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PROPERTIES OF A ($Mg^{2+}+Ca^{2+}$)-DEPENDENT ATPase OF BOVINE BRAIN CORTEX

EFFECTS OF DETERGENTS, FREEZING, CATIONS AND LOCAL ANESTHETICS

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SUMMARY

1. Microsomal fractions from bovine brain cortex contain a ouabain-insensitive, Mg^{2+}-dependent ATPase activity which is stimulated by low concentrations of $CaCl_2$ (65 μM) [($Mg^{2+}+Ca^{2+}$)-ATPase].

2. NaCl or KCl (100 mM) further activates the ($Mg^{2+}+Ca^{2+}$)-ATPase by 67%.

3. Sodium deoxycholate and lubrol-WX treatment of the microsomal fraction enhances the ($Mg^{2+}+Ca^{2+}$)-ATPase activity. This enhancement is less than that of (Na^+,K^+)-ATPase activity but greater than that of the basal Mg^{2+}-ATPase activity.

4. The Ca^{2+}-stimulated component of the ATPase activity is extremely labile. Ca^{2+} activation reaches a maximum 2 to 4 days after storage at -12 °C, but is greatly reduced after 7 days.

5. The presence of Ca^{2+} (65 μM) lowers the K_m for ATP from 240 to 40 μM.

6. Preparations containing an active ($Mg^{2+}+Ca^{2+}$)-ATPase have a ouabain-insensitive, Mg^{2+}-dependent *p*-nitrophenylphosphatase activity which is inhibited by $CaCl_2$ with an I_{50} of 65μM.

7. Neither adrenergic agonists nor α-adrenergic antagonists have effects on the ($Mg^{2+}+Ca^{2+}$)-ATPase. Tetracaine hydrochloride inhibits Ca^{2+} stimulation of the ATPase with an I_{50} of 3.1 mM and shifts the optimum Ca^{2+} concentration to 178 μM.

8. Since it has been suggested that the function of a ($Mg^{2+}+Ca^{2+}$)-ATPase may be to maintain low intracellular levels of Ca^{2+} and perhaps excitability of membranes in nervous tissue, the inhibition of ($Mg^{2+}+Ca^{2+}$)-ATPase by local anesthetics may account at least in part for their mechanism of action.

INTRODUCTION

It is well established, particularly in erythrocytes[1], that active Ca^{2+} transport is linked to a Ca^{2+}-stimulated Mg^{2+}-dependent ATPase (($Mg^{2+}+Ca^{2+}$)-ATPase).

Abbreviations: EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetate.

This enzyme has recently been purified form human erythrocytes[2], and has also been described in microsomal fractions from pig brain[3,4], synaptosomes[5], sarcoplasmic reticulum[6] and electric tissue[7]. Many tissues, including mammalian brain, also contain a Mg^{2+}- or Ca^{2+}-ATPase[8], but the activation of this enzyme by Ca^{2+} does not appear to require Mg^{2+} and occurs at relatively high (mM) concentrations of Ca^{2+}.

The present study describes the properties of a ($Mg^{2+}+Ca^{2+}$)-ATPase from microsomal fractions of bovine brain cortex. The properties of this enzyme are compared to properties reported for similar enzyme systems from other sources. The effects of local anesthetics and adrenergic amines on this enzyme were investigated, since these agents are known to influence the distribution of Ca^{2+} across biological membranes[9–11].

