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Aluminum Inhibition of Human Serum Cholinesterase

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Toxic effects of aluminum are a serious consideration for medical, industrial and environmental scientists. A variety of studies have suggested that aluminum may be sequestered from the environment and ingested via the food and water supply (CAMPBELL et al.; ONDREICKA et al., 1966; ABRAHAMSEN, 1976). Chronic ingestion of Al^{3+} by experimental animals has been reported to retard growth, concentrate Al^{3+} in certain organs (including brain), perturb phosphate balance, and induce fetal resorption (MAYOR et al., 1977; McCORMACK et al., 1979). The biochemical basis of Al^{3+} toxicity is not well-defined but may involve the proteins of cholinergic transmission, in particular the enzyme acetylcholinesterase (AChE) (PATOCKA, 1971; MILLER & LEVINE, 1974; YATES et al., 1980). We have shown previously that Al^{3+} is a noncompetitive inhibitor of membrane-bound AChE from eel electric organ and bovine caudate nucleus (MARQUIS & LERRICK, 1982; MARQUIS & BLACK, unpublished data). Preliminary data also suggest a relationship between increased Al^{3+} and decreased Ca^{2+} levels in brain tissue of experimental animals (MARQUIS, 1982) and a possible involvement of hyperparathyroidism in Al^{3+}-associated pathology (MAYOR et al., 1977). In dialysis patients, MAYOR et al. (1977) found that serum Al^{3+} and endogenous parathyroid hormone (PTH) concentrations were significantly correlated. It has been shown that Al^{3+} absorption from the gastrointestinal tract appears to be greatly enhanced by PTH, and that tissue distribution of the metal, including that to the brain, is increased by PTH (MAYOR et al., 1977), observations that may be particularly significant for patients with renal failure as they often show a compensatory rise in PTH levels as renal function declines (COOPER et al., 1978; COMMISSARIS et al., 1982). These data, and the suggestion that Al^{3+} may be a toxic agent in senile dementia of the Alzheimer type (SDAT), a disorder that appears to involve cholinergic neurotransmission (for a review, see BARTUS, 1982), led us to measure the effects of Al^{3+} in low and high Ca^{2+} media, on purified human serum cholinesterase (ChE; EC3.1.1.8). The data support the hypothesis that serum Ca^{2+} levels may be a limiting factor for Al^{3+} toxicity and suggest that Al^{3+} toxicity may be enhanced in disorders of Ca^{2+} mobilization and metabolism such as are likely to occur in parathyroid pathology or in aging, where PTH function is markedly decreased (KALU et al., 1982).

MATERIALS AND METHODS

For purification of ChE, citrated human plasma was obtained from whole blood that had been stored for 24-48 hours at 5°C. Fibrinogen was removed by adding 1 ml of 1 M $CaCl_2$ to each 100 ml of plasma and incubating at room temperature until a fibrin clot formed (about 2-3 hrs). The clot was removed with wooden applicator sticks and the serum centrifuged to remove residual precipitate. The serum was then dialyzed against 0.02 M Na acetate buffer, pH 4.0, until the pH was equilibrated, and precipitate was removed by centrifuging (DAS & LIDDELL, 1970; MUENSCH et al., 1976). A two-step purification method was used, resulting in a pure, homogenous enzyme preparation. In the first step, 75 ml of defibrinated, dialyzed plasma was chromatographed on a 150 ml column of DEAE-cellulose at pH 4.0, 5°C, and eluted with a linear salt gradient from 0.02 M to 0.2 M NaCl. The enzyme appeared between 0.04 M and 0.06 M NaCl in a small protein shoulder preceding the major peak. The peak enzyme fractions were pooled and purified to homogeneity by affinity chromatography on Concanavalin A-sepharose at 5°C. Typically, 5-10 ml of eluate was loaded onto a 10 ml column of affinity gel synthesized as per EDELMAN (1974). The enzyme-gel mixture was equilibrated 1/2 hour, then washed with 4 column volumes of 0.02 M NaAcetate buffer, pH 5.8. The wash fractions were reloaded onto the column, equilibrated 1/2 hour, and the column washed again with the same buffer. Ten ml 0.5 M α-methyl-D-mannoside (α-MM) in the same acetate buffer were loaded onto the column and equilibrated 1/2 hour. When 8 column volumes were eluted with 0.5 M α-MM, fractions #6 and #7 contained about 80% of the ChE activity. The ChE was pure by criteria of specific activity and evidence of a single protein band on polyacrylamide gel electrophoresis. Purification procedures and results are summarized in Table I.

