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The effects of aluminium, manganese and cadmium chloride on the methylation of phospholipids in the rat brain synaptosomal membrane

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It has been demonstrated that many heavy metals can exert neurotoxic effects. Thus, administration of aluminium salts is associated with neurofibrillary degeneration [1, 2], cadmium chloride damages the cells of the cerebellar cortex [3] and chronic manganese poisoning may adversely affect the extrapyramidal system [4]. The mechanism by which these metals act in the CNS is poorly understood (for reviews see [5, 6]). Work in this laboratory has shown that the three metals inhibit the uptake of choline [7], catecholamines [8], γ-aminobutyric acid and glutamic acid [9] by rat brain synaptosomes. They also inhibit synaptosomal sodium–potassium-activated adenosine triphosphatase, EC 3.6.1.3. [7]. Although these two inhibitory effects have been shown not to be directly related [7], nevertheless, they are consistent with earlier observations that interaction of polyvalent cations with molecules present in the cell membrane can lead to alterations in membrane function [10, 11].

Methylation of phospholipids may play a very important role in membrane function. The synthesis of phosphatidylcholine by phosphatidylethanolamine-*N*-methyltransferase (EC 2.1.1.17) is achieved in two stages [12]. Methyltransferase I methylates phosphatidylethanolamine to form the *N*-monomethyl derivative. This enzyme has a high affinity to the methyl donor, *S*-adenosylmethionine (SAM). Methyltransferase II successively methylates the product of the first reaction twice to form phosphatidylcholine. The second enzyme has a relatively low affinity to the methyl donor. The synthesis and translocation of these methylated derivatives affects many membrane properties in several systems (see [13]). In order to achieve a further understanding of the toxic action of aluminium, manganese and cadmium in neuronal function, we investigated the effects these metals have on rat brain synaptosomal phospholipid methyltransferases in the light of recent information on the putative role of these enzymes in the brain [14].

Synaptosomes were prepared from forebrains of adult male rats by fractionation on sucrose–Ficoll gradient [7]. The preparation, usually from four animals, was stored in 5 ml of 0.32 M sucrose, 10 mM Tris–HCl pH 7.4, at −20° for 24 hr. It was then thawed and centrifuged at 22,000 *g* for 15 min. The pellet was resuspended in 25 ml of 6 mM Tris–glycylglycine pH 8.0 and kept at 4° for 1 hr. The membrane fraction was sedimented and washed once with 10 ml of the Tris-glycylglycine buffer. It was finally suspended in 3 ml of the same buffer by gentle homogenization. This usually gave protein concentrations of about 5 mg/ml. The membrane fraction was stored in small aliquots at −20° and retained a constant enzyme activity for at least 1 week.

The methyltransferases were assayed by measuring the incorporation of the labelled methyl group from SAM into chloroform-methanol extractable lipids [14]. The reaction mixture, containing 25 mM Tris–glycylglycine pH 8.0, 2 mM $MgCl_2$, membrane fraction (0.2–0.4 mg of protein), 100 μM (2 μCi) or 3.4 μM (1.8 μCi) of *S*-adenosyl-L-[methyl-^{3}H]methionine (sp. act. 62 Ci/mmole; the Radiochemical Centre, Amersham, U.K.) and various concentrations of $AlCl_3$, $MnCl_2$ or $CdCl_2$ in a final volume of 100 μl, was incubated at 37° for 45 min. A zero-time sample was used as background control. This value was similar to that when the reaction mixture was incubated with a membrane fraction which had been heated at 100° for 5 min. The reaction products were identified by t.l.c. using a mixture of chloroform, propan-1-ol, n-propionic acid and water (2:2:3:1 v/v) as the developing solvent [14].

Phospholipid components in the rat brain synaptosomal membrane were found to be methylated at rates, as listed in Table 1, comparable to those reported [14]. At 3.4 μM SAM, $AlCl_3$, $MnCl_2$ and $CdCl_2$ affected the rates differently (Fig. 1). $MnCl_2$ as high as 1 mM, had little effect. $AlCl_3$, at concentrations above 0.5 mM, caused a slight but reproducible stimulation. This was not due to subsaturating conditions as addition of 1 mM $MgCl_2$ did not increase the reaction rate (Table 1A). $CdCl_2$, on the other hand, was a potent inhibitor. The concentration which caused 50 per cent inhibition (IC_{50}) was about 0.07 mM. This effect was probably not merely due to competition with Mg^{2+} as $CaCl_2$ (1 mM) was not inhibitory (Table 1A). Analysis of the products showed that at low concentration of SAM, about 40 per cent of the radioactivity was found to be associated with material co-chromatographed with phosphatidyl-*N*-monomethylethanolamine. Less than 10 per cent was found associated with phosphatidyl-*N*,*N*-dimethylethanolamine and phosphatidylcholine. The remaining radioactivities were found in unidentified substances which migrated with the solvent front. In the presence of 1 mM $CdCl_2$, the radioactivity in the phosphatidyl-*N*-monomethylethanolamine fraction was decreased by about 80 per cent. These results indicated that at low concentration of SAM, only the activity of methyltransferase I was measured and this was sensitive to inhibition by $CdCl_2$.

