PRATCTICAL BOOK OF PHARMACOLOGY EXPERIMENTS

(ISSN 978-93-5408-448-5)

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Expt. No.1

Title: introduction and appliances used for bioassay.

Theory:

Biological standardization (Bioassays)

Biological standardization or bioassay of drugs is defined as the assessment of the activity of a preparation by measuring its effect on living animals or tissues. It is essential that the amount of the active principle in a dose be uniform and of known potency. This enables the clinician to prescribe the dose that will always, as nearly as possible, induce the same magnitude of action. Thus, bioassay is a procedure for determining the quantative relationship between the dose (or concentration) of a drug and the magnitude of response it evokes. Such procedures utilize intact animals, isolated living tissues, animal preparation or microorganisms.

Biological standardization or bioassays are procedures by which the potency or the nature of the substance (constitution) is estimated by studying its effect on living matter. Bioassay are generally done using animal tissue or organs as in the case of use of g. pig ileum for the estimation of histamine or using the intact animal as in the case of bioassay of insulin using mouse or digitalis bioassay in g. pig.

Bioassay procedure are generally employed

1) When a chemical assay for the substance is not available or the substance gets inactivated by interacting with chemicals as the case with hormones.

2) When the quantity of the sample is too small. In such situation a matching type bioassay is continently done to compare the biological response with the standard drug.
3) To estimate the concentration of the active principle present in the tissue extract, the endogenous mediator like acetyl choline, 5 HT, prostaglandin.

4) To measure the pharmacological activity of new or chemically unidentified substances.
5) To measure the drug toxicity.

6) When the bioassay is more sensitive than the chemical assay.

The precision, reliability and reproducibility of a bioassay depends on the proper selection of the tissue or method with highest sensitivity and sensitivity for the drug. In spite of the tremendous and advancements made in the analytical chemistry and modern instrumentation, bioassay procedures continued to be used as successful tools not only I the estimation of the bioactive substances but also for the discovery of biologically active substances.

Bioassays are also essential in the development of new drugs. In the preclinical assessment of a new compound, the biological activity is compared with that of known (standard) compound (s) using appropriate test systems. In such studies the tests must be simple, reproducible and economical. Biological assessment of a new compound generally consists of carrying out a battery of such assays and based on these tests constructing a profile of activity. Clinical testing of drugs is guided by such profile of activity generated in animals.

Types of bioassays:

Bioassays are two types namely, quantal and graded. The quantal assay is a ‘all or none’ phenomenon. For example, insulin induced hypoglycemic convulsive reaction or the cardiac arrest caused by the digitalis. In both these cases the end point is an all or none response, i.e. either conclusions or no convulsions, similarly the cardiac arrest. On the other hand ‘the graded response’ assays are based on the observations that there is the proportionate increase in the observed response with a subsequent increase in the concentration or dose. The parameters employed in such bioassays are based on the nature of the effect the substance is expected to produce. For example, concentration of smooth muscle preparation for assaying histamine or the study of th blood pressure response in case of adrenaline.

The graded bioassays can be performed by employing any of the following techniques. The choice of the procedures depends on the 1) precision of assay demands 2) quantity of the sample available 3) availability of experimental animals.

a) **Matching bioassay**- It is the most simple type of bioassay. In this type of bioassay the response of the test substance is taken first and the observed response is tried to match with the response that is obtained with the standard drugs. Several responses of standard drug are recorded till a close matching response to that of test substance is observed. A corresponding concentration is thus calculated. This assay is employed when the sample size is very small. Since the assay does not involve the recording of concentration response curve, the sensitivity of the preparation is not taken into consideration. Therefore, the precision and reliability are not very good by this method.
b) **Interpolation method**- In this type of bioassay a concentration –response curve of a standard substance is first established. Then record 2-3 responses due to test substance. The selection of the test responses should be such that they lie on the linear portion of the concentration response curve of the standard drug. The precision and reliability of the assay is much better as compared to matching assay as the sensitivity of the preparation is assessed prior to testing the unknown sample.
c) **Bracketting method**-This method is made use of when the test sample is small. It is also a simple assay procedure. The response due to test substance is bracketted between two responses (greater and smaller) of standard substances. The precision and reliability of this procedure is also poor.
d) **Multiple point bioassay**- The multiple point bioassays could be: 1) three point bioassay, where two responses of standard drug and one response due to test sample are taken into consideration. The test response should be intermediate between the two responses due to standard drug. 2) Four point bioassay, where two responses of standard drug and two responses of test substance are made use of . The selection of two responses of the standard should be such that they lie on the liner portion of the concentration response curve and also the ration between the doses should be preferably 1:2. The selection of test response is determined by hit and trial method so that the responses fall on the liner part of the curve. Employing the Latin square design the responses are recorded in a random fashion. The precision, reliability and reproducibility of this assay method is very high. It is most common used for estimating the concentration of the unknown sample. 3) Six point bioassay, where three concentration of standard drug and three concentrations of test substances are used. The selection of doses is done as described under four-point assay. It is more time consuming procedure.

