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# Lipid Peroxidation and Aluminium Effects on the Cholinergic System in Nerve Terminals

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In the present study, we analyzed how aluminium and oxidative stress induced by ascorbate/$Fe^{2+}$ affect the mechanisms related with the cholinergic system in a crude synaptosomal fraction isolated from rat brain. [$^3$H]Choline uptake, [$^3$H]acetylcholine release, membrane potential and $Na^+/K^+$-ATPase activity were determined in the presence or in the absence of aluminium in control conditions and in the presence of ascorbate (0.8 mM)/$Fe^{2+}$ (2.5 μM). The extent of lipid peroxidation was measured by quantifying thiobarbituric acid reactive substances (TBARS). Under oxidizing conditions aluminium increased the formation of TBARS by about 30 %, but was without effect when the synaptosomal preparation was incubated in the absence of oxidants. Additionally, aluminium potentiated the inhibition of the high-affinity [$^3$H]choline uptake observed following lipid peroxidation and had the same effect on the $Na^+/K^+$-ATPase activity. [$^3$H]Acetylcholine release induced by 4-aminopyridine, and membrane potential were not significantly affected under oxidizing conditions, either in the absence or in the presence of aluminium. We can conclude that aluminium, by potentiating lipid peroxidation, affects the uptake of choline in nerve endings. This effect, occurring during brain oxidative injury, might contribute to the cholinergic dysfunction and neuronal cell degeneration known to occur in Alzheimer's disease.

*Keywords:* Aluminium toxicity, cholinergic system, crude synaptosomal fraction, lipid peroxidation, oxidative stress

## INTRODUCTION

There is evidence that aluminium, a metal without redox capacity in biological systems, has the capacity to stimulate lipid peroxidation induced by $Fe^{2+}$ in liposomes (Gutteridge et. al., 1985; Verstraeten and Oteiza, 1995), brain homogenates (Fraga et. al., 1990; Oteiza et. al., 1993), brain microsomes and myelin (Oteiza et. al., 1993; Verstraeten et. al., 1997) and bovine brain phosphatidylserine (Xie and Yokel, 1996). These findings suggest that the stimulatory effect of aluminium on lipid peroxidation plays an important role in various neurological disorders associated with increased levels of aluminium ion. Levels of reactive oxygen species were reported to be elevated in synaptosomes isolated from brain of animals treated with aluminium gluconate (Bondy et. al., 1998).

Reactive oxygen species and consequently lipid peroxidation have been suggested to be implicated in the development of neurological diseases (Halliwell and Gutteridge, 1985; Benzi and Moretti, 1995). Chronic dietary intoxication of mice with aluminium has been shown to

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cause the accumulation of aluminium in brain and to increase lipid peroxidation rates (Fraga et. al., 1990; Verstraeten et. al., 1997). Although highly controversial, aluminium has been suggested to be one of the neurotoxic factors implicated in neurodegenerative disorders, such as Alzheimer's disease (Deloncle and Guillard, 1990). Elevated levels of aluminium were found in the brain (Kruck and McLachlan, 1988), in neurofibrillary tangles (Perl, 1988) and in senile plaques (Tokutake et. al., 1995) of Alzheimer's patients. A syndrome of aluminium toxicity is well established in patients with chronic renal failure treated by haemodialysis (Wills and Savory, 1988).

An impairment of the cholinergic system in Alzheimer's patients brain, has also been reported (Whitehouse et. al., 1982; Coyle et. al., 1983). Several lines of evidence support the idea that accumulation of aluminium in the brain might contribute to the cholinergic deficiency observed (Meiri et. al., 1993; Bielarczyk et. al., 1998). Therefore, we investigated whether aluminium and oxidative stress, induced by the oxidant pair ascorbate/$Fe^{2+}$, affected [$^3$H]choline uptake and [$^3$H]acetylcholine release in a crude synaptosomal preparation. To evaluate the functional state of the preparation we also measured membrane potential and $Na^+/K^+$-ATPase activity. We observed a decrease of the choline uptake induced by lipid peroxidation in the presence of aluminium, as well as of $Na^+/K^+$-ATPase activity, suggesting that the reduction in choline uptake is due to an apparent energy loss and probable changes in the ionic gradients needed for transport.

