Bioassay of Oxytocin

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Oxytocin

- ✓ "Oxy – Rapid" & "Tocos – Labor"
- ✓ It is a polypeptide hormone, which release from **posterior pituitary glands** and stimulates the contraction of the uterine smooth muscle & mammary gland.
- ✓ It is synthesized in both men and women, but physiologically it has major role in women.
- ✓ "Prolactin hormone" is mainly responsible for production of milk in mammary glands but milk ejaculation require "**Oxytocin**"

Oxytocin

Major Physiological Effects:-

1. Contraction of the uterus during Labor and milk ejection in women.
2. It is also known as love & bonding hormone. It has a very special affect on mothering.
3. It promotes a feeling of well being and tranquility (sense of piece and calm).
4. It also suppresses the fear that would normally cause her to back off from threat.
5. Other- Ii also regulates Vasopressor and Blood pressure

BIOASSAY OF OXYTOCIN

Bioassay: Biological assays are a set of techniques for estimating the Potency or Strength of an "agent" by Using the "response" or "effect" on biological system or experimental living subjects.

Basic Principle: Potency of oxytocin is determined by comparing its specific activity with standard preparation of Oxytocin by using specific biological assay method.

Activity:

- ✓ Contraction of Uterus
- ✓ Depression of BP
- ✓ Milk Ejection Pressure

BIOASSAY OF OXYTOCIN

Standard Oxytocin: It contains freeze-dried synthetic oxytocin peptide with human albumin and citric acid (supplied in ampoules containing 12.5 Units)

BIOASSAY OF OXYTOCIN

Bioassay Methods:

1. **Method A:** Blood pressure lowering (BP depression) effects on Chicken
2. **Method B:** Contraction of uterine muscle on isolated rat uterus model
3. **Method C:** Milk ejection pressure in lactating rats

BIOASSAY OF OXYTOCIN

Methods A: Blood pressure lowering (BP depression) effects on Chicken

Step1. Young male chickens (1 to 2 kg) are selected and anaesthetized for experimental procedure.

Step 2. Expose gluteus primus muscle (thigh) & locate politeal artery & crural vein.

Step 3. Cannulate the politeal artery for BP monitoring and crural vein for infusion of Test/Standard Oxytocin

Step 4. Inject (0.1 ml to 0.5 ml) 1st dose of std dilute solution of oxytocin into cannulate vein and record B.P response. Required dose for decrease in BP-20-100 mU.

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Methods A: Blood pressure lowering (BP depression) effects on Chicken

Step 5. 3-10 min (depends on BP return Normalize) after 1st dose, 2nd dose of std solution of oxytocin injected and record the BP.

Step 6. Thereafter, test dilute solution is injected and record the BP so as to obtain responses similar to those obtained with the Standard.

- ✓ The ratio between the two dose of Test should be the same as Standard and this should be kept constant throughout the assay.
- ✓ Repeated the procedure at least 6 responses to each dose should be recorded.

BIOASSAY OF OXYTOCIN

Methods A: Blood pressure lowering (BP depression) effects on Chicken

Step 7. Calculate the mean result of each dose of Test and Standard. And mean test response is statistically compared with standard.

BIOASSAY OF OXYTOCIN

Methods B: Contraction of uterine muscle on isolated rat uterus model

Animals: Female albino rat (150-200 g) was used to isolated the uterus

Step 1. Inject oestradiol benzoate (100 ug, im) 18 to 24 hours before the assay.

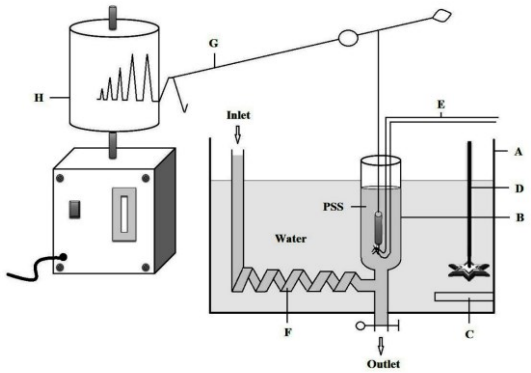
Step 2. Immediately before the assay, veginal smear is withdrawn and confirm the rats is in preoestrus or oestrus phase.

Step 3. Sacrificed the animal, isolate the horn of uterus and kept in Tyrode or suitable physiological salt solution (PSS Composition in % w/v: NaCl-0.662, KCl-0.045, CaCl2-0.007, NaHCO3- 0.256, Na2HPO4-0.029, NaH2PO4-0.003, MgCl2-0.01, and Dextrose-0.05) and maintain the bath at 32 ^{0}C.

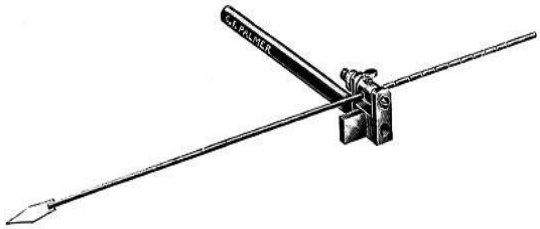
BIOASSAY OF OXYTOCIN

Methods B: Contraction of uterine muscle on isolated rat uterus model

Step 4. mountain the uterus in organ-bath and aerated/oxygenated the bath (PSS) solution with a mixture of 95% O2 and 5% CO2.



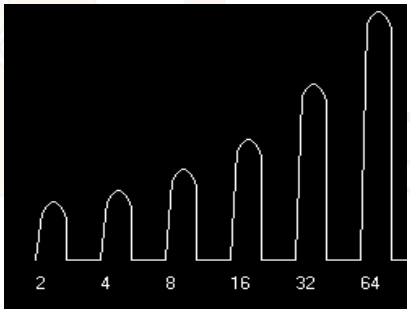
Isotonic Lever is used to record the contraction and load can not exceed 2 g



BIOASSAY OF OXYTOCIN

Methods B: Contraction of uterine muscle on isolated rat uterus model

Step 5. After setup the instruments and tissue, two dose of standard solution is injected and recorded the response (3-5 min interval with fresh solution) . Required dose is 10 to 50 microUnit/ml of bath liquid.



BIOASSAY OF OXYTOCIN

Methods B: Contraction of uterine muscle on isolated rat uterus model

Step 6. Thereafter, test dilute solution is injected and record the contraction so as to obtain responses similar to those obtained with the Standard.

- ✓ The ratio between the two dose of Test should be the same as Standard and this should be kept constant throughout the assay.
- ✓ Repeated the procedure at least 6 responses to each dose should be recorded.

Step 7. Calculate the mean result of each dose of Test and Standard. And mean test response is statistically compared with standard

BIOASSAY OF OXYTOCIN

Methods B: Contraction of uterine muscle on isolated rat uterus model

Apart from this procedure we can also estimated the potency of oxytocin by using: Matching, Interpolation, Bracketing, or Multipoint bioassay methods

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