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# Proton pump - key cell element in acid related digestive disorders

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**Abstract**. Proton pump is the final step in gastric acid secretion, its physiopathological and pharmacological importance being undeniable in acid related digestive disorders. The knowledge of the intimate mechanisms of gastric secretion and the interaction of ATP-ase (adenosine triphosphatase) with various cell proteins is essential in finding efficient and specific inhibitors in order to cure these diseases. The article proposes a synthesis of the most recent acquisitions in decoding gastric secretion mechanism, interaction between proton pump inhibitors (PPI) and the K/HATP-ase and in the main directions of studying the ATP-ase.
**Key Words**: proton pump, PPI, gastric secretion, second rank messengers

**Rezumat**. Pompa de protoni este elementul final al secreţiei acide gastrice, importanţa sa fiziopatologică şi terapeutică fiind de netăgăduit în afecţiunile digestive legate de acid. Cunoaşterea mecanismelor intime ale secreţiei gastrice, interacţiunea ATP-azei (adenozin trifosfataza) cu diverse proteine celulare este esenţială în găsirea unor inhibitori cât mai eficienţi şi cât mai specifici pentru tratarea acestor afecţiuni. Articolul îşi propune o sinteză a celor mai recente achiziţii în descifrarea mecanismelor secreţiei, a interacţiunii inhibitorilor de pompă de protoni (IPP) cu ATP-aza K/H dependentă şi a principalelor direcţii de studiu ale ATP-azei.
**Cuvinte cheie**: pompa de protoni, IPP, secreţie gastrică, mesageri de ordin II

**Introduction**. **General aspects**. In order to achieve gastric secretion, a wide complex of nervous and humoral effector systems are involved, the final result being the activation of K/H proton pump which secretes H outside the cell and this H bonds with Cl in order to form HCl (Schubert et al 2008). The K/H ATP-ase belongs to a larger family of ATP-ases with different cell functions of ionic change through cytoplasm membrane and against proton gradient (Shin et al 2009). Gastric K/H ATP-ase belongs to the type P ionic transport ATP-ases, along with more widespread Na/K ATP from which borrows some structural and functional similarities. Both are dimeric structures, containing a bigger α catalytic subunit and a smaller regulatory β subunit (Beane et al 2011). Proton pumps are target for more classes of substances capable of inactivating these ATP-ases and consequently modifying the ionic transmembrane ratio, thus reducing or augmenting some cell functions in order to obtain the expected therapeutic result (Fass 2009). In this manner oabain and digoxine blocks Na/K ATP-ase of cardiac muscle fiber resulting in more intra-cell Na which must be evacuated by change with calcium and thus increased cytosolic calcium will be available more appropriately to contractile proteins and consequently the improved hearth contraction (Qui 2005). In the same manner, omeprazole and other PPI inactivate gastric K/H ATP-ase and the gastric secretion drops dramatically so the therapeutic effect will be tremendous in acid related disorders such as gastro-duodenal ulcers, gastritis, duodenitis, gastro -esophageal reflux disease (GERD) and their complications (Veev et al 2006). As a matter of fact the recent advances of the

therapy of these conditions reside exactly in the performance and efficiency of PPI developed in the last twenty years, beginning with the first member, omeprazole, a timoprazole derived drug and continuing with more recent and efficient lansoprazole, pantoprazole and esomeprazole (Niklasson et al 2010). It is obvious that in the not very far future more efficient and more binding site specific drugs will be developed. Disadvantages of these drugs like relatively short half time and the compulsory prior activation in gastric glands lumen created premises to the development of new PPI that would not share these shortcomings and candidates could be proton pumps potassium antagonists which directly attach to ATP-ase, but so far the bond is not covalent and the efficiency depends on a constant plasma drug level (Qui 2005). Current article proposes to review gastric secretion mechanisms and its pharmacological approaches as well as aspects concerning proton pump structure, functions and PPI coupling as well as binding sites.