## MATERIALS AND METHODS

### Materials

[Methyl-$^3$H]choline chloride (specific activity 3.07 TBq/mmol; 83.0 Ci/mmol) was purchased from Amersham International (UK); 3-hemicholinium, 4-aminopyridine and ouabain were obtained from Sigma Chemical Co. (USA). All other reagents used were of analytical grade.

### Preparation of crude synaptosomal fractions

Crude synaptosomal fractions ($P_2$) were prepared from brain of male Wistar rats according to the method of Hajós (1975), slightly modified (Carvalho and Carvalho, 1979). Total cerebral cortex was homogenized in 10 volumes of 0.32 M sucrose, 10 mM Hepes-Na, pH 7.4 and centrifuged at 1500 *g* for 10 min. After centrifugation of the supernatant at 9000 *g* for 20 min the pellets were washed and resuspended in 0.32 M sucrose 10 mM Hepes-Na, pH 7.4. The protein content of the suspension was estimated by the biuret method (Layne, 1957) using bovine serum albumin as standard.

### Measurement of lipid peroxidation

Ascorbic acid and $Fe^{2+}$ were used to induce lipid peroxidation (Wills, 1969), and the extent of the peroxidative process was determined by the thiobarbituric acid (TBA) test (Ernster and Nerdenbrand, 1967). $AlCl_3.6H_2O$ (0.5 mM), or ascorbic acid (0.8 mM) plus $FeSO_4$ (2.5 μM), or ascorbic acid and $FeSO_4$ plus $AlCl_3.6H_2O$ were added to 5 ml of the incubation medium containing: 128 mM NaCl, 3 mM KCl, 1.2 mM $MgCl_2.6H_2O$, 1 mM $CaCl_2.2H_2O$ 10 mM glucose 50 mM Hepes-Na, pH 7.4, and synaptosomal preparation (1 mg/ml of protein, final concentration). The samples were then incubated at 30 °C for 15 min in a water bath with continuous stirring. Controls were incubated at 30 °C during the same period of time, in the absence of ascorbic acid, $FeSO_4$ or $AlCl_3.6H_2O$. The reaction was stopped by placing the tubes in ice. To measure the extent of lipid peroxidation, 0.5 ml of cold 40% trichloroacetic acid (TCA) and 2 ml of 0.67%

TBA with 6.8 mM butylated hydroxy toluene (BHT), were added to 0.5 ml of sample material, and the tubes were treated for 10 min in a boiling water bath. The tubes were allowed to cool at room temperature, and centrifuged at 1500 *g* for 10 min. The supernatant was collected and the absorbance measured at 530 nm in a Philips PU 8630 UV/VIS/NIR spectrophotometer. The amount of thiobarbituric acid reactive substances (TBARS) formed was calculated using a molar extinction coefficient of $1.56 \times 10^5$ $cm^2 \times mol^{-1}$ and expressed as nmol TBARS/mg of protein (Ernster and Nerdenbrand, 1967). The TBARS levels presented correspond to the difference between TBARS levels quantified after incubation of $P_2$ in the presence or in the absence of the oxidant system.

### [$^3$H]Choline uptake

Crude synaptosomal fractions at a concentration of 0.5 mg/ml were equilibrated at 30 °C in incubation medium containing various concentrations of aluminium (0.1 mM, 0.2 mM, 0.4 mM and 0.5 mM), in control and peroxidizing conditions (with 0.8 mM ascorbic acid and 2.5 μM $FeSO_4$ at 30 °C for 15 min). After 15 min of preincubation the reaction was started by adding [$^3$H]choline (final concentration 0.125 μM, 10 mCi/ml). In some experiments the reaction was performed at 0–4 °C or in the presence of 10 μM 3-hemicholinium (3-HC). After various periods of time (1, 5, 10 and 15 min) the reaction was stopped by rapid filtration of samples (0.5 ml containing 0.25 mg of protein) under vacuum through Whatman GF/B filters, followed by two washes with 5 ml of cold $Na^+$ medium supplemented with 1 mM choline chloride. The radioactivity retained in the filters was measured using Universol cocktail (ICN, Irvine. CA. USA), in a Packard Tri-Carb 2500 TR spectrometer provided with disintegrations per minute correction. Results are expressed as pmol [$^3$H]choline uptake/mg protein.

