freezing and thawing, by sonic treatment, or by treatment with a variety of surface active agents or lysozyme. It was observed also that cell density was an important factor when sonic treatment was employed but not when toluene treatment was used (Table II). As shown in Table III, when sonic extracts were centrifuged at 18 000 × *g* for 30 min, the pellet contained about 50% of the enzyme activity. In contrast, all of the enzyme activity of the toluenized preparations was found in the 18 000 × *g* pellet. The procedure finally adopted in order to obtain maximum activities consisted of gentle shaking for 2 min at 0° with 0.5% (v/v) of toluene (Table IV), followed immediately by determination of the enzyme as outlined. This procedure usually gives about 5–10 times higher threonine dehydratase activity than that obtained by sonic treatment.

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Manganese activation of a (Na^+-K^+)-dependent ATPase in pig brain microsomes

ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity, which is dependent on Mg^{2+} and is activated by Na^+ and K^+, has been reported in membrane preparations from numerous tissues of higher animals[1–4]. These (Mg^{2+}-Na^+-K^+)-ATPases are inhibited by ouabain, which also inhibits the active transport of Na^+ and K^+ in many tissues. Membrane (Na^+-K^+)-activated ATPases are, therefore, believed to be part of the mechanism pumping Na^+ out of, and K^+ into, cells, particularly nerve cells.

In at least some enzymes involved in the metabolism of ATP, such as pyruvate kinase, Mn^{2+} can be substituted for Mg^{2+} as divalent metal activator[5,6]. This paper presents evidence that pig brain microsomal ATPase activity can be activated when Mn^{2+} replaces Mg^{2+}, and that this activity is enhanced by the joint addition of Na^+ and K^+.

Microsomes from pig brain cortex were obtained as described by SCHWARTZ, BACHELARD AND MCILWAIN[7]. The microsomal pellet was washed 3 times with 5 mM EDTA–Tris (pH 7.4), suspended in water, and stored at −20°.

Microsomal ATPase activities were determined by incubation at 37° for 10 min

TABLE I

EFFECTS OF CATIONS AND INHIBITORS ON THE MICROSOMAL ATPase

ATPase activities were measured at 37° in media containing 80 μg protein, 3 mM ATP–Tris, (pH 7.4), 50 mM Tris–HCl (pH 7.4). Also present, where indicated, were 3 mM $MgCl_2$, 2 mM $MnCl_2$, 100 mM NaCl, and 30 mM KCl. S.E. are for groups of 6 experiments. Apparent K_m values were determined from Lineweaver–Burk plots.

Cations present	*ATPase activity (μmoles Pi/mg protein per h)*	*Apparent K_m for Mg^{2+}–ATP or Mn^{2+}–ATP (mM)*	*Concentrations of inhibitors giving half-maximal inhibition (mM)*			
			Mg^{2+}	*Ca^{2+}*	*Mn^{2+}*	*Ouabain*
Mg^{2+}	18.0 ± 0.1	0.90	1.90 ± 0.10*	1.80 ± 0.05	1.85 ± 0.05	
Mg^{2+}, Na^+, K^+	59.3 ± 0.4	0.60	2.95 ± 0.10*	0.50 ± 0.05	0.60 ± 0.05	$2.0 (\pm 0.5) \cdot 10^{-4}$
Mg^{2+}, Na^+	21.6 ± 0.2					
Mg^{2+}, K^+	18.5 ± 0.1					
Mn^{2+}	14.5 ± 0.1	1.00		2.70 ± 0.10*	2.65 ± 0.10*	
Mn^{2+}, Na^+, K^+	38.6 ± 0.3	0.65		0.45 ± 0.05*	0.55 ± 0.05*	$2.0 (\pm 0.5) \cdot 10^{-4}$
Mn^{2+}, Na^+, K^+	42.3 ± 0.3**					
Mn^{2+}, Na^+	16.9 ± 0.1					
Mn^{2+}, K^+	14.8 ± 0.1					

* These figures are concentrations of Mg^{2+}, Ca^{2+} or Mn^{2+} in excess of the ATP concentration, *e.g.* half-maximal inhibition of (Mn^{2+}-Na^+-K^+)-ATPase activity occurred in the presence of 3 mM ATP, 2 mM $MnCl_2$ and 1.45 mM $CaCl_2$. No correction has been made for the formation of ATP–metal complexes.

