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Effects of Aluminum Chloride on Normal and Uremic Adult Male Rats

Tissue Distribution, Brain Choline Acetyltransferase Activity, and Some Biological Variables

G. CHERRORET,[1] D. DESOR,[2] M. F. HUTIN,[3] D. BURNEL,[3] B. CAPOLAGHI,[4] AND P. R. LEHR*,[2]

[1]*Centre des Sciences de l'Environnement, Toxicologie, Université de Metz (France),* [2]*Laboratoire de Biologie et Physiologie du Comportement, URA CNRS 1293, Faculté des Sciences, Université Henri Poincaré, Nancy 1,54500 Vandoeuvre les Nancy Cedex, France;* [3]*Service de Chimie Générale appliquée àla Médecine, Faculté de Médecine, Université Henri Poincaré, Nancy 1, Nancy, France,* [4]*Laboratoire de Biochimie, CHR Metz-Thionville, Thionville, France.*

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ABSTRACT

Normal and uremic adult male rats were given a daily ip injection of 20 mg Al (Al chloride)/kg for 14 d. The results indicate that Al induces a significant decrease in food ingestion, weight gain, and total protein concentration in the plasma. Compared with control animals, very high increases in Al levels were found in plasma and hepatic homogenates (about 36 and 19 times, respectively). In the brain homogenates, the Al increases were lower (about 23%). The brain cholineacetyltransferase activity was reduced: 10.6 and 14.9% in normal and uremic rats, respectively. The nephrectomy and the food restriction did not affect the total protein concentrations in plasma and the cerebral cholineacetyltransferase activity. Both were only found to be reduced in the rats treated by Al chloride.

Index Entries: Aluminum chloride; blood proteins, rat, ChAT.

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

INTRODUCTION

High levels of Al have been found in serum and various organs of patients suffering from chronic renal failure as a consequence of large oral loads of Al in the form of Al-containing phosphate binding gels *(1)* or the presence of this element in dialysis fluid *(2,3)*. The accumulation of this element is the most probable cause of dialysis encephalopathy *(1)* and may be of major importance in the pathogenesis of microcytic anemia and osteomalacia *(4)*. Nonuremic adults ingesting large amounts of Al carbonate have high Al concentrations in urine and in bone *(5)*. Some patients treated by parenteral nutrition develop osteomalacia probably owing to Al contamination of the infusate *(6)*.

A decrease in the activity of cholineacetyltransferase (ChAT) was found in cerebral cortex from dialysis encephalopathy victims, but this reduction was less marked than that observed in Alzheimer's disease (AD) *(7)*. The involvement of Al in the etiology of AD has been proposed by some authors *(8,9)*. King *(10)* hypothesized that elevated levels of Al contribute to the cholinergic deficits in AD.

Several animal studies demonstrate an Al effect on ChAT in vivo *(7,11)* and in vitro *(12)*. The oral administration of Al salts by mixing with a standard diet (Cherroret, unpublished results) or by gastric intubation *(13)* in adult rats for a short time had apparently no effect on ChAT brain activity. In these cases, the Al concentration in plasma was not significantly different from that observed in control adult rats.

In the present work, we studied the effect of ip administration of high dose of Al chloride on ChAT brain activity of normal and uremic adult rats for 14 d. The weight gain, the Al levels in plasma, brain, and liver, and some biological variables were also evaluated.

MATERIALS AND METHODS

Animals and Treatment

Adult male rats of the Wistar strain (Iffa Credo, L'Arbresle, France) weighing 230–260 g were used. The animals were housed in individual wire-mesh cages in an air-conditioned room maintained at a relatively constant temperature (22–23°C) and a 12-h light–dark cycle (lights off at 7 PM). Water and standard diet (food pellets, Extra Labo, Provins, France) were available ad libitum.