MATERIALS AND METHODS

Enzyme preparation

Microsomal preparations were isolated from the cortex of bovine brains obtained fresh from a local slaughterhouse. The cortex (40 g) was homogenized in a Waring blender for 2 min in 200 ml of 0.25 sucrose containing 1 mM EDTA, and after centrifugation at $40000\times g$ for 20 min to remove soluble material[12] the pellet was resuspended in 200 ml of the sucrose–EDTA medium in a Potter–Elvehjem homogenizer (2 min, 8–10 strokes). After centrifugation at $18000\times g$ for 12 min (twice), the supernatant was further centrifuged at $30000\times g$ for 30 min. The resulting pellet was suspended in the sucrose–EDTA medium with a Potter–Elvehjem homogenizer to a final concentration of 3–4 ml/g cortex[13]. This preparation will be referred to as the microsomal fraction. The microsomal fraction was frozen at -12 °C and used within 2 to 4 days (see Table I). This preparation is referred to as Preparation A and was used in most of the studies. A second microsomal preparation (Preparation B) was prepared essentially according to Nakamaru[3], and differed from Preparation A in that the microsomal pellet was washed once with 0.32 M sucrose – 1 mM EDTA medium and recentrifuged at $30000\times g$ prior to resuspension in the sucrose–EDTA medium. Detergent treatment was accomplished by mild agitation of the preparations at room temperature for 20 min with an equal volume of detergent in distilled water[3] (usually 0.16% sodium deoxycholate was used to give a final concentration of 0.08%). Aliquots were then used for ATPase assays without further treatment. The sodium deoxycholate-treated preparations were found to have the following average specific activities (after subtraction of the Mg^{2+}-ATPase activity when applicable): Na^+- and K^+-stimulated, Mg^{2+}-dependent ATPase ((Na^+,K^+)-ATPase), 1.74 μmoles P_i/mg protein per min; ($Mg^{2+}+Ca^{2+}$)-ATPase, 0.27 μmole P_i/mg per min; and Mg^{2+}-ATPase, 0.23 μmole P_i/mg per min. The composition of the membrane preparation was as follows*: phospholipid, 320 μg/mg protein; cholesterol, 194 μg/mg; and sialic acid, 53 nmoles per mg. Succinic dehydrogenase activity was only 0.1–0.2% of the specific activity in the $18000\times g$ (mitochondrial) fraction of the cortex. Protein concentration was determined by the method of Lowry *et al.*[14], using three times recrystallized bovine serum albumin as a standard.

* Determined by Dr D. V. Godin, University of British Columbia.

Assay of ATPase

The incubation mixture (3 ml) for the assay of $(Mg^{2+}+Ca^{2+})$-ATPase activity contained 67 mM glycylglycine–imidazole (pH 7.4), 5 mM $MgCl_2$, 0.125 mM ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetate (EGTA), and as indicated in some cases 100 mM NaCl, 5 mM KCl and 0.1 mM ouabain, unless otherwise stated. The enzyme preparation (150–300 μg protein) was incubated with 1.5 mM ATP (usually the disodium salt, unless the effect of sodium was being investigated) for 10 min at 37 °C. The release of P_i was found to be linear in both time and protein concentration.

Inorganic phosphate was measured either by the original method of Fiske and SubbaRow[15], or by a modification of this method described previously[16] when interfering hydrophobic amines were present, *p*-Nitrophenylphosphatase activity was measured by a spectrophotometric assay (see ref. 17).

Reagents

Materials were reagent grade wherever possible. The compounds were obtained from Sigma (imidazole (grade III), glycylglycine, EGTA, ouabain, ATP, L-epinephrine bitartrate, bovine serum albumin), from Nutritional Biochemicals (sodium deoxycholate), and from B.D.H. (digitonin). Lubrol-WX and Triton X-100 were gifts from the laboratory of Dr G. I. Drummond, University of British Columbia. 2-Bromo-2-phenyl-*N*,*N*′-dimethylethanolamine hydrochloride was a gift from Dr B. Belleau, University of Ottawa. Tetracaine hydrochloride (K&K) was recrystallized from ethanol–ether before use, m.p.147–148 °C.

