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REVIEW

Pump Proton and Laryngeal H^+/K^+ ATPases

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Purpose: The presence of extra-gastric H^+/K^+ ATPases may explain the clinically significant effect of proton pump inhibitor (PPI) pharmacotherapy in patients with chronic laryngitis related to laryngopharyngeal reflux disease (LPRD) but without gastroesophageal reflux disease (GERD) symptoms. Given the need for a better understanding of GERD and LPRD, we review the various proton pumps with respect to their classification, function, and distribution. We then consider the potential role of the laryngeal H^+/K^+ ATPase pump in LPRD.

Methods: We searched databases of PubMed, EMBASE, and Web of Science to achieve related published before September 15, 2020.

Results: There were only seven English-literatures meeting inclusive criteria about laryngeal H^+/K^+ ATPases. Some studies provide convincing evidence of a laryngeal H^+/K^+ ATPase in normal laryngeal tissues but also suggest the potential role of the proton pump in the abnormal mucus secretion frequently seen in patients with chronic laryngitis.

Conclusion: A laryngeal H^+/K^+ ATPase expresses in normal laryngeal tissues. These findings question the current understanding of GERD and LPRD.

Keywords: laryngopharyngeal reflux disease, gastroesophageal reflux disease, larynx, proton pump, H^+/K^+ ATPases, chronic laryngitis

Introduction

Laryngopharyngeal reflux disease (LPRD) comprises a group of symptoms and signs caused by reflux of the gastric contents into the upper esophageal sphincter and is therefore considered an extra-esophageal variant of gastroesophageal reflux disease (GERD).[1,2] However, whether the true pathogenesis of LPRD involves reflux of the gastric contents is unclear. The different symptoms of GERD and LPRD suggest that they are, at least clinically, two different diseases. For example, LPRD does not always include gastric reflux symptoms such as heartburn and eructation, and some patients report only the sensation of a foreign body in the throat and frequent throat clearing.[3] Therefore, LPRD is not caused solely by gastric reflux but may involve other factors as well.

Recent studies of the etiology of LPRD have focused on exogenous factors affecting the laryngopharynx. The detection of salivary pepsin has also been performed to diagnose LPRD[1,3–6] but is not applicable for all cases of LPRD.[4] Thus, the search continues for endogenous factors of the laryngopharynx that participate in the pathogenesis of LPRD. Nonetheless, the proton pump is central to both LPRD and GERD.[1–3,7,8] Whereas the gastric H^+/K^+ ATPase is a well-characterized proton pump mediating gastric acid secretion,[7–9] other H^+/K^+ ATPases may be present in organs outside the stomach, such as the larynx. The presence of extra-gastric H^+/K^+ ATPases

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may explain the clinically significant effect of proton pump inhibitor (PPI) pharmacotherapy in patients with chronic laryngitis related to LPRD but without GERD symptoms.[10,11] Given the need for a better understanding of GERD and LPRD, here we review the classification, function, and distribution of the various proton pumps, focusing on the laryngeal H^+/K^+ ATPase.

A proton pump refers to a transmembrane integrated glycoprotein that transports hydrogen ions across the membrane against a concentration gradient. The energy fueling the proton pump and H^+ efflux is provided by ATP hydrolysis.[9] As a byproduct of the pump activity, a pH gradient forms on either side of the membrane. Four types of proton pumps with ATPase activity have been recognized: P-type,[10–14] F-type,[15–17] V-type,[18–20] and ABC ATPases.[18]

