Biochimica et Biophysica Acta, 646 (1981) 457–464
Elsevier/North-Holland Biomedical Press

BBA 79369

STUDIES OF GASTRIC Ca^{2+}-STIMULATED ADENOSINE TRIPHOSPHATASE

I. CHARACTERIZATION AND GENERAL PROPERTIES

JOYTIRMOY NANDI, TUSHAR K. RAY and PARIMAL C. SEN

Department of Surgery and Physiology, The State University of New York Upstate Medical Center, Syracuse, NY 13210 (U.S.A.)

(Received November 24th, 1980)
(Revised manuscript received April 28th, 1981)

Key words: ATPase; Ca^{2+} stimulation; (Porcine gastric microsome)

Gastric microsomes do not contain any significant Ca^{2+}-stimulated ATPase activity. Trypsinization of pig gastric microsomes in presence of ATP results in a significant (2–3-fold) increase in the basal (with Mg^{2+} as the only cation) ATPase activity, with virtual elimination of the K^+-stimulated component. Such treatment causes unmasking of a latent Mg^{2+}-dependent Ca^{2+}-stimulated ATPase. Other divalent cations such as Sr^{2+}, Ba^{2+}, Zn^{2+} and Mn^{2+} were found ineffective as a substitute for Ca^{2+}. Moreover, those divalent cations acted as inhibitors of the Ca^{2+}-stimulated ATPase activity. The pH optimum of the enzyme is around 6.8. The enzyme has a K_m of 70 μM for ATP and the K_a values for Mg^{2+} and Ca^{2+} are about $4 \cdot 10^{-4}$ M and 10^{-7} M, respectively. Studies with inhibitors suggest the involvement of sulfhydryl and primary amino groups in the operation of the enzyme. Possible roles of the enzyme in gastric H^+ transport have been discussed.

Introduction

The importance of Ca^{2+} as a key regulator for many biological processes has become increasingly appreciated [1–3]. Among the various modes of transport for storage and redistribution of intracellular Ca^{2+} and consequent modulation of cell function, the plasma membrane located Ca^{2+} pump has been implicated to play a crucial role. The Ca^{2+}-pump, like other ion transport ATPase, hydrolyses ATP and is a Ca^{2+}-activated Mg^{2+}-dependent ATPase [4]. Ever since its first discovery in erythrocyte plasma membrane 2 decades ago [4–6] the evidence for the existence of this pump has been documented in such diverse tissues as liver [7,8], brain [9,10], kidney [11,12], uterus [13,14], platelet [15], pancreas [16], adipocytes [17,18] and intestinal brush border membranes [19]. In conjunction with other cellular mechanisms for the transport and storage of Ca^{2+}, the plasma membrane located Ca^{2+}-pump appears to work towards keeping the concentration of intracellular free calcium to a low steady-state level (about 10^{-6} M).

There are numerous lines of evidence in the literature to suggest that Ca^{2+} plays an important role in gastric acid secretion [20]. Besides its role in maintaining the integrity of the epithelial junctional complexes [21] Ca^{2+} has recently been implicated [22–24] to act at some critical intracellular sites for its effects in gastric acid secretion. Thus, Rehm and collaborators [22] demonstrated two phases in the effects of removal of Ca^{2+} from the bathing medium of chambered bullfrog gastric mucosa: in the first phase H^+ secretory rate and p.d. partially dropped with an increase in resistance, whereas in the second phase both H^+ and p.d. reached zero with a marked decline in resistance. While both phases were reversed

Abbreviations: Pipes, 1,4-piperazinediethanesulfonic acid; FDNB, fluorodinitrobenzene; MDPF, 2-methoxy-2,4-diphenyl-3-dihydrofuranone; TNBS, 2,4,6-trinitrobenzenesulfonic acid; pCMBS, *para*-chloromercuribenzenesulfonic acid.

by introducing Ca^{2+} into the nutrient solution, addition of Ca^{2+} into the luminal solution, which is in direct contact with the apical membranes of the acid secreting cells, reversed only the second phase. Recently Kasbekar and Chugani [24] demonstrated that gastric secretagogues stimulate the efflux of Ca^{2+} both in chambered bullfrog gastric mucosa and isolated oxyntic cells. These studies [21–24] not only demonstrated the dependency of gastric H^+ transport on Ca^{2+} but also suggested [22,24] some mechanism for the transport of Ca^{2+} at the plasma membranes of the acid secreting cells.

