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Aluminum Neurotoxicity: An Experimental Perspective

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Aluminum neurotoxicity represents a serious consideration for a number of 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. 1957; MAYER & ULLRICH 1976; ABRAHAMSON et al. 1976). As it has been demonstrated that Al^{3+} is not lost once it has been taken up into brain tissue (DEBONI et al. 1974; CRAPPER 1976), continued exposure may lead to a gradual increase in the brain burden of this metal cation.

The neurotoxicity of Al^{3+} was first established when Dölken (1877) injected aluminum tartrate into rabbits and found that they developed neuronal degeneration in different parts of the brain. Changes in the CNS following systemic administration of Al^{3+} salts were described by SEIBERT & WELLS (1929), and the epileptogenic effect of aluminum hydroxide paste was described by KOPELOFF et al. (1942). More recently, aluminum has been implicated as a toxic agent in senile dementia of the Alzheimer type (CRAPPER et al. 1973, 1976; PERL & BRODY 1980; HETNARSKI et al. 1980); it is a problem of therapeutic importance for dialysis patients receiving aluminum hydroxide gels (ALFREY et al. 1976); and the antiperspirant action of aluminum salts remains a subject of several opposing hypotheses to explain the mechanism of their drying action (HÖLZLE & KLIGMAN 1979). Furthermore, the neurotoxicity of aluminum is of particular importance to individuals in geographical regions where soluble Al^{3+} salts are believed to be leached out of the soil by acidic rain conditions resulting from atmospheric pollutants (JOHNSON 1979; CRONAN & SCHOFIELD 1979). Recent studies by YATES et al. (1980) demonstrate that intracisternal injection of aluminum salts in rabbits produced altered levels of cholinergic enzyme activity in selected nuclei of the brain.

Our laboratory has carried out a number of preliminary experimental studies on the neurotoxicity of aluminum chlorohydrate [$Al_2(OH)_5Cl \cdot 2H_2O$] and demonstrated significant alterations in several biochemical parameters of the nervous system, particularly the cholinergic neurotransmission system. These results are reviewed below in synopsis form and discussed subsequently with particular emphasis on the apparent necessity for a comprehensive study of the neurotoxicity of aluminum salts.

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1. Noncompetitive Inhibition of Purified Acetylcholinesterase by Aluminum Chlorohydrate:

Measurements of altered activity of soluble acetylcholinesterase from eel (E. electricus) electric organ by the inorganic cations aluminum, scandium and yttrium demonstrated that these ions are noncompetitive enzyme inhibitors. Al^{3+} inhibited enzyme activity at all substrate and inhibitor concentrations studied. Inhibition by Al^{3+} was not sensitive to the active site-specific, competitive ligand physostigmine or to calcium, a peripheral site-binding activator cation. Inhibition by another peripheral site-binding noncompetitive inhibitor, decamethonium, was not altered by Al^{3+}. Aluminum appears thus to interact with a distinct class of peripheral anionic sites on AChE. Recent studies by TEAGARDEN et al. (1981) on the physicochemical properties of Al chlorohydrate confirm our suggestion that the large predominantly polycationic species in this salt may interact with the γ-peripheral sites, thought to be highly hydrophobic sites (ROUFOGALIS & QUIST 1972) on AChE. These studies, discussed in detail in a recent publication (MARQUIS & LERRICK 1982), suggest a possible mechanism for Al^{3+} neurotoxicity via alterations of the major postsynaptic enzymes of cholinergic neurotransmission.

2. The Effects of Environmental Aluminum on the North American Bullfrog

This study was designed to examine the possible relationships between the effects of soluble aluminum salts and acidic pond water conditions on the cholinergic enzymes, choline acetyltransferase (ChAc) and AChE in brain homogenates of amphibians. North American bullfrogs (Rana catesbeiana) weighing between 100 and 300 g, were raised in pond water at pH 4.6, 5.6, and 6.6 containing aluminum chlorohydrate at 5×10^{-6}M and 5×10^{-5}M, and in a control environment, pH 5.6, without added aluminum.

The pH conditions were chosen to represent the normal acidity of rainwater, usually well below neutral because of atmospheric carbonic acid-producing gases, and one pH unit on either side of the control. The Al^{3+} levels were chosen on the basis of solubility limits and published concentrations measured in the acidified lakes of the Adirondack Mountains (SCHOFIELD 1978).