### [$^3$H]Acetylcholine release experiments

Crude synaptosomal fractions were preequilibrated at 30°C for 5 min in incubation medium and then were loaded with [$^3$H]choline (the precursor of acetylcholine, final concentration 10 mCi/ml, 0.125 μM) for 15 min. At this time [$^3$H]choline was shown to be carried into the nerve terminals and used for the acetylcholine synthesis. After the loading period the peroxidation (with 0.8 mM ascorbic acid and 2.5 μM $FeSO_4$, for 15 min at 30 °C) was induced in the presence or absence of 0.5 mM $AlCl_3.6H_2O$. Preparations were then superfused essentially according to the original technique described by Raiteri et al. (1974), with minor modifications (Santos et. al., 1987). Briefly, aliquots of 500 μl were aspirated and layered over Whatman GF/B filters by using a peristaltic pump (flow rate 0.6 ml/min, kept constant throughout the experiment). After setting up the preparations and before starting sample collection, a 5 min washout with incubation medium enriched with 1 mM choline chloride and 10 μM 3-HC was performed, followed by a second 10 min washout with the same medium but without choline chloride. During this period all the [$^3$H]choline not accumulated was removed and a baseline for [$^3$H]choline was determined. The effluent was then collected in 1 min fractions for scintillation spectrometry analysis (500 μl collected fraction in 4 ml Universal scintillation liquid). The preparations were stimulated with 100 μM 4-aminopyridine (4-AP) 2 min after starting sample collection. At the end of the experiments, the filters were removed from the superfusion chambers and analysed by scintillation spectrometry for determination of tritium retained in preparation. The fractional release was expressed in terms of the percentage of total radioactivity present in the preparation at the beginning of the collection period. Separation of [$^3$H]ACh from [$^3$H]choline of each collected sample from the superfusion experiment was carried out as described previously (Rand and Johnson, 1981; Santos et al., 1998).

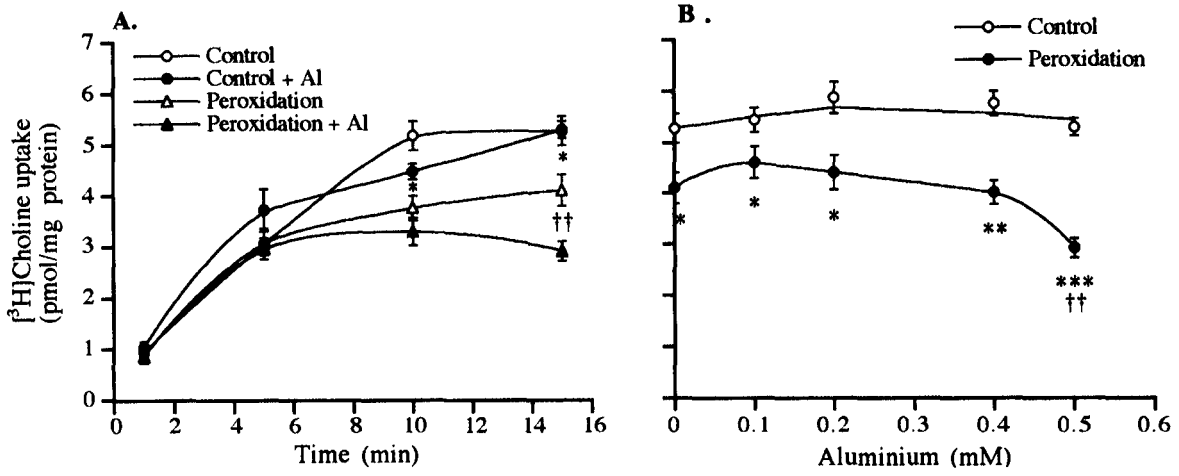


FIGURE 1 **A** - Effect of aluminium on [$^3$H]choline uptake by a crude synaptosomal fraction submitted to lipid peroxidation induced by ascorbate/$Fe^{2+}$. **B** - Effect of different aluminium concentrations on [$^3$H]choline uptake, at 15 min incubation, under control conditions or submitted to lipid peroxidation induced by ascorbate/$Fe^{2+}$. **A/B** -[$^3$H]Choline uptake experiments were carried out at 30 °C in a medium containing 0.8 mM ascorbic acid/2.5 μM $FeSO_4$ for 15 min in the absence or in the presence of 0.5 mM $AlCl_3.6H_2O$ (**A**) or in the presence of 0.1, 0.2, 0.4 and 0.5 mM $AlCl_3.6H_2O$ (**B**), as described in Materials and Methods. Results represent the mean ± SEM of four to nine animals. Values statistically different from respective controls $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; $^{\dagger\dagger}$values statistically different when compared to peroxidation conditions from peroxidized ($p < 0.01$)

