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Ca^{2+}-STIMULATED, Mg^{2+}-INDEPENDENT ATP HYDROLYSIS AND THE HIGH AFFINITY Ca^{2+}-PUMPING ATPase

TWO DIFFERENT ACTIVITIES IN RAT KIDNEY BASOLATERAL MEMBRANES

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The Mg^{2+}-dependency of Ca^{2+}-induced ATP hydrolysis is studied in basolateral plasma membrane vesicles from rat kidney cortex in the presence of CDTA and EGTA as Mg^{2+}- and Ca^{2+}-buffering ligands. ATP hydrolysis is strongly stimulated by Mg^{2+} with a K_m of 13 μM in the absence or presence of 1 μM free Ca^{2+}. At free Mg^{2+} concentrations of 1 μM and lower, ATP hydrolysis is Mg^{2+}-independent, but is strongly stimulated by submicromolar Ca^{2+} concentrations ($K_m = 0.25$ μM, $V_{max} = 24$ μmol P_i/h per mg protein). The Ca^{2+}-stimulated ATP hydrolysis strongly decreases at higher Mg^{2+} concentrations. The Ca^{2+}-stimulated Mg^{2+}-independent ATP hydrolysis is not affected by calmodulin or trifluoperazine and shows no specificity for ATP over ADP, ITP and GTP. In contrast, at high Mg^{2+} concentrations calmodulin and trifluoperazine affect the high affinity Ca^{2+}-ATPase activity significantly and ATP is the preferred substrate. Control studies on ATP-dependent Ca^{2+}-pumping in renal basolaterals and on Ca^{2+}-ATPase in erythrocyte ghosts suggest that the Ca^{2+}-pumping enzyme requires Mg^{2+}. In contrast, a role of the Ca^{2+}-stimulated Mg^{2+}-independent ATP hydrolysis in active Ca^{2+} transport across basolateral membranes is rather unlikely.

Introduction

It has been shown in several laboratories that the basolateral plasma membranes from kidney cortex contain a high-affinity, ATP- and calmodulin-dependent calcium transport system [1–4]. A high-affinity Ca^{2+}-ATPase activity with similar characteristics was also measured in this membrane preparation [4,5]. However, these measurements were complicated by a high background ATP hydrolysis, which was more than 10-fold higher than the Ca^{2+}-ATPase activity [4,5]. Similar problems of high background ATP hydrolysis were encountered in the measurements of Ca^{2+}-ATPase in liver plasma membranes, but it was found that the Ca^{2+}-ATPase can be conveniently measured when exogenous Mg^{2+} was omitted from the incubation medium [6–8].

In an attempt to apply this method to the kidney basolateral plasma membranes, we have measured the Mg^{2+}- and Ca^{2+}-concentration dependence of ATP hydrolysis over a wide range of well-defined bivalent cation concentrations. This study shows that at low Mg^{2+} concentrations the ATP hydrolysis is strongly stimulated by submi-

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

cromolar concentrations of Ca^{2+} in a Mg^{2+}-independent way, but in contrast to the high-affinity Ca^{2+}-ATPase, this activity is not dependent on calmodulin, shows no specificity for ATP and is not coupled to ATP-driven Ca^{2+} uptake. Thus, in the kidney basolateral plasma membranes ATP is hydrolysed in a Ca^{2+}-dependent manner by two different systems, only one of them being the Ca^{2+}-transporting ATPase.

Methods and Materials

Membrane isolation. Basolateral membranes were isolated from freshly prepared kidney cortex slices of male Wistar rats (160–200 g weight) as described previously [2]. The basolateral membranes were enriched 15–20-times in $(Na^+ + K^+)$-ATPase activity as compared to the initial homogenate, and contained no significant contamination with brush borders or mitochondria [2,5]. Finally, the basolateral membranes were resuspended in 100 mM KCl, 20 mM Hepes, 20 mM Mes (pH 7.0) and stored in liquid nitrogen. Storage in liquid nitrogen up to 3 months caused a decrease in Ca^{2+}- or Mg^{2+}-ATPase or ATP-dependent Ca^{2+}-uptake of maximally 20% without changing the affinities for these ions. Erythrocyte ghosts depleted from calmodulin were prepared from human red blood cells [9] and were stored in liquid nitrogen.

