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LONG-TERM EFFECTS OF POSTNATAL ALUMINIUM EXPOSURE ON ACETYLCHOLINESTERASE ACTIVITY AND BIOGENIC AMINE NEUROTRANSMITTERS IN RAT BRAIN

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Abstract : The long-term effects of early postnatal exposure to aluminium on acetyl choline esterase (AChE) activity and on biogenic amines were studied in different brain regions. The subjects were eight days old male Wistar rat pups. They were grouped into normal control and aluminium exposed groups. For aluminium exposure, the pups were gastric intubated with aluminium chloride (40 mg/Kg body weight) for two weeks. Control rats were given equal volumes of distilled water. After the treatment, they were rehabilitated for forty days. On the sixtieth day, the rats from both the groups were sacrificed and AChE activity, levels of dopamine, noraderenaline and serotonin were estimated in the cerebral cortex, hippocampus, septum, brainstem and striatum. In the aluminium exposed group : the AChE activity was significantly decreased in the hippocampus, septum, striatum and brainstem; serotonin levels were reduced by 20% in the cortex, hippocampus, septum and striatum; in brain stem, the serotonin level was decreased by 40%. A 60% reduction in noradrenaline levels was observed in the striatum whereas it was reduced by 25% in other regions except in hippocampus. Though dopamine levels were not altered in the cortex, septum and brainstem, they were reduced by 40% in the striatum. The study documents the long-term consequences of exposure to aluminium during the developmental periods.

Key words : postnatal exposure aluminium dopamine serotonin noradrenaline long-term effects AChE activity

INTRODUCTION

Aluminium, a non-essential element for life, is known to be a potential hazard to many life processes and the neurotoxic effects of this element have been described in great detail (1, 2). Clinically, the neurotoxicity of aluminium has been studied most extensively in dialysis encephalopathy (3, 4). There are also reports on selective and abnormal accumulation of aluminium in the brains of patients with certain neurological disorders such as Amyotrophic lateral sclerosis, Parkinson Dementia complex of Guam (5). Aluminium has been suggested to be a contributory factor in the

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pathology of Alzheimer's disease (6, 7). The administration of this metal to experimental animals have been shown to induce many neuropathological features similar to those observed in Alzheimer's disease, implicating its involvement either as a cause or a consequence of this disorder (8, 9). Moreover, the direct neurotoxicity of aluminium has been demonstrated in *in vitro* using various neuroglial cultures (10, 11) to understand the consequences of intracellular aluminium on neurodegeneration and cell death.

Although considerable attention has been given to the aluminium intoxication studies, information regarding the long-term effects of aluminium is considerably lacking. Varner et al. (12) have demonstrated that chronic administration of aluminium to rats in drinking water caused distinct morphological alterations in the brain. Their study provided evidence of neuronal injury in terms of reduced neuronal density, degenerative changes such as pyknosis, vacuolation, chromatin condensation and other cytoskeletal as well as cerebrvascular abnormalities. Similarly few studies (13, 14) have demonstrated that exposure to aluminium during developmental periods leads to impaired spatial memory and altered cholinergic functions in the adulthood. This is of particular concern as exposure of developing nervous system to insults may have more dangerous consequences than that of the adult nervous system. In view of the lack of information on long-term neurotoxic effects of aluminium on brain functions, the present study was designed to investigate the long-term effects of early postnatal exposure to aluminium. In this paper, the effects of aluminium on acetylcholine esterase (AChE) activity and on biogenic amine neurotransmitter (dopamine, noradrenaline and serotonin) levels in different brain regions are reported.

METHODS

Subjects

Pregnant Wistar rats were housed individually. They had ad libitum access to food and water. They were kept in well-aerated room and a twelve-hour light dark cycle was maintained at room temperature (26 ± 3°C). The day of delivery was considered as zero and the litter size per mother was maintained at eight. Only male pups of the litter were used. They were gastric intubated with 150 µl of aluminium chloride at a dose of 40 mg per kg body weight daily for two weeks from postnatal day 8 to 21. The control pups (from different litters) received the same volume of distilled water for the same periods. The pups were weaned on the twenty first day and were given free access to ad libitum food and water. When they attained sixty days of age, the rats from both the groups were subjected to biochemical measurements.

Biochemical studies

Animals from both groups (n = 6 per group) were killed by decapitation and the brain regions-cerebral cortex, hippocampus, septum, brainstem and striatum were dissected out according to the procedures described by Glowinsky and Iversen (15).