TABLE I. Summary of Purification of Human Plasma Cholinesterase

FRACTION	PROTEIN (mg)	PROTEIN (%)	ChE ACTIVITY SPECIFIC	ChE ACTIVITY TOTAL UNITS	ChE ACTIVITY RECOVERED
SERUM	3,614	100	1.2	4,336	100%
DEAE-CELLULOSE	18	5	180	3,240	75%
CON A-SEPHAROSE	0.13	0.7	5,600	728	17%

This preparation was obtained from 75 ml of citrated defibrinated plasma. Protein was determined by measuring the absorbance at A_{280}. ChE activity is defined as the amount of enzyme required to hydrolyze 1 μmol of propionylthiocholine (PSCh)/hr. Specific activity is expressed as μmoles of PSCh hydrolyzed/hr/mg protein.

Protein content was measured by the method of LOWRY et al.(1951) or by measuring the absorbance at 280 nm. Total ChE activity was measured spectrophotometrically by a modification of the technique of ELLMAN et al.(1961). The enzymatic hydrolysis of 1 mM propionylthiocholine (PSCh) was measured by monitoring the increase in absorbance at 412 nm due to the reaction of thiocholine with 5,5-dithio-2-bis-nitrobenzoate in 2 mM Tris buffer, pH 7.0, with or without 0.15 M NaCl added. Although enzyme activity was enhanced in lower pH buffers, Tris buffer was chosen to optimize the solubility of Al chlorohydrate.

RESULTS AND DISCUSSION

The data in Table II present the $K_{m(app)}$ and V_{max} values for enzyme reactions in physiological ionic strength Tris buffer. $K_{m(app)}$ and V_{max} were determined from double-reciprocal plots of reaction rate as a function of PSCh concentration (0.02 mM to 0.5 mM), and the data were analyzed and statistical parameters calculated on a PDP 11/23, using a Fortran IV program of CLELAND (1967).

TABLE II. Effects of Calcium, Magnesium, and Aluminum on the V_{max} and $K_{m(app)}$ of Purified Human Serum ChE at Physiological Ionic Strength

SAMPLE	V_{max} (μmoles/hr/mg/prot.)	$K_{m[app]}$(mM)
Control	4389 ± 103	0.16 ± 0.10
1 mM Ca^{2+}	6093 ± 206	0.10 ± 0.01
100 μM Al^{3+}	2294 ± 83	0.29 ± 0.02
Control	773 ± 42	0.04 ± 0.007
1 mM Ca^{2+}	949 ± 35	0.05 ± 0.01
1 mM Mg^{2+}	750 ± 56	0.05 ± 0.01
Control	560 ± 31	0.06 ± 0.01
2 μM Al^{3+}	681 ± 58	0.07 ± 0.02
100 μM Al^{3+}	416 ± 32	0.08 ± 0.02
200 μM Al^{3+}	342 ± 13	0.08 ± 0.01
Control	474 ± 43	0.14 ± 0.07
10 μM Al^{3+}	392 ± 6	0.10 ± 0.03
10 μM Al^{3+} + 1 mM Ca^{2+}	589 ± 81	0.13 ± 0.05
25 μM Al^{3+}	384 ± 25	0.23 ± 0.06
25 μM Al^{3+} + 1 mM Ca^{2+}	495 ± 9	0.25 ± 0.10
25 μM Al^{3+} + 10 mM Ca^{2+}	471 ± 38	0.21 ± 0.04
25 μM Al^{3+} + 1 mM Mg^{2+}	317 ± 24	0.11 ± 0.02
25 μM Al^{3+} + 10 mM Mg^{2+}	379 ± 8	0.19 ± 0.10

Experiments were carried out on 4 different enzyme preparations in 2 mM Tris buffer, pH 7.0, with 0.15 M NaCl added. N=5 for each double-reciprocal plot. The mean ± the standard error of the mean are indicated for each set of experimental values.