For studies on calmodulin sensitivity of the Ca^{2+}-ATPase activity, frozen membranes (1.5 mg protein) were diluted 50 times into a hypotonic solution containing 20 mM Hepes, 20 mM Mes (pH 7.0) and 5 mM EDTA, stirred on ice for 30 min and mixed with one volume of 1.2 M KCl, 20 mM Hepes, 20 mM Mes (pH 7.0) [2]. The membranes were washed, resuspended in 100 mM KCl, 20 mM Hepes, 20 mM Mes (pH 7.0) and were immediately used for Ca^{2+}-ATPase measurements.

ATP hydrolysis and Ca^{2+}-transport measurement. The hydrolysis of ATP and the ATP-dependent Ca^{2+} uptake were determined in a standard medium containing 100 mM KCl, 20 mM Hepes, 20 mM Mes (pH 7.0), 2 mM NaN_3, 10 μg/ml oligomycin, 0.5 mM EGTA, 1 mM CDTA, 5 mM theophylline, 2.5 mM ouabain, 1 mM DTT and 3 mM Na_2ATP. For studies of substrate specificity, ATP was replaced by 3 mM ADP, ITP, GTP or *p*-nitrophenylphosphate. $CaCl_2$ or $MgCl_2$ were added in different amounts to obtain the desired free Ca^{2+} or Mg^{2+} concentrations.

For ATP hydrolysis measurements the basolateral membranes containing 10 μg protein, or 70–100 μg erythrocyte ghost protein were incubated for 30 min at 37°C in 0.5 ml of the standard incubation medium. The reaction was stopped by adding 0.5 ml 10% (w/v) trichloroacetic acid. The liberated inorganic phosphate was analyzed according to Bonting and Caravaggio [10] by adding 1 ml of 4% $FeSO_4$ in 0.575 M H_2SO_4 (w/v) and determining the absorbance at 690 nm between 30 and 60 min after termination of the reaction. Under these conditions, ATP hydrolysis was linear with time (up to one hour) and with protein (up to 100 μg). The measurement of ATP hydrolysis by coupled enzyme assay as used in a previous study [5] was not possible because millimolar $MgCl_2$ concentrations are required for activation of pyruvate kinase. In experiments with *p*-nitrophenylphosphate the reaction was terminated by adding 1.5 ml 1 M NaOH to the assay medium and the hydrolysed *p*-nitrophenylphosphate was measured as the absorbance incresae at 410 nm [11]. When the effects of trifluoperazine on Ca-stimulated ATP hydrolysis were tested, freshly prepared trifluoperazine solutions were used and the incubation was carried out in the dark to prevent the formation for free radicals.

Ca^{2+} uptake was measured by a rapid filtration technique as described previously [2]. Basolateral membranes (50 μg protein) were preincubated in the standard medium (100 μl volume) with 1 μCi/ml $^{45}CaCl$ and without ATP. After 4 min a 20 μl sample was taken as ATP-independent control. The reaction was started by adding 10 μl 30 mM Na_2ATP and taking 20 μl samples 15, 60 and 105 s after addition of ATP, where Ca^{2+} uptake is linear [2]. The samples were immediately diluted into 1 ml of an ice-cold stop solution containing 100 mM KCl, 20 mM Hepes, 20 mM Mes (pH 7.0) and 1 mM $LaCl_3$, added just before use. 0.9 ml of this mixture was filtered on a 1.25 cm diameter Sartorius nitrocellulose filter (0.6 μm pore size) and washed once with 3 ml stop solution. The amount of ^{45}Ca retained on the filters was analyzed in a liquid scintillation counter.