AChE activity was measured by the method of Ellman et al (16). The levels of biogenic amine neurotransmitters [dopamine (DA), noradrenaline (NA) and serotonin (5HT)] were determined in the said brain regions of the second set of animals (n = 6 per group). The neurotransmitter levels were estimated isocratically, using HPLC system with electrochemical detection by the method of Wanger et al (17). The data were analyzed statistically by Student's two-tailed 't' test (18).

RESULTS

During the period of aluminium treatment, the pups looked weak and the mortality rate was quite high (40%). Those survived the treatment looked normal by two months of age. Body and brain weight did not differ significantly from that of controls (data not included).

When compared to the control group, the aluminium-treated group showed a significant reduction in the AChE activity of the hippocampus ($t = 9.6$, with ten degree of freedom, $P<0.001$), septum ($t = 7.51$, $P<0.001$), striatum ($t = 7.88$, $P<0.001$) and brainstem ($t = 32.55$, $P<0.001$) with an average reduction of 25% in these regions except in brain stem where the decrease was 40%. The enzyme activity however, remained unaffected in the cortex. Whereas the serotonin levels were significantly decreased in cortex ($t = 15.57$, $P<0.001$), hippocampus ($t = 13.04$, $P<0.001$), septum ($t = 8.18$, $P<0.001$), brain stem ($t = 25.3$, $P<0.001$) and striatum ($t = 6.49$, $P<0.001$). The average reduction in serotonin levels was 20% except in the brain stem where the decrease was 40%. Noradrenaline levels were reduced significantly (cortex: $t = 10.69$, $P<0.001$; septum: $t = 5.10$, $P<001$; brain stem: $t = 8.58$, $P<0.001$ and striatum: $t = 27.83$, $P<0.001$) with an average reduction of 25% in all the regions except in the striatum where the decrease was above 60%. Dopamine levels were decreased significantly only in the striatum ($t = 13.01$, $P<0.001$) and hippocampus ($t = 4.9$, $P<0.001$) and the decrease was 40 and 20% respectively. The dopamine levels remained unaltered in the cortex, septum and brainstem (Fig. 1).

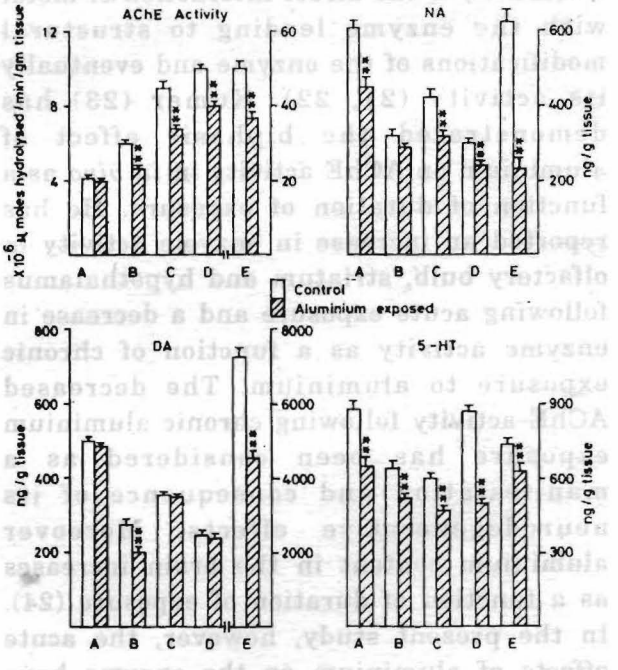

Fig. 1: Acetyl cholinesterase (AChE) activity, levels of NA (noradrenaline), DA (Dopamine) and 5-HT (serotonin) from different brain regions in control and Aluminium exposed rats (A = cortex, B = hippocampus, C = septum, D = brainstem And E = striatum). **P<0.001

