ACTIVATION OF BUTYRYLCHOLINESTERASE ENZYME BY MAGNESIUM IONS

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Abstract

Human serum Butyrylcholinesterase (BChE) is an important enzyme in detoxification with its capacity for hydrolyzing esters. the inhibition of this enzyme lead to ill health. The reaction of enzyme with metals ions have grown in recent years,. in our study we examine the reaction of Mg^{+2} with normal and toxic form of enzyme .we collected samples as two groups for normal and toxic plasma, treated with different concentration of Mg^{+2} , compare the reading of plasma cholinesterase before and after treating , there are significant changing for that two groups at certain Mg^{+2} concentration. this changing may be for the allosteric effect of cholinesterase enzyme , reformation the binding site as its environment changing .

Keywords: Butyrylcholinesterase, allosteric effect , bio-ions , Mg^{+2}

Introduction

There are two different types of cholinesterase enzymes in human body, which differ in their location in tissues, substrate affinity, physiological function and sensitivity toward various inhibitors: the first type is acetyl cholinesterase (AChE; E.C. 3.1.1.7) . it plays a major role in the regulation of several physiological events by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses. The second type is Butyrylcholinesterase (BChE; E.C. 3.1.1.8) [1-4], it is a major detoxification enzyme found abundantly in serum with the ability to hydrolyze a wide variety of choline esters, ester containing compounds [5-7].BChE can also take the place of AChE in the acetylcholine degradation, when acetyl cholinesterase is inhibited or absent [8] The enzyme acetylcholine esterase breaks down the neurotransmitter ,acetylcholine(substrate). which is released at nerve and muscle junctions, in order to allow the muscle or organ to relax [9]. A cholinesterase inhibitor (or "anticholinesterase") suppresses the action of the enzyme. The result of enzyme inhibition is that

acetylcholine(substrate) builds up and continues to act so that any nerve impulses are continually transmitted and muscle contractions do not stop.[9] there are chemicals that interfere with the action of cholinesterase ,that are potent neurotoxins. causing muscle spasms and ultimately death. as some snake venoms, and the nerve gases sarin and (VX)nerve agent [9]. and many substances used in insecticides have been shown to act by combining with a residue of serine in the active site of acetylcholine esterase, inhibiting the enzyme completely.[9] Among the most common acetylcholinesterase inhibitors are phosphorus-based compounds,.[9] ill-health following exposure to organophosphorus compounds has been attributed to the inhibition of cholinesterases enzyme. [10] Therefore, the knowledge of the cholinesterase status is crucial for the early diagnosis of organophosphate pesticides (OPs) exposure or intoxication and for monitoring the therapeutic effects of reactivates [11]. The inhibiting power on AChE and BChE varies widely among the different Organophosphates compounds. Some (Ops) inhibit AChE more strongly and some others BChE. Some others are stronger inhibitors of BChE than AChE[1]. In exposure to these substances, plasma BChE determination is a more sensitive indicator of exposure than AChE. [1] Interest in the interaction of the ChE with metal ions has grown in recent years[12], and where Life occurs in electrolyte solutions made of mixtures of 'bio-ions' (sodium Na^{+}) , potassium K^{+}, calcium Ca^{2+}, and chloride Cl^{-}), along with many other charged components. The precise composition of biological solutions is important. Gradients of Na^{+}, K^{+}, Ca^{2+}, and Cl^{-} ions provide energy to drive signals through the nervous system, and energize transport in most cells. The ions in biological mixtures carry information that controls biological systems. The selective flow of some of these ions are the signals of the nervous system. [13] Depending on the interaction between the enzyme , pesticides (OP) and ions, we designed laboratory experiment to demonstrate the effect of ions in the reconstruction efficiency of the enzyme.

Samples and method

in this study we classified samples to two groups normal group , and toxic group. For normal group, Blood samples were obtained from healthy volunteers (ages 20 to 50 years). who were not exposed to organophosphates, carbimates, or any ChE inhibitors. Heparinized blood samples were centrifuged at 6,000 rpm, to separate erythrocytes from plasma. the supernatant layer was used. Activity of cholinesterase was assayed by method described below. Then treated plasma by different concentration of Mg^{+2} , incubate mixture for half hours at 37°C . again measure the activity of enzyme for treated samples . there are sample blank (composed of Ellman's reagent and Mg^{+2} for each concentration without plasma)

