Modulation of gastric H^+, K^+-transporting ATPase function by sodium

Tushar K. Ray and Joytirmoy Nandi

Department of Surgery, The State University of New York, Upstate Medical Center, 750 East Adams Street, Syracuse, NY 13210, USA

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Gastric H^+, K^+-ATPase activity is not affected by Na^+ at pH 7.0 but is significantly stimulated by Na^+ at pH 8.5. For the stimulation at the latter pH, the presence of both Na^+ and K^+ were essential. Contrary the H^+, K^+-ATPase, the associated K^+-pNPPase was inhibited by Na^+ at both pH values. Sodium competes with K^+ for the K^+-pNPPase reaction. Also, unlike the H^+, K^+-ATPase activity the ATPase-mediated transport of H^+ within the gastric microsomal vesicles was inhibited by Na^+. For the latter event only the extravesicular and not the intravesicular Na^+ was effective. The data suggest that the K^+-pNPPase activity does not represent the phosphatase step of the H^+, K^+-ATPase reaction. In addition, the observed inhibition of vesicular H^+ uptake by Na^+ appears to be due to the displacement by Na^+ of a cytosolic (extravesicular) H^+ site responsible for the vectorial translocation of H^+.

H^+, K^+-ATPase *K^+-pNPPase* *Na^+, K^+-ATPase* *Na^+/H^+ antagonism* *Na^+/K^+ antagonism* *Vesicle*

1. INTRODUCTION

Inorganic cations are known to play important roles in biological processes. In fact, proper maintenance of ionic milieu is essential for survival and function of all cells. Hence the living cells have various hemeostatic mechanisms such as ion pumps, exchange diffusion, simple diffusion, etc. for maintaining proper ionic environment.

Among all the monovalent inorganic cations, Na^+, K^+ and H^+ are the most abundant in the biological system. Two of the well defined ion-pumping systems are Na^+,K^+-ATPase and H^+,K^+-ATPase, which have been demonstrated to share many common features [1].

The present study deals with the effects of Na^+ on the activities of the gastric microsomal H^+,K^+-ATPase and associated K^+-pNPPase as well as H^+ transport mediated by the gastric ATPase system. The data demonstrate that Na^+ does not affect the H^+,K^+-ATPase activity at neutral pH but significantly stimulates the enzyme at an alkaline pH. Sodium, however, inhibits the K^+-pNPPase reaction at both pH values by competing with K^+. Also, unlike the H^+,K^+-ATPase activity, the intravesicular transport of H^+ mediated by the gastric ATPase is inhibited by extravesicular Na^+ at neutral pH. The data are consistent with our recent report [2] suggesting that the K^+-pNPPase is not a partial reaction of the H^+,K^+-ATPase. The data also suggest a cytosolic site for binding of H^+ which appears critical for the H^+,K^+-ATPase-mediated vectorial translocation of H^+. Na^+ appears to be able to replace, at least in part, the H^+ from its specific site and thus inhibits H^+ transport. The data have been discussed in terms of the Na^+ and H^+ regulation of the monovalent cation-transporting ATPase systems.

2. MATERIALS AND METHODS

2.1. *Isolation of gastric microsomes*

Fresh pig stomachs were purchased from the local slaughterhouse. The gastric microsomal membranes, highly enriched in K^+-stimulated

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ATPase and pNPPase activities, were harvested as described [3,4]. No ouabain-sensitive Na^+,K^+-ATPase activity could be detected in these membranes. The microsomes are derived primarily from the apical and tubulovesicular membranes of the parietal cells [1]. Isolated microsomes appear in the form of tightly sealed vesicles with the ATP hydrolytic site facing the vesicle exterior and a high-affinity K^+ site responsible for the K^+-stimulated hydrolysis of ATP facing the vesicle interior [4,6,7].

2.2. *Assay of ATPase and* p-*nitrophenyl phosphatase*

The ATPase was assayed as previously described [4]. Briefly, the incubation mixture contained, in a total volume of 1 ml, 50 μmol Pipes (1,4-piperazinediethanesulphonic acid) buffer (pH 6.8), 1 μmol $MgCl_2$, 2 μmol Tris/ATP and 20 μg membrane protein, with or without 100 μmol KCl in the presence and absence of 5 μM valinomycin. After 10 min incubation at 21°C the reactions were stopped by 1 ml of 12% trichloroacetic acid. P_i was assayed by the procedure of Sanui [5].