Gastric Proton Pump H^+/K^+ ATPase and Its Functions

Gastric acid secretion is a physiological process common to all vertebrates. Its primary function is the digestion of food.[9] H^+ secretion is mediated by the gastric proton pump, H^+/K^+ ATPase, a member of the P2 ATPase family that also includes Ca^{2+} and Na^+/K^+ ATPases. The gastric H^+/K^+ ATPase mediates the transport of K^+ ions in the extracellular fluid into the cell, through coupling of phosphorylation and dephosphorylation, while intracellular H^+ ions are simultaneously pumped out of the cell against a 2.5-million-fold concentration gradient to complete ion transport and gastric acid secretion.[9,21] H^+/K^+ ATPase activity is stimulated by inflammatory and other factors and by the bacterium *Helicobacter pylori*, leading to excessive gastric acid secretion and GERD.[9,21] This is the mechanism targeted by PPI therapy.[7,8,16] H^+/K^+ ATPases contain the catalytic α-subunit and non-catalytic glycosylation β-subunit. The α-subunit of the H^+/K^+ ATPases of different species is made up of 1035 amino acids and includes the catalytic center, which resides in the large cytoplasmic domain of the enzyme. ATP-binding, acyl-phosphorylation, inhibitor-binding, and ion-recognition sites are also included in the α-subunit.[9,21] In addition to these functions, the α-subunit is important for the structure and function of H^+/K^+ ATPases, as it mediates ion transport and energy supply for transmembrane ion transport and confers stability to the holoenzyme. The 290-amino-acid β-subunit is responsible for enzyme assembly and plays a role in enzyme activity (Figure 1).[9,21]

Laryngeal H^+/K^+ ATPases

We searched databases of PubMed, EMBASE, and Web of Science to achieve related published before September 15,

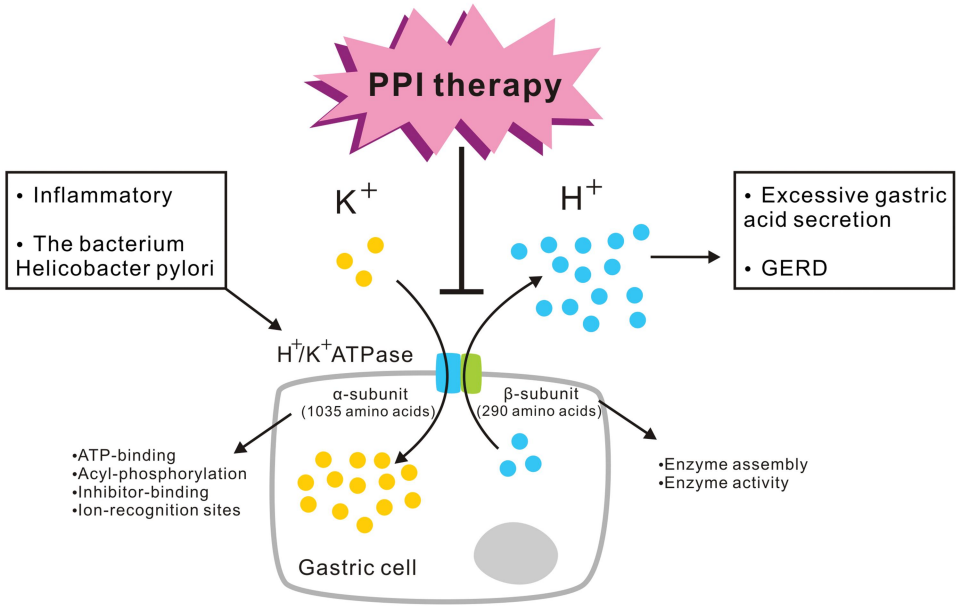

Figure 1 Gastric proton pump H^+/K^+ ATPase and its functions. The gastric H^+/K^+ ATPase mediates the transport of K^+ ions in the extracellular fluid into the cell, while intracellular H^+ ions are simultaneously pumped out of the cell against a high concentration gradient to complete ion transport and gastric acid secretion. H^+/K^+ ATPase activity is stimulated by inflammatory and other factors by the bacterium *Helicobacter pylori*, leading to excessive gastric acid secretion and GERD. This is the mechanism targeted by PPI therapy. H^+/K^+ ATPases contain the α-subunit and β-subunit. The α-subunit is made up of 1035 amino acids. ATP-binding, acyl-phosphorylation, inhibitor-binding, and ion-recognition sites are included in the α-subunit. The 290-amino-acid β-subunit is responsible for enzyme assembly and plays a role in enzyme activity.

