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Myelin Is a Preferential Target of Aluminum-Mediated Oxidative Damage

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The capacity of Al^{3+} to promote oxidative damage to brain membranes was investigated both *in vitro* and *in vivo*. *In vitro*, Al^{3+} and related metals (Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+}) stimulated Fe^{2+}-initiated lipid and protein oxidation in brain myelin and synaptic membranes. Al^{3+}, Sc^{3+}, Y^{3+}, and La^{3+} significantly promoted protein-associated carbonyl production in myelin, while in synaptic membranes, the stimulatory effect was observed in the presence of Ga^{3+}, In^{3+}, Y^{3+}, Sc^{3+}, and La^{3+}. In myelin the magnitude of the stimulation of lipid oxidation followed the order Sc^{3+}, Y^{3+}, La^{3+} > Al^{3+}, Ga^{3+}, In^{3+} > Be^{2+}. When compared to mitochondria and microsomal and synaptic membranes, myelin showed a marked susceptibility to Al^{3+}-mediated lipid peroxidation. The differential susceptibility of myelin compared to synaptic membranes could not be explained by differences in membrane composition, since the relative content of negatively charged phospholipids (binding sites) was similar for both membranes, and myelin had a lower content of poly-unsaturated fatty acids (substrates of lipid oxidation) and a higher concentration of α-tocopherol compared to synaptic membranes. In a model of Al^{3+} intoxication imposed to mice during pregnancy and early development, a 72% higher content of lipid peroxidation products was found in brain myelin. The fluidity of myelin evaluated by the polarization fluorescence of 1,3-diphenylhexatriene was significantly higher in the Al^{3+}-intoxicated mice than in controls. Since myelin has a high relative content of lipid:protein compared to other membranes, these results support our hypothesis that ions without redox capacity can stimulate *in vitro* and *in vivo* lipid oxidation by promoting phase separation and membrane rigidification, thus accelerating lipid oxidation.

Key Words: **aluminum; myelin; lipid peroxidation; free radicals; neurotoxicity.**

Aluminum (Al^{3+}) is one of the most abundant metals in the environment and is proposed to be involved in several neuropathologies (1, 2). Golub *et al.* (3) reported that low-level exposure to Al^{3+} during development and early adulthood in mice affected neurobehavioral parameters and reduced trace element concentrations in the central nervous system. Previous studies from our laboratory demonstrated that Al^{3+}, a metal without redox capacity, stimulates lipid peroxidation in the presence of Fe^{2+} in brain membranes (4) and in liposomes (5). This effect of Al^{3+} on Fe^{2+}-initiated lipid peroxidation was shared by other chemically and physically related metals, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} (6).

Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} have no redox capacity in biological systems but can stimulate lipid peroxidation by interacting with membrane phospholipids causing changes in membrane physical properties. In fact, Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} cause liposome aggregation, fusion, and permeabilization and those effects were positively correlated with the capacity of the metals to promote Fe^{2+}-initiated oxidative damage to membrane lipids (6). Recent evidence (7) indicates that Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} promote the formation of clusters of negatively charged phospholipids, where the mobility of the acyl chains from fatty acids could be restricted, thus accelerating lipid peroxidation.

In mice chronically fed high Al^{3+} diets, higher levels of lipid peroxidation products were found in brain (8). Since myelin is a membrane with a high relative content of phospholipids, it constitutes a potential target for membrane oxidative damage mediated by Al^{3+} and

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related metals. In the present work we evaluated the effect of Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} on Fe^{2+}-initiated lipid and protein oxidation in membranes isolated from rat brain. We also characterized the *in vivo* susceptibility of myelin to Al^{3+}-mediated oxidative damage in mice fed high Al^{3+} diets during pregnancy and early development.

MATERIALS AND METHODS

Chemicals. Aluminum(III) potassium sulfate, gallium(III) chloride, indium(III) chloride, yttrium(III) chloride, scandium(III) chloride, and beryllium(II) sulfate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Lanthanum(III) chloride was from Sigma Chemicals Co. (St. Louis, MO). Diphenylhexatriene was from Molecular Probes Inc. (Eugene, OR).

