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Chronic, Oral Aluminum Administration to Rats: Cognition and Cholinergic Parameters

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CONNOR, D. J., R. S. JOPE AND L. E. HARRELL. *Chronic, oral aluminum administration to rats: Cognition and cholinergic parameters.* PHARMACOL BIOCHEM BEHAV 31(2) 467–474, 1988.—Administration of aluminum sulfate in the drinking water of male Sprague-Dawley rats for thirty days resulted in an impairment of both consolidation and extinction of a passive avoidance task. No impairment of performance was observed on an active avoidance task, radial arm maze or open field activity measure. Biochemical analysis indicated a slight (<10%) but significant increase in hippocampal muscarinic receptor number after aluminum treatment as determined by tritiated quinuclidinyl benzilate (^{3}H-QNB) binding. No changes were found in choline acetyltransferase (ChAT) activity, phosphoinositide hydrolysis, ^{3}H-QNB binding in the cortex or tritiated pirenzepine (^{3}H-PZ) binding in the hippocampus or cortrex. These results indicate that cholinergic degeneration was not the cause of the observed cognitive impairments.

Aluminum Behavior Passive avoidance Active avoidance Radial arm maze Muscarinic Phosphoinositides Neurotoxin Rats

ALUMINUM neurotoxicity has been implicated in the pathology of several neurological disorders associated with cognitive impairments (9,35). The amyotrophic lateral sclerosis and parkinsonism-dementia of Guam have been correlated with high environmental levels of aluminum and the prevalent neurofibrillary tangles found in the central nervous system (CNS) of patients with these disorders contain high concentrations of aluminum (6, 15, 25). The neurological dysfunction observed in patients with dialysis encephalopathy has been linked to aluminum toxicity induced by high aluminum concentrations in the dialysate and to the use of phosphate-binding gels containing aluminum (1, 16, 23). Aluminum has also been suggested to play a role in Alzheimer's Disease (10). Recent studies have shown that both the neurofibrillary tangles (24) and the core of the neuritic plaques (5) found in the brains of patients with Alzheimer's disease contain high concentrations of aluminum. The associations between these dementing disorders and aluminum suggest that aluminum toxicity may be involved in the pathogenesis of the cognitive impairments observed in these disorders.

Few studies have assessed the effect of aluminum administration on cognitive function in animals. Intracranial aluminum injection impaired the performance of rabbits in a water maze task (28), a passive avoidance task (26) and impaired learning of a conditioned avoidance task by cats (8). Studies with rats have given more variable results. Injection of aluminum chloride into the hippocampus of rats resulted in a transient deficit in acquisition of a conditioned avoidance response (19). Chronic intubation with aluminum chloride ($AlCl_3$) produced no difference in performance on a shuttle box avoidance task (4). Aluminum chloride administered in the diet produced varying deficits on shuttle-box avoidance behavior depending on rat strain, sex, and whether or not parathyroid hormone was administered (7). In rats fed three doses of aluminum hydroxide (1500 mg/kg, 2500 mg/kg, and 3500 mg/kg) a significant correlation was found between brain aluminum content and impaired performance on a single trial passive avoidance task and on a visual-discrimination with reversal task (32).

In the present study we used a battery of behavioral tasks to investigate the effect of chronic oral aluminum sulfate administration on cognitive function in rats. This treatment has been shown to cause significant biochemical alterations in the CNS of rats and provides a chronic, rather than acute, model of aluminum toxicity (18). Because a close association exists between cholinergic activity and cognitive function (11,30), biochemical markers of presynaptic (choline acetyl-

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transferase activity; ChAT) and postsynaptic (muscarinic receptor binding) cholinergic function were measured. We also measured receptor-coupled inositol phospholipid hydrolysis after in vivo aluminum administration because this second messenger system has been shown to be inhibited by aluminum added in vitro (17).

METHOD

Aluminum Treatment

Male, Sprague-Dawley rats (initially weighing 120–130 grams) were housed communally in a temperature- (25°C) controlled room, illuminated 0600 to 1800 hours. Treated animals were maintained on drinking water containing 0.3% aluminum (as a 3.7% aluminum sulfate octadecahydrate solution; Mallinckrodt) ad lib for one month. Standard rat chow was provided to control and treated animals ad lib. During the last three weeks of aluminum administration, the treated animals consumed 18±0.8 ml of aluminum solution per day per rat (2.0 mmoles of aluminum/day/rat) and controls consumed 28±1.7 ml of water per day per rat. Animals used in the biochemical determinations were not exposed to any behavioral task.