RESULTS AND DISCUSSION

Effect of detergents

The order of effectiveness of detergents in eliciting both $(Mg^{2+}+Ca^{2+})$-ATPase and (Na^+,K^+)-ATPase activities in a crude homogenate of bovine brain cortex was: lubrol-WX (most effective), followed by Triton X-100 and sodium deoxycholate (equally effective). Digitonin in our hands was the least effective (contrast Nakamaru and Konishi[4]). Sodium deoxycholate, which in contrast to nonionic detergents does not interfere with phosphate determinations[16], was used in further studies. The effect of sodium deoxycholate on ATPase activities of microsomal fractions was investigated and the results are shown in Fig. 1. The activities of all three ATPases were enhanced by exposure of the enzyme preparation to sodium deoxycholate for 20 min at room temperature. The degree of enhancement of $(Mg^{2+}+Ca^{2+})$-ATPase activity was between that of (Na^+, K^+)-ATPase and Mg^{2+}-ATPase. Roelofsen *et al.*[18] have shown that detergents not only disrupt the structure of intact human erythrocyte cells, but at sub-lytic concentrations detergents can alter the molecular organization of the membrane structure in a more subtle manner. As well as disrupting the membrane vesicle structure, as shown in kidney microsomes[19], detergents must also alter the membrane structure of bovine brain microsomal preparations by removal of phospholipids[20]. Hence there are a number of possible explanations for the differential effects of sodium deoxycholate in eliciting the ATPase activities in this preparation. Nevertheless, the results suggest a difference in the organization of the $(Mg^{2+}+Ca^{2+})$-ATPase, (Na^+,K^+)-ATPase and Mg^{2+}-

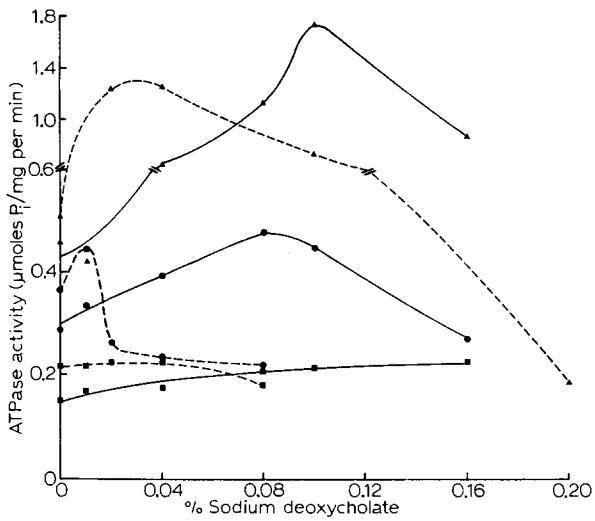

Fig. 1. Effect of sodium deoxycholate on ATPase activities. Deoxycholate treatment was made as described in Materials and Methods. The assay medium contained 67 mM glycylglycine–imidazole buffer (pH 7.4), 1.5 mM ATP, 5 mM $MgCl_2$, 0.125 mM EGTA, 100 mM NaCl, 5 mM KCl and other additions as described below. Results are shown for microsomal Preparation A (——) and Preparation B (– – –), which are described in Materials and Methods. ATPase activities are in the presence of: ■—■, 0.1 mM ouabain, (Mg^{2+}-ATPase); ●—●, 0.1 mM ouabain+0.2 mM $CaCl_2$(($Mg^{2+}+Ca^{2+}$)-ATPase, not corrected for basal Mg^{2+}-ATPase activity); and ▲—▲, no further additions ((Na^+,K^+)-ATPase, corrected for basal Mg^{2+}-ATPase activity).

ATPase within the phospholipid membrane matrix and perhaps within the vesicle structure. The significance of this in the respective functions of the ATPases is not clear.

The concentrations of detergent producing maximum enhancement differed markedly in two microsomal preparations prepared by slightly different methods. The activity of Preparation A (see Materials and Methods) was enhanced maximally by 0.1% sodium deoxycholate in the case of (Na^+,K^+)-ATPase (4-fold) and by 0.08% in the case of ($Mg^{2+}+Ca^{2+}$)-ATPase (1.6-fold). Higher concentrations of detergent were inhibitory. The Mg^{2+}-ATPase activity was only weakly enhanced and did not exhibit inhibition at higher concentrations of sodium deoxycholate (up to 0.16% was examined). In contrast, the activity of Preparation B was enhanced maximally by 0.02 to 0.04% sodium deoxycholate in the case of (Na^+,K^+)-ATPase (2.6-fold) and by 0.01% in the case of ($Mg^{2+}+Ca^{2+}$)-ATPase (1.2-fold). Concentrations of detergent greater than optimum were particularly inhibitory against this latter activity. Differences in the concentrations of detergent required to enhance ATPase activities of Preparations A and B were also found when the nonionic detergent lubrol-WX was used (at concentrations which did not interfere with the assay for P_i). The enhancement of the ATPase activities of both preparations by lubrol–WX was about 30% greater than that obtained with sodium deoxycholate treatment.