** Activity with 3 mM $MnCl_2$ and 4.5 mM ATP.

in volomes of 1 ml. Other conditions are shown in Table I. Reactions were terminated by addition of 0.5 ml 8% perchloric acid. Liberated orthophosphate was determined by a modification of the method of Fiske and SubbaRow and protein by the method of LOWRY *et al.*[8].

The pig brain microsomes catalysed hydrolysis of ATP with liberation of inorganic phosphate in the presence of either Mg^{2+} or Mn^{2+}. These ATPase activities were both greatly enhanced in the presence of Na^+ and K^+, whereas either Na^+ or K^+ singly had little effect (Figs. 1 and 2, Table I). The (Mg^{2+}-Na^+-K^+)- and (Mn^{2+}-Na^+-K^+)-ATPases both had the same pH optimum of 7.4, were inhibited by ouabain, and had similar apparent K_m's. They were also both inhibited by free Mn^{2+} and Ca^{2+} with similar sensitivities (Table I). The two (Na^+-K^+)-dependent ATPases are, therefore, probably activities of one enzyme, in which the usual Mg^{2+}-ATP substrate can be replaced by Mn^{2+}-ATP. The slightly higher apparent K_m for Mn^{2+}-ATP as substrate, compared with Mg^{2+}-ATP, may partly account for the lower (Na^+-K^+)-dependent ATPase activity in the presence of Mn^{2+}.

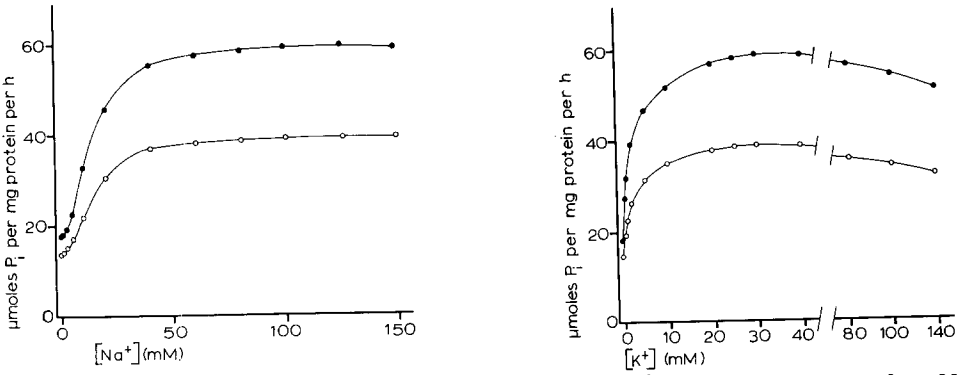

Fig. 1. ATPase activities measured at varying concentrations of Na^+ in the presence of 3 mM $MgCl_2$ and 30 mM KCl (●—●) or 2 mM $MnCl_2$ and 30 mM KCl (○—○). Other conditions are as shown in Table I.

Fig. 2. ATPase activities measured at varying concentrations of K^+ in the presence of 3 mM $MgCl_2$ and 100 mM NaCl (●—●) or 2 mM $MnCl_2$ and 100 mM NaCl (○—○). Other conditions are as shown in Table I.