BEHAVIORAL METHOD

Activity

General motor activity was measured in an open field apparatus consisting of an 80×80 cm square divided into sixteen 20×20 cm blocks, surrounded by a 45 cm high opaque wall. At the beginning of the measurement period, the animal was placed in the middle of the field and activity was measured from 0 to 1 minute and 1 to 5 minutes. Horizontal activity was defined as the number of hindlimb crossings between blocks and vertical activity as the number of rearings. Groups were compared by Student's *t*-test.

Passive Avoidance

A Lafayette model 12970 passive avoidance device was utilized for all passive avoidance tasks. The apparatus consisted of a runway (25×75 cm), illuminated by a 60-watt incandescent light, separated by a manually operated guillotine door from a black Plexiglas® chamber (46×40×17 cm) having a grid floor through which a shock (unconditioned stimulus; UCS) could be delivered.

Experiment 1. Animals were placed on the runway facing the closed guillotine door. After a 30 second delay the guillotine door was raised and the rats were allowed to enter the dark chamber and habituate in the apparatus for 3 minutes. Twenty-four hours later the procedure was repeated except the door separating the arm and the chamber was closed after entry of the rat and a 1.0 second, 0.8 mA scrambled foot shock (UCS) was administered through the floor grid. Retention was tested 1, 2, 3, 4 and 7 days after training.

Experiment 2. In the second experiment, rats were not prehabituated to the apparatus prior to shock and the guillotine door was open at the beginning of the trial. In order to eliminate the possibility of entry as a result of a startle response from being placed on the runway, the rat was positioned facing away from the open guillotine door and was required to turn and enter the dark chamber. When the rat had entered the chamber, the door was closed and a 4.0 second, 0.8 mA scrambled foot shock was administered. Thirty seconds later, the subject was returned to the home cage. The shock duration was increased in order to insure that all of the control animals acquired the task and to extend the extinction period so that any difference between groups would be more easily detected. Animals were tested at 24 hour intervals until extinction criteria was achieved.

Experiment 3. In order to increase the strength of the initial association, the final passive avoidance experiment involved the same conditions as Experiment 2 except that the animals were prehabituated to the apparatus for a 3 minute period, 24 hours prior to shock. Testing was done at 24 hour intervals until extinction criteria was reached.

For all paradigms, retention and extinction were assessed by the latency to enter (LTE) the dark chamber. Entry was defined as all four paws of the animal being on the grid floor of the dark chamber. The maximum latency was set at 5 minutes. Acquisition criterion was achieved when the rat withheld entry from the dark chamber for 5 minutes. Extinction criterion was achieved when the rat entered the dark chamber in less than 1 minute on 3 out of 4 consecutive days. The acquisition of the task and the number of days to reach extinction criteria were compared between groups using Fisher's exact test and by ANOVA with unequal n.

Active Avoidance

A Lafayette model 85150 step-up active avoidance device was used for all active avoidance tasks. The device consisted of a lower (shock) chamber with a grill floor and an upper (safe) chamber with a retractable back wall. The conditioned stimulus (CS) consisted of a 5-watt light and the unconditioned stimulus (UCS) was a scrambled foot shock. Concurrent with the presentation of the CS, the back wall of the upper chamber retracted to allow access to the safe chamber. To make an avoidance response, the animal had to climb into the upper chamber within the 10 second CS interval. If an avoidance response was not made, the UCS was administered for 10 seconds or until an escape response was made by the animal climbing into the upper chamber. The rat was then given a 10 second rest period. Upon conclusion of the trial, the back wall of the upper chamber was moved forward and the animal returned to the lower chamber. After a 10 second intertrial interval, the procedure was repeated.

To determine the effect of aluminum on the acquisition of the task, animals were treated with aluminum (0.3% for one month) prior to training. The acquisition schedule consisted of 10 trials per session with one session every other day and a UCS shock level of 0.3 mA. Acquisition criterion was defined as 9 avoidance responses in 10 trials (with the first 5 responses being avoidances) on 2 out of 3 consecutive days. In order to assess the strength of acquisition, 20 trials without shock were given 24 hours after acquisition criterion was reached. Data were analyzed by ANOVA with unequal n and by Student's *t*-test.

