## PHARMACOLOGY

1. **EFFECT OF SALINE PURGATIVE ON FROG INTESTINE**

**AIM:** To study the effect of saline purgative on frog intestine.

**PRINCIPLE:** Saline purgatives are the salts comprising of highly charged ions and do not crosses cell membrane freely. They remain inside the lumen and retain water through osmotic forces. They increase the volume of the contents of the bowel, stretch the colon and produces normal stimulus for contraction of the muscle that leads to defecation. The aim of the present study is to examine the effect of saline purgative on frog intestine.

**REQUIREMENTS Animal :** Frog Reagents : 0.9% to 0.45% of saline (hypotonic), 27% Magnesium sulphate (hypertonic), Frogs Ringer solution (isotonic) Instruments used: Frog’s board, pithing needle, dissecting instruments, needle with thread, tuberculin syringe with needle.

**PROCEDURE:** Pith the frog and place it on a dissecting board. Expose the abdominal cavity and carefully trace the small intestine. Make the small intestine into three compartments by tying threads of different colours in such a way that no fluid can move from one compartment to the other. Inject 0.2 ml of each hypotonic solution into first compartment, 0.2 ml of hypertonic solution to second compartment and 0.2 ml of isotonic solution into third compartment. Wait for 20 minutes and the observations are to be recorded.

## Observation:

Hypotonic solution causes the fluid to move from lumen into circulation by process osmosis thereby shrinks the intestine. Hypertonic solution (saline purgative) moves the fluid from cells into the lumen and swells the intestine and isotonic solution did not show any fluid movement across the intestinal membrane.

|  |  |  |
| --- | --- | --- |
| **Solution** | **Compartment** | **Effect** |
| **HypotonicSolution**  **(0.2 Ml of 0.9% Sodium Chloride)** | **First** | **Shrinking** |
| **Hypertonicsolution – Saline Purgative** | **Second** | **Swelling** |

|  |  |  |
| --- | --- | --- |
| **(0.2 Ml of 27% Magnesium Sulfate)** |  |  |
| **Isotonicsolution**  **(0.2 Ml of Frog Ringer)** | **Third** | **No change** |

## Conclusion:

**Hypertonic solution (saline purgative) moves the fluid from cells into the lumen and swells the intestine and shows purgative effect.**

# Test for pyrogens (rabbit method)

**Aim:** Learn how to determine the pyrogen in parenteral preparations by injecting the sample in rabbits for pyrogen testing.

**Introduction:** Pyrogen test is performed to check the presence or absence of pyrogens in all aqueous parenterals. Rabbits are used to perform the test because their body temperature increases when pyrogen is introduced by the parenteral route. For this test, three healthy rabbits are selected each weighing at least 1.5 kg. No rabbit should be selected if: 1. It has a normal temperature greater than 49.8°C.

1. It was used in a positive test during last two weeks or negative test during last two days.

**Method for Pyrogen Test:** The pyrogen testing is performed in an air- conditioned room. The food and water is withheld to rabbit overnight. A clinical thermometer is inserted in the rectum of each rabbit to a depth of not less than

7.5 cm. Two readings of the temperature of rabbit in normal conditions should be taken at the interval of half an hour before start the test and mean of the both should be calculated to determine the initial temperature. The equipment, injectors and needles used in the test should be pyrogen-free. These should be washed with water for injection and then heated at 260°C for two hours. The injection is warmed to 38°C before injecting to the rabbits. 0.5 to 1.0 ml per kg dose should be injected through the ear vein. Six reading of temperature is recorded at an interval of half an hour.

**Pyrogen Test Results:** The response of each rabbit is detected by the difference of initial temperature and the highest temperature recorded. The response of all three rabbits gives the sum of responses and can be concluded as: i) If the sum of responses does not greater than 1.4°C and any of rabbit shows the response less than 0.6° C, the product passes the test. ii) I sum of responses is greater than 1.4 °C or any of rabbit shows the response 0.6 or greater, continue the test using 5 rabbits.

iii) If the test is done using 5 rabbits, then if the sum of responses of all 5 rabbits is greater than 3.7°C and the individual response of not more than three rabbits is greater than 0.6°C, the product passes the test.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group Selected** | **Readings** | **Rabbit No.1** | **Rabbit No.2** | **Rabbit No.3** | **Total** |
| Vehicle Treated | Temperture Noted | 38.21 | 38.5 | 38.89 | 115.60 |
| DrugTreated(Pyrogen added) | Temperture Noted | 38.8 | 39.1 | 39.5 | 117.40 |
|  | Temperture Increased | 0.59 | 0.60 | 0.61 | 1.80 |

## Conclusion:

As rise in body temp of each rabbit is more than 0.6, so it shows that the test sample is not pyrogen free.

