

The role of carboxyl esterase and acid phosphatase in aged and lithium treated rats in regulation of neuronal function

Prabha M, Bhavana G, Sunitha P, Channarayappa, Lokesh KN

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Abstract

Acid Phosphatase (ACP) and Carboxyl Esterase (CE) were assayed for four different groups of young and old aged male Albino Sprague dawley rats (2-4 and 16-18 months) in brain and liver. Among them both aged rats were treated with 37 mg of lithium chloride (LiCl₂) per kilogram of body weight orally for 10 days. LiCl₂ showed completely positive effect on releasing carboxyl esterase in both young and old groups of brain (36.1 and 89.3 nmoles/min/mg of protein) and liver (114.93 and 91.73 nmoles/min/mg of protein) respectively than control. The young and old rat brain samples treated with LiCl₂ showed intense band for CE in Native PAGE gel than untreated groups. Acid phosphatase showed more activity in normal young rats than old aged rats however, old rats treated with LiCl₂ showed more significant ACP activity (65.3 nmoles/min/mg of protein) and young rats showed elevated ACP activity (55.1 nmoles/min/mg of protein) respectively than untreated groups. Thus LiCl₂ is involved in activation of enzymatic function and potential to be integrated into drug-development programs complexed with CE neuroprotective factor to facilitate the neuronal function.

Keywords: Hydrolases, Brain, Liver, Lithium Chloride, Specific activity and Native PAGE

Introduction

Hydrolytic enzymes break down biomolecules into their simpler units. Earlier studies have highlighted the role of hydrolytic enzymes in a many pathological conditions such as brain tumors, neurodegenerative diseases like Alzheimer's disease (Cataldo et al. 1996) and inherited metabolic disorders (Futerman et al. 2004).

Prabha M*, Bhavana G, Channarayappa, Lokesh KN

Department of Biotechnology, MSRT, Bangalore-560054

*Email: prabhamg@gmail.com

Sunitha P

Department of Biotechnology, Maharani Lakshmi Ammani College for Women, Bangalore-560012.

Among hydrolase's, acid phosphatase(s) and esterase's were extensively studied (Guner et al. 1985).

In esterase's family, mainly the carboxylesterases (EC 3.1.1.1) members of the serine hydrolase superfamily can efficiently hydrolyze a variety of ester, amide and carbamate-containing xenobiotics to their respective free acids (Yan-hui Yang et al. 2011). They are present in many parts of the body, including intestines, blood, brain, skin and tumor. Carboxyl esterase was reported in endothelial cells and blood brain barrier of human brain (Zhang W et al. 2002). Biochemical studies have proved that carboxyl esterases are efficient in hydrolyzing small substrates, but show vast differences with increasingly larger substrates. For example, the human liver hCE1 was 100 to 1000-fold less efficient in metabolizing CPT-11 than rabbit liver rCE, even though the two enzymes have over 80% amino acid identity (Wadkins et al. 2001). Therefore carboxylesterases play a critical role in the activation of various antiviral, anticancer, and antibiotic prodrugs (Potter et al. 2006).

Another hydrolase acid phosphatase (EC 3.1.3.2) is a phosphomonoesterase, different forms are found in various organs and their serum levels used to evaluate the success of the surgical treatment of prostate cancer. High concentrations of acid phosphatase are found in the prostate gland. Acid phosphatase activity, localized in the form of coarse granules in the lysosomes was found to be increased in reactive glial cells of gemistocytic and microglial origin (Friede et al. 1965).

Change in the activity of hydrolytic enzymes causes Lysosomal storage disorders (Bonten et al. 2004) and neurodegenerative diseases (Lisha Zhang et al. 2009). The deficiency of carboxylesterase results in obesity, hepatic steatosis and hyperlipidemia (Ariel et al. 2012). Hence it is necessary to regulate the activity of hydrolases in pathological and clinical conditions for the physiological function of a cell. Currently there is a great demand for methods, which help to modulate the function of these enzymes with chemicals. These methods with modulators might help to find out a potential drug leads (Troitskaya et al. 2004) for improvement in enzyme activity which inturn responsible for drug activation. Thus protein/enzyme modulation plays a central role in the regulation

of biological processes. The activity regulated by modulators often result in a conformational change or change in enzyme properties, which increases the effectiveness of the enzyme. Approaches towards this goal have been made extensively, for monitoring and enhancing enzyme activity with modulators of proteins (Amos Baruch et al. 2004) and metals such as zinc, iron and lithium.