TABLE I Effect of aluminium on lipid peroxidation in rat brain synaptosomal preparations

| | *TBARS (nmol/mg protein)* |
|---|---|
| Control | |
| -Al | 2.24 ± 0.15 |
| +Al | 2.13 ± 0.19 |
| Oxidizing system | |
| -Al | 11.80 ± 0.52[a] |
| +Al | 15.83 ± 0.73[ab] |

Results represent the mean ± SEM for duplicates measurements on nine to fifteen animals.

a. Values statistically different from controls ($p < 0.001$).
b. Values statistically different from peroxidized preparations in the absence of Al ($p < 0.001$).

## Determination of membrane potential

Membrane potential was estimated by calculating the transmembrane distribution of the tetraphenylphosphonium cation ($TPP^+$) with an electrode selective for $TPP^+$using a calomel electrode as reference (Muratsugu et. al., 1977; Kamo et. al., 1979). The reactions were performed at 30 °C in an open thermostated reaction chamber, with continuous magnetic stirring, in 0.75 ml incubation medium, pH 7.4, supplemented with 3 μM $TPP^+$ and 0.25 mg protein. $TPP^+$ uptake was measured from the decreased $TPP^+$ concentration in the medium and was followed during time for about 15 min. The effects of lipid peroxidation and/or aluminium were evaluated by the addition of synaptosomal fraction, treated immediately before with the oxidant pair, ascorbic acid (0.8 mM)/$FeSO_4$ (2.5 μM), in the presence or in the absence of aluminium or by aluminium alone, for 15 min at 30 °C, to the reaction medium. Membrane potential was determined by using the equation (at 25 °C): $\Delta\Psi$ (mV) = 59 log (v/V) − 59 log ($10^{\Delta E/59}$ − 1) as described by Muratsugu et. al., (1977) and Kamo et. al., (1979), in which v, V and ΔE stand for mitochondrial volume, volume of incubation

medium, and deflection of the electrode potential from the baseline, respectively. An internal synaptosomal volume of 3.23 µl/mg protein was assumed (Scott and Nicholls, 1980). The values given for the membrane potential were determined after correcting for the $TPP^+$ taken up in the absence of a $K^+$ gradient (medium containing 131 mM KCl, 1.2 mM $MgCl_2.6H_2O$, 1 mM $CaCl_2.2H_2O$, 10 mM glucose and 50 mM Hepes-Na, pH 7.4), which gives the mitochondrial contribution, and that of $TPP^+$ binding for the total $TPP^+$ uptake.

### Measurement of $Na^+/K^+$-ATPase activity

$Na^+/K^+$-ATPase activity was determined in crude synaptosomal fractions presubmitted to aluminium treatment and to lipid peroxidation, in the absence or in the presence of aluminium. Following experimental treatment the preparation was centrifuged, washed in incubation medium and the final pellet stored at –80 °C for later ATPase activity measurements. Samples, at a concentration of 0.1 mg protein/ml, were preincubated for 10 min in an assay medium containing: 132 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 0.1 mM EGTA and 10 mM Hepes-$Na^+$, pH 7.4, in the absence or in the presence of 0.5 mM ouabain. The reaction was initiated by adding 1 mM ATP-Mg, and after 15 min of incubation ice-cold 10% TCA was added to the samples to stop the reaction. The samples were kept on ice, centrifuged and the supernatant analysed for inorganic phosphate released using the colorimetric method of Taussky and Shorr (1953). $Na^+/K^+$-ATPase activity was taken as the difference between the total ATPase activity and the activity measured in the presence of 0.5 mM ouabain.

### Analysis of data and statistics

Results are presented as means ± SEM of the number of animals indicated in the figures legends. Statistical significance was determined using one-way Anova or by the two-tailed Student's *t*-test.