For *p*-nitrophenyl phosphatase the incubation mixture contained, in a total volume of 1 ml, 50 μmol Pipes buffer (pH 6.8), 2 μmol $MgCl_2$, 5 μmol *p*-nitrophenyl phosphate and 10 μg membrane protein, with and without 20 μmol KCl. After 20 min incubation at room temperature the reactions were stopped by 1 ml of 1.5 M NaOH. After a brief centrifugation, the absorbance of the supernatant was read at 410 nm.

2.3. *Study of vesicular* H^+ *uptake*

Vesicular accumulation of H^+ was measured at room temperature by the method of Lee and Forte [6] as in [4,7,8]. The amount of acridine orange taken up is a sensitive measure of intravesicular H^+ concentration. Wavelengths used were 493⟶530 nm (excitation⟶emission) in an Aminco Bowman spectrofluorimeter.

2.4. *Materials*

Pipes, Tris, ATP, *p*-nitrophenyl phosphate and valinomycin were purchased from Sigma. Acridine orange was purchased from Aldrich. All other chemicals used were the best grades available on the market.

3. RESULTS AND DISCUSSION

Sodium shows a differential response in H^+,K^+-ATPase activity depending on the pH of the reaction medium. Thus Na^+ does not have any appreciable effect on the enzyme activity at pH 7.0 (fig.1). However, at pH 8.5, when sensitivity of the H^+,K^+-ATPase to K^+ is totally lost, the presence of both Na^+ and K^+ significantly stimulated the enzyme (fig.2). It is unlikely that the affinity of the enzyme to K^+ has been changed to Na^+ at pH 8.5 since Na^+ alone cannot stimulate the H^+,K^+-ATPase activity (fig.2). It is highly likely, however, that the affinity of the enzyme to H^+ has been changed to Na^+ at elevated pH. Since both Na^+ and K^+ become obligatory cations for subsequent hydrolysis of ATP at pH 8.5, the enzyme behaves essentially like a Na^+,K^+-stimulated ATPase at such pH. It is noteworthy that the Na^+,K^+-ATPase of kidney has been reported by Skou and Esmann [9] to be stimulated by K^+ alone at low pH (6.0), thus behaving essentially like a H^+,K^+-ATPase. Hence, it appears that two distinct monovalent cation-transporting ATPases having well-defined physiological functions can

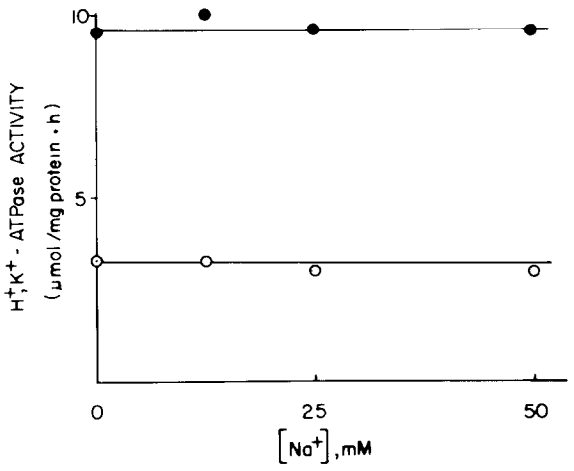

Fig.1. Effects of increasing concentrations of Na^+ on gastric microsomal H^+,K^+-ATPase activity at neutral pH. The assay was conducted in Pipes buffer (pH 6.8) at 21°C for 10 min using 25 μg membrane protein in the presence and absence of 100 mM KCl with and without valinomycin (details of the assay are given in section 2). The data represent the K^+-stimulated activity minus the basal (with Mg^{2+} as the only cation) rate. Without (○—○) and with (●—●) valinomycin.