To test the effect of aluminum on the retention of an active avoidance task, rats were trained on the active avoidance task prior to aluminum administration. Training sessions consisted of 20 trials per day with a UCS shock level of 0.8 mA. Training criterion was achieved when the rat established 18 avoidance responses in 20 trials with the first 5 responses being avoidances. Beginning 48 hours after reaching criterion, aluminum was administered for 30 days. To assess retention without new learning or recall being primed by the UCS, the first test session consisted of 20 trials without shock. No-shock trials continued until a stable extinction criteria was achieved (5 or fewer avoidance responses per session with none of the first 5 responses being

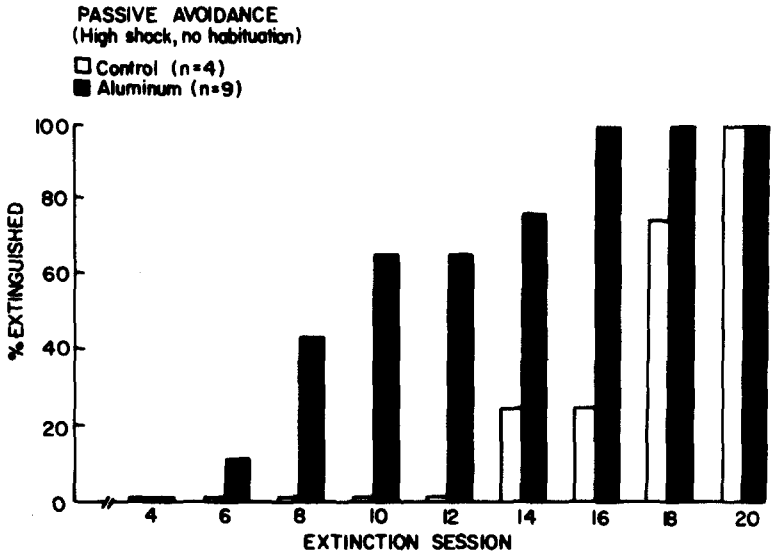

FIG. 1. Effects of aluminum sulfate administration (30 days) on extinction of a learned passive avoidance response (Experiment 2). Animals were trained without prehabituation to the apparatus as described in the Method section. Bars represent percent of each group to reach extinction criteria. Open bars represent control rats (n=4) and shaded bars represent aluminum-treated rats (n=9).

an avoidance). Following this, shock trials were resumed until criterion was reacquired. Data were analyzed by Student's *t*-test.

Radial Arm Maze

A standard, wooden open eight-arm radial maze was used to assess working memory in an appetitive task paradigm (21). This apparatus consisted of a black wooden eight arm maze elevated 100 cm above the floor. The center platform was octagonal and 30 cm in diameter with eight arms, 75 cm long and 7 cm wide, spaced at equal distances. At the outer end of each arm a 2 cm wide and 1 cm deep hole served as the reward cup.

Animals were deprived of food for a 24 hour period prior to the beginning of initial training. The prealuminum training consisted of a habituation, a shaping, and an acquisition session. In the habituation session four baits (dry fruit loops) were placed equidistant along each of the eight arms and the rats were allowed access to the baits for a 5 minute period. This procedure was repeated 24 hours later. The shaping sessions were then begun by reducing the number of baits per arm over the next 4 days until only the bait in the reward cup was presented.

In acquisition training, water deprivation was used as the motivating variable with water-soaked fruit loops as the reward. Food was provided ad lib, but access to water was restricted to 1 hour after each session. During the acquisition sessions, rats were placed in the center of the maze and allowed to enter the arms and eat the bait. Arm entries and reentries were recorded until the rat had obtained all baits or a 5 minute period had elapsed. Acquisition criterion was reached when all 8 baits were obtained within 5 minutes with less than 2 arm reentries (errors) per trial on 4 out of 5 consecutive days. Aluminum administration began 48 hours after acquisition criterion was reached.

After 30 days of aluminum administration, animals were retested. Procedures were the same as described in acquisition training. Rats were allowed access to water or to the aluminum sulfate solution for 1 hour and 2 hours, respectively, after each daily session. The number of days to reacquire the task and the number of arm reentries (errors) per day were analyzed by Student's *t*-test and ANOVA.

BIOCHEMICAL METHOD

Choline Acetyltransferase

ChAT activity was measured using the method of Fonnum (14). Homogenates of brain regions were incubated in triplicate for 20 minutes at 37°C in buffer containing [^{14}C] acetyl-CoA (0.2 mM; ICN Radiochemicals), choline (10 mM) and eserine (1 mM). The reaction was terminated by extraction of the synthesized [^{14}C]-acetylcholine into an organic phase containing scintillation fluid (14).

