EFFECT OF LITHIUM ON ACETYLCHOLINE ESTERASE ACTIVITY, AND ISOZYME PATTERN IN DEVELOPING CHICK BRAIN.

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Acetylcholine Esterase is an enzyme, which hydrolyses acetylcholine and is used as a marker for cholinergic neural function. It is known to be involved in synaptogenesis.

While on one hand it is known to be a marker for the developing chick brain it is also implicated in neurodegenerative diseases. In vertebrates the protein is synthesized by a single gene and undergoes alternative splicing to give 6-8 isoforms. Isozyme patterns of acetylcholine esterase have been suggested to be useful prognostic markers of neuronal degeneration.

Lithium a well-known teratogen is known to induce apoptosis in the developing chick brain. Understanding the dynamics of acetylcholine esterase isoform pattern in lithium induced neural tissue damage would help elucidating the role of these isoforms in frank neurodegenerative diseases. We have therefore studied activity and isozyme pattern of acetylcholine esterase in lithium treated and control 7 day old developing chick brain and report the same.

INTRODUCTION

Acetylcholine Esterase (AChE) is an enzyme, which hydrolyses the neurotransmitter acetylcholine and is used as a marker for cholinergic function in neural tissue.

AChE is an interesting enzyme because on one hand it is implicated in synaptogenesis while on the other hand it is known to be involved in neurodegeneration in adult tissue.

Elucidating AChE activity and Isozyme pattern in embryonic tissue undergoing development and degeneration is of interest to understand the differential role of the enzyme in these two states.

Using lithium-induced apoptotic developing chick brain as a model, we have studied AChE activity and Isozyme pattern in developing and degenerating embryonic neural tissue.

MATERIALS AND METHODS

1) Procuring and Inoculation of the Eggs:

The eggs of *Gallus domesticus* of the white leghorn species variety were procured from Central Poultry and Breeding Farm, Goregaon, Mumbai. After 24 hours of incubation, using a sterile needle, 0.125 M and 0.06 M LiCl was inoculated under aseptic conditions. Eggs were further incubated at 37^0C and harvested on day 7. Morphometric Measurements were carried out to ascertain lithium action.

2) Determination of AChE Activity: -

AChE activity in chick brain was determined by Ellman's Method [2]. Briefly, 2% of brain homogenate in Phosphate Buffer (pH 8) was reacted with substrate Acetylthiocholine Iodide and colour developer 5,5 Dithiobis (2-nitro benzoic acid) (DTNB). Absorbance was measured at 405nm. Folin-Lowry Method determined protein concentration. Specific Activity of AChE was calculated.

3) Electrophoresis:-

Combining two protocols (K.S.Krishnan [3] and V.Ashwani[1]), standardized in our laboratory, electrophoresis was carried out for AChE Isozyme Pattern. Briefly, homogenized brain with and without triton was centrifuged and electrophoresis of the supernatant was carried out on a 6% Polyacrylamide Gel at constant current. The gels were stained for AChE activity with Acetylthiocholine Iodide as the substrate, Copper Sulphate and Pottasium Ferricyanide as the colouring agents [1].

RESULTS

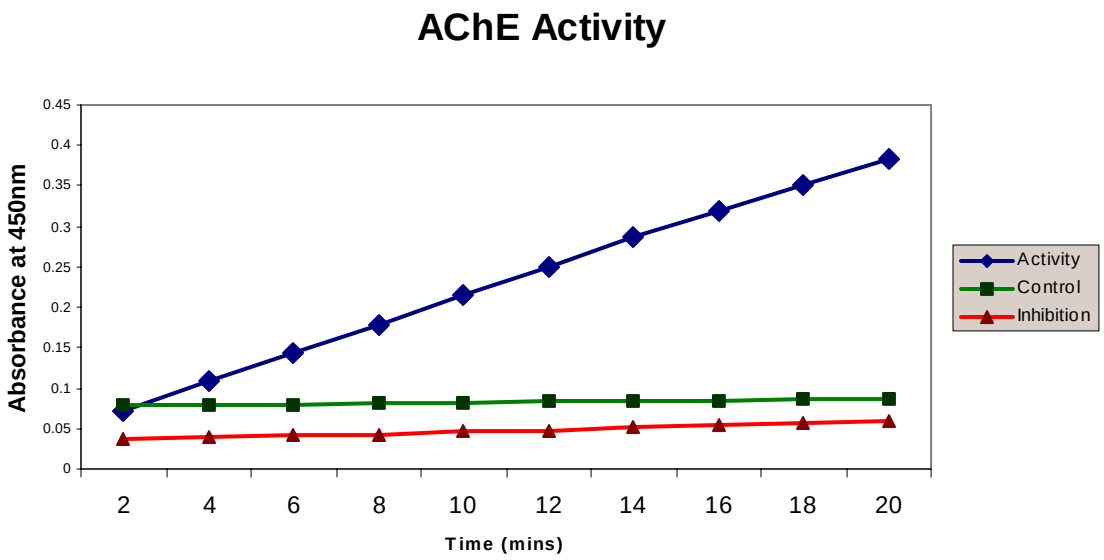
Effect of Lithium on gross morphology:

