EXPERIMENT NO.: 15 **DATE:**

AIM: TO STUDY THE LOCAL ANESTHETICS BY DIFFERENT METHODS

INTRODUCTION:

- Local anesthesia is any technique to induce the absence of sensation in a specific part of the body, generally for the aim of inducing local analgesia, that is, local insensitivity to pain, although other local senses may be affected as well.
- They are used in various techniques of local anesthesia such as:
  - Topical anesthesia (surface)
  - Topical administration of cream, gel, ointment, liquid, or spray of anaesthetic dissolved in DMSO or other solvents/carriers for deeper absorption
  - Infiltration
  - Brachial plexus block
  - Epidural (extradural) block
  - Spinal anesthesia (subarachnoid block)
  - Iontophoresis

PROPERTIES OF IDEAL ANAESTHETICS AGENTS:

- It should not irritate the tissue to which it is applied.
- It should not make any long-lasting changes on nerve structure.
- Its systemic toxicity should be minimal.
- It must be effective regardless of whether it is injected into tissue or applied locally on mucous membranes.
- The time of onset of anesthesia should be minimal.
- Duration of action must be sufficiently long to allow the procedure to be completed but not so long as to necessitate extended recovery.
- It should have enough potency to administer full anesthesia without supplementing additional concentrated solutions that are potentially damaging.
- It should not produce allergic reaction.
- It should be stable in solution and should spontaneously undergo biotransformation in the body.
- It should be sterile or capable of being sterilized by heat without deterioration.

SOME SCREENING METHODS FOR LOCAL ANAESTHETICS FOR PRECLINICAL STUDY:

1. CONDUCTION ANAESTHESIA

A. CONDUCTION ANESTHESIA IN THE SCIATIC NERVE OF THE FROG

Procedure

- Frogs (*Rana temporaria*) of either sex are used and are kept at 4 °C. The frog is decapitated with a pair of scissors. The skin is incised in the thigh region at both sides and the sciatic nerves are carefully exposed in the thigh, avoiding any stretching and injury of the nerve.
- The frog is suspended on a vertical board. Small pieces of white cotton are soaked with different concentrations of the test preparations (between 0.05% and 1%) or the standard and placed gently around the sciatic nerve for 1 min.
- Then the cotton swab is removed and the frog is placed with its extremities into a bath with 0.65% NaCl solution. This allows testing for duration and reversibility of the local anesthetic effect.
- One side is used for the test preparation and the other for the standard (e.g., 0.25% butanilicaine). Every 3 min the frog is removed from the bath and the toes of the legs or the ankle joint are pinched three times with a small forceps.
- The reflex contraction is abolished when conduction anesthesia is effective. The stimuli are repeated every 3 min until anesthesia vanishes. Two to 5 frogs are used for each concentration.

Evaluation

- Time of onset and duration of anesthesia are recorded for each concentration. Time-response and dose-response curves can be established.

B. CONDUCTION ANESTHESIA IN THE SCIATIC NERVE OF THE RAT

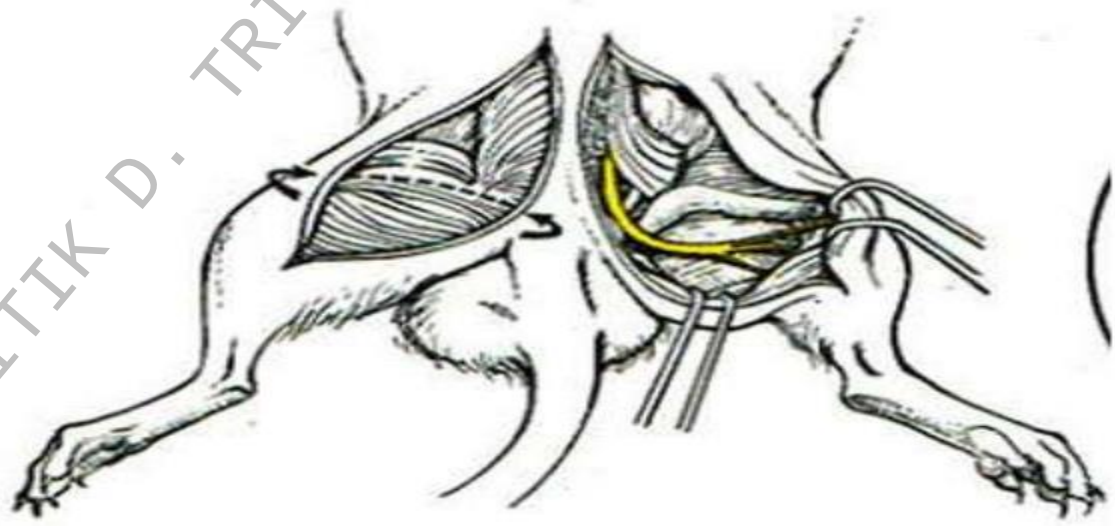
Procedure:

