

PHARMACOLOGY-II

UNIT 5 NOTES

ENDOCRINE SYSTEM

- Androgens and Anabolic Steroids
- Estrogens, Progesterone and Oral Contraceptives
- Drugs Acting on Uterus

BIOASSAY

- Principles and Applications
- Types of Bioassay
- Bioassay of insulin, Oxytocin, Vasopressin, ACTH, d-tubocurarine, Digitalis, Histamine and 5-HT



NOTES AVAILABLE AT :



WWW.IMPERFECTPHARMACY.IN

ANDROGENS

- Androgens are group of hormones that play a key role in the development and maintenance of male characteristics.
- They are primarily responsible for promoting the development of male reproductive tissues & secondary sexual characteristics such as increased muscle mass, facial hair and a deep voice.

Classification of Androgens

Androgens are classified into two classes:

- ① Naturally Occurring Androgens
 - Testosterone
 - Dihydrotestosterone
 - Androsterone
 - Dehydroepiandrosterone
- ② Synthetic Androgens
 - Methyltestosterone
 - Fluoxymesterone
 - Mesterolone

SYNTHESIS

- The production of male hormones & sperm is regulated by Pituitary Gland & Hypothalamus.
- Luteinizing Hormone (LH) & Follicle Stimulating Hormone (FSH) are two essential messenger hormones produced by pituitary gland which act on the testes.
- LH is required by Leydig cells in testes for producing testosterone.
- Testosterone along with FSH acts on seminiferous tubules for producing sperm.

Functions of Androgens

- Androgens are essential for development of male reproductive organs (testes, prostate, seminal vesicles) during fetal development and puberty.
- Testosterone plays a key role in sperm production & sexual function.
- Androgens stimulate the development of male secondary sexual characteristics, including increased muscle mass, body hair, deepening of voice & broader shoulders.
- Androgens contribute to development of bone mass & strength.
- They also influence fat distribution, particularly promoting fat loss from abdomen in men.
- Androgens also affect mood, aggression & overall mental health.

ANABOLIC STEROIDS

- Anabolic steroids are synthetic derivatives of testosterone designed to enhance its anabolic (muscle-building) effects while minimizing its androgenic (masculinizing) effects.
- They are primarily used to promote muscle growth, enhance athletic performance & for various medical conditions.

Examples

- Oxymetholone
- Nandrolone
- Stanozolol
- Methandienone
- Oxandrolone

Mechanism of Action

- Anabolic steroids enter cells and bind to androgen receptors.
- The steroid-receptor complex translocates to the nucleus, binds to androgen response elements in DNA & regulates gene expression involved in protein synthesis, leading to muscle hypertrophy & repair.

PHARMACOKINETICS

- Absorption : They are available in both oral & injectable form.
- Distribution : They are distributed throughout the body.
- Metabolism : It is metabolised by Liver by CYP450 enzyme.
- Excretion : Excreted primarily in urine.

Therapeutic Uses

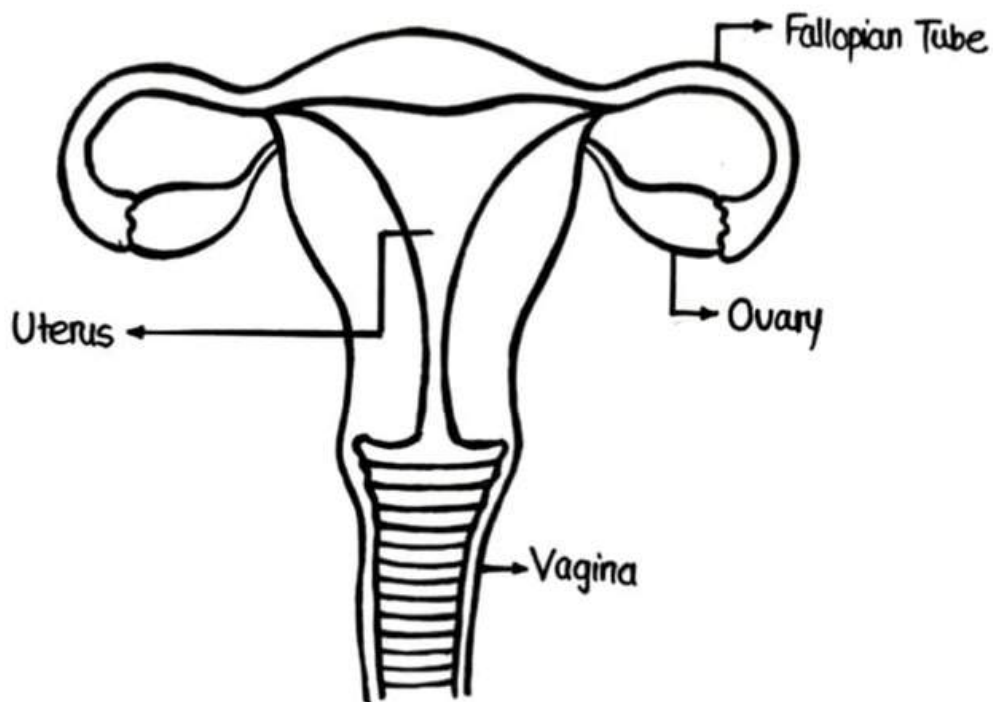
- Hypogonadism
- Anemia
- Delayed Puberty
- Osteoporosis

