

Review article

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FROM NERVES AND HORMONES TO BACTERIA IN THE STOMACH; NOBEL PRIZE FOR ACHIEVEMENTS IN GASTROLOGY DURING LAST CENTURY

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Rapid progress in gastroenterological research, during past century, was initiated by the discovery by W. Prout in early 18th century of the presence of inorganic, hydrochloric acid in the stomach and by I.P. Pavlov at the end of 19th century of neuro-reflex stimulation of secretion of this acid that was awarded by Nobel prize in 1904. Then, J. W. Black, who followed L. Popielski's concept of histamine involvement in the stimulation of this secretion, was awarded second Nobel prize in gastrology within the same century for the identification of histamine H₂-receptor (H₂-R) antagonists, potent gastric acid inhibitors, accelerating ulcer healing. The concept of H₂-R interaction with other receptors such as muscarinic receptors (M₃-R), mediating the action of acetylcholine released from local cholinergic nerves, and those mediating the action of gastrin (CCK₂-R) on parietal cells, has been confirmed both *in vivo* studies and *in vitro* isolated parietal cells. The discovery of H₂-R antagonists by Black and their usefulness in control of gastric secretion and ulcer healing, were considered as real breakthrough both in elucidation of gastric secretory mechanisms and in ulcer therapy. Discovery of even more powerful gastric acid inhibitors, proton pump inhibitors (PPI), also highly effective in acceleration of ulcer healing was, however, not awarded Nobel prize. Unexpectedly, two Australian clinical researchers, R.J. Warren and B.J. Marshall, who discovered in the stomach spiral bacteria, named *Helicobacter pylori*, received the third in past century Nobel prize in gastrology for the finding that this *bacterium*, is related to the pathogenesis of gastritis and peptic ulcer. They documented that eradication of *H. pylori* from the stomach, using antibiotics and potent gastric inhibitors, not only accelerates healing of ulcer but also prevents its recurrence, the finding considered as greatest discovery in practical gastrology during last century. Thus, the outstanding achievements in gastroenterology during last century have been awarded by three Nobel prizes and appreciated by millions of ulcer patients all over the world.

Key words: *Gastroenterology Nobel prize, vagal nerves, histamine, peptic ulcer, Helicobacter pylori*

INTRODUCTION

Since the discovery by W. Prout (1) in 18th century of the presence of hydrochloric (muriatic) acid in the stomach and ingenious observations by W. Beaumont (2) in 1822 of gastric secretory functions in Alexis St. Martin, a French Canadian traveler, with the permanent post-gun-shot gastric fistula (that served to Beaumont as a precious “human guinea pig”), it became generally accepted that gastric H⁺ (and pepsin) secretion occurs in the stomach to contribute to gastric digestion as originally proposed for the first time by Spallanzani (3). Further studies on animals and humans, confirmed that gastric acid secretion is required for normal digestion and that it results from the interplay of stimulatory and inhibitory influences on parietal cells as suggested for the first time by Beaumont (2).

GASTRIC ACID SECRETORY MECHANISMS

The most impressive basic research related to the physiological mechanisms of gastric acid secretion originated at the end of 19th century from the fascinating experiments performed in St-Petersburg by I. P. Pavlov on dogs prepared with esophageal and gastric fistulas (4). Pavlov was the first to demonstrate that gastric acid secretion in fasted dogs starts almost immediately following exposure to appetizing food even without the entrance of this food into the stomach (“sham-feeding”). He proposed the concept of *nervism* or entire neural control of gastric secretion (as well as salivary and pancreatic secretion) that was widely recognized and proponent of this concept was awarded Nobel prize in 1904 for the first time in gastroenterology. The importance of vagal nerves was proved by showing that vagotomy eliminated sham-feeding-induced gastric acid secretion, and this became widely accepted later on, after Dragstedt's (5) pioneering work on the role of section of vagus nerves in treatment of acid-pepsin disorders and peptic ulcers. With discovery by J.S. Edkins in 1905 of gastrin (6) followed, about half century later, by isolation and synthesis of “antral hormone” by R. Gregory and H. Tracy (7), the new concept of gastric secretory mechanism i.e. “hormonal” rather than nervous, gained support and some acceptance. The discovery in 1920 by L. Popielski, Polish pharmacologist at the Lvov University, a fervent supporter of Pavlov's *nervism*, that histamine (8), a non-nervous and non-gastrin compound, produces powerful gastric acid stimulation in animals and humans, opened an alternative, humoral, concept explaining oxyntic cell stimulation and becoming an important contribution to the development of gastric physiology, depreciating the role of *nervism*, but favoring humoral, namely histamine concept, championed later on by C.F. Code (9) (*Fig. 1*).

At this point, it should be mentioned, that thanks to the studies of Uvnas (10) and Olbe (11), gastrin was recognized as an important mediator of vagally stimulated gastric secretion because removal of gastric antrum, the major source of gastrin, greatly attenuated gastric secretory response to sham-feeding. On the









✓ The presence of HCl in the stomach by <u>W. Prout</u> in 1823		
✓ Vagus plays crucial role in gastric HCl secretion by <u>I.P. Pavlov</u> in 1895	★	
✓ Gastrin is gastric HCl stimulant by <u>J.S. Edkins</u> in 1905		
✓ <i>Dictum</i> ; NO ACID NO ULCER by <u>K. Schwartz</u> in 1910		
✓ Histamine as gastric HCl secretagogue by <u>L. Popielski</u> in 1916		
✓ H ₂ -receptor antagonists by <u>J.W. Black</u> in 1972 and proton pump inhibitors by <u>G. Sachs</u> in 1980 showing strong inhibition of HCl secretion stimulated by various secretagogues and anti-ulcer efficacy	★	 
✓ <i>Helicobacter pylori</i> and its role in gastritis and peptic ulcer disease by <u>B.J. Marshall & R.J. Warren</u> in 1983	★	

Fig. 1. Historical background; major discoveries in gastrology (star indicates Nobel prize)

other hand, vagal stimulation by Bugajski J. and Kaulbersz J. (12) and Sandvik A.K. and Waldum H.L. (13) was found to increase gastric secretion and, interestingly, also to cause an excessive release of histamine by the gastric mucosa. These results could be interpreted that either stimulation of vagal nerves cause the release of both gastrin and histamine from the G-cells and ECL-cells, respectively, or that such vagal stimulation results in the release of gastrin that, in turn, stimulates the ECL cells to release histamine. The fact that certain form of histamine such as an alpha-methyl histamine, present in *H. pylori* infected stomach enhances gastrin release (14), shows the complexity of the vagus-gastrin-histamine interaction, forming a self-stimulation loop acting on positive feedback basis to stimulate gastric acid secretion.