Surprisingly many numbers of drugs contain metals. This theme relies on the study of the design and mechanism of action of metal-containing pharmaceuticals and compounds that interact with endogenous metal ions in enzyme active sites. This diverse metal group includes the platinum and ruthenium anti-cancer drugs, gold drug chaperones and Lithium. Lithium is the lightest elemental metal and in human tissue it is found in low concentrations. Earlier researchers have highlighted its role in enhancement of hippocampus neurogenesis (Chen et al. 2000) and it is a mood stabilizer (Trevor Young et al. 2004).

Therefore the present study has been done by selecting the brain and liver to assay carboxyl esterase and acid phosphatase enzymes for monitoring and enhancing enzyme activity with modulators such as lithium chloride. Thus Determination of specific activity for two hydrolytic enzymes in brain and liver of rat model (young and old aged) treated with lithium chloride showed elevated activity for both enzymes as compared to their control. This may contribute a valuable therapeutic agent to the field of pharmaceuticals, which helps in regulation of enzymes, which are believed to play an important role in regulation neuronal cell function, neurodegenerative diseases and liver disorders.

Methods

Procuring of the rat models

Male albino Sprague dawley of young rats (weight of 150-200 grams) and old aged rats (weight of 300-350 grams) were purchased from Vekateshwara agency, Bangalore and were housed five per cage had free access to water and food (Libitum) and were exposed to a 12-hour light/dark cycle. After a two week accommodation period the rat models were used for research at MLACW Bangalore.

Therapeutic rat groups were treated daily with 37mg of Lithium chloride (LiCl₂) per kilogram of body weight orally for 10 days at 24 hours interval time and then protein estimation and enzyme assays were carried out similar to that of the control group.

Collection of rat tissue and processing of tissue specimens

Table: 1 Weight of Rat tissue samples

Group	Age (months)	Body weight of rats (g)	Weight of Brain (g)	Weight of Liver (g)
I (young control)	2-4	150-220	1.93	6.11
II (young therapeutic)	2-4	150-220	1.72	6.37
III (old control)	16-18	300-350	1.85	15.50
IV (old therapeutic)	16-18	300-350	2.06	10.33

The samples of brain and liver were collected by sacrificing the rats. The model was first anesthetized using Xylazine and Ketamine in the ratio 1:4. The models were then prepared for dissection by securing them on wax boards and a cut was made near the dorsal line of skin to takeout the liver and brain, which was removed by making a cut near the skull region. Both the liver and the brain were then subjected to wash with 0.9% Phosphate Buffer Saline (PBS) to remove the residues and blood, then tissues weight was noted down.

Preparation of tissue extraction from specimens for biochemical studies

Specimens (brain and liver) were homogenized using pestle and mortar followed by glass homogenization, with tris buffer saline (TBS pH=7.4 containing 1% (v/v) triton-X 100) and protease inhibitors (10 µl 0.5 mM phenyl methyl sulphonyl fluoride in ethanol). Tissue to buffer ratio was 1:10 w/v (wet weight). Denaturation was prevented by maintaining specimens at low temperature (5°C-10°C). Then homogenized sample was centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatants were collected for biochemical analysis and stored at 4°C until the enzyme activity was assessed.

Protein Estimation

Protein estimation was carried out according to Lowry's method (Lowry et.al 1951; Hartee 1972) using Bovine Serum Albumin solution (BSA) as a standard.

Estimation of Hydrolytic Enzymes

Activities of the hydrolytic enzymes in supernatant of tissue extracts were assayed spectrophotometrically.

