Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 03, pp. 649-651, March, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ACETYL CHOLINE ESTERASE ACTIVITY IN MUSCLE TISSUE OF EPILEPTIC ALBINO MICE

***Maleeka Begum, S. F., Srinivasan, J., Chandrasekar, S., Savitha, C. and Preethy Mani, M.**

Department of Biosciences, Sri Krishna Arts and Science College, Kunniamuthur Post, Coimbatore

ARTICLE INFO

Article History:
Received 17th December, 2012
Received in revised form
19th January, 2013
Accepted 24th February, 2013
Published online 19th March, 2013

Key words:
Pilocarpine, Muscarinic
and Glutamate.

ABSTRACT

Albino mice weighing approximately 60±0.5 gm were taken as experimental and control animals. Both the control and experimental mice were injected intraperitonally with atropine to reduce peripheral activity. Pilocarpine was dosed along with phosphate buffer against the muscarinic and glutamate receptors to the experimental mice to induce seizures in the mice. Seventeen days after the injection, the experimental and control mice were sacrificed. The brain parts and other body parts were dissected. The muscle of the experimental and control mice were taken for the experiment. Acetyl choline esterase (AchE) assay was done in muscle tissue of epileptic mice by spectrophotometric method. The kinetic parameter Vmax, the maximum rate of reaction and Km, Michaelis menten constant of acetyl choline esterase enzyme were studied in the membrane fraction of muscle tissue in epileptic condition. The result shows increase in the Vmax during epileptic condition than that of the control. Michaelis Menten constant was found to be decreased.

INTRODUCTION

Epilepsy is a brain disorder and one of the most common neurological disorders affecting 1% of the world population and is defined as a condition characterized by recurrent seizure (two or more) a clinical manifestation presumed to result from an abnormal and excessive discharge of a set of neurons in the brain. The clinical manifestation consists of a sudden and transitory abnormal phenomenon which may include alterations of consciousness, motor, sensory, Autonomic or Psychic events perceived by the patient or an observer. Epileptic Seizures are a sign of cerebral dysfunction. A substance found in chemical synapses that are released from the presynaptic terminal in response to depolarization by an action potential, diffuses across the synaptic cleft and binds a ligand gated ion channel on the post synaptic cell. This alters the resting potential of the post synaptic cell and thus its excitability.

Acetyl choline is a neurotransmitter found in cholinergic synapsis provides a stimulatory transmission in the nervous system. Acetylcholine is synthesized from choline and acetyl CoA by the enzyme choline acetyl transferase (EC 2.3.1.6) to form acetylcholine, which is immediately stored in small vesicular compartments closely attached to the cytoplasmic side of presynaptic membranes via a vesicular acetylcholine transporter (Foye *et al.*, 1995).The skeleton and muscle function together as the musculoskeletal system. This system plays an important homeostatic role: allowing the animal to move to more favorable external conditions. Rapid muscular contraction is important in generating internal heat, another homeostatic function. Acetyl choline esterase is one of the most prominent constituents of central cholinergic pathways. It terminates the synaptic action of acetyl choline through hydrolysis and yields the choline moiety that is necessary for transmitter recycling. Hydrolysis of acetylcholine by AChE is rapid enough to explain the observed changes in Na^+ conductance and electrical activity during synaptic transmission. AChE can be inhibited resulting in the over stimulation neuromuscular junctions. This leads to spasms and death by suffocation because the heart muscles experience severe arrhythmia at that condition. Skeletal muscle fibers contract only under the control of the nervous system. Communication between the nervous system and a skeletal muscle fiber occurs at a specialized intercellular connection known as a neuromuscular junction or myoneural junction. Acetylcholine is a neurotransmitter released by a neuron to change the membrane properties of another cell. In this case the release of acetylcholine from the synaptic terminal can alter the permeability of the sarcolemma and trigger the contraction of the muscle fiber.

***Corresponding author:** maleekailliasbiotech@gmail.com*

MATERIALS AND METHODS

Inducing Epilepsy in Albino Mice

Swiss Albino mice that weighed about 60±0.5 gm were taken as experimental and control were injected during early hours of the day (7 am to 9 am). Both the control and experimental mice were injected intraperitonally with atropine (182mg/kg of body weight) to reduce peripheral activity. Pilocarpine was also dissolved in phosphate buffer and injected 25 minutes after the first injection (182 mg/kg of body weight) to induce seizures in the mice. Both mice were then kept under observation. After 20 minutes the experimental mice started showing seizures and salvation. The experimental and control mice were observed every day for one hour. Seventeen days after the injection, the experimental and control mice were sacrificed by cervical dislocation. The brain parts and body parts were dissected. The muscle of the experimental and control mice were taken for the experiment. Visceral parts include heart, liver, kidney, pancreas and muscle were collected at the time of sacrifice and that stored at –70°C. The six regions of brain – cerebral cortex, cerebellum, hypothalamus, corpus striatum, brain stem and hippocampal regions were dissected out on a chilled glass plate. They were washed in physiological saline to remove blood.