The effects of Ca^{2+}, Mg^{2+} and Al^{3+} were assayed on four different enzyme preparations. While 1 mM Mg^{2+} had no effect on enzyme activity, 1 mM Ca^{2+} produced a noncompetitive, possibly "allosteric", increase in activity, i.e., unaccompanied by any alteration in substrate affinity. Al^{3+} is a noncompetitive inhibitor of serum ChE, in agreement with the results of similar assays on purified electric eel and bovine caudate nucleus AChE (MARQUIS & LERRICK, 1982; MARQUIS & BLACK, unpublished observations). 100 μM Al^{3+} reduced the V_{max} by 48% in the first, highly purified enzyme preparation and 26% in the second, less active, enzyme preparation. Further studies are needed to determine the significance of these differences.

The effects of multivalent cations on ChE activity were found to be fully reversed upon 100-fold dilution of pretreated enzyme protein into the substrate-reagent mixture. When ChE activity was measured in media of increasing ionic strength (0.2 to 500 mM), optimal activity was expressed at 2 mM with 20% less activity measurable at 0.15 M, the normal ionic strength of human serum. Nevertheless, experiments were carried out in buffer with 0.15 M NaCl added to approximate the physiological milieu of the enzyme.

Experiments with simultaneous addition of Ca^{2+} or Mg^{2+} and Al^{3+} to the enzyme assay medium demonstrated that 1-10 mM Ca^{2+} protects the ChE from inhibition by Al^{3+}, while Mg^{2+} has no measurable influence on the decreased V_{max} with concentrations of Al chlorohydrate of 10 μM or greater. The effect of Ca^{2+} may, thus, involve a specific Ca^{2+}-binding site on the enzyme rather than a generalized electrostatic phenomenon.

MARQUIS (1982) proposed that serum Ca^{2+} levels may influence the biochemical toxicity of Al^{3+}, and it has been shown by others that several factors may contribute to elevated tissue content of Al^{3+} and to the potential toxicity of the cation. It has been demonstrated that in both human patients and laboratory animals, plasma and brain Al^{3+} content may be elevated in subjects with dialysis dementia and as a consequence of renal failure, oral loading with Al, or abnormal permeability of the blood-brain barrier such as may occur in renal failure, metastatic cancer or hepatic encephalopathy (ALFREY et al., 1976; ARIEFF et al., 1979). In addition, in aluminum-loaded rats, PTH may act both to increase intestinal absorption of Al^{3+} and to increase tissue content of Al^{3+} in bone, cerebral cortex and muscle (MAYOR et al., 1977).

In light of these observations, several hypotheses are suggested by the results of these studies with serum ChE demonstrating that Ca^{2+} and Al^{3+} compete for interaction with a circulating plasma protein. First, elevated dietary Al^{3+} may reduce Ca^{2+} absorption and lower free circulating Ca^{2+} concentrations, producing an increase in PTH secretion and a clinical syndrome largely involving distrubed parathyroid function. Second, hyperparathyroidism, accompanied bydisturbed gastrointestinal absorption of Al^{3+}, may lead to deposition of Al^{3+} at Ca^{2+}-binding sites in

neural tissue and a toxicological syndrome resembling SDAT. Third, normal parathyroid function may be masked by elevated serum Al^{3+} levels, i.e., with Al^{3+} triggering a normal Ca^{2+} parathyroid response and "fooling" the gland into functioning as though elevated Ca^{2+} concentrations existed. Thus, the parathyroid may see Ca^{2+} plus Al^{3+} as total Ca^{2+}, resulting in a clinical condition similar to hypercalcemia. Finally, alteration of serum ChE kinetics, demonstrated here, may contribute directly or indirectly to the central cholinergic dysfunction associated with SDAT and may account in part for the frequent association of Al^{3+} with the etiology of the disorder. These hypotheses provide several lines of investigation for further study of Al^{3+} toxicity.

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