard.**
- Animals are placed in restrainer & vehicle/test/standard drug (0.1 ml) is injected on **both sides of tail root.**
- After 10 min of injection animals are subjected to radiant exposure.
- The **area of heating** is about **1.5 cm distal** to the **injection site.**
- For each individual animal, the **reaction time is noted before and after treatment.**

❖ **Interpretation:-** The average reaction time is calculated and **compared with pre-test value.**

❑ IN VITRO MODEL



I. Voltage clamp experiments for sodium and potassium conductance:

- Local anesthetics generally block the voltage-gated cation channels. Sodium, potassium and leakage currents (Ina, IK, I) can be measured

❖ **Methodology:-**

- **Single myelinated nerve** fibers are **dissected from** the sciatic nerve of the frog.
- The fibers are immersed in a Ringer solution
- The **nodal membrane is clamped** to a holding potential of -30 mV for **measurement** of **sodium, potassium and leakage currents**.
- Hyperpolarizing pre-pulses (-40 mV, 50 ms) afterward test pulses are applied in every 5 s to record peak sodium currents and to avoid the influence of accumulation of frequency-dependent block.
- Every second **pulse is followed by** after pulse (120 mV, 20 ms) to measure **steady state I_K**.
- The leakage current, **I_L is measured** at the **end of hyperpolarizing** pre-pulse and recorded every 10 s.

❖ **Interpretation:-** **After super fusion** of local anesthetic with the nodal membrane, **quick and then slow decrease** of peak **sodium current** observed. **potassium current (I_K)** and **leakage current** remains unaffected.

II. Isolated sciatic nerve preparation

- Isolated sciatic nerve are placed between stimulating and recording electrodes in solution containing local anesthetic and observed electrophysiological changes.
- The administration of local anesthetics retards action potential generation.