For the quantitative ion analyses and enzyme studies, the animals were sacrified by decapitation, the brains removed on ice, suspended in 116mM Tris buffer, pH 7.3 (0.2 mg wet weight/ml) and homogenized with a teflon/glass homogenizer. The homogenate was used for ChAc and AChE assays and for quantitative analysis of Na^+, K^+ and Ca^{2+} by ion-specific electrodes and elemental Al by atomic absorption spectroscopy of nitric acid digests of the tissue homogenates (YATES et al. 1980). In vitro studies in which Al^{3+} was added directly to the brain tissue homogenates (pH 7.3) of unexposed bullfrogs, were compared to the in vivo treatments. Protein determinations

were carried out by the method of LOWRY et al. (1951); AChE was assayed by the pH-stat method (MARQUIS & WEBB 1974); and ChAc by the method of FONNUM (1975).

TABLE 1

Acetylcholinesterase activity in brain tissue homogenates of bull frogs raised in bathing environments of varied pH and Al chlorohydrate concentrations

pH	BATH Al^{3+}(M)	AChE Activity (μmols/hr/mg protein)	
In Vivo Studies			
4.6	0	8.2 ± 1.3	(6)
	5×10^{-6}	7.7 ± 0.8	(2)
	5×10^{-5}	8.5 ± 0.2	(3)
5.6	0	13.2 ± 0.4	(2)
	5×10^{-6}	13.1 ± 0.9	(2)
	5×10^{-5}	9.3 ± 0.5	(2)
6.6	0	5.2 ± 0.7	(3)
	5×10^{-6}	6.0 ± 0.5	(3)
	5×10^{-5}	7.4 ± 0.4	(3)
In Vitro Studies			
7.3	0	8.5 ± 0.1	(2)
	5×10^{-6}	7.7 ± 0.6	(3)
	5×10^{-5}	10.0 ± 0.2	(2)

(n) = number of frog brains assayed

Non-paired t-tests indicate significant changes in enzyme activity, $p < 0.05$, in 5×10^{-5}M Al^{3+}, *in vitro*, as well as in the *in vivo* studies at pH 5.6 and pH 6.6, 5×10^{-5}M Al^{3+}, $p < 0.02$.

The Al^{3+}-enriched pond water environment produced no measurable changes in the levels of ChAc in the brain tissue of homogenates in either the *in vivo* or *in vitro* treatments. Brain AChE activity, however, was altered in a pH-dependent and concentration-dependent fashion. The results are summarized in Table 1. The animals raised at pH 4.6 showed generally very poor health and survived only 7-10 days. Nonetheless, brain AChE levels in these animals were not altered by the presence of Al^{3+} in the bathing

environment. Exposure to pH 5.6 and 6.6 did not significantly reduce the viability of the animals. At either pH level, Al^{3+} did not alter the level of CNS AChE activity. Exposure to 5 x 10^{-5}M aluminum chlorohydrate at pH 5.6 resulted in a 25% decrease in AChE activity, while exposure to this higher dose at pH 6.6 resulted in a slight elevation of enzyme activity over the unexposed controls. Notably, control enzyme levels were markedly lower at the higher pH.

TABLE 2

Quantitative analysis of ionic calcium and elemental aluminum in brain tissue homogenates of bullfrogs maintained in bathing environments with varied pH and Al chlorohydrate concentrations

pH	Bath Al^{3+}(M)	Brain Ca^{2+} (µg/g wet wgt.) Week 1	Week 4	Week 7	Brain Al (ng/g wet wgt.) Week 1	Week 4	Week 7
5.6	0	433	185	70	3	70	41
	5 x 10^{-6}	ND	971	60	113	442	ND
	5 x 10^{-5}	ND	260	100	ND	ND	138
6.6	0	455	ND	217	ND	ND	158
	5 x 10^{-6}	250	60	73	6	5	256
	5 x 10^{-5}						

ND = not determined

Quantitative analyses of ionic Ca^{2+} and elemental Al are summarized in Table 2. Each data point represents a single frog brain. While the concentrations of Al in the total brain appeared to increase with time of exposure to Al chlorohydrate, the concentrations of Ca^{2+} apparently decreased. Preliminary micrographs indicate that the brain burden of Al can be visualized by the fluorescent Morin stain (DEBONI et al. 1976) in these animals. Although too few animals and too few replicate measurements are available to indicate specific toxic effects of aluminum in these studies, the preliminary data are sufficiently suggestive to warrant a more extensive study.

3. Brain Aluminum and AChE in Adult Rats and Newborn Offspring Raised on Aluminum-Enriched Drinking Water

Studies were carried out on both adult and newborn rats to determine whether there is accumulation of Al and alterations of AChE activity in the brain of rats exposed to the cation via dietary intake as well as in the nursing offspring of these animals.

