

Experiment no. 15

Aim: Study of Local Anesthetics by Different Methods

References:

1. Ghosh, M. N. (2008). *Fundamentals of Experimental Pharmacology*. Hilton & Co.
2. Kulkarni, S. K. (2019). *Handbook of Experimental Pharmacology*. Vallabh Prakashan.
3. OECD Animal Testing Guidelines.
4. PubMed: Local anesthetic mechanism studies. <https://pubmed.ncbi.nlm.nih.gov>

Background and Principle:

Local anesthetics (LAs) are drugs that cause reversible loss of sensation in a localized area of the body without affecting consciousness. They act mainly by blocking voltage-gated sodium channels in the neuronal cell membrane, preventing nerve impulse conduction.

Their effectiveness can be studied using different models in animals by observing:

- Loss of pain response (nociception)
- Loss of corneal reflex (motor/sensory inhibition)
- Inhibition of nerve conduction
- Increased pain threshold

Common agents: Lidocaine, Procaine, Bupivacaine

Experimental Methods:

1. Intradermal Wheal Test (Guinea Pig Pinna Method)

Principle: When an acid (like HCl) is applied to the skin, it causes pain. Local anesthetics injected into the skin reduce this pain response by blocking sensory nerves.

Procedure:

1. Take a healthy guinea pig and restrain it gently to prevent injury.
2. Clip hairs from the ear (pinna) if necessary.

3. Apply a drop of 0.1N HCl to the skin of both ears. Observe the pain response like vocalization or twitching (this is the control response).
4. Inject 0.2 mL of 2% lidocaine intradermally into one ear (treated ear).
5. After 5 minutes, reapply HCl to both ears.
6. Record the absence or presence of pain response in the treated ear.

Observations:

Drug Used	Response Before Drug	Response After Drug	Duration of Anesthesia
Lidocaine	Positive (Pain)	Negative (No Pain)	~15 minutes

Interpretation:

Loss of pain response in the treated ear indicates effective local anesthetic action

2. Rabbit Corneal Reflex Test

Principle: Local anesthetics abolish the corneal reflex (blinking) by numbing the cornea and blocking sensory nerve endings.

Procedure:

1. Gently restrain a healthy rabbit.
2. Apply 1–2 drops of test drug (e.g., lidocaine or procaine) to one eye.
3. The other eye serves as a control.
4. After 1 minute, lightly touch the cornea with a cotton wisp.
5. If the rabbit does not blink, corneal reflex is lost.
6. Test at 1-minute intervals until reflex returns.

Observations:

Drug	Onset Time	Duration of Anesthesia	Time for Reflex to Return
Procaine	2 min	10 min	~12 min
Lidocaine	1 min	20 min	~22 min

Interpretation:

Shorter onset and longer duration indicate more potent anesthetic action. Lidocaine performs better than procaine.

3. Frog Sciatic Nerve-Gastrocnemius Muscle Preparation

Principle: Stimulation of a frog's sciatic nerve causes contraction of the gastrocnemius muscle. Application of a local anesthetic blocks the nerve impulse, and the muscle stops contracting.

Procedure:

1. Pith a frog and dissect to expose the sciatic nerve and gastrocnemius muscle.
2. Mount the nerve-muscle preparation on a stand or kymograph.
3. Stimulate the sciatic nerve electrically and observe muscle contractions.
4. Apply lidocaine directly on the sciatic nerve.
5. Observe time taken for abolition of contractions.
6. Rinse the nerve with saline and observe recovery of response.

Observations:

Drug	Time Applied	Time of Conduction Block	Recovery Time
Lidocaine	0 min	5 min	20 min

Interpretation:

Disappearance of muscle contraction shows blockade of nerve conduction by local anesthetic.

4. (Optional) Tail Immersion Method in Rats

Principle: A local anesthetic reduces pain sensation and increases the latency (reaction time) for tail withdrawal in response to heat.

Procedure:

1. Immerse 2–3 cm of the rat's tail in warm water ($50 \pm 0.5^{\circ}\text{C}$).
2. Record the tail-flick latency (seconds).
3. Administer test drug locally at tail base.
4. Measure latency at 5, 10, 15, and 20 minutes.

Observations:

Time (min)	Control Group	Lidocaine Group
0	2.2 sec	2.3 sec
5	2.1 sec	7.8 sec
10	2.0 sec	6.5 sec
15	2.1 sec	5.1 sec

Interpretation: Longer tail-flick latency indicates increased pain threshold due to local anesthetic action.

Results and Discussion:

- Lidocaine showed faster onset and longer duration compared to procaine.
- The corneal reflex test is useful for sensory nerve evaluation.
- Intradermal and tail immersion methods help assess peripheral nerve block and pain relief.
- The frog nerve-muscle setup demonstrates nerve impulse conduction block, a core mechanism of action.

Conclusion:

All tested methods confirm that local anesthetics like lidocaine produce reversible inhibition of pain or nerve conduction. Lidocaine is superior in potency and duration compared to procaine.

Precautions:

- Follow ethical and humane guidelines for animal use.
- Use freshly prepared and sterile solutions.
- Avoid injury or contamination of eyes, ears, or tissues.
- Dispose of biological waste safely.