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Altered calcium homeostasis: a possible mechanism of aluminium-induced neurotoxicity

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Abstract

The effect of aluminium, Al^{3+} (10 mg/kg body weight/day i.p.) for a period of 4 weeks was examined on the calcium homeostatic mechanisms in rat central nervous system. Incubation of synaptosomes prepared from rat brain, with aluminium in vitro had a detrimental effect on the activity of Ca^{2+} ATPase which could be reversed completely on exogenous addition of desferrioxamine (10 μM) and partially with glutathione (1 mM). In vivo administration also revealed a similar observation. A marked increase in the levels of intracellular calcium was observed after aluminium treatment. Concomitant to the increased levels of intracellular calcium, there was an increase in the levels of lipid peroxidation and a consequent decrease in fluidity of synaptic plasma membranes. In addition, aluminium also had an inhibitory effect on the depolarization-induced calcium uptake which was found to be of a competitive type. The biological activity of calcium regulatory proteins calmodulin and protein kinase C was considerably affected by aluminium. The results suggest that aluminium exerts its toxic effects by modification of the intracellular calcium messenger system with detrimental consequences on neuronal functioning.

Keywords: Calcium; Aluminium; Brain; Alzheimer's disease

1. Introduction

A steadily increasing amount of evidence reported in recent years has confirmed the fact that aluminium (Al^{3+}) can have severe neurotoxic effects [1]. Aluminium has been detected in both senile plaques [2] and neurofibrillary tangle bearing neurons [3] of patients with neurodegenerative diseases, particularly the Alzheimer's disease. It has also been shown that in some patients undergoing long-term hemodialysis, the accumulation of large amounts of Al^{3+} is directly responsible for the development of dialysis encephalopathy. Despite intense interest in the etiology of these neurological syndromes, there are very few reports regarding the effects of Al^{3+} on the metabolism of normal humans and animals.

The molecular mechanisms by which Al^{3+} exerts its neurotoxic effects remain unknown. However, several facts suggest that Al^{3+} can interact with Ca^{2+} binding sites and disrupt intraneuronal Ca^{2+} homeostasis, since in most of the neurodegenerative diseases, in which Al^{3+} has been implicated, a Ca^{2+} excess along with Al^{3+}, has been reported [4]. High concentrations of Al^{3+} and Ca^{2+} in the CNS tissue play a critical role in neurodegeneration, producing neurofibrillary tangles and inducing cell death.

Intracellular Ca^{2+} ions play an important role as second messengers that mediate the effects of a variety of extracellular signals. There is hardly any cellular function that is not affected directly or indirectly by the ubiquitous second messenger Ca^{2+} [5]. Homeostatic mechanisms maintain $[Ca^{2+}]_i$ at low concentrations and are a prerequisite for normal neuronal functioning. Alterations in this $[Ca^{2+}]_i$ homeostasis can lead to nervous system dysfunction, as the neuronal functions involved in long-term potentiation like memory and learning are thought to be linked to Ca^{2+}-triggered intracellular events [6].

In view of the altered state of Ca^{2+} metabolism during the course of neurodegenerative diseases and the fact that it has a role to play in altered neurological functions, a detailed investigation on brain Ca^{2+} homeostasis are imperative. Therefore, in the present study the effect of Al^{3+} on Ca^{2+} homeostatic mechanisms, viz., Ca^{2+} effluxing

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and influxing systems and eventually on the levels of $[Ca^{2+}]_i$ and the activity of calcium regulatory proteins, have been investigated.

2. Materials and methods

Calmodulin, activator-deficient cAMP phosphodiesterase (PDE), cyclic AMP (cAMP), 5'-nucleotidase (Snake Venom), EGTA, adenosine triphosphate, Dowex 1×2 (200–400 mesh) anion exchanger were obtained from Sigma Chemicals, St. Louis, MO, USA. Fura 2/AM was obtained from Molecular Probes, Inc., Eugene, USA. Desferrioxamine (DES) was obtained from Ciba Geigy, Basle, Switzerland. [2, 8-^{3}H]Adenosine 3'-5'-cyclic monophosphate (spec. act. 41.7 Ci/mmol) was obtained from Amersham Inc., UK and $^{45}Ca^{2+}$ (Spec. act. 105 mCi/mmol) from Bhabha Atomic Research Centre, Bombay, India. All other chemicals used were highest grade commercial products.

2.1. Animals and treatment

Male albino rats (wistar strain) weighing between 100–130 g were used throughout the course of study. Animals were housed in poly-propylene cages under hygienic conditions, fed standard rat pellet diet (Hindustan Lever Ltd., India) and water ad libitum. For in vivo studies, animals were divided into two groups of 8 animals each, which received either aluminium (10 mg/kg body weight/day) as aluminium lactate, for a period of 4 weeks (treated group), or an equal volume of 0.9% NaCl (control group), intraperitoneally. After the completion of the treatment, the animals were fasted overnight and then killed by decapitation. The brains were removed, rinsed in ice-cold normal saline and dissected into the different brain regions, viz., cerebral cortex, hippocampus, and corpus striatum, according to the guidelines of Glowinski and Iversen [7].