A colony of adult white rats (Charles River, CD, Sprague-Dawley derived) was fed ad lib standard lab chow (Agway RMH 3000) and ad lib drinking water. Half the animals drank normal filtered tap water (<1ppm Al) and half drank only tap water containing 0.06% (6.2mM) or 0.12% (12.4mM) Al chlorohydrate (Aldrich Chem. Co.).

TABLE 3

Quantitative analysis of elemental aluminum acetylcholinesterase activity in total brain tissue of newborn rats

Age (days)	$[Al^{3+}]$ maternal drinking water		Brain Al (total µg)	Protein+ (total mg in supernatant)	Brain Al (µg/mg protein)	AChE+ (mmols x 10^{-4}/ hr/mg protein in supernatant)	
8	0 (<1ppm)	(8)	3.0±.3	30.2±2.4	0.10	6.37±0.34	(8)
	0.06%	(2)	1.1±.1	26.8±6.0*	0.04	4.74±0.42	(6)
	0.12%	(3)	1.7±.1	24.7±6.7*	0.07	3.46±0.60	(6)
15	0 (<1ppm)	(8)	5.8±.9	53.4±1.6	0.11	5.90±0.31	(8)
	0.06%	(2)	3.6±.08	52.8±4.6	0.07	3.30±0.30	(5)
	0.12%	(6)	2.0±.15	57.2±3.4	0.04	4.10±0.29	(12)
22	0 (<1ppm)	(7)	3.7±.63	62.0±2.2	0.06	6.50±0.38	(12)
	0.06%	(3)	2.5±.14	74.4±5.6	0.03	3.30±0.25	(7)
	0.12%	(6)	3.0±.3	77.4±10.8	0.04	3.90±0.32	(12)

+Proteins and enzyme activity were determined on a 10,000 x g supernatant of the total brain homogenate.
*Due to a loss of samples, proteins were determined in duplicate on only 2 samples.
(n) = number of samples assayed.
ANOVA on brain Al levels = no significant differences
ANOVA on brain AChE levels = $F_{age} = 0.37$ (N.S.); $F_{dose} = 15.93$*

Animals on similar diets were mated and offspring were sacrificed at 8, 15 and 22 days of age. AChE activity and elemental Al were analyzed in whole brain homogenates using the same procedures described above for the frog brain studies, except that the enzyme and protein assays were done on a 10,000 x g supernatant of the whole brain homogenate. The data are summarized in Table 3. Analysis of variance on these data indicate no significant differences in the brain Al levels as a function of age

or maternal drinking water composition. There are also no significant differences in the brain AChE activity simply as a function of age; but there is a marked decrease in AChE activity within each age group as a function of maternal exposure to Al. It is not clear whether the altered enzyme levels can be correlated directly with exposure to Al or with altered developmental processes in these rat pups.

TABLE 4

Analysis of elemental aluminum and acetylcholinesterase activity in total brain tissue of adult rats maintained for 28 days with drinking water containing background levels, 0.06% or 0.12% aluminum chlorohydrate

$[Al^{3+}]$ of drinking water	Brain Al (total μg)		AChE (mmols x 10^{-4}/hr/mg protein in supernatant)+	
0 (<1ppm)	3.1	(1)	6.73 ± 0.09	(2)
0.06%	5.1±0.2	(3)	7.27 ± 0.17	(2)
0.12%	6.7	(1)	8.60 ± 0.49	(4)

+Proteins and enzyme activity were determined on a 10,000 x g supernatant of the total brain homogenate
(n) = number of brains assayed.

Similar analyses were carried out on brain tissue from adult rats maintained for 28 days with drinking water containing background levels (<1ppm), 0.06% or 0.12% Al chlorohydrate. These data are summarized in Table 4 and suggest a significant increase in both total brain Al and AChE activity with increased consumption of Al chlorohydrate. Also, the Al-drinking adults consumed, on the average, the same volume of water as did the controls. It is thus likely that Al can accumulate in the mammalian brain via dietary exposure and that this accumulation may result in a significant alteration of cholinergic neurotransmission.

The need for more comprehensive studies of aluminum neurotoxicity is strongly indicated by the results presented above. The effects of dietary exposure to Al salts should be examined in adult mammals, pregnant females, and their offspring. Al should be analysed in fetal and placental tissue, as well as in specific brain regions of the animals. These studies also suggest the need to carry out behavioral analyses for short term memory function in adults and for the development of motor and learning functions in the offspring. In addition to the enzymes of cholinergic neurotransmission, a variety of physiological ion levels should be monitored as well as renal, cardiac, and, in particular, parathyroid functions.

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