## RESULTS

### Influence of aluminium on lipid peroxidation

Lipid peroxidation was induced by the oxidizing system 0.8 mM ascorbic acid/2.5 µM $FeSO_4$ in the presence or in the absence of 0.5 mM $AlCl_3.6H_2O$, pH 7.4, during 15 min, at 30 °C. Table I shows that, in control conditions, there is no significant difference in the TBARS formation in the presence (2.13 ± 0.19 nmol TBARS/mg protein) or in the absence of aluminium (2.24 ± 0.15 nmol TBARS/mg protein). However, in the presence of oxidizing agents the addition of aluminium (0.5 mM) induced a statistically significant increase ($p < 0.001$) in TBARS formation (15.83 ± 0.73 nmol TBARS/mg protein) as compared to control (11.80 ± 0.52 nmol TBARS/mg protein), and this rise was even markedly higher than that observed with ascorbate/$Fe^{2+}$ alone.

### Influence of lipid peroxidation and aluminium on [$^3$H]choline uptake

The influence of lipid peroxidation, induced by ascorbate/$Fe^{2+}$and aluminium (0.1, 0.2, 0.4 and 0.5 mM) on [$^3$H]choline uptake was studied. We observed that [$^3$H]choline uptake (5.27 ± 0.28 pmol/mg protein) was not significantly altered in the presence of aluminium (5.30 ± 0.16 pmol/mg protein) for 15 min (Fig. 1A). However, lipid peroxidation induced a statistically significant reduction ($p < 0.05$) of [$^3$H]choline uptake, at 15 min (from 5.27 ± 0.28 to 4.10 ± 0.30 pmol/mg protein), when compared to the controls. A significant reduction ($p < 0.05$) was already present at 10 min (from 5.18 ± 0.28 to 3.75 ± 0.24 pmol/mg protein). When peroxidation was induced in the presence of alumi-

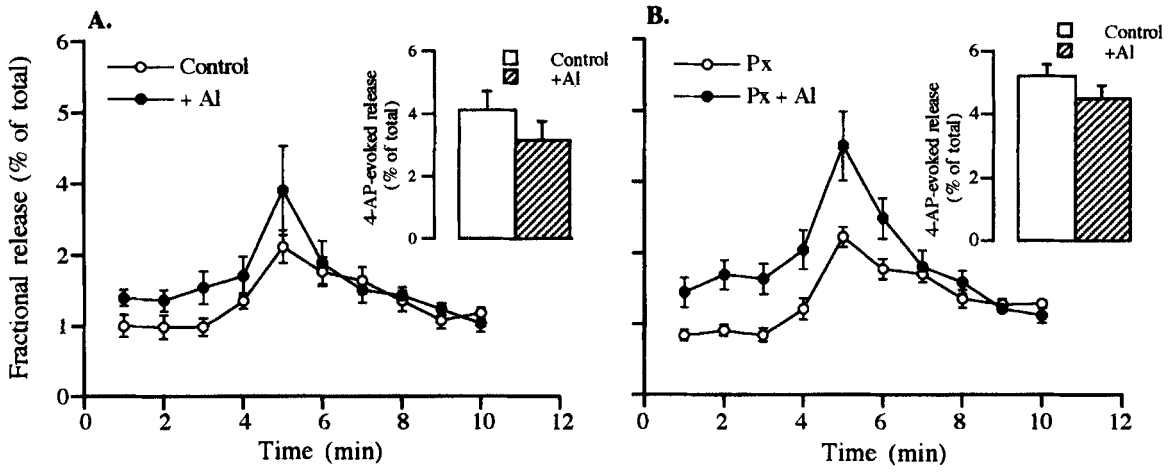


FIGURE 2 **A** – Effect of aluminium and **B** - Effect of lipid peroxidation and aluminium on tritium release by a crude synaptosomal fraction preloaded for 15 min at 30 °C with [$^3$H]choline and then peroxidized (Px) with 0.8 mM ascorbic acid/2.5 μM $FeSO_4$ for 15 min in the presence or absence of 0.5 mM aluminium. Samples (500 μl) were removed and placed in a perfusion system. The effluent was collected at a 1 min intervals. Two min after initiating of the collection, the preparations were stimulated with 100 μM 4-aminopyridine (4-AP), as described in Materials and Methods. The inserted histograms represent the effect of aluminium on the 4-AP induced release in oxidized preparations (percentage of total tritium release, which represents mainly [$^3$H]acetylcholine, released over the basal). Results represent the mean ± SEM for eight to ten animals

nium a statistically significant reduction ($p < 0.01$) in [$^3$H]choline uptake was observed (2.91 ± 0.19 pmol/mg protein) when compared to the uptake measured in peroxidizing conditions in the absence of aluminium (4.10 ± 0.30 pmol/mg protein) at 15 min (Fig. 1A). A 15 min incubation time was chosen for all further loading periods of with [$^3$H]choline, The presence of 0.5 mM aluminium caused a significant potentiation ($p < 0.01$) of lipid peroxidation mediated inhibition of [$^3$H]choline uptake (Fig 1B).

