Bull. Environ. Contam. Toxicol. (1985) 34:451–458

Bulletin of Environmental Contamination and Toxicology

Investigations of the Interaction of Aluminum with Bovine Plasma Monoamine Oxidase

Y. Tsuzuki and J. K. Marquis

Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118

Aluminum has been implicated as a toxic agent in senile dementia of the Alzheimer type and may be a significant environmental health hazard. In 1982, Marquis and Lerrick demonstrated that Al^{3+} is a noncompetitive inhibitor of the enzyme acetylcholinesterase, and that Al^{3+} binds to a peripheral anionic site distinct from that which binds Ca^{2+} or decamethonium. Siegel and Haug (1983) recently demonstrated that Al^{3+} inhibits calmodulin-stimulated phosphodiesterase. Wenk and Stemmer (1981) have shown that the toxic effects of ingested Al^{3+} on catecholaminergic transmitters are enhanced in animals fed diets deficient in Cu^{2+}, Zn^{2+}, Fe^{2+} or Mg^{2+}. Recent studies with human serum cholinesterase (Marquis, 1983) suggest that Al^{3+} and Ca^{2+} interact with the same anionic site and that aluminum toxicity may, in fact, be provoked by a hypocalcemic condition, since the enzyme is protected from Al^{3+} inhibition in high Ca^{2+} media.

The present studies were designed to examine the interaction of Al^{3+} with the catecholamine-metabolizing enzyme, monoamine oxidase (MAO).

MATERIALS AND METHODS

Enzyme activity was assayed as per Weissbach et al. (1960), a procedure based on the rate of disappearance of kynuramine as substrate. The assay medium (total volume: 3.0ml) contained 2.0mM potassium phosphate buffer (pH 7.0) and 0.167mg enzyme protein/ml. The reaction was started by adding 0.033mM kynuramine and incubation was continued at 37°C for 30 mins. The activity was expressed as the change in absorbance at 360nm per unit (nmoles/mg protein) of time. K_{mapp} and V_{max} were determined from Lineweaver-Burke plots of reaction rate as a function of kynuramine concentration (0.017mM - 0.1mM). Low and high ionic strength experiments were carried out as follows: Low ionic strength experiments were run in 2mM K_2HPO_4 buffer (pH 7.0) with 2mM KCl or NaCl added; high ionic strength experiments were run in the same buffer with 200mM KCl or NaCl added.

In order to determine enzyme inhibition by Al^{3+} and iproniazid, the I-50, or concentration required to produce 50% inhibition of enzyme activity was measured at a substrate (kynuramine) concentration of 33μM. Inhibition by Al^{3+} or iproniazid was measured by preincubating the enzyme and inhibitor for 5 mins then adding substrate to start the reaction.

Absorbance measurements of tyrosine (280nm), pyridoxal-phosphate (390nm) and a cysteine-DTNB complex (412nm) were performed on a Zeiss spectrophotometer, model PM2D.

For carbodiimide modification of MAO, 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDAC) was prepared in 2mM K_2HPO_4 buffer, pH 7.0. Reversible and irreversible effects of EDAC on enzyme activity were determined as follows (Means & Feeney, 1971): to determine reversible effects, the reaction was carried out in 0.5mM EDAC, 2mM K_2HPO_4 buffer, pH 7.0 and 0.017mM - 0.1mM kynuramine; to determine irreversible effects, the enzyme was pretreated with 0.5mM EDAC and the rate of disappearance of kynuramine was assayed in the same reaction buffer as above but without EDAC.

Monoamine oxidase (monoamine O_2:oxidoreductase, EC 1.4.3.4, MAO), partially purified from bovine plasma, with optimal specific activity of 23-43μmoles benzaldehyde oxidized per min per mg protein, was purchased from Sigma Chemical Co. Kynuramine dihydrobromide, pyridoxal-5-phosphate, iproniazid phosphate, dithiothreitol (DTT), ethylenediamine tetracetic acid (EDTA) and 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDAC) were also purchased from Sigma Chemical Co., and aluminum chlorohydrate ($Al_2(OH)_5Cl$) was purchased from Pfaltz & Bauer, Stamford, CT. Cysteine hydrochloride and tyrosine were purchased from Fisher Scientific Co.