Adverse Effect

- Hepatotoxicity
- Cardiovascular Risk
- Acne
- Alopecia
- Psychiatric Effects (Aggression, Mood swings etc.)

OVARIES

- Ovaries are female reproductive glands responsible for producing eggs (ova) and releasing hormones like Estrogen & Progesterone.
- They are present in a pair in Females.
- They are crucial for fertility & regulating Menstrual Cycle.
- They present inside the female body.



PROGESTERONE

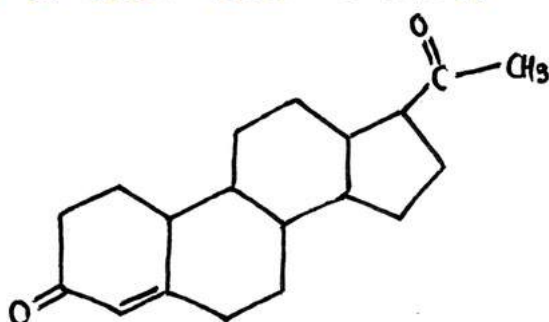
- It is also known as Pregnancy Maintaining Hormone.
- It prepares the uterus for pregnancy by thickening its lining.
- It is produced by corpus luteum in the ovaries.
- It also helps to regulate mood & promotes bone health.

ESTROGEN

- Estrogen (Oestrogen) is the major female sex hormone responsible for various primary & secondary sexual characteristics in females.
- It helps to regulate Menstrual Cycle.
- It is essential for growth & maintenance of reproductive tissues such as vagina, uterus.
- Estrogen levels rise significantly during pregnancy to support fetal development.
- It is responsible for various secondary sexual characteristics in females as follows :
 - High Pitch Voice
 - Pimple
 - Pelvic Girdle broadening
 - Breast Development
 - Mammary Glands Development
 - Pubic Hairs
 - Thickening of Uterus Lining

③ Progesterones

- Progesterone is a natural steroid hormone involved in the regulation of various functions in the body, particularly related to the menstrual cycle, pregnancy and development of fetus.
- It is produced mainly by ovaries after ovulation & by placenta during pregnancy.
- It is a 21 carbon steroid hormone.



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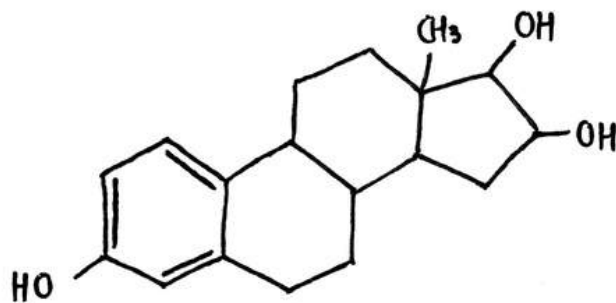
- Progesterone exerts its effects by binding to Progesterone Receptors.
- After binding it forms progesterone - receptor complex that regulates gene expression & perform various functions such as preparing the uterus for implantation, supports embryonic development & maintains pregnancy etc.

Uses

- Progesterone is used in combination with estrogens mainly in hormone therapy for menopausal symptoms & low sex hormone levels in women.
- It is also used in women to support pregnancy & fertility.

④ OESTRIOL

- Oestriol is a form of estrogen, a female sex hormone.
- It is one of the three main estrogens, along with estradiol & estrone.
- It is a major estrogen involved in pregnancy & is produced naturally by placenta & fetus.