For toxic group ,Blood samples were obtained from toxic patients (ages 20 to 50 years) who were t exposed to organophosphates or carbimates and ChE inhibited . Heparinized blood samples (not EDTA sample for minimize the chelation with metal) were centrifuged at 6,000 rpm, to separate erythrocytes from plasma. the supernatant layer was used. Activity of cholinesterase was assayed by Ellman's method described below. Then treated toxic plasma by different concentration of Mg^{+2} , incubate mixture for half hours at 37°C . again measure the activity of enzyme for treated sample . there are sample blank (from Ellman's reagent and Mg^{+2} for each concentration without plasma)

magnesium chloride anhydrous. $MgCl_2$. Magnesium treatment was done by adding certain concentration of metal stock solution , to prepare(0.1 , 0.5 , 1.0 , 1.5 mg /dl) Mg^{+2} /per volume of plasma.

Method for estimation Activity

The estimation of BChE activity was performed according to the method of *Ellman et al* [14] which has been modified by George and Abernethy [15] . The enzyme activity was calculated by measuring the increase in yellow color produced by thiocholine when it reacts with dithio-bis- nitro benzoate ions. A double beam spectrophotometer was used for estimating enzyme activity. The analyses were performed at 25 ± 1°C at wavelength of 440 nm. That was carried out by DTNB Kit. The assay of human serum cholinesterase with reaction involves of 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) with thiocholine liberated from its esters by enzymatic hydrolysis. The yellow 5-thio-2-nitro-benzoate (TNB) is formed that is detected by colorimetric substrates for the assay [16] .

Analysis of the Data

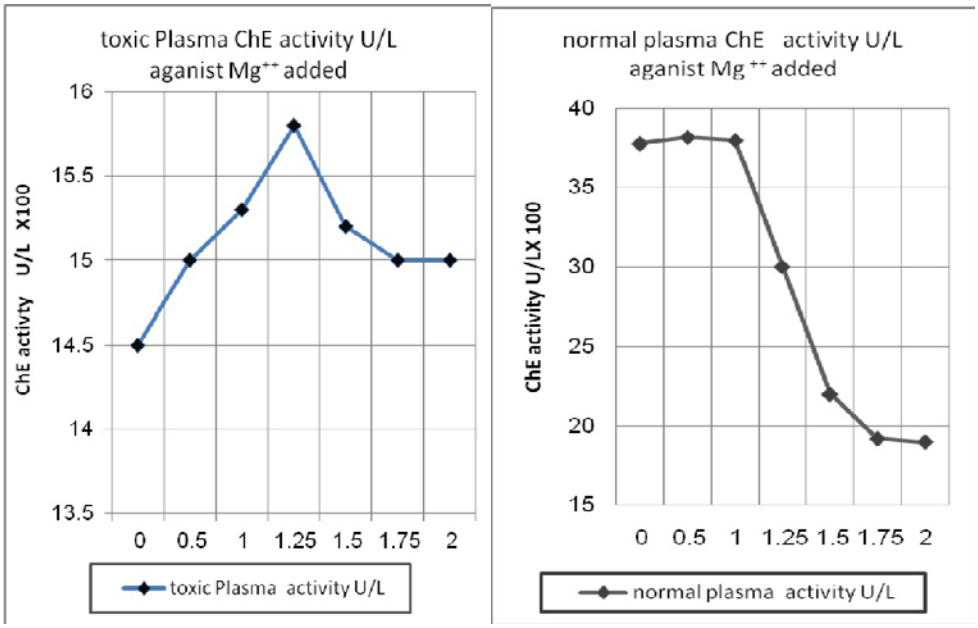
Statistical analysis was performed using Medcalc. Program for Windows (Ver. 11). We presented data as means ± standard deviation (SD). T-test was used for statistical analysis and $P<0.05$ was considered to be significant.

