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Characterisation of a high affinity Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase in the rat parotid plasma membrane

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Two Ca^{2+}-stimulated ATPase activities have been identified in the plasma membrane of rat parotid: (a) a (Ca^{2+} + Mg^{2+})-ATPase with high affinity for free Ca^{2+} (apparent K_m = 208 nM, V_{max} = 188 nmol/min per mg) and requiring micromolar concentration of Mg^{2+} and (b) a (Ca^{2+} or Mg^{2+})-ATPase with relatively low affinity for free Ca^{2+} ($K_{0.5}$ = 23 μM) or free Mg^{2+} ($K_{0.5}$ = 26 μM). The low-affinity (Ca^{2+} or Mg^{2+})-ATPase can be maximally stimulated by Ca^{2+} alone or Mg^{2+} alone. The high-affinity (Ca^{2+} + Mg^{2+})-ATPase exhibits sigmoidal kinetics with respect to ATP concentration with $K_{0.5}$ = 0.4 mM and a Hill coefficient of 1.91. It displays low substrate specificity with respect to nucleotide triphosphates. Although trifluoperazine inhibits the activity of the high affinity (Ca^{2+} + Mg^{2+})-ATPase only slightly, it inhibits the activity of the low-affinity (Ca^{2+} or Mg^{2+})-ATPase quite potently with 22 μM trifluoperazine inhibiting the enzymic activity by 50%. Vanadate, inositol 1,4,5-trisphosphate, phosphatidylinositol 4,5-bisphosphate, Na^+, K^+ and ouabain had no effect on the activities of both ATPases. Calmodulin added to the plasma membranes does not stimulate the activities of both ATPases. The properties of the high-affinity (Ca^{2+} + Mg^{2+})-ATPase are distinctly different from those of the previously reported Ca^{2+}-pump activity of the rat parotid plasma membrane.

Introduction

It is well established that cytosolic free Ca^{2+} is an important regulator of cellular metabolism [1]. In the parotid acinar cell, many aspects of its metabolism are influenced by changes in the intracellular level of free Ca^{2+} [2]. The extracellular free Ca^{2+} concentration is approximately four orders of magnitude greater than the submicromolar cytosolic free Ca^{2+}. Movement of Ca^{2+} ions into the cell down its steep electrochemical gradient is countered by an active extrusion of intracellular Ca^{2+}. In the rat parotid, an ATP-dependent Ca^{2+}-pump, responsive to submicromolar concentrations of free Ca^{2+}, has been identified in the plasma membrane fraction [3–5]. In addition, Takuma et al. [3] described a Na^+/Ca^{2+} exchanger associated with the basolateral membrane of the parotid cell. This Na^+/Ca^{2+} exchanger in parotid plasma membrane vesicles, however, showed no ability to accumulate Ca^{2+} against a concentration gradient indicating that the ATP-

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; CDTA, *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*',*N*'-tetra-acetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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dependent transport system is the physiologically more important one [3].

A high-affinity Ca^{2+}-stimulated ATPase has been reported to be associated with the plasma membrane fraction of a number of animal tissues [6–16]. A common feature of this ATPase is that it is stimulated by submicromolar concentrations of free Ca^{2+} and is dependent on low concentrations of Mg^{2+} [6–16] and is therefore a $(Ca^{2+} + Mg^{2+})$-ATPase. The high-affinity plasma membrane $(Ca^{2+} + Mg^{2+})$-ATPases of most tissues are regulated by calmodulin and acidic phospholipids [12,15–19] although these compounds were found to have no effect on plasma membrane high-affinity $(Ca^{2+} + Mg^{2+})$-ATPases of a number of tissues [9–11,15]. A number of investigators have suggested that this high-affinity plasma membrane $(Ca^{2+} + Mg^{2+})$-ATPase activity and the plasma membrane ATP-dependent Ca^{2+}-pumping activity are expressions of the same protein [7–9,11, 14–17,20,21].

Although the rat parotid plasma membrane ATP-dependent Ca^{2+}-pump has been characterised [3–5], there is no report of a Ca^{2+}-stimulated ATPase in this membrane fraction. We report here the detection and characterisation of a high-affinity Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase on the plasma membrane of the rat parotid. Our investigations indicate clearly that this ATPase possesses properties which are quite different from those reported for the rat parotid plasma membrane ATP-dependent Ca^{2+}-pump.