The plasma membranes derives primarily from the apical and tubulovesicular membranes of the acid secreting cells have been well characterized [25–28]. The gastric microsomes are highly enriched in a K^+-stimulated ATPase activity [26,28]. The Ca^{2+}-stimulated ATPase activity is very low and may be considered to be virtually absent in these membranes. However, we have recently demonstrated that mild trypsin treatment of the gastric microsomes in presence of ATP results in the manifestation of a highly active Ca^{2+}-stimulated ATPase [29,30]. Although the K^+-stimulated ATPase activity was inactivated by treatment with trypsin both in presence and absence of ATP [29], the enzyme activity could be fully restored [29] by the endogenous activator protein [31]. The data [29,30] suggested that the unmasked ATPase activity associated with microsomes trypsinized in presence of ATP is not an altered state of the microsomal K^+-stimulated ATPase but a true manifestation of a Ca^{2+}-stimulated ATPase activity. In the present report we have studied the properties of this Ca^{2+}-stimulated ATPase in detail. The possible significance of this microsomal Ca^{2+}-stimulated ATPase in gastric H^+ transport has been discussed.

Methods and Materials

Isolation of gastric microsomes

Purified microsomal membranes from pig gastric mucosa were prepared by the method described previously [31]. Briefly, the fundic mucosa of pig was desquamated and scraped [32] to collect the oxyntic cell enriched fractions. The mucosal cells were homogenized in 0.25 M sucrose containing 0.2 mM EDTA and 0.2 mM Pipes buffer (pH 6.8) by using a loose fitting pestle in a Dounce homogenizer. The homogenate was centrifuged at 8 000 × *g* for 5 min. The process was repeated three times. All supernatants were pooled together and layered over 40 ml of 37% (w/v) sucrose in 84 ml screw-cap tubes and centrifuged at 100 000 × *g* for 5 h in a type 35 Beckman angle rotor. The microsomal membrane band appeared at the interface of the clear soluble supernatant and 37% sucrose. The membrane bands were collected, diluted with homogenizing medium and centrifuged at 100 000 × *g* for 90 min. The pellet was resuspended in homogenizing medium at an appropriate protein concentration and used for the study. Proteins were assayed by the method of Lowry et al. [33] using bovine serum albumin as standard.

Digestion with trypsin

Treatment with trypsin was carried out at 30°C in 50 mM Tris-HCl buffer (pH 7.5) containing 250 mM sucrose with a weight ratio of trypsin to membrane protein 1 : 100 in the presence and absence of 2 mM ATP. Control membranes were incubated in parallel without trypsin. After a preincubation period of 3 min, trypsin digestion was carried out for 10 min and terminated by adding soybean trypsin inhibitor equalling twice the weight of trypsin. About 0.5–1 mg membrane protein was used for trypsin digestion. After the treatment the membranes were isolated by centrifugation for 60 min at 100 000 × *g* in the cold, resuspended in sucrose (250 mM sucrose/0.2 mM EDTA/0.2 mM Pipes buffer (pH 6.8)) and used for the present study. While all the proteins of the control (without trypsin) membranes were recovered in the pellet, a significant portion (12.6 ± 1.1%) of the microsomal proteins remained in the supernatant after the high-speed centrifugation of the trypsinized microsomes.

Assay of ATPase

The ATPase was assayed as previously described [31]. The incubation mixture contained, in a total volume of 1 ml, 50 μmol Pipes (pH 6.8) 1 μmol $MgCl_2$, 2 μmol Tris · ATP and 10 μg membrane protein in the presence or absence of 2 μmol KCl. After 10 min preincubation at 37°C the reactions were started with 2 μmol of Tris · ATP and incubated for 15 min. The reactions were stopped by adding 1 ml of 12% (w/v) trichloroacetic acid. The P_i was assayed

by the procedure of Sanui [34]. The K^+-stimulated ATPase activity was calculated by subtracting the basal rate (with Mg^{2+} as the only cation) from the rate of hydrolysis of ATP in the presence of both Mg^{2+} and K^+.

For the assay of Ca^{2+}-stimulated ATPase, the reaction mixture generally contained in a total volume of 1 ml 50 μmol Tris-Pipes buffer (pH 7), 1 μmol $MgCl_2$, 1 μmol Tris · ATP, 10 μg membrane protein 1 μmol EGTA in the presence and absence of different concentrations of Ca^{2+}. All other incubation conditions were the same as mentioned above. A K_{app} of $1 \cdot 10^7$ was used to calculate the concentrations of free Ca^{2+} in Ca^{2+}/EGTA buffer [35] as described by Reddy and Wynborny [36]. For the Ca^{2+}/EGTA buffer a fixed (1 mM) concentration of EGTA (pH 7.0) with variable amounts (0.244–0.99 mM) of $Ca(NO_3)_2$ in 50 mM Tris-Pipes buffer (pH 7.0) was used. Under such conditions, the presence of 1 mM ATP and 1 mM Mg^{2+} in the incubation mixture does not appreciably alter the concentration of free Ca^{2+} in the assay medium [37].