In a first experiment (E1), rats were divided into four experimental groups: normal rats (group 1, $n = 6$), normal rats treated by Al chloride (group 2, $n = 6$), uremic rats (group 3, $n = 6$), and uremic rats treated by Al chloride (group 4, $n = 6$). The rats of groups 3 and 4, anesthetized with Na pentobarbital (50 mg/kg body wt), were subjected to 5/6 nephrectomy using the method of Chanutin and Ferris *(14)*, slightly

modified. The upper and lower branchs of the renal artery from the left kidney were ligated, and the right kidney was removed. The mortality rate was about 10%. The plasma concentrations of creatinine were evaluated in groups 1 and 2 and in groups 3 and 4, 10 d after the nephrectomy. A blood sample was taken by ocular puncture of the cavernous sinus for creatinine evaluation; the values (µmol/L) were 56 ± 8, 61 ± 7, 99 ± 9, and 102 ± 8 in groups 1, 2, 3, and 4, respectively. Five days later, the rats in groups 2 and 4 were given a daily ip injection of 20 mg Al/kg (solution of Al chloride, pH 3.8, in NaCl 0.9%) for 14 d. The control rats of groups 1 and 3 were injected with an equivalent volume (1–1.5 mL) of vehicle (0.9% NaCl, pH 5.0). All the animals were sacrified by decapitation 24 h after the last injection.

In a second experiment (E2), rats were divided into four experimental groups: normal rats (group 5, $n = 6$), normal restricted rats (group 6, $n = 6$), uremic rats (group 7, $n = 6$), and uremic restricted rats (group 8, $n = 6$). The restricted rats (groups 6 and 8) received the standard diet in an amount equivalent to the daily one taken up by the intoxicated rats (groups 2 and 4). The plasma concentration of creatinine was evaluated in groups 5 and 6 and in groups 7 and 8, 10 d after the 5/6 nephrectomy. Five days after creatinine evaluation, all the rats received a daily ip injection of 0.9% NaCl (pH 5.0) for 14 d. They were sacrified by decapitation 24 h after the last injections. The rats were weighed and the daily food intakes recorded throughout the experiments.

Cholineacetyltransferase Activity

ChAT activity was measured by the micromethod of Fonnum *(15)*. After decapitation of the rats, the basal forebrain and the neostriatum were quickly isolated in a cold room by dissection *(16)* and then weighed. The brain areas from each rat were homogenized at 0°C in a Potter-Elvejhem apparatus with a Teflon pestle (A.H. Thomas Co, Philadelphia, Size A) in a 10-m*M* EDTA solution (pH 7.4). Ten minutes after adjunction of an equal volume of a 10-m*M* EDTA solution (pH 7.4) containing 0.5% Triton X100 (v/v), a 10-m*M* EDTA solution (pH 7.4) was used to complete the treatment. The final concentration of the homogenate was 5% (w/v). This homogenate was then divided into three aliquots to evaluate ChAT activity, proteins and Al concentrations, respectively.

For the evaluation of ChAT activity, the incubation mixture contained (final conc.): 0.2 m*M* acetylCoA, 50 m*M* sodium phosphate buffer (pH 7.4), 300 m*M* NaCl, 8 m*M* choline chloride, 20 m*M* EDTA (pH 7.4), and 0.1 m*M* physostigmine. The incubation solution (5 µL) and 2 µL of the labeled acetylCoA solution (acetyl 1 ^{14}C CoA, New England Nuclear, 48.1–59.3 mCi/mol) were placed in a microtube (23 × 2 mm) and the homogenate (2 µL) was added. The solution was mixed and incubated for 15 min at 37°C. The microtube was placed in a scintillation vial containing 5 mL of 10 m*M* sodium phosphate buffer (pH 7.4). Then 2 mL of

acetonitrile containing 10 mg of sodium tetraphenylborate (Kalignost) and 10 mL of toluene scintillation mixture (0.05% PPO, 0.02% POPOP, toluene q.s.p.) were added to the vial, which was slightly shaken. The radioactivity of acetylcholine (ACh), which was extracted in the toluene phase, was measured with a liquid scintillation spectrometer (Minibeta 1211, LKB). The enzyme activity was calculated from the specific activity of a given batch of ^{14}C acetylCoA. The specific activity of ChAT was expressed as nmol ACh synthesized/h/mg protein.

Protein Evaluation

The protein content of the brain homogenates was determined using the method of Lowry et al. *(17)* modified by Markwell et al. *(18)* with bovine serum albumin (fraction V) as a reference.