Animals. The *in vitro* experiments were carried out using adult Wistar rats (250 g) fed a semipurified commercial chow. For the *in vivo* experiments NIH mice (seven animals per group) were fed purified diets containing either 3 or 1000 μg Al/g diet (Al 3 and Al 1000, respectively) from Day 0 of pregnancy (9). At Day 40 of age, their offspring were killed by overexposure to carbon dioxide and brains were quickly excised and placed at 4°C.

Subcelullar fractionation. Myelin, synaptic membranes, microsomes, and mitochondria were isolated from brain as described by Lapetina *et al.* (10). Protein concentration in the subcellular fractions was measured according to Lowry *et al.* (11) using bovine serum albumin as standard.

Incubations. Myelin (0.25 mg protein/ml), synaptic membranes (0.5 mg protein/ml), microsomes (0.5 mg protein/ml), and mitochondria (0.5 mg protein/ml) in 20 mM Tris–HCl, 140 mM NaCl buffer, pH 7.4, were incubated for 90 min at 37°C in the presence of 0.1 to 1 mM Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, or La^{3+} and 0.1 mM $FeSO_4$. $FeSO_4$ stock solution (2.5 mM) was prepared in Milli-Q water immediately before addition. After incubation, lipid peroxidation was evaluated by measuring 2-thiobarbituric acid reactive substances (TBARS)[2] and protein oxidation by determining the content of protein-associated carbonyls.

TBARS determination. Incubations were stopped by addition of 0.1 ml of 4% (w/v) butylated hydroxytoluene in ethanol and TBARS production was measured as described by Fraga *et al.* (8).

Carbonyl determination. The concentration of protein-associated carbonyls was measured in myelin and synaptic membranes. Membranes were disrupted by addition of 1% (v/v) of Triton X-100 and further sonication for 1 min in a Branson 250 sonifier (Branson Ultrasonics Corp., Danbury, CT) at 100 W, keeping the samples at 4°C during sonication. Carbonyl content was determined basically as described by Levine *et al.* (12) by reaction with 2,4-dinitrophenylhydrazine and calculated from the maximum absorbance (360–390 nm) using a molar absorption coefficient of 22,000 $M^{-1}.cm^{-1}$.

Phospholipid determination. Lipids were extracted from myelin and synaptic membranes according to Folch *et al.* (13) and phospholipids were separated by thin-layer chromatography on HPTLC plates (Sigma Chem. Co) using the following solvent systems: chloroform:methanol:acetic acid:water (40:10:10:1) and chloroform:methanol:acetic acid:water (120:46:19:3) (14). Individual phospholipids were identified by I_2 exposure, and the content of lipid phosphorus was determined according to Chen *et al.* (15).

Analysis of fatty acids. Membrane lipids were extracted according to Folch *et al.* (13), dried under N_2 stream, and esterified with methanol:sulfuric acid (100:2, v/v) at 60°C for 2 h. The fatty acid methyl esters were extracted with chloroform and distilled water. The lower phase was washed twice with water, evaporated under N_2, and resuspended in *n*-hexane. Fatty acid analysis was performed by gas–liquid chromatography (Shimadzu GC-8A gas chromatograph) on a DB-23 column (J & W Scientific, Folsom, CA) with temperature programming at 5°C/min between 140 and 220°C. Fatty acids were identified by retention time and cochromatography with commercial standards. Peroxidation index (PI) was calculated as PI = (monoenoic % × 0.025) + (dienoic % × 1) + (trienoic % × 2) + (tetraenoic % × 4) + (pentaenoic % × 6) + (hexaenoic % × 8) (16).

Lipid-soluble antioxidants. Myelin (0.4 mg protein) was added with 1 ml of methanol and extracted with 4 ml of *n*-hexane. Samples were vortexed for 1 min and centrifuged for 5 min at 1000*g*. Aliquots (3 ml) of the upper layer were evaporated under N_2, resuspended in 200 μl of methanol:ethanol (1:1, v/v), and filtered through a 0.22-μm pore membrane. α-Tocopherol concentration was determined in the methanol:ethanol extracts by HPLC on a C_8 reversed-phase column with an in-line BAS LC4C electrochemical amperometric detector with a glassy-carbon working electrode at an applied oxidation potential of 0.6 V and ultraviolet detection (275/290 nm) (17). For ubiquinol determination, the extracts were reduced for 30 min in the dark with BH_4Na. After reduction, ubiquinols were extracted as described for α-tocopherol. Antioxidant concentration was quantified using commercial standards. Values are expressed as nanomoles per milligram of phospholipid.

