- INTRODUCTION

DOSE RESPONSE CURVE (DRC) FOR ACETYLCHOLINE ON FROG RECTUS ABDOMINIS MUSCLE

Dose Response Curve (DRC) demonstrate graded response to drugs/ agonist where increase in response is recorded. Its curve is generally sigmoid or S-shaped. Its response is observed between Log-dose versus Response curve for Acetylcholine. The study of DRC helps to understand the concept of threshold concentration, ceiling concentration and half maximal effective concentration (EC50).

The study of DRC indicates :-

1) Relative potency of drug/ agonist, where curves shift towards left results that drug is more potent.

2) Steeper slope of curve represents precision in bioassay.

Rectus abdominis muscle is skeletal muscle, and acetylcholine response is considered as nicotinic response.

- EQUIPMENT REQUIRED

Animal :- Frog

Drug:- Acetylcholine stock solution (1mg/ml)

Instrument:- Student Organ Bath, kymograph.

Physiological salt solution:- Frog Ringer's

- PRINCIPLE

Frog Rectus abdominis muscle is voluntary muscle wherein at neuromuscular junction, a nerve impulse releases acetylcholine from synapse into the cleft between nerve fibre and muscle. Acetylcholine is neurotransmitter which causes depolarization of muscle fibre resulting into muscle contraction. The muscle fibre of frog leads to continuous depolarization and prolong slow contraction of muscle. Frog rectus abdominis muscle contains nicotinic receptor and Acetylcholine acts as an agonist.

PROCEDURE:

1) Pith the frog and lay it on its back on the frog- dissecting board. Pin the four limbs to the frog board.

2) Remove the skin of the abdomen region and expose the rectus abdominis muscle.

3) Cut and prepare two rectus muscle preparation from each frog.

4) Tie thread to the top and bottom of each muscle preparation before detaching the muscle from the body of the frog.

5) Tie ends of tissue to aeration tube and isotonic lever respectively in up-right position, student organ bath containing, frog Ringer's solution under a tension of 1g. There is no need of maintain bath temperature and bubble the organ bath with air.

6) Relax the tissue for 30 min, during which period wash the tissue with fresh ringer's solution at least four times at every 10 minutes interval.

7) Record the contractions due to acetylcholine using either simple side way or frontal writing lever, for proper reading of response the contact time should be 90 Seconds and 5 min time cycle.

8) Record four responses of acetylcholine in increasing dose response or till the maximum response achieved. Wherein properly label the graph, mention the date, your name, and fix the tracing with the help of fixing solution.

9) Measure the height of response (mm) and draw DRC graph.

CALCULATION & INTERPRETATION:

1. Concentration of Acetylcholine (Ach) (µg/mL): Constant at 100 µg/mL for each entry.

2. Amount Added in Organ Bath: Given in mL

3. Amount Added in Organ Bath (in µg): This will be calculated using the formula

Amount in µg = Conc. Of Ach X Amount Added in mL

4. Concentration of Acetylcholine in µg/mL (in Organ Bath contains 20mL Solution):

Calculated as $\frac{\text{Amount Added (µg)}}{\text{20mL}}$

5. Response (in mm): Newly provided values.

6. % Response

Calculated as : Response / Maximum Response X 100, using the maximum response from the provided.

CONCLUSION

The dose-response curve (DRC) for acetylcholine on frog rectus abdominis muscle demonstrates how increasing concentration of acetylcholine, results in greater muscular contractions up to a ceiling effect. It helps to determine the muscle's sensitivity to acetylcholine, with critical values such as EC50 reflecting cholinergic potency. The curve can help you comprehend receptor activation and muscle response in neuromuscular physiology.

- IDEAL OBSERVATION

Sr. No	Conc. Of Ach (µg/mL)	Amount Added in Organ Bath (in organ bath contains 20ml solution)		Conc. of Ach in µg/mL	Response (in mm)	%Response
		In mL	In µg			
1.	100	0.1	10	0.5	20	21.98
2.	100	0.1	10	0.5	20	21.98
3.	100	0.2	20	1	45	49.45
4.	100	0.4	40	2	76	83.52

5.	100	0.8	80	4	90	98.90
6.	100	1.6	160	8	91	100.00

RESULT:

- The dose-response curve (DRC) for acetylcholine on the frog rectus abdominis muscle shows a sigmoidal shape.
- As acetylcholine concentration increases, muscle contraction intensifies until it reaches a plateau, indicating receptor saturation.

DISCUSSION:

- The DRC reveals that at low acetylcholine concentrations, muscle contraction is minimal, but as concentration rises, the response increases sharply, reaching a maximum when all receptors are occupied.
- The curve's steep rise reflects effective receptor binding, while the plateau indicates maximal muscle response.
- The EC50 value, the concentration for half-maximal response, helps assess acetylcholine potency.
- Shifts in the curve can indicate changes in receptor sensitivity or muscle function, providing insights into cholinergic signalling and neuromuscular dynamics.