1. **EFFECT OF DRUGS ON CILIARY MOTILITY OF FROG'S OESOPHAGUS**

**Aim:** EFFECT OF DRUGS ON CILIARY motility OF FROG'S OESOPHAGUS

**Animals:** Frog

**Drugs and solutions:** Acetylcholine 100µg/ml Physostigmine 100µg/ml Atropine 1µg/ml Frog's Ringer Apparatus: A pair of scissors, forceps, poppy seeds, cotton, droppers, frog board, stop-watch.

**PROCEDURE:** Pith a frog. Slit open the oesophagus from the buccal cavity to the stomach. Wipe the blood gently using a cotton swab dipped in Frog's Ringer solution, proceeding from cephalic to caudal end. Moisten the surface with Ringer solution. Place two pins at a distance of 2-3 cm. Place one seed on the groove near the pin at cephalic end. Start the stopwatch and observe the time taken for the seed to reach the pin at the caudal end. Take 2 such readings and calculate the average. Repeat the experiment using acetylcholine, physostigmine and atropine. Take control readings with Frog's Ringer between the drugs.

**Presentation of data and analyses:** Pool the data from other groups and formulate appropriate table(s) to display the data. Analyze the data using appropriate statistical test(s) to find out whether the given drugs produce significant effects in comparison with Ringer. Draw conclusions and record them.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Vehicle Treated** |  |  | **Drug Treated** |  |
| 28 | | | 19 | | |
| 32 | | | 20 | | |
| 29 | | | 16 | | |
| 14.833333333333334 | | | 9.166666666666666 | | |

Average

## Conclusion:

The drug (acetylcholine) shown increased motility(movment).

# Effect of Certain Agonists and Antagonists on Isolated Guinea- pig Ileum

**Aim:** Effect of Certain Agonists and Antagonists on Isolated Guinea-pig Ileum

Guinea pig ileum is a smooth muscle receive dual nerve supply from autonomic nervous system of both sympathetic and parasympathetic. Parasympathetic tone is dominant in ileum which Ach cause contraction by stimulating M3-R.

## Why Guinea pig ileum is used?

* 1. No myogenic contraction so any drug induce contraction can be seen.
  2. Very sensitive to histamine unlike the rabbit due to the presence of histaminase enzyme.

## Types of receptors present in guinea-pig ileum

* + - Cholinergic receptors.
    - Serotonergic or tryptaminergic receptors (5-HT1→5-HT7).
    - Histaminergic receptors.
    - Adrenergic receptors.

**Procedure:**

* + - 1. Arrange organ bath and rotating drum assembly as per standard protocol
      2. Dissect guinea pig & Isolate guinea pig ileum as per standard protocol
      3. Mount the ileum in organ bath assembly
      4. Apply agonist (Acetylcholine & Histamine) and record the response.
      5. Determine a 'standard dose' (one which produces approximately 75% of the maximum response) for acetylcholine and histamine. Select a low concentration to begin with and increase the dose in a geometric manner.
      6. Apply antagonist Atropine (Antagonise Acetylcholine) & Mepyramine (Antagonise Histamine) and record the response.
      7. Test the action of the antagonists atropine and mepyramine against exogenously applied acetylcholine and histamine. The concentrations of ACh and histamine are pre-selected as those which produce a sub-maximal (75%) contraction as determined in 'Part 1' (i.e. acetylcholine 8x10-8 M; histamine 8x10-7 M). In each experiment antagonists are added to the bathing fluid and left for 5 minutes before the agonist is added.

**Inference:**

Atropine at a specific concentration almost totally blocks the response to acetylcholine but has no effect on the histamine-induced contraction.

Mepyramine at a specific concentration almost to

# EFFECT OF SKELETAL MUSCLE RELAXANTS USING ROTA- ROD APPARATUS

**AIM**: To study the effect of CNS suppressant and muscle relaxant drug on mice using rotarod apparatus

**INTRODUCTION**: Rota-rod apparatus has a horizontal grooved rod rotating at a fixed speed, the mice are made to balance on this rod. Dependent upon their motor coordination. Central nervous activity and grip strength the animal either stay on the rotating rod for specific time and after that fall down on platform. The floor of each compartment has sensor, that deactivates the tissue and the exact fall off time for each rat is displayed on respective display.

**PRINCIPLE:** Reduction of motor co-ordination CNS depression and skeletal muscle relaxation leads to decrease in the fall off time and less no. of free riding indicates that administered drug has CNS depressant or muscle relaxant activity that either lead to decrease in the motor co-ordination or decrease in the gripping power.