Al Determination

The blood of each rat was collected in a heparinized plastic tube and centrifuged at 900*g* for 10 min. The plasma was divided into two aliquots to evaluate Al and some biological variable concentrations. The liver of each rat was homogenized at 0°C in a Potter-Elvejhem apparatus with a Teflon pestle (A.H. Thomas Co., Philadelphia, Size A) in distilled water. Al concentration in plasma, cerebral and liver homogenates was determined using an atomic emission spectrometer (Spectra Span V, Beckman).

Biological Variables

The concentrations of total proteins, urea, uric acid, and creatinine were evaluated in the second plasma aliquot using an analyzer Hitachi 717. Proteins were determined by the Biuret method, urea with glutamate dehydrogenase as indicator enzyme *(19)*, creatinine by the Jaffe's method without deproteinization *(20)*, and uric acid by a colorimetric enzymatic method using a microbial uricase and a sensitive peroxydase-catalyzed chromogenic system *(21)*.

Statistical Analysis

ChAT activity, Al concentrations, and various biological data were compared using single-factor analysis of variance (ANOVA). Multiple comparisons were performed using Fisher PLSD test *(22)*.

RESULTS

Analysis of food consumption of the rats in E1 (Fig. 1) indicated an overall significant Al effect starting on the first treatment day $F(3.22) = 180.9$ $p < 0.0001$. Multiple comparisons using Fisher's PLSD test indicated on the subsequent days differences in the comparisons of G1 vs G2 ($p < 0.001$) and

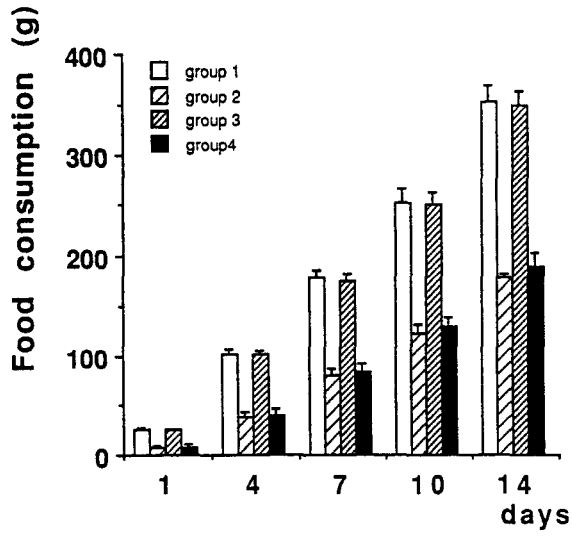

Fig. 1. Food weight ingested daily by rats in E1 (number of rats in each group: $n = 6$).

G3 vs G4 ($p < 0.001$). However, the weight of daily food ingested was similar, on the one hand, in the control groups G1 and G3, and on the other hand, in the intoxicated groups G2 and G4. In order to avoid the possible effect of undernutrition, the restricted rats (G6 and G8) in E2 received the standard diet in an amount equivalent to the daily one taken up by the Al-intoxicated rats (G2 and G4).

Figure 2 (A and B) shows the weight evolution of the rats in E1 and E2. The weight differences at day 0 of treatment between normal and uremic rats could be explained by the weight reduction of the uremic rats after the operation. Analysis of body weight data of the rats in E1 (Fig. 2A) indicated an overall significant effect of Al starting on the second treatment day—$F(3.22) = 198.3$ $p < .0001$. The significant decrease in the weight gain of the normal and uremic rats treated by Al vs normal and uremic controls agrees with a significant lower food ingestion for these animals. The same weight gain reduction was observed in groups G6 and G8 in comparison to control groups G5 and G7 after food restriction (Fig. 2B).

The Al concentrations in plasma, hepatic and cerebral homogenates in both experiments are shown in Table 1. ANOVA applied to plasma Al data (Table 1) indicated an overall significant effect of Al treatment—$F(3.22) = 163.57$, $p < 0.0001$. In groups 2 and 4, the Al data were 30 and 43 times higher than in groups 1 and 3, respectively. The 5/6 nephrectomy also induced a plasma Al increase (27.3%) in group 4 vs group 2. ANOVA applied to Al data in the liver homogenates (Table 1) indicated an overall significant effect of Al treatment—$F(3.22) = 184.58$, $p < 0.0001$. In groups 2 and 4, the Al data were 19.8 and 18.7 times higher than in groups 1 and 3, respectively. ANOVA applied to Al data in the cerebral