Acetyl Choline Esterase Enzyme activity

Acetyl choline esterase (AchE) assay was done in muscle tissue of epileptic mice using the spectrophotometric method of Ellman *et al.* (1961).

Prepapration of Homogenate

The frozen body parts of muscle tissues were weighed and 10% homogenate was prepared using sodium phosphate buffer (30mM/

pH -7). To this 1.0 ml of 1% Triton X 100 was added to release the membrane bound enzymes. It was then shaken gently and centrifuged at 10000 rpm for 30 minutes at 4°C. The supernatant contain the soluble acetyl choline esterase enzyme was then removed and stored in the deep freezer (-75 °C) without loss of activity until assayed. The substrate used in the assay system is acetyl choline iodide the ester of thiocholine and acetic acid. The activity of the enzyme can thus be measured by following the increase in the absorbance at 412 nm in a double beam spectrophotometer.

Assay Procedure

As 5, 5^1-dithoibis (2-nitro benzoic acid) is unstable at alkaline pH the stock solution was prepared by dissolving 39.6 mg in 10ml of 0.1 m sodium phosphate buffer containing 15 mg of sodium bi carbonate. Different concentrations of acetyl thiocholine iodide were prepared fresh by dissolving it in 0.1 m sodium phosphate buffer of pH 8. 5 ml of enzyme was added to 1 ml of sodium phosphate buffer (0.1m of pH 8) and incubated at room temperature for 5 minutes. To this 10 µl of DTNB (10mM) was added followed by 20 µl of acetyl thiocholine iodide of a certain molar concentration. Both DTNB and acetyl thiocholine iodide were added just before the spectrophotometric study was done. The increase in absorbance at 412 nm was recorded against blank in which enzyme was replaced by an equal volume of phosphate buffer.

Enzyme activity

If the change in extinction is E per minute then the activity in international unit is

AChE activity = (ΔE x 1000 x 1) / 1.36×10^4 x 0.05.
E = Extinction change per minute.
1.0 = Total volume of reaction mixture in ml.
0.05 = Volume of enzyme in ml.
1.36×10^4 = Molar Extinction coefficient of chromatophore at 412 nm (Litre $mol^{-1}cm^{-1}$) = E x 1.47 litre $moles^{-1}$ min^{-1}
AChE activity = E x 1.47 47 litre $moles^{-1}$ min^{-1}
The protein estimation was performed following lowry's method

RESULT AND DISCUSSION

In the present study kinetic parameters of acetylcholine esterase activity was analyzed in the epileptic conditions. Epilepsy is characterized by unprovoked, recurring seizures that disrupt the nervous system and can cause mental and physical dysfunction. Acetylcholine is a neurotransmitter in the cholinergic system, which is important for learning and memory, and is of interest in epileptic seizures. The present study was undertaken to examine possible changes in the activity of acetylcholine esterase after pilocarpine induced epilepsy in mice (Table 1). The animals injected with pilocarpine showed recurrent seizures during the chronic period.

Enzyme analysis

Kinetic parameters Vmax and Km of AChE in Epileptic Rat Muscle tissue

The kinetic parameter Vmax, the maximum rate of reaction and Km, Michaelis menten constant of acetyl choline esterase enzyme were studied in the membrane fraction of muscle tissue in epileptic condition. The result shows increase in the Vmax during epileptic condition than that of the control. Michaelis Menten constant was found to be decreased. (Table 2 and Fig.1)

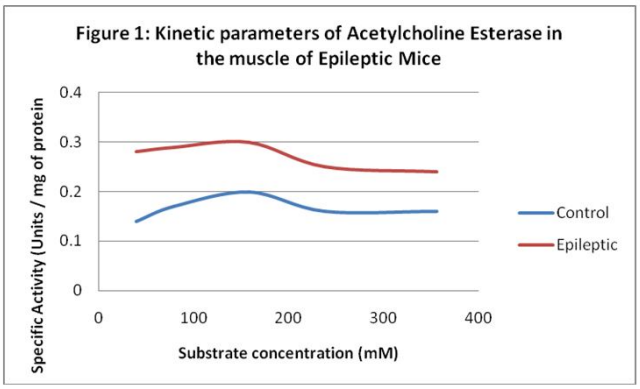

Table 2. Kinetic parameters of Acetylcholine Esterase in the muscle of Epileptic Mice

