1044-7393/92/1701—0079 $02.20

Aluminum-Induced Acute Cholinergic Neurotoxicity in Rat

JENG-HSIUNG F. PENG,*[,1] ZHI-CHUNG XU,[2] ZHI-XIN XU,[2] JOSEPH C. PARKER, JR.,[1] EDWARD R. FRIEDLANDER,[1] JIAN-PING TANG,[2] AND SRIKUMARAN MELETHIL[2]

University of Missouri–Kansas City, Department of Pathology, School of Medicine,[1] and School of Pharmacy,[2] Kansas City, MO 64108, USA

Received July 24, 1991; Accepted December 16, 1991

ABSTRACT

In the present study the acute effect of intravenous aluminum chloride (1 mg/kg) on choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities of rats was investigated. Aluminum was found to cross the blood-brain barrier (BBB) as indicated by the detection of aluminum in the cerebrospinal fluid (CSF) 30 min after femoral vein injection. Two hours following aluminum injection, ChAT activity in the basal forebrain and hippocampus was significantly reduced by 30% and 22%, respectively, whereas no change was observed in the caudate nuclei. On the other hand, AChE activity was significantly increased by 45% in the caudate nuclei, whereas little change was observed in other brain areas. This report demonstrates that rapid transport of Al across the BBB, and the acute nature of Al neurotoxicity in rats.

Index Entries: Aluminum transport; blood-brain barrier; cerebrospinal fluid aluminum; acute aluminum effect; cholinergic neurotoxicity; neurofibrillary degeneration; choline acetyltransferase; acetylcholinesterase.

INTRODUCTION

Aluminum (Al) has long been considered a neurotoxin owing to its link to many human diseases, such as dialysis encephalopathy and anaemia

*Author to whom all correspondence and reprint requests should be addressed.

(Alfrey et al., 1976). Al is one of the most ubiquitous elements in our environment, and humans have constantly been exposed to this element by the use of Al-containing utensils, foods, antiperspirants, and drugs (such as antacids). Al, which has been implicated in the etiology of Alzheimer's disease (AD) and other neurodegenerative disorders (Crapper McLachlan and De Boni, 1980; Perl and Brody, 1980), has been found to accumulate in the brains of AD patients (Perl and Good, 1990) and experimental animals (Gulya et al., 1990). Recently, investigators have further noted the elevation of Al in neuronal nuclei, neurofibrillary tangles, and senile plaques in the AD brain (Candy et al., 1984; Crapper McLachlan et al., 1989). The presence of ChAT has been demonstrated in senile plaques (Kitt et al., 1980; Armstrong et al., 1986). However, there is no agreement on whether Al is present in senile plaques and neurofibrillary tangles (Perl and Brody, 1980; Davis and Wolozin, 1987; Jacobs et al., 1989; Chafi et al., 1991).

Even though the role of Al in many neurodegenerative diseases is still uncertain (McDermitt et al., 1979; Markesbery et al., 1981), the mechanisms of Al accumulation in AD and experimental mammalian brains and the cholinergic dysfunction have continued to receive much attention. Attempts have been made to develop an animal model of AD that could mimic the neuropathological changes and cholinergic deficit in the AD brain using Al as a cholinergic neurotoxin (Kosik et al., 1983; Wisniewski et al., 1990). Results have, however, been conflicting. Al was noted to cause neurofibrillary degeneration in animals (DeBoni et al., 1976; Hetnarski et al., 1980), but some investigators reported Al had no effect on ChAT and AChE activities in the brain and spinal cord (Hetnarski et al., 1980; Johnson and Jope, 1987). Many other reports, however, favored Al causing reduction of cholinergic enzyme activity (Yates et al., 1980; Kosik et al., 1983; Boegman and Bates, 1984; Gulya et al., 1990). These data were obtained after chronic treatment of animals with Al administered through oral, intracisternal, intraventricular, and intraperitoneal routes. Since biochemical alterations usually precede morphological manifestations in most diseases, we sought to study the effect of Al on cholinergic enzyme activity during the early stage of Al treatment. This communication reports our findings on a time-course study of Al accumulation in the CSF, and the effect of this metal on cholinergic enzymes in the serum and various areas of the brain following a single Al dose.