As described in detail (see Materials and Methods), Preparation B differed

from Preparation A in that in the former case the microsomal pellet was washed once with 0.32 M sucrose and 1 mM EDTA prior to final resuspension in this same medium. The marked sodium deoxycholate inhibition of the $(Mg^{2+}+Ca^{2+})$-ATPase activity of Preparation B was partially delayed (to 0.04%) when 0.3 ml of the supernatant from the microsomal pellet wash was added to the enzyme incubation assay mixture. These results suggest that a single washing of the microsomal pellet with sucrose–EDTA medium removed a membrane constituent which in some way affects the degree of interaction of the enzymes and the lipid matrix. Removal or alteration of this membrane constituent could alter the membrane structure and hence its susceptibility to activation and inhibition by detergents. Although the protein contents and specific activities of Preparations A and B were similar (Fig. 1), EDTA is known to selectively solubilize membrane proteins[22]. It is thus possible that the membrane constituent removed or altered is protein in nature. A membrane protein (nectin) has been shown to control the interaction of ATPase with bacterial membranes[21]. This aspect is under further investigation.

Stability

The Ca^{2+}-stimulated component of the $(Mg^{2+}+Ca^{2+})$-ATPase activity was found to be extremely labile. Table I shows the effect of storage at -12 °C on both

TABLE I

STABILITY OF ATPase ACTIVITIES WITH RESPECT TO TIME AND FREEZING

Microsomal Preparation A was assayed immediately after isolation and without freezing (zero time), and after freezing at -12 °C at intervals up to 7 days. The assay medium (pH 7.4) contained 1.5 mM ATP, 4 mM $MgCl_2$, 0.125 mM EGTA and 0.2 mM $CaCl_2$ (when required). The microsomal preparation was treated with sodium deoxycholate (0.08%) prior to assay.

Days after preparation	*Specific activity (μmole P_i/mg per min)*		*Ca^{2+} stimulation (%)*
	Mg^{2+} – ATPase	*$(Mg^{2+}+Ca^{2+})$ – ATPase*	
0	0.145*	0.217*	50*
1	0.156	0.299	92
2	0.145	0.309	113
3	0.158	0.357	126
7	0.165	0.250	52

* Assayed in the presence of 2 mM $MgCl_2$.

$(Mg^{2+}+Ca^{2+})$-ATPase and Mg^{2+}-ATPase activities. Ca^{2+} stimulation was 1.5-fold greater in frozen preparations than in fresh unfrozen preparations. Peak Ca^{2+} stimulation (2.3-fold) of the frozen preparations occurred 2–4 days after storage. This effect of freezing might reflect disruption of vesicle structure[23] and perhaps of membrane organization, which could facilitate Ca^{2+} and ATP accessibility to the enzyme active site. After 7 days of storage at -12 °C Ca^{2+} stimulation decreased markedly. The Mg^{2+}-ATPase activity remained largely unaltered during this time interval (Table I). The lability of the Ca^{2+}-stimulated component was not decreased by storage at -76 °C or treatment with dithiothreitol (a sulfhydryl reducing agent), but the presence of 1 mM $MgCl_2$ and 1 M sucrose increased the stability of a similar

preparation from rabbit brain cortex to at least 11 days. The (Na^+, K^+)-ATPase activity in these preparations is stable for many weeks. A similar lability of the Ca^{2+}-stimulated component in electric tissue was reported by Wins and Dargent-Sallée[7]. The explanation for the instability of the ($Mg^{2+}+Ca^{2+}$)-ATPase is at present unknown, but the results showing protection against instability by $MgCl_2$ and sucrose and the studies of Bond[24] on erythrocyte ($Mg^{2+}+Ca^{2+}$)-ATPase suggest that unfavorable conformational changes may be responsible for this instability.

Effect of Ca^{2+}

The effect of various $CaCl_2$ concentrations (in the presence of 0.125 mM EGTA) on Preparations A and B was investigated. The results are shown in Fig. 2.

