Bull. Environ. Contam. Toxicol. (1994) 52:487–491

Environmental Contamination and Toxicology

In Vitro Effect of Aluminum Chloride on Choline Acetyltransferase Activity of the Rat Brain during Postnatal Growth

G. Cherroret,[1] D. Desor,[2] P. R. Lehr[1]

[1]Centre des Sciences de l'Environnement (Neurotoxicologie) Université de Metz, 1 rue des Récollets, B.P. 4025, 57040 Metz Cedex 1, France
[2]Laboratoire de Bilogie du Comportement, Faculté des Sciences, Université de Nancy 1, Nancy, France

Received: 15 January 1993/Accepted: 1 August 1993

A decrease in the activity of choline acetyltransferase (ChAT) has been well documented in brains from individuals with Alzheimer's disease (AD) (Bird et al., 1983; McGeer, 1984). Decreased ChAT activity was also found in dialysis encephalopathy victims, but this reduction was less marked than that observed in AD (Yates et al., 1980). The involvement of aluminum in the etiology of AD has been proposed by some authors on the basis of abnormal concentration of aluminum in autopsied brain samples from AD patients (Krishnan et al., 1987), in the neurofibrillary tangles (Perl and Pendlebury, 1986) and the neuritic plaques (Candy et al., 1986). King (1984) hypothesized that elevated levels of aluminum contribute to the cholinergic deficits in AD. Aluminum is considered to be the causal factor in dialysis encephalopathy (Alfrey et al., 1976), particularly in young children with azotemia (Andreoli et al., 1984). Several animal studies demonstrate in vivo an aluminum effect on ChAT (Yates et al., 1980; Hofstetter et al., 1987). The distribution of the cholinergic perikarya in the rat CNS has been established immunohistochemically using antisera to ChAT (Sofroniev et al., 1982). From the basal forebrain, ChAT positive fiber bundles could be followed to the olfactory bulb, neocortex and hippocampus (Ichikawa and Hirata, 1986). This paper examines the influence of aluminum chloride at different concentrations on the activity of ChAT in homogenates from basal forebrain and neostriatum of rats during postnatal growth.

MATERIALS AND METHODS

(Acetyl 1-^{14}C) acetyl CoA (48.1 - 59.3 mCi / mol) was purchased from New England Nuclear. Acetyl CoA was obtained from Boehringer Mannheim. Albino rats of the Wistar strain (Iffa Credo, L'Arbresle, France) used in these experiments were obtained from a breeding program maintained in our department. Standard food (Extra Labo, Provins, France) and water were given ad libitum. Young rats were weaned at 21 days postpartum. Younger rats were left with their mothers until the experiment's day. All animals were housed in plastic cages in an air-conditioned room having a relative constant temperature (20-22°C); the light cycle was 7.00 a.m. to 7.00 p.m.
The experiments were performed on rats of both sexes at 2 and 7 post-natal days, and only on males at 13, 15 , 30, 60 and 90 days of age (adult stage).
ChAT activity was measured by the micromethod of Fonnum (1975). The rats were sacrified by decapitation. The basal forebrains and the neostriatum were quickly isolated in a cold room by dissection as described by Glowinsky and Iversen (1966), then weighed. To determine the effect of aluminum on ChAT activity, parts of 4 brains from 15, 30, 60 days and adult rats were pooled for each