MATERIALS AND METHODS

Animal Preparation

Sprague-Dawley rats ($n = 7$) weighing 250–300 g were housed in stainless steel wire-bottom cages in rooms maintained at 24°C with a 12 h light 12 h dark cycle. Rats were fed commercial rat chow and tap water ad libi-

tum. Animals were allowed an acclimatization period of 4 d or more prior to the experiment. Detailed surgical procedure is described as follows. Rats were kept anesthetized with pentobarbital (50 mg/kg, ip) and maintained at 37°C. With the animal on its back, the left femoral artery (for blood collection) and vein (for Al administration) were cannulated with silicone tubing (0.012 in. id by 0.025 in. od) containing heparin (100 U/mL). Then the animal was placed on its stomach and the skull was exposed. A small hole was then drilled by hand using a dental bur on the sagittal midline immediately rostral to the interparietal-occipital bone structure. A 5-cm-long cannula (PE-50) tubing was gently placed in the cisterna magna to a depth of 0.6–0.8 cm. The skull was cleaned of any escaping CSF via the gap at the cannula/skull interface and the gap sealed with epoxy glue (Krazy glue, Krazy Glue Inc., Ithaca, IL). Then the animal was placed on its back again, and CSF flow via the cannula aided by gravity. Following injection of Al via the left femoral vein, a 50:50 mixture of normal saline and 5% glucose was infused (2 mL/h) for the first 2 h and then reduced to 1 mL/h. This infusion was found necessary to permit survival of the animal over the duration of the experiment (8–12 h), and also to maintain normal hematocrit (42–46%) and blood pressure. CSF was collected over a 2-h period. Al at a dose of 1.0 mg/kg as the chloride salt ($AlCl_3$) was dissolved in saline, and a total volume of 250–300 μL (pH 3.84) was injected as bolus (<5 s). Continuous CSF samples were collected at predose (1 h) and hourly intervals for a 4-h period. Usually, 80–100 μL/h of CSF were collected, and 30 min is the midpoint of the collection interval. CSF samples were immediately stored at −20°C until analysis.

In a separate study on the effect of Al on ChAT and AChE activities, one group of animals ($n=4$) each received 1 mg/kg Al as the chloride salt administered via the tail vein, and the control group received saline as sham treatment. Cumulative collections of 0–2 h CSF sample were undertaken. Blood samples were collected before and 2 h after Al administration for determination of serum AChE levels. Two hours after Al administration, animals were sacrificed, their brains removed, and various areas of the brain were dissected on ice.

Determination of Al Levels

Al levels in CSF were determined using a flameless atomic absorption spectrophotometer (AAS) according to the procedures described by Xu et al. (1991). Al-free plasticware, which was cleaned with 5% ultrapure aqueous nitric acid (v/v), 0.6% sodium EDTA (v/v), and Al-free deionized water (to avoid sample contamination) were used. The atomic absorption system consisted of a spectrophotometer (model 3030), graphite furnace (HGA 400) with pyrocoated tubes and L'vov platforms, autosample (AS40), and a graphics printer (PR 100). Measurements were made at 309.3 nm employing a hollow cathode lamp. Furnace conditions were dried at 100°C

for 25 s with a temperature ramp of 5 s; charring at 1100°C for 25 s with a temperature ramp of 20 s; and atomization of 2400°C for 4 s in the maximum heating mode.

Preparation of Enzyme Samples

For determination of cholinergic enzymes, rat brain tissues were homogenized for 1 min in 10 vol (w/v) of 0.32*M* sucrose solution containing 10 m*M* potassium phosphate (KPO_4) buffer, pH 7.4 and 2 m*M* EDTA using Omni mixer homogenizer. The homogenate was diluted fivefold with 10 m*M* KPO_4 buffer, pH 7.4 containing 1% Triton X-100 (final conc.) and left on ice for at least 1 h to solubilize the membrane bound enzymes before assay for cholinergic enzyme activities. Blood samples were collected before and 2 h after Al treatment, left at 4°C overnight, and then centrifuged at 3000 rpm for 15 min. After centrifugation, serum was carefully removed with a Pasteur pipet for assay of AChE activity.

