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# THE EFFECTS OF CADMIUM, MANGANESE AND ALUMINIUM ON SODIUM–POTASSIUM-ACTIVATED AND MAGNESIUM-ACTIVATED ADENOSINE TRIPHOSPHATASE ACTIVITY AND CHOLINE UPTAKE IN RAT BRAIN SYNAPTOSOMES

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**Abstract**— The effects of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ on rat brain synaptosomal sodium–potassium-activated and magnesium-activated adenosine triphosphatase (Na–K–ATPase and Mg–ATPase) activity and choline uptake were studied. All three types of metal ions inhibited Na–K–ATPase activity more markedly than Mg–ATPase activity. The rank order of inhibition of Na–K–ATPase was: $Cd^{2+}$ ($IC_{50}$ = 5.4 $\mu$M)>$Mn^{2+}$ ($IC_{50}$ = 955 $\mu$M) ≫$Al^{3+}$ ($IC_{50}$ = 8.3 mM). The rank order of inhibition of Mg–ATPase was: $Cd^{2+}$ ($IC_{50}$ = 316 $\mu$M)>$Mn^{2+}$ ($IC_{50}$ = 5.5 mM)>$Al^{3+}$ ($IC_{50}$ = 21.9 mM). $Al^{3+}$ was most potent in inhibiting synaptosomal choline uptake ($IC_{50}$ = 24 $\mu$M in the absence of $Ca^{2+}$ and 123 $\mu$M in the presence of 1 mM $Ca^{2+}$). $Cd^{2+}$ ($IC_{50}$ = 363 $\mu$M) was a more effective inhibitor of choline uptake than $Mn^{2+}$ ($IC_{50}$ = 1.2–1.5 mM). The presence of 1 mM $Ca^{2+}$ did not alter choline uptake, nor did it antagonize the inhibitory actions of the three metals. Our observations that $Cd^{2+}$ and $Al^{3+}$ inhibited synaptosomal choline uptake, but did not show parallel inhibitory effects on Na–K–ATPase activity directly contradicts the ionic gradient hypothesis. These results are also discussed in relation to the *in vivo* neurotoxicity of cadmium, manganese and aluminium.

The sodium–potassium-activated adenosine triphosphatase [ATP phosphohydrolase, EC 3.6.1.3 (Na–K–ATPase)] has been considered to be an integral part of the sodium pump and is responsible for the transport of potassium into and sodium out of a variety of cell types (for reviews see Rf. [1, 2]). In the ionic gradient hypothesis, it has been postulated that the energy available from the inward-directed sodium electrochemical gradient, which is maintained by the Na–K–ATPase, may be coupled to the different synaptosomal uptake systems [3]. Recently several studies have shown that certain divalent metal ions of toxicological interest are potent inhibitors of brain Na–K–ATPase [4–6]. Furthermore, the results of Prakash *et al.* [5] appear to lend additional support to the ionic gradient hypothesis, in that they found that metal ions (particularly $Hg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$), which inhibited synaptosomal Na–K–ATPase activity, showed parallel inhibitory effects on the synaptosomal uptake of choline and noradrenaline. Trace metals may have diverse roles in modulating brain function and behaviour, although the underlying biochemical mechanisms have not yet been elucidated [7]. We have investigated the effects of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ on the synaptosomal Na–K–ATPase and Mg–ATPase activity and choline uptake. We present evidence that all three metals (in their ionic forms) show selective effects on the inhibition of synaptosomal Na–K–ATPase and Mg–ATPase activity as well as choline uptake.

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## MATERIALS AND METHODS

Where possible analytical grade (AR) chemicals were used and obtained from either BDH Chemicals Ltd., Baird Rd., Enfield, Middlesex, U.K. or from Sigma (London) Ltd., Fancy Rd., Poole, Dorset, U.K., or from Bochringer Corporations (London) Ltd., Bell Lane, Lewes, East Sussex, U.K. Ficoll-400 was obtained from Pharmacia, Uppsala, Sweden, and dialysed against deionized glass-distilled water for at least 4 hr before use. [Methyl-$^3$H]-choline chloride (sp. act. 8.4 Ci/mole, final concentration $10^{-7}$M) was obtained from the Radiochemical Centre, Amersham, Buckinghamshire, U.K. Acid-washed and deionized-glass-distilled water-rinsed glassware was used. All solutions were prepared with deonized glass-distilled water.