Muscarinic Receptors

The method of Vickroy *et al.* (33) was employed to measure muscarinic receptor binding in homogenates of brain regions. Triplicate samples of homogenate were incubated for one or two hours at 25°C in 2 ml of 10 mM phosphate buffer containing 2.5 nM [^{3}H]-pirenzepine (^{3}H-PZ; New England Nuclear) or 0.60 nM [^{3}H]-quinuclidinyl benzilate (^{3}H-QNB; New England Nuclear), respectively. Incubations were terminated by rapid filtration through Whatman GF/B filters followed by a wash with 8 ml of ice cold buffer. Blanks contained 1 μM atropine sulfate. Protein concentration in the incubation tubes were 0.06 mg/ml and 0.03 mg/ml for ^{3}H-PZ and ^{3}H-QNB binding, respectively (33).

Inositol Phospholipid Hydrolysis

Phosphoinositide metabolism was determined by measurement of [^{3}H]myo-inositol-1-phosphate (M1P; accumula-

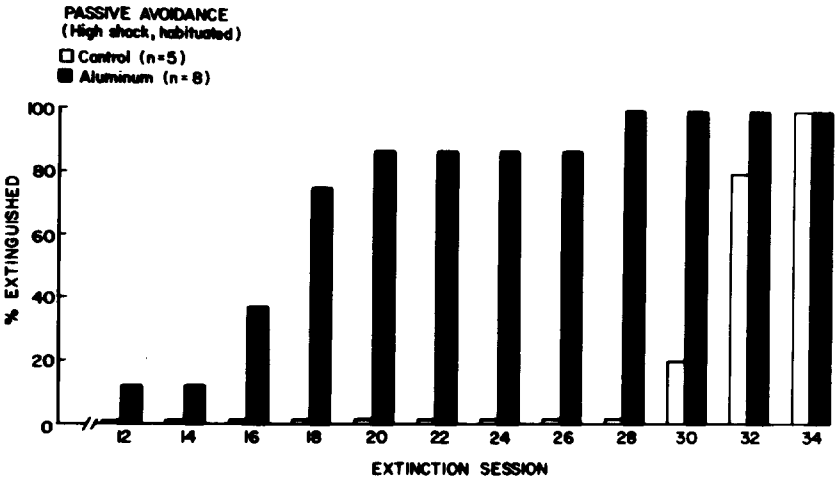

FIG. 2. Effect of aluminum sulfate administration (30 days) on extinction of a learned passive avoidance response (Experiment 3). Rats were trained with prehabituation to the apparatus as described in the Method section. Bars represent percent of each group to reach extinction criteria. Open bars represent control rats (n=5) and shaded bars represent aluminum-treated rats (n=8).

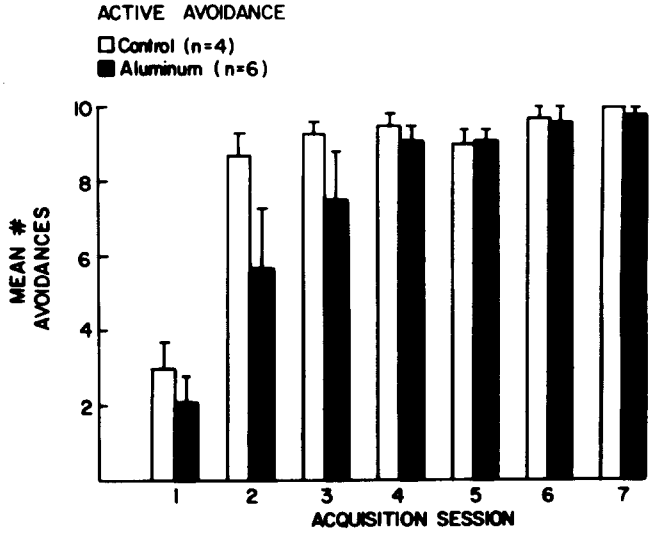

FIG. 3. Effect of aluminum administration (30 days) on acquisition of the active avoidance task. Data are expressed as the mean number of avoidance responses per session ±S.E.M. Open bars represent control rats (n=4), and shaded bars represent aluminum-treated rats (n=6).