Evaluation of membrane fluidity. The fluidity of myelin membranes was evaluated using the fluorescent probe diphenylhexatriene (DPH). Myelin resuspended in 20 mM Tris–HCl, 140 mM NaCl buffer, pH 7.4 (0.05 mg protein/ml), was added with 4 μl of 5 mM DPH in dimethyl sulfoxide. The probe was incorporated into the membranes by incubation for 30 min at 37°C. After incubation, fluorescence polarization was measured at 450 nm ($\lambda_{excitation}$: 360 nm) at 37°C. Lipid order parameter (Sp) was calculated according to Jähnig (18).

Statistics. One-way analysis of variance (ANOVA test) was performed using Statview 512+ (Brainpower Inc., Calabazas, CA).

RESULTS

In vitro effects of Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} on Fe^{2+}-initiated lipid and protein oxidation in brain membranes. The *in vitro* effect of Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} on Fe^{2+}-initiated lipid peroxidation was evaluated in both myelin and synaptic membranes isolated from rat brain. In the presence of 100 μM Fe^{2+}, 200–400 μM Al^{3+}, Ga^{3+}, In^{3+}, and Be^{2+} significantly stimulated TBARS production in myelin in a dose-dependent manner (Fig. 1A). Similarly to that observed in liposomes (6), Sc^{3+}, Y^{3+}, and La^{3+} showed a markedly higher stimulatory capacity at 100 to 200 μM concentration compared to the rest of the metals, reaching the maximum effect at 200 μM. When synaptic membranes were incubated under similar conditions, Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} (200–400 μM) significantly stimulated Fe^{2+}-initiated TBARS production (Fig. 1B). Unlike the results obtained in myelin membranes, the stimulation of lipid peroxidation in synaptic membranes was similar for all the metals.

Based on the greater susceptibility of myelin than synaptic membranes to lipid peroxidation, we investigated whether myelin was also more sensitive than

[2] Abbreviations used: TBARS, 2-thiobarbituric acid reactive substances; PI, peroxidation index; DPH, diphenylhexatriene.

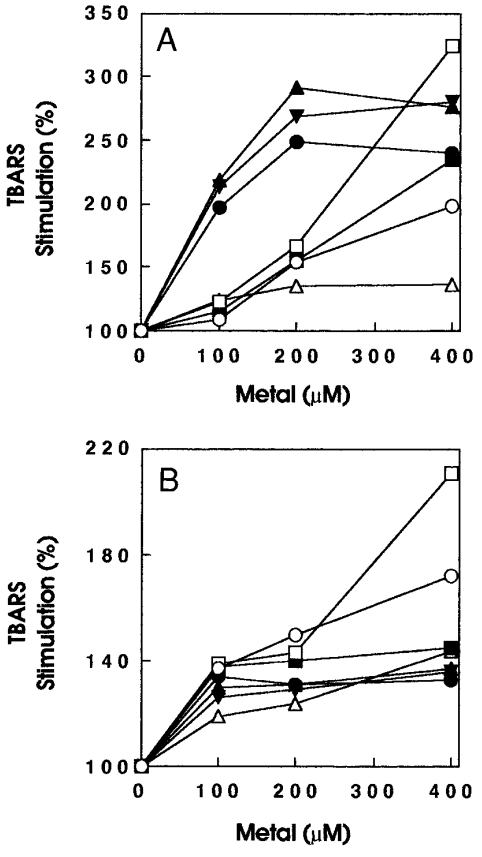

FIG. 1. Effect of Al^{3+} and related metals on Fe^{2+}-initiated lipid peroxidation in myelin and synaptic membranes. Myelin (A) and synaptic membranes (B) isolated from rat brain were incubated for 90 min at 37°C in the presence of 100 μM Fe^{2+} and 100–400 μM Al^{3+} (○), Sc^{3+} (●), Ga^{3+} (□), In^{3+} (■), Be^{2+} (△), Y^{3+} (▲), or La^{3+} (▼). At the end of incubation, TBARS production was evaluated as described under Materials and Methods. Results are shown as means of five independent experiments. Stimulation (%) = 100 × ($TBARS_{Al}$ − $TBARS_{no\ additions}$)/($TBARS_{Fe^{2+}}$ − $TBARS_{no\ additions}$).