2020. Search words included "laryngeal/larynx/head and neck" and "proton pump"; or "laryngeal/larynx/head and neck" and "H^+/K^+ ATPases"; or "laryngeal/larynx/head and neck" and "non-gastric H^+/K^+ ATPases"; or "non-gastric H^+/K^+ ATPases";

There were only seven English-literatures meeting inclusive criteria about laryngeal H^+/K^+ ATPases. In 1995, H^+/K^+ ATPases were discovered outside the stomach, first in kidney tissues[22] and subsequently in human lung mucus glands,[23,24] rat and human kidney tissues,[25–27] and rat rectum,[28,29] uterus,[29] heart,[30] inner ear, vestibule,[31–34] thymus,[35,36] and prostate.[37–41]

In 2003, Altman et al used immunohistochemical staining to identify H^+/K^+ ATPase α- and β-subunits in the serous cells and ducts of the minor seromucinous glands of larynges from two human cadavers.[42] In a follow-up study in 2005, those authors reported seromucinous glands in laryngeal surgical specimens without evidence of carcinoma and with an otherwise normal architecture on pathology. Of the 27 specimens obtained from 15 patients who underwent laryngeal surgery (11 total laryngectomies, 2 partial laryngectomies, 1 excision of a laryngeal mass, and 1 arytenoidectomy), the rates of positive staining of the α- and β-subunits were 96.3% (26/27) and 85.2% (23/27), respectively. The staining was observed mainly in the laryngeal seromucinous glands and ducts.[43] In 2011, the same authors demonstrated that the immunohistochemical staining of α- and β-subunits in the larynx was consistent with the presence of a gastric proton pump (H^+/K^+ ATPase).[43] Using immunohistochemical techniques and Western blotting, they also detected α- and β-subunits in three submandibular gland specimens, four normal laryngeal specimens (three benign and one invasive squamous cell carcinoma) without carcinoma and with an otherwise normal architecture confirmed by pathology, and three normal gastric specimens. Both the α- and β-subunits were identified in the submandibular gland ducts, together with high or strong expression in seromucinous glands and even stronger expression in seromucinous ducts. Although an anti-α-subunit antibody resulted in a somewhat stronger immunostaining intensity, the staining pattern was identical in location and distribution with those of both the H^+/K^+ ATPase α- and β-subunits.[40] Western blotting confirmed the presence of the H^+/K^+ ATPase α- and β-subunits in the laryngeal mucosa and submandibular gland. It was therefore suggested that symptoms of chronic laryngitis are not solely the result of acid produced by parietal cells of the stomach, but that acid-producing cells in the larynx also play a role.[44] In 2015, Stevanović et al evaluated expression of the β-subunit of the proton pump in 50 cadaver larynges and 11 larynges from laryngectomies performed on patients with laryngeal carcinoma not treated with perioperative chemoradiotherapy.[45] They found no β-subunit expression in the cadaver larynges, and 36% (4/11) of the laryngectomy specimens showed weak positivity in the seromucinous glands located in the supraglottis. The β-subunit was also evaluated in the chondrocytes of all surgical specimens and in most cadaver larynges evaluated.[53] In cadavers, the rates of β-subunit positivity in chondrocytes in the supraglottis, glottis, and subglottis were 74%, 60%, and 74%, respectively.[45] However, the role of H^+/K^+ ATPases in laryngeal chondrocytes is unclear.[45] In 2015, Becker et al detected H^+/K^+ ATPases in biopsied laryngeal tissues from patients with LPRD symptoms who underwent Dx-pH and pH/MII measurements. The tissues were also analyzed by real-time RT-PCR and immunohistochemical techniques.[46] Among the 20 patients, 14 had pathological Dx-pH results and 6 pathological pH/MII results, with 4 patients having pathological results on both tests. In the one patient with positive H^+/K^+ ATPase α- and β-subunit expression, as detected by immunohistochemistry, and pathological results on both the Dx-pH and pH/MII tests, PPI treatment was effective. PPI treatment was also effective in another patient positive for α- and β-subunit expression, as detected by real-time RT-PCR, and with pathological pH/MII test results. The low rate of H^+/K^+ ATPase positivity in that study can be explained by the fact that the proton pump is located mainly in the laryngeal seromucinous glands rather than in the squamous epithelium, whereas the biopsies were performed only in the superficial tissues, under endoscopy.[46] Consequently, the submucosal glands, expressing the H^+/K^+ ATPase proton pump, were probably missed.[54] Recently, McCormick CA et al detected alpha and beta subunits (ATP4A and ATP4B) of H^+/K^+ ATPase proton pump in the larynx of LPR and laryngeal carcinoma patients. They found that the positive expression of ATP4A and ATP4B was in 3/3 LPR, 4/8 laryngeal carcinoma-tumor and 3/8 laryngeal carcinoma-adjacent specimens. Although its small sample size and absence of a reflux and laryngeal cancer-free control cohort, they suggested that acid secretion by functional H^+/K^+ ATPase proton pumps expressed in laryngeal mucosa may elicit laryngeal mucosa cells and molecular changes associated with inflammation and cancerogenesis.[47] However, Herrmann et al detected significant expression

