GASTRIC K^+-STIMULATED ADENOSINE TRIPHOSPHATASE. DEMONSTRATION OF AN ENDOGENOUS ACTIVATOR

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1. Introduction

A K^+-stimulated, ouabain insensitive ATPase activity associated with the apical and tubulovesicular membranes of the acid secreting cells of fundic mucosa from a number of different species has recently been reported [1,2] and has been strongly implicated to be involved in H^+ transport [3–5]. During isolation of purified microsomal membrane vesicles enriched in K^+-stimulated ATPase from the fundus of various species, we observed that the degree of stimulation of the enzyme by K^+ in the presence of gramicidin varied considerably from one preparation to another. The ionophore, gramicidin, has been shown to be very effective for maximal activation of the microsomal K^+-stimulated ATPase [2]. The variability in enzymatic activity observed in presence of gramicidin indicated that there could be some dissociable regulatory factor(s) bound to the enzyme under in vivo conditions. The degree of dissociation of the factor(s) during isolation of the purified membranes might account for the variable activity of the enzyme.

In this report, we present evidence that there is a heat-labile, non-dialysable and protease-sensitive activator in the soluble supernatant fraction from rabbit fundic mucosal cells, which markedly activates the K^+-stimulated ATPase and *p*NPPase activities of rabbit, frog, and hog gastric microsomes. Furthermore, Ca^{2+} at low concentrations has been shown to obliterate the activating effects of the activator(s).

Abbreviations: PIPES, Piperazine-*N'*-bis (2-ethanesulfonic acid); *p*NPPase, *p*-nitrophenyl phosphatase; Tris, Tris (hydroxymethyl) amino-methane

The data suggest a critical interplay among the enzyme, factor(s) and Ca^{2+} in the regulation of the gastric K^+-stimulated ATPase.

2. Materials and methods

The oxyntic cell enriched fractions from fundic mucosa of frog, rabbit and hog were harvested by the NaCl desquamation procedure [6]. The mucosal cells were homogenized in 0.25 M sucrose containing 0.2 mM EDTA and 0.2 mM PIPES (pH 6.8) using a loose pestle Dounce homogenizer. The homogenate was centrifuged at 8000 × *g* for 5 min. The process was repeated 3 times. All the supernatants were pooled together and layered over 40 ml of 37% sucrose in the 84-ml capacity screw cap tubes and centrifuged at 100 000 × *g* for 5 h in type 35 Beckman angular rotor. The membrane bands appeared at the interface of soluble supernatant and 37% sucrose. The soluble supernatants were collected, dialysed exhaustively for 4 days against 50 vol. of 0.2 mM PIPES, 0.2 mM EDTA (pH 6.8) buffer with 4 changes. The dialysed supernatants were assayed for ATPase activator(s). The membrane bands were collected from the interface, diluted with homogenizing medium and centrifuged at 100 000 × *g* for 90 min. The pellet was suspended in the homogenizing medium with a protein concentration of 0.5 mg/ml. We call these membranes as purified microsomes. Proteins were assayed by the Lowry procedure [7].

The ATPase and *p*NPPase were assayed as previously described [8]. All assays were conducted at 37°C. For *p*NPPase the incubation mixture contained in a

total volume of 1 ml 50 μmol Tris–HCl (pH 7.4), 2 μmol $MgCl_2$, 5 μmol *p*NPP, 10 μg membrane protein, with and without 20 μmol KCl, various amounts of rabbit dialysed supernatant (activator) and other test substances. After 20 min the reactions were stopped by 1 ml 1.5 N NaOH. After a brief centrifugation, the supernatant was read at 410 nm. The incubation mixture for ATPase reaction contained in a total volume of 1 ml, 50 μmol of Tris–HCl (pH 7.4), 1 μmol $MgCl_2$, 2 μmol Tris–ATP, 5 μg membrane protein and various amounts of dialysed supernatant (activator) in presence and absence of 2 μmol of KCl. After 15 min the reactions were stopped by 1 ml 12% CCl_3COOH. The P_i was assayed by the procedure of Sanui [9].