DISCUSSION

In the present study, aluminium induced changes were measured 40 days after the stoppage of aluminium treatment, in order to assess the long-term effects of early exposure to aluminium. The data obtained showed that exposure to aluminium caused a significant decrease in the AChE activity in the hippocampus, septum, brainstem and striatum. Effect of early exposure to aluminium on AChE activity has not been reported. However, the biphasic effects of aluminium; an increase and decrease in enzyme activity with increase in concentration has been demonstrated in *in vitro* (19, 20). The biphasic activity has been attributed to the direct interaction of metal with the enzyme leading to structural modifications of the enzyme and eventually its activity (21, 22). Kumar (23) has demonstrated the biphasic effect of aluminium on AChE activity in *in vivo* as a function of duration of exposure. He has reported an increase in enzyme activity in olfactory bulb, striatum and hypothalamus following acute exposure and a decrease in enzyme activity as a function of chronic exposure to aluminium. The decreased AChE activity following chronic aluminium exposure has been considered as a manifestation and consequence of its neurodegenerative effects. Moreover aluminium content in the brain increases as a function of duration of exposure (24). In the present study, however, the acute effects of aluminium on the enzyme have not been measured. Yet the long-term effects were almost similar to those reported by Kumar following chronic exposure (23). Even though the aluminium content has not been measured, in the light of other studies, the observed decrease could be attributed to the degenerative effect of aluminium either on the cholinergic system, or to its direct interaction with the enzyme. Another interesting observation of the study is that the AChE activity of the cortex has been spared reflecting possible regional differences in the vulnerability to aluminium. Regional vulnerability to aluminium has been reported by Szerdahelyi and Kasa (25). In their study, the cholinergic hypofunctioning by cholinotoxin AF64A has promoted the accumulation of aluminium in parietal cortex and hippocampus but not in the frontal cortex. Moreover, the concentration of aluminium salts administered, the metal chemical speciation etc. are also of a paramount importance in defining the toxicity (22) as has been reported by Zubenko et al. (26) that higher concentrations of aluminium chloride are more effective in mediating cholinotoxic effects than that with aluminium citrate.

Aluminium exposure has resulted in a decrease of the individual neurotransmitter levels in brain regions, indicating the regional vulnerability. Even though serotonin levels were decreased in all regions, the brainstem exhibited higher susceptibility when compared to cortex, striatum, septum and hippocampus. The noradrenaline levels of striatum showed high vulnerability followed by cortex, brainstem and septum whereas that of hippocampus was least affected. Dopamine levels in cortex, brainstem and septum were unaffected by aluminium but the levels were decreased considerably in striatum. Reports on the specific regional vulnerability to aluminium of individual transmitter levels

are not available. Significant reduction of total catecholamine content in the cortex, midbrain and cerebellum following chronic aluminium treatment has been reported by Mostagie et al (27). However, in their study, analysis has not been performed to study the effect of aluminium on individual catecholamine neurotransmitters. They have also measured the aluminium content from these regions are have attributed this to a reduction in the synthesis of catecholamine following aluminium accumulation in brain regions. Accumulation of aluminium in the brain (28) and reduced catecholamine content in brain regions suggest its role in neurodegenration of specific neurotransmitter circuitry/synapses, neurotransmitter biosynthetic and degradative pathways and synaptic release mechanisms. The present observation of different regional vulnerability could also suggest the possibility of its interference with specific regions of the brain, which are more susceptible to aluminium.

In conclusion the present study shows that early postnatal exposure of low doses of aluminium is able to induce long-term deficits in acetylcholine esterase activity as well as in biogenic amine neurotransmitter levels in different brain regions. Further studies are necessary to confirm the long-term accumulation of aluminium in brain regions following early exposure and its consequential influence on neuronal morphology, pathology etc.

REFERENCES

1. Strong MJ, Garruto RM, Joshi JG, Mundy WR, Shafer TJ. Can the mechanisms of aluminium neurotoxicity be integrated into a unified scheme ? *J Toxicol Environ Health* 1996; 48: 599–613.
2. Environmental health criteria-194 for aluminium. *World Health Organisation, geneva* 1997.
3. Alfrey AC, LeGendre GR, Kaehny WD. The dialysis encephalopathy Syndrome: Possible aluminium intoxication. *N Eng J Med* 1976; 294: 184–188.
4. Salusky IB, Foley J, Nelson P, Goodman WG. Aluminium accumulation during treatment with aluminium hydroxide and dialysis in children and young adults with chronic renal disease. *N Egnl J Med* 1991; 3: 527–531.
5. Perl DP, Gajdusck DC, Garruto RM, Yanagiharaa RT, Gibbs CJ. Intraneuronal aluminium accumulation in amyotrophic lateral sclerosis and Parkinsonism-dementia of Guam. *Science* 1982; 217: 1053–1055.
6. Rifat SL. Aluminium hypothesis lives. *Lancet* 1994; 343: 3–4.
7. Yokel RA, Provan SD, Meyer JJ, Campbell SR. Aluminium intoxication and the victim of Alzheimer's disease. Similarities and differences. *Neurotoxicology* 1988; 9: 429–442.
8. Savory J, Huang Y, Herman MM, Reyes MR, Wills MR. Tau immunoreactivity associated with aluminium maloate-induced neurofibrillary degeneration in rabbits. *Brain Res* 1995; 669: 325–329.
9. Scott CW, Fieles A, Sygowsky LA, Caputa CB. Aggregation of tau protein by aluminium. *Brain Res* 1993; 628: 77–84.
10. Xie CX, Mattson MP, Lovell MA, Yokel RA. Intraneuronal aluminium potentiates iron-induced oxidative stress in cultured rat hippocampal neurons. *Brain Res* 1996; 743: 271–277.
11. Suarez-Fernandez MB, Soldado AB, Sanz-Medel A, Vega J, Novelli A, Fernandez-Sanchez MT. Aluminium-induced degernation of astrocytes occurs via apoptosis and results in neuronal death. *Brain Res* 1999; 835: 125–136.
12. Varner JA, Jensen KF, Hovarth W, Isaacson RL. Chronic administration of aluminium-flouride or sodium-flouride to rats in drinking water: alterations in neuronal and cerebroavascular integrity. *Brain Res* 1998; 784: 284–298.
13. Cherroret G, Bernuzzi V, Desor D, Hutin MF, Burnel D, Lehr PR. Effects of postnatal aluminium exposure on choline acetyl transferase activity and