of the H^+/K^+ ATPase α- and β-subunits in the stomach among different human tissues evaluated, including the larynx.[48]

The above-cited studies provide convincing evidence of a laryngeal H^+/K^+ ATPase[42–47] in normal laryngeal tissues but also suggest the potential role of the proton pump in the abnormal mucus secretion frequently seen in patients with chronic laryngitis. The discovery of a laryngeal proton pump may explain the pathologically acidic environment of the oropharynx in patients without gastroesophageal reflux, with profound implications for PPI therapy, as the larynx is one of the extragastric targets of these drugs.[42–46] The discovery of a laryngeal H^+/K^+ ATPase may also explain some of the clinical controversies regarding LPRD or GERD,[42–46] such as the inconsistent pH measurement in patients with significant LPRD symptoms,[49] abnormal laryngeal seromucinous secretion, and reduced MUC5AC gene expression seen in patients with chronic laryngitis associated with LPRD,[50] presence of pachydermia as a nonspecific finding associated with LPRD,[4] efficacy of a placebo on LPRD,[51] and high rate of failed laparoscopic Nissen fundoplication in patients with symptoms of LPRD.[52]

The function of the laryngeal H^+/K^+ ATPase proton pump is unclear. However, Altman et al suggested that H^+/K^+ ATPases are transmembrane enzymes mainly responsible for the transport of ions, similar to V-type ATPases.[42–44] A similarity of the laryngeal proton pump H^+/K^+ ATPase to the V-type ATPase in rodent seromucinous glands has also been reported.[42–45] These findings may be of clinical significance, given the indispensable role of the seromucinous glands in the normal and pathological functions of the larynx. For example, in some pathophysiological conditions, especially those associated with acidic environments, proton pumps in the laryngeal seromucinous glands and ducts may be responsible for regulating seromucinous secretion.[42–45] These conditions include (1) direct acid reflux in LPRD, (2) first-stage reflux, when reactivation during subsequent acid exposure leads to pepsin binding and thus to tissue autodigestion, which together with cellular necrosis regulates the pH of the interstitium and may lead to laryngopharyngeal reflux-induced acid exposure. These findings imply that the laryngeal H^+/K^+ ATPase is activated by laryngopharyngeal reflux (similar to esophageal Na^+/H^+ exchangers) and other infectious or inflammatory stimuli (similar to tracheal epithelial proton secretion) to maintain the intracellular pH and cell viability.[53,54] Thus, the symptoms of chronic laryngitis may not be caused solely by the acid produced by gastric parietal cells but rather also that produced by laryngeal seromucinous glands and ducts positive for H^+/K^+ ATPase. The larynx is susceptible to