3. Results and discussion

Table 1 shows the activating effects of dialysed supernatant fraction from rabbit fundic mucosa on the K^+-stimulated ATPase and *p*NPPase activities associated with rabbit gastric microsomes and effects of various agents on the activator(s) and enzyme activities. Both enzymes are significantly stimulated by the dialysed supernatant and the effects are completely abolished by NaF, the well known inhibitor of the K^+–ATPase system [1]. The lack of any effects of RNAse or DNAse on the dialysed supernatant demonstrates that the active principle is not a nucleic acid. The activating effect of the supernatant is lost by heating and pronase or trypsin digestion demonstrating that the factor is protein in nature.

Figure 1 shows that both K^+-stimulated ATPase and *p*NPPase are activated in a linear fashion up to 144 μg of activator(s) protein suggesting some kind of stoichiometric interaction between the enzyme and the activator(s). The K^+-stimulated *p*NPPase activity has previously been suggested [10] to be a partial reaction of the K^+-stimulated ATPase. This suggestion is further strengthened by our data demonstrating that both enzymes are stimulated by the activator(s) and the stimulations are completely inhibited by agents like NaF (table 1).

Table 2 shows that the activator(s) from rabbit activates the K^+-stimulated ATPase and *p*NPPase from frog and hog. In separate studies we have demonstrated that the activator(s) from hog can stimulate

Table 1
Activation of K^+-stimulated ATPase and *p*NPPase by dialysed supernatant of rabbit fundic cells and partial characterization of the 'Activator Factor'

System	K^+-stimulated	
	ATPase	*p*NPPase
Membrane alone	8.6	12.9
Dialysed supt. alone	0	0 2
Membrane + dialysed supt. (85 μg)	30.6	21 8
Membrane + dialysed supt. + NaF (2 mM)	0	0
Membrane + bovine serum albumin (80 μg)	9 3	12.5
Membrane + heated (85°C, 10 min) supt.	8.6	13.1
Membrane + pronase digested supt.	10.1	12.7
Membrane + pronase digested supt. + dialysed supt. (85 μg)	28.9	21.0
Membrane + trypsin digested supt.	9.0	12.1
Membrane + DNAse treated supt.	29.1	22.5
Membrane + RNAse treated supt.	28.8	21 6

Each value represents average of duplicate determinations. The enzyme activities have been expressed as μmol/mg protein·h. The K^+-stimulated activities have been calculated from the K^+-stimulated rate minus the basal rate (Mg^{2+} alone) 2 and 20 mM K^+ were used for ATPase and *p*NPPase respectively; 10^{-5} M gramacidin was included in ATPase assay. Pronase, trypsin, DNAse and RNAse were used at concentrations of 5 μg/ml for digestion of dialysed supernatant 3–4 h at 37°C. Details are given in section 2

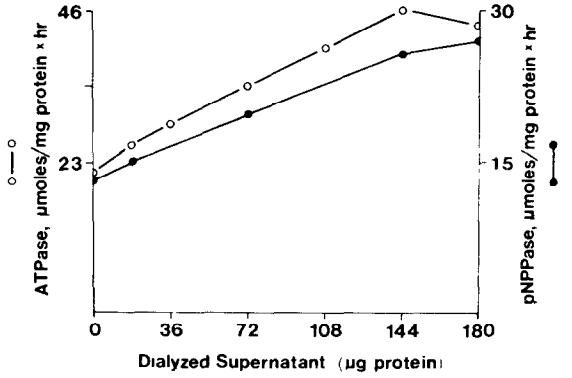

Fig.1. Effects of different amounts of dialysed supernatant from rabbit fundic cells on the rabbit gastric microsomal K^+-stimulated ATPase (○–○) and K^+-stimulated *p*NPPase (●–●) activity. For details of assay see table 1 and section 2.