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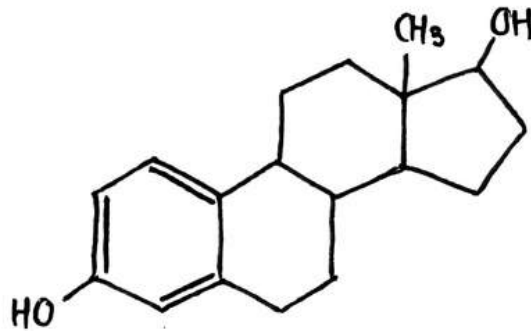
Oestriol is an estrogen receptor agonist, acting primarily on estrogen receptors (ER- α , ER- β) in estrogen responsive tissues such as uterus, vagina & breast.

Uses

- Vaginal Atrophy
- Urinary Tract Infections
- Hormone Replacement Therapy

⑤ OESTRADIOL

- Oestradiol is a form of Estrogen, produce naturally in human body by ovaries.
- It is the most potent & abundant form of estrogen in human body.



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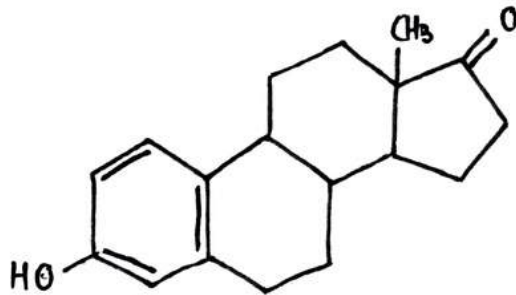
Oestradiol acts by binding to Estrogen receptors, where it regulates transcription of estrogen-responsive genes.

Uses

- Hormone Replacement Therapy
- Contraception
- Osteoporosis

⑥ OESTRONE

Oestrone is a weak steroid hormone and a minor female sex hormone.



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Oestrone binds to nuclear estrogen receptors in estrogen responsive tissue, although it has lower affinity and potency than estradiol.

Uses

- Hormonal Replacement Therapy.
- Premature Ovarian Failure.

ORAL CONTRACEPTIVES

- An Oral Contraceptive, often referred as "the pill" is a medication taken by mouth to prevent pregnancy.
- They prevent pregnancy by inhibiting ovulation and also by preventing sperm from penetrating through the cervix.

Types Of Oral Contraceptive

They are of mainly two types:

- ① Combined Oral Contraceptive
- ② Progestin - Only Pills

① COMBINED ORAL CONTRACEPTIVE

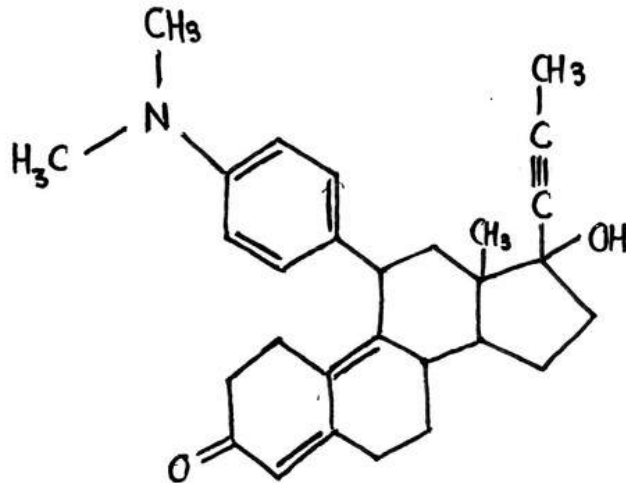
- If consist a combination of Estrogen & Progestin
- They work by :
 - Preventing Ovulation
 - Thickening cervical mucus to block sperm from reaching egg.
 - Thinning the uterine lining to reduce the likelihood of implantation.

② PROGESTIN ONLY PILLS (MINI PILLS)

- They are often used by individuals who cannot take estrogen.
- They primarily work by thickening cervical mucus & sometimes inhibit ovulation.

① MIFEPRISTONE

Mifepristone is a synthetic steroid hormone with both antiprogestogenic & antiglucoocorticoid properties.