2.2. Subcellular fractions

Synaptosomal plasma membranes (SPMs) were prepared from different regions of rat brain according to the method of Jones and Matus [8]. Briefly, the crude mitochondrial fractions were suspended in hypotonic buffer (5 mM Tris-HCl, pH 8.0) at 0°C for 30 min, followed by homogenization. The lysate was made 34% (w/v) with sucrose by the addition of an appropriate volume of 48% (w/v) sucrose. An upper phase of 28.5% (w/v) sucrose was layered over the sample phase and a small volume of 10% (w/v) sucrose layered over the upper phase. The density gradient was centrifuged at $60\,000 \times g$ for 110 min. The SPMs were collected from interface between the layers 10% and 28.5% and resuspended in 40 mM Tris-HCl, pH 7.4.

Synaptosomes were prepared on a discontinuous sucrose gradient according to the procedure of Gray and Whittaker [9]. Briefly, the crude mitochondrial pellet was suspended in 0.32 M sucrose in 20 mM Tris-HCl (pH 7.4) and the suspension layered on a stepwise density gradient consisting of layers of 0.8 M, 1.0 M and 1.2 M sucrose. After centrifugation at $80\,000 \times g$ for 2 h, the interface over 1.0 M sucrose was recovered and constituted the synaptosomal fraction (synaptosomes). Synaptosomes were suspended in physiological buffer of the following composition, 20 mM Tris-HCl (pH 7.4), 130 mM NaCl, 4.9 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 11 mM glucose.

2.3. Analytical methods

Synaptosomal K^+-stimulated $^{45}Ca^{2+}$ uptake was measured in unstimulated (low K^+ medium = 4.9 mmol KCl) and stimulated (high K^+ medium = 106 mmol KCl) synaptosomes by the method of Edelfors and Ravn-Jonsen [10]. Uptake was terminated with 3 ml of stop solution (1.8 mM $LaCl_3$ and 1 mM EGTA in 0.15 M NaCl, pH 7.4) followed by filtration of the sample through 0.45 μm nitrocellulose filters (Whatman, UK). Non-specific binding was determined by measuring the uptake at zero time. The net potassium induced $^{45}Ca^{2+}$ uptake (ΔK) was expressed as the difference in nmol of $^{45}Ca^{2+}$ taken up per mg of protein between $^{45}Ca^{2+}$ uptake across the synaptosomal membrane under unstimulated (low K^+) and stimulated (high K^+) conditions.

Ca^{2+} ATPase was assayed in the synaptic plasma membranes by the method of Desaiah et al. [11]. The reaction mixture (1.5 ml) contained 40 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 0.05 mM $CaCl_2$ and 2.5 mM ATP. The Mg^{2+}-ATPase activity was determined in the presence of 1 mM EGTA and this was subtracted from the total activity in order to obtain the net Ca^{2+} ATPase activity. The inorganic phosphate released by the action of ATPase was estimated by the method of Fiske and SubbaRow [12].

Intrasynaptosomal free Ca^{2+} was determined in synaptosomes with the calcium-sensitive indicator dye fura 2/AM according to the method of Adamson et al. [13]. Briefly, synaptosomes (in physiological buffer) were loaded with 1 μM Fura-2/AM at 37°C for 1 h. The excess Fura 2/AM was removed by centrifugation. The fluorescence (F) at 340 to 380 nm excitation and 510 nm emission was measured and $[Ca^{2+}]_i$ was calculated according to the formula.

$$[Ca^{2+}]_i = (F - F_{min})/(F_{max} - F) \times K_d$$

The K_d for Fura-free acid is 225 mM [14]. Maximal fluorescence was measured after lysis of synaptosomes with SDS and minimal fluorescence (F_{min}) was measured in the presence of 5 mM EGTA.

Lipid peroxidation (LPx) was ascertained by measuring malondialdehyde, an index of lipid peroxidation, according to the method of Wills [15]. The malondialdehyde in the samples was reacted with thiobarbituric acid (TBA) and

the chromophore was read at 532 nm. The results were expressed as nmoles of MDA per mg of protein using the molar extinction co-efficient of the MDA-TBA chromophore (1.56×10^5).

Membrane fluidity of SPMs was assessed by the method of Shinitzky and Inbar [16], using the fluorescent probe diphenyl-1,3,5-hexatriene (DPH). Briefly, the membranes (in 0.1 M Tris-HCl, pH 7.0) were incubated with 10 μM DPH. Mixture was incubated at 37°C for 1 h. and centrifuged at $10000 \times g$ for 15 min. Pellet obtained was washed with and suspended in PBS. Fluorescence was measured on a spectrofluorimeter (Kontron SFM 25) at 445/350 nm emission and excitation wavelengths respectively.

Calmodulin (CaM) was assayed in the heat-treated tissue extract by its ability to stimulate activator-deficient cAMP phosphodiesterase activity according to the method of Wallace et al. [17].