other membranes (mitochondria, microsomal) to Al-stimulated lipid peroxidation. For that purpose, brain myelin, synaptic membranes, mitochondria, and microsomes, at a similar phospholipid concentration, were incubated for 90 min at 37°C in the presence of 100 μM Fe^{2+} and 100–1000 μM Al^{3+} (Fig. 2). The basal levels of TBARS were 1.7 ± 0.3 nmol/mg protein for myelin, 1.61 ± 0.09 nmol/mg protein for synaptic membranes, 3.2 ± 0.7 nmol/mg protein for mitochondria, and 3 ± 1 nmol/mg protein for microsomes. After incubation in the presence of 100 μM Fe^{2+}, TBARS concentration was 6.9 ± 0.4 nmol/mg protein for myelin, 40 ± 2 nmol/mg protein for synaptic membranes, 40 ± 2 nmol/mg protein for mitochondria, and 39 ± 5 nmol/mg protein for microsomes. Al^{3+} significantly stimulated Fe^{2+}-supported lipid peroxidation in myelin, mitochondria, microsomes, and synaptic membranes at concentrations higher than 200 μM. At 800 μM Al^{3+} concentration, the stimulatory effects of Al^{3+} were 3.5-, 2.1-, and 2.2-fold higher in myelin compared to synaptic membranes, mitochondria, and microsomes, respectively.

To evaluate whether the differential susceptibility of myelin relative to synaptic membranes to metal-mediated lipid peroxidation is due to differences in membrane composition, the content of negatively charged phospholipids, fatty acids, and lipid-soluble antioxidants was characterized. The relative content of phosphatidyl serine and phosphatidyl inositol (preferential metal-binding sites) was similar for both membranes (Table I). Synaptic membranes had a higher ($P < 0.001$) relative content of poly-unsaturated fatty acids, which are substrates of lipid peroxidation (Table II). The concentration of α-tocopherol was 47% higher in myelin than in synaptic membranes (Table III), while the concentrations of ubiquinol-9 and ubiquinol-10 were 110 and 96% higher in synaptic membranes than in myelin, respectively. The capacity of Al^{3+} and related metals to induce oxidative modifications to membrane proteins was investigated. Myelin and synaptic membranes were incubated for 90 min at 37°C in the presence of 100 μM Fe^{2+} and 200 μM Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, or La^{3+}. Al^{3+}, Sc^{3+}, Y^{3+}, and La^{3+} significantly ($P < 0.005$) promoted protein carbonyl formation in myelin (Fig. 3). In synaptic membranes the stimulatory effect was observed when membranes were incubated in the presence of Ga^{3+}, In^{3+}, Y^{3+}, La^{3+}, or Sc^{3+} ($P < 0.01$).

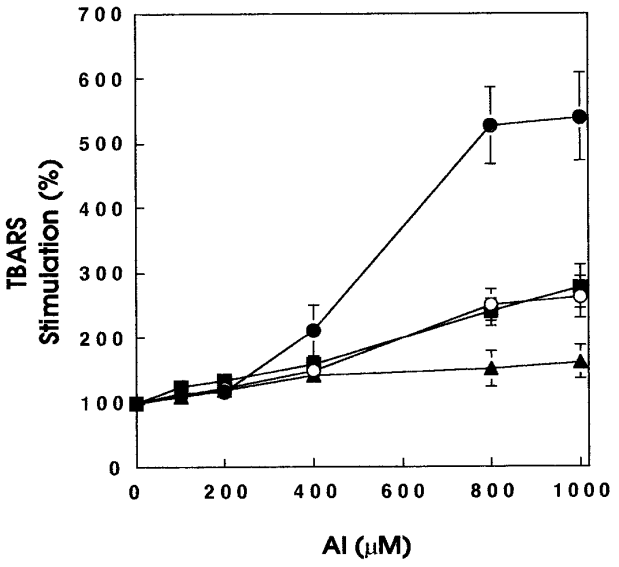

FIG. 2. Effect of Al^{3+} on Fe^{2+}-initiated TBARS production in brain membranes. Myelin (●), mitochondria (○), microsomes (■), and synaptic membranes (▲) isolated from rat brain were incubated for 90 min at 37°C in the presence of 100 μM Fe^{2+} and increasing amounts of Al^{3+} (100–1000 μM). The TBARS production stimulation was calculated as described in the legend to Fig. 1. Results are expressed as means ± SE of four independent experiments.