### *Subcellular fractionation*

Four adult male Wistar rats (Porton Strain) (150–180g, aged 40–55 days) were usually employed for the isolation of the synaptosomal fraction. Animals were decapitated. The brain was trans-sected at the level of the superior and inferior colliculi, and the part rostral to this trans-section (except the olfactory bulbs) was taken for the subcellular fractionation. The subcellular fractionation procedure was essentially the same as that of Lai and Clark [8, 9] except that the density gradient used for separating synaptosomes from 'free' mitochondria and myelin consisted of 3 ml of 0.32 M sucrose, 10 mM Tris–HCl, pH 7.4 overlaid on 6 ml of 7.5% (w/w) Ficoll, 0.32 M sucrose, 10 mM Tris–Hcl, pH 7.4 overlaid on a

suspension of the crude mitochondrial fraction in 10 ml of 12 per cent (w/w) Ficoll, 0.32 M sucrose, 10 mM Tris–HCl, pH 7.4. This gradient was modified from the procedure of Booth and Clark [10]. None of the media used contained EDTA.

*Choline uptake*

The procedure for determining synaptosomal choline uptake was basically that of Nicklas *et al.* [11]. Sets of tubes were set up containing Krebs–Ringer phosphate (135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2.6H_2O$, 10 mM D-glucose, 1 mM sodium phosphate buffer pH 7.4 and 1 mM Tris–HCl, pH 7.4, with or without 1 mM $CaCl_2$), $10^{-7}$M[$^3$H]choline chloride and the snyaptosomal preparation, in the absence or presence of $CdCl_2$, or $MnCl_2$, or $AlCl_3$. Uptake was carried out at 37° for 4 min (with a 3-min pre-incubation) in a shaking water-bath. The content of each tube was then filtered through a Millipore filter (pore size 0.65 $\mu$m). The filter was washed three times with 1 ml of ice-cold 0.15 M NaCl. The radioactivity on each filter was determined by liquid scintillation counting after solubilization with 2 ml of ethoxyethanol. The difference between the counts in the zero-time sample and the counts retained by subcellular particles after the 4-min incubation was usually used for computing the uptake rates.

### ATPase AND OTHER ASSAYS

The assays of Na–K–ATPase and Mg–ATPase activity were performed according to the method of Bonting [1]. The final concentrations of reagents were as indicate in Table 1. The synaptosomal fraction was suitably diluted with 6 mM Tris–HCl, pH 7.4 to give 35 $\mu$g of protein per assay tube. Assay tubes (final volume 1 ml per tube) were set up as shown in Table 1 with or without the addition of $CdCl_2$ or $MnCl_2$ or $AlCl_3$. Two tubes containing medium A were set up in each group of tubes. The tubes were pre-incubated at 37° for 10 min and, after the addition of ATP, the tubes were incubated for a further 10 min. The reaction was stopped with the addition of 2 ml of ice-cold 10 per cent (w/w) trichloroacetic acid. Then 1 ml from each tube was used for phosphate determination. To 1 ml of the sample was added 1 ml of acid molybdate solution

Table 1. Composition of incubation media for ATPase assays

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| ATP(diNa salt) | 1 | 1 | 1 | 1 | 1 | — |
| $MgCl_2$ | 2 | 2 | 2 | 2 | 2 | 2 |
| KCl | 5 | — | 5 | 5 | — | 5 |
| NaCl | 60 | 60 | — | 60 | 60 | 60 |
| Tris–HCl,pH7.4 | 92 | 92 | 151 | 92 | 92 | 92 |
| Ouabain | — | — | — | 0.1 | 0.1 | — |

All concentrations are in mmole/l. Each tube contains 35 $\mu$g of synaptosomal protein. The average activity in media B, C, D and E is the Mg–ATPase activity; the difference between activity in medium A and the Mg–ATPase activity is the Na–K–ATPase activity [1].

Medium F acts as the blank.