Assay of Cholinergic Enzymes

ChAT was assayed radiochemically as described by Peng et al. (1980). The reaction mixture contained (final conc.) 50 m*M* KPO_4, 12.5 m*M* choline chloride, 2 m*M* EDTA, 0.9% NaCl, 0.1 m*M* eserine sulfate, 50 μM [acetyl-1-^{14}C]acetyl-CoA, and an enzyme preparation in a total volume of 80 μL at pH 7.4. The mixture was incubated at 37°C for 15 min, and the reaction was terminated by adding 1 mL of perchloric acid at the end of incubation, and buffered with 1 mL of 0.5*M* Tris-acetate, pH 7.0 (A). The mixture was poured into Amberlite CG-50 column (1×5cm), which was preequilibrated with 2 mL of A buffer. The column was then washed extensively with 20 mL of distilled water. The bound [^{14}C]acetylcholine (ACh) was eluted with 3 mL of 4*M* acetic acid into a scintillation vial, followed by the addition of 12 mL of liquid scintillation cocktail, and the radioactivity was counted using a Packard scintillation counter. One unit of enzyme activity was defined as the amount of enzyme required to synthesize 1 nmol ACh/15 min.

AChE activity was assayed radiochemically as described by Sung (1980) using Dowex 50W-X8 resin (Na^+ form, 100–200 mesh). The sodium form of the resin was prepared from the commercial Dowex 50W-X8 hydrogen form by soaking the resin with 1.5*M* sodium hydroxide for 1 h, followed by washing with deionized water repeatedly until the pH of the resin was below 8.0. The reaction mixture for AChE assay contained 2.8 m*M* [acetyl-1-^{14}C]ACh iodide, 50 m*M* KPO_4, 2 m*M* EDTA, 0.2*M* NaCl, 3×10^{-5} *M* iso-OMPA for inhibition of nonspecific cholinesterase activity, and an enzyme preparation in a total volume of 80 μL at pH 7.4. The mixture was incubated at 37°C for 15 min, and the reaction was terminated by adding 0.6 mL of 4°C chilled distilled water. Labeled acetate and ACh were then separated by pouring the mixture into a Dowex column, which

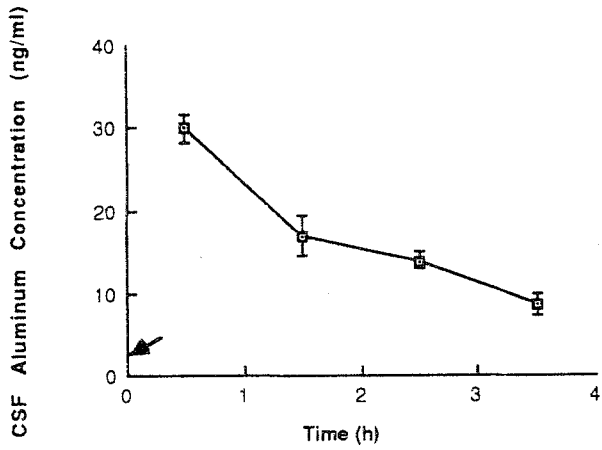

Fig. 1. Time-course study of Al accumulation in CSF of experimental rats. CSF was collected continuously for 4 h and Al levels in CSF was monitored as described in Materials and Methods. Data are means ± SD (error bars) of three determinations from three adult rats. Arrow indicates predose CSF Al concentration (2.6 ± 0.5 ng/mL).

was eluted twice with 0.6 mL of chilled distilled water. One unit of AChE activity is defined as the amount of enzyme required to hydrolyzed 1 nmol of substrate/min.

Determination of Proteins

Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Statistical Analysis

All data were analyzed by Student's *t*-test for their significance. The data were considered significant if $p < 0.05$.

RESULTS

Al Level in the CSF

Measurement of Al levels in the CSF following intravenous injection was used as a direct indicator for the Al that crosses the blood-brain barrier. Injection of Al into the femoral vein of rats resulted in significantly increased Al concentration in the CSF, which indicates that Al readily crosses the blood-brain barrier. At 30 min (midpoint of 0–1 h collection) after injection, the Al concentration elevated to 30 ± 1.8 ng/mL, and then slowly declined to the level of 8.5 ± 1.3 ng/mL at the end of the 4 h experiment (Fig. 1).