Materials

ATP, CTP, GTP, ITP, UTP, trypsin, trypsin inhibitor, Tris base, pCMBS, *N*-ethylmaleimide, EGTA, sucrose and Pipes were purchased from Sigma Chemical Co. TNBS and FDNB were purchased from Aldrich Chemical Company. MDPF was a generous gift from the laboratory of Dr. Weigele and kindly sent by Dr. W.E. Scott, Hoffman-LaRoche, Inc., Nutley, NJ. All other reagents were the best grade available from the market.

Results

Fig. 1 shows the effects of K^+ and/or Ca^{2+} on the ATPase activity associated with control and trypsinized gastric microsomes. The control membranes demonstrate very low stimulation by Ca^{2+} and a large stimulation by K^+ of the microsomal ATPase. The K^+-stimulated activity of the control microsomes is abolished by 1 mM Ca^{2+}. The trypsinized microsomes, on the other hand, showed a significantly higher level of Ca^{2+} stimulation and virtually no stimulation by K^+. Furthermore, the stimulation of ATPase by Ca^{2+} in the digested membrane was independent of the presence of K^+ in the assay medium.

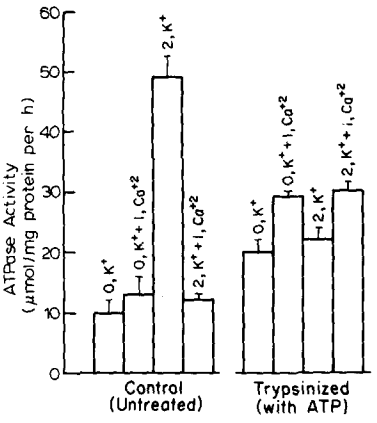

Fig. 1. Effects of K^+ and/or Ca^{2+} on the ATPase activity associated with control (untreated) and trypsinized pig gastric microsomes. Trypsinization was done in presence of 2 mM ATP. Details of trypsinization and assay of ATPase are given in Methods and Materials. Whenever used, the concentrations of K^+ and Ca^{2+} were 2 and 1 mM, respectively. No EGTA was present in the assay medium. Data are mean ± S.E. (n = 3).

Fig. 2 shows the effects of different pH on the Ca^{2+}-stimulated ATPase activity associated with trypsinized microsomes. pH 6.8 appears to be optimal for the Ca^{2+}-stimulated ATPase. Rather large changes in activity between the narrow range of pH 6.5 and 7.0 demonstrates pH 6.8 to be critical for the enzyme function under the conditions of our assay.

Effects of different divalent cations on the Ca^{2+}-

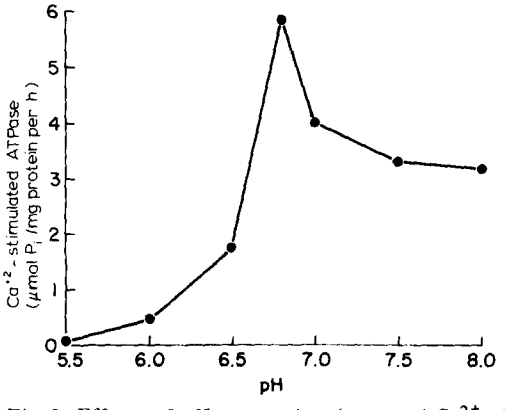

Fig. 2. Effects of pH on gastric microsomal Ca^{2+}-stimulated ATPase activity. Tris/Pipes buffers of different pH values and a fixed concentration of Ca^{2+} (1 mM) without EGTA were used. Details of the assay are given in Methods and Materials.

stimulated ATP hydrolysis by the trypsinized pig gastric microsomes are shown in Table I. While Ba^{2+} and Sr^{2+} act as partial inhibitors of the Ca^{2+}-stimulated ATPase; Zn^{2+} and Mn^{2+} completely inhibit the Ca^{2+}-stimulated activity. It should be mentioned in this connection that when tested alone at 1 mM concentration; none of the divalent cations except Ca^{2+} was effective in stimulating the hydrolysis of ATP (Table I).