Materials and Methods

Materials. Calmodulin was a gift of Dr. J.H. Wang, University of Calgary. All other fine chemicals and biochemicals were from Sigma Chemical Company, St. Louis.

Preparation of rat parotid plasma membrane-enriched fraction. Parotid glands were dissected from male Wistar rats of about 200 g which had been starved overnight. The glands were placed in ice-cold buffer containing 0.25 M sucrose, 5 mM Hepes-KOH (pH 7.4) (Buffer A), cleaned of extraneous tissue and minced finely with a pair of scissors. The tissue was homogenised in Buffer A using an Arthur Thomas homogeniser size AA driven at 500 rpm. The homogenate was filtered through wire mesh of 0.5 mm^2 pore size to remove connective tissue and unhomogenised materials and then made up to 6% (w/v) with Buffer A. A plasma membrane-enriched fraction was prepared from this homogenate as previously described [4]. The homogenate was centrifuged at $1500 \times g$ for 10 min. The $1500 \times g$ pellet was then resuspended in Buffer A and a 90% (w/v) solution of Percoll in 0.25 M sucrose was added to give a final concentration of 30% (w/v) Percoll. The mixture was centrifuged at $35000 \times g$ for 30 min. A band containing the plasma membranes which forms near the top was aspirated and resuspended in a hypotonic medium consisting of 5 mM Hepes-KOH (pH 7.4). The membrane fraction was then pelleted by centrifugation at $35000 \times g$ for 10 min. The final hypotonic wash served to wash away excess Percoll. The pellet containing the plasma membranes was resuspended in buffer A and stored frozen at $-80°C$ in small aliquots. The Ca^{2+}-stimulated ATPase showed negligible loss of activity for up to one month when stored in this manner.

Washing of plasma membranes with buffer containing EGTA. The plasma membrane fraction, obtained by the procedure described above, was pelleted by centrifugation at $100000 \times g$, 4°C for 20 min. The pellet was resuspended in 10 mM Hepes-KOH (pH 7.4) buffer containing 1 mM EGTA and homogenised by four strokes of a hand-driven Arthur Thomas homogeniser size AA. The membranes were then pelleted by centrifugation and washed two more times in the same manner with buffer containing 1 mM EGTA and once more with the same buffer minus the EGTA. The membranes were finally resuspended in 10 mM Hepes-KOH (pH 7.4) buffer.

Assay of ATPase activity. ATPase activity was determined by measuring the rate of release of inorganic phosphate. ATPase assays were carried out in 0.5 ml assay mixture containing 20 mM Hepes-KOH (pH 7.4), 1 mM Na_2ATP, 2 μg/ml oligomycin, 1 mM EGTA plus an amount of $CaCl_2$ added to give the desired free Ca^{2+} concentration and about 80 μg of plasma membrane. The membranes were preincubated for 10 min at 37°C and the reaction initiated by the addition of the di-sodium salt of ATP. After incubation for 10 min at 37°C, 0.25 ml of 5% trichloroacetic acid

(w/v) was added to terminate the reaction. Precipitated proteins were removed by centrifugation and inorganic phosphate present in the supernatant measured by the spectrophotometric method described by Baginski et al. [22].

The high-affinity Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity in the standard assay was calculated as the difference in the rate of ATP hydrolysis by membranes incubated in assay buffer containing 1 mM EGTA with no added Ca^{2+} (basal activity) compared to that in 1 mM Ca^{2+}-EGTA buffer containing 300 nM free Ca^{2+} (high-affinity $(Ca^{2+} + Mg^{2+})$-ATPase activity). The calculation of free Ca^{2+} values was according to Pershadsingh and McDonald [20]. At pH values other than 7.4 and where CDTA was used as chelator, free Ca^{2+} and free Mg^{2+} values were calculated also according to Pershadsingh and McDonald [20].

When the ATPase activity was measured in the presence of high concentrations (0.3 mM) of free Ca^{2+} or Mg^{2+}, the ATPase activity obtained (total ATPase) was assumed to be the sum of the activities of the high-affinity $(Ca^{2+} + Mg^{2+})$-ATPase plus the low-affinity (Ca^{2+} or Mg^{2+})-ATPase. Thus:

Low-affinity (Ca^{2+} or Mg^{2+})-ATPase activity = total ATPase activity − high-affinity $(Ca^{2+} + Mg^{2+})$ATPase activity

Other assays. Lactate dehydrogenase activities of the parotid cell extracts were assayed by a kinetic method [23]. The concentration of protein was determined by the method of Lowry et al. [24]. K^+-dependent *p*-nitrophenylphosphatase activity was assayed by the method of Arvan and Castle [25].