Protein kinase C was assayed according to the method of Kikkawa et al. [18], wherein the transfer of ^{32}P from (γ-^{32}P) ATP to lysine rich histone in the presence of Ca^{2+} and phosphatidyl serine, was measured with or without diolein. Basal activity (due to other kinases), measured by omission of Ca^{2+} and phosphatidyl serine and addition of excess of EGTA, was deducted from the total enzyme activity.

Aluminium analysis was carried out in the tissues by the wet acid digestion method of Zumkley et al. [19]. The samples were digested in the acid mixture of 90% HNO_3, 70% $HClO_4$ and 10 N H_2SO_4 in the ratio 2:2:1. The residue obtained was reconstituted in 0.1 M HCl. Aluminium was estimated on a Direct Current Plasma Emission Spectrophotometer (Spectraspan, V. Beckman).

Proteins in the samples were quantitated by the method of Lowry et al. [20].

2.4. Statistical analysis

Values in the tables and figures are means ± S.D. Comparison between two means have been made using Student's *t*-test, *P*-values higher than 0.05 have been considered insignificant.

3. Results

Ca^{2+} ATPase, a CaM-regulated membrane-bound enzyme is responsible for expelling calcium from the neurons and thus maintaining low intracellular calcium levels. Varying concentrations of aluminium (0–100 μM) caused a dose-dependent decrease in the activity of the enzyme, with the 50% inhibitory concentration (IC_{50}) for aluminium being calculated to be approx. 10 μM (Fig. 1).

Exogenously added (10 μM) desferrioxamine (DES), an Al^{3+} and Fe^{3+} chelator was able to almost completely restore the enzyme activity. However, addition of exogenous glutathione (1 mM) could only partially restore the enzyme activity (Fig. 2). Ca^{2+} ATPase activity, when measured, following in vivo aluminium exposure (10 mg/kg body weight, for a period of four weeks) revealed a significant decrease in the treated group as compared to the control group (Fig. 3). Maximum inhibition was observed in corpus striatum (56.44%), followed by hippocampus (52.52%) and cerebral cortex (50%).

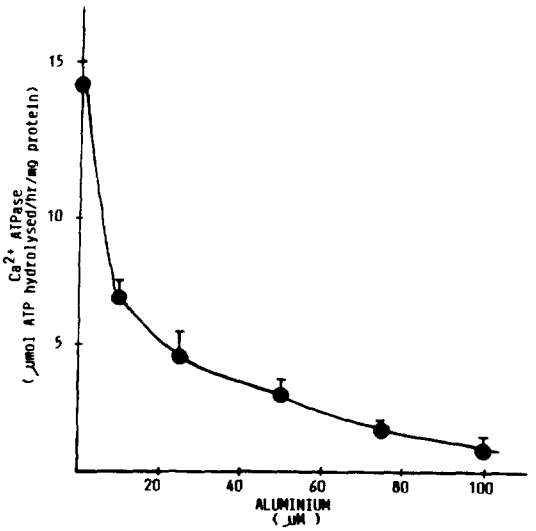

Fig. 1. Dose response curve of Ca^{2+} ATPase activity against aluminium concentration. The Ca^{2+} ATPase was assayed in the presence of aluminium (0–100 μM) in SPMs prepared from rat brain. Values are mean ± S.D. of four sets of experiments.

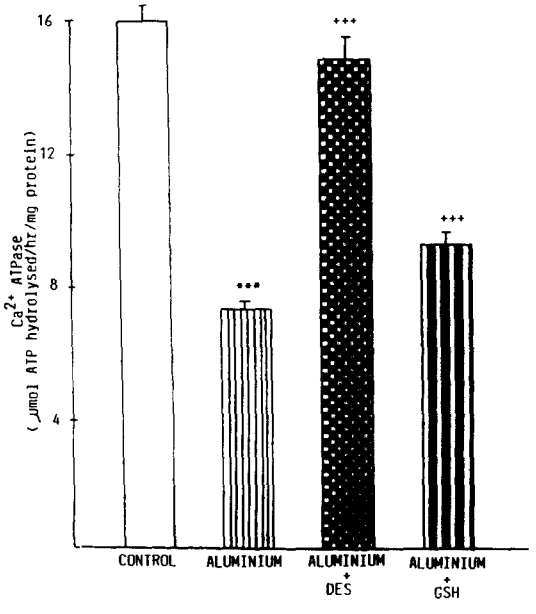

Fig. 2. Reversal of Ca^{2+} ATPase activity by exogenous addition of desferrioxamine and glutathione. The Ca^{2+} ATPase activity inhibited by IC_{50} concentrations of aluminium was determined in the presence of desferrioxamine (10 μM) and glutathione (1 mM). Values are mean ± S.D. of four sets of experiments *** $P < 0.001$, statistically significant from that in the absence of aluminium, $^{+++}$ $P < 0.001$, statistically significant from that in the presence of aluminium.