The Ca^{2+}-stimulated ATPase activity is completely dependent on the presence of Mg^{2+}. Effects of different concentrations of Mg^{2+} on the gastric microsomal Ca^{2+}-stimulated ATPase is shown in Fig. 3. The optimum concentration of Mg^{2+} was found to be 1.0 mM. 2 mM Mg^{2+} was found to be inhibitory for the Ca^{2+}-stimulated ATPase. The Lineweaver-Burk plot of the data (Fig. 3, inset) shows the apparent K_d for Mg^{2+} to be about 0.4 mM. However, the real K_d for Mg^{2+} will be less than 0.4 mM due to binding of some Mg^{2+} by EGTA under the conditions of our assay (Fig. 3).

Effects of different concentrations of free Ca^{2+} into the assay medium on the activity of microsomal Ca^{2+}-stimulated ATPase are shown in Fig. 4. About $5 \cdot 10^{-6}$ M Ca^{2+} appears to be needed for the maximal activation of the Ca^{2+}-stimulated ATPase of the trypsinized microsomes. Not shown in Fig. 4 is the fact

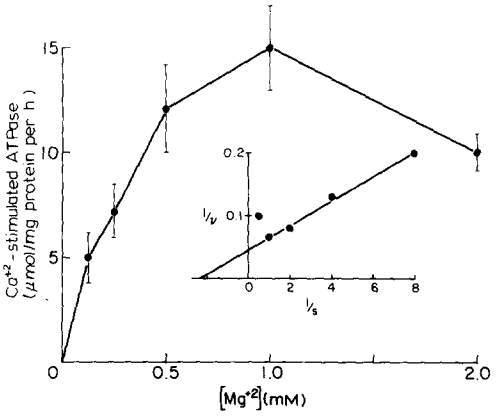

Fig. 3. Effects of different concentrations of $MgCl_2$ in the assay medium on gastric microsomal Ca^{2+}-stimulated ATPase activity. The concentration of free Ca^{2+} was maintained at 10^{-5} M using 1 mM EGTA. The inset shows the Lineweaver-Burk plot of the data. Details of the assay are given in Methods and Materials. Data are mean ± S.E. (n = 4).

that the Ca^{2+}-stimulated activity remains unaltered with higher (up to 1 mM) Ca^{2+}. The control membranes did not show any significant Ca^{2+}-stimulated activity under all those conditions of varied concentrations of free Ca^{2+} (Fig. 4).

Fig. 5 shows the effects of different concentrations of ATP on the Ca^{2+}-stimulated ATPase activity associated with the trypsin-treated gastric micro-

TABLE I

EFFECTS OF DIFFERENT DIVALENT CATIONS ON THE Ca^{2+}-STIMULATED ATPase ACTIVITY OF PIG GASTRIC MICROSOMES

The concentation of free Ca^{2+} in the assay medium was 10^{-5} M. The microsomes were preincubated for 15 min at 37°C in Tris/Pipes buffer (pH 7.0) containing 1 mM EGTA, 1 mM Mg^{2+} and other test substances in the presence and absence of an appropriate amount of Ca^{2+}. At the end of the preincubation period the reactions were started by adding 1 mM ATP and incubated further for 15 min. Details of the assay procedure are given in Methods and Materials. Data are mean ± S.E. (n = 8).

Cation tested (1 mM)	ATPase activity (μmol/mg protein per h)		
	without Ca^{2+}	with Ca^{2+}	ΔCa^{2+}
None (control)	22.96 ± 0.65	32.23 ± 0.62	9.27
Ba^{2+}	20.68 ± 0.35	25.46 ± 0.40	4.60
Sr^{2+}	21.34 ± 1.03	23.99 ± 1.41	2.65
Mn^{2+}	23.25 ± 0.53	22.20 ± 0.48	−1.05
Zn^{2+}	21.09 ± 0.44	20.64 ± 1.2	−0.45

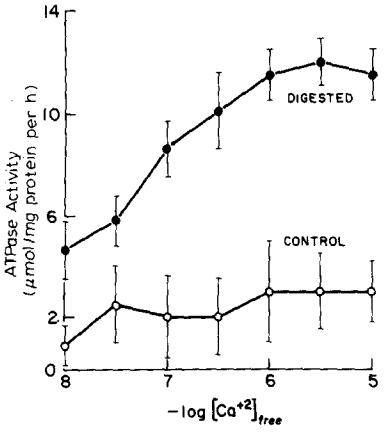

Fig. 4. Effects of different concentrations of free Ca^{2+} on the Ca^{2+}-stimulated ATPase activity associated with control and trypsin-digested microsomes. A fixed concentration (1 mM) of EGTA and variable amounts of Ca^{2+} were used to obtain the desired free Ca^{2+}. The Ca^{2+}-stimulated ATPase was taken as the rate in the presence of both Ca^{2+} and Mg^{2+} minus the basal (only Mg^{2+} as cation) rate. Values are mean ± S.E. (n = 3).