Further pharmacological studies performed by J.W. Black (15) through the modulation of the structure of histamine, resulted in the synthesis of specific antagonists of histamine H₂-receptors (H₂-R). These H₂R antagonists were found to cause a potent and side-effect free gastric acid inhibition in animals and humans and then were found to accelerate ulcer healing that brought to their inventor, Black, a second within last century Nobel prize in gastrology. Black's studies were carried on the assumption that histamine plays a key role in gastric acid secretory mechanism despite erroneous opinion till 1971 (16), few months before synthesis

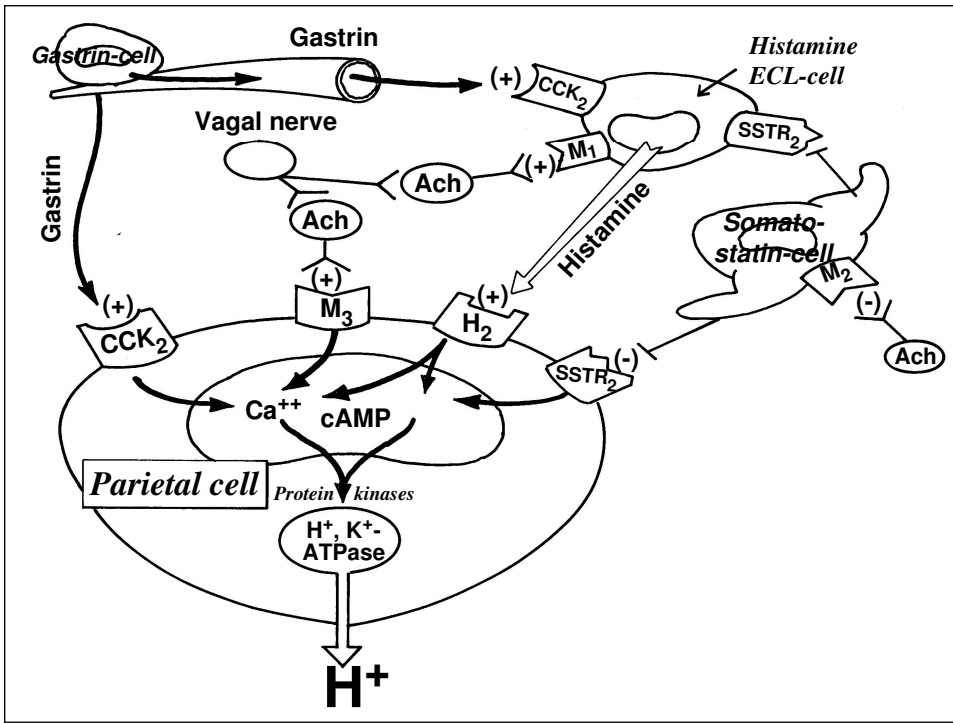


Fig. 2. Control of gastric H⁺ secretion; Role of gastrin, histamine and acetylcholine (Ach), each acting via specific receptors

of H₂-R antagonists that there is “no room for histamine” in the secretory mechanisms controlling gastric secretion, presumably because of ubiquity of this amine in various organs and the failure of inhibiting gastric secretion in rats by suppressing histidine decarboxylase (HDC) activity.

Interestingly, these new agents H₂-R antagonists, such as burimamide, metiamide and then cimetidine and ranitidine were found to inhibit not only histamine-induced gastric secretion, but also that provoked by ordinary meal or even vagal excitation (17). These results were initially explained by the interaction of H₂-R with others, such as muscarinic (M₃-R) and gastrinic (CCK₂-R), receptors at the oxyntic cell membrane on gastric secretory mechanism and this was supported, at least in part, by *in vitro* studies on isolated oxyntic cells by Lloyd and Soll (17). The discovery of H₂-R and their specific histamine H₂-antagonists should be considered as the major breakthrough in the physiology of gastric secretion, reinforcing the significant role of histamine in the regulation of gastric secretion as the “final common chemostimulator” of oxyntic cells (9) (Fig. 2). More recent achievements in pharmacological research led to discovery by G. Sachs and his associates (18) of Na⁺,K⁺-ATPase (proton pump) that was found to be incorporated in active form into the tubulovesicles membrane of intracellular canaliculi of oxyntic cells when

stimulated by vagal nerves, gastrin or histamine, acting *via* the membrane receptors and intracellular mediators such as cyclic AMP (histamine) or Ca^{2+} ions gastrin, acetylcholine and protein kinase pathways. Then inhibitors of this pump, called “proton pump inhibitors” (PPI), such as omeprazole, lansoprazole or pantoprazole, have been obtained and soon found to be the most powerful inhibitors of gastric acid secretion, independent of the way of oxyntic cell stimulation and clinically useful in the control of gastric acid secretion due to higher gastric acid inhibitory efficacy than that of H_2 -R antagonists (18, 19, 20). Moreover, their biological half life was found to be remarkable long, ranging from 14 h for lansoprazole to 46 h for pantoprazole and several times longer when compared to that for histamine H_2 -R antagonists. These discoveries apparently undermined, at least from practical point of view, the significance of both vagal nerves, as well as gastrin, in the stimulation of gastric secretory activity. The question remained whether vagus and gastrin control is less important than histamine in the physiological regulation of gastric secretion. To answer this question, have a quick survey on the history of research related to the origin and mechanism of production of gastric H^+ .

DISCOVERY AND IMPORTANCE OF GASTRIC ACID SECRETION.