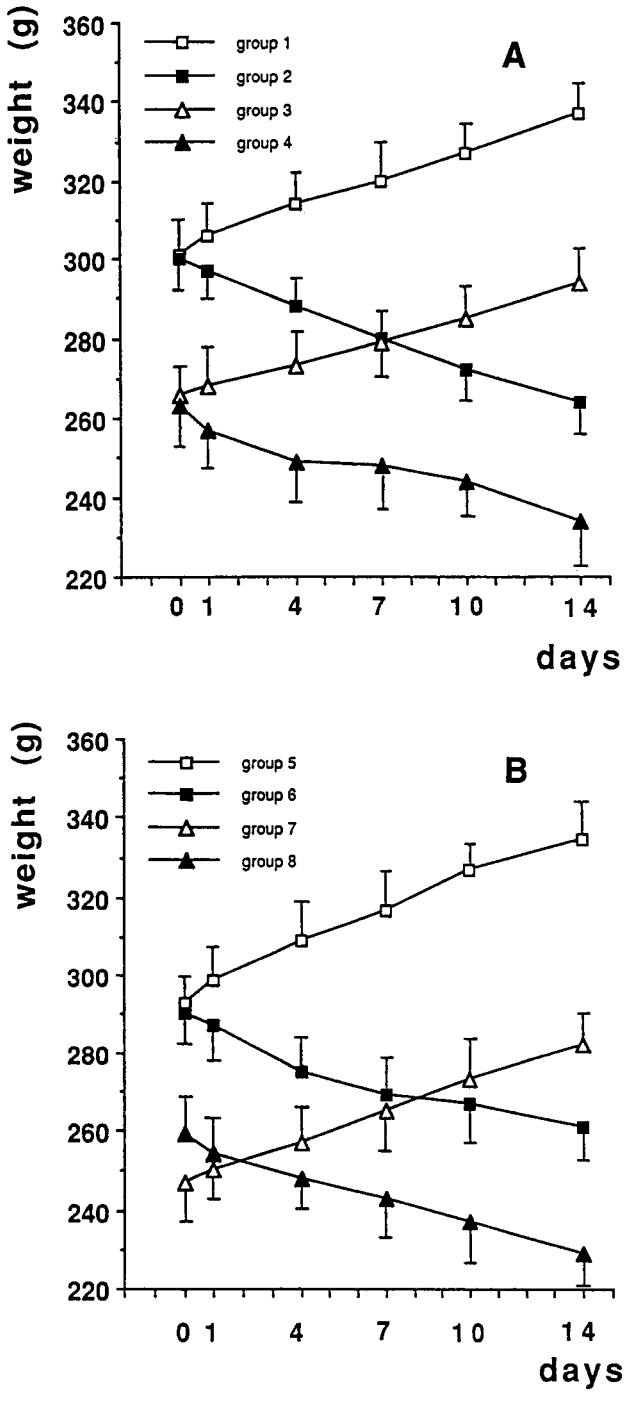

Fig. 2. Weight evolution of the adult male rats (number of rats in each group: $n = 6$) A: E1, B: E2.

homogenates (Table 1) indicated an overall significant effect of Al treatment—$F(3.22) = 11.27$, $p < 0.0001$. In groups 2 and 4, the Al data were 20.6 and 26.3% higher than in groups 1 and 3, respectively. For the Al

Table 1
Al Concentrations in Plasma, Liver, and Brain of Rats in the Two Experiments

	Plasma, μg/L	Hepatic homogenate, μg/g wet wt	Cerebral homogenate, μg/g wet wt
E1			
Group 1 (6)	27 ± 4	3.17 ± 0.37	3.64 ± 0.31
Group 2 (6)	812 ± 42[b]	63.06 ± 11.25[b]	4.39 ± 0.38[a]
Group 3 (6)	24 ± 5	3.20 ± 0.32	3.57 ± 0.35
Group 4 (6)	1034 ± 199[b]	59.88 ± 5.68[b]	4.51 ± 0.43[a]
E2			
Group 5 (6)	32 ± 45	3.01 ± 0.82	3.40 ± 0.44
Group 6 (6)	26 ± 6	3.22 ± 0.92	3.25 ± 0.25
Group 7 (6)	30 ± 3	2.98 ± 0.45	3.10 ± 0.31
Group 8 (6)	29 ± 4	3.12 ± 0.37	3.32 ± 0.36

Values are means ± SD. Numbers in parentheses indicate number of rats.
[a]$p < .01$, [b]$p < .001$ (Fisher's PLSD test).