RESULTS AND DISCUSSION

The double-reciprocal plots in Fig. 1 demonstrate that MAO activity is enhanced by 3.3μM Al^{3+}, while at higher concentrations (0.1mM and 0.5mM) Al^{3+} is a noncompetitive enzyme inhibitor, producing a decrease in the V_{max} with no change in the K_m for kynuramine.

The effects of Al^{3+} on MAO activity were measured in both high and low ionic strength buffer. As shown in the data in Table 1, total enzyme activity is equivalent at 2mM and 200mM ionic strength, but activity is somewhat enhanced (10-20%) in the presence of K^+ relative to Na^+ ions. Table 1 also presents the concentrations for 50% inhibition of MAO and the V_{max} and $K_{m(app)}$ values determined from the double-reciprocal plots shown in Fig. 1. In low ionic strength buffer, with 2mM KCl or NaCl added, the I-50 for Al^{3+} inhibiti of MAO is 0.8mM and 1.3mM, respectively, while at high ionic strength with 200mM KCl and NaCl added the I-50 for Al^{3+} is increased to 1.4mM and 2.2mM, respectively.

Table 2 presents double-reciprocal analysis of the effects of Ca^{2+} and iproniazid on MAO activity and the effects of Al^{3+} in the presence of these ligands. While the hydrazine derivatives such as iproniazid are generally known as competitive inhibitors of MAO (Nagatsu, 1973), Ca^{2+} (600nM) has been reported to effect no changes in mitochondrial MAO activity in rat liver (Demisch, 1981). As seen in Table 2, iproniazid is indeed a competitive inhibitor of the enzyme, with a $K_i = 5.4 \times 10^{-7}$M, in the control buffer. However, in the presence of Al^{3+} iproniazid becomes a noncompetitive plasma MAO inhibitor. In addition, Ca^{2+} increased enzyme activity

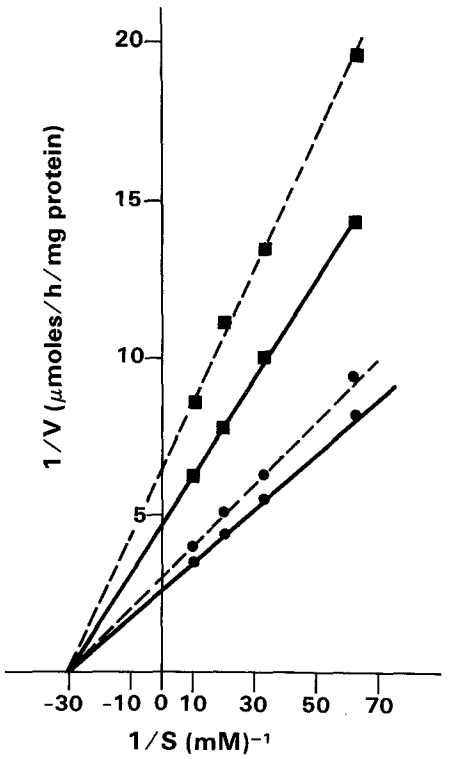

Figure 1. Double-reciprocal plots of enzyme activity and substrate concentration demonstrate the effects of different concentrations of Al^{3+} on the MAO reaction rate in the presence of varying concentrations (0.17mM - 0.1mM) of kynuramine. Control: - -●- -●- - 3.3μM Al^{3+}: —●—●— 0.1mM Al^{3+}: —■—■— 0.5mM Al^{3+}: - -■- -■- -

in the absence of Al^{3+}, with no effect on substrate affinity, but in the presence of Al^{3+}, calcium becomes a competitive MAO inhibitor. In 2mM K_2HPO_4 buffer, pH 7.0 with no NaCl or KCl added, the I-50 for Al^{3+} is 0.46mM with 95% inhibition at 2mM, while the I-50 for MAO inhibition by iproniazid is 0.066mM in the same buffer.