**Gastric acid secretion**. **Pharmacological approach**. Gastric secretion is the result of interaction of different substances with specific receptors that activate some enzymes implicated in the synthesis of several second messengers, everything conducting to the final step of activating and trafficking of the proton pump from basal-lateral pole to the apical pole of the cell (Kazuhiro et al 2011). The histamine, liberated as a pre-synthesized mediator from mast cells and enterochromaphile- like cells of gastric mucosa via degranulation, fixes on type H2 histamine receptors on the surface of parietal gastric cell then subsequently stimulant G protein (Gs) is activated, which leads to the activation of adenylate cyclase (Feldman 2006). Under its influence, the ATP (adenosine triphosphate) is converted into cAMP (cyclic adenosine monophosphate) which activates proteinkinases enzyme families and these directly activate the proton pump which is powered by the hydrolysis of ATP into AMP and inorganic phosphate and will move to the apical pole of the cell (Samuelson 2003; Kazuhiro 2011). The pump will then evacuate an H in change of inserting a K into cytosol (Feldman 2006). H on lumen will bind with separately specific channel secreted Cl to form HCl and this outer cell binding is especially designed to protect gastric cell from auto digestion (Forte et al 2010). H is obtained through previous activation of carbonic anhydrase (CA) which makes possible the obtaining HCO3 and H from H2O and CO2. Na-K gradient is maintained by the Na/K ATP-ase situated at the lateral-basal pole of the cell which powered by the same ATP hydrolysis inserts 2 K and evacuates 3Na from cell. Inhibitory G protein (Gi) has also modulator effect on AC, inhibiting its activity thus decreasing cell cAMP levels and stopping H secretion (Samuelson et al 2003). Gi protein is activated by somatostatin and prostaglandins via specific receptors situated on basal cell layer. Proton pump could be alternatively stimulated by increasing levels of calcium-calmodulin complex (Ca-C) achieved secondary to the activation of muscarinic M1, gastrine G and β2 adrenergic receptors by acetylcholine, gastrine and cathecolamines respectively (Caplan 2007). Acethylcholine (Ach) is liberated from postganglionary parasympathetic fibers of vague nerve and gastrine from G cells present in gastric antrum and delta pancreatic isle cells. Cathecolamines could reach the gastric cells through blood flood when of adrenal source or via postganglionary fibers belonging to abdominal sympathetic system (Kazuhiro et al 2011). Many of these steps were subject to various pharmacological approaches during time but the most reliable and logical therapeutic target is the final step, K/H ATP-ase (Thomson et al 2010).