Buffering of free Ca^{2+} and Mg^{2+} concentra-

tions. In addition to EGTA as Ca^{2+}-buffering ligand, CDTA was included in the standard incubation medium to control the free Mg^{2+} concentrations in the micromolar range. The total Ca and Mg concentrations needed to obtain the desired free metal ion concentrations were calculated with a pocket calculator (Texas, TI-58) by using a matrix-vector multiplication program as described by Van Heeswijk et al. [4]. The calculated values were verified by applying the iterative method to calculate the free metal ion concentrations at given total concentrations as used previously [5]. The results calculated by these two methods differed maximally by 10%. The following association constants of EGTA, CDTA and ATP with H^+, Ca^{2+} and Mg^{2+}, corrected for the temperature of 37°C and ionic strength of 0.15 were used [12,13]: H-EGTA $1.70 \cdot 10^9$ M^{-1}; H-HEGTA $4.07 \cdot 10^8$ M^{-1}; Ca-EGTA $4.37 \cdot 10^{10}$ M^{-1}; Ca-HEGTA $2.14 \cdot 10^5$ M^{-1}; Mg-EGTA $2.57 \cdot 10^5$ M^{-1}; Mg-HEGTA $2.34 \cdot 10^3$ M^{-1}; H-CDTA $1.38 \cdot 10^{12}$ M^{-1}; H-HCDTA $1.15 \cdot 10^6$ M^{-1}; Ca-CDTA $1.10 \cdot 10^{13}$ M^{-1}; Mg-CDTA $1.51 \cdot 10^{11}$ M^{-1}; H-ATP $9.47 \cdot 10^6$ M^{-1}; H-HATP $9.12 \cdot 10^3$ M^{-1}; Ca-ATP $5.37 \cdot 10^3$ M^{-1}; Ca-HATP $6.30 \cdot 10^1$ M^{-1}; Mg-ATP $1.55 \cdot 10^4$ M^{-1}; Mg-HATP $1 \cdot 10^2$ M^{-1}.

Materials. Bovine brain calmodulin was obtained from Fluka, trifluoperazine from Smith, Kline and French Laboratories. $^{45}CaCl_2$ (21 mCi/mg) was from New England Nuclear. All other chemicals used were of purest grade available and were obtained from Sigma and Merck.

Results

Buffering of free Ca^{2+} and Mg^{2+}

In the presence of 1 mM $MgCl_2$ a stimulation of ATP hydrolysis in renal basolateral membranes by submicromolar Ca^{2+} has been shown by using EGTA as Ca^{2+}-complexing ligand [4,5]. When studying the Mg^{2+}-dependence of high-affinity Ca^{2+}-ATPase, CDTA is included in the medium to control the free Mg^{2+} concentrations as well. CDTA binds both Ca^{2+} and Mg^{2+} with high affinities (see Methods and Materials) and should chelate endogenous Mg^{2+} present in the membrane preparation. It could be calculated that under standard assay conditions, e.g. with 0.5 mM EGTA, 1 mM CDTA and 0–1.4 mM $CaCl_2$ (see Methods and Materials), free Mg^{2+} concentrations are buffered quite well in the micromolar range. In addition, free Ca^{2+} concentrations can be controlled at submicromolar levels.

Mg^{2+}-dependence of the Ca^{2+}-stimulated ATP hydrolysis

The Mg^{2+} concentration dependence of ATP hydrolysis by the kidney basolateral plasma membranes in the presence and absence of 1 μM free Ca^{2+} is shown in Fig. 1. In the absence of Ca^{2+} the ATP hydrolysis is practially zero at free Mg^{2+} concentrations of 1 μM and lower. At higher concentrations, Mg^{2+} progressively stimulates the ATP hydrolysis to reach a maximum at about 0.5 mM free Mg^{2+}. 1 μM Ca^{2+} stimulates strongly the ATP hydrolysis at very low Mg^{2+} concentrations. As the concentration of Mg^{2+} is increased, the stimulation by Ca^{2+} decreases and stabilises at a level of about 10% of the original value when the free Mg^{2+} concentration exceeds 100 μM (see Fig. 1B). The apparent K_m for Mg^{2+} of the ATP hydrolysis is about 13 μM, both in the presence and the absence of Ca^{2+}. The maximal rates of ATP hydrolysis (at high Mg^{2+} concentrations) are 52 and 56 μmol P_i/h per mg protein in the absence and presence of 1 μM Ca^{2+}, respectively.