TABLE I

Relative Content of Phospholipids in Rat Brain Myelin and Synaptic Membranes

Phospholipid	Myelin (%)	Synaptic membranes (%)
Sph	5.9 ± 0.9[a]	1.4 ± 0.3[b]
PC	31 ± 2[a]	45 ± 2[b]
PS + PI	14.7 ± 0.8	12.0 ± 0.9
PE	49 ± 1[c]	42 ± 2[d]

Note. Rat brain myelin and synaptic membrane lipids were extracted and separated by TLC as described under Materials and Methods. Results are expressed as means ± SE of four independent experiments. Sph, sphingomyelin; PC, phosphatidyl choline; PS, phosphatidyl serine; PI, phosphatidyl inositol; PE, phosphatidyl ethanolamine.

[a] Significantly different from [b], $P < 0.01$.

[c] Significantly different from [d], $P < 0.05$ (one-way ANOVA test).

Lipid peroxidation in Al^{3+}-intoxicated mice. The *in vivo* susceptibility of myelin to Al^{3+}-mediated oxidative damage to lipids was investigated. For that purpose, mice were fed diets containing 3 or 1000 μg Al/g during pregnancy and until Day 40 postnatal. At 40 days of age, excess dietary Al^{3+} did not affect offspring body or brain weights (data not shown).

Lipid peroxidation was evaluated in the 40-day old pups in brain homogenates and myelin membranes. TBARS content in brain homogenates was similar between groups (Fig. 4). In myelin, TBARS concentration (nmol/g fresh tissue) was 72% higher ($P < 0.01$) in the Al 1000 group, compared to the Al 3 mice (Fig. 4). TBARS content calculated as μmol TBARS/mg phospholipids was 38% higher ($P < 0.01$) in the Al 1000 mice than in controls (0.64 ± 0.04 and 0.88 ± 0.04 for Al 3 and Al 1000, respectively). Myelin concentration of α-tocopherol was significantly higher ($P < 0.01$) in the Al-intoxicated mice (0.23 ± 0.03 and 0.33 ± 0.02 nmol α-tocopherol/mg phospholipid, for Al 3 and Al 1000, respectively). Al was present in the myelin fraction, and although values were not statistically different, a tendency ($P < 0.07$) toward higher concentrations of Al^{3+} was observed in myelin from the Al 1000 group (22 ± 2 ng Al/g fresh tissue) compared to the control group (16 ± 2 ng Al/g fresh tissue).

The fluidity of myelin membranes was evaluated by a fluorescence polarization method, using DPH as a probe. The order parameter (Sp) measured in myelin from the Al 1000 mice was significantly higher ($P < 0.01$) than in the Al 3 group (0.660 ± 003 and 0.676 ± 0.003 for Al 3 and Al 1000, respectively).

TABLE II

Fatty Acid Content in Rat Brain Myelin and Synaptic Membranes

Fatty acid	Myelin (%)	Synaptic membranes (%)
16:0	14.0 ± 0.7[a]	23.9 ± 0.8[b]
18:0	36 ± 4	33 ± 2
18:1	30 ± 4[a]	10 ± 2[b]
18:2	0.8 ± 0.2	0.9 ± 0.1
20:1	3.5 ± 0.4[a]	0.6 ± 0.1[b]
20:4	9.5 ± 0.9	11.4 ± 0.4
22:4	3.0 ± 0.3	3.2 ± 0.4
22:6	3.4 ± 0.4[a]	16.3 ± 0.9[b]
Peroxidation index	79.1 ± 0.6[a]	190 ± 8[b]

Note. Results are expressed as means ± SE of four independent experiments.

[a] Significantly different from [b], $P < 0.001$ (one-way ANOVA test).