### Influence of lipid peroxidation and aluminium on [$^3$H]acetylcholine release

Figure 2 shows that 4-AP caused a transient increase in tritium release from crude synaptosomes. In control conditions [$^3$H] ACh accounted for 90 ± 7% (n = 3) of the 4-AP evoked tritium release and 30 ± 2% (n = 3) of the basal tritium release that occurred before stimulation. This indicates that the evoked tritium release from preparations loaded with [$^3$H] choline is a good measure of the induced release of [$^3$H]ACh (see Rodrigo et al., 1994). It has been shown that 4-AP induces only the vesicular release of neurotransmitters by promoting the firing of spontaneous action potentials (Santos et al., 1992). The 4-AP induced [$^3$H]ACh release from preparations treated with aluminium (3.09 ± 0.46 %) was not statistically different from that determined in controls (4.15 ± 0.59 %) (Fig. 2A). In the presence of oxidizing agents no significant difference in the release of [$^3$H]acetylcholine release was observed either in the absence or in the presence of aluminium (Fig. 2B). The amount of [$^3$H]ACh released upon peroxidation (5.21 ± 0.36 % total release/5 min) was similar to that observed in peroxidation conditions in the presence of aluminium (4.51 ± 0.39 % total release/5min). However, in the presence of aluminium, a significant increase in the basal release of tritium was observed when compared to the basal tritium release observed in the absence of aluminium.

**Influence of lipid peroxidation and aluminium on membrane potential and on $Na^+/K^+$-ATPase activity**

The influence of lipid peroxidation and aluminium on membrane potential was evaluated by using a $TPP^+$ sensitive electrode. In control conditions the membrane potential was –52.83 ± 1.88 mV, similar to that calculated in the preparation treated with aluminium (-52.39 ± 1.50 mV), in peroxidizing conditions (-48.54 ± 2.96 mV) and in peroxidizing conditions in the presence of aluminium (-43.21 ± 3.79 mV) where a small difference, but statistically not significant, was observed (Table II).

Figure 3 shows that aluminium potentiates the peroxidation-induced inhibition of the $Na^+/K^+$-ATPase activity. The enzyme activity significantly decreases in peroxidized preparation (67.61 ± 8.25 nmol Pi/mg protein/min) as compared to controls (100.85 ± 6.78 nmol Pi/mg protein/min). Aluminium increases the inhibition observed in the presence of ascorbate/$Fe^{2+}$ by about 20% (from 67.61 ± 8.25 to 51.46 ± 4.63 nmol Pi/mg protein/min). The activity of $Na^+/K^+$-ATPase in the presence of aluminium (85.44 ± 10.06 nmol Pi/mg protein/min) is not statistically different when compared to controls.