Results

Identification of two types of Ca^{2+}-dependent ATPases

The reaction mixtures used for the assay of Ca^{2+}-dependent ATPase activities in plasma membranes fractions from various cells generally contain millimolar concentrations of Mg^{2+} [18,26–27]. In the presence of such high con-

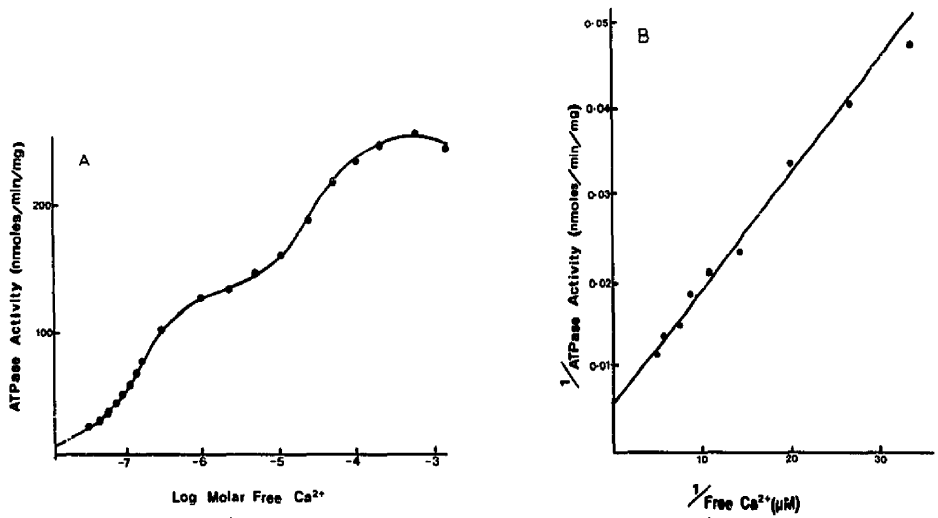

Fig. 1. (A) Dependence of Ca^{2+}-stimulated ATPase activity on free Ca^{2+} concentration. The Ca^{2+}-stimulated activity of the rat parotid plasma-membrane fraction was assayed in the presence of 1 mM EGTA and varying concentration of Ca^{2+} added to give the free Ca^{2+} shown on the abscissa. All other conditions are standard as described in Materials and Methods. Each point represents the mean of triplicate determinations. The standard errors of the means are all less than 4% of the value of the means. (B) Lineweaver-Burk plot of the high-affinity $(Ca^{2+} + Mg^{2+})$-ATPase activity. This plot is derived from the data of Fig. 1A for free Ca^{2+} values from 30 nM to 200 nM.

TABLE I

CO-PURIFICATION OF $(Ca^{2+} + Mg^{2+})$-ATPase WITH *p*-NITROPHENYLPHOSPHATASE ACTIVITY

The *p*-nitrophenylphosphatase and $(Ca^{2+} + Mg^{2+})$-ATPase activities were assayed as described in Materials and Methods. The $(Ca^{2+} + Mg^{2+})$-ATPase activity was measured in the presence of 1 mM Ca^{2+}-EGTA buffer (300 nM free Ca^{2+}). The values of the enzyme activities were the means ± S.E. of triplicate determinations.

	Specific activity (nmol/min per mg)	
	p-Nitrophenyl-phosphatase	$(Ca^{2+} + Mg^{2+})$-ATPase
Homogenate	19.4 ± 1.1	10.8 ± 0.4
1500 × *g*	25.7 ± 1.8	13.8 ± 0.3
Plasma membrane-enriched fraction	202.4 ± 8.1	102.6 ± 3.6

