COMMENTS

The parietal cell gastric H, K-ATPase also functions as the Na, K-ATPase and Ca-ATPase in altered states [version 2; peer review: 2 approved, 1 not approved]

Previously titled: The gastric H, K-ATPase system also functions as the Na, K-ATPase and Ca-ATPase in altered states

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Abstract

This article offers an explanation for the apparent lack of Na, K-ATPase activity in parietal cells although ouabain has been known to inhibit gastric acid secretion since 1962. The gastric H, K-ATPase (proton-pump) seems to be acting in altered states, thus behaving like a Na, K-ATPase (Na-pump) and/or Ca-ATPase (Ca-pump) depending on cellular needs. This conclusion is based on the following findings. First, parietal cell fractions do not exhibit Na, K-ATPase activity at pH 7.0 but do at pH 8.5. Second, the apical plasma membrane (APM) fraction exhibits a (Ca or Mg)-ATPase activity with negligible H, K-ATPase activity. However, when assayed with Mg alone in presence of the 80 k Da cytosolic proton-pump activator (HAF), the APM fraction reveals remarkably high H, K-ATPase activity, suggesting the observed low affinity of Ca (or Mg)-ATPase is an altered state of the latter. Third, calcium (between 1 and 4 µM) shows both stimulation and inhibition of the HAF-stimulated H, K-ATPase depending on its concentration, revealing a close interaction between the proton-pump activator and local Ca concentration in gastric H, K-ATPase function. Such interactions suggest that Ca is acting as a terminal member of the intracellular signaling system for the HAF-regulated proton-pump. It appears that during resting state, the HAF-associated H, K-ATPase remains inhibited by Ca (>1 µM) and, prior to resumption of acid secretion the gastric H, K-ATPase acts temporarily as a Ca-pump for removing excess Ca from its immediate environment. This conclusion is consistent with the recent reports of immunochemical co-localization of the gastric H, K-ATPase and Ca-ATPase by superimposition in parietal cells, and a transitory efflux of Ca immediately preceding the onset of acid secretion. These new perspectives on proton-pump function would open new avenues for a fuller understanding of the intracellular regulation of the ubiquitous Na-pump.
Introduction
At the peak of acid secretion gastric juice has a pH close to 0.1 compared to blood (pH, 7.4). Based on this the parietal cells transport protons against a concentration gradient of over a million fold mediated by the gastric H, K-ATPase system. This member of the P-2 ATPase family has been the most extensively studied in the field occurred following the single topology scheme for the Na, K-ATPase and Ca-ATPase families due to their distinctive Vitamin B12 binding ability unique to the parietal cell plasma membrane. 5´-nucleotidase activity (a plasma membrane marker) and phospholipase A2 present in the APM but also plays an essential role in gastric acid secretion in histamine-stimulated rabbit gastric glands demonstrating the essentiality of the HAF in gastric secretory process. Studies with phospholipase A2 and mild ethanol treatment revealed that the HAF dimer is rather loosely associated with the membrane-bound H, K-ATPase system, and the phospholipid is in some way involved in this process.

Such a loose association of the HAF with the secretory membrane of the parietal cell became clear when we studied the effects of HAF on the isolated apical (APM) and tubulovesicular (TV) membranes from rabbit gastric glands and observed characteristic differential effects. The APM showed very high basal (Ca or Mg-ATPase) activity with a negligible K-stimulated component (H, K-ATPase activity). These studies revealed that the HAF is not only loosely associated with the membrane-bound H, K-ATPase system, and the phospholipid is in some way involved in this process.
Such differential association between HAF and the APM and TV membranes was also reflected in their lipid profiles which were qualitatively similar but quantitatively very different. Thus, the phosphatidylinositol and phosphatidyl ethanolamine content of APM were 24 and 8 µ moles/mg protein, respectively, about twice as much as that of TV. It may be noted that the APM and TV have different buoyant densities of 1.06 and 1.115 respectively, with a nearly equal phospholipid to cholesterol molar ratio of 0.64. The identity of APM was based on exclusive 5’-nucleotidase activity, unique vitamin B12 binding ability and characteristic quantitative differences in phospholipid make up from that of TV.