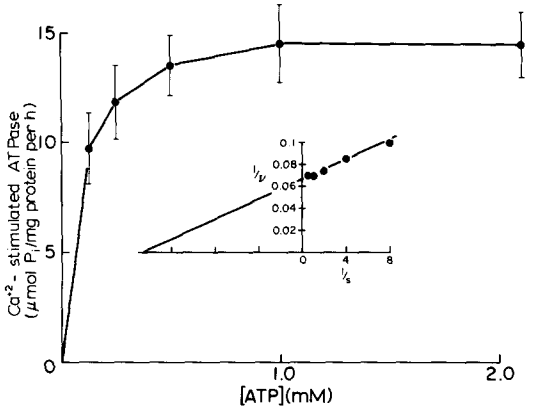

Fig. 5. Effects of different concentrations of ATP on the Ca^{2+}-stimulated ATPase activity associated with trypsinized gastric microsomes. The concentration of Ca^{2+} was maintained at 10^{-6} M using 1 mM EGTA in the assay medium. ATP does not have any significant effect on free Ca^{2+} under these conditions [37]. The inset shows the Lineweaver-Burk plot of the data. Details of the assay are given in Methods and Materials. Data are mean ± S.E. (n = 3).

somes. About 1 mM ATP appears to be optimal for the Ca^{2+}-stimulated activity. The Lineweaver-Burk plot of the data (Fig. 5, inset) demonstrates that the K_m for the enzyme is about 0.07 mM ATP.

Table II shows the efficacy of various nucleotide triphosphates to act as substrates for the Ca^{2+}-stimulated hydrolytic activity. ATP is the most and UTP the least preferred substrate; the order being ATP → ITP → GTP → CTP → UTP.

TABLE II

CALCIUM STIMULATION OF THE HYDROLYSIS OF DIFFERENT TRIPHOSPHONUCLEOTIDES BY THE TRYPSINIZED GASTRIC MICROSOMES

The microsomes were perincubated for 15 min at 37°C in Tris/Pipes buffer (pH 7.0) containing 1 mM EGTA, 1 mM Mg^{2+} and an appropriate amount of Ca^{2+} ($[Ca^{2+}_{free}] = 10^{-5}$ M). At the end of preincubation the reactions were started with 1 mM of different nucleotides and incubated further for 15 min. Details of assay are given in Methods and Materials. Data are mean ± S.E. (n = 8).

Nucleotide tested (1 mM)	Enzyme activity (μmol/mg protein per h)		
	without Ca^{2+}	with Ca^{2+}	ΔCa^{2+}
ATP	21.07 ± 0.44	30.88 ± 0.40	9.81
GTP	24.55 ± 1.20	27.35 ± 0.70	2.80
CTP	23.09 ± 0.19	25.22 ± 0.92	2.13
ITP	26.51 ± 0.14	30.01 ± 0.11	3.5
UTP	23.16 ± 0.48	22.80 ± 0.09	−0.36

TABLE III

EFFECTS OF VARIOUS INHIBITORS ON THE GASTRIC MICROSOMAL Ca^{2+}-STIMULATED ATPase ACTIVITY

The microsomes were preincubated for 15 min at 37°C in Tris/Pipes buffer (pH 7.0) containing 1 mM EGTA, 1 mM Mg^{2+}, an appropriate amount of Ca^{2+} ($[Ca^{2+}_{free}] = 10^{-5}$ M) and other test substances. At the end of preincubation the reactions were started by adding 1 mM ATP and incubated further for 15 min. Details of assay, are given in Methods and Materials. Data are mean ± S.E. (n = 4). NEM, N-ethylmaleimide.

Agent tested	ATPase activity (μmol/mg protein per h)		
	without Ca^{2+}	with Ca^{2+}	ΔCa^{2+}
None (control)	21.48 ± 0.41	30.97 ± 0.90	9.49
NaF (1 mM)	21.48 ± 1.04	23.48 ± 1.14	2.0
NEM (1 mM)	22.05 ± 0.38	24.82 ± 1.57	2.77
pCMBS (10 μM)	26.17 ± 1.44	27.41 ± 0.98	1.24
TNBS (1 mM)	20.67 ± 0.88	22.34 ± 0.70	1.67
FDNB (1 mM)	21.59 ± 1.40	21.65 ± 1.35	0.06
MDPF (20 μg)	23.55 ± 0.65	24.37 ± 0.53	0.71

Effects of various inhibitors on the gastric Ca^{2+}-stimulated ATPase activity are shown in Table III. Between the two sulfhydryl agents, pCMBS was nearly 100-times more potent than N-ethylmaleimide. NaF, the mechanism of action of which is unknown also inhibits about 80% at 1 mM concentration. Of the three amino (primary)-reactive agents, TNBS, FDNB and MDPF, MDPF is the most potent inhibitor. TNBS, which unlike FDNB is a membrane-impermeable reagent [27], is about 25-times less effective than FDNB at 1 mM concentration.

