Pharmacology & Toxicology (2) Lab.

Summer Semester
(2019 - 2020)

Presented by:

Dr. Khaled F. Al-Massri

Assistant Professor of Pharmacology and Toxicology,

Faculty of Medicine and Health Sciences,

University of Palestine, Palestine

Course outlines

Lab. 1: Introduction to experimental pharmacology

Lab. 2: To demonstrate the analgesic effect of drug in mouse using hot plate/tail flick method

Lab. 3: To study the effect of various tranquilizers and sedatives on motor co-ordination by Rota Rod test in rodents

Lab. 4: To demonstrate the anticonvulsant property of drug against pentylenetetrazole (PTZ) induced convulsions in mice

Lab. 5: To demonstrate amnesic effect of drug in rat using Morris water maze apparatus

Lab. 6: To demonstrate the anti-inflammatory property of drug against carrageenan induced paw edema

Lab. 7: To demonstrate analgesic effect of drug against acetic acid induced writhing in rat

Lab. 8: To demonstrate gastric ulcer induction/formation by different methods

Lab. 9: Revision

Lab. 10: Final Exam

References

- Medhi, B., & Prakash, A. (2010). Practical Manual of Experimental and Clinical Pharmacology: Jaypee Brothers, Medical Publishers Pvt. Limited

Lab. 1

Introduction in experimental pharmacology

Presented by:

Dr. Khaled F. Al-Massri

Assistant Professor of Pharmacology and Toxicology,

Faculty of Medicine and Health Sciences,

University of Palestine, Palestine

- Pharmacological experiments are designed to study the effects of drugs on tissues, organs, and other living subjects
- Find out new therapeutic agents
- Study the mechanism(s) by which the drug interact and affect the targets
- Clinical Trials: Study the effect (s)/side effect (s) of drugs on humans

Laboratory Animals

Rats were first used for experimental purposes

Carefully bred rats are used in animal testing for a number of reasons, including their frequent reproduction, genetic purity and similarities to human biology

Lifespan	2.5-3.5 years
Adult weight	M 300-500g, F 250-300g
Birth weight	5-6g
Heart rate	330-480 beats/minute
Respiratory rate	85 breaths/minute
Body temp.	35.9-37.5°C

Rats

Rats are generally fed a diet containing low fiber, protein and fat

Rat rooms are usually maintained at 30-70% relative humidity and a temperature of 18-26°C

Rats should be acclimatized to handling to reduce stress

Blood can be collected from several sites in the rat including tail vein, retro-orbital sinus, vena cava or cardiac puncture

Can receive oral, IP, IM, and IV

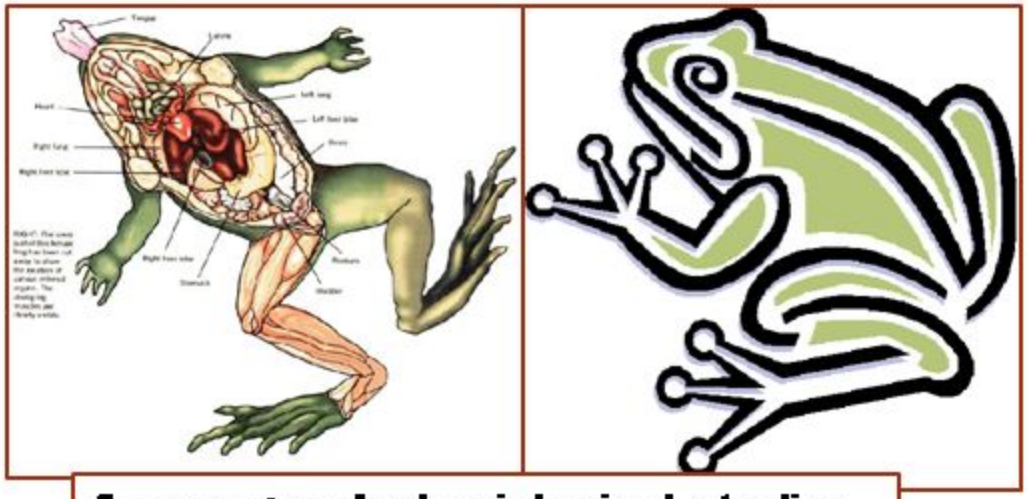
Mice

Lifespan	1-3 years
Adult weight	M 20-30 g, F 18-35g
Birth weight	1-2g
Heart rate	310-840 beats/minute
Respiratory rate	80-230 breaths/minute
Body temp.	36.5-38°C