Carboxyl Esterase

Assay was carried out by Gomori (Gomori G. 1941) and later modified by Van Asperen (Asperen VK. 1962). Enzyme reaction was initiated by adding 900 µl of 5 mM α-naphthyl acetate in phosphate assay buffer (pH 7.0) to 100 µl tissue extract then incubated for 15 min at 27°C. Subsequently, the reaction was stopped by the addition of 500 µl DBLS reagent and enzyme activity was measured at 600nm.

Acid phosphatase

Assay was carried out according to the method described earlier (Lam WK et al. 1978). The enzyme reaction was initiated by allowing 200µl of the tissue supernatant (obtained after tissue homogenization) to react with 800 µl of 5 mM pNPP (SIGMA) in 0.1 M-citrate buffer (pH 5.0) and incubated for 10 minutes. The reaction was stopped by the addition of 1ml of 2N NaOH and enzyme activity was measured at 450 nm.

Electrophoresis (Laemmli UK. 1970)

The brain tissue supernatant sample of 40µl was electrophoresed in a native polyacrylamide gel (10 %) at a constant voltage of 50 Volts. The run was stopped when the marker dye coomassive brilliant blue reached 1-2 mm above the lower edge of the plate. The gel was carefully transferred to the PBS solution. Then gel was incubated in substrate 5 mM α-naphthyl acetate solution for 10 minutes and then stopping reagent DBLS was added and incubated till intense bands were seen.

Results and Discussions

Estimation of total protein by Lowry's method

The protein content was estimated in brain and liver of young rats, old aged rats and other set of similar aged groups of these rats treated with lithium chloride for 10 days at a time interval of 24 hours.

Table 2: Total Protein present in 1ml of tissue supernatant of brain and liver of control and therapeutic groups

Tissues	Young Control	Young LiCl ₂ Treated Rats	Old Control	Old LiCl ₂ Treated Rats
Brain (nmoles /min/mg of protein)	35.2	11.79	29.92	8.8
Liver (nmoles /min/mg of protein)	36.52	20.94	39.6	24.2

The protein activity was found to be more in young brain than old aged rats. This may be one of the reason for the loss of neuronal cell function since protein activity related with cell function and cell number.

The rats which were treated with lithium chloride showed lower protein content than their control groups of both young and old aged rats (Table 2). This also may be one of the reasons for increased enzyme activities in rats treated with 37mg of lithium chloride for 10 days at a 24 hours interval time.

Specific Enzyme Activity

Specific activity was calculated for Carboxyl esterase and Acid phosphatase from tissue supernatant of brain and liver as shown in a graph and tables (Table 3 and 4 and Figures 1, 2 and 3).

Specific activity of Carboxyl esterase

Table 3: Specific activity of Carboxyl esterase from 1ml tissue supernatant of brain and liver samples of young and old aged rats.

Tissues	Young Control	Young LiCl ₂ Treated Rats	Old Control	Old LiCl ₂ Treated Rats
Brain (nmoles /min/mg of protein)	14	36.1	31.63	89.3
Liver (nmoles /min/mg of protein)	41.2	114.93	49.83	91.73

Carboxylesterase (CE) is a glycoprotein which showed significantly higher activity in both old brain and old liver samples of control rats as compared to young brain and young liver samples of similar groups (Table 3, Figure 1). However all groups with lithium treated rats showed significant carboxyl esterase elevated activity as compared to their control, whereas liver showed increased activity by 2.78 fold.

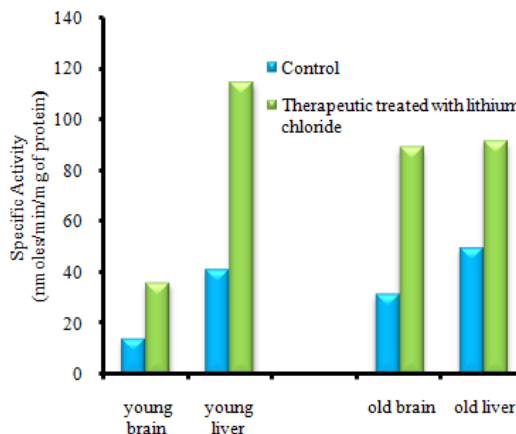


Figure 1: Specific activity of Carboxylesterase estimated in 1ml of tissue extract of brain and liver.