Discussion

The gastric microsomes used in the present study were derived primarily from the apical and tubulovesicular membranes of the acid-secreting cells and have previously been characterized as plasma membranes on the basis of enzyme data [25–28], lipid compositions including cholesterol-to-phospholipid molar ratio of 1.9 [31] and electron microscopy showing absence of mitochondrial and endoplasmic reticulum type membranes [32].

Trypsinization of gastric microsomes in presence of ATP results in the unmasking of a microsomal Ca^{2+}-stimulated ATPase activity (Fig. 1). The Ca^{2+}-stimulated ATPase activity, however, is not manifested when the microsomes are trypsinized in absence of ATP but otherwise under identical conditions [29,30]. Unmasking of the Ca^{2+}-stimulated ATPase is not due to an alteration of the microsomal K^+-stimulated ATPase by trypsin since the K^+-stimulated activity can be fully restored [29] by the addition of partially purified activator protein. Hence, these data (Fig. 1) suggest that there must be an endogenous inhibitor which keeps the microsomal Ca^{2+}-stimulated ATPase suppressed. The inhibitor can not be normally removed by mild trypsin treatment. However, when combined with ATP, the inhibitor becomes vulnerable to tryptic attack and hence results in unmasking of the Ca^{2+}-stimulated ATPase activity.

Like Ca^{2+}-stimulated ATPase of any other tissue [4–19], the enzyme is completely dependent on Mg^{2+} for its activity. The K_a for Mg^{2+} is about 0.4 mM (Fig. 3). The activity is strongly inhibited at 2 mM (Fig. 3) or higher (data not shown) concentrations of Mg^{2+}. It is possible that Mg^{2+} at higher concentrations may compete with Ca^{2+} for the Ca^{2+} site and thus exert its inhibitory effects. The inhibitory effects of other divalent cations such as Sr^{2+}, Ba^{2+}, etc. (Table I) may also be explained in that light. Alternatively, Sr^{2+}, Ba^{2+}, Mn^{2+} and Zn^{2+} may either compete with Mg^{2+} for their effects or may have combined effects on both Ca^{2+} and Mg^{2+} sites of the enzyme.

Of all the nucleotide triphosphates tested ATP is the most effective substrate (Table II); the enzyme has a K_m of about 70 μM ATP (Fig. 5), a value comparable to those of other Ca^{2+}-transport ATPases in the literature [9,13,18]. The $K_{0.5}$ for Ca^{2+} is about 10^{-8} M. Maximal activation occurs at Ca^{2+} concentrations of 10^{-6} M or higher. The demonstration of such high affinity Ca^{2+} sites for the gastric microsomal Ca^{2+}-stimulated ATPase makes the acid-secreting cells a candidate for calcium pumping mechanism, in analogy to red cell membrane calcium pump [40].

The pH optimum for the activation of Ca^{2+}-stimulated ATPase of gastric microsomes is sharp and lies within a very narrow range around pH 6.8; the activity being significantly reduced above pH 6.8. Such pH sensitivity of the Ca^{2+}-stimulated ATPase may have some important implications in terms of the role of Ca^{2+} in gastric acid secretion. Thus, after the onset of H^+ transport the intracellular pH of the acid secreting cells have been reported to show an alkaline shift [41]. Therefore, if a Ca^{2+} pump is somehow involved at the initiation stage of acid secretion, the pump activity could be greatly reduced during the steady-state condition of acid secretion due to the elevated intracellular pH.

Inhibition of the Ca^{2+}-stimulated ATPase (Table III) by the sulfhydryl agents (pCMBS and *N*-ethylmaleimide) suggest the involvement of some critical -SH groups in the enzyme function. This observation is consistent with the reports on the Ca^{2+}-stimulated ATPase of other systems [18,42]. Inhibitory effects of the primary amino group reactive agents such as TNBS, FDNB and MDPF (Table III) suggest participation of some free NH_2 groups in the gastric Ca^{2+}-stimulated ATPase function. It is noteworthy that some free NH_2 groups of the Ca^{2+}-stimulated ATPase have been demonstrated to be critical for its function in other systems as well [43].