Correspondence to: P. R. Lehr

experiment. The combined brain areas from the left hemispheres of rats 1 and 2 and from the right hemispheres of rats 3 and 4 were homogeneized at 0°C in a Potter Elvejhem apparatus with a Teflon pestle (A.H. Thomas Co, Philadelphia, Size A) in a 10 mM EDTA solution (pH 7.4). This homogenate, in which no aluminum was added, was the control. The aluminum concentration (4.5 10^{-7} M Al) was determined using an atomic emission spectrometer (Spectra Span V, Beckman). The combined brain areas from the right hemispheres of rats 1 and 2 and from the left hemispheres of rats 3 and 4 were homogeneized in a 10 mM EDTA solution (pH 7.4) containing Al_2Cl_3, $6H_2O$ at different concentrations: 10^{-4}, 10^{-3}, 5.10^{-3} and 10^{-2} M. Ten minutes after adjunction of an equal volume of a 10mM EDTA solution (pH 7.4) containing 0.5% Triton X100 (v/v), a 10mM EDTA solution (pH 7.4) was used to complete the treatment. The final concentration of the homogenate was 5% (w/v). The incubation mixture contained (final concentration : 0.2 mM acetylCoA, 50 mM sodium phosphate buffer (pH 7.4), 300mM NaCl, 8 mM choline chloride, 20 mM EDTA pH 7.4 and 0.1 mM physostigmine. The incubation solution (5µl) and the labelled acetylCoA solution (2µl) were placed in a microtube (23 mm x 2 mm) and the homogenate (2µl) 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 10mM sodium phosphate buffer pH 7.4. Then 2 ml acetonitrile containing 10 mg sodium tetraphenylborate and 10 ml of toluene scintillation mixture (0.05% PPO, 0.02% POPOP, toluene) were added to the vial which was slightly shaken. The radioactivity of acetylcholine (ACh) which was extracted into 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 / hr / mg protein. In a preliminary experiment, parts of 2 brains from 13, 15, 30, 60 days and adult rats were used in each experiment to estimate the variation of ChAT activity during postnatal development. At days 2 and 7, the enzyme activities were evaluated in the whole brain homogenates because the brain areas were not yet differentiated. No aluminum was added in these homogenates

The protein content of the homogenates was determined using the method of Lowry et al. (1951) modified by Markwell et al. (1978) with bovine serum albumin (fraction V) as a reference. For the effect of aluminum on ChAT activity, the data were analyzed with paired Student's t test. The overall significant effect of aluminum treatment was evaluated using ANOVA procedures. Multiple comparisons are computed with Scheffe's test.

RESULTS AND DISCUSSION

The ChAT specific activities estimated at pH 7.4 in the brain homogenates from rats at different stages of development are in Table 1.

The enzyme activity in the brain areas increased rapidly (126.4%) between days 13 and 30, then slowly between days 30 and 60 (15.2%). The value reached at postnatal day 60 was similar to that evaluated at the adult stage. This developmental profile for the ChAT activity determined in brain of control rats agrees with the findings of others for rat brain (Patel et al, 1987); the variation of specific activity was substantial during the first 25 days after birth.

Figure 1 indicated the effect of aluminum on the ChAT activity of homogenates from rat brain areas during postnatal development. At 10^{-4} M, aluminum chloride had no effect on ChAT activity at any age of the rat. At 10^{-3} M, aluminum inhibited the ChAT activity; this inhibition decreased with rat's age from 13.6% at day 15 ($p< 0.001$) to 8.2% at the adult stage (NS). The levels of significance of paired

Student's t test were p = 0.0503 and p = 0.0464 respectively in 30 and 60 day old rats. In adult rat, the inhibition of ChAT activity by aluminum increased at concentrations greater than 10^{-3} M : 9.7% at 5.10^{-3} M (NS) and 15.1% at 10^{-2} M ($p< 0.01$). On the contrary, at these last concentrations, aluminum activated the enzyme in homogenates from rats at postnatal day 15 : the values of activation were

Table 1. ChAT activity in rat brain

Age (days)	ChAT activity (nmol / mg protein / h)	Number of experiments
2	0.30 ± 0.03	10
7	2.52 ± 0.33	10
13	9.87 ± 0.32	10
15	16.40 ± 0.30	10
30	22.35 ± 1.50	10
60	25.76 ± 1.12	10
adult	25.31 ± 0.80	10

Values are means ± SD.

15.1% ($p< 0.05$) and 21.9% ($p< 0.001$) at 5.10^{-3} and 10^{-2} M respectively. This ChAT activation was seriously reduced on postnatal day 30 : 2% (NS) and 6.8% ($p< 0.05$) at 5.10^{-3} and 10^{-2} M respectively. At day 60, aluminum inhibited the enzyme: the values of inhibition were 9.6% ($p< 0.01$) and 13.0% ($p< 0.01$) at 5.10^{-3} and 10^{-2} M respectively. So, aluminum chloride at concentrations greater than 10^{-3} M activated the ChAT until about postnatal day 30. Afterwards the enzyme was inhibited.The high aluminum doses used in this study are justified by the presence of EDTA and phosphate buffer which induced a decrease of free aluminum concentration in the incubation solution. Elsewhere, a part of free aluminum was adsorbed by various membrane structures. Aluminum chloride was also found to inhibit ChAT of chick brain homogenates in vitro (MILLAND et al., 1986).

