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# ASSAY FOR SCREENING OF ACETYLCHOLINESTERASE INHIBITORS

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## ABSTRACT

A new experimental model using isolated frog rectus abdominus muscle preparation, acetyl choline and brain homogenate was used to screen acetylcholinesterase inhibitors. When brain homogenate was given along with sub maximal dose of acetylcholine, the response of acetyl choline was found to be reduced and this can be attributed to the presence of acetylcholinesterase enzyme in rat brain homogenate. When revastigmine a proved acetylcholinesterase inhibitor was added to the above mixture of ach and brain homogenate the responses recorded were similar to the sub maximal dose of acetylcholine and effect of brain homogenate was effectively nullified.

**Key words**: Acetylcholinesterase inhibitors, Brain homogenate, Rivastigmine.

## INTRODUCTION

Many recent behavioral, pharmacological, neurobiological studies have provided evidence for a cholinergic involvement in learning and memory[1]. Cholinergic hypothesis claims that the decline in cognitive function in dementia predominantly related to a decrease in cholinergic neurotransmission[2]. Acetyl cholinesterase (AchE) plays an important role in many pathological conditions by decreasing the availability of acetylcholine in the synaptic cleft[3]. Treatments for memory deficit problems like alzheimer's disease in humans often involve enhancing the retention of acetylcholine in brain synapses. Rivastigmine is a drug marketed to prevent memory problems act by inhibiting the action of enzyme in the brain that breaks down the acetylcholine. Hence, estimation of AchE activity is useful in screening of new molecules for their possible anticholinesterase activity. Here is an experimental model, which is sensitive, selective, specific, and reliable for the screening of the drugs which act centrally by inhibiting acetylcholine in brain.

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Isolated Frog rectus abdominus muscle is selected for the study because it is sensitive to acetylcholine and contracts slowly. Responses of acetylcholine are expected to be inhibited due to the presence of AchE in the rat brain homogenate. If the test drug is inhibitor of AchE centrally, the response regains. Potentiation effect was observed in presence of the acetylcholine. By calibrating the decline in response of acetylcholine on isolated frog rectus abdominis muscle due to the presence of AchE in rat brain homogenate, we can assess the AchE enzyme inhibitor activity of a test drug.

**Requirements**

Animals: Frog**,** Male Wistar rat weighing 150-180 g.

Chemicals: Acetylcholine (1 mg/mL), Rivastigmine

Physiological solution: Frog ringer solution

## EXPERIMENTAL

### Preparation of the rat brain homogenate (Acetyl cholinesterase enzyme)

Male rat of Wistar strain weighing 150 g was sacrificed by cervical dislocation. The total brain was immediately excised and homogenized with 2 mL of ice cold saline for 10 mins. The homogenate was centrifuged at 3000 RPM for 30 minutes and stored at -20$^{o}$C till use.

### Dose response curve of acetylcholine using rectus abdominis muscle preparation of frog

Thread was tied to the top and bottom of each muscle preparation before detaching the muscle from the body of the frog. The preparation was mounted in up-right position in the organ bath containing frog ringer solution under a tension of 1 g. Bubble the organ bath with air. Relax the tissue for 45 min, during which period wash the tissue with fresh quantum of ringer solution for at least 4 times. Dose response curve was recorded for graded concentrations of acetylcholine on muscle preparation by taking 60 seconds as base line and 90 seconds as contact time and 3 minutes for washing[4]. Selected sub maximal dose of the acetylcholine and graded doses of brain homogenate were incubated at 37$^{o}$C for 15 min. The incubated preparations were added to tissue bath and responses were recorded. In the next phase standard central anti cholinesterase drug rivastigmine was added to the above preparations and incubated as given above. Responses were recorded separately using the same tissue. To confirm the results same procedure was repeated with higher doses of acetylcholine.

## RESULTS AND DISCUSSION

Upon addition of graded doses of acetylcholine in the Frog rectus abdominis muscle (FRAM) preparation increased contractions were observed. When prepared rat brain homogenate was added to the selected sub maximal dose of the acetylcholine, showed decline in the response of acetylcholine revealing that the acetylcholine being degraded by the AchE enzyme, which is present in the rat brain homogenate. This was conformed from the above observed responses. In the first phase the responses of acetylcholine were found to be declined in dose dependent manner of rat brain homogenate. In the next phase there is the restoration of responses of acetylcholine due to anti cholinesterase drug which neutralizes AchE.

Ach (µg/mL)

| 1 | 2 | 4 | 8 | 16 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 16 | 16 | 32 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B.H (mL) | | | | | | 0.1 | 0.2 | 0.4 | 0.1 | 0.2 | 0.4 | 0.4 | 0.8 | 0.4 | |
| AchE I (mL) | | | | | | | | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | | |

B.H.: Brain Homogenate

**Fig. 1 : Effect of rat brain homogenate on acetyl choline responses on FRAM**

## CONCLUSION

Acetylcholine is a vital excitatory parasympathomimetic neurotransmitter, which causes stimulatory effect on isolated frog rectus abdominis muscle. The anti cholinesterase drug reversed the effect of AchE and restored the responses of Ach. This confirms the

presence of AchE in rat brain homogenate and it is useful as an assay model for screening of novel drugs or test drugs. Although there exist a number of models like *in vitro* chemical models Elman's, modified Elman's but this is a simple, reproducible, reliable model for screening and confirmation of the anticholinesterase activity of the test drug by using isolated FRAM and muscle contraction as the parameter. This assay can be considered as an alternative, economic and effectively working method with principle of inhibition of central AchE by the test drug.

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