**PROCEDURE:** Animals are divided into 2 groups

* Administer one group with the drug to be tested and other with vehicle by

intraperitoneal route.

* When the animal falls off respectively time will be displayed on the timer.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group Selected | Readings | Mouse No.1 | Mouse No.2 | Mouse No.3 | Average |
| **Vehicle Treated** | **Fall off time (Sec)** | **52** | **50** | **55** | **52.333333333333336** |
|  | **Free Ridings** | **52** | **50** | **55** | **52.333333333333336** |
| **Diazepam Treated** | **Fall off time (Sec)** | **42** | **39** | **45** | **42** |
|  | **Free Ridings** | **42** | **39** | **45** | **42** |

## Conclusion:

The observed reduction in fall off time and free riding shows that Diazepam at 1 mg/kg i.p. dose produces decrease in motor co-ordination and decrease in muscle strength.

# Effect of drugs on isolated frog heart

**Aim:** To study the effect of drugs(inotropic and chronotropic actions) on perfused frog heart.

## Principle:

Drugs may influence the rate (chronotropy) and force (inotropy) of contraction of the

heart.An increase in the heart rate is called a “positive chronotropic”

response,while a

“negative chronotropic” response is a decrease in the heart rate.Similarly,an

increase in the

force of contraction is called a „positive inotropic‟ response and a decrease in the

force of

contraction is called a „negative inotropic‟ response.

Sympathomimetic amines such as adrenaline and noradrenaline produce positive inotropic

and positive chronotropic response.whereas parasympathomimetics such as acetylcholine

produce negative inotropic and negative chronotropic response.

## Requirements:

**Animal :**frog

**Apparatus**:frog‟sringersolution,reservoir,tubing,screwclip,syme,cannula,clamp,

bosshead ,thread ,syringe and needle. **Drugs :** Adrenalin (stock solution 10 µg/ml) Noradrenaline (stock solution 10µg/ml) Acetylcholine(stock solution 10µg/ml) Calcium chloride(stock solution 10µg/ml)

Potassium chloride(stock solution 10µg/ml) **Physiological solution :** Frog ringer **Procedure:**

1. Pith the frog and pin it to the frog board.
2. Give a mid line incision on the abdomen.Remove the pectoral girdle and expose the heart.
3. Carefully remove the pericardium and put a few drops of frog ringer over the heart.
4. Trace the inferior vena cava,put a thread around it and give a small cut in order to insert the

venous cannula which is in turn connected to a perfusion bottle containing frog ringer.Insert a

cannula in the vein and tie the thread to assure the cannula in place.

1. Give a small cut in one of the aortae for the perfusate to come out.
2. Adjust a proper venous pressure of 2-4 cm by altering the height of perfusion bottle.The

effective venous pressure is the height in cms from level of the venous cannula and the ringer

level in the perfusion bottle.Use of marriott‟s bottle helps in attaining the constant

pressure.

Start the perfusion by opening screw clamp attached to the tube.

1. Pass a thin pin hook through the tip of the ventricle,and with the help of a fine thread

attached to the hook,tie it to the free limb of the universal lever,which is fixed to a

stand.Adjust proper tension and magnification by altering the height of the lever.Record the

normal contraction of the heart on the smoked drum.

1. Inject 0.1,0.2,0.5 and 1 ml of the stock solution of each drug in a sequential order and note

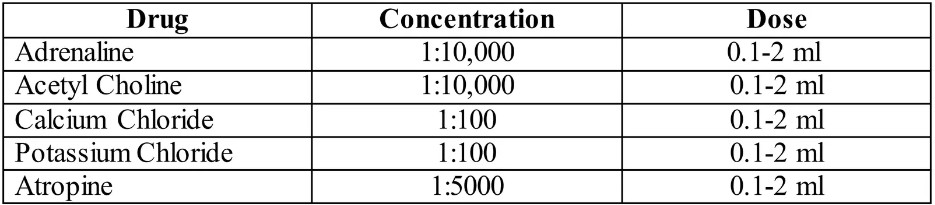
the change in the rate and amplitude of contraction.Keep at least 5 min gap between the

administration of each dose of the drug.The drug is administered by injecting the drug into

the perfusion tube very close to the venous cannula.Take precautions to avoid any leakage of

the drug from the tube,and the injection of air bubbles.

1. Label and fix the tracing with the fixing solution.



## Conclusion:

We completely detached the heart from the rest of the body and recorded its heart rate.The heart is beating on its own.