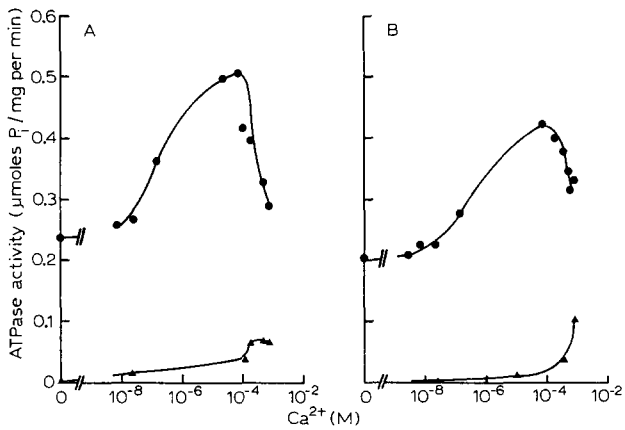

Fig. 2. Effect of $CaCl_2$ on ATPase activities of microsomal preparations. A refers to results with Preparation A and B refers to results with Preparation B (see Materials and Methods). The assay medium does not contain NaCl, KCl or ouabain, but $CaCl_2$ activation curves were virtually identical when these were present. Preparations A and B were treated with 0.08% and 0.01% sodium deoxycholate, respectively, prior to their assay. ●—●, in the presence of 5 mM $MgCl_2$; ▲—▲, in the absence of $MgCl_2$.

In the presence of 5 mM $MgCl_2$ both Preparations A and B gave asymmetrical bell-shaped Ca^{2+} stimulation dependence curves. Threshold levels of stimulation appear at free Ca^{2+} concentrations as low as 0.01 μM (calculated by the method of Katz *et al.*[25]). Peak Ca^{2+} stimulation occurred at a free Ca^{2+} concentration of 65 μM. This compares with 30 μM found by Nakamaru[3] for pig brain microsomes and 25 μM found by Wolf[2] for purified ($Mg^{2+}+Ca^{2+}$)-ATPase from human erythrocytes. The presence of sodium deoxycholate had no detectable effect on the position of the Ca^{2+} stimulation curve.

In the absence of added $MgCl_2$ a Ca^{2+}-stimulated ATPase (Ca^{2+}-ATPase) of low activity (less than 0.1 μmoleP_i/mg per min) was present (Fig. 2). Maximum activity of this enzyme occurred at around 0.77 mM Ca^{2+}, but higher Ca^{2+} concentrations were not tested . Similar Ca^{2+}-ATPase activities in the absence of $MgCl_2$ have been reported in mammalian brain[8] (neurostenin), erythrocytes[26] (spectrin), guinea-pig placenta[27] and *Escherichia coli*[28].

Effect of Mg^{2+}

The concentration of $MgCl_2$ was varied in the absence or presence of $CaCl_2$ (65 μM) using both 'fresh' (Fig. 3A) and 'aged' (Fig. 3B) fractions of Preparation A. Ca^{2+} stimulation of Preparation A increased with increasing concentration of $MgCl_2$ and was absolutely dependent on the presence of $MgCl_2$ (Fig. 3A). $MgCl_2$ activation was hyperbolic in these preparations. In contrast, fractions of Preparation A which were aged at -12 °C for greater than one or two weeks showed asymmetric bell-shaped Mg^{2+} activation curves in the absence of $CaCl_2$, maximum $MgCl_2$ activation occurring at 1 mM (Fig. 3B). Inhibition of these aged preparations by $MgCl_2$ was prevented by the presence of $CaCl_2$ (Fig. 3B). The mechanism of this apparent Ca^{2+} stimulation is not known, but it can be readily detected since both basal Mg^{2+}-ATPase and 'Ca^{2+}-stimulated' activities are considerably lower than in fresh preparations.