Table 2
Effect of Al on ChAT activity (nmol/mg proteins/h)

E1		E2	
Group 1 (6)	28.47 ± 1.34	Group 5 (6)	27.2 ± 0.68
Group 2 (6)	25.43 ± 1.31[a]	Group 6 (6)	27.5 ± 0.76
Group 3 (6)	28.82 ± 0.89	Group 7 (6)	27.5 ± 1.57
Group 4 (6)	24.50 ± 1.59[a]	Group 8 (6)	27.9 ± 0.82

Values are means ± SD. Numbers in parentheses indicate number of rats. Significantly different from control group 1.
[a]$p < .01$ (Fisher's PLSD test).

concentrations in plasma, liver, and cerebral homogenates in E2, no significant differences were found among the four groups (Table 1).

ANOVA applied to ChAT activity data in E1 (Table 2) indicated an overall significant effect of the Al treatment—$F(3.22) = 18.95$, $p < 0.0001$. Therefore, the administration of Al inhibited ChAT activity in the cerebral areas: 10.6 and 14.9% in the normal (group 2) and uremic (group 4) rats, respectively. In the E2, no significant differences were found among the four groups.

The concentrations of total proteins, urea, uric acid, and creatinine evaluated in the rat plasma 1 d after the end of treatment in E1 and E2 are shown in Tables 3 and 4, respectively. ANOVA applied to total proteins data (Table 3) indicated an overall significant effect of Al treatment—$F(3.22) = 79.27$, $p < 0.0001$. In groups 2 and 4, the total proteins data were 17.6 and 19.2% lower than in groups 1 and 3, respectively. They showed no differences, on the one hand, in groups 1 and 3, or on the other hand, in groups 2 and 4. The administration of Al to normal and uremic rats

Table 3
Evaluation of Some Variables in the Plasma of the Rats in E1

Variables	Group 1, 6	Group 2, 6	Group 3, 6	Group 4, 6
Total proteins (g/L)	65.3 ± 1.7	53.8 ± 2.7[a]	65.0 ± 2.1	52.5 ± 1.3[a]
Urea (nmol/L)	8.5 ± 0.1	8.3 ± 0.8	16.5 ± 3.0[a]	15.8 ± 1.2[a]
Uric acid (μmol/L)	235 ± 44	244 ± 27	210 ± 31	225 ± 22
Creatinine (μmol/L)	58.5 ± 11	60.0 ± 7	109.0 ± 6.5[a]	112.0 ± 11[a]

Values are means ± SD. Numbers indicate number of rats. Significantly different from control group 1.
[a]$p < .01$ (Fisher's PLSD test).

Table 4
Evaluation of Some Variables in the Plasma of the Rats in E2

Variables	Group 5, 6	Group 6, 6	Group 7, 6	Group 8, 6
Total proteins (g/L)	65.0 ± 2.2	65.5 ± 2.0	67.0 ± 1.5	65.4 ± 2.5
Urea (nmol/L)	8.9 ± 0.4	9.2 ± 0.3	14.6 ± 1.3[a]	13.9 ± 1.6[a]
Uric acid (μmol/L)	220 ± 45	210 ± 18	227 ± 32	188 ± 25
Creatinine (μmol/L)	57.0 ± 4	56.5 ± 4	105.0 ± 5.4[a]	101.0 ± 3.2[a]

Values are means ± SD. Numbers indicate number of rats in experiments.
Significantly different from control group 5.
[a]$p < .01$ (Fisher's PLSD test).

had no significant effect on plasmatic urea, creatinine, and uric acid concentrations (Table 3). In E2 (Table 4), the food restriction had no significant effect on the four plasmatic variables; particularly the total proteins concentrations did not differ among the four groups.