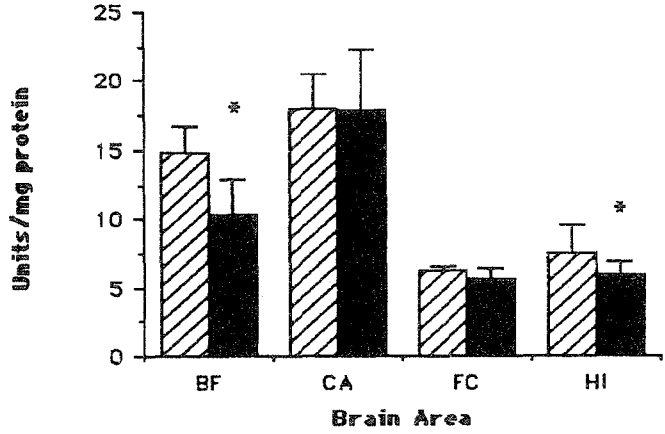

Fig. 2. Effect of Al treatment on ChAT activity. ▨=control; ■=Al. Values are means (control=3, Al=4)±SD (error bars) expressed as nmol ACh formed/mg protein/15 min. Assays were performed in triplicate. BF=Basal forebrain; CA=Caudate nucleus; FC=Frontal cortex; HI=Hippocampus. *significant $p<0.05$.

Effect of Al on Cholinergic Enzyme Activity

The effect of aluminum on cholinergic function was determined by measuring the ACh synthesizing and degrading activities of the cholinergic enzymes ChAT and AChE, respectively. The cumulative value of CSF Al was 41.6±21.2 ng/mL (midpoint of 0–2 h collection), which was about 40% higher than the peak level at 30 min during pharmacokinetic studies as described earlier. After 2 h of treatment, ChAT activity was significantly reduced in the hippocampus and basal forebrain by 22% (control, 7.53±2.05 vs Al, 5.9±0.96 U/mg protein) and 30% (control, 14.8±2.6 vs Al, 10.3±1.9 U/mg protein), respectively (Fig. 2). Little or no appreciable change in ChAT activity was observed in the caudate nucleus and frontal cortex. The effect of Al on cholinergic enzymes observed in this study was not related to the Al concentration in the vascular volume of the brain tissue when it is homogenized because a brain of 2 g has 40 μL of blood according to the blood volume of 2% in the brain. At 2 h after rat received 1 mg/kg Al injection, the blood Al concentration was approximately 5,000 ng/mL, i.e., 5 ng/μL. Therefore, a 2 g brain has 200 ng blood Al, and thus the blood Al in the treated animal was calculated to be about 0.1 ng/mg brain, which is insignificant when compared to 1.7 ng/mg brain Al in the untreated controls.

AChE activity in the caudate nucleus was significantly increased by almost 45% 2 h after treatment with aluminum (control, 491±161 vs Al, 711±91 U/mg protein), whereas the same enzyme showed little or no change in other brain areas (Fig. 3).

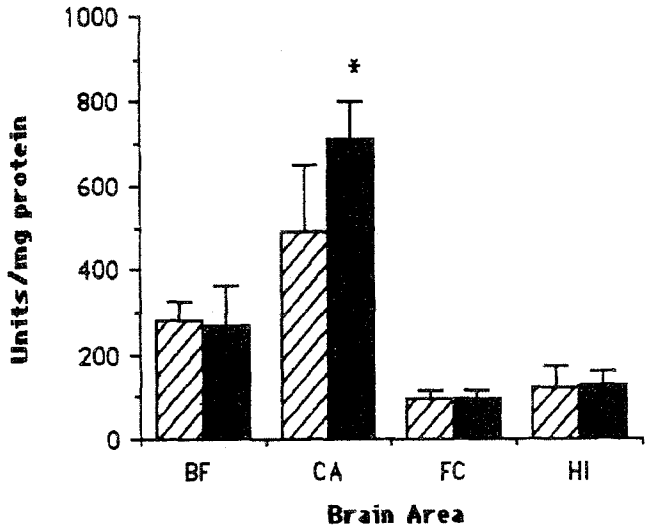

Fig. 3. Effect of Al on AChE activity. ▨=control; ■=Al. Values are means (control=3, Al=4)±SD (error bars) expressed as nmol substrate hydrolyzed/mg protein/min. Assays were performed in triplicate. *significant $p<0.05$.