Secretion of gastric acid, constituting major “parietal” component of gastric juice (21), is considered as a result of the interaction of numerous stimulatory and inhibitory neuro-hormonal influences, acting on oxyntic cells after ingestion of food. It should be mentioned at this point that already A. Vesalius in 1543 (22) identified the unusually “wandering” (vagal) nerves among various intra-abdominal structures. The question was then raised what functions could be attributed to these “wandering” nerves in gastric secretion and food digestion. It required almost three centuries before W. Prout in 1823 (1) identified HCl in the gastric content of humans and other species, and four centuries before basic studies on gastric secretory mechanisms began. I.P. Pavlov (4) was the first to prove experimentally on sham-fed dogs the functional significance of vagal nerves in the stimulation of gastric acid secretion by showing that transection of vagal nerves abolished gastric secretion induced by sham feeding (cephalic phase) and reduced that provoked by ordinary feeding. These animal studies served later on as a background of clinical usefulness of vagotomy in acid-pepsin diseases initiated by L. Dragstedt (5). With the introduction of microscopy, it was demonstrated that the gastric glands comprise the parietal (oxyntic or acid producing) cells and peptic (pepsinogen producing) cells. R.P. Heidenhain (23) of Breslav University, characterized a “third” type of cells, which adhere to the external surface of epithelial cells, later identified as enterochromaffin-like cells (ECL-cells) and then found to express the histidine decarboxylase (HDC), the major enzyme responsible for the biosynthesis in the oxyntic mucosa of histamine, the final stimulant of parietal cells.

The discovery of gastric acid and pepsinogen initiated endless research related to various aspects of gastric functions, particularly to the mucosal protection against corrosive action of this acid, mainly at the level of intracellular canaliculi of parietal cells where acid concentration reaches extremely high level (about 170 mmol/L). This acid, somewhat diluted by concomitantly secreted water, passes into the lumen of oxyntic glands and then into the gastric lumen to be finally transferred (due to gastric propulsive activity) to the upper duodenum. The question of autodigestion of gastric mucosal lining exposed to the corrosive products of its own acid-pepsin secretion, revitalized after famous Spallanzani's "experiments" in 1782 (3), showing that gastric juice *in vivo* is capable of digesting a variety of food including meat. The existence of putative vital forces in gastric wall, that were initially thought to maintain the viability of the gastric mucosa despite its permanent exposure to highly concentrated acid-pepsin secretion, was soon abandoned and the active mucus-alkaline secretion was proposed to explain the mucosal resistance to acid-pepsin aggression on gastro-duodenal mucosa (24). Gastric oxyntic cells exposed to high concentration of H^+ at the intracellular canaliculi level defend themselves from acid damage by constitutively expressed cyclooxygenase-2 (COX-2) and local production of protective prostaglandins (PG), mainly of E series (25).

Next question, still vital, is as to whether such active mucosal protection based primarily on mucus- HCO_3^- secretion combined with rich mucosal blood flow (26) is merely a local mucosal response to topical acid or whether it can involve intramural or extramural neuro-hormonal mechanisms. In the early 1930s, T. Teorell (27) suggested that H^+ secreted into gastric lumen shows negligible "back-diffusion" into the mucosa in exchange for Na^+ ions and that this results from the permeability characteristics of the gastric mucosa that is hydrophobic in nature. It has been proposed that the gastric surface epithelium, which is covered by adherent mucus gel layer, secretes constantly HCO_3^- into this mucus layer to neutralize luminal H^+ back-diffusing from gastric lumen towards the mucous epithelial cells, thus creating pH-gradient within this mucus layer and preventing surface epithelial cell from irritant effect of luminal noxious substances, including gastric acid (24, 26). In the stomach, the tight junctions between adjacent epithelial cells and continuous secretion of mucus- HCO_3^- on surface epithelium form an efficient protective mucosal barrier, whose thickness reaches about 200-300 μm . Teorell (27) proved, that surface epithelial cells and adherent mucus containing bipolar phospholipids, prevent, due to their high polarity, the ionized mineral acids, such as HCl, from back-diffusing from gastric lumen into the mucosa, but unionized organic compounds such as bile salts or acetylsalicylic acid (aspirin), with a relatively low pKa, can rapidly reach surface mucosal cells by non-ionic diffusion to accumulate in their interior, dissociate in neutral *milieu* and to cause the cell damage. The barrier concept was further developed by H.W. Davenport (28) and C.F. Code and J.F. Scholer (30) and proposed that breaking

the barrier represents an initial step in the process of mucosal injury with a subsequent cascade liberation of histamine-like substances, overt mucosal bleeding, and acute gastritis. This can be observed by gastroscopy in humans after ingestion of aspirin or concentrated ethanol, but also in experimental animals exposed to various irritants and used as animal models of gastric damage for studying gastro-protective efficacy of various drugs (30, 31).

Kaunitz J.D. and Akida Y. (26), Bukhave and his associates (31) and our group (30, 32-34) found that the gastric mucosal barrier, with its tight junctions at the surface epithelial cell layer, and the duodenal mucosal barrier, with its rather leaky epithelial cells, operate due to an active HCO_3^- - mucus secretion, particularly in response to topical application of HCl. The mediators of gastro-duodenal mucus- HCO_3^- secretion appear to be the same, including, constitutively expressed COX-1 (PG) system, the nitric oxide (NO) synthase (NOS)-NO system and capsaicin-sensitive afferent nerves releasing calcitonin-gene related peptide (CGRP) (31-33), all activated by the action of aggressive H^+ -pepsin secretion. The excitation of afferent nerves triggers the extramural cholinergic, nitroergic and peptidergic nerves (releasing NO, VIP, PACAP), leading to enhanced mucus- HCO_3^- secretion. Thus, neuro-hormonal regulation concern not only gastric acid secretion but also mucus- HCO_3^- secretion.

Several anti-ulcer and gastro-protective drugs including sucralfate, bismuth salts (e.g. De-Nol), antacids (e.g. Maalox), and exogenous stable PGE analogs, such as misoprostol, have been found to be effective in the stimulation of gastro-duodenal mucus- HCO_3^- secretion when applied in anti-ulcer therapy (33). Recent studies using isolated parietal cells revealed that cholinergic agonists, such as carbachol, induce expression of COX-2 in these cells *via* several signaling pathways leading to an abundant production of PG, protecting these cells and the entire surface epithelium of gastric mucosa against the damage provoked by secreted H^+ (25). Constitutively expressed COX-1, normally present in gastric mucosa, generates PG, providing the day-to-day maintenance of gastric mucosal integrity, gastric mucosal protection against gastric acid and any other irritants and tonically inhibiting COX-2. The inflammatory process occurring in this mucosa due to infection with e.g. *H. pylori* may induce COX-2 by inflammatory mediators and its products, PG, limit the extent of mucosal damage *via* enhancing the mucosal defense system.