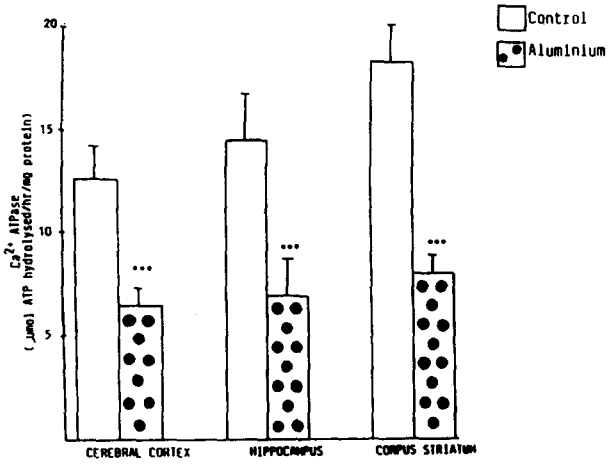

Fig. 3. Effect of in vivo aluminium exposure on the Ca^{2+} ATPase activity in SPMs. Values are mean ± S.D. of 8 animals/group. *** $P < 0.001$, statistically significant from control group.

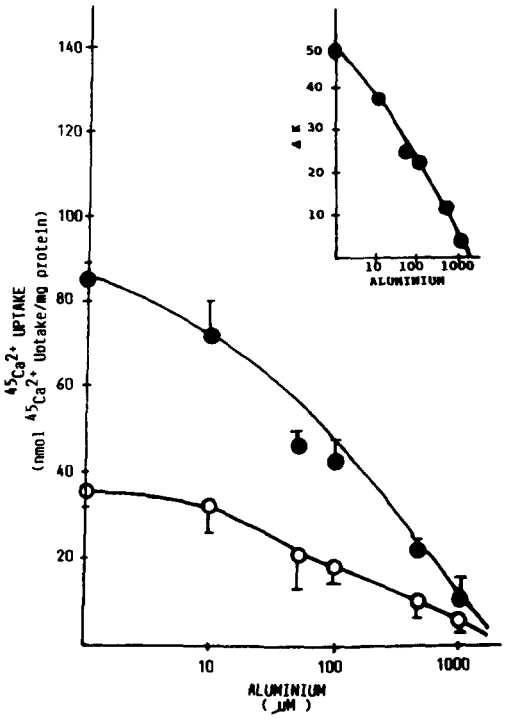

Fig. 4. Effect of in vitro aluminium exposure on $^{45}Ca^{2+}$ uptake in synaptosomes. $^{45}Ca^{2+}$ uptake was measured in unstimulated (○) and stimulated (●) synaptosomes following incubation with aluminium (0–1000 μM) for 30 min. Inset depicts the net uptake (ΔK). Values are mean ± S.D. of four sets of experiments.

As evident from the above data, Al^{3+} significantly alters Ca^{2+} handling in the nerve terminals in terms of Ca^{2+} efflux which may have dramatic consequences on the intrasynaptosomal Ca^{2+} concentration ($[Ca^{2+}]_i$), a sensitive indicator of neurotoxicity. Our results presented in Table 1, revealed a nearly 2-fold increase in the levels of resting $[Ca^{2+}]_i$ in the treated rats as compared to the control animals. Maximum increase was observed in the cerebral cortex > hippocampus > corpus striatum, in that order, suggesting thereby that Al^{3+} exposure alters the ability of nerve terminals to expel Ca^{2+}, leading to an elevation of intrasynaptosomal Ca^{2+} levels.

One of the major consequences of increased $[Ca^{2+}]_i$ levels is the enhanced production of free radicals, which can have detrimental effects on the integrity of cellular membranes in terms of lipid peroxidation. Therefore, the effect of Al^{3+} exposure on the levels of malondialdehyde, an index of lipid peroxidation, was investigated. In vivo Al^{3+} exposure resulted in a significant accentuation in the levels of LPx (Table 1) and the maximum increase was observed in the cerebral cortex (1.66-fold), followed by hippocampus (1.41-fold) and corpus striatum (1.21-fold). The consequence of enhanced peroxidation is the loss of membrane phospholipids which is eventually responsible for alterations in membrane fluidity. Therefore, the fluidity of synaptic plasma membranes was assessed using the fluorescent probe DPH. The results presented in Table 1 indicate that the fluorescence polarisation, which is inversely proportional to membrane fluidity, increased following Al^{3+} exposure.