- Male Wistar or Sprague Dawley rats weighing 125 to 175 g are used. The animal is suspended in a prone position by grasping the base of the tail and thoracic cage.
- A hind limb is extended to its full length and the depression for needle insertion is located by palpation with the left index finger. The site of injection is the area under the skin at the junction of the biceps femoris and the gluteus maximus muscles.
- The sciatic nerve is blocked in the midthigh region with 0.2 ml of the drug solution administered by a 24- to 25-gauge needle attached to a 0.25 ml tuberculin syringe.

- Usually a 1% solution of the test drug in 0.9% NaCl is used as a test solution. The other leg is used for a control drug (e.g., procaine or lidocaine).
- Immediately after the injection, repeated checks of the digit of the foot and the walking behavior are performed.
- In the normal foot, the digits are wide apart, while in the blocked leg the digits of the foot are close together. Also the successful block is evidenced by dragging of the leg and an inability of the animal to use the leg in walking up the inclined wire mesh cover of the cage.
- After the time of block for each leg is noted, each animal is examined every 5 to 10 min in order to note the time of recovery.

Evaluation

- From the data, averages for onset and duration of action are calculated, plus the frequency of blocks are noted. Using various doses of test compound and standard, dose-response curves can be established and potency ratios calculated.



C. CONDUCTION ANESTHESIA ON THE MOUSE TAIL

Procedure

- Groups of 10 mice (NMRI-strain) of both sexes with a weight between 18 and 22 g are used for each dose. Before administration of the test compound or the standard the normal reaction time is determined.
- The animal is placed into a small cage with an opening for the tail at the rear wall. The tail is hold gently by the investigator. By opening of a shutter, a light beam exerting radiant heat is directed to the proximal third of the tail.

- After about 6 s, the reaction of the animal is observed by the investigator. The mouse tries to pull the tail away and turns the head. The shutter is closed with a switch when the investigator notices this reaction.
- Mice with a reaction time of more than 6 s are not used in the test. The test compounds and the standard are injected in a volume of 0.1 ml on both sides in the area of the tail root.
- The animals are submitted to the radiant heat again after 10 min. The area of heating is about 1.5 cm distal to the injection site. For each individual animal the reaction time is noted.

Evaluation

- There are two possibilities for evaluation:
  i. The average values of reaction time after each time interval are calculated and compared with the pretest value by analysis of significance.
  ii. At each time interval only those animals which show a reaction time twice as high or higher as the pretest value are regarded as positive. Percentages of positive animals are counted for each time interval and each dose and *ED*50 values are calculated according to LITCHFIELD and WILCOXON.

D. RETROBULBAR BLOCK IN DOGS

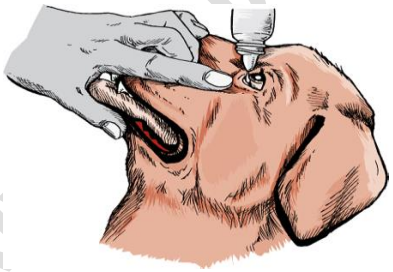
Procedure:

- Young female mongrel dogs weighing 13–15 kg are used. Twenty-four h before the test, 0.25% eserine (Physostigmine) ointment is placed in each conjunctival sac of the dog. Pentobarbital (25 mg/kg) is administered intravenously, then repeated with 10 mg/kg at hourly intervals, thus maintaining the animal in light anesthesia (corneal reflex present).
- Ten min after induction, the dog is put into 30-degree head-down position, and 20 ml of 0.05% tetracaine is forced into the epidural space through the interarcuate ligament. Horner's syndrome occurs within 5 min.
- A 150-watt surgical lamp is now focused upon the eye from 1 meter distance. 15 min later, a retrobulbar block is performed: The sclera is seized with an ophthalmic forceps and the eyeball is pulled downward and medially; a 23-gauge needle is then introduced through the superior rectus muscle, tangentially to the globe.