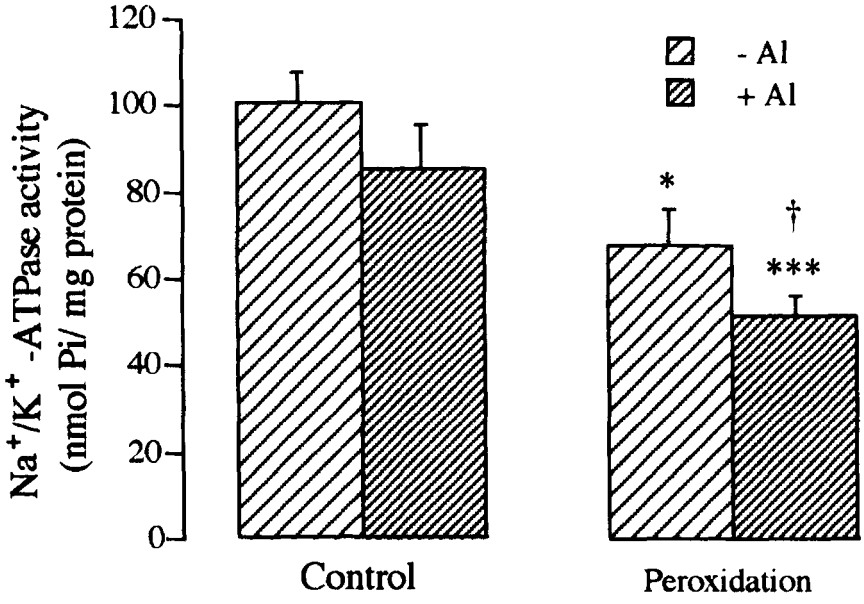


FIGURE 3 Effect of lipid peroxidation and aluminium on $Na^+/K^+$-ATPase activity. Crude synaptosomal fractions were preincubated by peroxidation with 0.8 mM ascorbic acid/ 2.5 μM $FeSO_4$ for 15 min at 30 °C in the presence or absence of 0.5 mM aluminium. Following preincubation the $Na^+/K^+$-ATPase activity was determined as described in Materials and Methods. Results represent the mean ± SEM for six animals. Values statistically different as compared with the respective controls, $^*p < 0.05$, $^{**}p < 0.001$; $^\dagger$values statistically different when compared with preparations peroxidized in the absence of Al ($p < 0.05$)

TABLE II Effects of lipid peroxidation and aluminium on membrane potential in rat brain synaptosomal preparations

| | | *Membrane Potential (mV)* |
|---|---|---|
| Control | | |
| | -Al | − 52.83 ± 1.88 |
| | +Al | − 52.39 ± 1.50 |
| Oxidizing system | | |
| | -Al | − 48.54 ± 2.97 |
| | +Al | − 43.21 ± 3.79 |

Membrane potential was measured at 30 °C by following the uptake of $TPP^+$ in incubation medium supplemented with 3 μM $TPP^+$, as described in Materials and Methods. Results represent the mean ± SEM of four animals.

## DISCUSSION

In the present study it was shown that aluminium was not able to induce lipid peroxidation by itself, but promotes lipid peroxidation initiated by the oxidizing system ascorbate/$Fe^{2+}$, which is in agreement with reports of several authors (Gutteridge et. al., 1985; Oteiza et. al., 1993; Xie and Yokel, 1996). It has been suggested that aluminium ions produce an alteration in membrane structure that facilitates lipid peroxidation (Gutteridge et. al., 1985). Van Rensburg et. al. (1995) hypothesized that aluminium ions bind to phosphate groups of membrane phospholipids, the positive charge of aluminium ions interact with the polar groups of these lipids, causing gaps to develop in the membrane; so fatty acids, thus exposed, could be attacked by iron-induced free radicals. Aluminium could also potentiate the pro-oxidant properties of iron by stabilizing ferrous ions in its more damaging ferrous ($Fe^{2+}$) form, which can promote the Fenton reaction (Yang et al., 1999). Recently, we reported that in the presence of an oxidizing system aluminium accumulation was significantly increased in synaptosomes, suggesting a possible role for aluminium in the promotion and enhancement of oxidant-induced damage believed to occur in neuronal cell degeneration (Amador et. al., 1999).

As reported previously, we observed that [$^3$H]choline is accumulated by a high affinity transport mechanism mediated by a membrane carrier, since it is inhibited by 3-HC and when the uptake assay was performed at 0–4 °C (data not shown; Worrall and Williams, 1994). Lipid peroxidation decreased significantly the [$^3$H]choline uptake and this decrease was enhanced when the synaptosomal preparation was peroxidized in the presence of aluminium (Fig. 1). A decrease on [$^3$H]choline uptake was also observed with $Fe^{2+}$-induced oxidative stress (Cancela et. al., 1994), during aging (Fong et. al., 1995), following traumatic brain injury (Dixon et. al., 1995a) and in acidosis conditions (Cancela et. al., 1994). The alteration in high affinity choline uptake has been previously reported to be responsible for the decreased acetylcholine levels in different brain regions following aluminium exposure (Julka et. al., 1995). On the other hand, using rat synaptosomes Lai et al. (1980), observed that higher aluminium concentrations ($IC_{50} = 224$ μM in the absence of $Ca^{2+}$and $IC_{50} = 123$ μM in the presence of $Ca^{2+}$) were needed to inhibit choline uptake. As the high-affinity choline transporter is coupled to an electrochemical sodium ion gradient (Worrall and Williams, 1994) it is probable that, the mechanism through which aluminium interferes with the uptake of choline might be dependent on the sodium pump activity. In fact, several authors have described the inhibitory effect of aluminium on the activity of $Na^+/K^+$-ATPase (Lai et. al., 1980; Rao, 1990; Caspers et. al., 1993; Sarin et. al., 1997). Our results demonstrated that aluminium by itself was without effect on the enzyme activity (Fig. 3). However, in the presence of ascorbate/$Fe^{2+}$, which increases the level of reactive oxygen species with consequent lipid peroxidation, aluminium potentiated the inhibition of $Na^+/K^+$-ATPase activity. It was reported that the inhibition of $Na^+/K^+$-ATPase may contribute to produce the neuronal dysfunction