Results

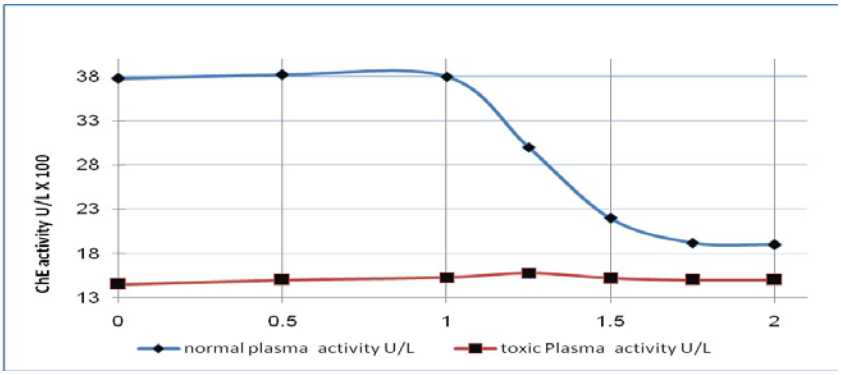
For normal samples group ;There were some changing in values of activity for Butyrylcholinesterase after treated plasma with magnesium ions, related to start point which untreated sample . this changing not significant in all concentrations of Mg^{+2}/plasma , but the significant changing appear where 1.5, 1.75 and for 2.00 mg/dl Mg^{+2} added to normal plasma , where the changing significant in toxic plasma appear at 1.0 , 1.25 and 1.5 mg/dl Mg^{+2} to toxic plasma ,where P value <0.05 ,but they return to nonsignificant changing at 1.75 , 2.00 mg/dl Mg^{+2} added . As shown in the table

Table (1) statistical data for the enzyme activity for toxic and normal plasma, before and after treatment with Mg^{+2} ions.

concentration Mg^{+2} Added mg /dl	BuChE activity U/L normal plasma ± SD	P value	BuChE activity U/L toxic Plasma ± SD	P value
0 (start reading)	3780 ± 52		1450 ± 24	
0.5	3820 ± 42	0.36	1500± 30	0.087
1	3800 ± 45	0.64	1530±35	0.031
1.25	3000 ± 35	<0.0001	1580± 30	0.004
1.5	2200 ± 36	<0.0001	1520 ±30	0.034
1.75	1920 ± 36	<0.0001	1500 ±24	0.063
2	1900 ± 29	<0.0001	1500±26	0.071



Figures(1,2) show the changing in ChE activity as Mg ion added in normal plasma and toxic plasma

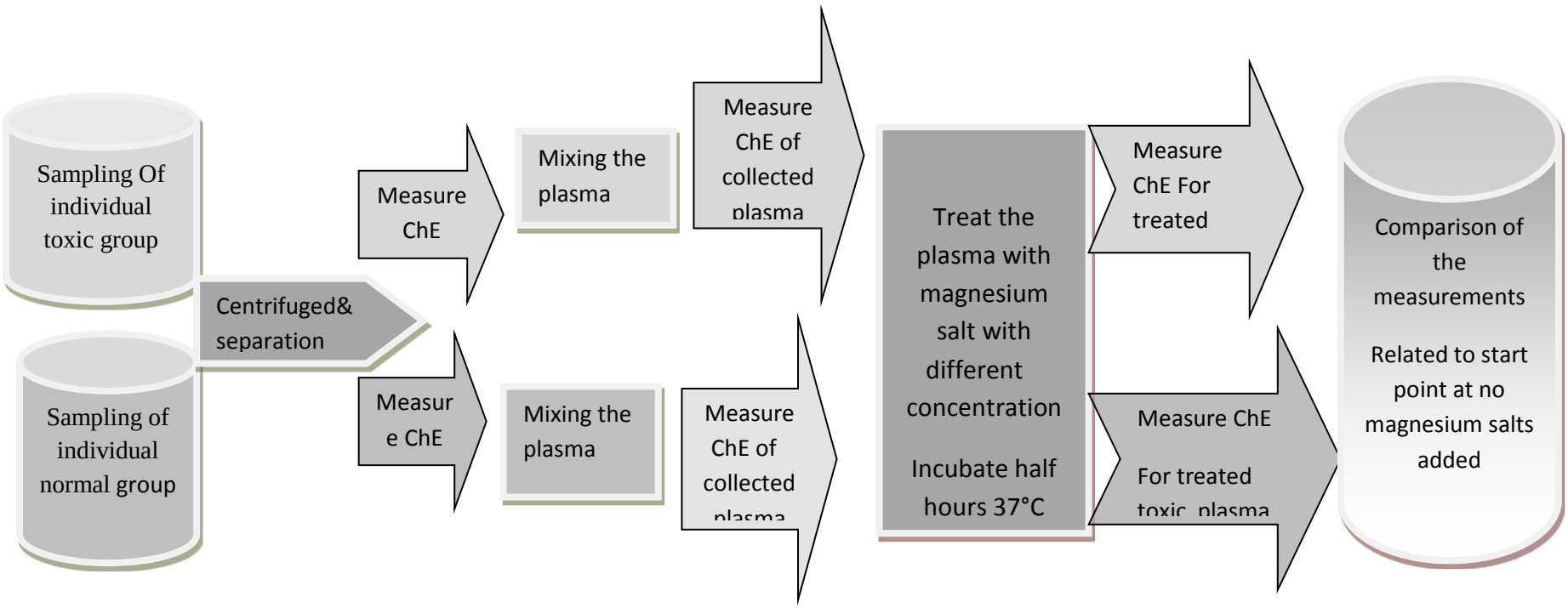


Figures(3) show the changing in ChE activity as Mg ion added in normal plasma and toxic plasma .