Fig. 2 shows the Ca^{2+} concentration dependence of the ATP hydrolysis, estimated at 1 μM free Mg^{2+}, i.e., at the lowest Mg^{2+} concentration which could be effectively buffered in the standard assay. An Eadie-Hofstee plot of the data suggests a one-affinity mechanism with a K_m of 0.25 μM Ca^{2+} and a V_{max} of 24 μmol P_i/h per mg protein. This high affinity for Ca^{2+} is very similar to the affinity of the Ca^{2+}-ATPase determined at mM $MgCl_2$ concentration as reported previously [5]. However, the rate of Ca^2-stimulated Mg^{2+}-independent ATP hydrolysis is 6-fold higher than the V_{max} of the Ca^{2+}-ATPase (see also Fig. 1B).

Characterization of Mg^{2+}-independent ATP hydrolysis

In studies on Ca^{2+}-induced ATP hydrolysis and ATP-dependent Ca^{2+}-pumping in renal basolateral membranes, both activities appeared to be calmodulin-sensitive when measured in the presence of millimolar Mg^{2+} concentrations [2,5,14]. The effects of calmodulin on Ca^{2+}-stimulated ATP

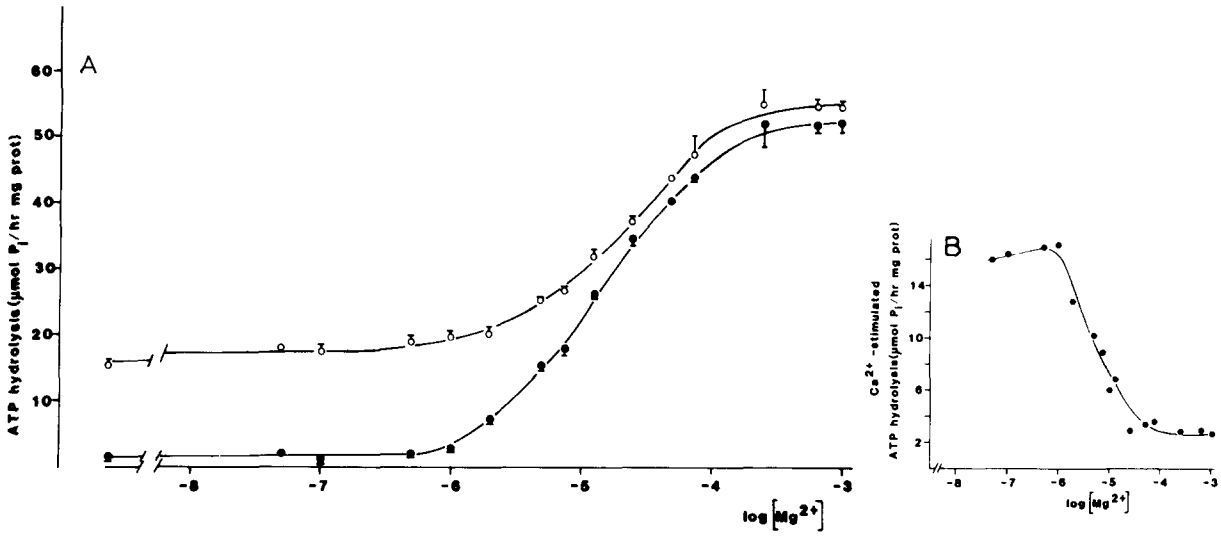

Fig. 1. Mg^{2+} concentration-dependence of ATP hydrolysis in renal basolateral membranes in the absence and presence of 1 μM free Ca^{2+}. ATP hydrolysis is measured under standard assay conditions (see Methods). (A) ATP hydrolysis at zero Ca^{2+} (●), in the presence of 1 μM free Ca^{2+} (○). Means ± S.E. of 2–5 experiments are given. (B) The differences between ATP hydrolysis at 1 μM Ca^{2+} and at zero Ca^{2+} are replotted.