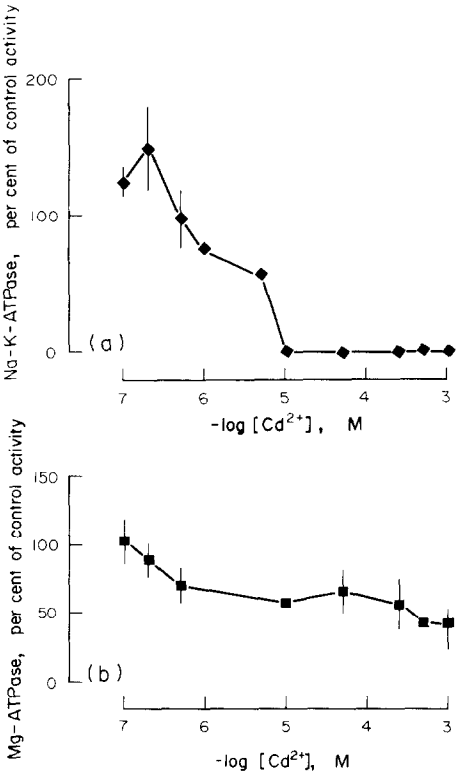


Fig. 1. Effects of $Cd^{2+}$ on rat brain synaptosomal Na–K–ATPase and Mg–ATPase activity. (a) The activity of Na–K–ATPase was determined in the presence of various concentrations of $CdCl_2$ and expressed as per cent of control (i.e. in the absence of $Cd^{2+}$) values as described in the Methods section. Data were Mean ± S.D. of at least six experiments. Synaptosomal Na–K–ATPase activity in the absence of $Cd^{2+}$ was 397 ± 76 nmol Pi/min/mg protein (Mean ± S.D. of 16 experiments). (b) The activity of Mg–ATPase was measured in the presence of different concentrations of $CdCl_2$ and expressed as per cent of control (i.e. in the absence of $Cd^{2+}$ values as described in the Methods section). Data were Mean ± S.D. of at least six experiments. Synaptosomal Mg–ATPase activity in the absence of $Cd^{2+}$ was 389 ± 61 nmol Pi/min/mg protein (Mean ± S.D. of 16 experiments). (Only the S.D.s greater than the heights of the symbols were drawn).

[from Sigma (London) Ltd.], followed by 0.25 ml of Fiske and Subbarow Reducer [from Sigma (London) Ltd.], and the optical density of the resultant mixture was read at 660 nm after incubating for 10 min at room temperature. $KH_2PO_4$ (20 $\mu$g Pi/ml) was used to construct the standard curve of inorganic phosphate.

Protein was determined by the method of Lowry *et al.* [12].

Statistical analyses of significance of differences were done with non-paired *t*-test.

### RESULTS

*Effects of* $Cd^{2+}$ *and* $Al^{3+}$ *on synaptosomal* Na–K–ATPase *and* Mg–ATPase

In 16 rat brain synaptosomal preparations the activity of Na–K–ATPase was 397 ± 76 nmol Pi/min per mg of protein (Mean ± S.D.) and that of Mg-ATPase was 389 ± 61 nmol Pi/min per mg of protein

(Mean ± S.D.). These values are comparable to those quoted in the literature [5, 16].

Figure 1 shows the effects of $Cd^{2+}$. $Cd^{2+}$ at 100 – 200 nM significantly ($P < 0.05$) stimulated Na–K–ATPase activity (by 20–40 per cent). At 1 $\mu$M, $Cd^{2+}$ clearly inhibited the activity of this enzyme, and at 10 $\mu$m, $Cd^{2+}$ virtually completely inhibited its activity (Fig. 1a). The concentration of $Cd^{2+}$ which gave rise to 50 per cent inhibition of Na–K–ATPase activity ($IC_{50}$) was 5.4 $\mu$M. However, the inhibitory effect of $Cd^{2+}$ on Mg–ATPase was much less marked ($IC_{50}$ = 316 $\mu$M; see Fig. 1b). Significant inhibition of Na–K–ATPase activity by $Mn^{2+}$ ($IC_{50}$ = 955 $\mu$M; see Table 2) was achieved at concentrations greater than 1 mM (data not shown). Similarly Mg–ATPase was inhibited by $Mn^{2+}$ ($IC_{50}$ = 5.5 mM; see Table 2) at concentrations exceeding 1 mM (data not shown).