Principles of bioassay of drug:

1) All bioassays (laboratory studies, toxicity studies, clinical trials) must be comparative against a standard drug or preparation.

2) The standard and the new drug should be , as far as possible identical to each other.
3) The method for comparing the unknown and the standard drug should preferably (but not essentially) test the therapeutic property of the drug.
4) The method should estimate, as far as possible, and allow an estimate of the error due to biological variation in different animals/ persons at any one time, and in the same animal / person at different times.

Application of bioassay methods:

The various applications of bioassay methods are:

1) Standardization of drugs of natural origin.
2) Estimation of biological substances like acetylcholine, adrenaline, noradrenaline and serotonin in body fluids or tissue extracts.
3) Screening of new compounds for biological activity. Even synthetic products are subjected to these methods, as chemical structure does not truly predict pharmacological activity.
4) Bioassay is employed if the drug is composed of a complex mixture of substances of varying structure and activity. E.g. digitalis, posterior pituitary.
5) Diagnosis and research. The concentration of gonadotropin in the blood or in urine may be estimated by injecting these fluids in animals (chemical methods may be employed, if available).
6) Estimation of the dose of a drug required to produce a therapeutic or toxic response, e.g. ED50 (effective dose 50) or LD 50 (Lethal dose 50).

Summary of experimental details for some common isolated preparations (ON LEFT PAGE)

Preparation	**Salt solution**	**Temp. (^{O}C)**	**Gas**	**Lever**	**Tension (g)**	**Magnification**
Guniea pig trachea	Krebs	37	Carbogen (5% CO2 in O2)	Isotonic frontal	0.2-0.5	12-20
Goat trachea	Krebs	37	Carbogen (5% CO2 in O2)	Isotonic frontal	0.2-0.5	12-20
Rat ileum	Tyrode	37	O2	Isotonic frontal	0.25	7

Rat uterus	De Jalon	25-36	Carbogen (5% CO_2 in O_2) or O_2	Isotonic frontal	0.5-1	4
	Krebs	37	Carbogen (5% CO_2 in O_2)	Isotonic frontal	4	4
	Mc Ewen	34	O_2	Isotonic frontal	4	4
	Locke	37	Carbogen (5% CO_2 in O_2)	Isotonic frontal	4	10
Frog rectus	Frog ringer	Room	O_2 or air	Simple or gimbal	1-1.5	6-10

Experiment no.3

Aim : To record concentration response curve of acetylcholine using Chicken ileum

Requirement :

Animal : Hen/Rat

Apparatus : Student Thermostatic organ bath,areation tube,areator,frontal writing lever,forceps.

Physiological salt solution: Tyrode Solution

Drug : Acetylcholine

Stock Solution (100 µg/ml)

Principle :

Dose response curve demonstrate graded responses to drugs where increase in response is recorded with a subsequent increase in dose or concentration of drug. The dose response curve is sigmoid or "S - shaped" curve. The first 25% portion of curve has poor discrimination where as middle portion shows greater sensitivity to different concentration & responses to increasing concentration are linearly differentiated, last part of curve shows ceiling effect where no more increase in response is seen with further increase in dose, when doses are increased in geometric progression (logarithmic intervals) & responses are plotted against logarithm of doses, the relationship is called as log dose response curve. The logarithmic transformation of dose offers some advantages such as:

1. Linear portion of sigmoid curve become straight.
2. A comparison of two dose response curve is much simpler.
3. Large dose ranges can be plotted.
4. Error is described all through the graph independent of dose.

Procedure:

1. Remove the attached tissue of ileum & the ileum should be aerated with aerator.

2. Tie the thread to top & bottom of each ileum in upright position in the organ bath containing tyrode solution under the tension of 0.5mg.

