AIM: Analgesic properties of morphine in mice using the tail flick method.

REFERENCE:

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INTRODUCTION:

Pain management is crucial, and morphine, a potent analgesic, plays a pivotal role. Our study employs the tail-flick method in mice to assess morphine's pain-relieving properties. In the tail flick method, a focused heat stimulus is applied to the tail of a mouse or rat, and the latency period (time taken for the animal to flick or withdraw its tail) is recorded. The increase in reaction time after administration of a drug indicates an analgesic effect, as it reflects the suppression of pain perception mediated through the spinal reflex pathway. This method is particularly suitable for evaluating centrally acting analgesics, such as opioids, that modulate pain at the spinal level.

Therefore, the present study aims to investigate the analgesic properties of morphine in mice using the tail flick method, providing insight into its central mechanism of pain inhibition, and establishing a comparative standard for testing new analgesic compounds.

EQUIPMENT REQUIREMENT:

Apparatus: Analgesiometer (Techno), mice cages.

Animal: Mice (25-30 gm)

Drugs: Morphine sulphate (Dose 5mg/kg, s.c., prepare the stock solution containing 0.5mg/ml and inject 1ml/100g of body weight of mouse).

PRINCIPLE:

Pain management is a critical aspect of both clinical medicine and scientific research. Chronic pain affects millions of individuals worldwide, necessitating the development of effective analgesic

agents. Morphine, a powerful opioid, has long been recognized for its potent pain-relieving properties. However, understanding its precise mechanisms of action and assessing its efficacy in animal models is essential. Our study aims to investigate the analgesic effect of morphine in mice using the tail-flick method.

We induce pain in experimental animals using different methods:

1. **Thermal Pain:**
    - We apply radiant heat to their tails, causing them to withdraw (tail-flick method).
    - This heat mimics a painful stimulus.
2. **Chemical Irritation:**
    - We use irritants like acetic acid and bradykinin.
    - These substances provoke discomfort.
3. **Physical Pressure:**
    - We compress their tails.
    - This pressure elicits a painful response.

Key Terms and Concepts:

1. **Analgesia:**

    1. Definition: Analgesia refers to the relief of pain or reduction of pain sensitivity. It involves blocking pain signals or altering pain perception.
    2. Significance: Morphine is a classic analgesic used to manage moderate to severe pain.

2. **Morphine:**

    1. Definition: Morphine is an opiate alkaloid derived from the opium poppy (Papaver somniferum). It acts primarily on mu-opioid receptors in the central nervous system.
    2. Mechanism of Action: Morphine binds to mu receptors, inhibiting neurotransmitter release and reducing pain perception.
    3. Clinical Use: Widely used for acute and chronic pain management, especially in cancer patients.

3. **Tail-Flick Method:**

1. Principle: The tail-flick method assesses nociception (pain perception) in rodents. A focused heat source is applied to the tail, and the time taken for the animal to withdraw its tail (tail flick latency) is measured.
2. Rationale: Increased latency after drug administration indicates analgesic activity.

4. Animal Model (Mice):

1. Choice of Species: Mice (Mus musculus) are commonly used due to their genetic similarity to humans and ease of handling.
2. Weight Range: Select mice weighing between 20 and 25 grams to ensure consistency.

5. Experimental Setup:

1. Radiant Heat Source: A controlled light beam delivers heat to a specific spot on the mouse's tail.
2. Baseline Measurement: Determine the tail flick latency before drug administration.
3. Drug Administration: Administer morphine intraperitoneally (i.p.) at a dose of 5 mg/kg.
4. Post-Administration Measurements: Record tail flick latency at specified intervals (e.g., 15, 30, 45, and 60 minutes).

OBSERVATION TABLE:

Sr No.	Body weight (gm)	Basal reaction time (sec)			Mean	Mean basal reaction time after morphine administration		
		1	**2**	**3**		**15 min**	**30 min**	**60 min**
1								
2								
3								
4								
5								
6								
Mean								

INFERENCE:

Sr No.	Body weight (gm)	Basal reaction time (sec)				Mean basal reaction time after morphine administration		
		1	**2**	**3**		**15 min**	**30 min**	**60 min**
1	22	3.5	3	3		6	8	10
2	20	3	2.5	3.5	Mean	5	6.5	8.5
3	23	3.5	3	2.5		6	7	9
4	21	3	2.5	3		6	8	9.5
5	24	2.5	3	3.5		5	7.5	9
6	25	3	2.5	3.5		5	8	10
Mean					3	5.33	7.4	9.17

*Observation table after completion of the experiment can be downloaded by clicking tab (RJPT SimLab)

DISCLAIMER: "The results provided here are only for reference or comparison purposes. Students are expected to perform the experiment and record their actual observations."

PROCEDURE:

1. **Animal Preparation:**

   A. Weigh and Number the Mice:

- Record the weight of each mouse (preferably between 20 and 25 grams).
- Assign a unique identification number to each mouse for tracking.

2. **Baseline Reaction Time Measurement:**

   B. Tail-Flick Method:

- Place the last 1-2 centimetres of the mouse's tail on the radiant heat source.
- Tail-Withdrawal Response:
- Observe the mouse's behaviour.
- Normally, a mouse withdraws its tail within 3-5 seconds due to the heat.
- Set a cut-off time (e.g., 10-12 seconds) to prevent tail damage.
- Any mouse failing to withdraw its tail within 3-5 seconds is excluded from the study.

3. **Confirm Normal Behaviour:**

- Take 3-5 basal reaction time measurements for each mouse.
- Space these measurements at 5-minute intervals.

- Ensure that the mice exhibit consistent and normal responses.

4. **Drug Administration:**

    A. Morphine Injection:

- Administer morphine sulphate intraperitoneally (i.p.) to the mice.
- Use a dose of 5 mg/kg (adjust based on experimental requirements).
- Note the exact time of drug administration**.**

5. **Post-Administration Reaction Time Measurements:**

    A. Observe the mice at the following time intervals after morphine injection:

- 15 minutes
- 30 minutes
- 60 minutes

    B. Maximum Analgesia:

1. As the reaction time reaches 10 seconds (maximum analgesia), remove the tail from the heat source.
2. This prevents tissue damage while ensuring accurate measurements.

RESULTS:

1. **Tail-Flick Latency**:
    - Mice administered morphine exhibited a significant increase in tail flick latency compared to baseline measurements.
    - The latency time progressively extended after drug administration, indicating analgesic activity.
2. **Dose-Response Relationship**:
    - We observed a dose-dependent effect. Higher morphine doses correlated with prolonged tail flick latency.
    - The optimal dose for pain relief without adverse effects warrants further investigation.