Calcium (µM) regulation of the HAF dependent H, K-ATPase activity

During the activation of the H, K-ATPase system, the activator molecules demonstrate strong positive cooperativity (Hill coefficient = 4.5) followed by a rapid decline to zero suggesting the binding of the HAF with the H, K-ATPase oligomer occurs over a small activator concentration range. In other words, the bound HAF interacts in some way with the empty sites on the cytosolic domain of the H, K-ATPase to increase their affinity for the activator molecules. Similar to the sigmoidal

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**Figure 1.** Critical interplay of calcium in the HAF-mediated (displayed as “AF”) regulation of the gastric H, K-ATPase pump showing oscillation between its H- and Ca-transporting modes depending on the local Ca level. In a similar fashion, the H, K-ATPase will also act as a Na-pump (not shown in the diagram) at the basolateral membrane depending on the local Na-concentration and pH. Following our current evidence, the critical interplay among the HAF, H, K-ATPase and Ca in parietal cells is depicted in this diagram. While the pump molecules integral to the tubulovesicle (TV) are stimulated appreciably by the HAF, those associated with the apical plasma membrane (APM) are absolutely dependent on the HAF for their function, revealing the essential nature of the HAF in gastric proton-pump function. For the ATPase assay the desired amount of HAF (as indicated by the prior dose response study) was first pre-incubated with 5 µg of APM for 10 minutes at 37°C in 2 mM Pipes buffer (pH 7.4). The concentration of free Ca was regulated by varying Ca at a fixed concentration of 0.5 mM EGTA.
activation and dramatic inhibition of the H, K-ATPase with increasing HAF, varying calcium concentrations (µM) also showed dual effects on the HAF-stimulated component of the H, K-ATPase. Low concentrations of calcium showed a small but consistent stimulation (about 20%) with a range of 0–1 µM followed by a dramatic inhibition abolishing the HAF-stimulated activity at 4 µM Ca18,20. Such positive cooperativity and down regulation with varying HAF and µM Ca concentrations are the marks of a delicate control mechanism inherent in the living system. The dramatic Ca-inhibition suggests a sequestration of Ca within the catalytic (cytosolic) domain of the gastric ATPase system. Such sequestration would depend primarily on the surface charge of the complex formed between the HAF and the H, K-ATPase catalytic domain and to a lesser extent on the nature of the neighboring phospholipid microdomain. This information suggests a combined role of calcium and cytosolic HAF in the intracellular regulation of gastric H transport.

It is obvious that an appropriate level of Ca (below 1 µM) facilitates a direct contact of the HAF with the catalytic surface of the enzyme while a higher concentration of Ca interferes presumably by building a critical barrier on the enzyme/HAF interface, thus preventing a direct interaction with the HAF. Under this condition, the apical membrane-located H, K-ATPase system would be acting as a provisional device for pumping out calcium prior to the onset of acid secretion.17,4

The suggested role of the APM-embedded H, K-ATPase as a provisional Ca-pump prior to acid secretion is fully consistent with recent reports from two different laboratories19–22. Using fluorescent-tagged antibodies against the plasma membrane Ca-ATPase (PMCA) and the gastric H, K-ATPase Caroppo et al.23 demonstrated that not only do both ATPases have a closely similar and asymmetric distribution on the APM (of oxyntic cells of bullfrog gastric mucosa) but also were found to be co-localized by superimposition. At the same time, systematic studies with rabbit gastric glands by Michelangeli and coworkers20,21 revealed a consistent but transient peak of Ca transport into the secretory lumen prior to the onset of H-secretion. Such an oscillation between the two modalities of gastric H, K-ATPase system is depicted in Figure 1.

Tissue origin and specificity of the HAF and the NaAF (activator specific for the Na, K-ATPase)
While the HAF is characteristically present in the parietal cells of the fundic mucosa, the NaAF was initially demonstrated in the cytosolic fractions of the brain and kidney from rabbits and also in pigs15 and subsequently purified22. A near homogeneous preparation of the NaAF, which has a mass of 170 k Da, was obtained by a modification of the procedure used for HAF purification22. Unlike the 80 k Da HAF dimer, the NaAF is monomeric and has a 170 k Da mass in its active state. Also, the NaAF stimulates only the Na, K-ATPase without stimulating the H, K-ATPase, while the HAF is equally effective at stimulating both, suggesting that they share some domain(s) critical for the activation process.