$Al^{3+}$ was a very poor inhibitor of Na–K–ATPase ($IC_{50}$ = 8.3 mM; see Table 2) and Mg–ATPase ($IC_{50}$ = 21.9 mM; see Table 2).

The synaptosomal Na–K–ATPase activity was more sensitive to inhibition by all three metal ions in comparison with the synaptosomal Mg–ATPase. The rank order of inhibition of both types of ATPase activities was: $Cd^{2+} > Mn^{2+} \gg Al^{3+}$ (see Table 2).

*Effects of* $Cd^{2+}$, $Mn^{2+}$ *and* $Al^{3+}$ *on the time-course of rat brain synaptosomal choline uptake.*

The effects of the metals on synaptosomal choline uptake were examined in the presence or absence of 1 mM $Ca^{2+}$ with a view to ascertaining whether $Ca^{2+}$ antagonized the inhibitory effects of heavy metals (see [13] for discussion).

*In the absence of calcium.* Without addition of the metals, the time-course of synaptosomal choline uptake appeared to be biphasic: it increased linearly for the first 4 min followed by a further period of 11 min of linear increase but at a reduced rate (see Fig. 2A). In the presence of 500 $\mu$M $Cd^{2+}$ (a concentration around its $IC_{50}$ value), the time-course of choline uptake was markedly affected in that the initial rate of increase of uptake was much reduced over the first 4 min and the more gradual increase (seen in the control) was abolished. With the addition of 1.5 mM $Mn^{2+}$, the biphasic nature of choline uptake was maintained but the relative increases in uptake were greatly reduced (Fig. 2A). From Fig. 2A, it was apparent that $Al^{3+}$, at 200 $\mu$M, shortened the initial period of linear uptake from 4 to 2 min and thereafter inhibited the linear increase of choline uptake.

*In the presence of* 1 mM *calcium.* In the absence of added metal ions, the inclusion of 1 mM $Ca^{2+}$ in

Table 2. Summary of $IC_{50}$ values

| | | $IC_{50}$ $Cd^{2+}$ | $IC_{50}$ $Mn^{2+}$ | $IC_{50}$ $Al^{3+}$ |
|---|---|---|---|---|
| Synaptosomal choline uptake | Without $Ca^{2+}$ | 363 $\mu$M | 1.5 mM | 224 $\mu$M |
| | With 1 mM $Ca^{2+}$ | 363 $\mu$M | 1.2 mM | 123 $\mu$M |
| Synaptosomal Na–K–ATPase | | 5.4 $\mu$M | 955 $\mu$M | 8.3 mM |
| Synaptosomal Mg–ATPase | | 316 $\mu$M | 5.5 mM | 21.9 mM |

Some $IC_{50}$ values were determined from the data shown in Figs. 1 and 3. The others were derived from the data of six experiments where 6–7 different concentrations of metal ions were employed in each experiment.