How the Ca^{2+}-stimulated ATPase may be related to the gastric H^+ transport mechanism is a subject of much speculation. Ca^{2+} has been suggested to be a final common mediator for the effects of gastric secretagogues [23] in H^+ transport. Our data (Fig. 1) show that Ca^{2+} (1 mM) strongly inhibits the microsomal K^+-stimulated ATPase, which has recently been demonstrated to be the enzymatic pumping mechanism for the neutral antiport of H^+ and K^+ (K^+-H^+ exchange) in gastric microsomal vesicles [44–46] and probably in intact mucosa as well [47,48]. It is likely that the influence of Ca^{2+} on gastric acid secretion may lie in its direct effect on the gastric K^+-stimulated ATPase system. Recent reports [49] on the inhibitory effects of μM (10–20 μM) concentrations of Ca^{2+} on the endogenous activator-stimulated ATPase of gastric microsomes strongly suggests such a possibility. The apical membrane-located Ca^{2+} pump may therefore work by simply removing Ca^{2+} from the K^+-stimulated ATPase environment, thus ensuring optimal functioning of the K^+/H^+ exchange system. The second possible role of Ca^{2+} may be in the well-known phenomenon of membrane transformation, which is known to accompany the secretagogue-induced gastric acid secretion. It is believed [50] that

the intracellular tubulovesicular membranes fuse with the apical plasmalemma to provide the greater surface area needed to meet the secretory demand of the tissue. Ca^{2+} may be needed for the activation of any type of phospholipase A, located at the point of fusion of the secretory membranes. The high concentration of Ca^{2+} needed for the activation of the phospholipases is subsequently pumped out of the cell by the Ca^{2+}-stimulated ATPase after completion of the membrane fusion process.

Another question of fundamental importance that remains to be answered is why the gastric microsomal Ca^{2+}-stimulated ATPase remains in a latent form. Does the dependency on the proteolytic activation of the Ca^{2+}-stimulated ATPase in presence of ATP carry any special significance in terms of regulation of the gastric secretory process? The answer awaits future investigation in this area. However, proteolytic activation of the gastric microsomal Ca^{2+}-stimulated ATPase is not a unique phenomenon, since trypsin activation of a latent ATPase in *Mycobacterium phlei* has recently been reported [51]. Furthermore, unmasking of a latent Ca^{2+}-stimulated ATPase by trypsin has been reported in the membrane vesicle preparation from *Azotobacter vinelandii* [52] where the trypsinized membranes were demonstrated to extrude Ca^{2+}, mediated by the unmasked ATPase.

Acknowledgements

We gratefully acknowledge the skilful technical assistance of Miss Susan Huybensz and Miss Valerie Seabold. We thank Miss Susan Golden for typing the manuscript. This work was supported in part by a grant from U.S. Public Health Service, AM 25544 and Surgical Fund, Department of Surgery, Upstate Medical Center. T.K.R. is a recipient of Research Career Development Award, AM 00623.