Gropus	Vmax (U/mg protein)	Km (mM)
Control	0.20	22.5
Epileptic	0.30	17.5

DISCUSSION

Atropine is an anti-cholinergic drug extracted from Belladonna. It blocks acetylcholine which is a neurotransmitter that facilitated the movement of impulses from one nerve to another. Atropine blocks acetylcholine centrally, in the brain, in a region called the Nucleus Basalis. The nucleus basalis contain cells, neurons that secrete acetylcholine and through long radiating fibers these cells communicate with hippocampus, which stores the brain memories. Acetylcholine is metabolized by an enzyme called Acetylcholine esterase. Pilocarpine model of epilepsy in mice is well suited to the study of several kinds of epilepsy that mimic human epileptic fits. Pilocarpine induced status epilepticus (SE) can be achieved by high doses of intraperitoneal injections on the order of 300 mg/kg; however relatively high mortality rates (up to 50%) are generally associated with this method (Heidi *et al*., 2005) prior to pilocarpine induced SE, essential preinjection protection from peripheral effects of pilocarpine treatment can be provided by anticholinergies which do not cross the blood brain barriers (Andre *et al*., 2000), lithium pre treatment, followed by one or several low doses of pilocarpine produces SE and chronic epilepsy with much lower mortality rates than a single dose of pilocarpine (Glien *et al.*, 2001) Evidence obtained from invitro studies have shown that several cellular mechanisms may lead to epileptic form activity. Reduced GABAergic inhibitory synaptic activity (e.g. Federico and Mac Vicar, 1996; Bain, 1994; Rodriguez *et al*., 1997; Wong and Miles, 1994) or enhanced glutamatergic excitatory synaptic activity (Federico and Mac Vicar, 1996; Johnson and Brown, 1981; traub *et al.*, 1993) may lead to epileptic – like phenomenon. Acetylcholine esterase is a serine esterase and has a catalytic trial

Table 1a. Specific Activity of Actylcholine Esterase In Muscle Tissue Of Experimental Mice- Control & Epileptic

Substrate concentration (mM)	Control Enzyme Unit (U/Min)	Control Specific activity (Enzyme unit/mg protein)	Epileptic Enzyme Unit (U/Min)	Epileptic Specific activity (Enzyme unit/mg protein)
39.6	0.048	0.14	0.063	0.28
79.25	0.057	0.17	0.065	0.29
158.25	0.062	0.20	0.066	0.30
237.75	0.052	0.16	0.057	0.25
356.00	0.055	0.16	0.054	0.24

similar to that found in serine proteases, trypsin and chymotrypsin. The trial consists of Ser 200, His 400, and Glu 237. The enzyme is located on the surface of the post synaptic membrane and linked by a GPI anchor. Acetylcholine esterase is an enzyme which occurs at high specific activity in the brain. It is present in conducting fibers throughout the animal kingdom and localized inexcitable membrane. According to Nachmonsohn's theory (Leuzinger *et al*., 1967) the action by acetylcholine induce a conformational changes of the receptor protein, which initiates a series of reaction is excitable membrane associated with electrical activity.

The present study has been aimed at determining kinetic parameters of AChE in muscle tissue of epileptic mice. Epilepsy was induced in Swiss Albino mice by injecting pilocarpine intrapritoneally on the order of 182 mg/kg. After 20 minutes the experimental mice were observed every day. Seventeen days after the injection the experimental and control mice were sacrificed. The muscle of the mice was taken for the assay procedure. The kinetic parameter Vm (maximum rate of reaction) and Km (Michaelis Menton constant) were studied. The result showed an increase in the Vmax and decrease in Km during epileptic condition than that of control (Table 2 and Fig.1). In epileptic condition because of the seizures, neuronal firing occurs and these impulses have to pass all over the body and for this purpose the ACh produces more. The released ACh rapidly diffuses across the synaptic cleft and binds with specific ACh receptors located in the post synaptic membrane found at neuro-muscular functions. These receptors initiate an action potential event in the muscle cell membrane causing a massive influx of extracellular calcium thereby triggering muscle contractions.