LOCAL vs EXTRAMURAL MECHANISMS INVOLVED IN INDUCTION OF GASTRO-DUODENAL LESIONS BY *H. PYLORI* AND OTHER IRRITANTS

The spiral *bacterium*, has been identified for the first time in human gastric content and depicted under microscope in humans with various gastric diseases, including gastric ulcer and cancer, by W. Jaworski, professor of Cracow Medical Academy in his voluminous Handbook of Gastric Diseases (in polish) over 100

years ago as *Vibrio rugula* (34, 54) (Fig 3). Definitive microbiological characterization of this spiral bacteria named initially *Campylobacter pylori* and then as *Helicobacter pylori* should be, however, attributed to Australian clinical researchers, R. J. Warren, pathomorphologist, and B. J. Marshall (35), who in

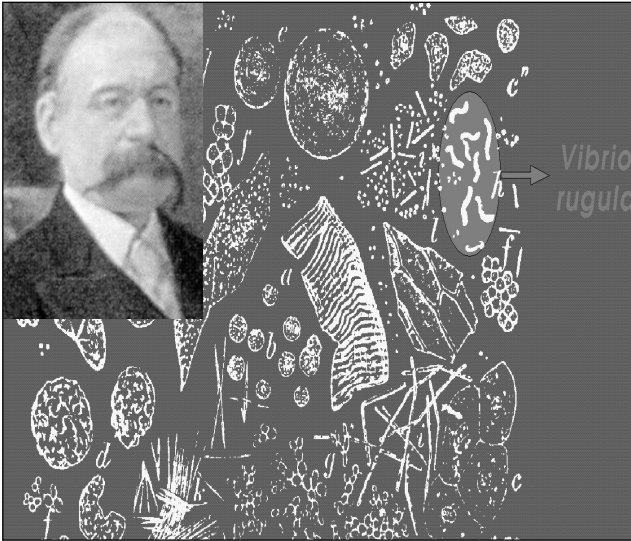


Fig. 3. W. Jaworski discovered in 1896 spiral bacteria in gastric sediment obtained from humans and called them *Vibrio rugula*

	
Barry J. Marshall	J. Robin Warren
Australia	Australia
NHMRC <i>Helicobacter pylori</i> Research Laboratory, QEII Medical Centre; University of Western Australia Nedlands, Australia	Perth, Australia
born 1951	born 1937

Fig. 4. Nobel prize in physiology and medicine in 2005 year

early 1980s proved that bacteria is pathogenic by fulfilling all three Koch's criteria (isolation of *bacterium*, its culture and demonstration of its pathogenicity) and who provided clinical evidence that it causes gastritis and peptic ulcer (34, 35) (Fig 4). It is now evident that the stomach of more than 50% of world adult population, exhibits *H. pylori* infection and ability to damage of gastric mucous cells by "injecting" into these cells ("Trojan horse") of its cytotoxins such as CagA, thereby, turning into mucosal damage accompanied by enhanced expression and release of proinflammatory cytokines, including interleukin-8 (IL-8), interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). This strongly affects gastric acid secretion and alters gastrin-somatostatin ratio as well as mucous surface cell mucus/HCO₃⁻ secretory activity and the quality of adherent mucus gel, resulting in acute and then chronic gastritis. The induction of COX-2 by this *bacterium* may disturb the mucosal protection by the generation of proinflammatory substances, including reactive oxygen species (ROS), though generation of PG that initially has been claimed to belong to damaging proinflammatory "team", but appear to attenuate the damage caused by other proinflammatory substances. It became evident that the eradication of the *bacterium* may restore, at least in part, the disturbed mucosal integrity and reverse the course of gastritis. Administration of mucosal barrier breaker such as aspirin or ethanol usually results in bleeding erosions or exacerbation of chronic gastric ulcerations. Considering duodenal mucus-HCO₃⁻ secretion in response to topical H⁺, delivered into duodenal bulb by *H. pylori* infected stomach, it should be emphasized that the duodenal mucosa behaves somehow differently than gastric mucosa in response to topical acid. Isenberg and his colleagues (32, 36) and Kaunitz et al. (26) using chambered human or animal duodenum, confirmed earlier proposal that, unlike gastric mucosa, where tight epithelial cells constitute the main component of gastric mucosal barrier against H⁺, in the duodenum, H⁺ easily penetrates the duodenocytes, but does not damage them, though transiently decreases their intracellular pH (pH). This strongly activates the basolateral Na⁺-HCO₃⁻ cotransporters, followed by massive inward movement through basolateral membrane of HCO₃⁻ from the extracellular space, leading to activation of the HCO₃⁻/Cl⁻ exchangers in apical membrane of duodenocytes and resulting in a marked stimulation of HCO₃⁻ secretion together with mucus gel that is capable of neutralization of H⁺ ions entering the duodenal lumen, thereby, securing duodenal mucosal neutrality and integrity (Fig. 5).

Flemstrom and his group (37) and Glad et al. (38) provided evidence that, neurally released melatonin that neuronal VIP, participate in the mechanism of the stimulation of duodenal mucus-alkaline secretion by topical H⁺-activating the vago-vagal reflexes and their brainstem centers, also essential for the control of gastric or pancreatic secretion (39). The neuronal pathway involved in activation of gastro-duodenal mucus-alkaline secretion with the contribution of melatonin was proposed by Reiter (40), just reinforcing Flemstrom's idea implicating melatonin in gastro-duodenal protective system. As shown by Isenberg and his