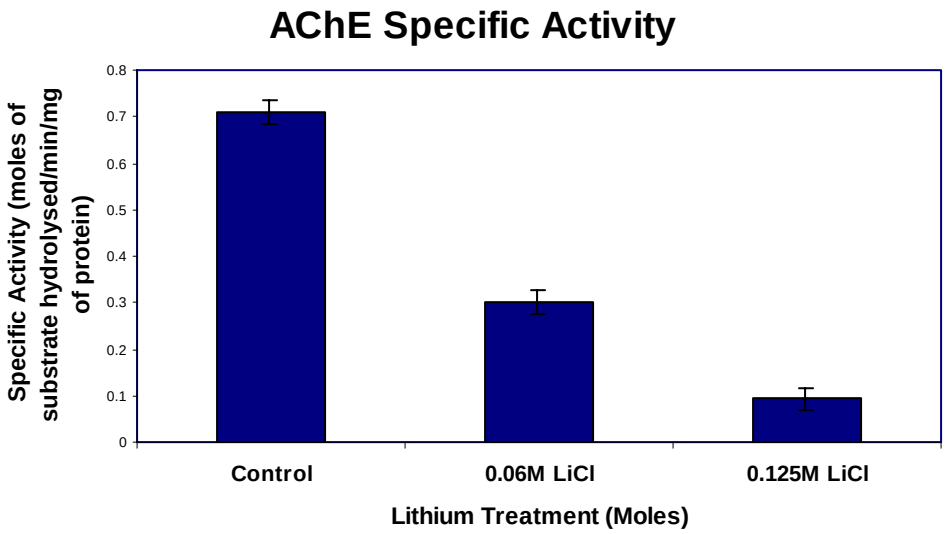
Effect of Lithium on 4 to 6/7 days old chick embryos: (Upper row : Control; Middle row : 0.06M LiCl; Lower row: 0.125M LiCl)

Treatment	**Brain/Body**
Control	0.37 ± 0.02
0.06M LiCl	0.41 ± 0.01
0.125M LiCl	0.44 ± 0.02

Specific Activity of AchE (moles/min/mg)

Cortex	**Whole Brain**
0.1642×10^{-4}	0.1237×10^{-4}
0.1176×10^{-4}	0.1502×10^{-4}
0.16×10^{-4}	0.1584×10^{-4}
Mean = 0.1473×10^{-4}	**Mean** = 0.1441×10^{-4}
SD = 0.03	**SD** = 0.02


AChE Activity
Absorbance at 450nm
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
2
4
6
8
10
12
14
16
18
20
Time (mins)
Activity
Control
Inhibition


AChE Specific Activity
Specific Activity (moles of substrate hydrolysed/min/mg of protein)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
0.06M LiCl
0.125M LiCl
Lithium Treatment (Moles)

DISCUSSION

Lithium a drug in use for bipolar disorders, is also known as a teratogen and causes apoptosis in embryonic nervous system. In the present study lithium was seen to significantly affect the morphology of 7 day old chick embryo. A dose dependent reduction in AChE activity was seen in brain of lithium treated chick embryos as compared to controls. In addition the isozyme pattern was also altered.

We report 3 bands (R_{V1}=1,R_{V2}=3,R_{V3} =5.4) representing the isofoms of AChE in normal embryonic 7 day old chick brain. E1 appears to be the membrane bound enzyme, which is solubilized by triton and is reflected as increased proportion in triton containg assay.

Lithium appears to increase the proportion of the E1 (R_v=2.1), which corresponds to the membrane bound isoform. Our preliminary data suggests that this increase in relative proportion of E1is because of lithium inhibiting membrane binding of this isoform. Lithium is known to modulate cytoskeletal element. The change in isoform pattern is probably because of poor anchoring of E1 in the cell membrane and needs to be further investigated.

REFERENCES

1) Ashwani V. (1992), Ph.D. thesis, University of Mumbai.
2) Ellman G.L., et al. (1961) Biochem. Pharmacol.7,88-95.
3) Krishnan K.S and Manjrekar K. (1987), Annal. Biochem. 160, 409-413.

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