hydrolysis at 1 μM and at 650 μM free Mg^{2+} are compared in Fig. 3. For these studies, the basolateral membranes were shocked hypotonically in the presence of EDTA, and washed with hypertonic KCl (see Methods). In the presence of 1 μM Mg^{2+} no effect of calmodulin on Ca^{2+}-stimulated ATP hydrolysis is observed. At high Mg^{2+}, a small but significant stimulatory effect of calmodulin is visible which is Ca^{2+} concentration-dependent and optimal at 0.5 μM Ca^{2+} (2.72 ± 0.31 μmol P_i/h per mg protein). The effects of trifluoperazine on Ca^{2+}-dependent ATP-hydrolysis in native basolateral membranes at 1 and 650 μM Mg^{2+} are shown in Fig. 4. Added in a concentration of 30 μM where trifluoperazine should act predominantly as a calmodulin antagonist [15], no effect of this drug on the Ca^{2+}-stimulated Mg^{2+}-independent ATP hydrolysis is observed. In contrast, the ATP hydrolysis is inhibited by 35% at high Mg^{2+}. The results shown in Figs. 3 and 4 indicate that the calmodulin-dependent Ca^{2+}-ATPase requires Mg^{2+} for its activity.

The substrate specificity of the high-affinity

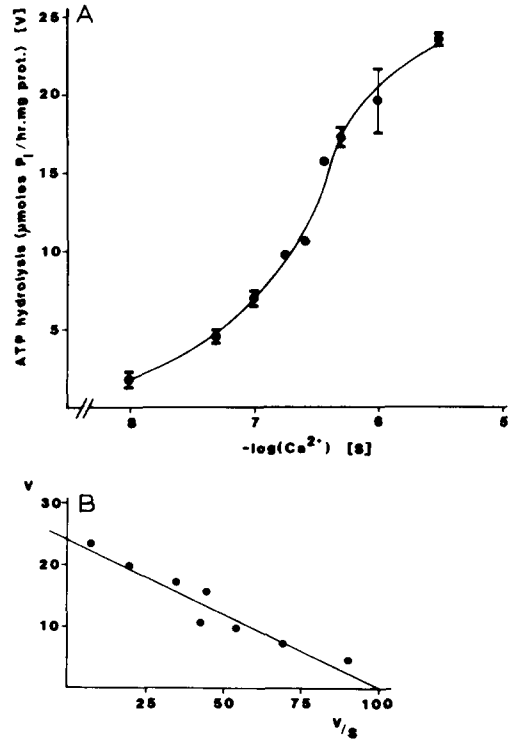

Fig. 2. Ca^{2+} concentration-dependence of ATP hydrolysis at low Mg^{2+}. ATP hydrolysis is measured in the standard assay at a constant free Mg^{2+} concentration of 1 μM (A). Means ± S.E. of two or three experiments are given. Kinetic parameters are estimated from the Eadie-Hofstee plot of the data (B).

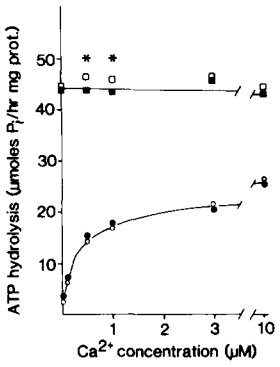

Fig. 3. Effects of calmodulin on ATP hydrolysis at low and high Mg^{2+} concentrations. ATP hydrolysis is measured at 1 μM free Mg^{2+} (○, ●) or 650 μM free Mg^{2+} (□, ■) in the absence (closed symbols) and presence of 1 μg/ml calmodulin (open symbols). Means of three experiments are given. The standard error of the mean is maximally 2.2%. * $P < 0.1$.