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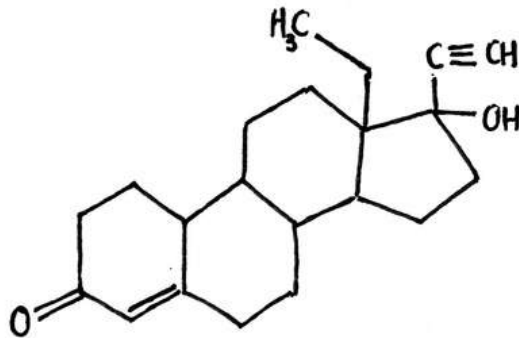
- Mifepristone is an antiprogestin that blocks the action of progesterone, a hormone necessary to maintain a pregnancy.
- By blocking the action of progesterone, mifepristone alters the endometrium, induces bleeding & causes uterine lining to shed.

Uses

Mifepristone is used in combination with misoprostol to end an early pregnancy.

② NORGESTREL

- Norgestrel is a synthetic progestin, a derivative of progesterone primarily used in hormonal contraception.
- It is composed of racemic mixture of two stereoisomers, dextro-norgestrel & levo-norgestrel.



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- It binds to progesterone receptors & suppresses ovulation by inhibiting gonadotrophin release (primarily Luteinizing Hormone)
- Thickens cervical mucus, making it difficult for sperm to penetrate.

Uses

- Birth control pill
- Endometrial Cancer

③ LEVONORGESTREL

Levonorgestrel is a synthetic progestogen widely used in hormonal contraception & emergency contraception.

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Same as Norgestrel

Uses

- Contraception
- Emergency Contraception

DRUGS ACTING ON UTERUS

- Drugs acting on uterus refers to study of how various medications affect uterine function, including contractions, tone and blood flow.
- Drugs affecting the uterus can be categorized based on their mechanism of action as follows:

① UTEROTONIC AGENTS (STIMULATE UTERINE CONTRACTIONS)

These drugs stimulate uterine contractions, promoting labor and ~~facilitate~~ controlling postpartum bleeding.

- **Oxytocin** : It is a natural hormone that stimulate uterine contractions, promoting labor and facilitating milk ejection. it is used to induce labor & control postpartum hemorrhage.
- **Prostaglandins** : These compounds increase uterine contractions and are used for labor induction or to manage abortion.
- **Ergot Alkaloids** : These drugs induce uterine contractions by stimulating smooth muscle, commonly used to prevent or treat postpartum hemorrhage.

② Tocolytics (Inhibit Uterine Contractions)

- These drugs suppress premature uterine contractions to prevent preterm labour.
- They are used to delay labour in certain situations, allowing time for fetal maturation or transportation to a facility with advanced neonatal care.
- β_2 -Agonist : These drugs relax the uterine smooth muscle by stimulating β_2 -adrenergic receptors, thereby inhibiting uterine contractions eg.: Terbutaline, Ritodrine etc.
- Calcium Channel Blockers : They inhibit calcium entry, reducing uterine contractions
ex- Nifedipine.
- Magnesium Sulfate : It is used for preterm labor
- NSAIDs : They inhibit prostaglandin synthesis, reducing uterine contractions.

③ PROGESTIN

- It is used to maintain pregnancy and prevent preterm labor.
- It inhibits uterine contractions by reducing the sensitivity of uterus to oxytocin and prostaglandins.

④ ANTI-PROGESTIN

- An antiprogesterone can be used in combination with prostaglandin for medical abortion.
- It works by blocking the effects of progesterone, leading to termination of pregnancy and softening of cervix for delivery or abortion.

⑤ UTERINE RELAXANTS

These agents are used for reducing uterine tone.

- Nitroglycerin : A vasodilator that can also reduce tone by relaxing smooth muscle.

BIOASSAY

- A bioassay is a scientific experiment or test used to evaluate the biological activity or potency of a substance/drug by observing its effect on living organisms/cells.
- The main purpose of bioassay is to determine how strong or effective a substance is in relation to its biological activity, which might include toxicity, therapeutic effect or other physiological responses.

PRINCIPLE OF BIOASSAY

The basic principle of bioassay is to compare test substance with the standard preparation of same substance and to find out how much test substance is required to produce same biological effect, as produced by standards.

Application of Bioassay

- They are used for standardization of drug of natural origin.
- They are used for selection of new compounds for determining their biological activity.
- They are used for various diagnostic & research purposes.
- They are used for determining the dose of a drug required for generating a therapeutic or toxic response.

