

AIM: To study the bioassay of serotonin using rat fundus strip by three point bioassay.

APPARATUS:

- Reservoir, tubing, hemostatic forceps, isolated organ bath, aeration tube, isotonic frontal writing lever and recording drum.

EXPERIMENTAL CONDITION:

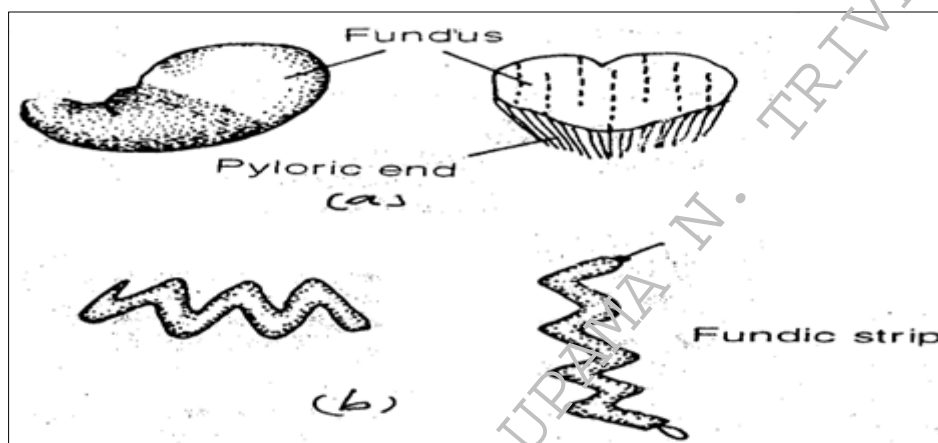
- Physiological Salt solution (PSS) : Krebs solution
- Animal : Rat [120-150 gm, overnight fasted]
- Temperature : 37 (+ or -) 10C
- Aeration : Carbogen (95% O₂ and 5% CO₂)
- Basal tension on the tissue : 1 gm
- Magnification of the response : 10 times
- Drug : Serotonin (stock solution 10 µg/mL)

PRINCIPLE:

Rat fundus is a very sensitive tissue for the study of the action of several naturally occurring substances like 5-hydroxy tryptamine, histamine, acetyl choline and bradykinin. Unlike the intestinal smooth muscle (ileum) this preparation is slow contracting and slow relaxing type. Rat fundus is generally employed for the bioassay of serotonin. The fundus (the upper part of the stomach) is grey in colour and therefore, easily identified from pyloric part (pink in colour). A zig-zag preparation of the fundus strip is prepared so as to expose maximum portion of the tissue to drug. The tissue is sensitive to 1 ng/ml of serotonin, 0.05-1 ng/ml of histamine and 0.2-0.5 ng/ml of acetylcholine, respectively.

Rat Fundus Strip:

The Fundus strip is suitable for the assay of 5-HT as it is very sensitive to the drug. It is 10 times less sensitive to Acetylcholine. It is also used for the assay of PGE₂.



Preparation of fundus strip from rat fundus

PRINCIPLE OF 3 POINT METHOD

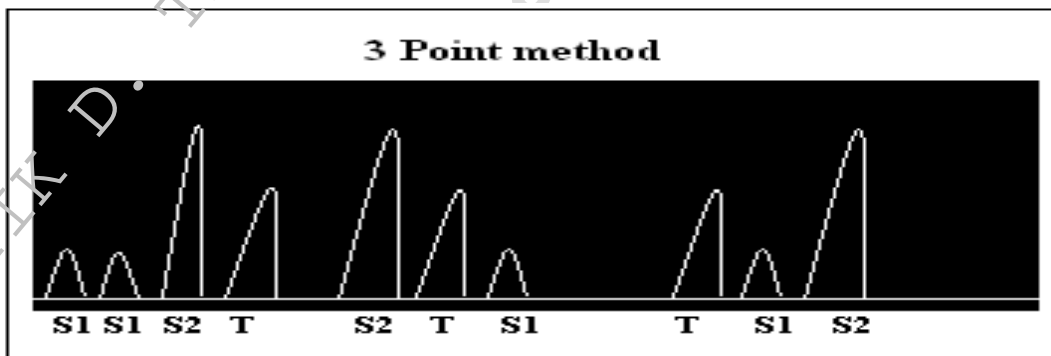
Serotonin produces contractions of smooth muscle of rat ileum. In 3-point assay method, 2 doses of the standard acetylcholine (S1, S2) and one dose of the test acetylcholine (T) are used. The test dose is fixed in such a way that it gives the response between the responses produced by S1 & S2. These three selected doses are repeated by the Latin Square design method i.e. S1, S2, T – S2, T, S1 – T, S1, S2. The mean responses are calculated and plotted against log-dose and amount of standard producing the same response as produced by the test is determined mathematically.

PROCEDURE:

1. Sacrifice the rat by a blow on the head and carotid bleeding.
2. Cut open the abdomen and expose the stomach.
3. Identify the fundus of the stomach (upper part) .Incise it from the junction of pyloric part and put it in the dish containing Krebs solution.
4. Incise the fundus from the lesser curvature and open it longitudinally. Give alternate zig zag cuts to make a fundal strip preparation. Tie both the ends with the thread and mount in the organ bath contain krebs solution at 37⁰ C. Aerate the tissue.
5. Apply 1 gm load and allow the preparation to equilibrate for 30 mins. Using frontal writing lever with 10-12 magnification record the contractions due to increase concentrations of serotonin. Since the muscle contracts slowly and relaxes slowly,a contact time of 90 sec ,and 5 min time cycle.

6. Select two doses from the DRC of standard drug, eliciting sub-maximal responses and bearing a dose ratio 1:2 preferentially and designate them as S1 and S2 and respectively.
7. Select one dose from the DRC of test solution in such a way that the response due to this dose lies preferentially between S1 and S2 and designate it as T.
8. Record 3 sets of responses due to S1 S2 and T adding them to organ bath in a randomized fashion as per Latin square design mentioned in the principle. The Latin square design of addition of doses is followed to ensure good randomization and to account for the fluctuating sensitivity of the tissue.
9. Measure various response to calculate the mean of each response(S1,S2,T)
10. Plot the graph with log dose on X-Axis and % of response on Y –axis and interpolate the T response on to the DRC of standard in between S1 and S2 so as to find the standard dose that gives an equivalent response of that of test.
11. Calculate the potency of the test drug by converting the log of the standard dose that has produced an equivalent response as that of test in to anti-log and report the potency as number of $\mu\text{g/ml}$.

GRAPH



DRAW GRAPH

CALCULATIONS:

$$\text{Conc. of Unknown} = \frac{n_1}{t} \times \text{antilog} \left\{ \frac{T - S_1}{S_2 - S_1} \times \log \frac{n_2}{n_1} \right\} \times \text{Cs} \times \text{dil. factor}$$

n_1 = Lower std. dose

n_2 = higher std. dose

t = test dose

S_1 = response of n_1

S_2 = response of n_2

T = response of t

Cs = conc. of std.

RESULT: The concentration of given unknown sample is _____ $\mu\text{g/ml}$.

QUESTIONS:

1. Give the principle of 3 point method.
2. Discuss mechanism of Serotonin.