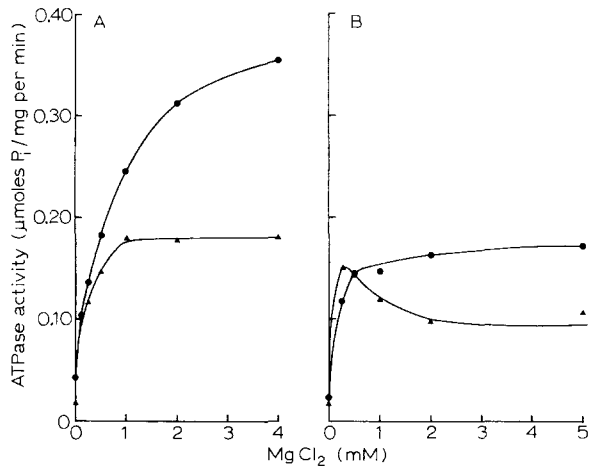

Fig. 3. Effect of $MgCl_2$ on the ATPase activities of microsomal Preparation A. A refers to a preparation stored at -12 °C and used within three days of isolation. B refers to the same preparation aged for more than a few weeks at -12 °C. The preparations were treated with 0.08% sodium deoxycholate prior to assay. The assay medium does not contain NaCl, KCl or ouabain. Data points are means of duplicate determinations. ●—●, in the presence of 0.2 mM $CaCl_2$+0.125 mM EGTA; ▲—▲, in the presence of 0.125 mM EGTA alone.

Effect of substrate

ATP (both disodium and Tris salt) was varied at a constant level of $MgCl_2$ (5 mM) in the presence or absence of $CaCl_2$ (65 μM), in order to determine the K_m for substrate hydrolysis. The results were analyzed according to the method of Lee and Wilson[29], in order to minimize possible non-linearity at the low ATP concentrations. In addition, incubation times were decreased to 5 min at the low ATP concentrations. The presence of $CaCl_2$ (65 μM) decreased the K_m for ATP from 240 (in its absence) to 40 μM. This is the same as that of the high substrate affinity ($Mg^{2+}+Ca^{2+}$)-ATPase from human erythrocytes obtained by Wolf[2,30].

Effect of monovalent cations

The ($Mg^{2+}+Ca^{2+}$)-ATPase activity was increased by either NaCl or KCl. At 100 mM concentrations these monovalent cations increased both Mg^{2+}-ATPase

and ($Mg^{2+}+Ca^{2+}$)-ATPase activities. However the ($Mg^{2+}+Ca^{2+}$)-ATPase was preferentially increased by 67% over that of the Mg^{2+}-ATPase. The high Ca^{2+} affinity ($Mg^{2+}+Ca^{2+}$)-ATPases from human erythrocytes and electric tissue are also activated by NaCl or KCl at relatively high concentrations[30,31,7]. The effect of monovalent cations does not appear to be simply an ionic strength effect, but rather appears to be related to changes in protein conformation induced by Na^+ or K^+, but not Li^+ (ref. 24).

p-Nitrophenylphosphatase activity

The K^+-stimulated *p*-nitrophenylphosphatase reaction is considered to be a partial reaction corresponding to the K^+-stimulated dephosphorylation step in ATP hydrolysis by (Na^+,K^+)-ATPase. It was thus of interest to study the effect of Ca^{2+} on the ouabain-insensitive *p*-nitrophenylphosphatase activity in these preparations. In this study Ca^{2+} did not stimulate the Mg^{2+}-dependent, ouabain-insensitive *p*-nitrophenylphosphatase activity in microsomal preparations which had an active ($Mg^{2+}+Ca^{2+}$)-ATPase activity. Rather a low concentration of free $CaCl_2$ (65 μM) inhibited this activity by 50%, both in the presence of NaCl, KCl and ouabain or in their absence. This is in contrast to results obtained from sarcoplasmic reticulum, where Ca^{2+} has been found to stimulate the *p*-nitrophenylphosphatase activity in the presence of KCl[34]. There is no evidence from these results to suggest that a ouabain- and K^+-independent, Mg^{2+}-dependent *p*-nitrophenylphosphatase is a partial reaction of the ($Mg^{2+}+Ca^{2+}$)-ATPase in brain microsomes. This is of interest in the light of the report by Cha *et al.*[35] that Ca^{2+} stimulated the ouabain-insensitive dephosphorylation step of erythrocyte ($Mg^{2+}+Ca^{2+}$)-ATPase.