centrations of Mg^{2+}, no Ca^{2+}-stimulated ATPase activity could be detected in the rat parotid plasma membrane-enriched fractions. The presence of Ca^{2+}-stimulated ATPase activity was revealed only when Mg^{2+} was omitted from the reaction mixtures. As shown in Fig. 1A, in the absence of added Mg^{2+} and using a Ca^{2+}-EGTA buffer, two types of Ca^{2+}-stimulated ATPase activities were detected: a Ca^{2+}-stimulated ATPase requiring submicromolar concentrations of free Ca^{2+} (the high-affinity Ca^{2+}-ATPase), and a Ca^{2+}-ATPase stimulated by millimolar concentrations of free Ca^{2+} (the low-affinity Ca^{2+}-ATPase). The concentration of free Ca^{2+} required for maximal activity of the high-affinity Ca^{2+}-ATPase was 300 to 500 nM while the concentration of free Ca^{2+} required for optimal activity of the low-affinity Ca^{2+}-ATPase was 0.2 to 0.5 mM. A Lineweaver-Burk plot (Fig. 1B) of the high-affinity Ca^{2+}-ATPase activity was linear with a K_m of 208 ± 14 ($n = 3$) nM free Ca^{2+} and a V_{max} of 188 ± 11 ($n = 3$) nmol/mg per min. Table I shows that this high-affinity Ca^{2+}-ATPase co-purified with K^+-stimulated *p*-nitrophenylphosphatase activity, which is widely accepted as an enzymic marker of plasma membranes.

In order to demonstrate Mg^{2+}-dependence, we employed the chelator, *trans*-cyclohexane-1,2-diamine-*N*,*N*,*N'*,*N'*-tetraacetic acid (CDTA), which possesses high affinities for both Mg^{2+} and Ca^{2+}, unlike EGTA which has a selectively high-affinity for Ca^{2+} only. Fig. 2 shows that in the presence of Ca^{2+}-CDTA buffered reaction mixtures, the high-affinity Ca^{2+}-ATPase activity was abolished as compared to the activity in the Ca^{2+}-EGTA buffered system. In a separate experiment using a Ca^{2+}-Mg^{2+}-CDTA buffered system (results not shown) where the free Ca^{2+} concentration was maintained at 200 nM and the free Mg^{2+} concentration was varied, it was observed that the high-affinity $(Ca^{2+} + Mg^{2+})$-ATPase required free Mg^{2+} in excess of 3 μM for expression of its activity. Thus, this enzyme is a Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase referred to in this paper as the high-affinity $(Ca^{2+} + Mg^{2+})$-ATPase. The expression of the activity of this enzyme in the Ca^{2+}-EGTA system is presumably a result of the presence of a low concentration of endogenous Mg^{2+} in the plasma membrane preparation. The assay medium contained 5 to 6 μM Mg^{2+} as measured by atomic absorption spectrophotometry and the plasma membrane preparation used in our ATPase assays added a further 1.6 μM Mg^{2+} giving a total of 6.6 to 7.6 μM Mg^{2+}.

The low-affinity ATPase could be stimulated by Mg^{2+} alone or by Ca^{2+} alone (Fig. 3) and is therefore a $(Ca^{2+}$ or $Mg^{2+})$-ATPase. Concentra-

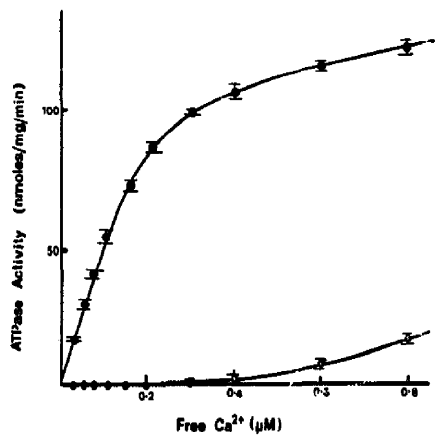

Fig. 2. Effect of Ca^{2+}-EGTA and Ca^{2+}-CDTA buffer systems on the parotid plasma membrane high-affinity $(Ca^{2+} + Mg^{2+})$-ATPase activity. Total buffer ligand concentration was maintained at 1 mM. Assay procedures were as described in Materials and Methods. ●, Ca^{2+}-EGTA system; ○, Ca^{2+}-CDTA system. Each point represents the mean ± S.E. of triplicate determinations.