TYPES OF BIOASSAY

There are mainly two types of bioassay

- ① Quantal Bioassay
- ② Graded Bioassay

① Quantal Bioassay

- Quantal Bioassay is a type of bioassay that measures an all or nothing response.
- In other words, it assesses whether a specific effect occurs or not, rather than measuring the degree or magnitude of the effect.
- In this type of bioassay, there is no intermediate response.
Example : Digitalis , D- tubocurarine , Insulin
- This method of bioassay is not very accurate, but can be employed for :
 - (1) Comparison of threshold responses
 - (2) Comparison of ED₅₀ or LD₅₀

② Graded Bioassay

- Graded bioassay is a type of bioassay that measures the magnitude or degree of a biological response to a substance, rather than just an all-or nothing effect.
- It is used in pharmacology, toxicology and physiology to determine the potency, efficacy and relative strength of a substance.

① BIOASSAY OF INSULIN

- Bioassay of Insulin is a laboratory method used to measure the biological activity of insulin in a sample.
- Insulin is a hormone that regulates blood sugar levels, and this bioassay assesses its ability to stimulate glucose uptake in cells.

Principle

The bioassay of Insulin is based on the ability of Insulin to stimulate the uptake of glucose by cells, typically isolated adipocytes or myocytes.

Standard Insulin & Unit

- It is a pure, dry & crystalline insulin.
- One unit contains 0.04082 mg.
- The unit is specified by Ministry of Health, Government of India and is equivalent to international unit.

Preparation of Standard Solution

- Accurately weigh 20 units of Insulin and dissolve in normal saline.
- Acidify it with HCl ~~with~~ to pH 2.5.
- Add 0.5% phenol as preservative.
- Add 1.4% to 1.8% glycerin.
- Final volume should contain 20 units/ml.

Preparation Of Test Sample

The solution of test sample is prepared in same way as standard solution.

Methods Of Bioassay Of Insulin

There are mainly two methods of bioassay of Insulin

- Rabbit Method
- Mouse Method

RABBIT METHOD

Selection Of Rabbits :

- Rabbits should be healthy, weighing about 1800-3000 grams.
- They should be maintained on uniform diet but are fasted for 18 hrs. before assay.

Procedure

- Rabbits are generally divided into 4 groups of 3 rabbit each, 2 groups for standard insulin, 2 for test insulin.
- Measure the baseline blood glucose level using a glucometer or blood sampling.
- Now the test consist of two parts :
 - First Part : Inject the insulin subcutaneously at a predetermined dose.
Now from each rabbit, a sample of blood is withdrawn upto 5 hrs. at the interval of 1 hrs each.

- Second Part : For second part same animals are used
 - The experiment can be carried out after 1 week.
 - Again the rabbits are fasted & initial blood glucose level is determined.
 - The grouping is now reversed, means those animals which received the standard are given the test & those which received the test are now given the standard.

MOUSE METHOD

- Mice shows characteristics convulsion after s/c injection of insulin at elevated temperature due to hypoglycemic effect.
- The percentage convulsions produced by test and standard preparations are compared.

Procedure

- Use healthy mice of similar weight (around 20-25 grams).
- Mice are fasted for 18 hours to stabilize their blood glucose levels before the test.
- Mice are divided into 4 groups each containing 25 mice & insulin is injected.
- Monitor the mice, for hypoglycemic convulsions over a specific time period (usually 30-60 minutes)
- ~~Can~~ Record the number of mice showing convulsion at each dose

② BIOASSAY OF VASOPRESSIN

- The bioassay of vasopressin is a biological test used to determine the potency & biological activity of vasopressin by measuring its effect on physiological functions, such as antidiuretic action or blood pressure regulation.
- It is also known as Antidiuretic Hormone (ADH)

METHODS

① Antidiuretic Action in Rats :

- Principle : Vasopressin reduces urine output by increasing water reabsorption in the kidneys.
- Procedure :
 - Animals are administered vasopressin intravenously or subcutaneously.
 - Urine output is measured over a set period.
 - The test sample is compared with standard vasopressin sample.
 - Potency is determined by reduction in urine volume or increase in urine osmolality.

② Blood Pressure Assay in Dogs or Cat

- Principle : Vasopressin causes vasoconstriction, leading to increase in blood pressure.
- Procedure :
 - Vasopressin is injected intravenously.
 - Blood pressure changes are recorded using a pressure transducer.
 - The test sample is compared with standard sample.