found in neurodegenerative disorders (Lee, 1991). The decrease of choline uptake may also be due to modifications on the membrane transporter protein induced by lipid peroxidation (Palmeira et. al., 1993). Aluminium increases this effect by potentiating the lipid peroxidation process (Table I). Cancela et al. (1994), have shown that $Fe^{2+}$-induced lipid peroxidation leads to a decrease in the high-affinity choline uptake, but a direct interaction of $Fe^{2+}$with the carrier is unlikely.

Results reporting membrane lipid peroxidation and $Na^+$, $K^+$-ATPase activities may be affected by the presence of myelin in synaptosomal preparations. Recently, Verstraeten et al. (1997) reported that myelin showed a higher susceptibility to $Al^{3+}$-mediated lipid oxidation as compared to that of synaptic membranes. Since myelin has a higher relative content of lipids (lipid:protein 70%) compared to other membranes it could be a preferential target for $Al^{3+}$-induced changes in membrane physical properties namely membrane rigidification, phase separation and enhancement of lipid oxidation rates (Oteiza et al., 1993; Verstraeten and Oteiza, 1995; Verstraeten et. al., 1997)

To induce acetylcholine release we used 4-AP, in order to induce only the vesicular release by promoting the firing of spontaneous action potentials. We found that lipid peroxidation and /or aluminium did not modify the 4-AP induced [$^3$H]acetylcholine release, which suggests that the vesicular release of ACh, in our experimental conditions, is not affected by oxidative stress conditions and aluminium (Fig. 1). However, a decrease in the release of acetylcholine after ascorbate/$Fe^{2+}$ induced lipid peroxidation (Meyer and Judkins, 1993), aluminium treatment (Bielarczyk et al., 1998), during aging (Meyer et. al., 1986) and following traumatic brain injury (Dixon et. al., 1995b) has been reported. These differences on acetylcholine release are probably due to different experimental conditions used. Recently, it has been reported that the inhibition of the $Ca^{2+}$-dependent acetylcholine release by aluminium was caused by the inhibitory interaction of Al-Pi anionic forms with the calcium influx through the plasma membranes (Bielarczyk et al., 1998). The increase in the basal release that we observed when preparations were peroxidized in the presence of aluminium may represent the release of cytoplasmic [$^3$H]choline (not vesicular [$^3$H]ACh), which was not used for [$^3$H]acetylcholine synthesis, probably due to the reversal of the plasma membrane carrier responsible for the uptake of choline, since under these conditions (peroxidation in the presence of aluminium) the membrane structure might be altered.

We found that, the membrane potential of our crude synaptosomal preparation was maintained after lipid peroxidation and/or aluminium treatment (Table II). This evidence suggests that synaptosomes maintain the membrane functional integrity, in the conditions studied. Previous work by other investigators and by using different biological preparations, is consistent with our results (Palmeira et. al., 1993; Pereira et. al., 1996; Tretter and Vizi 1996).

According to the results presented, we can conclude that aluminium by itself can not induce any alteration in the studied parameters. However, aluminium potentiates the alterations induced by lipid peroxidation. The alteration of the cholinergic system, observed under our experimental conditions, results from a reduction in the choline uptake, probably due to an alteration of the choline membrane carrier activity resulting from a change in membrane fluidity. Also, the observed decrement in $Na^+/K^+$-ATPase activity and choline uptake inhibition, under oxidizing conditions, suggests that the reduction in the uptake is due to a change in the ionic gradients needed for the transport.

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