The parietal cell gastric H, K-ATPase also functions as the Na, K-ATPase and Ca-ATPase in altered states
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.
Changes from Version 1

I want to thank both reviewers for their time and constructive comments. Based on their suggestion I have slightly changed the title specifying “parietal cells” to avoid misperception. I am grateful to Dr. Silvana Curci for her comments raising critical issues such as the nature of the gastric P-2 ATPase system associated with the parietal cells connected to the secretary canneliculi of a gastric gland compared to those away from the secretary canneliculi. Since the activities and functions of the gastric P-2 ATPase members are dependent on the HAF, I have focused my response on the activities and turnover of the HAF in actively secreting parietal cells as opposed to those sluggish cells in glandular base. The question of Dr. Curci reminded me of a unique feature of the HAF that we recorded long ago and was discussed previously in reference 13 (review article). The HAF possesses a very rare ability to phosphorylate histone without being phosphorylated by itself under all conditions we tested. This strongly implicates that the HAF could control its intracellular level by influencing the gene transcription through feedback mechanism in the parietal cells. Furthermore, the P-2 ATPase systems in the non-parietal cells could be similarly controlled by the ubiquitous NaAF in a tissue specific manner. Such a mode of NaAF self-regulation at the gene level will have a dominant outcome in overall cell metabolism and function. These critical aspects have now been included appropriately towards the end of the article.

I am equally grateful to Dr. Gabrielle Planelles for her input. In response to Dr. Planelles’ review I have included the details on ATPase assay. I also included the important features of the isolated apical plasma membranes (APM) of the parietal cells based on which we differentiated the APM from the intracellular tubulovesicles (TV). Thus, compared to the intracellular TV the APM had lower buoyant density, almost twice the amount of the individual phospholipids compared to TV, exclusive 5'-nucleotidase activity (a plasma membrane marker) and distinctive Vitamin B12 binding ability unique to the parietal cell plasma membrane.

See referee reports

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 millionfold mediated by the gastric H, KATPase system. This member of the P-2 ATPase family has been the most extensively studied along with the Na, KATPase and Ca-ATPase families due to their prominent roles in health and disease. Major developments in the field occurred following the single topology scheme for the Na, KATPase reaction proposed by Post and Albers in the early 1960s, which was subsequently extended to the H, KATPase system. The Post-Albers (PA) scheme visualizes Na-dependent phosphorylation of the 100 kDa α-subunit by ATP (a kinase step) and a sequential K-dependent dephosphorylation (a phosphatase step) during each reaction cycle. The activity of K-dependent p-nitrophenyl phosphatase (K-pNPPase), which is always co-purified with the Na, KATPase system was assumed to represent the phosphatase step of the total ATPase reaction. However this assumption was subsequently proven to be erroneous since K-pNPPase activity reflects the ion channel activity across the membrane rather than being a partial reaction of the ATPase. Based on the orientation of the ATP hydrolytic sites and the associated pNPPase sites together with their corresponding K regulatory sites (of high and low affinity respectively) across the membrane a mirror image orientation of the two α-subunits (within the functional H, K-ATPase complex) was proposed. The dual topology model fits well with numerous reports in the literature.

This unified dual topology model helped to clarify the demonstrated lack of Na, K-ATPase activity in parietal cell fractions so far using the conventional assay procedure even though ouabain (a Na-pump inhibitor) has long been known to eliminate gastric acid secretion. However, gastric H, K-ATPase activity shows stimulation by sodium manifesting a Na, K-ATPase activity when assayed at pH 8.5. In addition, the purified cytosolic activator protein (of 80 k Da mass) for the H, K-ATPase system stimulates the gastric H, K-ATPase and the renal Na, K-ATPase to the same degree. These data revealed that the catalytic α-subunit (which faces cytosol) of the functional dual topology H, K-ATPase can also bind Na at an alkaline pH thus acting like a Na, K-ATPase. Such encouraging high pH neighboring the H, K-ATPase catalytic site would be attained during peak gastric acid secretion. This paper reviews the evidence pointing to the conclusion that the cytosolic regulation of the active transport of H/K, Na/K and Ca/H occurs in a tissue-specific manner where an individual P-2 ATPase subspecies is capable of transporting any of the other cations depending on the local ionic milieu in order to maintain optimal housekeeping conditions.