3. There is no need to maintain the bath temperature bubble organ bath with air.

4. Relax tissue for 30 mins,during this wash the tissue 3 times with tyrode solution.Record the contraction due to acetylcholine using frontal writing lever.

5. The time cycle should be 5 mins in which 30 sec baseline,90 sec contact time & 3 min. washing.Cycle may be used for proper recording of response.

6. Record atleast 4 responses to increasing doses of acetylcholine or till you get maximum response.Maximum response is achieved,label the graph & major the height of response & draw a dose (concentration response curve).

References: S.K. kulkarni, Handbook of experimental pharmacology,3rd edition,Vallabh Prashan,Delhi 2005.pg no. 85

Result:

From concentration response curve of acetylcholine using chicken ileum, it is observed that as the concentration of acetylcholine increases then concentration response also increases.

Conclusion: From above result it is concluded that chicken ileum is highly sensitive to acetylcholine.

Table: concentration response curve of acetylcholine using chicken ileum (ON LEFT SIDE PAGE)also Graph.

Sr. no.	Drug	Conc.	Dose	Response
1	Acetyl choline	100µg/ml	0.1ml	
2	Acetyl choline	100µg/ml	0.2ml	
3	Acetyl choline	100µg/ml	0.4ml	
4	Acetyl choline	100µg/ml	0.6ml	
5	Acetyl choline	100µg/ml	0.8ml	

EXPERIMENT NO : 04

Aim:- To carry out bioassay of acetylcholine using isolated chicken ileum prepared by interpolation method.

Requirements:-

Animal :- Hen/Rat

Apparatus: - Student thermostatic organ bath, aeration tube, aerator, frontal writing lever, forceps,

Physiological salt solutions: Tyrode solution.

Drug: Acetyl choline – Standard solution

Acetyl choline – Test solution

Stock solution (100µg/ml)

Principle:

Dose response curve demonstrate graded response to drug where increase in response is recorded with a subsequent increase in dose or concentration of drug. The drug response curve is sigmoid or "s" shaped curve. The first 0.5% position of curve has poor discrimination whereas middle portion shows greater sensitivity to different concentration and responses to increasing concentration are linearly differentiated, last portion of curve shows ceiling effect where no more increase in response is seen with further increase in dose, when doses are increased in geometric progression (logarithmic intervals) &responses are plotted against logarithm of doses, the relationship is called as log dose response curve

The logarithmic transformation of dose affects some advantages such as:

1) Linear portion of sigmoid curve become straight.

2) A comparison of two dose response curve is much simpler.

3) Large dose ranges can be plotted.

4) Error is described all through the graph independent of dose.

Procedure:-

A) Remove the attached tissue of ileum and ileum should be aerated with aerator.

B) Tie the thread to top and bottom of each ileum in upright position in organ bath containing thyroid solution under the tension of 0.5mg

C) There is no need to maintain the bath temperature bubble organ bath with air

D) Relax tissue for 30min during this wash the tissue 3 times with tyroidsolution, record the contraction due to Ach using frontal writing lever

E) Thetime cycle should be 5min in which 30sec baseline 90sec contact time and 3min washing. Cycle may be used for proper recording of response

F) Plot concentration response curve due to standard Ach. Solution measured height of response due to different doses of test solution and read the corresponding concentration from standard curve.

Result: - The concentration of unknown sample of Acetyl choline by interpretation method using chicken ileum was found to be ----------µg/ml.

Reference: -S.K. Kulkarni, Handbook of Experimental pharmacology, 3rd edition. Vallabh Prakashan, Delhi 2005. Page no 85.

EXPERIMENT NO : 05

Aim:- To study the effect of atropine on CRC of acetylcholine using isolated chicken ileum.

Requirements:-

Animal :- Hen/Rat

Apparatus: - Student thermostatic organ bath, aeration tube, aerator, frontal writing lever, forceps,

Physiological salt solutions: Tyrode solution.

Drug: Atropine (100µg/ml)

Principle:

Dose response curve demonstrate graded response to drug where increase in response is recorded with a subsequent increase in dose or concentration of drug. The drug response curve is sigmoid or "s" shaped curve. The first 0.5% position of curve has poor discrimination whereas middle portion shows greater sensitivity to different concentration and responses to increasing concentration are linearly differentiated, last portion of curve shows ceiling effect where no more increase in response is seen with further increase in dose, when doses are increased in geometric progression (logarithmic intervals) &responses are plotted against logarithm of doses, the relationship is called as log dose response curve

The logarithmic transformation of dose affects some advantages such as:

1) Linear portion of sigmoid curve become straight.