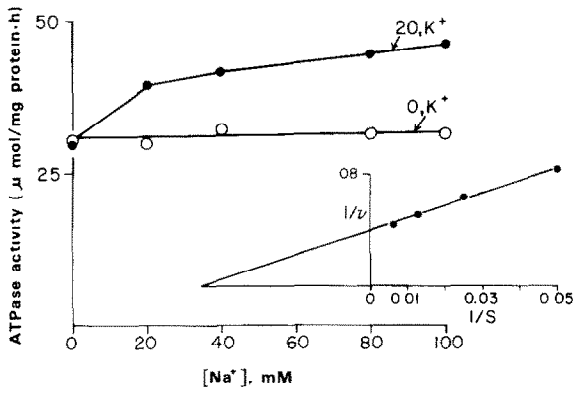

Fig.2. Effects of increasing concentration of Na^+ on gastric microsomal H^+,K^+-ATPase activity at alkaline pH. The assay was conducted in 50 mM Tris-Pipes buffer (pH 8.5) at 37°C using 10 μg membrane protein in the presence and absence of 20 mM KCl. Not shown here is that under the conditions of alkaline pH of our hypotonic assay medium, as above, the membranes were completely leaky to K^+, hence unresponsive to valinomycin. At pH 8.5, the membranes become leaky even in an isotonic medium as in fig.1. Without (○—○) and with (●—●) K^+. Inset shows the Lineweaver-Burk plot of the $(Na^+ + K^+)$ values. The K_a value for Na^+ is about 22 mM.

change their affinities to either H^+ or Na^+ depending on the surrounding pH. The observation might suggest that the two ATPases have similar basic structural organization with respect to the orientation of the catalytic site, the transcytosolic K^+ ligand site and some cytosolic Na^+ or H^+ site. The latter site shows pH-dependent specificity to either Na^+ or H^+, suggesting the involvement of an ionizable group, presumably an imidazolium group with a pK_a around 7 [10] at such ligand-binding sites. Recently, the involvement of a histidine imidazole group at or near the catalytic site of the gastric H^+,K^+-ATPase has been strongly suggested from studies with diethylpyrocarbonate [11], a site-specific probe [12]. Under physiological conditions a strict specificity to either of the cations (depending on the ATPase) could be maintained by an influence from the surrounding electrostatic environment provided by the neighboring phospholipids, glycoproteins and other proximal charges of the same peptide molecule.

Unlike the H^+,K^+-ATPase, the associated K^+-pNPPase is inhibited by Na^+ in a dose-dependent manner. Thus, Na^+ competes for K^+ in the K^+-pNPPase reaction (fig.3). Furthermore, such Na^+/K^+ antagonism for K^+-pNPPase is observed over the whole range (6.5–8.5) of pH studied (not shown). Since our previous data (see figs 1 and 2 and discussion above) demonstrated that the K^+ site is independent of the H^+ (at pH 7.0) or Na^+ (at pH 8.5) site, it is clear that the K^+ sites regulating the H^+,K^+-ATPase and associated K^+-pNPPase are distinctly different. These observations are consistent with our recent observation [3] with spermine which competes with K^+ for the K^+-pNPPase without inhibiting the H^+,K^+-ATPase reaction [13]. These and other [2] observations clearly suggest that the K^+-stimulated pNPPase activity does not represent a partial step of the H^+,K^+-ATPase reaction. It is interesting that the Na^+ effects on the H^+,K^+-ATPase and K^+-pNPPase activities are nearly the same as those reported for the effects of this cation on Na^+,K^+-ATPase and associated K^+-pNPPase activities [14]. This observation again points out to the similarities underlying the operation of the H^+,K^+-ATPase and Na^+,K^+-ATPase as mentioned in this paper (see above) and elsewhere [1].

The intravesicular transport of H^+ mediated by the gastric microsomal H^+,K^+-ATPase system is

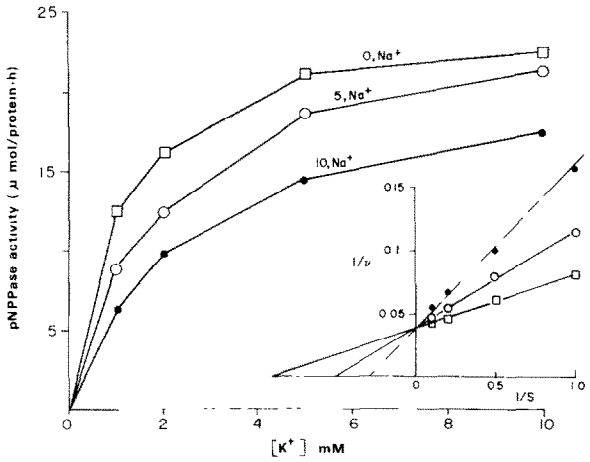

Fig.3. Competition of Na^+ with K^+ for the K^+-stimulated pNPPase reaction at neutral pH. Similar competition was also observed at pH 7.5 and 8.5 (not shown). Inset shows the Lineweaver-Burk plot of the data. The K_a value for K^+ is increased from 1 to 2 and 3.5 mM in the presence of 5 and 10 mM Na^+, respectively. Details of the assay are given in section 2.