Easy to make disease models

The mouse and human genomes are about 85 percent the same, and those similarities have made the mouse a powerful model for studying human biology and disease

Handling, blood collection, and drug administration: same as rats



frog or toad: physiological studies

guinea pig: hypersensitive test (allergic reaction) or the screening of anti-asthmatic drug

Rabbit: Expensive, the effect of some drug

Other: Cat, Dog, pig, and Monkey

How to write a laboratory report

Each student should write his report by himself using his own words to improve his language. **DO NOT COPY.**

The report should be brief, precise and including the following items:

1) The name of the student and the experiment.

2) The principle of the experiment.

3) The aim of the experiment.

4) Materials and methods used.

5) Results, including the measurements and observations

during the experiment that sometimes should be arranged in a form of table.

6) Discussion and conclusion

Lab. 2

To demonstrate the analgesic effect of drug in mouse using hot plate/tail flick method

Presented by:

Dr. Khaled F. Al-Massri

Assistant Professor of Pharmacology and Toxicology,

Faculty of Medicine and Health Sciences,

University of Palestine, Palestine

Analgesic activity study of drugs by hot plate (Eddy's hot plate) method

BACKGROUND

Algesia (sensitivity to pain) can be induced by various ways to the animals. Painful response can be noted with and without pain killers (analgesics). Analgesic drugs inhibit the sensitivity to pain.[1] In this method, heat is the source for pain. A constant heat (55 °C) will be given to the animal by hot plate and the response of the animals e.g., jumping, paw licking and withdrawal will be observed. Aim of this experiment is to study analgesic activity of drug by Eddy's analgesiometer.

REQUIREMENTS

Animals	:	Mice (20-25 g) or Rat (150-180 g)
Drugs	:	Morphine sulphate (5 mg/kg), inject i.p. 1 ml/100 g body weight,
Other drugs:		Pentazocin (20 mg/kg, i.p.), Aspirin (100 mg/kg, p.o.)
Equipment :		Eddy's hot plate (55 °C heat for painful stimulus) analgesiometer

Analgesic activity study of drugs by hot plate (Eddy's hot plate) method

1 Weigh and mark the animals

2 Observe the basal reaction time with a constant temperature (55 °C)

3 Inject Morphine sulphate (5 mg/kg), i.p.

4 Note down the reaction period at different time intervals (15, 30, 60 and 120 min)

5 Observation table.

S.No.	Animal Weight	Basal reaction time (sec)		Reaction time after treatment	
		Paw licking	Jump response	Paw licking	Jump response
1.					
2.					
3.					
4.					

PROCEDURE

Weigh and mark the animals. Observe the basal reaction time by placing the animals on the hot plate and note the jumping or paw licking (any of the first reaction) in animals with a constant temperature (55 °C). Usually in 6-8 sec animals show the response. A cut off period of 15 sec will be considered to avoid any damage to the paws.[2] Inject drug to animals and note down the reaction period of animals on the hot plate apparatus at different time intervals including 15, 30, 60 and 120 min after the drug treatment. As the reaction time increases with drug treatment, 15 sec will be taken as maximum analgesia and the animals are to be taken from the analgesiometer to avoid injury. Calculate percent increase in reaction time at each time interval.

Observation table.

S.No.	Animal Weight	Basal reaction time (sec)		Reaction time after treatment	
		Paw licking	Jump response	Paw licking	Jump response
1.					
2.					
3.					
4.					

CONCLUSION

From this experiment this can be concluded that, the drug inhibits pain stimulus and with increase in time after administration which has analgesic property.

Analgesic activity study of drugs by tail-flick method

BACKGROUND

Algesia (sensitivity to pain) can be induced by various ways to the animals. Painful response can be noted with and without pain killers (analgesics). Analgesic drugs inhibit the sensitivity to pain.[1] Pain is induced by radiant heat applying on a selected spot generated by a beam of light.