Table 1. Effects of metal ions on the incorporation of [^{3}H]methyl into membrane phospholipids

Metal ion	Enzyme activity (pmoles/mg/45 min)	Per cent of control
(A) *S*-adenosylmethionine = 3.4 μM		
Control	4.57	100
$MgCl_2$	4.82	106
$CaCl_2$	5.21	114
(B) *S*-adenosylmethionine = 100 μM		
Control	13.72	100
$AlCl_3$	13.42	98
$MnCl_2$	11.30	82
$CdCl_2$	11.40	83

The membrane fraction from rat brain synaptosomes was incubated with *S*-adenosyl-L-[methyl-^{3}H]methionine in the presence of 1 mM of the metal ions. In the case of $MgCl_2$, the total Mg^{2+} concentration in the assay would be 3 mM. All values given are means of duplicate determinations which differed in all cases by less than 5%. These values show the metals to be without marked effect under the conditions of the experiment. Similar results are obtained upon repeated experiments.

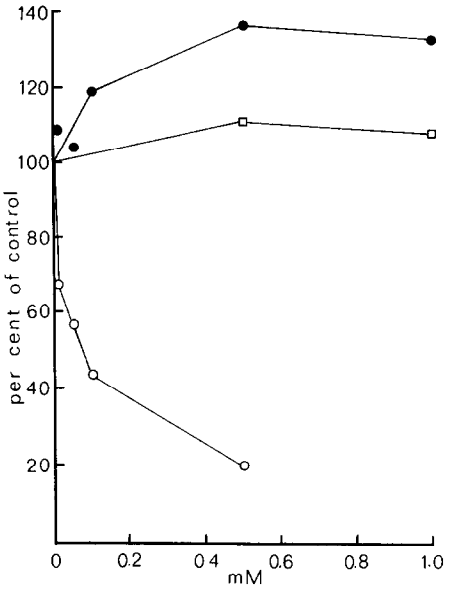

Fig. 1. The membrane fraction from rat brain synaptosomes was incubated for 45 min with 3.4 μM (1.8 μCi) of *S*-adenosyl-L-[methyl-^{3}H]methionine in the presence of various concentrations of $CdCl_2$ (○), $AlCl_3$ (●) or $MnCl_2$ (□). The radioactivity incorporated into the lipid fraction was measured. The values given are the means of duplicate determinations which differed in all cases by less than 5%. The results expressed as percentages of the control activity, which was 6.47 pmoles/mg/45 min, show $CdCl_2$ to have a marked inhibitory effect. Results of a similar nature are obtained upon repeated experiments.

At high concentration of SAM (100 μM), none of the three metals altered the rate of methylation to any appreciable extent (Table 1B). They were also ineffective if freshly prepared intact synaptosomes were used in place of the membrane fractions which indicated that the lack of effect was probably not due to disruption of membrane integrity.

In the erythrocyte ghost, methylation by methyltransferase I only was sufficient to change the fluidity of the membrane [15]. An increase in the rate of phospholipid methylation was also associated with enhancement in β-adrenergic-adenylate cyclase coupling in the rat reticulocyte (16). Binding of β-adrenergic agonists to astrocytoma cells stimulated both methyltransferase I and II [13]. These effects have led to speculations that in the neurones, methyltransferases may play a role in the functions of synapses [14]. One possibility is that these enzymes may be linked to neurotransmission processes such as release and uptake of the transmitter substances. Our previous results showed that $AlCl_3$, $MnCl_2$ and $CdCl_2$ were all inhibitors of neurotransmitter uptake in rat brain synaptosomes [7–9]. Although they inhibit with varying degrees of potency, the general trend was that $AlCl_3$ was slightly more potent than $CdCl_2$ and $MnCl_2$ was the least effective. However, these effects are clearly not parallel to that on methyltransferase I as shown in this study. It can be concluded that the action of the metals on the methylation of phospholipids in the synaptosomal membrane is quite distinct from that on neurotransmitter uptake into synaptosomal particles. Since Cd^{2+} is toxic to many tissues, it would be interesting to determine if it affects methylation of phospholipids in tissues other than the brain.

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