Effect of drugs

In view of the suggested role of L-epinephrine in stimulating Ca^{2+} influx during smooth muscle contraction[36] and previous speculation about the role of ATPase structures in adrenergic mechanisms[37], the effects of L-epinephrine (an adrenergic agonist) and dimethylphenylaziridinium ion (an irreversible α-adrenergic antagonist[38]) on the ($Mg^{2+}+Ca^{2+}$)-ATPase were investigated. Neither L-epinephrine (10^{-6}–10^{-3} M) nor dimethylphenylaziridinium ion (10^{-6}–10^{-3} M) had any significant effects on this activity. These results do not provide experimental support for previous speculation involving direct interaction of adrenergic drugs with ($Mg^{2+}+Ca^{2+}$)-ATPase[39].

The studies of Feinstein[10] indicated that Ca^{2+} and local anesthetics depress membrane excitability in similar ways, and it was suggested that both these agents may have a common locus of action, possibly phospholipid in nature. Local anesthetics have been shown to displace membrane bound Ca^{2+} from human erythrocytes[40], and it was suggested that inhibition of a Ca^{2+}-stimulated ATPase may be a possible mechanism of local anesthetic action[40]. It was thus of interest to examine the effect of local anesthetics on the brain ($Mg^{2+}+Ca^{2+}$)-ATPase. It was first established that tetracaine inhibited the ATPase system with a greater potency than procaine, which is consistent with the order of potency found physiologically. The I_{50} for the inhibition of ($Mg^{2+}+Ca^{2+}$)-ATPase by tetracaine was 3.1 ± 0.05 mM. The effect of various $CaCl_2$ concentrations on the inhibition

of $(Mg^{2+}+Ca^{2+})$-ATPase by tetracaine is shown in Fig. 4A, and the effect of various $MgCl_2$ concentrations in Fig. 4B. Tetracaine shifted the concentration of free Ca^{2+} required for maximum stimulation from 65 to 178 μM (Fig. 4A). The inhibition by tetracaine was greatest near optimum Ca^{2+} concentrations. These results indicate that tetracaine interferes with Ca^{2+} stimulation of the $(Mg^{2+}+Ca^{2+})$-ATPase. Higher concentrations of $CaCl_2$ (0.8–2.4 mM) antagonized tetracaine inhibition, possibly at a second Ca^{2+} site. The hyperbolic nature of the curve for Mg^{2+} activation of $(Mg^{2+}+Ca^{2+})$-ATPase was altered in the presence of tetracaine (Fig. 4B). Tetracaine inhibition was noncompetitive towards ATP.

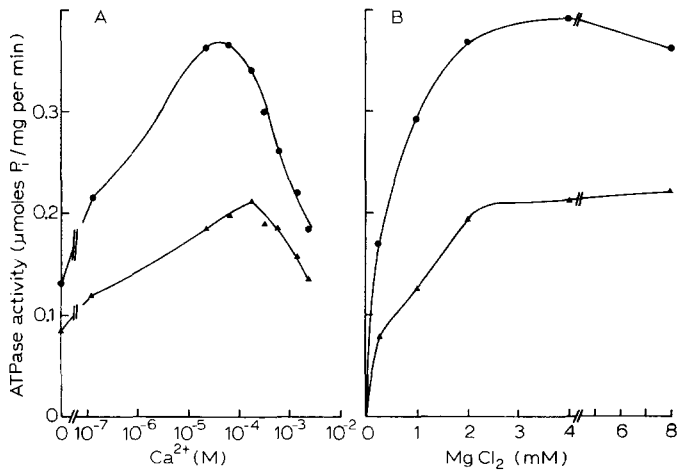

Fig. 4. Effect of $CaCl_2$ (A) and $MgCl_2$ (B) on the inhibition of ATPase activities by tetracaine. Microsomal Preparation A was treated with 0.08% sodium deoxycholate prior to assay. The assay medium contains 0.125 mM EGTA, 100 mM NaCl, 5 mM KCl and 0.1 mM ouabain. A, assayed with 5 mM $MgCl_2$ and B, assayed with 0.2 mM $CaCl_2$. Data points are means of duplicate determinations. ●—●, in the absence of tetracaine; ▲—▲, in the presence of 3.3 mM tetracaine hydrochloride added 10 min prior to the addition of ATP.