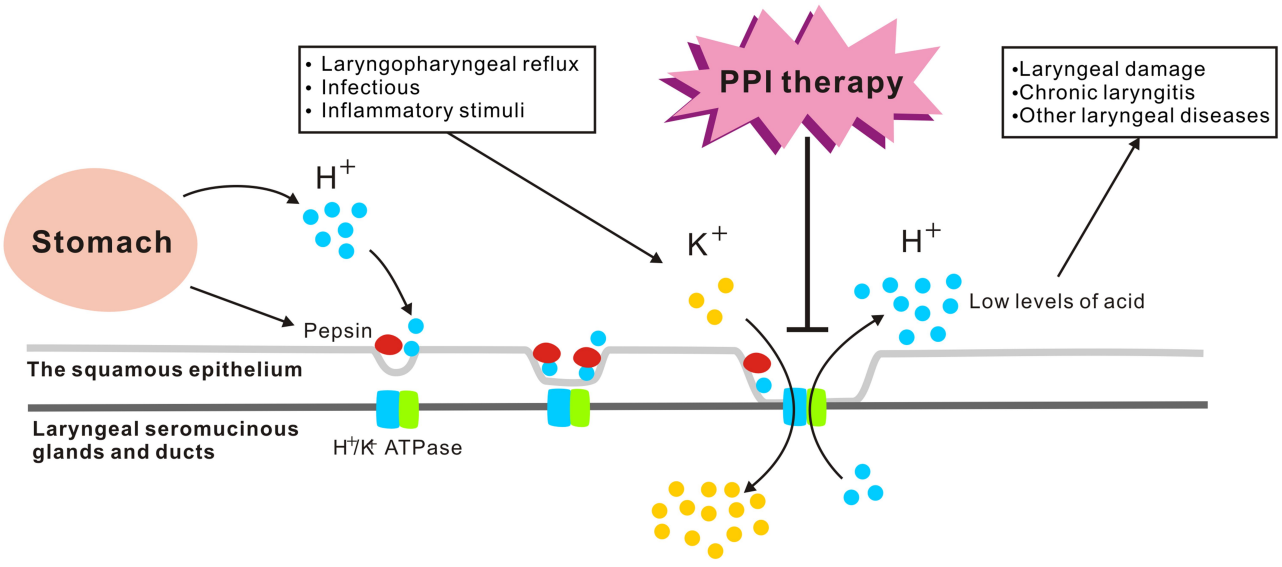

Figure 2 Laryngeal H^+/K^+ ATPases. The H^+/K^+ ATPase proton pump is located mainly in the laryngeal seromucinous glands and ducts rather than in the squamous epithelium, which may be responsible for regulating seromucinous secretion in two conditions: (1) direct acid reflux in LPRD, (2) first-stage reflux, when reactivation during subsequent acid exposure leads to pepsin binding and thus to tissue autodigestion, which together with cellular necrosis regulates the pH of the interstitium and may lead to laryngopharyngeal reflux-induced acid exposure. The laryngeal H^+/K^+ ATPase is activated by laryngopharyngeal reflux and other infectious or inflammatory stimuli. The laryngeal H^+/K^+ ATPase secretes low levels of acid, which may induce laryngeal damage and, subsequently, chronic laryngitis or other laryngeal diseases. The pathologically acidic environment of the oropharynx in patients without gastroesophageal reflux is effective for PPI therapy, as the larynx is one of the extragastric targets of these drugs.

an altered pH. Thus, we suggest that the laryngeal H^+/K^+ ATPase secretes low levels of acid, similar to acid release in the stomach. This low level of acid in the larynx may induce laryngeal damage and, subsequently, chronic laryngitis or other laryngeal diseases. It may also explain why patients without apparent LPRD are sometimes responsive to PPI pharmacotherapy (Figure 2).

Conclusions and Expectations

The detection of a H^+/K^+ ATPase proton pump in laryngeal nononcological patients suggests a mechanism for LPRD attributable to the larynx alone. However, the function of the laryngeal H^+/K^+ ATPase remains unclear. Further studies are needed to determine whether its activities include acid secretion, and whether there is a correlation between laryngeal H^+/K^+ ATPase and laryngeal chronic laryngitis. The PPI binding site in laryngeal H^+/K^+ ATPases also remains to be identified.

Disclosure

The authors report no conflicts of interest in this work.

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