

METALLIC IONS AS NEGATIVE MODIFIERS OF CHOLINESTERASE

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Summary : Copper and Zinc have been found to be potent inhibitors of pseudo-cholinesterase of human plasma and true-cholinesterase of erythrocytes and rat brain.

Key words : cholinesterase copper zinc

INTRODUCTION

Several metallic ions have been described as positive and negative modifiers of various enzyme systems (2 to 8). It is further known that metals form a part of active site of enzyme and participate in catalysis directly (3 and 5). Additionally they are known to interact also with allosteric sites leading to modulation of enzyme activity (5 and 7). In some cases, metals are found to chelate some regulatory substances like amino acids and thus alter the activity of enzymes. In present study effect of copper and zinc on pseudo and true cholinesterase activity was studied in detail and it was found that the two ions are powerful inhibitors of cholinesterase enzyme.

MATERIALS AND METHODS

The enzyme activity was measured in Warburgs apparatus by method of Ammon (1), in terms of microlitres of carbondioxide liberated. Acetylcholine of 0.11 M and 0.165 M concentration was used as substrate for the measurement of pseudo and true cholinesterase activity respectively. Copper sulphate and Zinc sulphate solutions varying from 0.1 mM to 2.4 mM concentration was used for the study to find out their effect on enzyme activity. Human plasma and erythrocytes were separated out and the enzyme was further purified by the method of Nachman-sohn and Wilson (5) by ammonium sulphate precipitation. Rat brain homogenate was prepared in Phosphate buffer (pH 7.2) and was used to determine the effect of copper on enzyme activity. The enzyme samples were preincubated with metallic salts for 30 minutes. The readings in the Warburgs manometers were taken after 15,30 and 60 minutes intervals.

RESULTS AND DISCUSSION

The results are presented in Fig. 1 and Table I and II. The effect of copper ions on pseudo-cholinesterase of plasma and true cholinesterase activity of rats brain is presented in Fig. 1. Inhibitory effect of copper was found to be dose dependent while percentage of inhibition was greater in case of pseudo-cholinesterase of plasma as compared to true cholinesterase of rat brain.

TABLE I : Effect of different concentrations of Zn^{++} on cholinesterase activity of plasma. The sample is preincubated for 30 minutes with zinc sulphate. The figures are average of three sets of observations in each case. The results are expressed in terms of μL CO_2 liberated.

Zn^{++} mM	μL CO_2			Percent decrease		
	Period of incubation in minutes					
	15	30	60	15	30	60
Control	66	143	242	—	—	—
0.2	44	88	142	33.4	38.4	41.4
0.4	33	66	92	50.0	53.9	62.0
1.2	22	44	77	66.7	69.2	68.4
2.4	16	33	55	76.0	77.0	77.0

TABLE II : Effect of different concentrations of Zn^{++} on cholinesterase activity of erythrocytes. The sample is preincubated for 30 minutes with zinc sulphate. The figures are average of three sets of observations in each case. The activity is expressed in terms of μL CO_2 liberated.

Zn^{++} mM	μL CO_2			Percent decrease		
	Period of incubation in minutes					
	15	30	60	15	30	60
Control	63	99	220	—	—	—
0.1	44	65	138	30.1	34.5	37.4
0.2	33	55	132	47.5	44.5	40.0
1.2	16	33	77	74.5	66.5	65.0
2.4	11	22	44	82.5	77.5	80.0

It is evident from Table I that zinc has inhibitory effect on cholinesterase activity of plasma. The activity was markedly reduced in the concentration of 0.2 mM. When the concentration was raised to 2.4 mM the inhibition was observed to the extent of 76 percent during first fifteen minutes of incubation.

It was observed that zinc produces inhibitory effect on cholinesterase activity of erythrocytes. Even in a concentration of 0.1 mM, 30.1 percent decrease in enzyme activity during fifteen minutes of incubation was noted. When the concentration was raised to 1.2 mM the activity was reduced by more than sixty percent. Zinc was found to be more effective against the true cholinesterase of erythrocytes activity as compared to the effect on pseudocholinesterase of plasma.

The two metallic ions i.e. copper and zinc have been found to be potent inhibitors of both pseudo as well as true cholinesterase activity. Metals may act as modifiers of enzyme activity in several ways. It is difficult to assess the exact nature of inhibition. The possibility of

complex formation with some modifiers and allosteric modification of enzyme activity could be one of the mechanism suggested. Further work (unpublished data) revealed that histidine is capable of protecting the enzyme against the metallic inhibition.

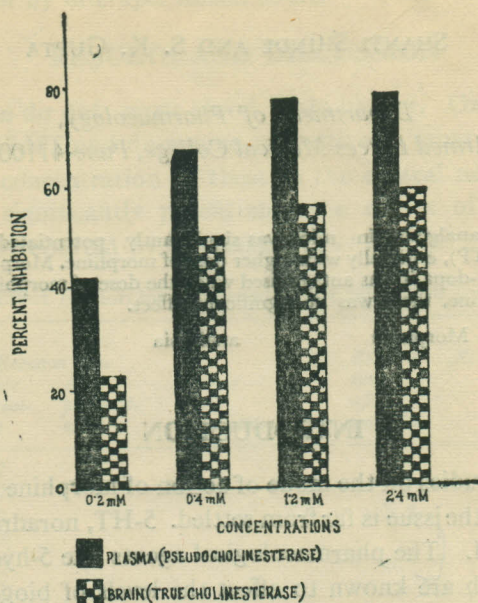


Fig. 1 : Effect of different concentrations of Cu^{++} on plasma and true cholinesterase activity of brain. The sample is preincubated for 30 minutes. The results are expressed in terms of percent inhibition.

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