2) A comparison of two dose response curve is much simpler.

3) Large dose ranges can be plotted.

4) Error is described all through the graph independent of dose.

Procedure:-

A) Remove the attached tissue of ileum and ileum should be aerated with aerator.

B) Tie the thread to top and bottom of each ileum in upright position in organ bath containing thyroid solution under the tension of 0.5mg

C) There is no need to maintain the bath temperature bubble organ bath with air

D) Relax tissue for 30min during this wash the tissue 3 times with tyroide solution, record the contraction due to Ach using frontal writing lever

E) The time cycle should be 5min in which 30sec baseline 90sec contact time and 3min washing. Cycle may be used for proper recording of response.

F) Record atleast 4 responses to increasing doses of ach or till u get max response.

G) Add 0.1 ml of atropine along with 0.1ml Ach and record the response for 30 sec.

Result: - From The graph it was found that atropine reduces the contractions of ileum preparation.

Reference: -S.K. Kulkarni, Handbook of Experimental pharmacology, 3rd edition. Vallabh Prakashan, Delhi 2005. Page no 85.

Expt: 6

Comment on Special instruction, any Drug interaction of following these examples and ADR in prescription

(**Amoxicillin and clavulanic acid**, **Metronidzole and ethyl alchohol, Ciprofloxacin and theophyline, Aspirin and warfarin**, Chloroquine and alkali mixture, **Sucralfate and antacid**, **L-dopa and pyridoxine**, Propanolol and verapamil, **Digoxin and hydrochlorthiazide**, Chlorpropamide and dicommurol, Gentamycin and gallamine, Lithium and thiazide, **Propanolol and insulin**, Enapril and spironolactone)

Theory :

A drug interaction is a reaction between two (or more) drugs or between a drug and a food, beverage, or supplement. Taking a drug while having certain medical conditions can also cause a drug interaction. For example, taking a nasal decongestant if you have high blood pressure may cause an unwanted reaction.

There are three types of drug interactions:

- **Drug-drug interaction**: A reaction between two (or more) drugs.
- **Drug-food interaction**: A reaction between a drug and a food or beverage.
- **Drug-condition interaction**: A reaction that occurs when taking a drug while having a certain medical condition. For example, taking a nasal decongestant if you have high blood pressure may cause an unwanted reaction. A drug interaction can make a drug less effective, increase the action of a drug, or cause unwanted side effects.

A) Drug: Ciprofloxacin and Theophyline

I. Theophylline : Anti asthmatic agent, Respiratory smooth muscle relaxant , Bronchodailator agent, Cardiovascular Agent

Mechanism of Action:

Theophylline Relaxes the smooth muscle of bronchial air way and pulmonary blood vessels and reduces air way responsiveness to histamine methacholine adenosine and allergens. Theophylline inhibits the types 3 and 4 phosphodiesterase(PDE) enzyme responsible for the breaking down AMP in smooth muscle cells.

II) **CIPROFLOXACIN :** Antibacterial**,** Enzyme inhibitor**,** Anti infective agent

Mechanism of action

Ciprofloxacin acts on topoisomeraseII(DNA gyrase)&topoisomerase IV. Ciprofloxacin targeting of the alpha subunits of DNA gyrase prevents if form supercoiling the bacterial DNA which prevents DNA replication

III) **DRUG DRUG INTERACTION**

Ciprofloxacin may significant increases blood levels of theophylline which may leads to potentially serious & life threatening side effects. nausea ,vomiting, confusion, restlessness ,loss of appetite, headache tremor, insomnia seizures, heart palpitations& irregular heartbeat as these may be signs & symptoms of excessive theophylline levels.

B) SUCRALFATE AND ANTACID:

I) SUCRALFATE: Ulcer protective

MOA: Sucralfate complex of aluminium hydroxide and sucrose octasulafte .It stomach to its anionic form which binds to the ulcer base. This creates a protective barrier to pepsin and bile and inhibits the diffusion of gastric acid.

II) ANTACIDS: Milk of Magnesia

MOA: when excessive amount of acids are produced. In the stomach the natural mucous barrier of that protects the lining of stomach can damage the esophagus in people with acid reflux.