BIOASSAY OF OXYTOCIN

- The bioassay of oxytocin is a biological test used to determine the potency and biological activity of oxytocin by measuring its effect on uterine contractions or milk ejection.
- Oxytocin is a hormone that plays a central role in stimulating uterine contractions during labor and promoting milk release during lactation.

METHODS

① Uterine Contraction Method (Rat or Rabbit)

- Principle : Oxytocin causes uterine smooth muscle contraction, which can be measured in Rat or Rabbits.
- Procedure :
 - The test involves injecting the oxytocin preparation into female animal, such as rat or rabbit, that has been fasted or anesthetized.
 - Uterine contractions are observed and recorded over time.
 - Potency is determined by comparing the test preparation's response to that of standard oxytocin preparation.

② Milk Ejection Test (In Lactating Animals)

- Principle : Oxytocin promotes the release of milk from the mammary glands.
- Procedure :
 - In lactating animals, the test sample of oxytocin is injected and milk ejection is observed.
 - The volume of milk produced or the time taken for milk to appear at the nipple is measured.

BIOASSAY OF ACTH

- The bioassay of ACTH is a biological test used to determine the potency and biological activity of ACTH by measuring its ability to stimulate the Adrenal Cortex to produce corticosteroids, primarily cortisol.
- ACTH is hormone secreted by pituitary gland that regulates the function of adrenal glands.

Method

Adrenal Ascorbic Acid Depletion Test

Principle

ACTH stimulates the adrenal cortex, leading to depletion of adrenal ascorbic acid, which can be measured.

Procedure

- Rats are injected with ACTH.
- After a set time, the rats are sacrificed and the adrenal glands are removed.
- The ascorbic acid content in adrenal glands is measured.
- The degree of ascorbic acid depletion is compared between test and standard ACTH preparations.

BIOASSAY OF d- Tubocurarine

- The bioassay of d- tubocurarine is a biological test used to determine potency and neuromuscular blocking activity of d- tubocurarine.
- d- Tubocurarine works by blocking acetylcholine receptors at neuromuscular junction, leading to skeletal muscle relaxation.

Method

Rat or Rabbit Skeletal Muscle Assay

- Principle : d- Tubocurarine causes skeletal muscle relaxation by blocking neuromuscular transmission.
- Procedure :
 - An anaesthetized rat or rabbit used.
 - d- Tubocurarine is administered intravenously, and muscle contraction response is measured.
 - The degree of muscle relaxation is recorded.
 - The potency determined by comparing the muscle relaxation effect of the test sample to a standard d- tubocurarine preparation.

BIOASSAY OF DIGITALIS

- The bioassay of digitalis is a biological testing method used to measure the potency & therapeutic activity of digitalis glycosides (eg. digoxin, digitoxin) by evaluating their effects on heart muscle.
- It is primarily used to treat heart failure & arrhythmias by increasing force of myocardial contraction & slowing heart rate.

Method

Cat or Dog Heart Method

- Principle: Digitalis increases the force of contraction of the heart until cardiac arrest occurs at high doses.
- Procedure:
 - The animal (usually a cat or dog) is anesthetized.
 - Digitalis is administered intravenously.
 - The heart rate and contraction force are monitored until cardiac arrest occurs.

BIOASSAY OF HISTAMINE

- The bioassay of histamine is a biological testing method used to determine the potency & pharmacological activity of histamine.
- Histamine is a naturally occurring biogenic amine involved in allergic reactions, inflammation & gastric acid secretion.

Method

Blood Pressure Response in Anaesthetized Animals (Cats, dogs etc.)

Principle: Histamine causes a characteristic drop in blood pressure due to vasodilation.

Procedure:

- An anesthetized animal is connected to a blood pressure monitoring system.
- Histamine is injected intravenously.
- The drop in blood pressure is recorded & compared b/w standard & test preparations.

BIOASSAY OF SEROTONIN

Serotonin plays a key role in neurotransmission, vasoconstriction, gastrointestinal motility & platelet aggregation.

Method

Blood Pressure Response In Anaesthetized Animals

- Principle: Serotonin can cause both vasoconstriction & vasodilation, leading to characteristic changes in blood pressure.
- Procedure:
 - An anesthetized animal is connected to a blood pressure monitoring system.
 - Serotonin is administered intravenously.
 - The changes in blood pressure are recorded & compared.

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