In order to at least partially elucidate the mechanism of action of Al^{3+} effects on plasma MAO, complexes of the cation with tyrosine, pyridoxal phosphate and cysteine were assayed spectrophotometrically. As seen in Table 3, the absorbances of tyrosine at 280nm, pyridoxal phosphate at 390nm and cysteine-DTNB at 412nm were decreased 13.7%, 15% and 20.7%, respectively, by addition of 1mM Al^{3+}.

The data in Table 4 demonstrate that enzyme inhibition by Al^{3+} can be completely reversed by 5mM EDTA, while the sulfhydryl (SH) reagents, cysteine and DTT, partially reverse enzyme inhibition by

Table 1. Effects of aluminum on the K_{Mapp} and V_{Max} of MAO from bovine plasma in low and high ionic strength media

A. Low Ionic Strength Experiments: The enzyme reactions were carried out in 2mM K_2HPO_4 buffer, pH 7.0, 2mM KCl or NaCl, 0.167 mg protein/ml and 0.017 - 0.1mM kynuramine.

SAMPLE	K_{Mapp} (μM)	V_{Max} (nmoles/h/mg prot.)	I_{50} (mM)
KCl (2mM)	27.0 ± 0.5	400.0 ± 14.9	
+0.5mM Al^{3+}	26.3 ± 0.7	250.0 ± 7.6	
+1.3mM Al^{3+}	26.0 ± 2.0	279.1 ± 1.2	0.8 ± 0.1
NaCl (2mM)	32.3 ± 1.4	333.3 ± 4.4	
+0.5mM Al^{3+}	32.3 ± 1.5	277.8 ± 7.9	
+1.3mM Al^{3+}	31.8 ± 1.1	166.7 ± 2.6	1.3 ± 0.1

B. High Ionic Strength Experiments: The enzyme reactions were carried out as in A. above but with 200mM KCl or NaCl added.

KCl (200mM)	25.0 ± 0.9	396.0 ± 13.2	
+0.5mM Al^{3+}	24.4 ± 0.7	344.8 ± 4.7	
+1.3mM Al^{3+}	24.1 ± 0.4	206.2 ± 8.6	1.4 ± 0.2
NaCl (200mM)	29.0 ± 3.8	350.9 ± 7.5	
+0.5mM Al^{3+}	28.5 ± 3.7	322.6 ± 1.3	
+1.3mM Al^{3+}	28.2 ± 2.8	243.9 ± 3.2	2.2 ± 0.1

Experimental data are expressed as the mean ± standard error of between 3 and 5 kinetic assays.

0.5mM Al^{3+}. As spectrophotometric assays indicate that Al^{3+} also binds to cysteine in such a manner as to reduce SH interaction with DTNB, it is suggested that Al^{3+} may interact directly with SH groups on the MAO molecule or that SH groups are situated in close proximity to the Al^{3+}-binding sites.

Earlier studies on the interaction of Al^{3+} with the enzyme acetylcholinesterase (AChE) demonstrated that pretreatment of the enzyme with chemical modifying reagents selective for carboxyl groups can block the interaction of aluminum ions with peripheral anionic sites (Marquis & Black, 1984). It was, thus, of interest to determine whether the carbodiimides would similarly aid in

TABLE 2. Effects of calcium and iproniazid on MAO inhibition by aluminum

	SAMPLE	K_{Mapp} (μM)	V_{Max} (nmoles/h/mg prot.)	K_i
A.	Control	33.4 ± 1.8	333.4 ± 6.0	
	+5mM Ca^{2+}	55.6 ± 4.7	476.2 ± 2.2	
	+0.01mM Iproniazid	50.0 ± 7.0	333.4 ± 9.8	0.54 ± 0.02uM
B.	0.5mM Al^{3+}	33.4 ± 1.5	152.9 ± 3.7	0.42 ± 0.01mM
	+5mM Ca^{2+}	56.2 ± 2.3	303.0 ± 7.0	
	+0.01mM Iproniazid	38.5 ± 5.0	107.0 ± 0.1	

Experimental data are expressed as the mean ± standard error of between 2 and 3 kinetic assays in 2mM K_2HPO_4 buffer, pH 7.0.