III) Drug Drug Interaction: Some antacids can make it harder for sucralfate to absorb in stomach. So avoid taking an antacid within 30 min before taking sucralafate.

C) METRONIDAZOLE AND ETHYL ALCOHOL

I) Metronidazole :-Antibacterial, Antiprotozoal

MOA- It acts by inhibiting nucleic acid synthesis by distrupting the DNA of a microbial cell.

II) Ethyl alcohol: Antiseptic, Antidote(Antidote to methanol and ethylene glycol poisioning)

MOA-It promotes GABAa receptor mediated synaptic inhibition (through ca^{-} ion channel opening) Inhibit NMDA and kinate type of excitatory amino acid receptor (operating through cation channel) Ethanol indirectly reduce neurotransmitter release by inhibiting voltage sensitive neuronal channel.

III) DRUG DRUG INTERACTION:

Metronidazole can produce a reaction similar to that of disulfiram when administered to patients drinking ethanol. This drug/chemical interaction results in accumulation of

acetaldehyde in the blood. Acetaldehyde is hepatotoxic, cardiotoxic, and arrythmogenic.

D) L-DOPA AND PYRIDOXINE:

I) L- dopa :- Anti-Parkinson drug

Mechanism of action:- L-Dopa is prodrug. Levodopa is converted into dopamine by action of DOPA decarboxylase enzyme by decarboxylation within presynaptic terminals of dopaminergic neurons in the stratium. The dopamine produced is responsible for the therapeutic effectiveness of the drug.

II) **PYRIDOXINE: Vitamin B6 (Water soluble)**

Mechanism of action:-Pyridoxine is converted in erythrocytes to pyridoxal phosphate and to a lesser extent pyridoxamine phosphate which act as coenzyme for various metabolic functions. It is involved in conversion of tryptophan to niacin or serotonin.

III) **DRUG-DRUG INTERACTION**

Pharmacologic doses of pyridoxine (VIT B6) enhance the extracerebral metabolism of levodopa; decreases therapeutic effect of levodopa

E) DIGOXIN AND HYDROCHLORTHIAZIDE

I) **DIGOXIN: cardiotonic agent**

Mechanism of action : Digoxin increases the force of contraction of the muscle of the heart by inhibiting the action of the enzyme ATPse (na+, k+) that controls movement of calcium ,sodium ,and potassium into heart muscle. Calcium controls force of contraction.

II) **Hydrochlorthiazide: (Thiazide Diuretics**

Mechanism of action: It belongs to class of diuretics it reduces blood volume by acting on thr kidneys to reduce sodium reabsorption in the distal convoluted tubule thiazides increases the reabsorption of calcium in this segment in amanner unrelated to sodium transport.

III) **DRUG-DRUG INTERACTION**

Hydrochlorthiazide interact to digoxin diuretic interaction can lead to the development of serious cardiac arrhythmia.

F) ASPIRINS AND WARFARIN

I) **Aspirin**- (At low dose: Antiplatelet and At high dose: Analgesic)

Mechanisms of action: Aspirin is the only irreversible inhibitor cox enzyme apart from antipruritic, analgesic and anti-inflammatory effect aspirin has several other indication, Aspirin is used to inhibit niacin induced flushing.

II) **Warfarin**: (anticoagulant)

Mechanism of Action: Warfarin is interfering with clotting factor synthesis by inhibition of the C1 subunit of the vitamin K epoxide reductase (VKORC1) enzyme complex, thereby reducing the regeneration of vitamin K1 epoxide.

III) DRUG-DRUG INTERACTION: A combination of warfarin and aspirin is associated with increased bleeding compared with warfarin monotherapy.

G) Propranolol and Insulin

I) **Propranolol (** beta blockers)

Mechanisms-Propranolol is a competitive antagonist of beta-1-adrenergic receptors in the heart. It competes with sympathomimetic neurotransmitters for binding to receptors, which inhibits sympathetic stimulation of the heart

II) **Insulin**

Mechanisms- Insulin lowers blood glucose by stimulating peripheral glucose uptake primarily by skeletal muscle cells and fat, and by inhibiting glucose production and release by the liver. It also increases protein synthesis and conversion of excess glucose into fat.

Drug -Drug Interaction:- Oral propranolol impair glucose recovery way insulin induced hypoglycemia in insulin dependent diabetes mellitus .Thus are at increased risk Of prolonged hypoglycemia if treated with non selective beta adrenergic antagonist such as propranolol.

