EXPERIMENT NO:-06 **DATE**

AIM: - To study the effect of Physostigmine on DRC of acetylcholine using isolated frog rectus abdominis muscle preparation.

APPARATUS:

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

EXPERIMENTAL CONDITION:

- Physiological Salt solution (PSS) : Frog's ringer
- 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 : Physostigmine (2 µg/ml)
  Acetylcholine Chloride (1, 10 or 100 µg/mL)

PRINCIPLE:

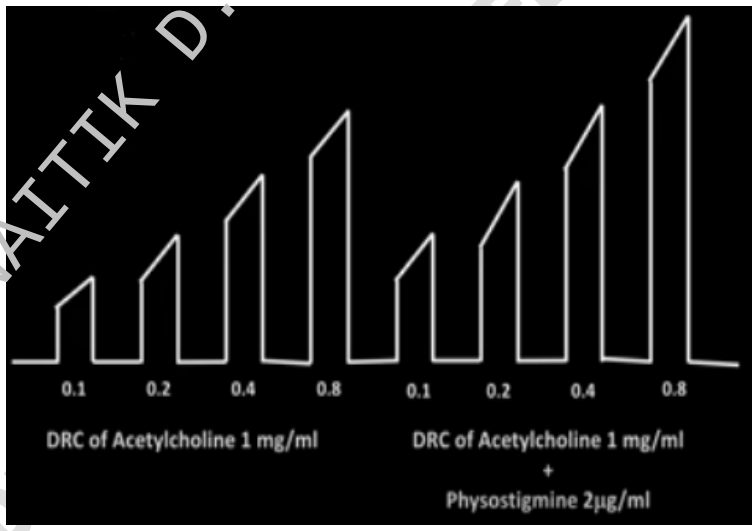
Physostigmine is an anticholinesterase drug and it inhibit the breakdown of Ach. As a result the action of Ach is potentiated. The DRC of Ach will shifted to left in the presence of Physostigmine.

PROCEDURE:

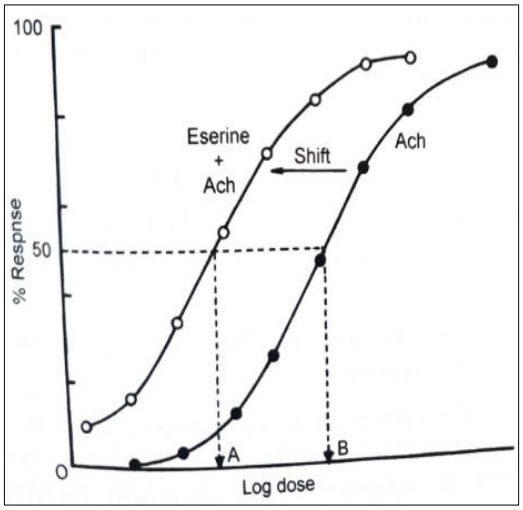
1. The assembly is set up and the arrangements are made for the above mentioned condition.
2. A frog is sacrificed as per CPCSEA recommended guidelines.
3. The frog is placed in a tray with ventral side facing up. The skin is incised longitudinally in the middle of abdomen from pubic symphysis to the sternum. Two recti are situated on the either sides of the midline. They are dissected out by cutting its attachment from pubic symphysis from below, sternum from above and abdominal muscles from sides. The recti can be easily differentiated from other muscles because the recti are white and shiny whereas other muscles are pinkish in color.

4. Two recti are separated from the midline and one rectus muscle is mounted in the organ bath. One end of the muscle is tied to the aeration tube and the other is connected to the isolated frontal writing lever.
5. The tissue is allowed to stabilize for half an hour. During this period the PSS is changed after every ten min. once the tissue is stabilize, graded doses of Ach are added to at defined time period of interval for obtain contractile responses.
   - 00 sec: Start the drum and record a base line for 30 sec.
   - 30 sec: Add the first dose of drug in organ bath and take the response for another 30sec.
   - 60: Stop the drum and give wash until the tip of lever rich to baseline.
6. Continue above procedure for next doses and record DRC of Ach.
7. Add Physostigmine 22 µg/ml to the reservoir containing frog ringer and irrigate the tissue with Physostigmine ringer for 30 min.
8. Repeat the DRC of Ach in presence of Physostigmine
9. Label and fix the DRC
10. Plot the both DRC of Ach that is in presence & in absence of Physostigmine.
11. Note the potentiation in the response of Ach and calculate the relative EC 50 values.

GRAPH:



Potentiation of effect of Ach in presence of Physostigmine



Shift in DRC

DRAW GRAPH

OBSERVATION TABLE

Sr. No	Drug Name	Conc. of drug	Dose of drug in mL	Response in mm	% Response
1.	Ach	1mg/mL			
2.					
3.					
4.					
5.	Ach + **Physostigmine**	1mg/mL + 2 µg /mL			
6.					
7.					
8.					
9.					

RESULT:

QUESTIONS:

1. What is potentiation?
2. Discuss mechanism of Physostigmine.