TABLE 3. Interactions of aluminum with tyrosine, pyridoxal phosphate and cysteine

SAMPLE	Tyrosine (280nm)	Pyridoxal Phosphate (390nm)	Cys-DTNB (412nm)
Control	0.809 ± 0.001	0.972 ± 0.005	1.350 ± 0.020
1.0mM Al^{3+}	0.698 ± 0.003	0.826 ± 0.010	1.070 ± 0.030
% - Control	86.3 ± 2.5	85.0 ± 2.7	79.3 ± 2.0

Experimental data are expressed as the mean ± standard error of between 3 and 4 assays in 2mM K_2HPO_4 buffer, pH 7.0.

identifying at least one subgroup of Al^{3+} binding sites on MAO. The results of these experiments are shown in Table 5. The water-soluble carboxyl group affinity reagent, EDAC, exerts a small but significant effect on the catalytic activity of MAO.

TABLE 4. Effects of EDTA, CYS, and DTT on MAO inhibition by aluminum

SAMPLE	ENZYME ACTIVITY (nmoles/h/mg prot.)	% - CONTROL
A. Control	159 ± 1	100
+5mM EDTA	140 ± 2	88.1 ± 4.2
+5mM CYS	160 ± 4	103.1 ± 2.8
+5mM DTT	160 ± 3	100.6 ± 1.7
B. 0.5mM Al^{3+}	84 ± 2	53.2 ± 1.8
+5mM EDTA	142 ± 2	101.4 ± 1.6
+5mM CYS	112 ± 3	68.3 ± 2.2
+5mM DTT	138 ± 2	86.3 ± 3.4

Experimental data are expressed as the mean ± standard error of between 3 and 4 assays in 2mM K_2HPO_4 buffer, pH 7.0.
Abbreviations: EDTA = ethylenediamine tetracetic acid; CYS = cysteine; DTT = dithiothreitol.

When assayed with increasing substrate concentrations (0.017mM - 0.1mM), in the presence of a fixed EDAC concentration, reversible competitive inhibition of MAO is evident. In addition, the EDAC treated enzyme is not activated by 3.3μM Al^{3+}. The inhibition of EDAC modified enzyme by low and high concentrations of Al^{3+} is competitive inhibition.

In summary, these data demonstrate that Al^{3+} is a reversible, noncompetitive inhibitor of the catecholamine-metabolizing enzyme, MAO. Unlike the Al^{3+}-serum cholinesterase interaction described previously (Marquis, 1983), Al^{3+} and Ca^{2+} appear to interact with separate binding sites on bovine plasma MAO. Siegel et al. (1983) have also reported that aluminum ions differ appreciably from calcium ions in their interactions with calmodulin. Previous studies demonstrated similar independence of Al^{3+} and Ca^{2+} actions on the membrane bound AChE from electric organ of Electrophorus electricus (Marquis & Lerrick, 1982), while chemical modification studies with purified bovine caudate AChE indicated that Al^{3+} and Ca^{2+} bind, at least in part, to the same sites on that enzyme (Marquis & Black, 1984)

TABLE 5. Carbodiimide modification of binding site reactivity of bovine plasma MAO

A. Reversible Effects: The enzyme reactions were carried out in the presence of 0.5mM EDAC*, 2mM K_2HPO_4 buffer, pH 7.0, with 0.017 - 0.1mM kynuramine.