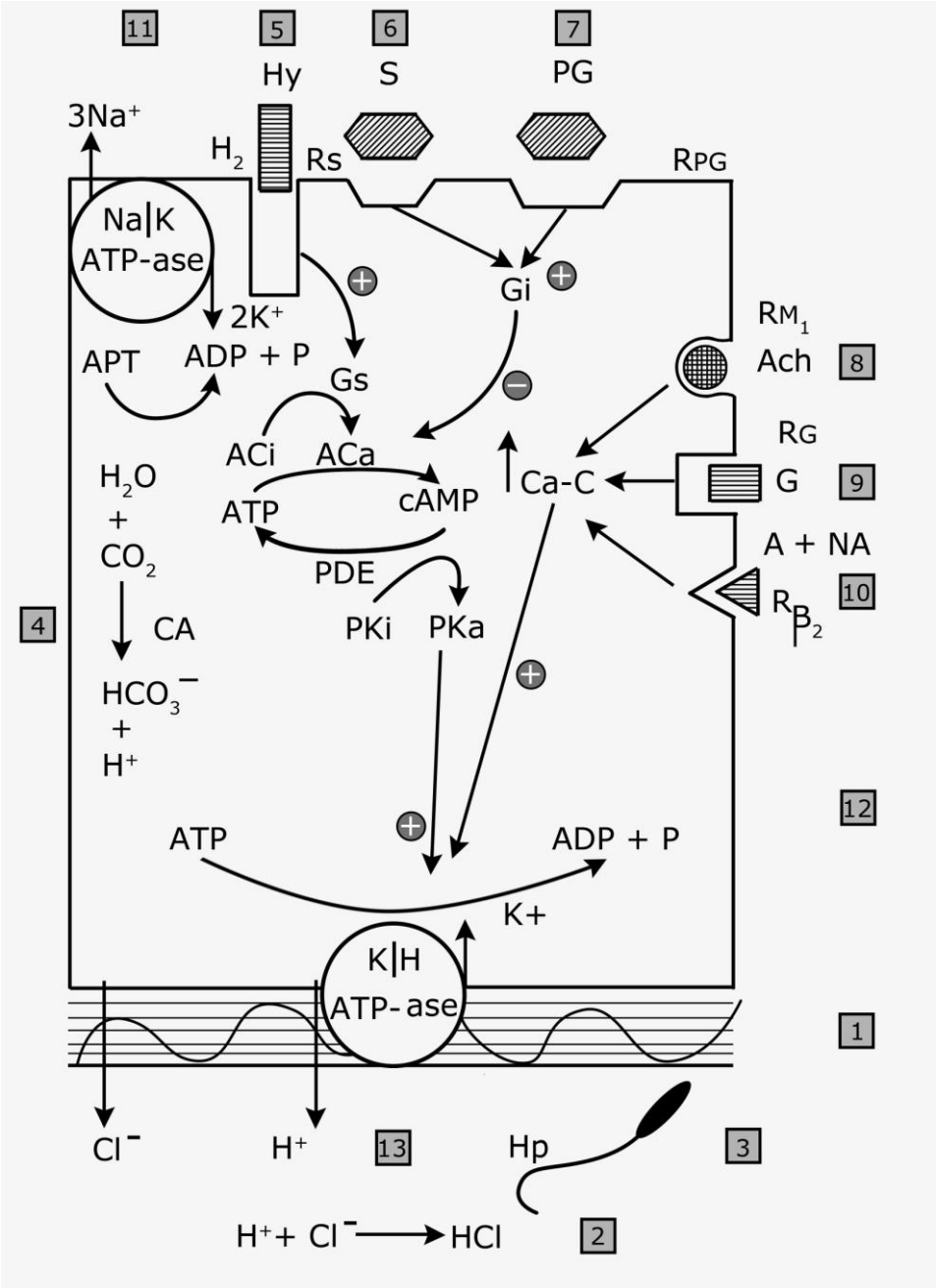


Figure 1. Gastric secretion and its pharmacological approach. H2=histamine type 2 receptor, Rs= somatostatin receptor, $R_{PG}$= prostaglandin receptor, RM1= muscarinic acetylcholine M1receptor, RG= gastrin receptor, Rβ2= β2 adrenergic receptor, Hy= histamine, PG= prostaglandin, Ach= acetylcholine, G= gastrin, A+NA= adrenaline and noradrenaline, Gs= stimulant G protein, Gi= inhibitory G protein, ACi= adenylate cyclase- inactive, ADa= adenylate cyclase- active, Ca-C= calcium-calmodulin complex, CA= carbonic anhydrase, PDE= phosphodiesterase, PKi= proteinkinase- inactive, PKa= proteinkinase- active, ATP=adenosine triphosphate, ADP= adenosinediphosphate, P= inorganic phosphate, Hp= Helycobacter pylori. Numbers indicate the medicine used for each sequence: 1=mucous and cell protectors (carbenoxolone, sucralfate, bismuth), 2=antiacids, 3= antibiotics for Hp, 3=CA inhibitors (acetazolamid), H2 blockers (ranitidine), S=somatostatin and its analogues, 7= prostaglandines, 8=M1 blockers (atropine, pirenzepine), 9=G blockers (proglumide), 10=β2 blockers (propranolol), 11= Na/K ATP-ase blockers (oabain), 12=others (timipramine, pepsin inhibitors), 13= PPI

**K/H ATP-ase - Structure. Functions. Perspectives**. K/H ATP-ase like all other members of type P ATP-ase family from which share a high degree of structure homology, is composed of two fragments with different biological functions (Sachs et al 2007). α catalytic subunit has a molecular weight of 10kD and passes ten times through cell membrane, having the role of pumping protons and of binding and performing hydrolysis of ATP (Abe et al 2010). β regulatory unit has a lighter molecular weight and passes only once through the membrane placing its NH2 end facing the cytoplasm and the COOH end facing the gastric lumen (Sachs et al 2007). Extracytosolic extremity is bigger and possesses an agglomeration of saccharide residues and is responsible for regulation and cell trafficking of the entire macromolecular complex (Abe et al 2010). Overall, this can adopt two conformations, type E1 and E2, during this conformational transition γ phosphorilated ATP binds covalently aspartic acid residues generating a phosphorilated intermediate from which the P type denomination comes (Abe et al 2009). As a consequence of this bonding protein chains, quaternary structure modification occurs in order to facilitate ionic convey (Sachs et al 2007). So far it has not been proven that covalent binding between ATP-ase and ATP take place, but membrane fusion processes regulate the secretive capacity of the pump (Abe et al 2010). After secretive stimuli stop, fragments of membrane are internalized forming a series of tubular vesicular elements, a veritable membrane system situated under cytosolic side of membrane (Trott et al 2010). During stimulation via second messengers like Ca, cAMP, IP3, DAG, the fusion between these tubular vesicle systems and cell membrane is reduced thus allowing pump secretion (Trott et al 2010). Different experiments (Nguyen et al 2004) on transgenic mice bearing punctual mutation of the gene encoding K/H ATP-ase reveal interesting data. For example, the replacement of tyrosine residue of β regulatory unit with alanine make enzyme re-internalization impossible and the result will be the development of chronic gastritis, but anyhow the secretion will be stopped if histamine, gastrine and nervous stimuli cease (Nguyen et al 2004). Ionic trafficking in gastric human cell is not an isolated phenomenon and it is not strictly related only with secondary messengers (Caplan 2007, Nguyen et al 2004). α subunits of ATP-ase are anchored on cytoskeleton proteins like ankyrine, spectrine and actine by the instrumentality of loops 2, 3, 4, 5 of polypeptide chain and they play the important role of maintaining cell polarity and of facilitating lateral- basal and apical migration of ATP-ase (Durr et al 2009). Protein antigen CD63 plays the fundamental role of separating lysosomes, peroxisomes and secretive vesicles easing their fusion with membrane and interacts with ATP-ase β unit modifying its degree of motility in cell environment (Espinoza 2011). A recent study based on computer assisted models of ATP-ase showed that there are a series of homology coordinating peptides of segments M5 and M6 and of E1 and E2 conformers, the domains N, A, P, M1 and M2 being also isolated as well as the active targets sites for PPI and APA (Sachs et al 2007). In order to allow K entrance and H expulsion, the pump must change conformation from E2 to E1 and the cytosolic domain containing lysine in 791 position must be dislocated from quaternary structure of pump macromolecule in order to make possible ion trafficking (Shin et al 2008). The PPI developed in 70's have benzimidazolic structure with various position substitutes, the first members being timoprazole and picoprazole, the predecessor of omeprazole (Munson et al 2007). Because all PPI are weak basis they rapidly accumulate in acid environment of pump interacting cytoplasmatic vesicles and they can no longer inhibit it (Shin et al 2008). This way was revealed that in order to achieve their goal PPI must be activated first and this activation implicates the formation of tetracyclic compounds, the sulphonamidic derivates of omeprazole (Munson et al 2007). Activated in gastric glands lumen, PPI binds directly on pump surface while it is active and transports protons (Shin et al 2008) .The bonding site consists in one or more cysteine residues and in spite of 60-90 minutes half life of PPI, the inhibition is permanent and in order to continue secretion a new pump must be sinthesized (Asano 2004). Nevertheless, inhibition is not achieved synchronous for all secretive units hence the need of several consecutively days