tion using a modification of the method of Berridge *et al.* (3). Cortices or hippocampi were sliced (0.3 mm) in 2 perpendicular directions using a McIlwain tissue slicer. The slices were washed several times and preincubated for 60 minutes at 37°C in media (NaCl, 122 mM; KCl, 4.9 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 3.6 mM; dextrose, 11 mM; HEPES, 30 mM; bubbled with 95% CO_2 for 20 min, pH adjusted to 7.3 with NaOH) to restore energy balance. Media was renewed after 30 min of preincubation. Slices were then washed several times and incubated in 50 μl of media containing 0.53 μM myo-[2-^{3}H]inositol (American Radiolabelled Chemicals) and 10 mM LiCl for 60 minutes at 37°C. Carbachol (5 mM), norepinephrine (200 μM), K^+ (25 mM), or media (basal) and $CaCl_2$ (1.3 mM) were then added to the prelabeled slices and incubated for 60 minutes. The reaction was stopped by rapidly washing the slices twice with 6 volumes of ice-cold media, with an addition of 1.5 ml of $CHCl_3$/MeOH/12 N HCl (1:2:0.01). After a 20 minute extraction period, 1.0 ml of chloroform and 0.5 ml of H_2O were added and the lipid and aqueous phases were separated by centrifugation. The lipid phase was dried overnight at room temperature and counted in 5 ml of scintillation fluid.

The aqueous phase was mixed with 0.5 ml of a 50% AG1×8 slurry (formate form; BioRad) and 1.0 ml H_2O. The mixture was vortexed and added to a 10 ml plastic column. The resin was washed with 8 ml of 5 mM Na-tetraborate/60 mM Na-formate and 6.0 ml of 200 mM NH_4-formate in 0.1 M formic acid (to elute inositol monophosphate; M1P). Ten ml of scintillation fluid was added to the final eluate and the radioactivity was determined.

Protein Determination

Protein concentration was determined by the method of Lowry *et al.* (20).

RESULTS

Behavior

Activity. No significant differences were observed between control and aluminum-treated rats in horizontal or vertical activity at 0–1 minute, 1–5 minutes, or 0–5 minute time intervals ($p>0.05$).

Passive avoidance. Initial (preshock) latency to enter the dark chamber was not different between the control and treated groups for any of the three passive avoidance experiments.

Experiment 1. Aluminum sulfate administration did not produce a deficit in acquisition or retention of the avoidance response in Experiment 1. All rats in both the control and treated groups withheld entry for the maximum time allowed (5 minutes) at 1, 2, 3, 4, and 7 days.

Experiment 2. All control animals demonstrated a LTE of 5 minutes on the first day after training while only 3 of the 9 aluminum-treated animals reached criterion on day one. The remaining 6 animals reached criterion on days 2–4 ($p<0.05$; Fisher's exact test). The learned response extinguished significantly faster in the aluminum-treated group than controls

TABLE 1

EFFECT OF ALUMINUM ADMINISTRATION ON RETENTION OF THE ACTIVE AVOIDANCE RESPONSE

Pretreatment	(n=6)	(n=4)
Days to Acquisition	3.1 ± 0.1	3.3 ± 0.3
30 Day Treatment Period	Aluminum	Control
Posttreatment		
Days to Extinction (no shock)	2.4 ± 0.6	2.5 ± 0.3
Days to Reacquisition (with shock)	1.8 ± 0.2	2.5 ± 0.3
Avoidances on Extinction Trial One (no shock)	13.5 ± 4.0	15.3 ± 3.5
Avoidances on Reacquisition Trial One (with shock)	15.2 ± 1.0	15.3 ± 1.5

Animals were trained to criteria on the active avoidance response. After thirty days of treatment with aluminum sulfate, the animals were tested for retention of the avoidance response in the absence of the UCS (shock). After the avoidance response was extinguished, the UCS was reinstated and reacquisition trials began. Data is expressed as means ± S.E.M.

TABLE 2

EFFECTS OF CHRONIC ORAL ALUMINUM SULFATE TREATMENT ON CHOLINERGIC MARKERS

Brain Region	ChAT (n=4–6)	[^{3}H]-QNB (n=8–9)	[^{3}H]-PZ (n=5)
Cortex			
Control	0.73 ± 0.075	796 ± 29	292 ± 27
Aluminum-Treated	0.73 ± 0.03	804 ± 20	288 ± 20
Hippocampus			
Control	1.13 ± 0.03	591 ± 10	243 ± 14
Aluminum-Treated	1.28 ± 0.06	642 ± 14*	246 ± 14

ChAT activity and radioligand binding assays were carried out as described in the Method section. Values are expressed as mean ± S.E.M. in nanomoles/minute/mg protein for ChAT and femtomoles/mg protein for radioligand binding assays. *$p<0.05$.