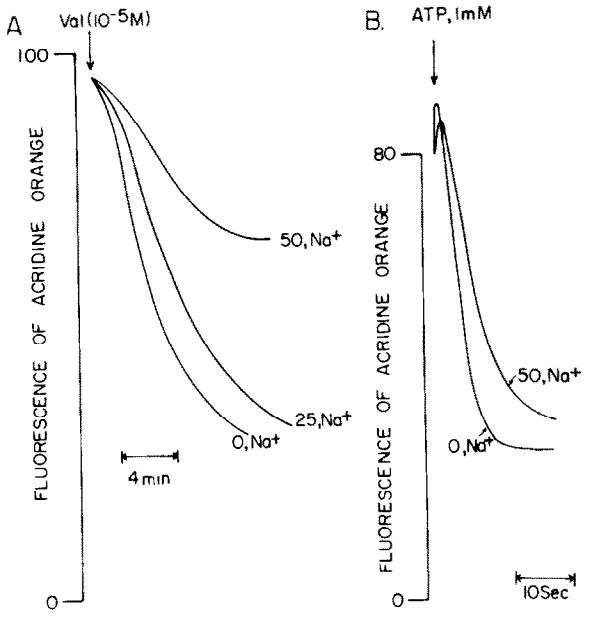

Fig.4. Effects of Na^+ on the H^+,K^+-ATPase mediated uptake of H^+ inside gastric microsomal vesicles. For A, the incubation medium (total volume 2 ml) consisted of 10 mM Pipes buffer (pH 7.0), 100 μg membrane protein, 1 mM $MgCl_2$, 1 mM ATP, 50 mM Tris-Cl (pH 7.0), 100 mM KCl, designated amount of NaCl and 0.01 mM acridine orange. The H^+,K^+-ATPase reaction was started by the addition of 10^{-5} M valinomycin at room temperature and the concomitant uptake of acridine orange was measured as described in section 2. For B, the vesicles were pre-loaded with K^+ at room temperature for 60 min in absence of ATP in the same medium as in A. The reactions were initiated with the addition of 1 mM ATP and the fluorescence of acridine orange was recorded.

strongly inhibited by extravesicular Na^+ both in the valinomycin-K^+ system and the K^+ pre-loaded vesicles (fig.4a,b). Such inhibition was not observed when Na^+ was present within the vesicles (not shown). This observation is in contrast to spermine which inhibits H^+ transport only from the vesicle interior [13]. Thus, the sites of action of Na^+ and spermine are clearly different; the former acts from the cytosolic side while the latter acts from the lumen side of the transbilayer H^+,K^+-transporting ATPase system. This observation of the inhibition of vesicular H^+ transport by extravesicular Na^+ would be consistent with the reported [15] inhibition of acid secretion by cytosolic Na^+ in isolated gastric glands. Na^+ (up to 50 mM), when present either inside or outside the vesicles, did not show any effect on the dissipation of the artificial ΔpH imposed across the gastric microsomal vesicles (not shown) suggesting the absence of any Na^+/H^+ exchange across the vesicles. Based on these and other observations discussed earlier it has been concluded that high concentrations of Na^+ at pH 7.0 can partially displace H^+ from a specific H^+ site located at the cytosolic part of the H^+,K^+-ATPase molecule and thus interfere with the vectorial translocation of H^+. The said H^+ site appears to be critical for ion transport or ion channel activity and is probably different than the one specified earlier for the pH-dependent cationic regulation since high concentration of Na^+ does not inhibit H^+,K^+-ATPase activity (fig.1) under conditions identical to those for H^+ uptake measurement (fig.4a). Hence, the catalytic activity and ion channel activity are likely to be functionally independent even though they are present within the same enzyme structure. A recent report [16] demonstrating that the voltage-stimulated uptake of Rb^+ through the erythrocyte Na^+,K^+-ATPase complex could be maintained under conditions of complete vanadate inhibition of the ion transport ATPase would be consistent with such a conclusion.

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