treatment, but the risk of tolerance like in case of H2 receptors blockers is practically zero (Chandrappa et al 2010). Recent substances like esomeprazole are absorbed in the small bowel and activated immediately after absorption so ATP-ase inhibition is more rapidly achieved and more prolonged (Asano 2004). The alternative, yet experimental, of benzimidazolic PPI had been discovered in the 80's and it is represented by protonatable amines acting as competitive K of ARP-ase (APAs=ATP-ase protonatable amines). The cornerstone in the development of APA was the observation that blocking K internalization will automatically block H secretion (Roepke et al 2006). Compound SCH 28080 possesses o high degree of selectivity in inhibiting K/H ATP-ase, due to its specific and close bonding with the last dodecapeptide of COOH end belonging to M1 domain and to the loop between M1 and M2 α catalytic subunit domain (Roepke et al 2006). A similar mechanism has been proposed for compound SKF 96067 (Chandrappa et al 2010). Without entering details must be stated that during the studying of ATP-ase it was discovered that in fungi cell membrane there exists a similar structure with the role of maintaining pH gradient in order to capture nutrients, in addition having functions in cell growth and pathogenicity (Perlin 1998). Development of these ATP-ase inhibitors on a large scale will open new perspectives of antifungal therapy (Witzke et al 2010). PPI unlike classic compounds such as fluconazole, itraconazole, amphotericin B would not induct drug resistance and would have antifungal effect channeled on more metabolic processes, as the pump has many physiological key roles (Vadlapudi et al 2011). PPI has also bactericidal effect on Helicobacter pylori (Hp), known for its implications in peptic ulcer, gastric cancer, gastric lymphoma, as the bacteria also has a similar P type ATP-ase that could be targeted by PPI (Siddaraju 2007). Cag A strains of Hp owns a vacuolating citotoxin weighting 120kD and they also have a V type ATP-ase which is not affected by PPI therapy (Hilleringmann et al 2006). Some other recent studies suggest that PPI have potential as anti-allergic asthmatic agents as they could reduce translationally controlled tumor protein (TCTP) secretion, associated with allergic reaction, by reducing calcium mobilization inside the cell (Chol et al 2009). IPP could also induce apoptosis of B cell tumors as they hold control of cellular pH in both normal and neoplasm cells (De Milito et al 2007). Last but not least, it has been suggested that PPI could act as chemosensitizer agents on some human tumor cells (Fais et al 2007). For instance, PPI could chemosensitize gastric adenocarcinoma cells to the effect of chemotherapic drugs as they affect cell homeostasis and acid trafficking (Min et al 2009). In addition, it has been proved that ATP-ases are over expressed in many types of metastatic cancer and positively correlated with invasion, metastases and poor surviving rates (De Milito et al 2007).

**Conclusions**. Type P K/H ATP-ases are ubiquitous structure among eukaryotic cells and have various functions and control systems. In gastric cells, K/H ATP-ase plays the key role in gastric acid secretion and it is the main target in modern therapy of peptic ulcer, gastritis and GERD. Solving the molecular structure of the pump and its intimate activation mechanisms as well as achieving to synthesize more powerful and specific inhibitors create prerequisites of improving therapy of the acid related disorders as well as of widening of PPI spectrum.

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