- It is immobilized as soon as a click indicates penetration of the retrobulbar space; correct placement is confirmed by free motion of the needle tip and protrusion-rotation of the eyeball upon injection of 1 ml of air.
- After aspiration, 2 ml of the tested anesthetic is then injected at a rate of 0.5 ml per second. The pupil dilates and reaches its maximal diameter (6 mm) within a few minutes.
- This apparent diameter is estimated with a 2-cm long ruler calibrated in millimeters, whose center is gently applied to the corneal center.
- The pupil is measured every 15 s for 5 min, then every 5 min until reappearance of maximal miosis (pinpoint and asymmetrical), a precise endpoint which generally coincides with corneal reflex and lacrimation.

Evaluation

- Drug latency (in min) and duration (in 5-min units) are averaged for both eyes of each animal, and the mean and standard deviation then calculated for all test animals. Analysis of variance is performed to find significant differences between various local anesthetics.



2. INFILTRATION ANESTHESIA

Procedure

- Adult guinea pigs of either sex weighing 250–300 g are chosen. On the day preceding the experiment the hair on the back is clipped and two areas of 4–5 cm diameter are shaved.
- This produces a certain amount of irritation which disappears overnight. The sensitivity of the skin is greatest in the midline and slightly more so in the front than in the back area. For this reason each concentration of a local anesthetic must be tested in both areas.
- Six tests using three guinea pigs can be performed simultaneously. The doses of local anesthetics are always injected intracutaneously in 0.1 ml saline. Three guinea pigs receive one dose in the front area and another dose in the back area; the size of the wheal is marked with ink.

- One side is used for the test preparation, the other side for the standard (e.g., 1% butanilicaine).
- The reaction to pin prick is tested 5 min after injection in the following way. After observing the animal's normal reaction to a prick applied outside the wheal, six pricks are applied inside the wheal and the number of pricks is counted to which the guinea pig fails to react.
- The pricks are applied at intervals of about 3–5 s. Six pricks are applied every 5 min for 30 min. Having completed the test on 3 guinea pigs, the same solutions are injected into 3 other guinea pigs, but the solution which was used for the front is now used for the back area and vice versa.

Evaluation

- The number of times the prick fails to elicit a response during the 30 min period is added up, and the sum, out of possible 36, gives an indication of the degree of anesthesia.
- Using various doses, dose-response curves can be established. For time-response curves, the prick tests are repeated every 10 min. Half-life times are calculated as the time, when after complete anesthesia 3 out of 6 pricks elicit again a response.

3. SURFACE ANESTHESIA

A. SURFACE ANESTHESIA ON THE CORNEA OF RABBITS

Procedure

- Albino rabbits of either sex weighing 2.5–3 kg are placed into rabbit holding cages. The upper and lower eyelashes are carefully clipped.
- The conjunctival sac of one eye is held open, thus forming a pocket. From a 1 ml syringe with a 22-gauge needle, 0.5 ml of a solution of the anesthetic is applied into the conjunctival sac for 30 s.
- Then the procedure is repeated, so that 1.0 ml is applied within 1 min. One ml of the standard (0.1% solution of tetracaine hydrochloride) is applied to the other eye.
- Effective local anesthetics extinguish the corneal reflex (blinking) elicited by any touch of the cornea. For quantitative purposes, the irritation with a bristle according to von Frey (1894, 1896, 1922) has been recommended.
- An equine hair bending at a load of 230 mg is attached perpendicularly to a glass rod.
- Within 25 s, the cornea is touched 100 times. The summation of many stimuli applied this way gives better results than a single touch with a glass rod. The test is started 5 min after application of the drug and repeated every 5 min until anesthesia vanishes and blinking

occurs again. The time between disappearance and reappearance of the corneal reflex is registered.

Evaluation

- Using the time of loss of the corneal reflex as parameter after application of different doses, dose-response curves can be established and potency ratios versus the standard calculated.