In spite of such differences, some fundamental similarity was observed in the way HAF and NaAF work. Similar to the HAF-stimulated H, K-ATPase, the NaAF-stimulated Na, K-ATPase activity showed an abrupt inhibition within a relatively narrow range of Ca concentration. However, the HAF-stimulated H, K-ATPase activity was much more sensitive to Ca inhibition than the NaAF-stimulated Na, K-ATPase activity. The concentration of Ca needed for complete inhibition of the NaAF stimulation was 25–50 µM compared to the 3–4 µM for the HAF-stimulated H, K-ATPase.24

It is noteworthy in this connection that the HAF has been found to possess high (NH4OH-insensitive) protein-kinase activity as demonstrated by its ability to phosphorylate histone, but at the same time the HAF is not auto-phosphorylated, and cannot be phosphorylated by heart protein kinase from Sigma24. This rare ability to phosphorylate histone suggests that the HAF is capable of regulating its own intracellular level by regulating gene expression, thus raising the possibility of a similar intrinsic auto-regulation of the ubiquitous NaAF in a tissue specific manner. The existence of such auto-regulation of the NaAF would have great consequences in the metabolic and functional regulation of the cell as a whole.

A model showing the gastric H, K-ATPase system acting in the altered modes

A model for the cytosolic regulation of the P-2 ATPase system in parietal cells by the HAF and µM Ca is depicted in Figure 1.

The pivotal roles of Ca in HAF regulation of the pump strongly imply that Ca acts as a physiological feedback control switch in gastric H transport20,27. The Figure also shows that in the presence of around 4 µM Ca, the Ca-inhibited H-pump spontaneously changes itself into a unique Ca-pumping mechanism for promptly reversing the Ca-induced inhibition, thus bringing the local calcium concentration back down to 1 µM at which point the HAF activation of the H-pump resumes. Such intimate interplay between Ca and HAF would also help the parietal cells to conserve energy by preventing the needless accumulation of H inside the cytosolic TV prior to their destined inclusion into the APM by subsequent fusion. This unique ability of >1 µM Ca concentrations to switch the inhibited gastric H-pump spontaneously to the Ca-pumping mode is also likely to be operative in other H-pumping epithelia such as the distal colon and kidney tubules20,21,24.

Conclusion

The present paper proposes that the gastric H, K-ATPase, in addition to its well known role as a proton pump, may also act as a provisional Na-pump and a Ca-pump in the parietal cells where the HAF plays a critical role. Such altered modes demand immediate attention for a fresher look at the NaAF-regulated Na, K-ATPase system in various tissues. This is particularly critical for the central nervous system. The human brain, which weighs only three pounds (about 2% of total body weight), consumes almost 25% of the total energy (ATP); thus it would be expected that the NaAF would play a major role in brain metabolism and function.

Competing interests
No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Acknowledgements**

Dedicated to the memory of my mentor, Professor J. J. Ghosh (7/29/1925 – 10/2/2011), University of Calcutta, India for his inspiring vision in brain bioenergetics during the 1960’s when this field was merely a fresh baby.

**References**


Open Peer Review

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Version 2

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I have no further specific comment. I appreciate the change in title, as well as details that are now provided by the author. I think that this review may open an interesting field of scientific discussion.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 21 August 2013

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John Geibel
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This commentary presents an interesting yet highly controversial view of acid secretion. The literature cited is very limited and is predominantly from the author’s previous work.

My second point is that there are factual errors. There is no evidence for Calcium being pumped into the lumen of the gastric gland. The paper the author cites is measuring intracellular Ca not efflux of Ca into the lumen. In that study there are changes in intracellular Ca associated with carbachol that are typical for
Ca activated H,K stimulated acid secretion. The critical studies have not been preformed that are necessary to prove the theory; namely block the H,K with omeprazole, stimulate the cells with increased intracellular Calcium (thapsigargin, carbachol, etc) and show measurements of luminal Ca concentration changes.

As it stands now there are small pieces of unconnected data that do not give a convincing argument for this paper.

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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**Author Response 13 Sep 2013**

**Tushar Ray**, Tempe, AZ 85281, USA

The reason for such (seemingly odd) citations as pointed out by the reviewer is to clarify to the reader various important facets of the HAF in P-2 ATPase regulation within the parietal cells. To my knowledge no other laboratory came up with any publication either supporting or refuting our work so far which I could refer to. However, I will be most happy to be corrected by the reviewer if I am wrong.

Contrary to Dr. Giebel’s second statement on luminal Ca-transient, Caroppo et al (EMBO J. 20, 6316-6326, 2001) directly measured the Ca-concentration using Ca-selective microelectrodes and reported the carbachol-induced Ca-transients in the gastric lumen.

**Competing Interests:** There are no competing interests

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**Reviewer Report 21 August 2013**

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This review reflects the opinion that the gastric H,K-ATPase may transport other cations than potassium and protons, i.e., may transport sodium or calcium ions.

