Check for updates

COMMENTARY

UPDATE 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

Tushar Ray^{1,2}

¹Ramakrishna Vedanta Ashrama of Phoenix, Tempe, AZ 85281, USA

²SUNY Upstate Medical Center, Syracuse, NY 13210, USA

v2 First published: 31 Jul 2013, 2:165
<https://doi.org/10.12688/f1000research.2-165.v1>
 Latest published: 12 Sep 2013, 2:165
<https://doi.org/10.12688/f1000research.2-165.v2>

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 μ M) 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 μ M) 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

Open Peer Review

Approval Status

	1	2	3
version 2 (update) 12 Sep 2013		 view	
version 1 31 Jul 2013	 view	 view	 view

1. **Silvana Curci**, Harvard Medical School, MA, USA
2. **Gabrielle Planelles**, Institut National de la Santé et de la Recherche Médicale (INSERM) UMRS-845, Paris, France
3. **John Geibel**, Yale University, New Haven, CT, USA

Any reports and responses or comments on the article can be found at the end of the article.

fuller understanding of the intracellular regulation of the ubiquitous Na-pump.

Corresponding author: Tushar Ray (Tray4381@gmail.com)

Competing interests: No competing interests were disclosed.

Grant information: This work was supported in part by a grant from NIH, AM 19531. The author was a recipient of the NIH Research Career Development 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.*

Copyright: © 2013 Ray T. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Ray T. **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]** F1000Research 2013, 2:165 <https://doi.org/10.12688/f1000research.2-165.v2>

First published: 31 Jul 2013, 2:165 <https://doi.org/10.12688/f1000research.2-165.v1>

UPDATED 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 secretory canaliculi of a gastric gland compared to those away from the secretory canaliculi. 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 possess 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 million fold mediated by the gastric H, K-ATPase system. This member of the P-2 ATPase family has been the most extensively studied along with the Na, K-ATPase 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, K-ATPase reaction proposed by Post and Albers in the early 1960s, which was subsequently extended to the H, K-ATPase system. The Post-Albers (PA) scheme visualizes Na-dependent phosphorylation of the 100 k Da 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, K-ATPase 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 ATPase¹. 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^{1,2} 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 literature³.

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 secretion⁴. However, gastric H, K-ATPase activity shows stimulation⁵ 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 degree⁶. 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 universally⁷⁻¹³ 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^{9,13}. The anti-HAF antibody completely blocks acid secretion in histamine-stimulated rabbit gastric glands demonstrating the essentiality of the HAF in gastric secretory process⁶. Studies with phospholipase¹⁴ and mild ethanol treatment^{15,16} 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 effects¹⁷. 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 HAF¹⁷. 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 HAF¹⁷. 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 TV¹⁷. 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 TV¹⁷.

Calcium (μ M) 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 range⁹. 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 function¹⁷. For the ATPase assay⁹ the desired amount of HAF (as indicated by the prior dose response study) was first pre-incubated with 5 μ g 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^{17,18}. 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^{17,18}.

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^{19–21}. Using fluorescent-tagged antibodies against the plasma membrane Ca-ATPase (PMCA) and the gastric H, K-ATPase Caroppo *et al.*¹⁹ 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^{20,21} 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 pigs¹³ 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 both⁶, 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^{10,22} 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-ATPase¹⁰.

It is noteworthy in this connection that the HAF has been found to possess high (NH_2OH -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 Sigma¹³. 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^{10,17}. 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^{23,24}.

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.

Grant information

This work was supported in part by a grant from NIH, AM 19531. The author was a recipient of the NIH Research Career Development

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.