SAMPLE	K_{Mapp} (μM)	V_{Max} (nmoles/h/mg prot.)
Control	58.5 ± 1.2	227.3 ± 2.8
0.05mM EDAC	52.6 ± 1.4	277.8 ± 2.4
0.1mM EDAC	37.7 ± 0.9	256.4 ± 8.3

B. Irreversible Effects: Enzyme was pretreated with 0.5mM EDAC* and the rate of disappearance of kynuramine (0.017 - 0.1mM) was determined in the same buffer as above but without EDAC.

Control (no EDAC)	33.4 ± 1.8	333.4 ± 6.0
+0.5mM EDAC	69.0 ± 1.2	333.4 ± 3.5
+3.3μM Al^{3+}	33.4 ± 0.9	384.6 ± 2.6
Control +0.5mM EDAC	60.6 ± 0.4	238.1 ± 0.9
+3.3μM Al^{3+}	66.7 ± 3.0	232.6 ± 1.8
+0.1mM Al^{3+}	111.1 ± 10.9	227.3 ± 2.9
+0.5mM Al^{3+}	222.2 ± 3.2	222.2 ± 4.0

*EDAC = 1-ethyl-3(3-dimethylaminopropyl) carbodiimide

Experimental data are expressed as the mean ± standard error of between 2 and 4 kinetic assays.

The binding sites for Al^{3+} on bovine plasma MAO appear to be distinct from the substrate binding site, as evidenced by the noncompetitive nature of the inhibition, but Al^{3+} exerts an effect on the enzyme active site that indirectly influences both substrate oxidation and interaction of the competitive inhibitor iproniazid. Carboxyl-group modification with a carbodiimide reagent eliminates Al^{3+} interaction at peripheral or non-substrate binding sites in a manner similar to that observed for Al^{3+} interaction (Marquis & Black, 1984). It is proposed that aluminum neurotoxicity *in vivo* may result at least in part from specific effects on neurotransmitter metabolizing enzymes. Preliminary data suggest that Al^{3+} exerts little or no effect *in vitro* on the synthetic enzymes, choline acetyltransferase and tyrosine hydroxylase (Tsuzuki & Marquis, unpublished data). The neurotoxicity

of this multivalent cation may, thus, involve primarily regulatory interactions of postsynaptic enzyme proteins.

Acknowledgements. This work was supported in part by a grant from the U.S. Army Research Office (#DAAG-29-82-0042).

REFERENCES

Demisch L (1981) Short-term alteration of MAO activity after translocation of calcium in human blood platelets. Life Sci 28: 1995-2002

Marquis JK (1983) Aluminum inhibition of human serum cholinesterase. Bull Environment Contamin & Toxicol 31: 164-169

Marquis JK, Black EE (1984) Aluminum activation and inactivation of bovine caudate acetylcholinesterase. Bull Environment Contamin Toxicol In Press

Marquis JK, Lerrick AJ (1982) Noncompetitive inhibition by aluminum, scandium and yttrium of acetylcholinesterase from Electrophorus electricus. Biochem Pharmacol 31: 1437-1440

Means GE, Feeney RE (1971) Chemical Modification of Proteins. Holden-Day, Inc., San Francisco, CA pp 144-148

Nagatsu T (1973) Biochemistry of catecholamines. Univ. of Tokyo Press, Tokyo, p. 130.

Siegel N, Coughlin R, Haug A (1983) A thermodynamic and electron paramagnetic resonance study of structural changes in calmodulin induced by aluminum binding. Biochem Biophys Res Commun 115: 512-517

Siegel N, Haug A (1983) Aluminum interaction with calmodulin. Evidence for altered structure and function from optical and enzymatic studies. Biochim Biophys Acta 744: 36-45

Weissbach H, Smith TE, Daly JW, Witkop B, Udenfriend S (1960) A rapid spectrophotometric assay of monoamine oxidase based on the rate of disappearance of kynuramine. J Biol Chem 235: 1160-1163

Wenk GL, Stemmer KL (1981) The influence of ingested aluminum upon norepinephrine and dopamine levels in the rat brain. Neurotoxicol 2: 347-353

Received May 7, 1984; accepted May 25, 1984