TABLE III

Lipid-Soluble Antioxidant Concentration in Rat Brain Myelin and Synaptic Membranes

Antioxidants	Myelin (nmol/μmol PL)	Synaptic membranes (nmol/μmol PL)
α-Tocopherol	0.30 ± 0.01[a]	0.20 ± 0.01[b]
Ubiquinol-9	0.39 ± 0.03[a]	0.82 ± 0.06[b]
Ubiquinol-10	0.077 ± 0.005[a]	0.150 ± 0.010[b]

Note. Lipid-soluble antioxidant concentration was evaluated in rat brain myelin and synaptic membranes as described under Materials and Methods. PL, phospholipids. Results are expressed as means ± SE of five independent experiments.

[a] Significantly different from [b], $P < 0.001$ (one-way ANOVA test).

DISCUSSION

Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} have been reported to stimulate lipid peroxidation in liposomes

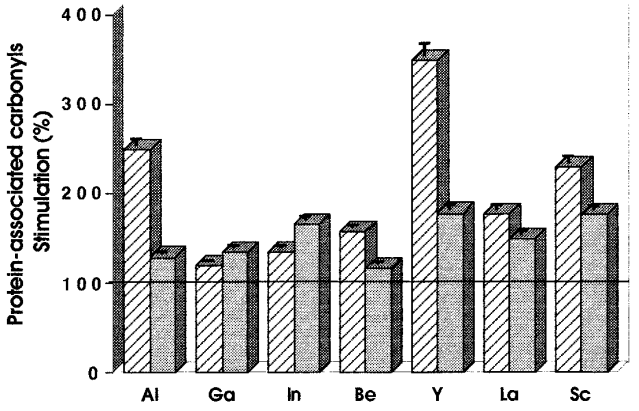

FIG. 3. Oxidative damage to proteins mediated by Al^{3+} and related metals. Myelin (▨) and synaptic membranes (■) isolated from rat brain were incubated for 90 min at 37°C in the presence of 100 μM Fe^{2+} and 200 μM Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, or La^{3+}. After incubation the oxidative damage to membrane proteins was evaluated by measuring the content of protein-associated carbonyls. Results are expressed as means ± SE of four independent experiments.

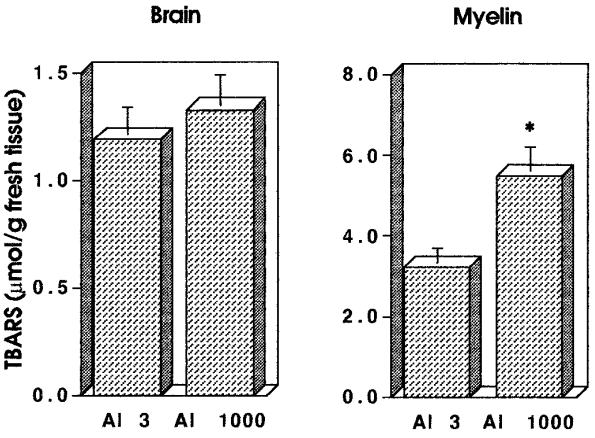

FIG. 4. TBARS content in brain homogenates and myelin from Al-intoxicated mice. Brain homogenates (left) and myelin (right) from mice fed diets containing 3 or 1000 μg Al/g were analyzed for Al^{3+}-mediated lipid peroxidation. Results are expressed as means ± SE ($n = 6$). *Significantly different ($P < 0.01$).

in the presence of Fe^{2+} (6). Since these metals have no redox capacity in biological systems, they cannot initiate lipid peroxidation per se. Evidence from our laboratory showed that these metals could promote the formation in the membrane of clusters of negatively charged phospholipids (7). Since the mobility of the phospholipid acyl chains was restricted, results indicated that metal-mediated membrane rigidification and phase separation could act synergistically to facilitate the propagation of lipid peroxidation initiated by Fe^{2+}.