Tetracaine is not specific for inhibition of $(Mg^{2+}+Ca^{2+})$-ATPase, but also inhibits Mg^{2+}-ATPase with less potency ($I_{50}=4.3$ mM) and (Na^+,K^+)-ATPase with greater potency ($I_{50}=1.8$ mM), the latter inhibition being competitive with K^+ activation[41]. Nakamaru[3], Ohashi *et al.*[5] and Ohtsuki[42] suggested that the function of the $(Mg^{2+}+Ca^{2+})$-ATPase system in brain cells may be to maintain a low intracellular concentration of Ca^{2+} and hence maintain the excitability of the nerve membrane. Whereas muscle[6], brain[42] and erythrocytes[1,31] appear to maintain low intracellular Ca^{2+} levels by a Ca^{2+} pump, the squid axon appears to utilize a Na^+–Ca^{2+} exchange mechanism[43]. Inhibition of $(Mg^{2+}+Ca^{2+})$-ATPase by local anesthetics might be expected to lead to accumulation of intracellular Ca^{2+} in muscle, brain and erythrocytes. This Ca^{2+} accumulation in turn could cause changes in cell volume[40], K^+ permeability[44] and inhibition of excitability[5,42] and (Na^+, K^+)-ATPase activity. Inhibition of (Na^+,K^+)-ATPase by tetracaine in nerve axons could indirectly lead to similar effects through increase in Ca^{2+} influx caused by stimulation of the Na^+–Ca^{2+} exchange mechanism. It has been shown recently that local anesthetics act predominantly near the inside surface of nerve

axons (Narahashi *et al.*[45]), and at intracellular sites in skeletal muscle[11]. Hence it is likely that local anesthetic inhibition of the ($Mg^{2+}+Ca^{2+}$)-ATPase activity at the internal membrane surface or stimulation of the Na^+–Ca^{2+} exchange mechanism discussed above may contribute at least in part to the increase in threshold for action potential generation produced by local anesthetics[46].

CONCLUSION

Microsomal fractions from bovine brain cortex contain a ouabain-insensitive Mg^{2+}-dependent ATPase activity which is stimulated by low concentrations of Ca^{2+}. This ($Mg^{2+}+Ca^{2+}$)-ATPase appears similar to that reported by Nakamaru[3] in pig brain, by Wins and Dargent-Sallée[7] in electric tissue and Schatzmann and Rossi[31] in human erythrocytes, since it is further activated somewhat non-specifically by either NaCl or KCl. The Ca^{2+}-stimulated component is extremely labile, but can be elicited by detergents and freezing. The organization of ($Mg^{2+}+Ca^{2+}$)-ATPase in its membrane environment may be different to that of the (Na^+,K^+)-ATPase, since detergents elicit this latter activity to a greater extent than the ($Mg^{2+}+Ca^{2+}$)-ATPase activity. Adrenergic stimulants and inhibitors have little effect on the ($Mg^{2+}+Ca^{2+}$)-ATPase activity. The local anesthetic tetracaine interferes with Ca^{2+} stimulation of the ATP and alters the kinetics of Mg^{2+} activation. High concentrations of $CaCl_2$ protect against tetracaine inhibition. Brain ($Mg^{2+}+Ca^{2+}$)-ATPase has been shown to follow a similar distribution to (Na^+, K^+)-ATPase[3], and is probably localized in synaptic membranes[5]. The function of the ($Mg^{2+}+Ca^{2+}$)-ATPase in these membranes is not fully understood, but since brain microsomes exhibit an ATP-dependent Ca^{2+} uptake[42], and since a Ca^{2+} pump with similar properties to this enzyme has been shown to operate in human erythrocytes[1,31] and sarcoplasmic reticulum of muscle[47], it is tempting to suggest that the function of this enzyme is to maintain a low intracellular Ca^{2+} level, and hence excitability in nervous tissue.

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