DISCUSSION

In many experiments, a two-stage 5/6 nephrectomy was performed *(14,23)*. During the first surgical procedure, two-thirds of the left kidney was removed by cutting off both poles; exactly 14 d after this operation, the right kidney was removed. In our study, a single operation was necessary to realize the 5/6 nephrectomy: the two branches of the renal artery from the left kidney were ligated, and the right one was removed. The diagnosis of acute renal failure is based on functional plasma parameters, like creatinine and urea; the concentrations of these two compounds increase after the 5/6 nephrectomy *(23)*. Therefore, the partial nephrectomy realized in our study produces a renal insufficiency in rats of groups 3 and 4 (Table 3) and groups 7 and 8 (Table 4). Otherwise, the weight of daily food ingested and the body weight gain were similar in

the control groups (normal and uremic rats). Our observations are in agreement with those of Gretz et al. *(23)*.

The present results concerning the effects of Al are in agreement with those of Ondreicka et al. *(24)*, who reported that a high oral intake of Al sulfate (2835 ppm) in male adult rats for 8 d led to a reduction in food intake and in average body weight. This weight loss was also observed after gastric intubation or ip administration of Al salt in adult rats *(13,25,26)*. The body weight decrease was attributed to a reduction of the food ingestion, which is similar in normal and uremic rats treated by Al. However, the weight reduction is dose-dependent. Therefore, the ip administration of Al chloride (0.05 or 0.5 mg · kg^{-1} body wt) for 12 wk to partially nephrectomized or intact female rats did not result in any statistically significant effects on the body weight *(27)*.

The food restriction and the 5/6 nephrectomy have no significant effect on the concentration of total proteins in the rat plasma. On the contrary, the administration of Al induced a similar reduction of this total proteins concentration in the normal and uremic rats, but had no significant effect on plasma urea and creatinine contents. Therefore, catabolism and urine excretion of the proteins were not affected. The liver regulates protein metabolism, so the significant decrease of total protein in plasma could be attributed to a reduction of the protein synthesis in this organ. Indeed in the normal and uremic rats treated by Al, our data indicated a very high accumulation of Al in the hepatic homogenates (Table 1). Berlyne et al. *(25)* observed, in rats exposed to high Al concentrations, high levels of Al in the hepatic tissue leading to a reduction of the protein synthesis. The hepatocytes ultrastructure abnormalities induced by Al accumulation, rarefaction of mitochondria, and of rough endoplasmic reticulum are a severe alteration in the functional value of the hepatocytes (intramitochondrial metabolism, synthesis, and protein secretion functions) *(28)*. Two previous studies compared liver Al concentration of normal and uremic rats with apparently conflicting results: Arieff et al. *(29)* reported increased values in uremic rats, whereas Thurston et al. *(30)* found no change. In these cases, the Al was administered orally to the normal and uremic rats. Chan et al. *(31)*, after ip administration of Al chloride to normal and uremic adult rats, observed that uremic-Al-treated rats had lower Al concentrations in the liver than the nonuremic, Al-treated animals. In our study, the Al levels in the liver homogenates showed no significant difference between the groups 2 and 4 (Table 1).

The Al level also increased significantly in the cerebral homogenates in groups 2 and 4 in parallel to plasma Al (Table 1), but the Al values were not significantly different in the two groups. These elevated Al levels could explain the significant decrease in ChAT activity in the cerebral areas of the normal and uremic rats (groups 2 and 4) (Table 2). On the contrary, the food restriction had no significant effect on Al level (Table 1) and ChAT activity (Table 2) in cerebral areas. In male rats treated with Al salt by gastric intubation for 10 d *(13)*, the brain ChAT activity was

not affected. Indeed in this case, the Al concentrations in the plasma and cerebral homogenates did not differ between control and treated animals. On the contrary, in young rats treated by gastric intubation with Al lactate (200 mg Al/kg/d) from postnatal days 5–14, the Al concentrations in the cerebral areas increased in parallel to plasma Al and the brain ChAT activity was reduced *(32)*. The elevation of Al concentration observed in the cerebral homogenate could be attributed to an increase, in young rats, in the permeability of the blood–brain barrier.

In conclusion, the nephrectomy and the food restriction did not affect the total proteins concentration in plasma and the cerebral ChAT activity. Both of them were only reduced in the rats treated by Al chloride.

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