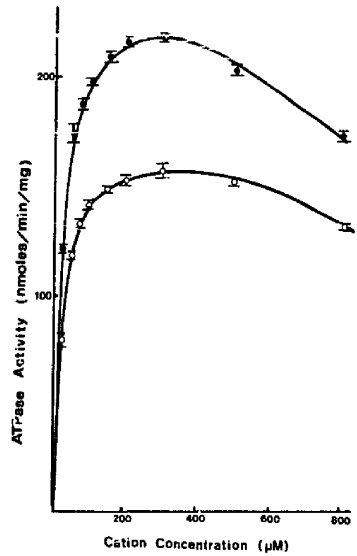

Fig. 3. Dependence of the parotid plasma membrane low-affinity (Ca^{2+} or Mg^{2+})-ATPase on free Ca^{2+} or free Mg^{2+} concentration. The enzyme activity was assayed in the presence of 1 mM CDTA and total Ca^{2+} or Mg^{2+} added to give the concentration of the free Ca^{2+} (○) or free Mg^{2+} (●) shown on the abscissa. All other assay conditions were as described in Materials and Methods. Each point represents the mean ± S.E. of triplicate determinations.

tions ($K_{0.5}$) of free Ca^{2+} or free Mg^{2+} required for half-maximal activation of this low-affinity ATPase were 23 μM and 26 μM, respectively. 0.2 to 0.5 mM free Ca^{2+} or free Mg^{2+} maximally activate the enzyme while concentrations of free Ca^{2+} or Mg^{2+} in excess of the optimal concentration resulted in partial inhibition. In the presence of optimal concentration of Mg^{2+}, the enzyme was approximately 40% more active than in the presence of corresponding concentration of Ca^{2+}. Ca^{2+} and Mg^{2+} did not exhibit synergistic stimulation of the enzyme (results not shown) indicating that the two divalent cations were acting on the same enzyme.

Oligomycin (2 μg/ml) and Ruthenium red (2 mM) had no effect on the high-affinity or low-affinity Ca^{2+}-stimulated ATPases. This indicates that any mitochondrial contaminant that may be in the plasma membrane preparation made no measurable contribution to the observed high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase and the low-affinity (Ca^{2+} or Mg^{2+})-ATPase activities. Oligomycin (2 μg/ml) was routinely included in the buffer used for ATPase assay.

Effect of ATP concentration

The effect of ATP concentration on the rate of ATP hydrolysis by the high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase was investigated in the concentration range of 0.1 mM to 3 mM ATP. The velocity vs. substrate plot (Fig. 4A) was sigmoidal and the Lineweaver-Burk plot (Fig. 4B) was non-linear indicating either the presence of two enzymes or one enzyme exhibiting positive co-operativity. A Hill plot of the data shows a straight line (results not shown) with a Hill coefficient of 1.91. $K_{0.5}$ for ATP was 0.4 mM.

Nucleotide specificity

The ability of the parotid plasma membrane fraction to hydrolyse nucleotides other than ATP is shown in Table II. The high affinity ($Ca^{2+} + Mg^{2+}$)-ATPases exhibited wide substrate specificity. GTP, CTP, UTP and ITP were hydrolysed at approximately the same rate as that of ATP. AMP and ADP were hydrolysed at rates which were much lower compared to ATP.

TABLE II

NUCLEOTIDE SPECIFICITY OF THE ($Ca^{2+}+Mg^{2+}$)-ATPase

The nucleotidase activity was assayed as described for ATPase in Materials and Methods. Concentration of the nucleotides were all at 1 mM. The incubation mixtures contained 1 mM Ca^{2+}-EGTA buffer (300 nM free Ca^{2+}). The values of the enzyme activities were means ± S.E. of triplicate determinations.

Nucleotide	Nucleotidase activity (nmol/min per mg)
ATP	102 ±3.2
GTP	104 ±4.3
CTP	103 ±3.7
UTP	110 ±3.3
ITP	101 ±2.8
ADP	66 ±3.6
AMP	8.9±0.7