learning disabilities in the rat. *Neurotoxicol Theratol* 1992; 14: 259–264.

14. Santucci D, Rankin J, Laviola G, Aloe E, Alleva E. Early exposure to aluminium affects eight arm maze performance and hippocampal nerve growth factor levels in adult mice. *Neurosci Lett* 1994; 166: 89–92.
15. Glowinsky J, Iversen LL. Regional studies of catecholamiines in the rat brain-1. The disposition of ^{3}H norepinephrine, ^{3}H dopamine and ^{3}H DOPA in various regions of the brain. *J Neurochemistry* 1966; 13: 655–669.
16. Ellman GL, Courtney KD, Andres V, Feathrstone RM. A new and rapid colorimetric determination of acetylcholine esterase activity. *Biochem Pharmacol* 1961; 7: 88–95.
17. Wanger J, Vitali P, Palfreyman MG, Zraika M, Huot S. Simultaneous determination of 3, 4-dihydroxyphenylalanine, 5-hydroxytryptophan, dopamine, 4-hydroxy 3-methoxyphenylalanine, norepinephrine, 3, 4-dihydroxyphenylacetiic acid and homovanillic acid, serotonin and 5-hydroxyindoleacetic acid in rat cerebrospinal fluid and brain by HPLC with electrochemical detection. *J Neurochem* 1982; 38: 1241–1254.
18. Zar JH, Biostatistical analysis, Prentice-Hall International, New Jersey, USA. 1984.
19. Gulya K, Rakonzay Z, Kasa P. Cholinotoxic effects of aluminium in rat brain. *J Neurochem* 1990; 54: 1020–1026.
20. Zatta P, Zambenedetti P, Bruna V, and Filippi B. Activation of acetylcholinesterase by aluminium (III): the relevance of the metal species. *Neuroreport* 1994; 5: 1777–1780.
21. Zatta P, Zambenedetti P, Pizziuti A, Perazzolo M. Different effects of aluminium upon carbonic anhydrases and Na^+/K^+-ATPase activities in rat. *Neurosci Lett* 1995; 197: 65–68.
22. Marquis JK, Black EE. Aluminium activation and inactivation of bovine caudate acetylcholinesterase by trivalent cations. *Biochem Pharmacol* 1985; 34: 533–538.
23. Kumar S. Biphasic effect of aluminium on cholinergic enzyme of rat brain. *Neurosci Lett* 1998; 248: 121–123.
24. Bilkei-Gorzo A. Neurotoxic effect of enteral aluminium. *Food Chem Toxicol* 1993; 31: 357–361.
25. Szerdahelyi P, Kasa P. Intraventricular administration of the cholinotoxin AF64A increases the accumulation of aluminium in the rat parietal cortex and hippocampus, but not in the frontal cortex. *Brain Res* 1988; 444: 356–360.
26. Zubenko GS, Hanin I. Cholnergic and noradrenergic toxicity of intraventricular aluminium chloride in the rat hippocampus. *Brain Res* 1989; 498: 381–384.
27. Mostaghie AA, Rahimi S, Messripour M. Changes in catecholamine levels of cerebellum, midbrain and brain-cortex in aluminium intoxicated rats. *Ind J Pharmacol* 1996; 28: 244–248.
28. Petit TL. Aluminium in human dementia. *Am J Kid Dis* 1985; 6: 313–317.