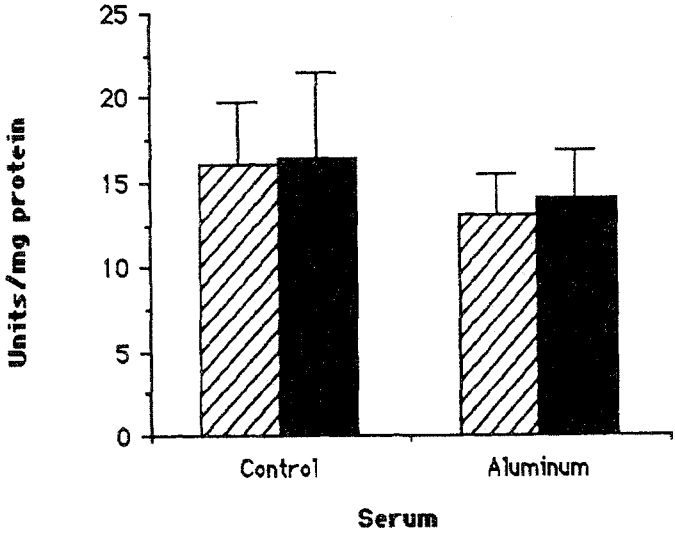

Fig. 4. Effect of Al on serum AChE activity. ▨=0h AChE; ■=2h AChE. Values are means ($n=3$)±SD (error bars) expressed as nmol substrate hydrolyzed/mg protein/min. Assays were performed in triplicate.

Effect of Al on Serum AChE Activity

Serum AChE activity was measured before and 2 h after Al treatment. Neither Al treatment nor control groups showed any significant change in AChE activity (Fig. 4). This indicated that Al treatment did not have much effect on serum AChE.

DISCUSSION

The possible etiological role of Al in the pathogenesis of AD is currently a subject of intense debate. Even though Al has been extensively investigated, the route by which Al enters the brain, and the mechanism of Al transport into the brain are unknown. Furthermore, the Al dose that causes neuronal damage in the brain is also unknown. Animals in previous studies in which various forms of Al salt were administered via various routes were usually treated multiple times (DeBoni et al., 1976; Hetnarski et al., 1980; Kosik et al., 1983; Gulya et al., 1990). Subsequently, the Al concentrations and neurochemical changes in the brain were measured. Thus, Al has been reported to be elevated or accumulated in various areas of the CNS (DeBoni et al., 1976; Yates et al., 1980; Gulya et al., 1990; Wisniewski et al., 1990). The present investigation shows that Al readily crosses the BBB and enters into the CSF after intravenous injection. The 42% higher CSF Al value in the second experiment as compared to the first experiment in this study could be a result of individual animal variation and different route of Al injection. The mechanism for Al crossing the BBB is unknown, but the same high affinity receptor-ligand system that has been hypothesized for iron (Fe^{3+}) could also be used to explain the Al transport (Roskams and Connor, 1990).

Since neurofibrillary degeneration was observed both in the brains of AD patients and experimental animals subjected to chronic Al treatment (DeBoni et al., 1976; Hetnarski et al., 1980; Yates et al., 1980; Boegman and Bates, 1984; Wisniewski et al., 1990), it was proposed that alterations of the cholinergic neurotransmission could be the underlying biochemical mechanism of Al neurotoxicity (Kosik et al., 1983; Gulya et al., 1990). In an attempt to elucidate the mechanism of Al-induced neurotoxicity, most investigators have studied the chronic effect of Al in vitro, in tissue culture (Singer et al., 1990) or metal-enzyme interaction in vitro (Gulya et al., 1990). We studied the effect of Al on the CNS at an early stage (2 h) of treatment, when the Al concentration in the CSF was found to be 12 times higher than that of predose levels. The effect of Al on ChAT and AChE activities was carried out at 2 h postinjection, assuming that the induction of Al neurotoxicity could be longer than its entrance into the CSF. The Al may or may not have greater effect on cholinergic enzymes if measured at an early times. The effect of Al on rats does not seem to be caused by acid pH shock, since only 250–300 μL of $AlCl_3$ solution, which is approximately equal to 1% of the total blood volume, was injected in each animal. The buffering action of the blood will bring it to pH 6.0 soon after Al injection. Our results indicate that as early as 2 h after Al injection, ChAT activities in the basal forebrain and hippocampus are significantly reduced, but no significant difference are observed in the frontal cortex and caudate nucleus. These results confirm other reports, except some variance in the affected areas (Yates et al., 1980; Kosik et al., 1983;