I) AMOXICILLIN and CLAVULANIC ACID

I. **AMOXICILLIN**: Antibacterial, Anti infective.

MOA-Competitively inhibit penicillin binding protein, penicillin bind proteins are responsible for glycosyltransferase and transpeptidase reactions that lead to cross linkage of D-alanine and D-aspartic acid in bacterial cell wall.

II. **CLAVULANIC ACID** : Antibacterial, Anti infective.

MOA- It contains Beta-lactum ring in its structure that binds in an irreversible fashion to beta-lactumase, preventing them from inactivating certain beta-lactum antibiotics.

II) **Drug Drug interaction-** Amoxicillin is in a class of medications called penicillin-like antibiotics. It works by stopping the growth of bacteria. Clavulanic acid is in a class of medications called beta-lactamase inhibitors. It works by preventing bacteria from destroying amoxicillin.

J) ENALAPRIL AND SPIRONOLACTONE

Enalapril:, ACE-Inhibitors

Mechanism of action: It competes with angiotension I for binding at the angiotensin –converting enzyme, blocking the conversion of angiotension I to Angiotension II.

Spironolactone: Diuretics

Mechanism of action : It blocks action of aldosterone so increase sodium excretion while decreasing potassium excretion.

DRUG-DRUG INTERACTION: Using enalapril together with spironolactone may increase the levels of potassium in blood (hyperkalemia), especially if patient have kidney disease, diabetes, heart failure.

K) PROPRANOLOL AND VERAPAMIL

Propranolol:Beta Blocker.

Mechanism of action: It is competitive antagonist of Beta-1 adrenergic receptor in the heart. It is complete with sympathomimetic neurotransmitter for binding to receptor, which inhibits sympathetic stimulation of heart. It works by relaxing blood vessels and slowing heart rate to improve blood flow and decrease the blood pressure.

Verapamil: Calcium Channel Blocker.

Mechanism of action : Block L- type of Calcium Channel in cardiac tissue decreases inward Calcium-current in SA Node and AV-Node. **Also** decrease conduction velocity and increase effective refractory period.

DRUG-DRUG INTERACTION – Synergistic or additive effect also involve negative (Chronotropic), (Ionotropic), (Dromotropic) Effect.

Experiment no.7

Aim:- To record the dose response curve of histamine on goat tracheal chain .

Principle: Guinea Pig ileum or goat trachea is a smooth muscle. It is very sensitive to histamine. Histamine acts on H1 receptor. Histamine acts as an agonist and produces contraction of Guinea Pig ileum or goat trachea. Histamine action on H1 receptor is blocked by H1 receptor antagonist like chlorpheneramine, mepyramine, estimazole, terfenadine etc. Acetylcholine acts as an agonist and produces contraction of Guinea Pig ileum or goat trachea. Acetylcholine action on muscarinic receptor is blocked by atropine and other parasympatholytic drugs like Homatrpopine, hyocine etc.

Requirements:

Apparatus: Student thermostatic organ bath, aeration tube, aerator, frontal writing lever, rubber tubes, tuberculin syringe, scissor, forceps.

Animal required: Guinea Pig ileum or goat trachea

Physiological salt solution: Krebs solution.

Drug solution: Histamine (1 mg/ml)

Procedure:

1) A goat is killed by stunning and cutting the throat as near the head as possible.
2) Trachea is dissected out and placed in a dish containing Krebs's solution kept at 37^0C with aeration.
3) Trachea is cleared off any connective tissue. Trachea consists of cartilaginous surface on the anterior surface and strip of smooth muscle on posterior aspect.
4) Cut the trachea transversely between the segments of cartilage so as to obtain rings of trachea muscle. These rings are usually 3-5 are tied with cotton thread so as to form chain.
5) This chain is them mounted in Krebs solution in organ bath. Here outer jacket is filled with water and maintained for 35-37^0C throughout the experiment.
6) Now one end of chain is attached to the aerator tube and another is attached to the lever. The solution in organ tube is bubbled with aerator i.e. 1 bubble/sec.
7) Avoid any stretching. Now apply the small load i.e. about 0.2-0.5gm and magnification should be high i.e. 10-12 fold.

8) Relax the preparation for 30 min. during this period wash the tissue with fresh Krebs solution at interval of 10 min.