Discussion

Where Life occurs in electrolyte solutions made of mixtures of 'bio-ions' (Na^{+}, K^{+}, Ca^{2+},$Mg^{+2}$and Cl^{-}), along with many other charged components. The precise composition of biological solutions is important. Gradients of Na^{+}, K^{+} Ca^{2+}, and Cl^{-} ions provide energy to drive signals through the nervous system. the ions in biological mixtures carry information that controls biological systems. The selective flow of some of these ions are the signals of the nervous system [13]., Magnesium is important mineral, It maintains and balances the electrical signals in the body. . " Magnesium deficiency certainly qualifies as a principal cause of disease. there is simply nothing we can do to adequately enhance our state of health when magnesium supplies are less than adequate in our bodies" says Sircus [17]. Interest in the interaction of the ChE with metal ions has grown in recent years[12]. Among the bivalent metal ions, the effects of Hg^{2+} [18], Cd^{2+} [19], Cu^{2+}[20], Mg^{2+} [21] and Ca $^{2+}$ [22] on BChE from different sources have been investigated. For brain BChE purified from sheep, it has been shown that $Cd^{2+}$and $Zn^{2+}$are hyperbolic mixed-type inhibitors of the enzyme. and Ca^{2+} or Mg^{2+} reactivates the enzyme after Cd^{2+}or Zn^{2+} inhibition[23]. Al^{3+} also inhibits human serum cholinesterase [3]. Inhibition with Cd^{2+}or Zn^{2+} showed hyperbolic mixed-type inhibitors[23],but Al^{3+} was a linear mixed-type in-The changing in activity of enzyme in the normal plasma related to the charges of magnesium which can poisoned the active site of the enzyme . inhibitor of the sheep brain BChE [24]. Cd^{2+}, Zn^{2+} and Al^{3+} are the linear mixed-type inhibitors of BChE in human and their kinetic parameters have been reported recently[12]. The effect of magnesium ions on the activity of butyryl cholinesterase enzyme in the normal and toxic forms was the aim of this study .

Where we examine the effect of magnesium on plasma cholinesterase, in two cases in normal& toxic plasma. In normal plasma there are changing (decrease in activity of ChE) due to effect of magnesium ion on the normal allosteric form of cholinesterase . as Mg^{+2} converted this form to other allosteric form have bind site with less reactive form . in toxic plasma the changing (increase activity) coincide with the results of *(A.N. Cokugras et all*)[23] where Ca^{+2} and Mg^{+2} activate the enzyme after poisoned by Cd^{+2} , Zn^{+2}. not by pesticides as in our study , this increase in activity due to the enzyme after poisoned by Cd^{+2} and Zn^{+2} or by pesticides converted to allosteric form (poisoned allosteric form),that less reactive than normal allosteric form. the poisoned allosteric form can convert to other allosteric form more reactive in presence of magnesium ions. In the higher concentration than 1.5 mg/dl of Mg^{+2} in toxic plasma, the more reactive allosteric form be poisoned again by high charges of Magnesium ions as effect of Cd^{+2} ,Zn^{+2} .


Sampling Of individual toxic group
Sampling of individual normal group
Centrifuged& separation
Measure ChE
Measur e ChE
Mixing the plasma
Mixing the plasma
Measure ChE of collected plasma
Measure ChE of collected plasma
Treat the plasma with magnesium salt with different concentration
Incubate half hours 37°C
Measure ChE For treated
Measure ChE
For treated toxic plasma
Comparison of the measurements
Related to start point at no magnesium salts added

Conclusion

We concluded in vitro , magnesium ions can convert the allosteric form of Butyrylcholinesterase enzyme at certain concentration. By this way ,can use magnesium ions in activation of cholinesterase, in the case of temporary hypoenzyme (inhibition) , as poisoning by pesticides or nerve gases.

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