Neurotransmitters bind to specific receptor proteins and channels and initiate signaling events mainly related to electrical transmissions such as action potentials or stimulating calcium waves in the cytoplasm. The neurotransmitters and hormones are synthesized mainly in neurons and specialized glands of which the liver and skeletal muscles can utilize the aminoacid degradation for its energy production. The enzymes, acetylcholine esterase clears the neurotransmitter acetylcholine into its constituent's acetate and choline. The choline molecules are then transported into the axon terminal of the pre synaptic neuron where they are efficiently acetylated to acetyl choline. ACh has few therapeutic applications owing to this diffuse action and rapid hydrolysis by acetyl choline esterase (AChE). The structural brain damage insulted by the pilocarpine may underline or be associated with recurrent spontaneous seizures in mice. We found that epileptic conditions leads to a remarkable change in the activity of acetylcholine esterase enzyme in the muscle tissue compare to the control.

In epileptic muscle tissue the acetyl choline esterase enzyme showed increased activity compared to that of control tissue. The released acetylcholine rapidly diffuses across the cleft and binds with specific acetylcholine receptors located in the post synaptic membrane found at neuro-muscular junction. These receptors initiate an action potential event in the muscle cell membrane causing a massive influx of extracellular calcium, thereby triggering muscle contractions. AChE can be inhibited resulting in the over stimulation of neuromuscular junctions. This leads to spasms and death by suffocation because the heart muscles experience severe arrhythmia at that condition. In the present study, the results showed an increase in AChE activity with a decreased affinity which is an index for acetylcholine function. Thus it is suggested acetylcholine has a definite function in epileptic condition.

REFERENCE

Andre, V., A, Ferrandon, C. Marescaux., A. Nehlig, (2000). Lesional and Epiletogenic consequences of Lithium - pilocarpine induced status epilepticus are affected by previous exposure to isolated seizure: affect of amygdale kinelling and maximal electroshock, N*eurosciences* Vol 99 pp 469-481.

Avolim M., (1984) Molecular mechanism of anti epilogical findings in primary generalized epilepsy. *Epilpesia* Vol 25 pp 8 – 21.

Calabresi, P D, Centonze., P, Gubellini, A, Pisani G, Bernard (2000). Acetyl choline mediated modulation of striated function. *Trends Neuroscience* Mar Vol23 (3) pp 120 – 126

Chen, B.M and A.D, Grinnell (1995) integrins and modulation of transmitter release from motor nerve terminals by stretch *Science* Vol 269 pp 1579 – 1580.

Chubb, I. W. Ranirie G.H White., and A. Hodgson (1980). Acetylcholine esterase hydrolyses substances P *Neuroscience S*, Vol 12 pp 2065 – 2072.

Heidi, l Grabenster., J, Damien., A, Ferroro., Philip., William L Chapman., Edward, Dudek (2005) Absence – like seizures in adult rats following pilocarpine induced status epileptius early in life *Science* Vol 20 pp 165 – 168.

Johnston, D., and T.H. Brown., (1981) Giant synaptic potential hypothesis for epileptic activity. *Science* Vol 21 pp 294 – 297.

Lemos, Alheiro, E.A (1995) Supression of pilocarpine – induced status epilepticus, the late development of epilepsy in rats. *Epilepsia* Vol 102 pp 423 – 428.

Leuzinger, W., A.L Baker., (1967) Acetylcholine esterase, large scale purification, homogeneity and aminoacid analysis. *Proc. Nat Acad Sci, U.S.*, Vol 57 pp 446 – 451.

Mac Bain, C.J (1994) Hippocampel inhibitory neuron activity in the elevated potassium model of epilepsy. *J. Neurophysiol* Vol 72 pp 2853 – 2863.

Rodriguez – Moreno, A.O Hurreras., and J, Lerma., (1997). Kainate receptors presynaptically down regulate GABAergic inhibition in the rat hippocampus. *Neuron* Vol 4 pp 893 – 901.

Traub, R.D., R, Miles and Jefferys (1993) Synaptic and intrinsic conductance shape Picrotoxin – induced synchronized after discharges in the guinea pig hippocampal slice *J. Physiol* Vol 461 pp 525 – 547.

Wong R.K.s, and R. Miles (1994) Study of GABAergic inhibition and GABA receptors in experimental epilepsy. In epilepsy, Models, mechanism and concepts, edited by schwortzkroin, P.A Cambridge, U.K Cambridge University Press pp 424 – 436.

****