Our results could be attributed to the physiochemical characteristics of the enzyme particularly to the molecular forms that occur. Indeed, the brain possesses multiple forms of ChAT which differ in isoelectric points (Malthe-Sorenssen and Fonnum, 1972). So both mature and 7 days old brain of rat contained three molecular forms of ChAT with isoelectric points of pH 7.3, 7.9 and 8.3; the immature brain appeared to contain smaller concentrations of the most basic form of ChAT (Atterwill and Prince, 1978). The results obtained from synaptosomal fractions of rat brain (Badamchian et al., 1986) showed that the soluble fraction of ChAT had only one isoelectric point at pH 7.8, whereas the ionically membrane bound ChAT fraction had two isoelectric points at pH 8.1-8.15 and 7.45-7.5. The possibility arises therefore of differences in the multiple forms of ChAT. The activation of ChAT activity observed in homogenates from 15 days old rats by high concentration of aluminum could be attributed to one or both of the more acidic forms which may be precursors of the basic enzyme predominant in adult rats according to Atterwill and Prince, 1978. In contrast, the inhibition of ChAT activity in homogenates from adult rat could be attributed to the isoenzyme with the isoelectric point at pH 8.3. It would also be interesting to see if the soluble and membrane bound ChAT fractions differ in their response to aluminum. A subsequent study using synaptosomal fractions of rat brain might be necessary to settle this question.

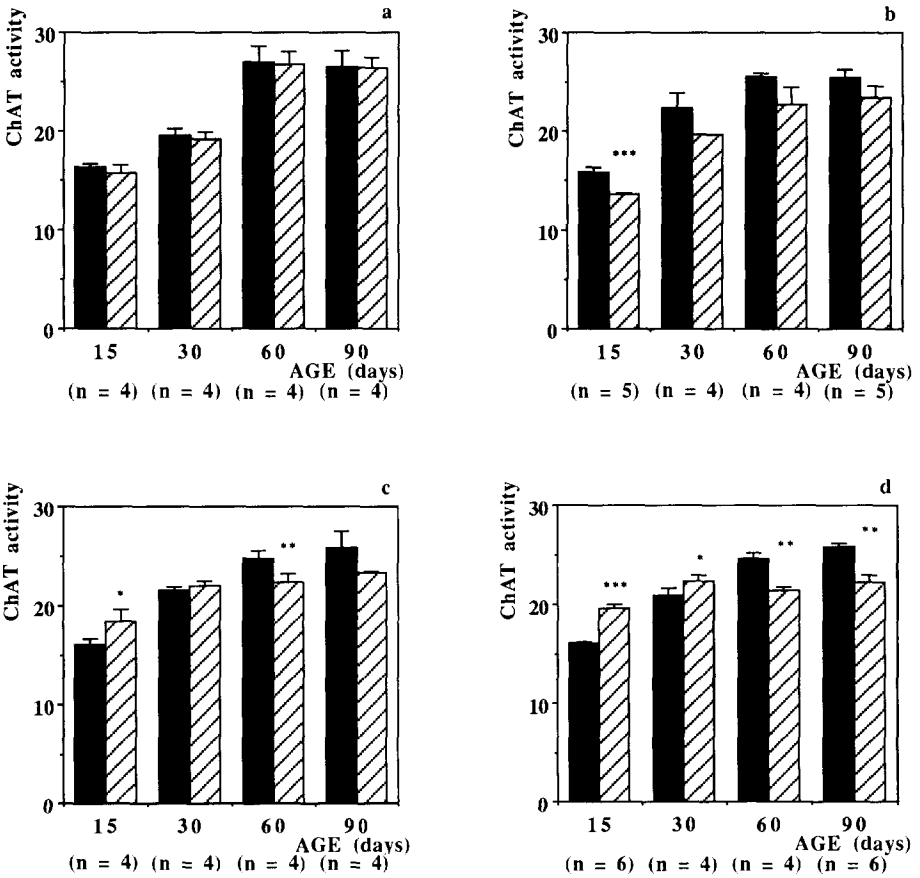

Figure 1. Effect of aluminum on ChAT activity (nmol / mg protein / h).
Means ± SD. Numbers in brackets indicate numbers of experiments .
Control ■ Aluminum ▨
Aluminum concentrations :
a = 10^{-4} M ; b = 10^{-3} M ; c = 5.10^{-3} M ; d = 10^{-2} M.
Significant different from control : *p< 0.05 **p< 0.01 ***p< 0.001

Acknowledgments. This research was supported by grants from the Fondation pour la Recherche Médicale (Comité Lorraine).