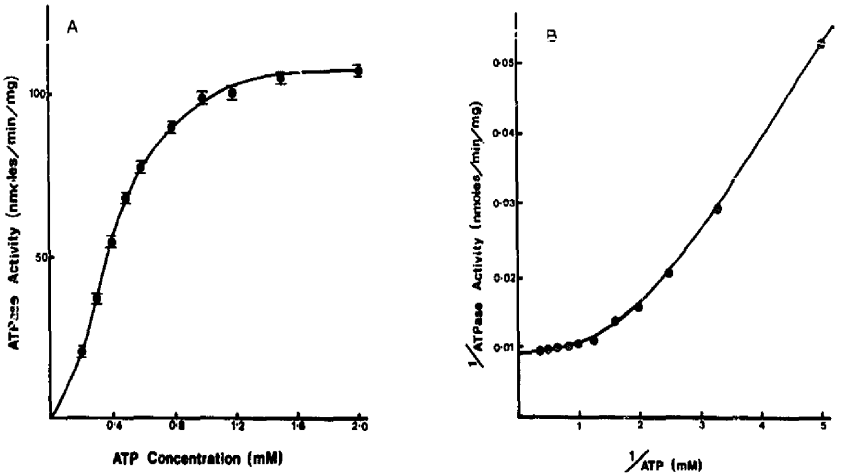

Fig. 4. (A) Dependence of the parotid plasma membrane high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase on ATP concentration. Enzyme activity was assayed in the standard incubation medium (1 mM Ca^{2+}-EGTA buffer, free Ca^{2+} = 300 nM) as described in Materials and Methods with the exception that the ATP concentration was varied between 0.1 mM and 3 mM. Each point represents the mean ± S.E. of triplicate determinations. (B) Lineweaver-Burk plot of the dependence of the high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase activity on ATP concentration. This plot is derived from the data of Fig. 4A.

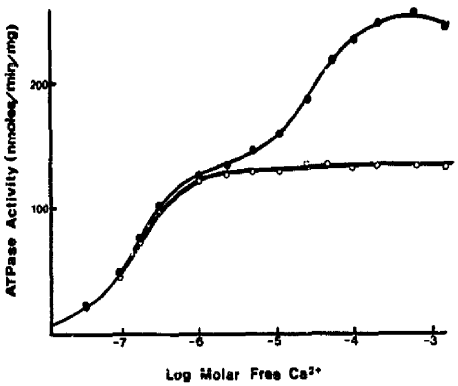

Fig. 5. Effect of 60 μM trifluoperazine on the Ca^{2+}-stimulated ATPase activities of parotid plasma membrane fraction. The Ca^{2+}-stimulated ATPase activity was assayed in the presence of 1 mM EGTA and varying concentration of Ca^{2+} added to give the free Ca^{2+} shown in the abscissa. All other conditions were standard as described in Materials and Methods. ●, No trifluoperazine; ○, Ca^{2+}-stimulated ATPase activity assayed in the presence of 60 μM trifluoperazine. Each point represents the mean of triplicate determinations. The standard errors of the means are all less than 4%.

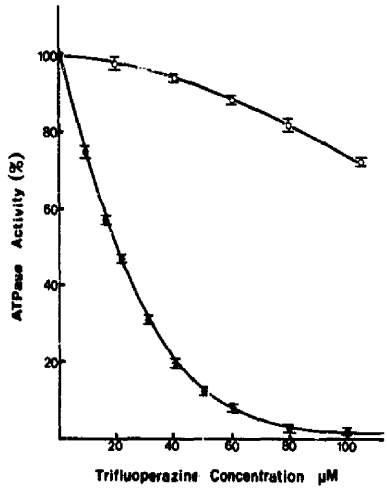

Fig. 6. Dose-reponse of the effect of trifluoperazine on the high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase and the low-affinity (Ca^{2+} or Mg^{2+})-ATPase activities. The Ca^{2+}-stimulated ATPase activities of the parotid plasma membrane fraction were assayed under standard conditions as described in Materials and Methods. ○, high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase activity; ●, low-affinity (Ca^{2+} or Mg^{2+})-ATPase activity. Each point represents the mean ± S.E. of triplicate determinations.

Effects of trifluoperazine and calmodulin

Fig. 5 shows that while 60 μM trifluoperazine had little effect on the activity of the high-affinity (Ca^{2+} + Mg^{2+})-ATPase, it inhibited the low-affinity (Ca^{2+} or Mg^{2+})-ATPase quite effectively. A plot of the ATPase activity at various concentrations of trifluoperazine (Fig. 6) shows that while 60 μM trifluoperazine inhibited the high-affinity (Ca^{2+} + Mg^{2+})-ATPase by 12%, it inhibited the low-affinity (Ca^{2+} or Mg^{2+})-ATPase by 92%. 50% inhibition of the low-affinity (Ca^{2+} or Mg^{2+})-ATPase was observed in the presence of 22 μM trifluoperazine. Calmodulin, added at concentrations of up to 150 nM, did not stimulate the activities of both ATPases. This lack of stimulation by added calmodulin was also observed with plasma membranes that had been washed with buffer containing EGTA (see Materials and Methods).