In order to evaluate the effect of Al^{3+} on voltage-operated calcium channels (VOCC), which play a pivotal role in coupling electrical activity to neurotransmission, the Ca^{2+} uptake under stimulated (high K^+ medium) and unstimulated (low K^+ medium) conditions was measured in the synaptosomes. Fig. 4 shows the effect of varying concentrations of Al^{3+} (0–1000 μM) on $^{45}Ca^{2+}$ influx. The net K^+ induced Ca^{2+} influx, ΔK, (inset) was inhib-

Table 1
Effect of aluminium exposure on the levels of $[Ca^{2+}]_i$, lipid peroxidation and on the membrane fluidity in different regions of rat brain

	$[Ca^{2+}]_i$ (nM)		Lipid peroxidation (nmol MDA/mg protein)		Fluorescent polarisation (P)	
	Control	Al-treated	Control	Al-treated	Control	Al-treated
Cerebral cortex	104.03 ± 13.83	298.94 ± 63.34 ***	5.09 ± 0.26	8.46 ± 0.51 ***	0.29 ± 0.01	0.34 ± 0.01 ***
Hippocampus	157.36 ± 18.21	300.02 ± 25.82 ***	5.07 ± 0.26	7.16 ± 0.23 ***	0.26 ± 0.05	0.35 ± 0.02 ***
Corpus striatum	178.28 ± 7.17	240.37 ± 18.18 ***	5.20 ± 0.58	6.33 ± 0.40 ***	0.240 ± 0.05	0.34 ± 0.01 ***

Rats were exposed to aluminium (10 mg/kg body weight, i.p.) for four weeks.
Values are mean ± S.D. of 8 animals/group.
*** $P < 0.001$, statistically significant from control group.

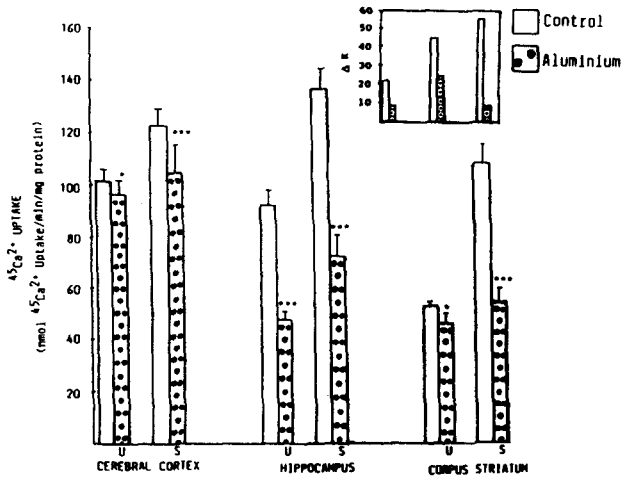

Fig. 5. Effect of in vivo aluminium exposure on the depolarization induced calcium influx in synaptosomes. $^{45}Ca^{2+}$ uptake was measured after 1 min in unstimulated (U) and stimulated (S) synaptosomes. Inset indicates the values of ΔK (net K^+-induced Ca^{2+} influx). Values are mean ± S.D. of 8 animals/group * $P < 0.05$, *** $P < 0.001$, statistically significant from control group.

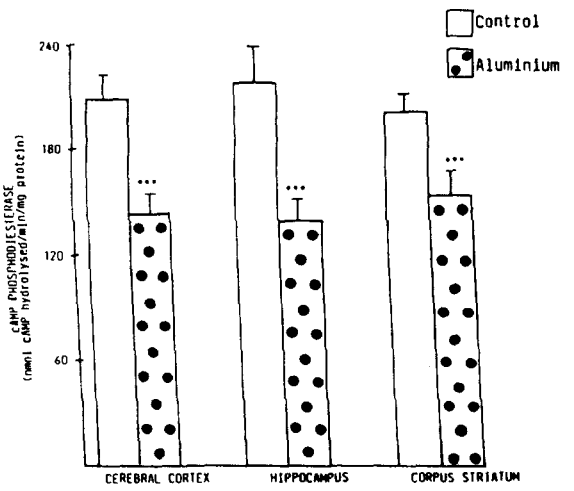

Fig. 7. Effect of in vivo aluminium exposure on the activity of calmodulin in different regions of rat brain. Calmodulin was assayed in the heat-treated supernatant in terms of its ability to stimulate cAMP phosphodiesterase. Values are mean ± S.D. of 8 animals/group *** $P < 0.001$, statistically significant from control group.

ited at all the concentrations of Al^{3+} with IC_{50} of Al^{3+} for the net uptake being at 50 μM concentration of Al^{3+}. At higher concentrations, the uptake was completely abolished.

In order to determine the type of inhibition of $^{45}Ca^{2+}$ uptake by Al^{3+}, a double reciprocal plot of $^{45}Ca^{2+}$ uptake vs external Ca^{2+} concentrations was plotted in the presence (50 μM) and absence of Al^{3+}. The results indicate that there was only a slight change in the values of J_{Ca}^{Max} (maximal Ca^{2+} uptake) in the presence of Al^{3+} (7.14) as compared to that in its absence (9.09) in terms of nM $^{45}Ca^{2+}$ uptake/min/mg protein, whereas the values of K_{Ca} (concentration of external Ca^{2+} at which $J_{Ca} = 1/2\ J_{Ca}^{Max}$, in mM extracellular calcium), increased in the presence of Al^{3+} (3.22), as compared to those in its absence (1.89), indicating the inhibition to be of a mixed type (i.e., a combination of competitive and non-competitive type). In vivo exposure to aluminium for a period of four weeks on the Ca^{2+} uptake via VOCC, as demonstrated in Fig. 5, revealed a significant decrease in $^{45}Ca^{2+}$ uptake in synaptosomes following incubation with ^{45}Ca for 1 min in both polarized and depolarized media. The net uptake (inset), i.e., ΔK, was also inhibited. The maximum inhibition was observed in corpus striatum (84.79%) followed by cerebral cortex (58.41%) and hippocampus (45.94%).