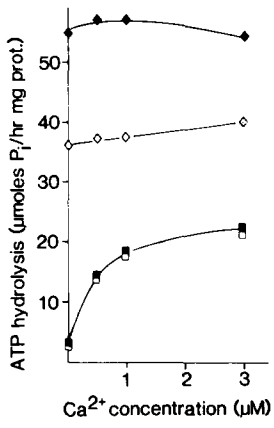

Fig. 4. Effect of trifluoperazine on ATP hydrolysis at low and high Mg^{2+} concentrations. ATP hydrolysis is assayed in the presence of 1 μM Mg^{2+} (□, ■) and 650 μM Mg^{2+} (◇, ◆) without (closed symbols) and with 30 μM trifluoperazine (open symbols). Means of two experiments are given.

Ca^{2+}-ATPase measured at low and high Mg^{2+} concentrations is shown in Table I. At 1 μM Mg^{2+} no preference exists for ATP over ADP, ITP and GTP. Almost no Ca^{2+}-induced *p*-nitrophenylphosphate hydrolysis occurs under these conditions which is probably due to the presence of 5 mM theophylline, an inhibitor of alkaline phosphatase [11] in the standard assay. At 650 μM Mg^{2+} a high specificity for ATP is observed, which is in agreement with the nucleotide specificity of ATP-dependent Ca^{2+} uptake shown previously [2].

To test whether the Ca^{2+}-stimulated Mg^{2+}-independent ATP hydrolysis might nevertheless be the expression of the Ca^{2+}-pumping ATPase, the Mg^{2+}-dependence of ATP-dependent Ca^{2+} uptake in the renal basolateral membranes is studied. The results are presented in Fig. 5A. ATP-dependent Ca^{2+} uptake is strongly Mg^{2+}-dependent and about 50 μM free Mg^{2+} is needed for maximal Ca^{2+} uptake. The half maximal activation of the ATP-dependent Ca^{2+}-pumping at about 15 μM Mg^{2+} is in close agreement with the apparent K_m for Mg^{2+} of 13 μM for the Ca^{2+}-induced ATP hydrolysis presented in Fig. 1.

Renal high-affinity Ca^{2+}-ATPase activity shares several properties with the well-studied erythrocyte Ca^{2+}-ATPase, i.e., affinity for Ca^{2+}, sensitivity to calmodulin and vanadate [3,5,16]. As an additional

TABLE I

SUBSTRATE SPECIFICITY OF Ca^{2+}-STIMULATED ATP HYDROLYSIS AT LOW AND HIGH Mg^{2+} CONCENTRATIONS

Nucleotide hydrolysis was measured as the difference between inorganic phosphate production in the presence and absence of 1 μM free Ca^{2+}. ATP is replaced by the other nucleotides in the standard incubation assay (see Methods). Means ± S.E. of 3 or 4 experiments are given.

	Ca^{2+}-dependent substrate hydrolysis (μmol P_i/h per mg protein)				
	ATP	ADP	ITP	GTP	*p*-Nitrophenyl-phosphate
1 μM Mg^{2+}	23.6 ± 0.6	27.4 ± 3.2	26.4 ± 1.0	26.9 ± 1.1	1.5 ± 0.4
650 μM Mg^{2+}	2.5 ± 0.6	n.s. *	n.s. *	0.6 ± 0.3	n.s. *

* n.s., no significant stimulation above nucleotide hydrolysis in the absence of Ca^{2+}.