($p<0.01$; Fig. 1). Rank analysis showed that the number of days to reach the initial 5 minute LTE acquisition criteria was inversely correlated to the number of days to extinction criteria (Spearman's r=−.78; $p<0.05$).

Experiment 3. The addition of a 3 minute prehabituation period 24 hours prior to shock resulted in all of the control animals and 7 out of the 8 aluminum-treated animals acquiring the 5 minute LTE criteria on day one. The remaining aluminum-treated animal reached criterion on day 2. As in Experiment 2, administration of aluminum caused a significantly faster extinction of the passive avoidance response compared to control animals ($p<0.001$; Fig. 2).

Prehabituation to the apparatus 24 hours prior to shock significantly increased the mean number of days to extinction for both the aluminum-treated ($p<0.01$) and control ($p<0.001$) animals (Experiment 2 vs. Experiment 3). Prehabituation increased the mean number of days to extinction by 84% for control animals (from 17 to 32 days) and by 70% for aluminum-treated animals (from 10 to 17 days).

Active avoidance. Pretreatment with aluminum sulfate did not affect the number of sessions required to reach acquisition criteria (control=5.72±1.05, aluminum=6.0±0.73). No significant differences were found between groups in the number of avoidance responses or on the interaction of group × days ($p>0.5$ and $p>0.1$, respectively; Fig. 3). A significant days-effect was found, reflecting learning of the task ($p<0.05$). The treated and control groups also behaved similarly in the no-shock condition (number of active avoidance responses: control=14.5±3.6, aluminum=16.0±2.6; n=4–6).

In the active avoidance retention paradigm, the mean number of days to initial acquisition criteria (prealuminum sulfate administration) did not differ between the group to be treated with aluminum and control animals. Aluminum treatment did not significantly alter the number of trials (without shock) to reach extinction criteria nor alter the reacquisition of the task. The number of avoidance responses during the first session of extinction trials and the first session of reacquisition trials were also similar between aluminum-treated and control animals (Table 1).

Radial arm maze. The mean number of days to reach initial acquisition criteria was not significantly different between the control animals and the animals to be treated with aluminum (5.8±1.5, and 4.2±0.13, respectively; n=4–10, $p>0.1$). After 1 month of aluminum sulfate treatment, the mean number of days to reacquire task criteria was not significantly different between the two groups (control=7.0±1.08; aluminum=5.2±0.42, $p>0.07$). Analysis of arm reentries also showed no significant interaction effect of group × sessions or any significant difference between groups.

Biochemistry

There were no significant differences of ChAT activity between the aluminum-treated animals and controls in either the cortex or the hippocampus (Table 2). Muscarinic receptor number as determined by [^{3}H]-PZ and [^{3}H]-QNB binding was similar in the cortex of control and aluminum-treated rats. Although the B_{max} of ^{3}H-PZ binding in the hippocampus was not different between groups, there was a small (10%), but significant ($p<0.05$) increase in the B_{max} of ^{3}H-QNB binding in the hippocampus of the aluminum-treated rats compared with controls.

Inositol phospholipid hydrolysis demonstrated a similar resistance to aluminum treatment. Basal, carbachol (5 mM), norepinephrine (200 μM), and K^+ (25 mM) stimulated production of [^{3}M]M1P were not significantly different in cortical or hippocampal slices from the aluminum-treated rats compared with controls (Fig. 4).

DISCUSSION

A battery of behavioral tests was employed to determine the neurotoxic effects of chronic oral aluminum sulfate administration in the male Sprague-Dawley rat. The results indicate that this administration paradigm produced specific impairments of cognitive function in rats, manifest as deficits in acquisition and retention of the learned response in a passive avoidance task. The observation of these impairments