Effects of a variety of compounds

The following compounds, with their concentrations tested in parenthesis, were found to have no significant effect on the activities of the high-affinity (Ca^{2+} + Mg^{2+})-ATPase and the low-affinity (Ca^{2+} or Mg^{2+})-ATPase: Na^+ (100 mM); K^+ (100 mM); ouabain (1 mM); vanadate (20–200 μM); NaF (1 mM); carbamylcholine (20 μM); phenylephrine (20 μM); isoproterenol (20 μM); cyclic AMP (20 μM); inositol 1,4,5-trisphosphate (20 μM) and phosphatidylinositol 4,5-bisphosphate (40 μg/ml). The insensitivity of the high-affinity (Ca^{2+} + Mg^{2+})-ATPase towards vanadate could be because Mg^{2+} was not present at millimolar concentration. It had been reported by Barrabin et al. [28] that vanadate requires the presence of Mg^{2+} in the millimolar range in order to inhibit the human erythrocyte (Ca^{2+} + Mg^{2+})-ATPase.

Discussion

A Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase has been identified in a rat parotid plasma membrane fraction. This enzyme copurified with the K^+-dependent *p*-nitrophenylphosphatase activity indicating that it is a plasma membrane enzyme. Like the plasma membrane (Ca^{2+} + Mg^{2+})-ATPase of a number of animal tissues [9,11,13–15,29], the parotid enzyme exhibits a high affinity for Ca^{2+} with an apparent K_m of 208 nM. In common with the (Ca^{2+} + Mg^{2+})-ATPases of liver [9–11], adipocytes [14,20], corpus luteum [15] and kidney [29], the parotid (Ca^{2+} + Mg^{2+})-ATPase requires a low concentration of Mg^{2+} (estimated to be in the micromolar range) in addition to submicromolar Ca^{2+}, for it to be optimally active.

The parotid plasma membrane high affinity (Ca^{2+} + Mg^{2+})-ATPase appears to belong to a class of plasma membrane (Ca^{2+} + Mg^{2+})-ATPase which exhibits common characteristics of insensitivity towards calmodulin [10,11,15,29], phenothiazines [10,11,29], Na^+ or K^+ [11,15,20,26] and lack of specificity towards nucleotide triphosphate substrates [11,12,29], in addition to requirements for Ca^{2+} plus Mg^{2+}.

The properties of this plasma membrane (Ca^{2+} + Mg^{2+})-ATPase are quite different from those of the previously reported parotid endoplasmic reticulum (Ca^{2+} + Mg^{2+})-ATPase [30]. Firstly, although both enzymes are stimulated by submicromolar concentrations of free Ca^{2+}, the endoplasmic reticulum enzyme requires high (millimolar) concentrations of free Mg^{2+} [30] whereas the plasma membrane enzyme does not require added Mg^{2+}, its requirement for Mg^{2+} being satisfied by its endogenous Mg^{2+} which is estimated to be in the micromolar level. Second, the parotid endoplasmic reticulum enzyme exhibits high specificity for its nucleotide triphosphate substrate [30] whereas the plasma membrane enzyme exhibits broad substrate specificity in respect of nucleotide triphosphates. Third, 100 mM K^+ activates the endoplasmic reticulum enzyme by 2-fold [30] whereas it has no effect on the activity of the plasma membrane enzyme. Fourth, trifluoperazine inhibits the endoplasmic reticulum enzyme quite effectively (50% inhibition at 52 μM trifluoperazine) [30] whereas the plasma membrane enzyme is relatively resistant to inhibition by trifluoperazine. Fifth, phosphatidylinositol 4,5-bisphosphate (40 μg/ml) stimulates the rat parotid endoplasmic reticulum (Ca^{2+} + Mg^{2+})-ATPase by 2-fold [31] whereas it has no significant effect on the plasma membrane enzyme. Thus the high-affinity (Ca^{2+} + Mg^{2+})-ATPase activity of this rat parotid plasma membrane fraction could

not be the expression of the activity of residual endoplasmic reticulum ($Ca^{2+} + Mg^{2+}$)-ATPase activity that could possibly be found as a contaminant in our plasma membrane preparation.