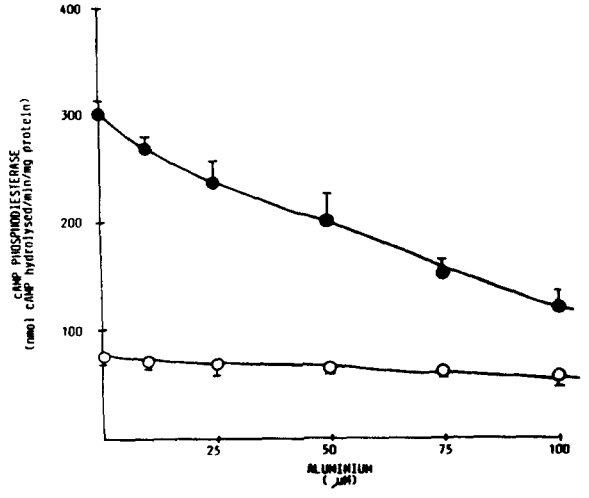

Fig. 6. Effect of in vitro aluminium on the biological activity of calmodulin. Aluminium ions (0–100 μM) were incubated with calmodulin-stimulated (●) and basal (○) cAMP phosphodiesterase for 30 min. Values are mean ± S.D. of four sets of experiments.

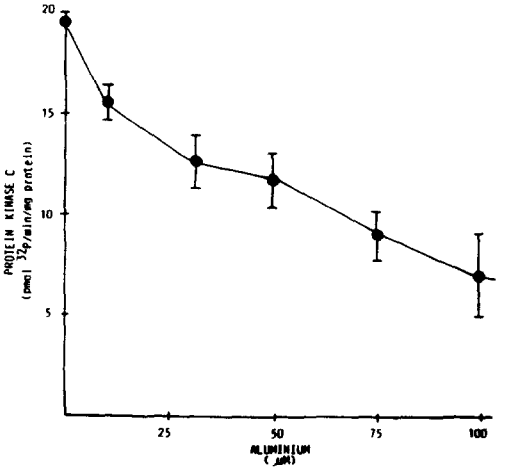

Fig. 8. Effect of in vitro aluminium on the activity of protein kinase C. The activity of PKC was assayed by its ability to transfer ^{32}P from ATP to histone in the presence of aluminium (0–100 μM). Values are mean ± S.D. of four sets of experiments.

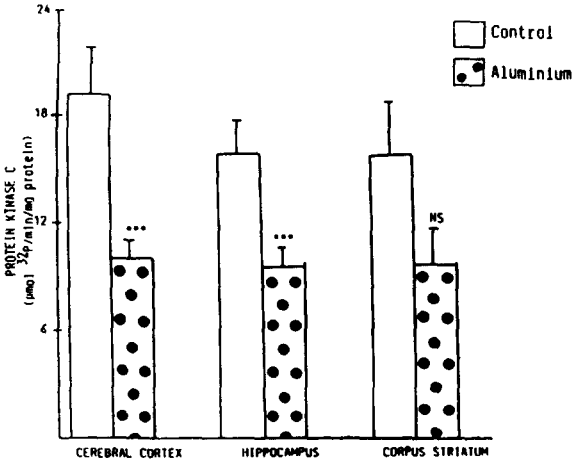

Fig. 9. Effect of in vivo aluminium exposure on calcium regulatory protein, PKC. Values are mean ± S.D. of 8 animals/group. * $P < 0.05$, *** $P < 0.001$, statistically significant from control group.

The activities of calmodulin and protein kinase C were also investigated following Al^{3+} exposure. In vitro incubation of Al^{3+} (0–1000 μM) with CaM had a detrimental effect on the biological activity of the protein. However, basal cAMP phosphodiesterase was only slightly affected by Al^{3+} ions (Fig. 6). In vivo administration of Al^{3+} also inhibited the CaM activity with the maximum decrease being in hippocampus (36.56%) followed by cerebral cortex (31.76%) and corpus striatum (22.49%) (Fig. 7).

Incubation with varying concentrations of aluminium (0–100 μM) inhibited the activity of PKC at all the concentrations of Al^{3+} (Fig. 8). In vivo Al^{3+} exposure (Fig. 9) further substantiated this result, where the maximum inhibition was observed in the cerebral cortex (47.73%) and hippocampus (45.95%) followed by corpus striatum (38.74%).