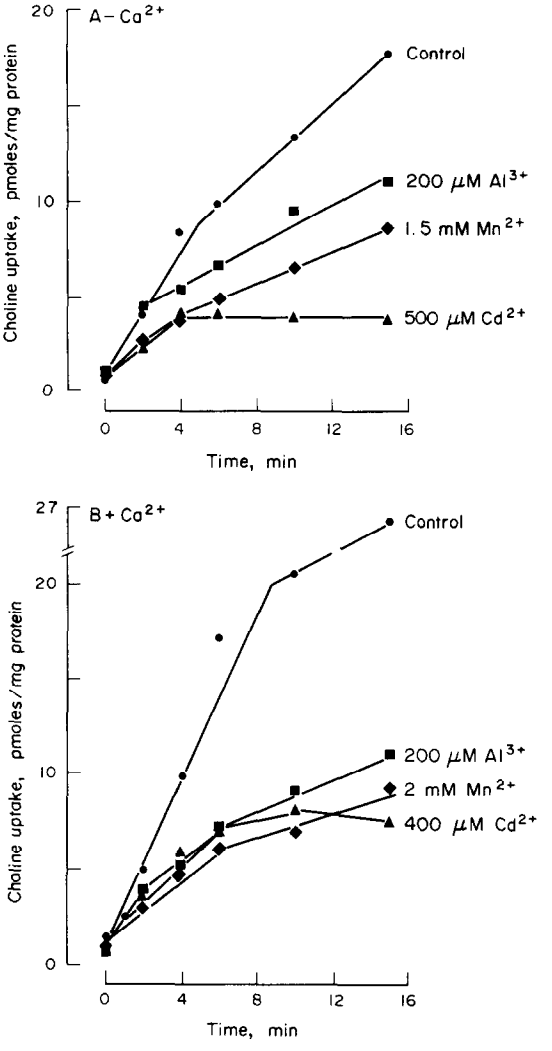


Fig. 2. A. Effects of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ on the time-course of [$^3$H]-choline uptake by rat brain synaptosomes in Krebs–Ringer phosphate without calcium. Choline uptake was determined in the presence of either 500 $\mu$M $Cd^{2+}$ (▲), or 1.5 mM $Mn^{2+}$ (◆), or 200 $\mu$M $Al^{3+}$(■), or in the absence of these three metal ions (i.e. the control, ●) in Krebs–Ringer phosphate without calcium at various times of incubation as described in the Methods section. The data were means of 6–8 determinations derived from 2 to 3 separate experiments.

B. Effects of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ on the time-course of [$^3$H]-choline uptake by rat brain synaptosomes in Krebs–Ringer phosphate with 1 mM $CaCl_2$. The experimental procedure was as described in the legend to A, except that the concentrations of metal ions used were: 400 $\mu$M $Cd^{2+}$ (▲), or 2 mM $Mn^{2+}$(◆), or 200 $\mu$M $Al^{3+}$(■). Control values (●) were means of three experiments; the others were means of 6–8 determinations derived from two to three experiments.

the medium seemed to prolong the initial linear phase of choline uptake from 4 to 10 min; thereafter the uptake rate decreased considerably (compare Figs. 2A and 2B). The addition of $Cd^{2+}$, or $Mn^{2+}$, or $Al^{3+}$, at concentrations around their $IC_{50}$ values (see Table 2), resulted in inhibition of choline uptake in similar ways to that observed in media containing no calcium (compare Figs. 2A and 2B).

*Effects of varying concentrations of* $Cd^{2+}$, $Mn^{2+}$ *and* $Al^{3+}$ *on synaptosomal choline uptake*

Since the uptake of choline by the synaptosomal fraction in the presence or absence of 1 mM $Ca^{2+}$ was linear for at least the first 4 min (see Figs. 2A and 2B), all subsequent uptake studies were carried out with a 4-min incubation time.

In the absence of $Ca^{2+}$, the rate of choline uptake by synaptosomes was 2.00 ± 0.22 pmol/min per mg of protein at 37° (Mean ± S.D. of 13 experiments), whereas in the presence of 1 mM $Ca^{2+}$ the choline uptake rate was slightly lower, at 1.94 ± 0.12 pmol/min per mg of protein at 37° (Mean ± S.D. of 9 experiments). However, the difference between these rates was not statistically significant ($P > 0.05$).

Under conditions described for high affinity choline uptake (choline at $10^{-7}$ M; see [13]) cadmium significantly inhibited, in a dose-dependent manner, the uptake of choline into synaptosomes in Krebs–Ringer phosphate medium without calcium ($IC_{50}$ for $Cd^{2+}$ = 363 μM; see Fig. 3A). However, the inclusion of 1 mM $Ca^{2+}$ in the medium did not overcome this inhibition ($IC_{50}$ for $Cd^{2+}$ = 363 μM; see Fig. 3A). In fact the inhibition of choline uptake by 1 mM $Cd^{2+}$ was more pronounced in the presence of 1 mM $Ca^{2+}$ than in its absence (see Fig. 3A).

In the absence of $Ca^{2+}$, the inhibitory effect of manganese was not observed until the $Mn^{2+}$ concentrations exceeded 1 mM ($IC_{50}$ = 1.5 mM; see Fig. 3B). However, with 1 mM $Ca^{2+}$ in the medium, some inhibition of choline uptake by $Mn^{2+}$ was evident even when its concentration was as low as $10^{-4}$ M ($IC_{50}$ = 1.2 mM; see Fig. 3B).