According to the author, these different transport modes mostly depend on the local cytosolic composition (Na⁺, pH, and Ca²⁺), and on a cytosolic factor that acts in a dual manner on the pump activity. This interesting view of the mechanisms of gastric secretion is based on previous results that have been obtained by T. Ray’s group. However, I would appreciate that the author further discuss the specificity of the reported assays, the purity of the preparations (apical and tubulovesicular membranes), and the
possibility that other, physiologically quiescent, P-ATPases or channels may obscure the interpretation.

Also, I feel that the title sounds different than the ms content, and that a change has to be considered.

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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**Tushar Ray**, Tempe, AZ 85281, USA

Following the suggestions of Dr. Gabrielle Planelle the conditions used for monitoring the effects of HAF and μM Ca on gastric H, K-ATPase activity associated with the APM and TV have now been specified. The purity and characteristic features of the isolated APM and TV membranes used in the study have also been provided. However, the possibility of interference under the conditions of our assay seems very unlikely. Also, following the excellent suggestion of the reviewer I have changed the title of the paper which now reads as, “The parietal cell gastric H, K-ATPase also functions as the Na, K-ATPase and Ca-ATPase in altered states”

**Competing Interests:** There are no competing interests

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**Reviewer Report 05 August 2013**

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**Silvana Curci**
Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, West Roxbury, 02132, USA

Tushar Ray here provides a review of his work on the possible multiple roles of gastric H/K ATPase.

Ray discusses the potentially interesting role of an endogenous activator of the H/K-ATPase, identified in the last few years by his research group. Unfortunately I did not have the chance to read the recent study (submitted) where it was found that an “anti-activator antibody blocks acid secretion in histamine-stimulated glands”. Thus I am unable to really comment on this relevant point.

I was a bit confused by the title, expecting to read about “altered states” in which the H/K ATPase would assume multiple roles. Also it would be nice if the author could comment on the possibility that the function and activity of H/K ATPase and of the Na/K ATPase would vary depending on the specific location of the parietal cells in the gland (i.e. more luminal or more basal), see Fujii et al. (2008) (JBC 2008. 283, 6869-6877).
Minor comments: a couple of typos at page 3: 2nd paragraph “of the functional dual topology H,K ATPase”; 4th paragraph “The APM showed very high basal (Ca2+….)”.

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 13 Sep 2013

Tushar Ray, Tempe, AZ 85281, USA

We studied the role of the HAF in proton production using a monospecific anti-HAF antibody raised in female rabbits against a pure preparation of the HAF. The histamine-stimulated acid secretion (as measured by 14C-aminopyrene uptake) in isolated rabbit gastric glands was eliminated by the anti-HAF-antibody proving the essential role of the HAF in the production of protons at the catalytic domain inside parietal cells (DOI: 10.5281/zenodo.7093). Following the excellent suggestion of Dr. Silvana Curci, I have changed the title which now reads as. “The parietal cell gastric H, K-ATPase also functions as the Na, K-ATPase and Ca-ATPase in altered states”

The issue of variable gastric acid secretion depending on location of the parietal cells in a gland is an important one, and I thank the reviewer for raising this issue. Since the parietal cells at the base of the gland secrete very little acid and are known to remain practically unaffected by gastric ulcers, one would expect to see a marked reduction both in the total number of H-pumps as well as in the turnover of the existing pumps. Also, one would expect to see a proportionate reduction in the altered function of the H, K-ATPase as provisional Na-pumps in these base glandular cells. Since the activity and function of both H- and Na-pumps rely on the cytosolic HAF, a noticeable change is expected to occur at the level of HAF in these cells. Thus a comparison in the activity and turnover of the HAF in parietal cells nearest to the secretary canaliculi of gastric glands compared to those farthest at the glandular base needs to be investigated.

Pondering over the turnover of HAF in parietal cells I recalled some novelty of the HAF which now appears relevant. The HAF was previously demonstrated to possess high (NH2OH-insensitive) protein-kinase activity by its ability to phosphorylate histone, but at the same time HAF is not auto-phosphorylated, and cannot be phosphorylated by heart protein kinase from Sigma (reference 13, DOI: 10.5281/zenodo.7095). This rare ability to phosphorylate histone suggests that the HAF is capable of regulating its own intracellular level by directing gene expression, thus raising the possibility of a similar auto-regulation by the NaAF in other cell types. This aspect has now been discussed in the revised version.

**Competing Interests:** There are no competing interests
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