EXPERIMENT NO:-05 **DATE**

AIM: - To study the DRC of acetylcholine using frog rectus abdominal muscle.

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 : Acetylcholine Chloride (1, 10 or 100 µg/mL)

THEORY:

Graded Dose Response Relationship Curve of Acetylcholine on Frog Rectus Muscle:

- Single biological unit, either a single animal or an isolated tissue is used.
- It depends upon an observation that graded increase (in geometric proportion) in the dose of drug gives proportional rise in the magnitude of biological response.
- Actually, beyond a specific dose level, biological response increases in proportion to the increase in dose. This dose level is known as 'Threshold dose'.
- Such proportional rise in biological response occurs only up to a dose level known as 'Ceiling dose', beyond which a steady biological response is achieved even after increasing the doses.
- Shape of Graded DRC, when plotted as 'dose Vs Response' is a 'Parabola'
- Shape of 'Log Dose Vs Response' curve is a 'Sigmoid' line or is having 'S' like shape.

- In another method the response to the graded doses are obtained by increasing the concentration of the drug in the bath fluid step by step without taking recovery of the preceding dose. The curve is obtained is called as **cumulative dose response curve.**

Drug-receptor interactions

Most drugs produce their actions by interacting with receptors.

Drug + receptor =drug-receptor complex = response

A receptor has the ability to bind a drug. This ability of a receptor makes us expect a response for each drug receptor complex formed, this efficiency of receptors varies & depends on the factors like,

- **Affinity:** ability of a drug to bind to a receptor
- **Occupancy:** fraction of receptors occupied to the total number of receptors
- **Efficacy:** (intrinsic activity): ability of a drug to change the receptor conformation to produce a response.

So, a pharmacological response depends on the affinity and efficacy.

Agonist concentration curves (Dose-response curves)

We usually use the log of a drug's concentration (instead of using the concentration value itself). This will help us to plot the curve even in extremely low concentrations of drugs.

- ✓ It makes a *sigmoid* (s-shaped) curve *(instead of hyperbolic)*
- ✓ It is called a log-dose response curve (LDR) by raising the dose above the "threshold dose level", there will be a gradual increase in the response of that drug. Thus, LDR of similarly active drugs produce parallel LDR curves, enabling us to compare between the potencies of qualitatively similar drugs.

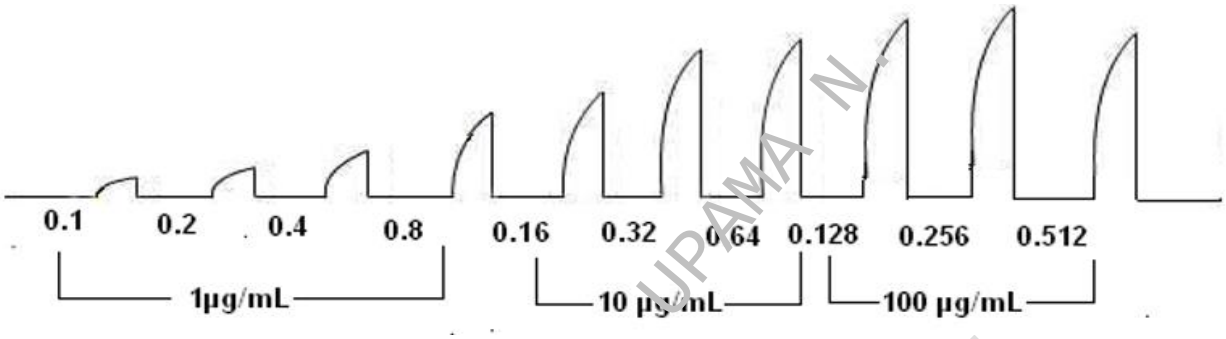
- ***Potency*** (EC50 or ED): the concentration or dose needed to produce a 50% maximal response.
- ***Efficacy*** (Emax 50) is the max response the drug can produce Efficacy of an agonist depends on both affinity (binding) & intrinsic activity. Concentration-response curves cannot be used for direct estimation of the **affinity** of the agonist to the receptor because:

The affinity of an agonist can be determined by finding out the pD_2 value. pD_2 is defined as –ve log of molar concentration of agonist which produces half (50%) of the maximum response or **pD_2= -log[EC_{50}].**

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.
7. Measure the height of concentration at different doses of Ach.
8. Tabulate the observations into three columns as Dose of Ach, Height of conc. (in mm) & % response.

GRAPH:



DRAW GRAPH:

OBSERVATION TABLE

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

9.					
10.					

RESULT:

QUESTIONS:

1. What is graded dose response curve?
2. What is cumulative dose response curve?
3. Define affinity, potency & efficacy.
4. Define and write formula of pD_2.