This shows that lithium has completely positive effect on releasing of carboxyl esterase, which plays an important role in drug metabolism and xenobiotics metabolism necessary for the chemoprotective functions of proteins in the liver and brain (Sompop Bencharit et al. 2003) samples. The role of lithium in neural drug design and development was well established before 1989 (Michael J. Berridge et al., 1989), but there were no earlier reports related to the significant increased specific activity of carboxyl esterase when treated with LiCl₂ in brain and liver. It is also known that LiCl₂ drug is an antidepressant and neural protective, similarly carboxyl esterase is also neuroprotective and drug metabolizing enzyme. Therefore carboxyl esterase is involved in metabolism of anticancerous drug activation.

Earlier study showed the activation of carboxyl esterase for the drug activation in treatment of tumor growth and deficiency of this enzyme leads to failure of drug metabolism in tumors. In support to this result, earlier thesis reports showed lower carboxyl esterase activity in brain tumors (Prabha et.al (2005) thesis) as compared to normal brain. Hence lower activity of carboxylesterase in tumors is one of the main reasons for the failure of chemotherapy. Therefore it is necessary to activate the CE with activators which was reported first time with LiCl₂ as modulator that showed higher CE activity with young and old aged rats treated with LiCl₂ of brain and liver samples. So the positive effect for the increased level of carboxyl esterase with LiCl₂ that acts as a modulator, if it is complexed with the drugs involved in treatment of cancer. This information supports with a report where HB1.F3.C1 cells were engineered to encode for a secreted form of rabbit carboxylesterase (rCE), an enzyme capable of activating the anticancer prodrug CPT-11. The in vivo-administered rCE secreting stem cells selectively migrate to disseminated metastases where the enzyme activates the CPT-11 prodrug, thus increasing antitumor activity (Riccardo Fodde 2006).

Native PAGE for carboxylesterase expression

The young and old aged rat brain samples treated with LiCl₂ showed an intense band pattern as compared to untreated groups. However old aged rats treated with LiCl₂ also showed more intense band pattern (Figure 2 panel D) which refers to the high expression of carboxyl esterase.

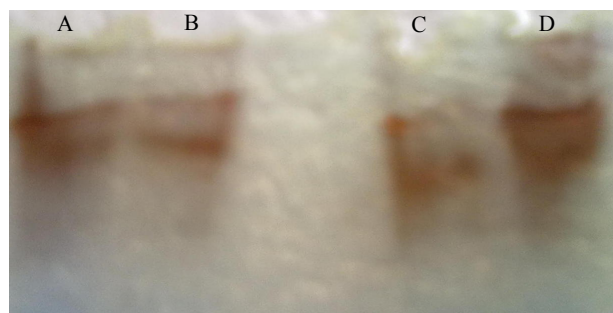


Figure 2: Native PAGE for carboxylesterase expression
A: Young rats Control B: Young rats treated with LiCl₂; C: Old aged rats control; D: Old aged rats treated with LiCl₂

Specific activity of Acid Phosphatase

Table 4: Specific activity of Acid Phosphatase from 1ml of tissue supernatant of brain and liver of young and old aged rats

Tissues	Young Control	Young LiCl ₂ Treated Rats	Old Control	Old LiCl ₂ Treated Rats
Brain (nmoles /min/mg of protein)	15.1	55.1	4.17	65.3
Liver (nmoles /min/mg of protein)	51	91.9	3.78	71.2

Specific activity was also calculated for acid phosphatase which was similar to CE. Acid phosphatase for LiCl₂ treated young brain showed increased activity by 4 folds when compared to untreated group and similar group in liver showed elevated enzyme activity by almost 2 folds as compared to its control (Table 4).

In case of old aged rat brain treated with LiCl₂ showed elevated activity of acid phosphatase by 22 fold as compared to untreated old brain (Figure 3). While old aged rat liver treated with LiCl₂ showed significant increased enzyme activity by 23.6 folds. The graph (Figure 3) showed old aged rat's brain samples with lower acid phosphatase activity as compared to young rat's brain and liver samples. However, it is significantly elevated after treated with LiCl₂ that showed positive effect in case of old samples. So, lithium chloride is a positive modulator for activating acid phosphatase in old aged brain, liver and young samples also.