REFERENCES

Alfrey AC, Le Gendre GR, Kaehny WD (1976) The dialysis encephalopathy syndrome. Possible aluminum intoxication. N Engl J Med 294: 184-188

Andreoli SP, Bergstein JM, Sherrard DJ (1984) Aluminum intoxication from aluminum-containing phosphate binders in children with azotemia not undergoing dialysis. N Engl J Med 17: 1079-1084

Atterwill CK, Prince AK (1978) Multiple forms of choline acetyltransferase and the high affinity uptake of choline in brain of developing and adult rats. J Neurochem 31: 719-725

Badamchian M, Morrow Jr KJ, Caroll PT (1986) Immunological, isoelectric, hydrophobic and molecular weight differences between soluble and ionically

membrane-bound fractions of choline o-acetyltransferase prepared from mouse and rat brain. Neurochem Int 9: 409-421
Bird TD, Stranahan BS, Sumi SM, Raskind M (1983) Alzheimer's disease: choline acetyltransferase activity in brain tissue from clinical and pathological subgroups. Ann Neurol 14: 284-293
Candy JM, Oakley AE, Watt F, Grime GW, Klinowski J, Perry RH, Edwardson JA (1986) A role for aluminum, silicon and iron in the genesis of senile plaques. Modern trends in aging research. 147: 443-450
Fonnum F (1975) A rapid radiochemical method for the determination of choline acetyltransferase. J Neurochem 24: 407-409
Glowinski J, Iversen LL (1966) Regional studies of catecholamines in the rat brain. I . The disposition of (3H) norepinephrine, (3H) dopamine and (3H) dopa in various regions of the brain. J Neurochem 13: 655-669
Hofstetter JR, Vincent I, Bugiani O, Ghetti B, Richter JA (1987) Aluminum-induced decrease in choline acetyltransferase, tyrosine hydroxylase, and glutamate decarboxylase in selected regions of rabbit brain. Neurochem Pathol 6: 177-193
Ichikawa T, Hirata Y (1986) Organization of choline acetyltransferase containing structures in the forebrain of the rat. J Neurosci 6: 281-292
King RG (1984). Do raised brain aluminum levels in Alzheimer's dementia contribute to cholinergic neural deficits? Med Hypotheses 14: 301-306
Krishnan SS, Harrison JE, Crapper McLachlan DR (1987) Origin and resolution of the aluminum controversy concerning Alzheimer's neurofibrillary degeneration. Biol Tr Elem Res 13: 35-42
Lowry OH, Rosebrough NJ, Farr A L, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265-275
Malthe-Sorenssen D, Fonnum F (1972). Multiple forms of choline acetyltransferase in several species demonstrated by isoelectric focusing. Biochem J 127: 229-236
Markwell MAK, Haas SM, Bieber LL, Tolbert NE (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 87: 206-210
McGeer PL (1984) Aging, Alzheimer's disease and the cholinergic system. Can J Physiol Pharmacol 62: 741-754
Milland JA, King RG, Rogers LJ (1986) Changes in aggressive behaviour and forebrain choline acetyltransferase activity in the chick following aluminum chloride administration. Asia Pacific J Pharmacol 1 : 105-110
Patel AJ, Hayashi M, Hunt A (1987) Selective persistent reduction in choline acetyltransferase activity in basal forebrain of the rat after thyroid deficiency during early life. Brain Res 422: 182-185
Perl DP, Pendlebury WW (1986) Aluminum neurotoxicity. Potential role in the pathogenesis of neurofibrillary tangle formation. Can J Neurol Sci 13: 441-445
Sofroniev MV, Eckenstein F, Thoenen H, Cuello AC (1982) Topography of choline acetyltransferase-containing neurons in the forebrain of the rat. Neurosci Lett 33: 7-12
Yates CM, Simpson J, Russell D, Gordon A (1980) Cholinergic enzymes in neurofibrillary degeneration produced by aluminum. Brain Res 197: 269-274