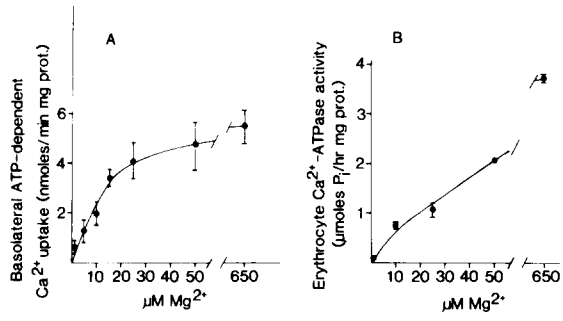

Fig. 5. (A) Mg^{2+} concentration-dependence of ATP-dependent Ca^{2+} uptake in renal basolateral membranes. (B) Mg^{2+} concentration-dependence of erythrocyte ghost Ca^{2+}-ATPase. Both activities are assayed in the standard incubation medium, Ca^{2+} uptake in the presence of 1 μM free Ca^{2+}, ghost Ca^{2+}-ATPase as the difference between ATP hydrolysis in the presence and absence of 1 μM free Ca^{2+}. Results are given as means $\pm$ S.E. of 2–4 experiments.

control, the Mg^{2+}-dependence of erythrocyte ghost Ca^{2+}-ATPase is determined, and the results are shown in Fig. 5B. Ghost Ca^{2+}-ATPase activity is measured in the presence of 1 μg/ml calmodulin, which stimulates Ca^{2+}-ATPase activity about 4-fold at high Mg^{2+} concentrations (not shown). At 1 μM Mg^{2+} hardly any Ca^{2+}-ATPase activity can be detected, and about 50 μM Mg^{2+} is needed for half maximal activation of the enzyme.

In conclusion, the results of the study described here suggest that Mg^{2+} is required for the Ca^{2+} transporting ATPase in renal basolateral plasma membranes. In addition, however, submicromolar concentrations of calcium stimulate the hydrolysis of nucleotide phosphates when the Mg^{2+} concentrations are low.

Discussion

The results presented in this study indicate that the renal basolateral high-affinity Ca^{2+}-ATPase which probably reflects the Ca^{2+}-pumping enzyme, needs Mg^{2+} for its functioning. Studies on Mg^{2+}-dependences of substrate specificity and calmodulin-sensitivity strongly suggest the Mg^{2+}-requirement of the enzyme. In addition, ATP-dependent Ca^{2+} uptake appears strongly Mg^{2+}-dependent, though possible change in vesicle size or permeability by varying the Mg^{2+} concentration in the uptake assay cannot be excluded. In the presence of millimolar Mg^{2+} concentration, ATP-dependent Ca^{2+} uptake has similar characteristics as the high affinity Ca^{2+}-ATPase measured in the same membrane preparation, except for the V_{max} of the Ca^{2+} uptake which is only 10% of the maximal Ca^{2+}-ATPase activity [2,5]. This difference is most likely due to the small part of the total vesicle population which is sealed and inside-out oriented and involved in the measured ATP-driven Ca^{2+} uptake, whereas the main part is leaky and right-side out oriented, as discussed previously [2].

The calmodulin stimulation of the high-affinity Ca^{2+}-ATPase activity in the presence of high Mg^{2+} concentrations is hardly visible against the high background ATP hydrolysis. Only by statistical evaluation of the differences between the activities plus and minus calmodulin one tends to conclude that calmodulin increases the enzyme activity as shown previously [5]. In absolute values, however, the calmodulin-dependent ATPase was maximally 6% of the total (Ca^{2+} + Mg^{2+})-stimulated ATPase in our studies, what makes kinetic studies rather difficult. This problem prompted us to study the Mg^{2+}-dependence of the high-affinity Ca^{2+}-ATPase in kidney cortex basolaterals in an attempt to dissociate better between the Ca^{2+}-stimulated ATPase and the Mg^{2+}-dependent background ATPase. As shown in this study, the background ATP hydrolysis appears strongly Mg^{2+}-dependent, but the same seems true for the specific Ca^{2+}-stimulated ATPase. Both activities have an apparent affinity for Mg^{2+} of 13 μM.