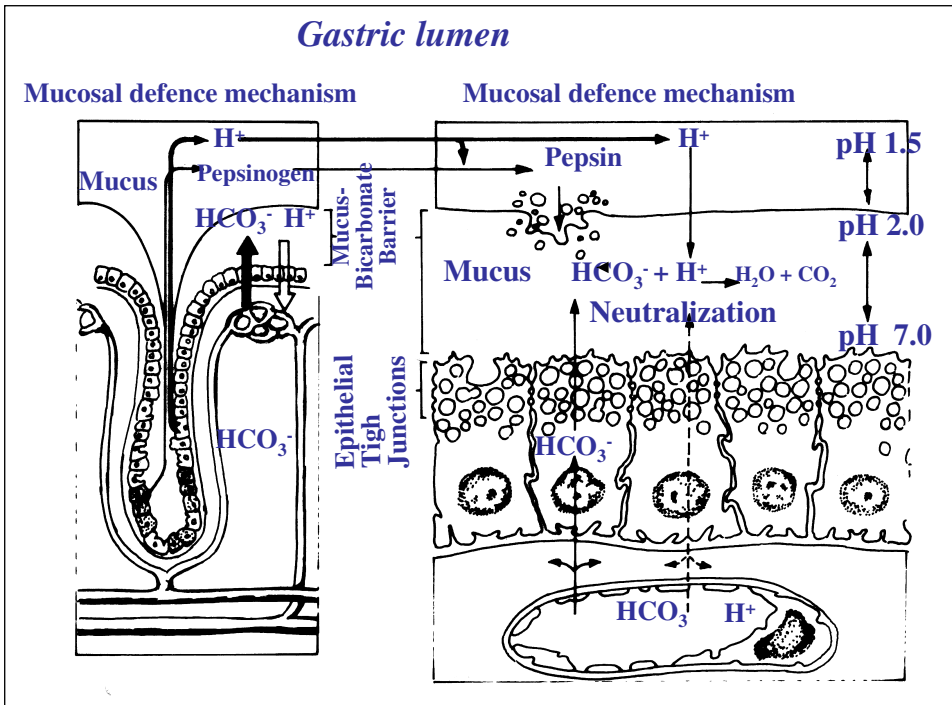


Fig. 5. The protective action of mucus- HCO_3^- secretion in the mechanism of gastric integrity (Konturek S.J. *et al.* 2004).

group (31, 36), *H. pylori* infection reduces duodenal HCO_3^- mucus secretion (despite of increasing mucosal PGE_2 generation) and this allows for excessive penetration of gastric H^+ and other irritants into the mucosa, causing damage of duodenocytes with subsequent formation of gastric metaplastic loci in duodenum. This mechanism leads to the formation of the “*locus minoris resistentiae*” for duodenal *H. pylori* infection and, finally, to the ulcer development. It appears that the *H. pylori* infection of the gastro-duodenal mucosa activates the vago-vagal reflexes (gut-brain axis) that together with direct damaging action on mucosal cells and inhibitory effect of *bacterium* on somatostatin release from D-cells, results in the hypergastrinemia and enhancement of gastric H^+ secretion, contributing to ulcerogenesis. When the *H. pylori* infection is limited to the antral mucosa and accompanied by marked rise in plasma gastrin, gastric acid secretion becomes highly elevated leading to duodenal ulcerogenesis (14, 17). With the *H. pylori* infection extending to the oxyntic gland area and the development of fundic atrophic gastritis, the gastric ulcerogenesis or cancerogenesis may occur and could be accompanied by alteration in plasma gastrin levels and decreased gastric acid secretion (41-43). However, in the majority of *H. pylori* infected humans only gastritis, usually in chronic active form occurs without alterations in gastric acid

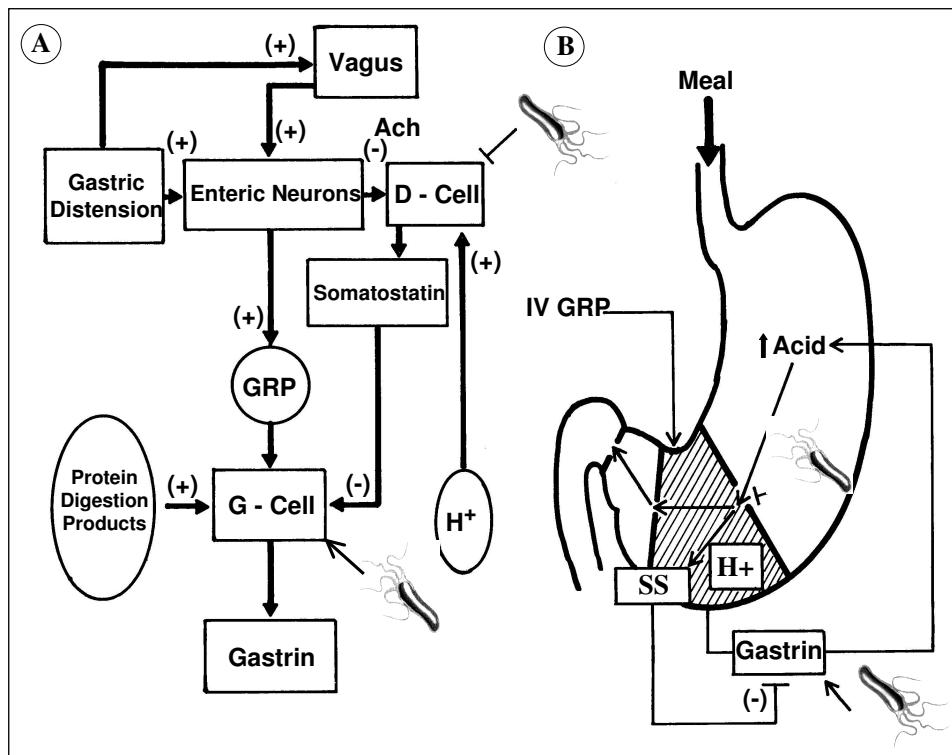


Fig. 6. Neuro-hormonal control of gastric acid secretion and the stimulatory \nearrow or inhibitory influence of *H. pylori* infection on this secretion.

secretion or plasma gastrin levels. Following the successful pharmacological eradication of *H. pylori*, increased basal and stimulated gastric H^+ secretion and elevated hypergastrinemia as well as altered duodenal HCO_3^- mucus secretion are quickly restored even despite of the reduction in mucosal PG generation (32).