In present study the acid phosphatase activity was lower in old aged rat brain than young brain this may be due to the localization and activation of lysosomes in young rat brain and liver. However the acid phosphatase showed elevated activity in LiCl₂ treated old aged rat brain and liver as compared to their control and young rat samples also. The mechanism of acid phosphatase activation with lithium chloride is unknown.

Acid phosphatase is a lysosomal marker (Honsi et al. 2000). Dysfunction lysosome system results in the formation of Tau insoluble aggregates in lysosomes of Alzheimer's disease (AD) (Hamano et al. 2008). Some insoluble aggregates is of particular interest because it is presumed to be a precursor molecule that may lead to formation of plaque amyloid. Histopathology of ACP lysosomal hydrolases activity in senile plaques has been documented long before regarding the plaque pathogenesis become available.

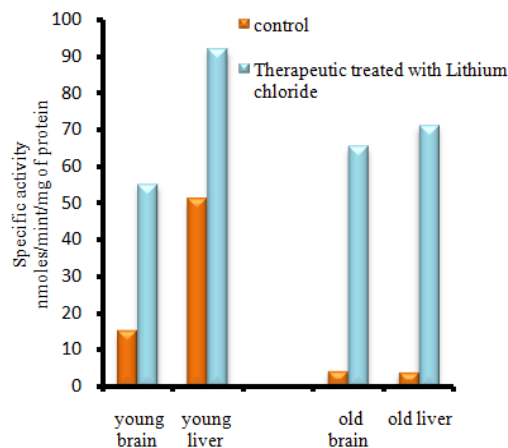


Figure 3: Specific activity of Acid phosphatase (ACP) from 1ml of tissue extract of brain and liver samples

The neuropathogenic contributions of lysosomal dysfunction (Ben et al 2002) may be key to Alzheimers Disease Pathology and alterations to the lysosomal system contribute to protein deposits associated with different types of age-related neurodegeneration. The residual bodies accumulate as an indicator of lysosome dysfunction (Cataldo et al., 1994). The changes in endolysosomes appear to play an important and early role in the pathogenesis of AD. Lysosomal abnormalities progressively worsen as neurons begin to degenerate.

Earlier report also suggest that reduced activity of the low-molecular-weight acid phosphatase, (Shimohama et al., 1993) which possesses phosphotyrosine protein phosphatase activity, might be linked to aberrant protein tyrosine phosphorylation found in Alzheimer brains.

But it is unknown that acid phosphatase are involved in AD because another report says that low molecular weight acid phosphatase is low in AD but not in control and exact data cannot be established based on these result alone. But Acid Phosphatase might have originated from neurons with oxidative stress of oxygen membrane or free radical mediated damage because membrane abnormalities lead to release of intracellular molecules and enzymes. This shows acid phosphatase is not involved in the Alzheimer's disease.

In supporting to this data, the present study showed that acid phosphatase elevation in the presence of lithium which is involved in neuronal regulation by activating hydrolytic enzymes in a cell. This was observed in old aged lithium treated rats which showed higher acid phosphatase activity than controls of old and young rats.

Earlier study showed selective enhancement of lysosomal responses clears Alzheimer's Disease (Butler and David 2008). So present study showed increased acid phosphatase activity in lithium treated old rat brain samples which is related to enhancement of lysosomal activation since acid phosphatase is a lysosomal marker.

Therefore lithium chloride activates acid phosphatase and carboxylesterase elevation since lithium is a neuroprotective that helps in the regulation of neuronal function by protecting neuronal death. This information supports with earlier reports that Lithium stimulates neuronal growth especially in hippocampus (Chen et al. 2000). Thus Lithium chloride is involved in the

activation of enzymes and has the potential to be integrated into drug-development programs complexed with carboxyl esterase which is neuroprotective factor to facilitate the function, validation of pharmaceutical targets related to drug metabolism and in the treatment of brain, liver and other human diseases.

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