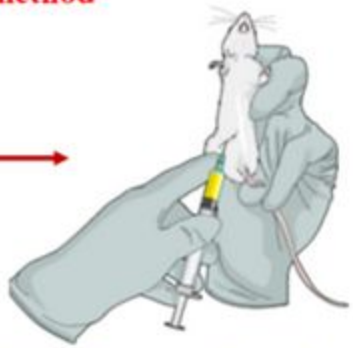
REQUIREMENTS

Animals	:	Mice (20-25 g) or Rat (150-180 g)
Drugs	:	Morphine sulphate (5 mg/kg), inject i.p. 1 ml/100 g body weight,
Other drugs	:	Pentazocin (20 mg/kg, i.p.), Aspirin (100 mg/kg, p.o.)
Equipment	:	Tail flick analgesiometer, Mouse restraint

Analgesic activity study of drugs by tail-flick method

Pharmacology

Analgesic activity study of drugs by tail-flick method



2 Allow the animals to acclimatize and calm and without movement of tail

1 Weigh and mark the animals

4 Inject Pentazocin (20 mg/kg, i.p.)

3 Apply heat not more than 3 cm from the tail tip.
Note the flicking time.
Consider 10 sec as maximum algesia.

5 Note down the reaction time after 30 min of drug injection at 15, 30, 45 and 60 min.

6 Observation table.

Sl. No.	Body Weight	Treatment	Basal reaction time (sec)	Reaction time after 30 min of drug treatment

Credit: Mindthegraph.com

PROCEDURE

Weigh and mark the animals. Allow the animals to acclimatize. Hold the animal, gently cover with a glove to restrain. Perform the experiment when the animal will be calm and without movement of tail. Ensure that the animals have no previous damage in the tail at the time of experiment. Hold the test animal under heat source and press the start button. Heat will be applied not more than 3 cm from the tip. After application of heat, the animal will withdraw its tail with sudden flick. Set a timer at the start of application of heat and note down the time of withdrawal of tail. The withdrawal of tail from the heat source is referred as tail flick latency. Check the basal reaction time of animals and note down. When the reaction time reaches 10 sec will be considered maximum analgesia to avoid damage. Start the experiment 30 min after drug treatment.[2] Reaction time after 15, 30, 45 and 60 min of drug treatment will be noted. Calculate the percentage of increase in reaction time or index of analgesia at above time interval.

Observation table.

Sl. No.	Body Weight	Treatment	Basal reaction time (sec)	Reaction time after 30 min of drug treatment

CONCLUSION

Prolonged latency time ensures relief of pain. From this experiment it can be concluded that the tested drug has analgesic ativity

Calculation for group 1

Mouse No	X	$X-\bar{x}$	$(X-\bar{x})^2$
1			
2			
3			
4			
5			
	Mean ($\bar{x}$) =		Total =

$$s^2 = \frac{\sum (X - \bar{X})^2}{N - 1}$$

$SD = \sqrt{S^2}$

$SE = SD \backslash \sqrt{n}$

$$T\,calc = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{SE_1^2 + SE_2^2}}$$

Ttab → 2 tail P = 0.05

Ttab → Degree of freedom (D.F) = $(n_1 - 1) + (n_2 - 1)$

If Tcalc > Ttab statistically significance

Two Tailed Significance						
Degrees of freedom (n-1)	$\alpha = 0.20$	0.10	0.05	0.02	0.01	0.002
1	3.078	6.314	12.706	31.821	63.657	318.300
2	1.886	2.920	4.303	6.965	9.925	22.327
3	1.638	2.353	3.182	4.541	5.841	10.214
4	1.533	2.132	2.776	3.747	4.604	7.173
5	1.476	2.015	2.571	3.305	4.032	5.893
6	1.440	1.943	2.447	3.143	3.707	5.208
7	1.415	1.895	2.365	2.998	3.499	4.785
8	1.397	1.860	2.306	2.896	3.355	4.501
9	1.383	1.833	2.262	2.821	3.250	4.297
10	1.372	1.812	2.228	2.764	3.169	4.144
11	1.363	1.796	2.201	2.718	3.106	4.025
12	1.356	1.782	2.179	2.681	3.055	3.930
13	1.350	1.771	2.160	2.650	3.012	3.852
14	1.345	1.761	2.145	2.624	2.977	3.787
15	1.341	1.753	2.131	2.602	2.947	3.733