Optimal (Mg^{2+}-Na^+-K^+)-ATPase activity occurred at a Mg^{2+}:ATP molar ratio of 1:1, while optimal (Mn^{2+}-Na^+-K^+)-ATPase activity occurred at a Mn^{2+}:ATP ratio of 0.67:1. These optimum ratios were unchanged at ATP concentrations ranging from 1.5 to 6.0 mM with both Mg^{2+} and Mn^{2+}. When either Mg^{2+} or Mn^{2+} was in excess of the ATP concentration, the (Mg^{2+}-Na^+-K^+)- and (Mn^{2+}-Na^+-K^+)-ATPases were inhibited, Mn^{2+} being a much more effective inhibitor than Mg^{2+} (Fig. 3 and Tables I and II). This inhibition by free Mg^{2+} and Mn^{2+} may reflect competition by these divalent ions for enzyme sites normally occupied by Na^+ or K^+, and kinetic evidence has in fact been obtained for competition between Ca^{2+} and Na^+, and between Mn^{2+} and Na^+ (unpublished results). On this basis the affinity of the enzyme Na^+-site for Mn^{2+} would be high relative to Mg^{2+}, and in this respect Mn^{2+} would resemble Ca^{2+}. Such a difference in affinities makes it possible to explain the difference in optimal Mg^{2+}:ATP and Mn^{2+}:ATP ratios. At a molar ratio of 1:1, Mn^{2+}:ATP, free Mn^{2+} would appear to be at a concentration sufficient to compete significantly with Na^+ for enzyme sites. Excess ATP would, therefore, be expected to induce the observed increase in ATPase activity by complexing Mn^{2+}, thereby lowering the concentration of free Mn^{2+}. In contrast, the concentration of free Mg^{2+} at a 1:1 ratio of Mg^{2+}:ATP appears

TABLE II

MAXIMUM EFFECTS OF ATPase INHIBITORS

ATPase activity	*% Activity remaining at maximal inhibition*			
Inhibitors:	*Mg^{2+}*	*Ca^{2+}*	*Mn^{2+}*	*Ouabain*
(Mg^{2+}-Na^+-K^+) — (Mg^{2+})	74	4	15	13
(Mg^{2+})	67	58	54	—
(Mn^{2+}-Na^+-K^+) — (Mn^{2+})		4	31	14
(Mn^{2+})		55	53	—

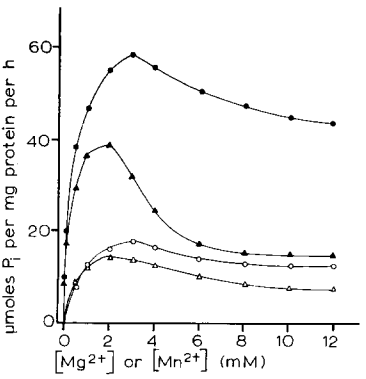

Fig. 3. ATPase activities at varying concentrations of Mg^{2+} (●—●, ○—○) or Mn^{2+} (▲—▲, △—△). Filled symbols are in the presence of 100 mM NaCl and 30 mM KCl. Other conditions are as shown in Table I.

to have no significant inhibitory effect and this is reflected in the high Mg^{2+} concentration required for half-maximal inhibition of (Mg^{2+}-Na^+-K^+)-ATPase activity.

The basal Mg^{2+}-ATPase and Mn^{2+}-ATPase activities were also inhibited by excess Mg^{2+}, or Mn^{2+}. In these cases, however, the concentration of Mn^{2+} required for half-maximal inhibition was much greater than for inhibition of the (Na^+-K^+)-dependent activities and maximal inhibitions were less than 50%. Here, inhibition may arise from competition, between metal–ATP and free metal ions for the enzyme substrate site, or from the formation of enzymically inactive dimetal–ATP complexes.

Further experiments, which will be published later, have shown that Mn^{2+} also catalyses the formation of a phosphorylated membrane derivative in the presence of Na^+ and ATP and that this derivative is labile to K^+. Such a membrane derivative is characteristic of (Mg^{2+}-Na^+-K^+)-ATPases[9,10] and constitutes further evidence for the enzymic identity of the (Mg^{2+}-Na^+-K^+)- and the (Mn^{2+}-Na^+-K^+)-ATPases.

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