Without the inclusion of $Ca^{2+}$ in the medium, $Al^{3+}$ appeared to inhibit choline uptake in a dose-dependent fashion ($IC_{50} = 2.24 \times 10^{-4}$M; see Fig. 3C). The addition of 1 mM $Ca^{2+}$ to the medium did not antagonize the inhibitory effect of $Al^{3+}$: in fact the presence of $Ca^{2+}$ seemed to slightly enhance this inhibition ($IC_{50} = 1.23 \times 10^{-4}$M; see Fig. 3C). Thus it is reasonable to conclude that the rank order of inhibition of choline uptake was: $Al^{3+} > Cd^{2+} \gg Mn^{2+}$. Although $Al^{3+}$ was the most potent inhibitor of choline uptake, it was still a relatively ineffective inhibitor in view of the comparatively high $IC_{50}$ (123–224 μM).

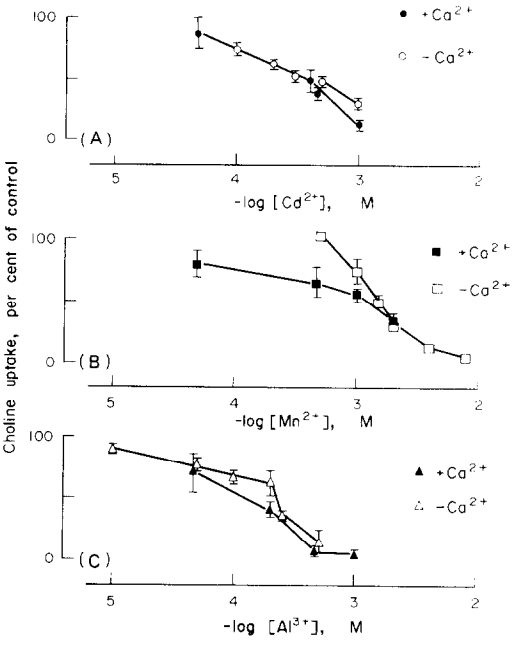


Fig. 3. Effects of varying concentrations of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ on rat brain synaptosomal choline uptake in Krebs–Ringer phosphate with or without 1 mM $CaCl_2$.

A. Effects of different concentrations of $Cd^{2+}$ on synaptosomal choline uptake were examined in Krebs–Ringer phosphate in the presence (●; Mean ± S.D. of three experiments) or absence (○; Mean ± S.D. of 6–8 determinations derived from two separate experiments) of 1 mM $CaCl_2$ as described in the Methods section.

B. Synaptosomal choline uptake was determined in the presence of different $MnCl_2$ concentrations in Krebs–Ringer phosphate without calcium (□; Mean ± S.D. of 6–8 determinations derived from two separate experiments) or with 1 mM $CaCl_2$ (■; Mean ± S.D. of three experiments) as described in the Methods section.

C. Synaptosomal choline uptake in Krebs-Ringer phosphate without calcium (△; Mean ± S.D. of 6–8 determinations derived from two experiments) or with 1 mM $CaCl_2$ (▲; Mean ± S.D. of 3 experiments) as described in the Methods section. All values were expressed as per cent of control (i.e. in the absence of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$). Control synaptosomal choline uptake rates were 2.00 ± 0.22 pmol/min/mg protein (Mean ± S.D. of 13 experiments) in the absence of $Ca^{2+}$ and 1.94 ± 0.12 pmol/min/mg protein (Mean ± S.D. of nine experiments) in the presence of 1 mM $CA^{2+}$. (Only the S.D.s greater than the heights of the symbols were drawn).

## DISCUSSION

It is widely accepted that the sodium-potassium-activated adenosine triphosphatase (Na–K–ATPase) is an integral part of the mechanisms involved in conduction and synaptic transmission within the nervous system [1–6]. The functional activities of this enzyme may be coupled to the sodium-dependent active transport system present in the synaptic membranes for the uptake of neurotransmitters and their precursors [3–5]. The inhibitory actions of metals on the activity of this enzyme have been extensively examined in brain microsomal preparations [6, 14]. These studies indicate that at a concentration of $10^{-6}$M, metal ions ($Cu^{2+}$, $Zn^{2+}$, $Fe^{2+}$ and $Pb^{2+}$) inhibit brain microsomal Na–K–ATPase activity competitively ($Fe^{2+}$ and $Pb^{2+}$) and non-competitively ($Cu^{2+}$ and $Zn^{2+}$) [6, 14]. Furthermore, Donaldson *et al.* [4] have found a good correlation between the ability of divalent metals ($Zn^{2+}$, $Cu^{2+}$, $Mn^{2+}$ and $Fe^{2+}$) to induce convulsions *in vivo* and their potency as an inhibitor of brain microsomal Na–K–ATPase *in vitro*. However, little is known about the effects of metals on the structure and function of synaptosomes and how these effects may