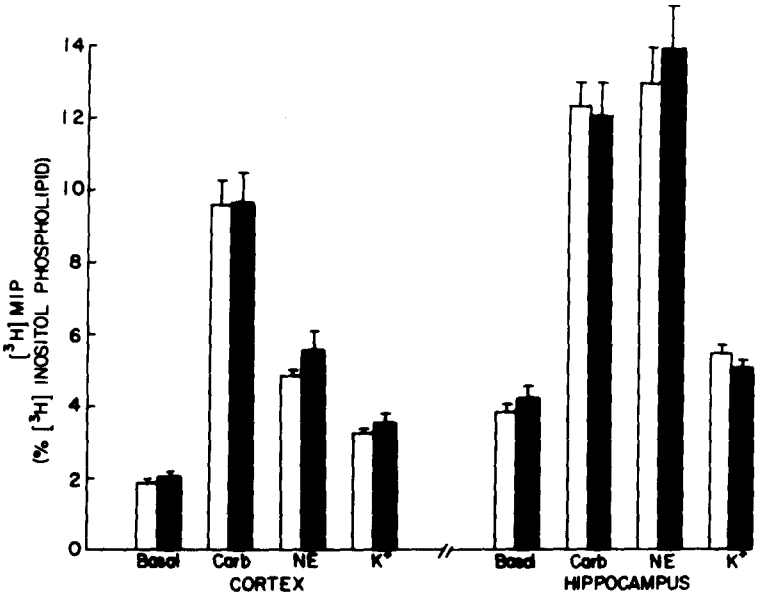

FIG. 4. Effect of aluminum sulfate administration (30 days) on phosphoinositide hydrolysis. Rat cortical or hippocampal slices were prelabeled with [^{3}H]-inositol and the release of [^{3}H]-M1P was measured as described in the Method section. Data are expressed as the percent [^{3}H]-M1P/[^{3}H]-lipids ±S.E.M. Open bars represent control rats (n=4), and shaded bars represent aluminum-treated rats (n=4).

was found to be dependent upon the experimental constraints under which the task was performed. Moreover, the cognitive impairments appear specific to this task because no deficits were observed in acquisition or retention of an active avoidance task or performance on a radial arm maze task.

Control and aluminum-treated animals behaved similarly when a standard passive avoidance paradigm was employed (Experiment 1). In order to assess more subtle influences of aluminum on behavior, the sensitivity of the task was increased by decreasing the saliency of the events (cues) through 1) omitting prehabituation to the apparatus, 2) eliminating the 30-second door delay and 3) continuing trials until extinction criterion was reached by each animal. Utilizing this protocol, deficits in both the acquisition/consolidation and retention of the tasks were evident (Experiment 2).

The longer period required for most of the aluminum-treated animals to acquire the passive avoidance behavior when compared to controls (Experiment 2), demonstrated one of the potential problems of measuring learning at only the 24 hour retention interval. That is, entry into the dark box in less than 5 minutes at the 24 hour test period may be an indication of an impaired acquisition/consolidation process rather than a retention deficit. In order to test retention of the task in the absence of a deficit in acquisition/consolidation, the initial strength of association between the CS and the UCS was increased by prehabituating the animals to the apparatus 24 hours before the training trial (Experiment 3). In this situation, all of the controls and all but one of the aluminum-treated animals reached acquisition criterion on day one and the remaining animal achieved criterion the next day. In the absence of a significant impairment of acquisition/consolidation, the aluminum-treated animals still extinguished significantly faster than controls, indicating that retention of the task was impaired independently of its initial acquisition.

An increase in general motor activity could potentially confound performance on passive avoidance and many other behavioral tasks. Aluminum administered by other methods has been reported to increase (4), decrease (7,32) or produce no change (4,7) of activity measures. In this study, activity measurements did not reveal any significant difference between groups when analyzed in 0–1, 1–5 or 0–5 minute intervals. The 0–1 minute interval was analyzed separately to exclude the possibility that an initial activity difference would be masked by the longer measurement period (0–5 minutes). That is, since extinction criterion in the passive avoidance task was set at an LTE of 1 minute or less, even a brief increase in the initial activity, which would be insignificant over the total interval (0–5 minutes), could affect the measurement of the extinction of the passive avoidance response.

Sensitivity to foot shock could also influence the performance of the rats on the behavioral tasks. Since the acquisition of the active avoidance task and of the passive avoidance task in Experiments 1 and 3 was similar between the aluminum-treated and control animals, altered sensitivity does not appear to account for the differences between the two groups in performance of the passive avoidance task. It is also doubtful that the lower fluid intake had a significant effect on the aluminum-treated animals since no obvious signs of dehydration were present (skin turgor, ungroomed fur, etc.) and recent work by our group indicates that control animals yoked by fluid intake to the aluminum-treated group, show no impairments on the passive avoidance task (to be published).