Gulya et al., 1990). Zubenko and Hanin (1989) have shown intraventricular administration of Al chloride, but not Al citrate, resulted in dose-related reduction in high affinity choline transport. They also showed that the same treatment had no significant effect on norepinephrine concentration in the hippocampus. However, synaptosomal uptake of norepinephrine, serotonin, γ-aminobutyric acid, and glutamic acid was shown to be inhibited by Al. On the other hand, synaptosomal uptake of dopamine was shown to be stimulated by Al at low concentrations but inhibited at high concentrations (Lai et al., 1981). For unknown reasons, aluminum treatment significantly increased ChAT activity in a neuroblastoma×glioma hybrid cell culture (Singer et al., 1990), in sharp contrast to the animal studies. An in vitro study showed that Al treatment did not inhibit ChAT activity, which suggested that the effect of Al on ChAT is via a mechanism other than metal-protein interaction (Gulya et al., 1990). The effect of Al on AChE followed quite a different pattern. AChE activity in the caudate nucleus was activated by 45% whereas little or no change was seen in other brain areas. The reason for the difference in regional response to Al treatment is that the decreased ChAT activity in the basal forebrain could be the result of decreased synthesis and increased breakdown of the enzyme. Also, the decreased axonal transport of the enzyme from the cell bodies in the basal forebrain could contribute to ChAT activity decreased in the hippocampus of the experimental animals (Boegman and Bates, 1984). It should also be noted that caudate nucleus that is adjacent to the third ventricle is partly bathed in the CSF and subjected to a different mode of Al effect. Yates et al. (1980) reported an increase of AChE in the frontal cortex of Al-treated rabbits after a single intracisternal injection, whereas Marquis (1982) observed an increase in rat brain AChE 28 d after Al exposure. Purified erythrocyte AChE was also reported to be activated by Al (Boegman and Bates, 1984). These reports were different from the observed decreasing AChE activity in AD brain (Davis and Maloney, 1976). In our studies, the effect of Al on serum AChE activity was also investigated, since it could provide us a potential marker for early diagnosis of AD. In short, our results show that Al could have a devastating effect on the overall brain cholinergic function by lowering ACh synthesis in some areas and increasing ACh degradation in other area of the brain. This establishes that Al has both the acute and chronic effect on cholinergic enzymes of experimental animals.

ACKNOWLEDGMENT

The expert statistical evaluation of the data by Lee Willoughby is greatly appreciated.

REFERENCES

Alfrey A. C., LeGendre G. R., and Kaehny W. D. (1976) The dialysis encephalopathy syndrome: Possible aluminum intoxication. *N. Engl. J. Med.* **294,** 184–188.

Armstrong D. M., Bruce G., Hersh L. B., and Terry R. D. (1986) Choline acetyltransferase immunoreactivity in neuritic plaques of Alzheimer brain. *Neurosci. Lett.* **71,** 229–234.

Boegman R. J. and Bates C. A. (1984) Neurotoxity of aluminum. *Can. J. Physiol. Pharmacol.* **62,** 1010–1014.

Candy J. M. Oakley A. E., Atack J., Perry R. H., Perry E. K., and Edwardson J. A. (1984) New observations on the nature of senile plaque cores, in *Developments in Neuroscience, Vol. 17: Regulation of Transmitter Function: Basic and Clinical Aspects* (Vizi E. S. and Magyar K., eds.), pp. 301–304, Elsevier, New York.

Chafi A. H., Hauw J. J., Rancurel G., Berry J. J., and Galle C. (1991) Absence of alumunim in Alzheimer's disease brain tissue: Electron microprobe and ion microprobe studies. *Neurosci. Lett.* **123,** 61–64.

Crapper McLachlan D R. and De Boni U. (1980) Aluminum in human brain disease—an overview. *Neurotoxicology* **1,** 3–16.

Crapper McLachlan D. R., Lukiw W. J., and Kruck T. P. A. (1989) New evidence for an active role of aluminum in Alzheimer's disease. *Can. J. Neuro. Sci.* **16,** 490–497.

Davis P. and Maloney A. J. F. (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2,** 1403.

Davis P. and Wolozin B. L. (1987) Recent advances in the neurochemistry of Alzheimer's disease. *J. Clin. Psychiatry* **48,** 23–30.

DeBoni U., Otvos A., Scott J. W., and Crapper McLachlan D. R. (1976) Neurofibrillary degeneration induced by systemic aluminum. *Acta Neuropath.* (Berl.) **35,** 285–294.