be related to the overall neurotoxicity of metals observed *in vivo* [13]. Inhibition of the high affinity uptake of choline and dopamine into synaptosomes is observed in rodents which have been chronically fed with lead, and studies using the *in vitro* model of neurotransmission produce similar results [13, 15]. In addition, the work of Silbergeld and Adler [15] indicates that the effects of lead on the neurotransmission of acetylcholine and dopamine may be the consequence of its interfering with the interactions of calcium with the synaptosomal as well as the mitochondrial membranes. An alternative explanation of their results [13, 15] may lie in the observations of Prakash *et al.* [5], who showed that several divalent metals ($Cu^{2+}$, $Zn^{2+}$ and $Hg^{2+}$) inhibited synaptosomal choline uptake and that these effects were apparently associated with inhibition of Na–K–ATPase by these ions.

We examined the effects of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ on synaptosomal Na–K–ATPase and Mg–ATPase. Apart from $Mn^{2+}$, the other two metals were not studied by Prakash *et al.* [5], nor did they report results on the effects of metals on synaptosomal Mg–ATPase. We found that all three metals ($Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$) inhibited the activity of the synaptosomal Na–K–ATPase to a much greater extent than the activity of synaptosomal Mg–ATPase (see Fig. 1 and Table 2). Our findings are consistent with the observations of Donaldson *et al.* [4], who found that transition metals, other than those studied by us, are poor inhibitors of brain microsomal Mg-ATPase. From our data it is evident that $Cd^{2+}$ is as potent an inhibitor of synaptosomal Na–K–ATPase as $Cu^{2+}$ and $Hg^{2+}$ (compare the results of Prakash *et al.* [5] with ours).

In order to test the ionic gradient hypothesis, which maintains that the activity of the Na–K–ATPase may be coupled to the synaptosomal uptake of neurotransmitters and their precursors [3], we have also examined the effects of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ on synaptosomal choline uptake.

The inhibitory actions of these three metals on choline uptake in the presence or absence of 1 mM $Ca^{2+}$ appear to be virtually identical (compare the results in Figs. 2 and 3 and Table 2) suggesting that $Ca^{2+}$ (at 1 mM) apparently does not antagonize the inhibitory effects of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$. Our results contrast with those of Silbergeld [13], who found that reduced $Ca^{2+}$ inhibited synaptosomal choline uptake and $Ca^{2+}$ partially counteracted the inhibition of choline uptake by lead. The discrepancy between our findings and hers could be attributable to our employing a fairly purified synaptosomal fraction containing less than 5 per cent of mitochondrial contamination (see [8–10]), whereas Silbergeld [13] used the post-nuclear supernatant, which was grossly contaminated with non-synaptosomal material, including all the 'free' mitochondria.

All three metals ($Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$) inhibit synaptosomal choline uptake in a concentration-dependent and time-dependent manner (see Figs. 2 and 3) with $Al^{3+}$ being the most potent. However, $Al^{3+}$ is the least effective amongst these metals in inhibiting the synaptosomal Na–K–ATPase activity (see Table 2). Furthermore, it is worth noting that at $10^{-5}$ M, $Cd^{2+}$ completely inhibits the activity of synaptosomal Na–K–ATPase and yet, at the same concentration, it appears to have no marked effect on synaptosomal choline uptake (see Fig. 3A). Since $Cd^{2+}$ and $Al^{3+}$ inhibit synaptosomal choline uptake but do not show parallel inhibitory effects on the synaptosomal ATPase activity, it follows that the electro-chemical potential gradient maintained by the Na–K–ATPase activity cannot be the sole driving force responsible for the uptake of choline by synaptosomes. Essentially the same conclusions may be deduced from the work of Hexum [6], who found that, at $10^{-4}$ M, lead vitrually completely inhibited brain microsomal Na–K–ATPase activity and the studies of Silbergeld [13] where she showed that synaptosomal choline uptake and dopamine uptake were only partially inhibited by lead at concentrations between $10^{-5}$ and $2.5 \times 10^{-4}$M. Our results as well as those of Hexum [6] and Silbergeld [13, 15] appear to contrast with those of Prakash *et al.* [5].