In brain homogenates, Al^{3+} and related metals added *in vitro* were previously shown to enhance Fe^{2+}-initiated lipid peroxidation (3, 19). In the present work, we extended these findings characterizing the susceptibility of different brain membranes to Al^{3+}-, Sc^{3+}-, Ga^{3+}-, In^{3+}-, Be^{2+}-, Y^{3+}-, and La^{3+}-mediated lipid and protein oxidation. Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, Be^{2+}, Y^{3+}, and La^{3+} significantly stimulated lipid peroxidation in the presence of Fe^{2+}, in both rat brain myelin and synaptic membranes. The magnitude of the effect followed the order Sc^{3+}, Y^{3+}, $La^{3+} > Al^{3+}$, Ga^{3+}, $In^{3+} > Be^{2+}$ which is in agreement with that previously observed in liposomes (6). For all the metals studied myelin was more susceptible to lipid oxidation than synaptic membranes. At 800 μM Al^{3+}, the stimulatory effect was 3.5, 2.1, and 2.2 times higher in myelin compared to synaptic membranes, mitochondria, and microsomes, respectively. The extent of the effect did not depend on differences in lipid concentration, since incubations were performed using similar amounts of phospholipids for all the membranes.

Proteins can undergo oxidative modifications through several mechanisms (20). Carbonyl residues are formed in proteins by redox metal-catalyzed oxidation of certain amino acids (histidine, proline, arginine, and lysine). Carbonyl residues are also incorporated into proteins through covalent binding with products of lipid peroxidation, like 4-hydroxynonenal (21). In both cases, the protein acquires new carbonyl groups that can be detected by reaction with 2,4-dinitrophenylhydrazine. However, the origin of the carbonyl group cannot be elucidated using this methodology. Al^{3+} and related metals significantly enhanced the content of protein-associated carbonyls in myelin and synaptic membranes. No significant correlations were found between the effect of metals on lipid and protein oxidation for either myelin or synaptic membranes. This lack of correlation suggests that the reaction of lipid peroxidation products with membrane proteins is not the only mechanism involved in the observed increase of protein carbonyls mediated by Al^{3+} and related metals.

In an attempt to explain the differential susceptibility of myelin to metal-mediated lipid peroxidation, factors that could affect the extent of lipid peroxidation were characterized in myelin and synaptic membranes. The concentration of negatively charged phospholipids (potential metal-binding sites) was similar for both membranes. The peroxidation index was lower and the concentration of α-tocopherol was higher in myelin than in synaptic membranes. These results indicated that differences in membrane binding sites or in the concentration of lipid peroxidation substrates or antioxidants do not account for the particular susceptibility of myelin to Al^{3+}-mediated oxidation. Since the relevance of the antioxidant role of ubiquinols in biological membranes is still under discussion, it is not possible to evaluate the impact of the different content of ubiquinols on how prone myelin and synaptic membranes are to oxidation.

The particular susceptibility of myelin to Al^{3+}-mediated oxidation is probably due to its characteristic high relative content of lipids (70%). In this environment, the action of Al^{3+} and related metals, causing membrane rigidification, phase separation, and enhancement of lipid oxidation rates, would be facilitated. This hypothesis is supported by the fact that the magnitude of the stimulatory effect of metals on Fe^{2+}-supported lipid peroxidation in myelin is similar to that previously observed in liposomes (6), in which the mentioned mechanism was demonstrated (7). Accordingly, in synaptic membranes, which contain a high proportion of proteins (70%), in addition to a low stimulatory effect on lipid oxidation, the stimulation of lipid oxidation was low and the magnitude of the effect was similar for all the metals.

Based on the *in vitro* results, we investigated the possibility that also *in vivo* myelin could be a preferential target for Al^{3+}-mediated lipid peroxidation. We found that mice fed diets containing 3 or 1000 μg Al/g during gestation and early development (40 days post-

natal) had similar contents of TBARS in brain. Similarly, it was previously shown that adult mice fed high Al^{3+} diets (1000 μg Al/g) for 5 or 7 weeks had similar levels of lipid peroxidation products in brain as mice fed low Al^{3+} diets (23). However, in the present study, a 72% higher concentration of lipid peroxidation products was found in myelin from the Al^{3+}-intoxicated mice than in controls.

Myelin isolated from the Al 1000 mice brains was found to be significantly more rigid than myelin from the Al 3 mice. In agreement with our results, in the presence of 20 μM Al^{3+}, erythrocyte ghost membranes showed a displacement of the Sp temperature-dependence curve equivalent to the rigidification caused by a 2°C decrease in the membrane temperature (24). Although the changes in Sp that we observed were small, they are similar to those found in other synthetic and biological membranes, when fatty acid composition is heterogeneous. Under these conditions there is not a sharp transition temperature (Tm), but a slight change in fluidity is observed (25). In fact, when rat brain myelin fluidity was evaluated, a smooth slope for Sp values was found in the studied temperature range (Sp 20°C, 0.805; 50°C, 0.670). The higher rigidity of Al 1000 myelin could be due to a direct effect of Al^{3+} on lipid ordering or to changes in membrane lipid composition, secondary to long-term Al^{3+} exposure.