BRAIN-GUT AXIS IN GASTRODUODENAL MUCOSAL DAMAGE CAUSED BY *H. PYLORI*, NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID) AND OTHER IRRITANTS

The question remains whether *H. pylori*-induced gastric and duodenal mucosal damage is merely a local phenomenon or whether it also involves the extragastric, namely neuro-hormonal mechanisms. To answer this question, several tools were employed; 1. inactivation of sensory afferent nerves that serve to signal the brain about the changes occurring in the gastrointestinal tract, using large neurotoxic dose of capsaicin in animals; 2. surgical vagotomy to eliminate the transmission of signals from the GI tract to central nervous system (CNS) through the sensory vagal fibers, which constitute over 90% of total vagal fibers, and which supply the gastro-

duodenal area that is crucial for the *H. pylori*-induced inflammatory changes and ulcerogenesis; 3. intracerebral application of various hormonal substances to determine whether the CNS centers can influence the gastric H^+ -secretory pattern and gastro-duodenal mucus- HCO_3^- responses to topical irritants such as *H. pylori* or aspirin application and 4. determination of expression of cFOS in intrinsic and extrinsic neurons involved in the transmission of gut-brain-gut signals (31). Using such wide spectrum of physiological and pharmacological tools, several researchers were able to reveal that the gastro-duodenal mucosa is equipped with a variety of neuronal sensors that respond to the action of luminal irritants such as *H. pylori* produced cytotoxic substances, especially cytotoxin CagA and VacA, luminal acid, ethanol, various drugs e.g. nonsteroidal anti-inflammatory agents (NSAID) or even physiological changes such as chemical ingredients of food, its osmolarity and pH as well as motility and tension of the GI tract wall. Through activation of chemo-, osmo-, mechano- and noci-receptors of gastroduodenal mucosa, the afferent nerves mediate short local or intramural and long, vago-vagal or extramural (axonal and spinal or cerebral) reflexes triggered by luminal H^+ or *H. pylori* and affecting, among others, also the mucus- HCO_3^- secretion, the gastro-duodenal mucosal barriers, and

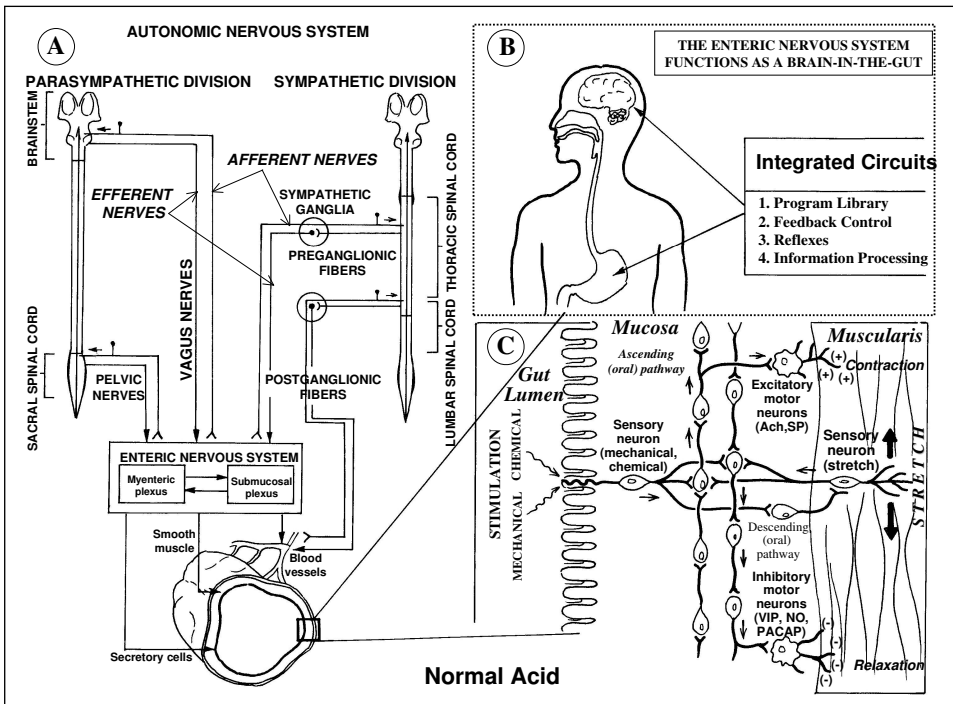


Fig. 7. Brain-gut axis. Enteric neuronal system (ENS) is considered as little brain connected by two pathways with CNS (B). ENS consists of about 100 million of sensory and motor neurons and interneurons in submucosal and muscular plexuses.

mucosal integrity as well as mucosal microcirculation (30) (Fig. 7). The space limitation of this review does not allow for the detailed presentation of the evidence, obtained, in part, from animals experimentations, supporting the involvement of extragastric neuro-reflexes in the maintenance of gastro-duodenal mucosal integrity but it is of interest that e.g. experimental chronic gastric ulcers in rats infected with *H. pylori* or with gastric mucosal damage by acidified aspirin or ethanol are greatly augmented following capsaicin-induced inactivation of afferent sensory nerves or vagotomy. This could be interpreted that both sensory afferent and vagal efferent nerves are involved not only in the pathogenesis of gastro-duodenal mucosal lesions but also are required for normal course of their healing and for the maintenance of mucosal integrity (44). In humans with *H. pylori* infected stomach, the course of post-eradication ulcer healing probably also involves the long vago-vagal reflexes initiated by activation of gastric mucosal sensors by *H. pylori*-released cytotoxins and inflammatory products as well as the reactive oxygen species (ROS) generated in gastric mucosa by *H. pylori* infection. Also the development and subsequent repair of aspirin- and ethanol-induced gastric mucosal lesions may involve the brain-gut axis, starting with the irritation of mucosal chemo-receptors by noxious chemicals and their mucosal toxic products such as ROS (34). This is supported by experimental evidence that inactivation of sensory afferent fibers with capsaicin or subdiaphragmatic vagotomy, eliminating the activity of both afferent and efferent vagal pathways, greatly delays ulcer healing and worsens the lesion induced by various local irritants (34). As mucosal lesions and ulcerations induced either by *H. pylori* infection and aspirin, ethanol or ischemia-reperfusion, that were thought to involve predominantly local mechanism, are greatly affected by inactivation of afferent sensory fibers with neurotoxic dose of capsaicin or by vagotomy, it is tempting to assume that brain-gut axis is involved in pathogenesis of *H. pylori* induced ulcer formation and in healing of these ulcers *via* neural mediation of local mucosal changes including gastric blood flow at ulcer area (31). This does not to exclude the contribution in mucosal repair processes of local anti-ulcer and protective humorals such as PG, gastrin, somatostatin, ghrelin, leptin etc., that may modify the final outcome of mucosal ulcer healing, and repair processes connected with brain-gut axis (45,46).