The lack of significant differences between the groups on radial arm performance, active avoidance acquisition or re-

tention, and acquisition or extinction in passive avoidance in Experiment 1 could have several possible interpretations. There are indications that different neuronal subsystems are associated with the performance of different behavioral tasks. Unlike tasks such as the radial arm maze which appear to be largely dependent upon intact cholinergic hippocampal function (12,31), performance of the passive avoidance task seems to be disrupted by lesions of several CNS regions. Hippocampal (34), striatal (27), and basal forebrain (2) lesions have all been shown to effect passive avoidance performance. It is possible that the neuronal systems involved in passive avoidance learning are more accessible or susceptible to aluminum toxicity than the systems underlying learning and retention of the other tasks.

The memory requirement of the task may also be important in considering the effects of aluminum. The radial arm maze is considered to be a measure of working memory and is dependent upon an appetitive reinforcer (22) while the passive avoidance task measures reference memory and utilizes a negative reinforcer. Also, in the passive avoidance paradigm, aluminum was administered prior to training while in several of the other tasks (active avoidance retention, radial arm maze), animals were trained prior to aluminum administration. Therefore, aluminum may interfere with the consolidation and retention of new memory rather than alter the retrieval of established memories acquired previous to aluminum exposure. It should be noted however, that aluminum did not affect active avoidance acquisition which may indicate that the different characteristics of each task (multi vs. single exposure to the UCS, go vs. no-go response, etc.) may draw on different systems.

Although no general effects of aluminum on cholinergic function were found, a small increase in hippocampal muscarinic receptor number was observed. This may reflect a slight decline in hippocampal cholinergic presynaptic activity. However, the results indicate that the aluminum treatment did not lead to cholinergic degeneration since ChAT activity, carbachol-stimulated hydrolysis and binding of another ligand (^{3}H-PZ) were unaffected. The inability of aluminum to affect other cholinergic parameters in the hippocampus is consistent with the normal performance by the aluminum-treated animals on the radial arm maze as this task has been shown to be dependent upon normal hippocampal cholinergic function (13, 22, 31).

Although in vitro aluminum inhibits carbachol-induced hydrolysis of inositol phospholipids in brain slices (17), no inhibition was observed following chronic in vivo administration of aluminum. This indicates that an irreversible alteration of this receptor-coupled system did not occur with this treatment. Preparation and washing of the brain slices during the assay may have removed the aluminum from the slices, masking a direct effect. Alternately, a longer treatment period may be required to expose the CNS to sufficient aluminum to induce an irreversible effect on this system. An initial shock or trauma to the tissue (such as changes in membrane fluidity or cell metabolic activity) as may occur in the preparation of brain slices for the phosphoinositide assay and in some clinical situations (e.g., Alzheimer's disease, dialysis encephalopathy) could also be necessary to make the neurons more susceptible to aluminum toxicity where an initial trauma may induce neuronal sensitivity to aluminum toxicity.

The variable behavioral responses to aluminum administration that have been reported could be due to different testing conditions of behavioral tasks between laboratories, variable modes of administration, and different susceptibilities of rats by age, sex and strain (4, 7, 19, 32). The chemical form of aluminum administered may also play a role in determining absorption (both intestinal and through the blood-brain barrier). For example, daily intubation with aluminum citrate significantly increased the cortical and hippocampal concentrations of aluminum, but intubation with aluminum hydroxide did not (29). The mode of administration and form of aluminum that was employed in the present study was chosen on the basis of previous research which showed CNS effects of this treatment paradigm (18). The chronic oral administration protocol reduces the stress and possible side-effects of other methods that have been used, such as daily administration by intubation with etherization, and is more comparable to the exposure of the general populace to aluminum than acute intracranial or subcutaneous injection. Moreover, aluminum sulfate has been used in water purification systems as a flocculant (9) and has been shown to be leached from the soil by acid rain (12).

This study demonstrates that chronic oral administration of aluminum sulfate induces cognitive impairments in the rat without producing major changes in the cholinergic system. These results and previous studies demonstrate the potential for neurotoxic effects of peripherally administered aluminum in the rat. It would be of interest to determine if the neurotoxic effects of aluminum could be potentiated by a compromised blood-brain barrier or by disruption of neuronal homeostasis in a system associated with clinical pathology (such as the cholinergic system in Alzheimer's disease). In any case, this treatment protocol provides a useful model for further study of aluminum toxicity.

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