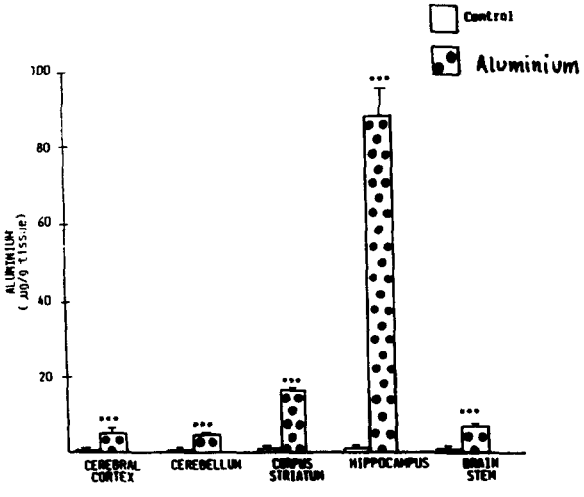

Fig. 10. Distribution of aluminium in different regions of rat brain. Values are mean ± S.D. of 8 animals/group. *** $P < 0.001$, statistically significant from control group.

The distribution of Al^{3+} revealed accumulation in all the regions, maximum being in the hippocampus followed by corpus striatum > cerebral cortex > cerebellum in that order (Fig. 10).

4. Discussion

The role of calcium and its homeostatic mechanisms as intracellular regulators of many physiological processes is an important area of study in different fields of biology. The results from the present study demonstrated that Al^{3+} affects Ca^{2+} homeostatic mechanisms at several loci in the nerve terminals. Aluminium exposure was seen to considerably alter the activity of the Ca^{2+} effluxing enzyme of the cell, Ca^{2+} ATPase. Following both in vitro and in vivo Al^{3+} exposure, a decrease in the activity of this enzyme was observed. Evidence suggests that a similar kind of decrease in the enzyme activity in neuronal cells of patients of Alzheimer's disease may be as a result of similar series of events in both the cases. Aluminium is known to displace Mg^{2+} from ATP to form an Al-ATP complex which is a dead-end inhibitor of Ca^{2+} ATPase [21]. However, a decrease in the biological activity of CaM, as observed in Fig. 7, may also account for the decreased Ca^{2+} ATPase activity, as CaM is a known activator of the enzyme. An altered CaM conformation on Al^{3+} exposure has also been reported by Seigal and Haug [22]. However, the possibility that Al^{3+} may be acting directly on the active site of the enzyme also cannot be ruled out. The reversal of inhibition of Ca^{2+} ATPase by exogenously added DES, a known Al^{3+} chelator, further substantiates that Al^{3+} might be responsible for impairing the activity of this enzyme, since chelation of Al^{3+} restores the activity back to normal. The partial reversal in the presence of glutathione may be attributed to the sulfhydryl groups present in GSH, that prevent the interaction of Al^{3+} with Ca^{2+} ATPase.

As evident from the data, there was a marked increase in the $[Ca^{2+}]_i$ levels as a result of decreased Ca^{2+} effluxing ability of the cell following Al^{3+} administration. Further, the elevation in levels of $[Ca^{2+}]_i$ following Al^{3+} exposure might also be due to the release of Ca^{2+} from intracellular stores, a process reported to be triggered in the presence of metals [23]. Increased $[Ca^{2+}]_i$ is a sensitive index of neurotoxic damage and is responsible for a wide variety of events, such as enhanced production of free radicals which potentiate lipid peroxidation [24]. The stimulation of lipid peroxidation by Ca^{2+} can be explained, based on their ability to liberate Fe^{2+} bound with negatively charged groups of lipids, thereby increasing the concentration of catalytically active Fe^{2+} in the system, which augment free radical release and eventually peroxidation of lipids. However, the possibility that the effect of Al^{3+} is probably due to a direct interaction with cellular components affecting the physical state of membranes

cannot be eliminated. Besides, raised levels of $[Ca^{2+}]_i$ are also responsible for increase in the spontaneous release of neurotransmitters, altered cytoskeletal organisation, impaired action of hormones and growth factors, activation of proteases, lipases and endonucleases and impairment of mitochondrial oxidative phosphorylation, etc. [25].

It was observed that concomitant to the increase in $[Ca^{2+}]_i$, there was an accentuation in the levels of LPx. A major consequence of enhanced LPx is the oxidative deterioration of cellular membranes. In the present study it has been demonstrated that Al^{3+} alters the neuronal membrane integrity in terms of increased fluorescence polarisation, with consequent decreased fluidity of membranes. Thus it can be suggested that peroxidation of membrane lipids might be responsible for rigidifying the membranes, a fact corroborated by Gutteridge and Halliwell [26] and may effect the functioning of membrane-bound enzymes, receptors and channels.