NEURO-HORMONAL REGULATION OF GASTRIC ACID SECRETION IN INTACT AND *H. PYLORI* INFECTED STOMACH

Besides mucus-HCO₃⁻ component, called “non-parietal component” and playing major protective role in maintaining of gastric mucosal integrity, there is also a very dynamic “parietal component” secreted by the oxyntic cells and characterized by highly concentrated H⁺ and variable volume flow depending upon the degree of gastric secretory stimulation. Postprandial secretion that is customary divided into three overlapping phases; cephalic, gastric and intestinal,

each including neural, usually vagal, and hormonal, predominantly gastrin-histamine, components, resulting in the excitation of oxyntic cells in the following steps; 1. the activation by secretagogues of parietal cell receptors such as H_2 -R, M_3 -R, CCK_2 -R and others; 2. the formation of intracellular stimulatory mediators such as cyclic AMP and Ca^{2+} , 3) the activation of protein kinases that phosphorylate the cytoplasmic protein involved in proton pump activity, 4) the incorporation of active cytoplasmic proton pumps into the membrane covering the intracellular canaliculi and transformation of parietal cells from resting to active state with rich cytoplasmic tubovesicles and active Na^+,K^+ -ATPase (or proton pump) shifted into the apical membrane of numerous intracellular canaliculi of oxyntic cells to increase their surface and ability to secrete H^+ in extremely high concentration into the gastric lumen (19, 46) (Fig. 8).

Phases of gastric secretion and their mechanisms

The mechanism of H^+ secretory stimulation by oxyntic cells in gastric glands is virtually the same during all three phases but the contribution of neural (vagal) and hormonal components varies depending upon the type of secretory stimulants and the phase of secretion (20). Pavlov (4) analyzed the secretory results obtained from his “sham-fed” dogs (with opened esophageal fistula to avoid the swallowed

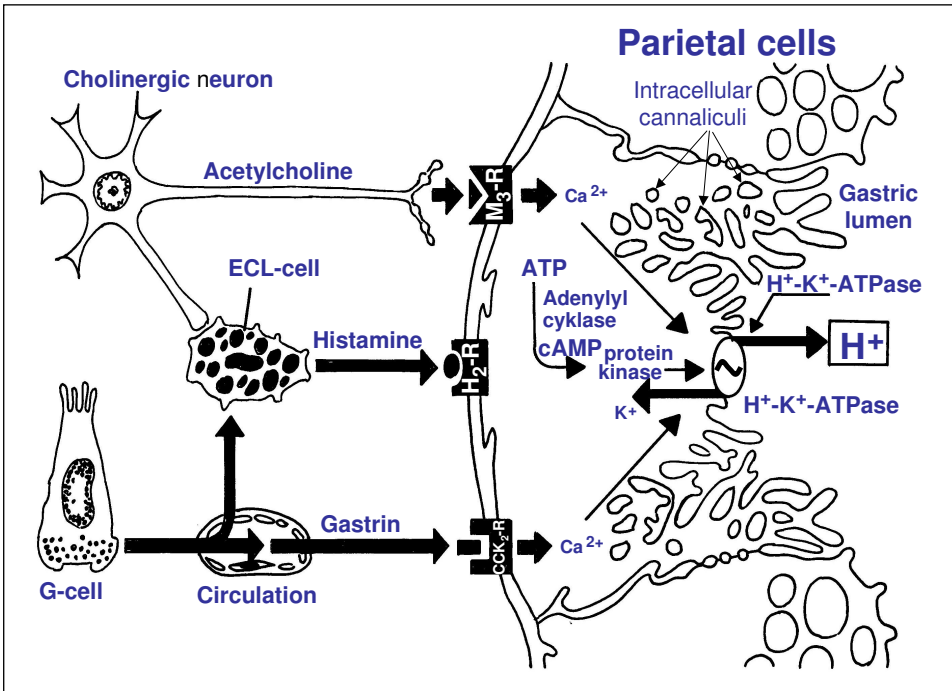


Fig. 8. Endogenous stimulants of parietal cells and their intracellular mediators.

food to enter the stomach) and gastric pouches fashioned from the oxyntic gland area with full vagal innervation to “mirror” the secretory activity of intact stomach, concluded that nervous (cephalic) phase of gastric secretion is entirely neurally mediated. It is initiated by stimulation of various receptors in the head area and followed by projection of sensory information to brain-stem vagal nuclei and then relayed to efferent fibers originating from two vagal complex nuclei; the dorsal motor nucleus and ambiguous nucleus, to supply the oxyntic glands. Since subdiaphragmatic vagotomy abolished the sham-feeding induced gastric secretory response and electrical stimulation of vagal nerves restored this secretion, the evidence for the most important role of vagal reflex stimulation in the mechanism of gastric secretion was considered at that time to be proven. As sham-feeding induced copious gastric acid secretion reaching over 50% of the total acid response to a meal and this was dramatically reduced by subdiaphragmatic vagotomy, Pavlov (4) concluded that this secretion is entirely vagally-mediated. Unfortunately, Pavlov at the end of his “neuro-gastroenterological” research, which gave him world acclaim and Nobel prize, was discouraged by undeniable evidence of hormonal (gastrin) control of gastric secretion (see *Fig. 8*), left classic gastrointestinal physiology research and focused his interest on higher CNS functions conditioned reflexes using salivary glands as effector organs (6, 12, 13, 51).

Vagal nerves, gastrin, GRP, TRH and histamine in the stimulation of gastric secretion

“Continuing” the original Pavlov's *nervism* idea, we used his model of secretory excitation i.e. sham-feeding in dogs with esophageal fistula and “modified sham-feeding” in humans before and after vagotomy and/or antral mucosectomy to eliminate, respectively, vagal component and major endogenous source of gastrin (16, 45-47). We confirmed that in both species (dogs and humans) sham-feeding caused very potent stimulation of gastric acid secretion, reaching about 50-60% of pentagastrin-maximum. Several interesting findings have been obtained in these experiments; 1. this neural stimulation of gastric secretion was accompanied, especially in dogs, by significant rise in plasma levels of gastric acid stimulatory hormones, including gastrin, gastrin-releasing peptide (GRP) (probably of neuronal origin) histamine, ghrelin, cholecystokinin (CCK) with small increment in plasma levels of gastric inhibitory hormones such as pancreatic polypeptide (PP), peptide YY (PYY), leptin and secretin (*Fig. 6*); 2. the removal of antral mucosa in dogs completely abolished plasma gastrin response and also significantly reduced acid response to sham-feeding and 3. anticholinergics such as atropine actually increased plasma gastrin response to sham-feeding, while causing almost complete suppression of acid response to this procedure (17). Our recent results with vagal stimulation obtained in dogs by various techniques including classic sham-