Since myelin has a high relative content of lipids (lipid:protein, 70:30) compared to other membranes, it could be a preferential target for Al^{3+}-induced changes in membrane physical properties (membrane rigidification, phase separation). We have previously demonstrated that Al^{3+} and other related metals without redox capacity can stimulate Fe^{2+}-initiated lipid oxidation by increasing lipid packing and by promoting the formation of rigid clusters, which favors the propagation of lipid oxidation. The present results show that this mechanism could be involved both *in vivo* and *in vitro* in Al^{3+}-mediated oxidation of myelin. The oxidation of myelin components, lipids and proteins, could be one of the mechanisms involved in the neurotoxicity of Al^{3+}.

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REFERENCES

1. Ganrot, P. O. (1986) *Environ. Health Perspect.* **65,** 363–441.
2. van der Voet, G. B., Marani, E., Tio, S., and de Wolff, F. A. (1991) *Prog. Histochem. Cytochem.* **23,** 235–242.
3. Golub, M. S., Han, B., Keen, C. L., Gershwin, M. E., and Tarara, R. P. (1995) *Toxicol. Appl. Pharmacol.* **133,** 64–72.
4. Oteiza, P. I., Fraga, C. G., and Keen, C. L. (1993) *Arch. Biochem. Biophys.* **300,** 517–521.
5. Oteiza, P. I. (1994) *Arch. Biochem. Biophys.* **308,** 374–379.
6. Verstraeten, S. V., and Oteiza, P. I. (1995) *Arch. Biochem. Biophys.* **322,** 284–290.
7. Verstraeten, S. V., Nogueira, L. V., Schreier, S., and Oteiza, P. I. (1997) *Arch. Biochem. Biophys.* **338,** 121–127.
8. Fraga, C. G., Oteiza, P. I., Golub, M. S., Gershwin, M. E., and Keen, C. L. (1990) *Toxicol. Lett.* **51,** 213–219.
9. Donald, J. G., Golub, M. S., Gershwing, M. E., and Keen, C. L. (1989) *Neurotoxicol. Teratol.* **11,** 345–351.
10. Lapetina, E. G., Soto, E. F., and De Robertis, E. (1967) *Biochim. Biophys. Acta* **135,** 33–43.
11. Lowry, D. H., Rosembrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
12. Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Ciment, I., Lenz, A. G., Ahn, B. W., Shattiel, S., and Stadtman, E. R. (1990) *Methods Enzymol.* **186,** 464–478.
13. Folch, P., Lees, M., and Sloane-Stanley, G. (1957) *J. Biol. Chem.* **226,** 449–509.
14. Weiss, S. J., McKinny, J. S., and Putney, J. W. (1982) *Biochem. J.* **204,** 4093–4099.
15. Chen, P. S., Jr., Toribara, T. Y., and Warner, H. (1956) *Anal. Chem.* **28,** 1756–1758.
16. Witting, L. A., and Horwitt, M. K. (1964) *J. Nutr.* **82,** 19–33.
17. Motchnik, P. M., Frei, B., and Ames, B. N. (1994) *Methods Enzymol.* **234,** 269–279.
18. Jähnig, F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6361–6365.
19. Oteiza, P. I., Verstraeten, S. V., and Adonaylo, V. N. (1995) *Ciência Cultura* **47,** 330–335.
20. Stadtman, E. R. (1990) *Biochemistry* **29,** 6323–6331.
21. Uchida, K., and Stadtmand, E. R. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4544–4548.
22. Bruce Martin, R. (1986) *Clin. Chem.* **32,** 1797–1806.
23. Oteiza, P. I., Keen, C. L., Han, B., and Golub, M. S. (1993) *Metabolism* **42,** 1296–1300.
24. Weis, C., and Haug, A. (1989) *Thromb. Res.* **54,** 141–149.
25. Brener, R. R. (1984) *Prog. Lipid Res.* **23,** 69–96.