B. SUPPRESSION OF SNEEZING REFLEX IN RABBITS

Procedure

- Groups of male rabbits weighing 3 kg are used. Using a cotton tampon, the test solution is applied to the mucous membrane of one nostril.
- The solution of a standard local anesthetic is administered to the nasal mucosa of the other nostril. After 2 min the mucous membrane is stimulated by a fine pencil. Loss of the sneezing reflex is regarded as sign of complete anesthesia.
- The stimulation is repeated after 3, 6, 10 and 15 min and continued every 5 min until the sneezing reflex reappears. Various concentrations of test compound and standard are applied.

Evaluation

- Using the loss of the sneezing reflex as parameter after application of different doses, dose-response curves can be established and potency ratios versus the standard calculated. Furthermore, the duration of activity can be evaluated.

4. EPIDURAL ANESTHESIA:

EPIDURAL ANESTHESIA IN GUINEA PIGS:

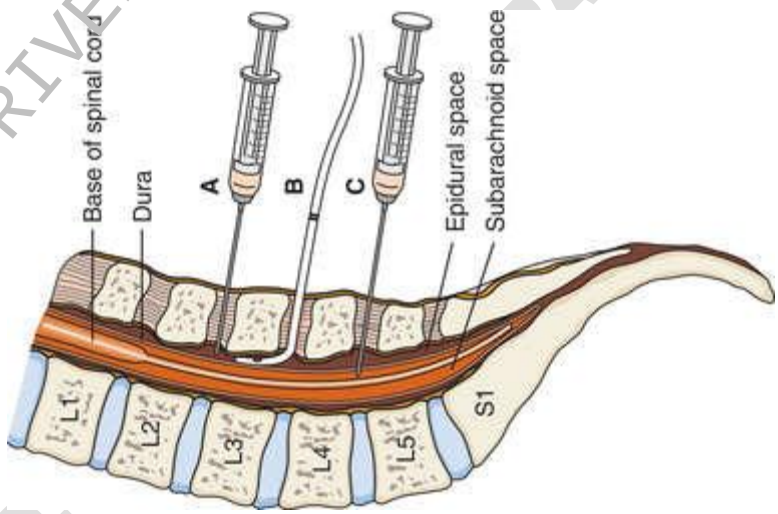
Procedure

- Male guinea pigs weighing 300–500 g are anesthetized by means of an intraperitoneal injection of an aqueous solution of chloral hydrate 42.5 g/l; ethanol 90 g/l; propylene glycol 428 g/l; sodium pentobarbitone 9.75 g/l; and magnesium chloride 21 g/l. A skin incision is made from the level of the lumbosacral fossa and approximately 1.5 cm down in order to expose the sacral area in the mid-line.
- With the vertebral column flexed, the lumbosacral intervertebral ligament is carefully incised. Through this small opening a polyethylene catheter (PE 10) is inserted maximally 1.5 cm along the roof of the vertebral canal to the L4–L5 region.

- The catheter is sutured to the overlying lumbar fascia which is then closed. The catheter is tunneled under the skin and exteriorized through an incision in the neck region.
- After fixation of the catheter to the fascia of the neck muscles and suturations of the incisions, the catheter is filled with saline and sealed.
- After a recovery period of at least 1 day, 0.1 ml of 2.0% lidocaine is injected over a period of 1 min, and the motor and sensory blocks are assessed.
- The injection of lidocaine which results in a bilateral, reversible blockade indicates a successful preparation. A minimum of 8 animals are used in the further experiments for each test solution.

Evaluation

- Mean time to onset of block and mean duration of block are calculated from number of legs blocked.



5. INTRATHECAL (SPINAL) ANESTHESIA:

SPINAL ANESTHESIA IN RATS

Procedure

- Male Sprague-Dawley rats weighing 50–75 g are used. The rat is held firmly by the pelvic girdle. A 30-gauge needle is attached to a 25-µl Hamilton syringe is inserted into the tissue on one side of the L5 or L6 spinous process at an angle of about 20°.
- The needle is advanced to the groove between the spinous and transverse processes and then moved forward the intervertebral space at an angle of about 10°. About 0.5 cm of the needle is then in the vertebral column.

- Correct placement of the needle is indicated by an arching of the tail. Drugs are dissolved in saline or water and administered in a volume of 5 µl.
- Antinociception is determined in a modification of tail flick assay in rats by placing the tail of the rat under a focused radiant heat source.

Evaluation

- The degree of antinociception is defined as the percentage of maximum possible effect. This percentage is determined for each dose at each time measured allowing to calculate *ED*50 values.