

AIM: To study the bioassay of acetylcholine using rat ileum/colon by four 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) : Tyrode solution
- Animal : Rat [120-150 gm,]
- 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 : Acetylcholine (stock solution 10 µg/mL)

PRINCIPLE:

In 4-point assay method, 2 doses of the standard and 2 dose of the test are used. Initially a graded dose response curve for the standard drug is taken. From this response two doses of the standard drug S1 & S2 are selected. The two doses should preferably be in the ration of 1:2. The test drug [T1 and T2] dose should be arranged in such a way that its T1 response should be in between S1 & S2 and T2 should be less than or equal to S2. These 4 selected doses are repeated by the Latin Square design method i.e. S1, S2, T2, T1 – S2, T2, T1, S1 – T2, T1, S1, S2, in order to avoid bias. The mean responses are calculated.

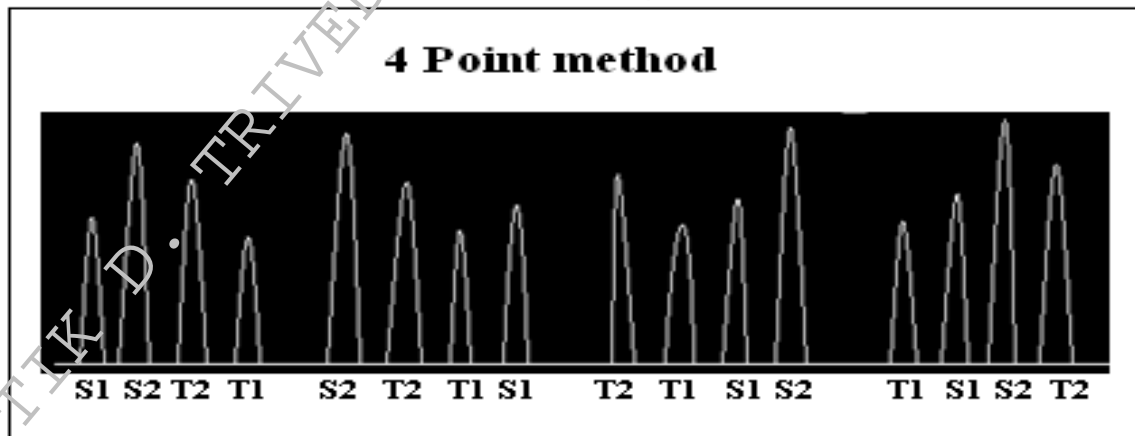
PROCEDURE:

1. The assembly is set up and arrangements are made for experimental conditions mentioned above.
2. A rat which is fasted overnight is sacrificed as per CPCSEA recommended guidelines. The abdominal cavity is quickly opened & a piece of ileum is isolated. It placed in a Petri dish containing Tyrode solution maintained at 37°C.
3. The mesentery of ileum is removed & the lumen of ileum is cleaned by passing warm

DR. NAITIK D. TRIVEDI & DR. UPAMA N. TRIVEDI

4. Tyrode solution through it from a pipette held at an angle of about 20-30 degrees.
5. The tissue is mounted in mammalian organ bath and connected to isotonic frontal writing lever. The tissue is allowed to stabilize for 30 min.
6. The responses of standard Acetylcholine solution are taken till the maximum effect is obtained.
7. The doses of S1 & S2 should be arranged such that response of n2 dose (S2) is twice than response of n1 dose (S1).
8. The doses of unknown sample (t1 and t2) should be arranged in such a way that its T1 response should be in between S1 & S2 and T2 should be less than or equal to S2.
9. Record four sets of responses due to n1, n2 & t1, t2 by adding them to the organ bath in a randomized fashion by following Latin square design.
10. Determine S1, S2 & T1, T2 Put their values in equation & find out concentration of unknown sample.

GRAPH



DRAW GRAPH

CALCULATIONS:

$$\text{Concentration of unknown} = n_1/t_1 \times \text{anti log} \left\{ \frac{(S_1 + S_2) - (T_1 + T_2)}{(S_2 + T_2) - (S_1 + T_1)} \right\} \log n_2/n_1 \times C_s$$

Where,

n1 = lower standard dose

n2 = Higher standard dose

t1 = Lower test dose

t2 = Higher test dose

S1= response of n1

S2 = response of n2

T1 = response of t1

T2 = response of t2

Cs= Concentration of standard

RESULT: The concentration of given unknown sample is _____ µg/ml.

QUESTIONS:

1. Give the principle of 4 point method.