Gulya K., Rackonczay Z., and Kása P. (1990) Cholinotoxic effects of aluminum in rat brain. *J. Neurochem.* **54,** 1020–1026.

Hetnarski B., Wisniewski H. M., Iqbal K., Dziedzic J. C., and Lajtha A. (1980) Central cholinergic activity in aluminum-induced neurofibrillary degeneration. *Ann. Neurol.* **7,** 489–490.

Jacobs R. W., Duong T., Jones R. E., Trap G. A., and Scheibel A. B. (1989) A reexamination of aluminum in Alzheimer's disease: Analysis by energy dispersive x-ray microprobe and flameless atomic absorption spectrophotometry. *Can. J. Neurol. Sci.* **16,** 498–503.

Johnson G. V. W. and Jope R. S. (1987) Aluminum alters cyclic AMP and cyclic GMP levels but not presynaptic cholinergic markers in rat brain in vitro. *Brain Res.* **403,** 1–6.

Kitt C. A., Price D. L., Struble R. G., Cork L. C., Wainer B. H., Becker M. W., and Moble W. C. (1980) Evidence for cholinergic neuritis in senile plaques. *Science* **226,** 1443–1445.

Kosik K. S., Bradley W. G., Goof P. F., Rasool C. G., and Selkoe D. J. (1983) Cholinergic function in lumbar aluminum myelopathy. *J. Neuropath. Exp. Neurol.* **42,** 365–375.

Lai J. C. K., Lim L., and Davison A. N. (1981) Differences in the inhibitory effect of Ca^{2+}, Mn^{2+}, and Al^{3+} on the uptake of dopamine by synaptosomes from forebrain and straitum of the rat. *Biochem. Pharmacol.* **30,** 3123–3125.

Lowry O. H., Rosebrough N. J., Farr N. J., and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

Markesbery W. R., Ehmann W. D., Hossain T. I. M., Alauddin M., and Goodin D. T. (1981) Instrumental neutron activation analysis of brain aluminum in Alzheimer disease and aging. *Ann. Neurol.* **10,** 511–516.

Marquis J. K. (1982) Aluminum neurotoxicity: An experimental perspective. *Bull. Environ. Contam. Toxicol.* **29,** 43–49.

McDermitt J. R., Smith A. L., Iqbal K., and Wisniewski H. M. (1979) Brain aluminum in aging and Alzheimer's disease. *Neurology* **29,** 809–814.

Peng J. H., McGeer P. L., Kimura H., Sung S. C., and McGeer E. G. (1980) Purification and immunochemical properties of choline acetyltransferase from human brain. *Neurochem. Res.* **5,** 943–962.

Perl D. P. and Brody A. R. (1980) Alzheimer's disease: X-ray spectrophotometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. *Science* **208,** 297–299.

Perl D. P. and Good P. F. (1990) Aluminum, environment and Alzheimer's disease: Active role or passive bystander? *Neurobiol. Aging* **11,** 83.

Roskams A. J. and Connor J. R. (1990) Aluminum access to the brain: A role for transferrin and its receptor. *Proc. Natl. Acad. Sci. USA* **87,** 9024–9027.

Singer H. S., Searles C. D., Hahn I. H., March J. L., and Troncoso J. C. (1990) The effect of aluminum on markers for synaptic neurotransmission, cyclic AMP, and neurofilaments in a neuroblastoma x glioma hybridoma (NG108-15). *Brain Res.* **528,** 73–79.

Sung S. C. (1980) Acetylcholinesterase solubilized from normal and dystrophic muscles by collagenase treatment. *Biochim. Biophys. Acta* **628,** 286–292.

Wisniewski H. M., Moretz R. C., Sturman, J. A., Wen G. Y., and Shek J. W. (1990) Aluminum toxicity in mammals. *Environ. Geochem. Health* **12,** 115–130.

Xu Z. X., Pai S. M., and Melethil S. (1991) Kinetics of aluminum in the rat. II; dose-dependent urinary and biliary secretion. *J. Pharmaceut. Sci.* **80,** 946–951.

Yates C. M., Simpson J., Russell D., and Gordon A. (1980) Cholinergic enzymes in neurofibrillary degeneration produced by aluminum. *Brain Res.* **197,** 269–274.

Zubenko G. S. and Hanin I. (1989) Cholinergic and noradrenergic toxicity of intraventricular aluminum chloride in the rat hippocampus. *Brain Res.* **498,** 381–384.