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

- Ray TK, Nandi J: **K⁺-stimulated p-NPPase is not a partial reaction of the gastric (H⁺ K⁺)-transporting ATPase: Evidence supporting a new model for the univalent cation transporting ATPase systems.** *Biochem J.* 1986; **233**(1): 231–238.
[PubMed Abstract](#) | [Free Full Text](#)
- Nandi J, Das PK, Levine RA, et al.: **Half of the (Na⁺ K⁺)-transporting-ATPase associated K⁺-stimulated p-nitrophenyl phosphatase activity of gastric epithelial cells is exposed to the surface exterior.** *Biochem J.* 1988; **252**(1): 29–34.
[PubMed Abstract](#) | [Free Full Text](#)
- Ray TK, Das PK, Zinkievich M, et al.: **Dual topology of the gastric proton pump: A bidirectional molecular motor for the simultaneous uphill transport of H and K.** In *Gastrointestinal Disorders: Symptoms, Treatment and Prevention*, Nova Science Publications, N. Y. (Editors, M. Battik and N. Grimaldi). 2012; pp. 175–200.
[Reference Source](#)
- Davenport HW: **Effect of ouabain on acid secretion and electrolyte content of frog gastric mucosa.** *Proc Soc Exp Biol Med.* 1962; **110**(3): 613–615.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ray TK, Nandi J: **Modulation of gastric H⁺, K⁺-transporting ATPase function by Sodium.** *FEBS Lett.* 1985; **185**(1): 24–28.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ray TK, Nandi J: **Calcium Signalling is pivotal to P2-ATPase function** --- Submitted to *Biochemistry*. 2013.
- Ray TK: **Gastric K⁺-Stimulated Adenosine Triphosphatase: Demonstration of an Endogenous Activator.** *FEBS Lett.* 1978; **92**(1): 49–52.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bandopadhyay S, Ray TK: **Purification and characterization of a cytosolic activator protein for the gastric H⁺, K⁺-ATPase system from dog fundic mucosa.** *Prep Biochem.* 1986; **16**(1): 21–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bandopadhyay S, Das PK, Wright MV, et al.: **Characteristics of a pure endogenous activator of the gastric H⁺, K⁺-ATPase system: Evaluation of its role as a possible intracellular regulator.** *J Biol Chem.* 1987; **262**(12): 5664–5670.
[PubMed Abstract](#)
- Ray TK, Bandyopadhyay S, Das PK: **Regulation of the gastric H⁺, K⁺-transporting ATPase by a cytosolic 80 kDa activator protein and calcium: Ca²⁺ as a possible physiological switch.** *Indian J Biochem Biophys.* 1988; **25**(1–2): 43–51.
[PubMed Abstract](#)
- Ray TK, Bandopadhyay S, Das PK: **Endogenous activator regulation of the gastric H⁺, K⁺-ATPase: Studies with pure preparations.** *Annals NY Acad of Sci.* 1988; **529**: 88–91.
[Publisher Full Text](#)
- Bandopadhyay S, Ray TK: **Purification and characterization of a cytosolic activator protein for the gastric H⁺, K⁺-ATPase system from dog fundic mucosa.** *Prep Biochem.* 1986; **16**(1): 21–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ray TK, Chakrabarti R: **Regulation of active transport of monovalent cation across the animal cell plasma membranes by cytosolic regulatory proteins.** *Indian J Biochem Biophys.* 1988; **25**(3): 219–225.
[PubMed Abstract](#)
- Sen PC, Ray TK: **Control of the potassium ion-stimulated adenosine triphosphatase of pig gastric microsomes: Effects of lipid environment and the endogenous activator.** *Arch Biochem Biophys.* 1980; **202**(1): 8–17.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Nandi J, Wright MV, Ray TK: **Effects of phospholipase A2 on gastric microsomal H⁺, K⁺-ATPase system: Role of "boundary lipids" and the endogenous activator protein.** *Biochemistry.* 1983; **22**(25): 5814–5821.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ray TK, Nandi J: **Regulation of the gastric (H⁺, K⁺)-transporting ATPase system by the endogenous activator. Effects of phospholipase A2 treatment.** *Biochem J.* 1983; **212**(3): 887–890.
[PubMed Abstract](#) | [Free Full Text](#)
- Ray TK, Das PK, Nandi J, et al.: **Characteristics of the isolated apical plasmalemma and intracellular tubulovesicles of the gastric acid secreting cells: Demonstration of secretagogue-induced membrane mobilization.** *Biochemistry.* 1988; **27**(25): 8958–8968.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ray TK, Bandopadhyay S, Das PK: **Regulation of the gastric H⁺, K⁺-transporting ATPase by a cytosolic 80 kDa activator protein and calcium: Ca²⁺ as a possible physiological switch.** *Indian J Biochem Biophys.* 1988; **25**(1–2): 43–51.
[PubMed Abstract](#)
- Caroppo R, Gerbino A, Debellis L, et al.: **Asymmetrical, agonist-induced fluctuations in local extracellular [Ca²⁺] in intact polarized epithelia.** *EMBO J.* 2001; **20**(22): 6316–6326.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Perez-Zoghbi JF, Mayora A, Ruiz MC, et al.: **Heterogeneity of acid secretion induced by carbachol and histamine along the gastric gland axis and its relationship to [Ca²⁺]_i.** *Am J Physiol Gastrointest Liver Physiol.* 2008; **295**(4): G671–G681.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Perez JF, Ruiz MC, Michelangeli F: **Simultaneous measurement and imaging of intracellular Ca²⁺ and H⁺ transport in isolated rabbit gastric glands.** *J Physiol.* 2001; **537**(Pt 3): 735–745.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Das PK, Chakrabarti R, Bandopadhyay S, et al.: **Demonstration of an endogenous activator for the Na⁺, K⁺-ATPase system.** *Mol Cell Biochem.* 1989; **91**(1–2): 123–129.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rajendran VM, Sangan P, Geibel J, et al.: **Ouabain sensitive H,K-ATPase functions as Na,K-ATPase in apical membrane of rat distal colon.** *J Biol Chem.* 2000; **275**(17): 13035–13040.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Cougnon M, Bouyer P, Planelles G, et al.: **Does the colonic H,K-ATPase also act as an Na,K-ATPase?** *Proc Natl Acad Sci U S A.* 1998; **95**(11): 6516–6520.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 2

Reviewer Report 16 September 2013

<https://doi.org/10.5256/f1000research.2352.r1837>

© 2013 Planelles G. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Gabrielle Planelles

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

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 confirm that I have read this submission and 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

<https://doi.org/10.5256/f1000research.1824.r1468>

© 2013 Geibel J. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

John Geibel

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

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 performed 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 confirm that I have read this submission and 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.

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

Reviewer Report 21 August 2013

<https://doi.org/10.5256/f1000research.1824.r1466>

© 2013 Planelles G. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Gabrielle Planelles

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

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 Ca^{2+}), 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 confirm that I have read this submission and 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

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

Reviewer Report 05 August 2013

<https://doi.org/10.5256/f1000research.1824.r1281>

© 2013 Curci S. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Silvana Curci

Department of Surgery, Harvard Medical School, MA, 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 confirm that I have read this submission and 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 secretory 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](https://doi.org/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

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research