In addition to the Mg^{2+}-dependent Ca^{+}-ATPase activity a Ca^{2+}-stimulated Mg^{2+}-independent ATP hydrolysis could be measured with a high affinity for Ca^{2+}. The specific activity of this Ca^{2+}-stimulated Mg^{2+}-independent ATP hydrolysis is about 6-fold higher than that of the Mg^{2+}-dependent Ca^{2+}-ATPase. This inhibition might be due to competition between Ca^{2+} and Mg^{2+} in activating non-specific ATP hydrolysis at these relative high Mg^{2+} concentrations as shown in corpus luteum plasma membranes [17]. Such a competition could also explain the inhibition of Ca^{2+}-induced Mg^{2+}-ATPase observed in crude kidney baslolaterals [18] and heart sarcolemma [19].

In the presence of high Mg^{2+}, calmodulin stimulates ATP hydrolysis by 6%, whereas 30 μM trifluoperazine inhibits this activity by 35%. This discrepancy can be explained by inhibition of Mg-dependent background ATPase activity by this drug in addition to the 'specific' calmodulin-sensitive ($Ca^{2+} + Mg^{2+}$)-ATPase as reported for the red blood cell Ca^{2+}-ATPase [20].

This study strongly suggests that the specific Ca^{2+}-pumping ATPase in renal basolateral membranes is Mg^{2+}-dependent, as shown for practially all Ca^{2+}-pumping ATPases studied so far. However, the Mg^{2+}-independent high-affinity Ca^{2+}-stimulated ATP hydrolysis presented in this study is quite unique. In studies on high-affinity ($Ca^{2+} + Mg^{2+}$)-ATPase in plasma membranes from rat liver and corpus luteum, a stimulation in ATP hydrolysis by submicromolar Ca^{2+} concentrations could be detected only when no external Mg^{2+} was added to the assay [6–8,17]. However, in the presence of the chelator CDTA almost no high-affinity Ca^{2+}-ATPase activity was measurable anymore, indicating that these enzymes require endogenous Mg^{2+}, introduced by the membranes and reagents in the assay [6,8,17]. In contrast, with CDTA and without external Mg^{2+} in the assay, a high affinity Ca^{2+}-stimulated ATP hydrolysis with a high specific activity is observed in renal basolateral membranes as shown in the present study. The enzymatic basis of this activity is not clear at present. This Ca^{2+}-stimulated Mg^{2+}-independent ATP hydrolysis is not from mitochondrial origin because of the presence of NaN_3 and oligomycin in the standard assay. Furthermore, it is not likely that it reflects alkaline phosphatase activity, an enzyme which also has a high-affinity for Ca^{2+} [11]. Theophylline present during incubation suppresses the Ca^{2+} activation of the enzyme, as shown by the virtual absence of Ca^{2+}-induced *p*-nitrophenylphosphate hydrolysis. The possibility that adenylate kinase is responsible for the observed Ca^{2+}-induced ADP hydrolysis at low Mg^{2+} concentration is rather unlikely because this enzyme requires millimolar Mg^{2+}, but is Ca^{2+}-independent [21]. Parallel studies on high-affinity Ca^{2+}-ATPase activity in basolateral plasma membranes of rat duodenal mucosa show a similar Mg^{2+}-requirement for the enzyme as for the renal enzyme reported here (unpublished results). At Mg^{2+} concentrations obviously required for optimal functioning of the enzyme, however, the Ca^{2+}-stimulation of ATP hydrolysis is hardly detectable due to the high background Mg^{2+}-ATPase activity, whose identity remains unknown [11].

A better separation between specific ($Ca^{2+} + Mg^{2+}$)-ATPase and non-specific background ATPase can possibly be obtained after solubilization and further purification of the enzyme, as has been reported for Ca^{2+}-ATPase from dog kidney cortex and rat liver plasma membranes [6,8,14], studies which are currently under investigation in our laboratory.

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