9) Mean while prepare stock solution of Histamine (1 mg/ml)

10) Record the normal baseline on the drum for 30 second. At the end of 30 sec. add 0.1ml of histamine in to the inner organ bath and record the response of drug for 90 sec. after recording the response for 90 sec. switch off kymograph immediately, open the outlet of the inner organ bath and remove the Krebs solution present in the inner organ bath. Again fill the inner organ bath with fresh Krebs solution. Repeat the washing procedure 3-4 times or till the writing point of frontal lever comes to the base line. Then record the dose dependent effect of histamine solution using higher doses.

Result:

Dose response curve of histamine is as follows

Sr. no.	**Drug**	**Dose (ml)**	**Response in mm**
1	Histamine	0.1	
2	Histamine	0.2	
3	Histamine	0.4	
4	Histamine	0.8	
5	Histamine	1.6	

References :

1) Kulkarni S.K., handbook of experimental pharmacology; 3rd edition; Vallabh Prakashan , Delhi: 2005. P no. 94-95.

Composition of Krebs solution:

Ingradient	Nacl	Kcl	$Cacl_2$	$Mgso_{4.7H2o}$	KH_2PO_4	$NaHCo_3$	Glucose	Distilled water
Quantity (gm)	6.9	0.35	0.28	1.28	0.16	2.1	1	1 lit

Experiment no. 8

To carry out the bioassay of histamine using isolated goat treachea preparation by interpolation method

Aim: To estimate the strength of an unknown solution of histamine by interpolation bioassay method by using smooth muscle preparation of goat trachea.

Principal: Interpolation method of bioassay is less time consuming and yet reliable as compared to matching type of bioassay. One of the main advantages of this assay is that the sensitivity of the tissue is first determining by prior plotting of conc. response curve with the known agonist. If the linearity of the curve is good, one can do very accurate estimate of the test substance. Isolated tracheal chain of goat is utilized to study the bronchodilatory activity of the drugs. Antagonists of acetylcholine, histamine can be studied by using this preparation. The response is slow to produce but lasts for a longer time. If large effects have been produced it may be necessary to wait for 30 min before the preparation recovers completely. If doses are chosen which produce small effects, it is possible to use a 15 min time cycle in which the drug is left in contact with the tissue for 5 min. Drug to be tested: acetylcholine, histamine and 5-HT produce contraction of tracheal chain while Adrenaline, Noradrenaline, aminophylline and theophylline antagonizes the effect of these substances.

Procedure:

Isolated preparation:- Isolated tracheal chain of goat

Physiological solution: Krebs's solution

Drug: Histamine (1 mg/ml)

Apparatus: Students organ bath, Sherrington revolving drum machine, frontal writing lever, kymograph paper, aerator, reservoir, I.V. Set, plastisin.

Procedure:

11) Step 1-9 same as previous experiments.

12) Record the responses given by 0.1, 0.2, 0.4, 0.8 and 1.6 ml of standard solution of histamine on kymograph paper. Since response is slow to develop but lasts for a longer time and necessary to give longer lasting period.

13) Time cycle

    a) Base line recording: 30 sec

    b) Contact time: 3 min

c) Washing period or recovery period: 15-30 min

14) Then give the unknown solution of histamine and record the response as above.

15) Plot the concentration response curve as log concentration Vs response in mm.

16) Read the corresponding conc. of unknown drug from graph and report the result.

Result:

Concentration of unknown sample of histamine by interpolation method using isolated preparation of tracheal chain of goat was found to be …….µgm/ml.

References :

2) Kulkarni S.K., handbook of experimental pharmacology; 3rd edition; Vallabh Prakashan , Delhi: 2005. P no. 95.

3) Staff of the department of pharmacology, university of Edinburgh; pharmacological experiments on isolated preparation; 2nd edition; E. & S. Livingstone Ltd, Great Britan: 1970 P.no. 100-102.

Composition of Krebs solution:

Ingradient	Nacl	Kcl	$Cacl_2$	$Mgso_{4.7H2o}$	KH_2PO_4	Glucose	$NaHCo_3$
Quantity (gm)	6.9	0.35	0.28	1.28	0.16	1	2.1

Distilled water 1 lit.

Calculations: (Stock solutions: 1mg/ml)

Sr. no.	**Drug**	**Dose (ml)**	**Concentration (µg)**	**Log conc.**	**Response (mm)**
1	Histamine standard	0.2			
2	Histamine standard	0.4			
3	Histamine	0.8			

	standard				
4	Histamine standard	1.6			
5	Unknown histamine	0.5			