We have observed an enhanced spontaneous release of neurotransmitters from nerve terminals following Al^{3+} exposure [27], an observation justified by the increased $[Ca^{2+}]_i$ since neurotransmitter release has been reported to be linked with the levels of $[Ca^{2+}]_i$ [28]. All Ca^{2+} regulated neuronal functions like synaptic transmission, dendritic induction, axonal transport and CaM-activated enzymes would be affected by increased $[Ca^{2+}]_i$. Enhanced $[Ca^{2+}]_i$ may have dramatic consequences on the developing brain, by decreasing neurite outgrowth, pruning dendrites and ultimately resulting in death of individual neurons.

Voltage-operated calcium channels open transiently in response to depolarization, to regulate the entry of calcium through the neuronal membranes [29]. This influx of Ca^{2+} then provides the stimulus for the release of neurotransmitters from the synaptic vesicles into the synaptic cleft. Aluminium was seen to have an inhibitory effect on both the depolarization and polarization dependent $^{45}Ca^{2+}$ influx, in a concentration-dependent manner. The IC_{50} concentration of Al^{3+} for net K^+-induced $^{45}Ca^{2+}$ influx was 50 μM, suggesting thereby that Al^{3+} is capable of displacing Ca^{2+} transport, in a manner consistent with the observation of Nachshen [30] who in a study on the effects of varying concentrations of Ca^{2+} (0–2.5 mM) in the extracellular medium, reported Al^{3+} to act as a partially competitive inhibitor, preventing Ca^{2+} ions from reaching a binding site on or within the channel. However, the inhibitory effect is not solely competitive, but is rather better described to be of a mixed type. It can be proposed that in addition to a competitive interaction with the Ca^{2+} binding site, there are Al^{3+}-induced perturbations, either in the structure of the channel molecule itself or in the phospholipid domain in close proximity to the channels as evident from the altered membrane fluidity of neuronal cells, demonstrated in the present study. Such a competition between Ca^{2+} and Al^{3+} has been observed in intestinal absorption of Ca^{2+} and deposition in bone [31]. In vivo Al^{3+} exposure was seen to decrease the fast phase of Ca^{2+} uptake by the VOCC, a fact supported by the observations of Koenig and Jope [1]. This observation suggests that Al^{3+}-induced reduction in the capacity of the neurons to take up Ca^{2+} could dramatically alter intraneuronal Ca^{2+} homeostasis with resultant impaired neurotransmission. The decreased depolarization-dependent Ca^{2+} uptake has also been reported in neuronal disorders and during the aging process [32,33]. Therefore, these results demonstrate that decreased Ca^{2+} uptake would significantly alter Ca^{2+} homeostasis with obvious detrimental consequences.

Our study has demonstrated a decreased Ca^{2+} extrusion capacity of the cell in terms of decreased Ca^{2+} ATPase activity resulting in an enhanced $[Ca^{2+}]_i$ and consequent altered neuronal function. Since altered concentrations of Ca^{2+} binding proteins have been reported in diseased states of CNS [34], therefore the effect of Al^{3+} on Ca^{2+} binding regulatory proteins was studied. Aluminium exposure was seen to alter the biological activity of CaM which may consequently have detrimental effects on the vital CaM-dependent processes, such as the regulation of disassembly of cellular cytoskeleton through inhibition of the polymerization of cytoskeletal proteins, neurotransmitter synthesis and release, metabolism of carbohydrates, lipids and amino acids and perhaps gene expression [35].

Protein kinase C is a Ca^{2+} phospholipid-dependent kinase. It is highly concentrated in the brain [36], localized in the presynaptic nerve terminal [37] and regulates neurotransmitter release in the mammalian CNS via a G protein-coupled mechanism [38]. Inhibition of PKC following Al^{3+} exposure, as observed in the present study, might induce perturbations in the phospholipid domains, consequently altering Al^{3+} to modulate the intramembranal G-protein, which regulates Ca^{2+} channel function and PKC activity. Thus learning and memory deficits might result from reduced neurotransmitter release due to Al^{3+} induced inhibition of PKC, and Ca^{2+} utilization at the presynaptic site. Moreover, our results demonstrate maximum Al^{3+} accumulation in the hippocampus (80-fold), a region associated with the processes of memory and learning, further substantiating the evidence that Al^{3+} plays a role in the etiopathogenesis of Alzheimer's disease and other neurodegenerative disorders.

The mechanisms responsible for the neurotoxic effects of Al^{3+} are not very well understood. As Al^{3+} is a multivalent cation with reported effects on membranes, membrane proteins and some Ca^{2+} binding proteins, the effect of Al^{3+} on synaptosomal Ca^{2+} homeostatic mechanisms was studied. Based on the present results, it can be implied reasonably, that Al^{3+} can effectively alter Ca^{2+} homeostatic mechanisms by interfering with the 'steady state' Ca^{2+} homeostasis, following an inhibition of Ca^{2+} ATPase, thereby altering levels of resting $[Ca^{2+}]$. Alternatively, it could also interfere with transient fluxes through Ca^{2+} channels in synaptic plasma membrane, thereby altering the excitability of neurons and eventually conduc-

tion of action potential along the axons. The action of calcium receptor proteins CaM and PKC was also impaired, thereby effecting neuronal functioning.

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