Sole dependency of the apical membrane associated H, K-ATPase on the cytosolic endogenous activator (henceforth called “HAF”) for activity

HAF is an 80 k Da (a dimer of two identical 40 k Da subunits) cytosolic protein that occurs universally in the parietal cells. Activation of the gastric H, K-ATPase by the HAF is rather complex, resulting in a substantial increase in the affinity of the enzyme to K. Interaction of the HAF results in both up- and down-regulation of the gastric H, K-ATPase system depending on its concentration, showing a strong positive cooperativity (Hill coefficient = 4.5) followed by rapid decline. The anti-HAF antibody completely blocks acid secretion in histamine-stimulated rabbit gastric glands demonstrating the essentiality of the HAF in gastric secretory process. Studies with phospholipase 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). When assayed with Mg, K and HAF, the K-stimulated component was greatly stimulated (over 100-fold) by the HAF. In contrast, the pure TV membranes exhibited a very low or negligible basal activity, but the very high K-stimulated ATPase activity only required a small amount of stimulation (only about 60%) by the HAF. These studies revealed that the HAF is not only loosely bound to the APM but also plays an essential role in gastric acid secretion thus supporting our earlier conclusion.
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 phosphatidyl choline content of APM and TV was 67 and 33 µ moles/mg protein respectively with corresponding phosphatidyl choline/phosphatidyl ethanolamine ratios being 1.38 and 0.87 for APM and TV. Also, the phosphatidyl inositol and phosphatidyl serine 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

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 Ca\textsuperscript{12,13}. 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\textsuperscript{12,14}.

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 laboratories\textsuperscript{15,16}. Using fluorescent-tagged antibodies against the plasma membrane Ca-ATPase (PMCA) and the gastric H, K-ATPase Caroppo et al.\textsuperscript{17} 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 coworkers\textsuperscript{18,19} 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 pigs\textsuperscript{20} and subsequently purified\textsuperscript{21}. 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 purification\textsuperscript{22}. 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\textsuperscript{19,22} 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\textsuperscript{19}.

It is noteworthy in this connection that the HAF has been found to possess high (NH\textsubscript{4}OH-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 Sigma\textsuperscript{23}. 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 transport\textsuperscript{20,21}. 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 tubules\textsuperscript{22,23}.

**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 weights 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.

**Grant information**

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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.

Award (AM00623) during the course of this investigation at SUNY-Upstate Medical Center, Syracuse, NY, USA.

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

Referee Responses for Version 2

Gabrielle Planelles
Institut National de la Santé et de la Recherche Médicale (INSERM) UMRS-845, Paris, France

Approved: 16 September 2013

Referee Report: 16 September 2013
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.

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.

Competing Interests: No competing interests were disclosed.

Referee Responses for Version 1

John Geibel
School of Medicine, Yale University, New Haven, CT, USA

Not Approved: 21 August 2013

Referee Report: 21 August 2013
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.

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

1 Comment

**Author Response**

**Tushar Ray**, RayIsles Inc., USA

Posted: 13 Sep 2013

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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**Gabrielle Planelles**

Institut National de la Santé et de la Recherche Médicale (INSERM) UMRS-845, Paris, France

Approved: 21 August 2013

**Referee Report:** 21 August 2013

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.

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.

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

Tushar Ray, RayIsles Inc. , USA
Posted: 13 Sep 2013

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

Silvana Curci
Department of Surgery, Harvard Medical School, MA, USA

Approved: 05 August 2013

Referee Report: 05 August 2013
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+….)”.

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.

Competing Interests: No competing interests were disclosed.
Author Response

Tushar Ray, RayIsles Inc., USA
Posted: 13 Sep 2013

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