Table 2
Stimulation of frog and pig gastric microsomal ATPase and *p*NPPase by rabbit 'Activator and Factor'

Additions	ATPase (mol/mg protein·h)		*p*NPPase (mol/mg protein·h)	
	without K^+	with K^+	without K^+	with K^+
Frog microsomes (gradient purified)	2.8	14.8	2.9	14.1
Frog microsomes + dialysed supt (rabbit), 136 μg protein	3 4	21.7	3.5	26.6
Pig microsomes (gradient purified)	10 8	21.3	0.8	13.4
Pig microsomes + dialysed supt. (rabbit), 136 μg protein	11.9	54.6	1.9	38.5

Details of assay are given in table 1

the enzymes from frog and rabbit (unpublished data). It has been demonstrated [10] that the catalytic subunit of the ATPase from all three species mentioned above has a molecular weight of about 100 000. Therefore, it is likely that the molecular characteristics of the activator(s) from different species may be very similar or the same and hence may cross react in ATPase activation. Thus, our data suggest that there are some soluble intracellular regulatory protein(s) in the oxyntic cells from a variety of species capable of controlling the K^+-stimulated ATPase under physiological conditions. Furthermore, the nature of the activator(s) and their mode of control on the ATPase system appears very similar since the factor from one species can stimulate the ATPase system of another species and vice versa.

Calcium in μM levels shows different effects on the K^+ and (K^+ + activator)-stimulated ATPase reaction (fig.2). Ca^{2+} shows a small but steady stimulation up to 20 μM – the highest stimulation being 25%. However, the (K^+ + activator)-stimulated activity is strongly inhibited by Ca^{2+} (10–20 μM) and the activation was completely abolished at 20 μM Ca^{2+}. These data suggest a critical interplay between the activator(s) and Ca^{2+} in the regulation of the K^+-stimulated ATPase. Purification of the factor(s) is in progress.

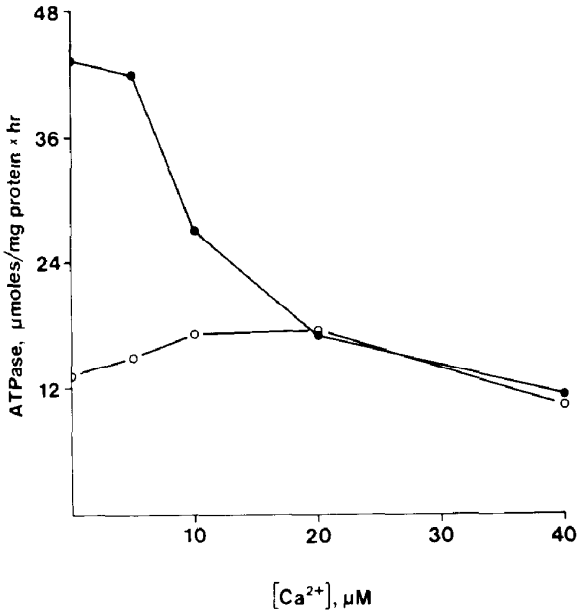

Fig.2. Effects of dialysed supernatant (rabbit) and different concentrations of Ca^{2+} on the pig gastric microsomal K^+-stimulated ATPase activity. K^+, (○–○) K^+ plus 144 μg dialysed supernatant protein, (●–●). Details of assay are given in table 1.

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References

[1] Forte, J. G., Ganser, A. L. and Ray, T. K. (1976) in: Gastric Hydrogen ion Secretion (Kasbekar, D., Sachs, G. and Rehm, W. S. eds) pp. 302–330, Marcel Dekker, New York.
[2] Forte, J. G., Ganser, A. L. and Tanisawa, A. S. (1974) Ann. NY Acad. Sci. 242, 255–267.
[3] Forte, J. G. and Lee, H. C. (1977) Gastroenterology 73, 921–926.
[4] Sachs, G., Chang, H., Rabon, E., Schackman, R., Sarau, H. M. and Saccamani, G. (1977) Gastroenterology 73, 931–940.
[5] Ray, T. K. and Tague, L. L. (1978) in: Proc. of the Intern. Symp. on Gastric Ion Transport; Supplement to Acta Physiol. Scand. in press.
[6] Forte, J. G., Ray, T. K. and Poulter, J. L. (1972) J. Appl. Physiol. 32, 714–717.
[7] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[8] Ray, T. K. and Forte, J. G. (1974) Biochim. Biophys. Acta 363, 320–339.
[9] Sanui, H. (1974) Anal. Biochem. 60, 489–504.
[10] Sachs, G., Spenney, J. G. and Rehm, W. S. (1977) in: International Reviews of Physiology (Crane, R. K. ed) vol. 12, pp. 127, University Park Press, Baltimore.