References

1 Rasmussen, H. and Tenenhouse, A. (1968) Proc. Natl. Acad. Sci. USA 59, 1364–1369
2 Scarpa, A. and Carafoli, E. (1978) Editors, Calcium Transport and Cell Function, Ann. NY Acad. Sci. Vol. 307
3 Racker, E. (1980) Fed. Proc. 39, 2422–2426
4 Dunham, E.T. and Glym, I.M. (1961) J. Gen. Physiol. 156, 274–281
5 Schatzman, H.J. (1966) Experientia 22, 364–365
6 Schatzmann, H.J. and Vincenzi, F.F. (1969) J. Physiol. (London) 201, 369–395
7 Lindsay, R. (1976) Q. J. Exp. Physiol. 61, 95–104
8 Moore, L., Chen. T., Knapp, H.R. and Landon, E.J. (1975) J. Biol. Chem. 250, 4562–4568
9 Robinson, J.D. (1976) Arch. Biochem. Biophys. 176, 366–374
10 Roufogalis. B.D. (1973) Biochim. Biophys. Acta 318, 360–370
11 Borle, A.B. (1969) J. Gen. Physiol. 63, 57–69
12 Moore, L., Fitzpatrick, D.F., Chen. T.S. and Landon, E.J. (1974) Biochim. Biophys. Acta 345, 405–418
13 Akerman, K.E. and Wikstrom, M.F. (1979) FEBS Lett. 97, 283–287
14 Janis, R.A., Crankshaw, D.J. and Daniel, E.E. (1977) Am. J. Physiol. 232, C50–C58
15 Robblee, L.S., Shepro, D. and Belamarich, F.A. (1973) J. Gen. Physiol. 61, 462–481
16 Formby, B., Capito, K., Egeberg, J. and Hedeskov, C.J. (1976) Am. J. Physiol. 230, 441–448
17 Bruns, D.E., McDonald, J.M. and Jarett, L. (1976) J. Biol. Chem. 251, 7191–7197
18 Black, B.L., Jarett, L. and McDonald, J.M. (1980) Biochim. Biophys. Acta 596, 359–371
19 Ghijsen, W.E.J.M. and Van Os, C.H. (1979) Nature 279, 802–803
20 Barreras, R.F. (1973) Gastroenterology 64, 1168–1184
21 Sedar, A.W. and Forte, J.G. (1964) J. Cell Biol. 22, 173–188
22 Jacobson, A., Schwartz, M. and Rehm, W.S. (1965) Am. J. Physiol. 209, 134–140
23 Kasbekar, D.K. (1974) Proc. Soc. Exptl. Biol. Med. 145, 234–239
24 Kasbekar, D.K. and Chugani, H. (1976) in Gastric Secretion (Kasbekar, D.K., Sachs, G. and Heinz, E., eds.), 187–211, Marcel Dekker, New York
25 Ray, T.K. and Forte, J.G. (1974) Biochim. Biophys. Acta 363, 320–339
26 Forte, J.G., Ganser, A.L. and Ray, T.K. (1976) in Gastric Hydrogen Ion Secretion (Kasbekar, D.K., Sachs, G. and Heinz, E., eds.), pp. 302–330, Marcel Dekker, New York
27 Ray, T.K., Meng-Ai, Z. and Sen, P.C. (1980) in Hydrogen Ion Transport in Epithelia (Schulz, I., ed.), pp. 203–215, Elsevier/North-Holland Biomedical Press, Amsterdam
28 Ganser, A.L. and Forte, J.G. (1973) Biochim. Biophys. Acta 307, 169–173
29 Ray, T.K. and Sen, P.C. (1981) Life Sci. 28, 1969–1974
30 Ray, T.K. and Sen, P.C. (1980) in Proceedings of the International Symposium on Calcium-binding Proteins and Calcium Function in Health and Disease, pp. 93–96, Elsevier Publishing Co., New York
31 Sen. P.C. and Ray, T.K. (1980) Arch. Biochem. Biophys. 202, 8–17
32 Forte, J.G., Ray, T.K. and Poulter, J.L. (1972) J. Appl. Physiol. 32, 714–717
33 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275

34 Sanui, H. (1974) Anal. Biochem. 60, 489–504
35 Martell, A.E. (1971) in Stability Constants of Metal Ion Complexes, Part II, Special Publication No. 25, p. 651, The Chemical Society, Burlington House, London, U.K.
36 Reddy, Y.S. and Wynborny, L.E. (1976) Biochem. Biophys. Res. Commun. 73, 703–709
37 Scharff, O. (1979) Anal. Chimica Acta 109, 291–305
38 Sen, P.C. and Ray, T.K. (1979) Arch. Biochem. Biophys. 198, 548–555
39 Ray, T.K. and Fromm, D. (1981) submitted
40 Schatzman, H.J. (1973) J. Physiol. Lond. 235, 551–569
41 Hersey, S.J. (1978) Acta Physiol. Scand. Special Suppl. 243–252
42 Yamada, S. and Ikemoto, N. (1978) J. Biol. Chem. 253, 6801–6807
43 Murphy, A.J. (1976) Biochem. Biophys. Res. Commun. 70, 1048–1054
44 Sachs, G., Spenney, J.G. and Lewin, M. (1978) Physiol. Rev. 58, 106–173
45 Forte. J.G., Machen, T.E. and Obrink, K.J. (1980) Annu. Rev. Physiol. 42, 111–126
46 Sen, P.C., Meng-Ai, Z. and Ray, T.K. (1980) Arch. Biochem. Biophys. 205, 340–351
47 Ray, T.K. and Tague, L.L. (1980) Biochem. Pharmacology 29, in the press
48 Ray, T.K. and Tague, L.L. 1978) Acta Physiol. Scand. Special Suppl., 283–292
49 Ray, T.K. (1978) FEBS Lett. 92, 49–52
50 Forte, J.G., Forte, T.M. and Ray, T.K. (1972) in Gastric Secretion (Sachs, G., Heinz, E. and Ullrich, K.J., eds.), pp. 37–68, Academic Press, New York
51 Karla, V.K., Lee, S.H., Ritz, C.J. and Brodie, A.F. (1975) J. Supramol. Struct. 3, 231–241
52 Bhattacharya, P. and Barnes, E.M., Jr. (1976) J. Biol. Chem. 251, 5614–5619