Many investigators have suggested that the plasma membrane high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase may be the same protein as the ATP-dependent Ca^{2+}-pump [9,11,14–16,20]. The biochemical properties of the rat parotid plasma membrane high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase described in this paper and the properties of the plasma membrane Ca^{2+}-transporter of the same tissue are however distinctly different: (a) trifluoperazine is a potent inhibitor of Ca^{2+}-transport (30 μM trifluoperazine inhibits Ca^{2+} transport by 50%) [4] but is ineffective against the ($Ca^{2+} + Mg^{2+}$)-ATPase; (b) the ($Ca^{2+} + Mg^{2+}$)-ATPase hydrolyses the different nucleotide triphosphates equally well whereas the Ca^{2+}-transporter exhibits high specificity in its requirement for nucleotide triphosphate [4]; (c) the Ca^{2+}-transporter requires millimolar concentrations of Mg^{2+} [3–5] whereas the ($Ca^{2+} + Mg^{2+}$)-ATPase does not require added Mg^{2+} for its reaction as the endogenous Mg^{2+} (free Mg^{2+} estimated to be in the micromolar level) is sufficient; and (d) the ($Ca^{2+} + Mg^{2+}$)-ATPase's apparent K_m for free Ca^{2+} is approx. 5-times higher that the Ca^{2+}-transporter's $K_{0.5}$ for free Ca^{2+} [4]. There is thus sufficient evidence to suggest that the observed high-affinity ($Ca^{2+} + Mg^{2+}$)-ATpase activity of the parotid plasma membrane may not be attributable to the previously reported ATP-dependent Ca^{2+}-pump. Undoubtedly this parotid membrane fraction also contains the ATP-dependent Ca^{2+}-pump as reported previously [4], but since it requires millimolar concentration of Mg^{2+} for optimal activity [3–5], it would be largely inactive in the absence of added Mg^{2+}. Since the high affinity ($Ca^{2+} + Mg^{2+}$)-ATPase activity described in this paper was assayed in a system where no Mg^{2+} was added (see Materials and Methods), the ATP-dependent Ca^{2+}-pump would therefore make negligible contribution to the observed ATPase activity.

Lin [32] solubilised the ATP-dependent Ca^{2+}-pump of liver plasma membrane and reconstituted it in phospholipid vesicles. He reported that the properties of the reconstituted Ca^{2+}-transporter indicated that it is not the same protein as the previously reported high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase. Ghijsen et al. [29], working with rat kidney basolateral membranes, came to a similar conclusion. In a recent report [21], however, the high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase purified from rat liver plasma membrane was found to be an active ATP-dependent Ca^{2+}-pump when it was reconstituted into phospholipid vesicles. The liver plasma membrane enzyme differs from the parotid plasma membrane ($Ca^{2+} + Mg^{2+}$)-ATPase with respect to their affinities for ATP and free Ca^{2+} [9,21] and are probably different enzymes.

The parotid plasma membrane fraction also possesses a divalent ion-stimulated ATPase which is distinctly different from the high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase in that it (a) exhibits low affinity for Ca^{2+} with a $K_{0.5}$ of 23 μM and (b) can be maximally stimulated by Ca^{2+} alone or by Mg^{2+} alone. This enzyme is therefore a low-affinity (Ca^{2+} or Mg^{2+})-ATPase. (Ca^{2+} or Mg^{2+})-ATPases which possess similar properties have been found in the plasma membrane fractions of a wide variety of animal cells [11,20,29,33–36] and have often been referred to as 'non-specific Mg^{2+}-ATPases'. The parotid low-affinity (Ca^{2+} or Mg^{2+})-ATPase is inhibited effectively by low concentrations of trifluoperazine whereas the high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase is relatively unaffected. This differential effects of trifluoperazine lends support to the suggestion that the two ATPases are distinct entities. Ghijsen et al. [29] reported similar differences in the effects of trifluoperazine on the low- and high-affinity Ca^{2+}-ATPases of rat kidney basolateral membranes. That trifluoperazine inhibits, while added calmodulin did not stimulate, its activity suggests the possibility that trifluoperazine can inhibit enzymes independently of calmodulin activation [37]. Interpretation of the data is complicated by the fact that the effects were observed in experiments carried out on native membranes which contain a mixture of the two ATPases. Work is in progress in our laboratory in the study of the properties of purified ATPases following solubilisation of the membranes and fractionation of the enzymes.

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