In conclusion, our investigations on the *in vitro* effects of $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ demonstrate that $Cd^{2+}$ is a potent inhibitor of synaptosomal Na–K–ATPase but it is far less effective as an inhibitor of synaptosomal choline uptake. Like $Cd^{2+}$ (but unlike $Mn^{2+}$), $Al^{3+}$ is also moderately effective as an inhibitor of synaptosomal choline uptake. Furthermore, we have also observed that $Cd^{2+}$, $Mn^{2+}$ and $Al^{3+}$ show some selective inhibitory effects on the synaptosomal uptake of dopamine, noradrenaline and 5-hydroxytryptamine (Lai, Lim & Davison, in preparation). However, despite the fact that all three metals are toxic to the nervous system *in vivo* [17, 19–21], it is exceedingly difficult to relate our *in vitro* observations to their *in vivo* toxic effects. Nevertheless, our results suggest that metal ions, such as $Cd^{2+}$ and $Al^{3+}$, may be useful and selective tools for elucidating the structure and function of not only the synaptosomal membranes but also other types of biological membranes as well.

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## REFERENCES

1. S. L. Bonting, in *Membranes and Ion Transport* (Ed. E. E. Bittar), Vol 1, p.257. Wiley-Interscience, London (1970).
2. J. L. Dahl and L. E. Hokin, *A. Rev. Biochem.* **43**, 327 (1974).
3. B. F. Bogdanski, in *Transport Phenomena in the Nervous System* (Eds. G. Levi, L. Battistin and A. Lajtha), p. 291. Plenum Press, New York (1976).
4. J. Donaldson, T. St.-Pierre, J. Minnich and A. Barbeau, *Can. J. Biochem.* **49**, 1217 (1971).
5. N. J. Prakash, J. Fontana and R. I. Henkin, *Life Sci.* **12**, 249 (1973).
6. T. D. Hexum, *Biochem. Pharmac.* **23**, 3441 (1974).
7. D. Oberleas, D. F. Caldwell and A. S. Prasad, *Int. Rev. Neurobiol.*, Suppl. I, 83 (1972).
8. J. C. K. Lai and J. B. Clark, *Meth. Enzymol.* **55**, Part F, 51 (1979).
9. J. C. K. Lai, J. M. Walsh, S. C. Dennis and J. B. Clark, *J. Neurochem.* **28**, 625 (1977).
10. R. F. G. Booth and J. B. Clark, *Biochem. J.* **176**, 365 (1978).
11. W. J. Nicklas, S. Puszkin and S. Berl, *J. Neurochem.* **20**, 109 (1973).

12. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 295 (1951).
13. E. K. Silbergeld, *Life Sci.* **20**, 309 (1977).
14. H. P. Ting-Beall, D. A. Clark, C. H. Suelter and W. W. Wells, *Biochim. biophys. Acta.* **291**, 229 (1973).
15. E. K. Silbergeld and H. S. Adler, *Brain Res.* **148**, 451 (1978).
16. A. Y. Sun, G. Y. Sun and T. Samorajski, *J. Neurochem* **18**, 1711 (1971).
17. R. B. Rastogi, Z. Mercli and R. L. Singhal, *J. Neurochem.* **28**, 789 (1977).
18. B. R. Nechay and J. P. Saunders, *J. Pharmac. exp. Ther.* **200**, 623 (1977).
19. G. C. Cotzias, P. S. Papavasiliou, J. Ginos, A. Stock and S. Düby, *A. Rev. Med.* **22**, 305 (1971).
20. H. L. Elliott, A. I. Macdougall and G. S. Fell, *Lancet* **i**, 1203 (1978).
21. D. R. Crapper, S. S. Krishman and A. J. Dalton, *Science* **180**, 511 (1973).