feeding as well as that induced by insulin hypoglycemia or 2-deoxy-d-glucose cyto-hypoglycemia confirmed that the highest response to vagal stimulation did not exceed 50% of that attained with exogenous stimulus such as histamine or gastrin applied in a dose inducing maximal gastric acid secretory response. This stimulation was, however, accompanied by the rise in plasma concentration of gastrin that probably contributed, at least in part, to this stimulation because the blockade of gastrin receptors (CCK₂-receptors) by agent S-0509 (48) caused significant reduction in gastric acid response to sham feeding. The contribution of CCK was probably negligible to this stimulation because the blockade of receptors for CCK (CCK₁-receptors) with specific blocker, L-364,718, failed to affect this neurally mediated stimulation of gastric acid secretion (33). The most effective inhibitor of this secretion was found to be atropine which almost completely eliminated the sham-feeding as well as insulin- and 2-deoxy-D-glucose-induced gastric acid secretion (*Fig. 9*). Therefore, there is no doubt that vagal-cholinergic component plays a most important role in cephalic stimulation, while gastrin, GRP, histamine and ghrelin represent only a minor component of vagally excited gastric acid secretion. Probably vagal-cholinergic

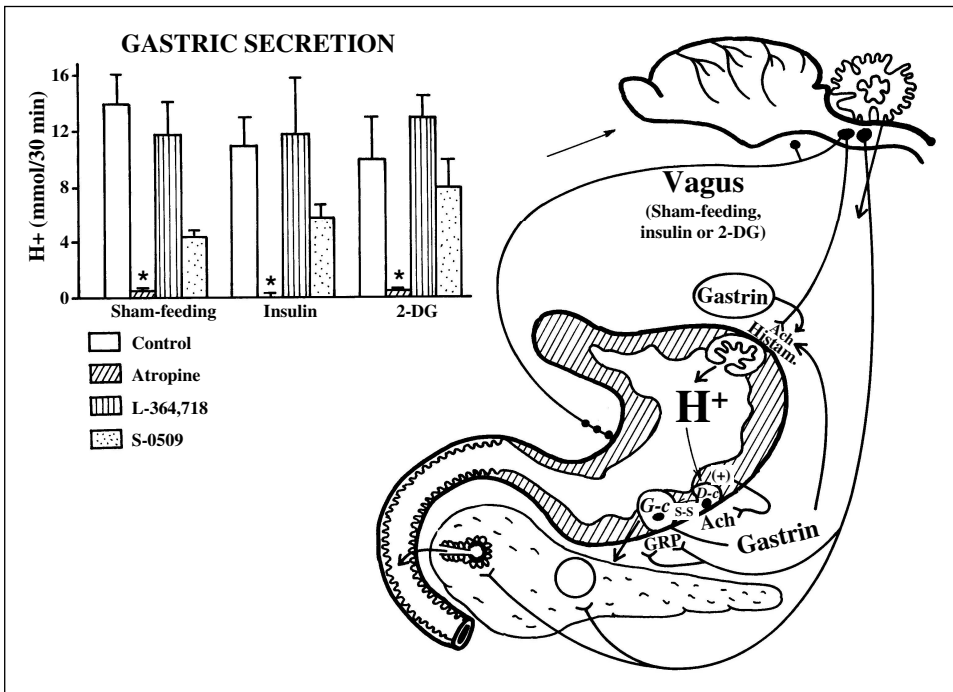


Fig. 9. Gastric acid response to vagal stimulation induced by sham-feeding, insulin hypoglycemia or 2-deoxy-D-glucose (2-DG) glucocytopenia in dogs under control conditions and following i.v. application of atropine (25 μ g/kg), L-364, 718, CCK₁-receptor blocker (10 mg/kg) or S-0509 (15 mg/kg), CCK₂-receptor blocker (unpublished data).

component interacts with neurally released gastrin and GRP on oxyntic glands leading to augmented gastric acid secretion.

Relation between the G-cells, D-cells and ECL-cells in control of gastric secretion

As indicated above this vagal-cholinergic stimulation of oxyntic cells never reaches maximal value that can be obtained with maximal dose of exogenous stimulants such as gastrin or histamine in humans and animals. This limitation of gastric acid response to vagal-cholinergic stimulation is probably caused by local paracrine action on oxyntic cells of somatostatin released from the D-cells in oxyntic mucosa by luminal H⁺ but remaining under inhibitory influence of cholinergic nerves and locally released histamine from the ECL cells acting *via* H₂-receptors as well as amylin colocalized in the G-cells to enhance their secretory activity by autocrine mechanism. In the antral mucosa the D-cells and somatostatin release are under inhibitory influence of cholinergic nerves but are stimulated by neuronal VIP, histamine released from enterochromaffin (EC) cells and antral natriuretic peptide (ANP) (16). It is of interest that sham-feeding causes a small but significant rise of plasma ghrelin that was elevated already before sham-feeding but quickly declined after this procedure suggesting that this hormone could be responsible for appetitive behaviour accompanying sham-feeding in fasted animals and might contribute to initial part of stimulation of gastric acid secretion (33).

These results seem to point out that the mechanism of cephalic or vagally stimulated sham-feeding is quite complex and this complexity is further enhanced by recent studies indicating that centrally injected or endogenously released in the brain (by cold) thyrotropin-releasing hormone (TRH) from the neurons projecting from the caudal medullary raphe nuclei to the dorsal vagal complex. TRH might participate in neural stimulation of gastric secretion and alterations in blood flow (49, 50). This is confirmed by the fact that intracisternal application of TRH causes an increase in gastric acid secretion and the formation of acute gastric lesions. Furthermore, the intracisternal administration of a thyrotropin-releasing hormone-1 receptor antisense oligonucleotide, can abolish sham-feeding induced gastric secretion in conscious rats. This stimulus for vagal excitation and gastric secretion may originate from the brain itself (20) and may contribute to vagally-induced stimulation of gastric secretion during the sham-feeding.

The discovery of gastrin in the *antrum* by Edkins and its publication in the Journal of Physiology (6), made only small impression on Pavlov, who just enjoyed Nobel prize awarded to him in 1904, but soon ordered the verification of Edkins (6) discovery in his lab. Under his supervision, the hormonal (gastrin) contribution to the regulation of gastric secretion, was amply confirmed (51). The verification of the "Edkins hypothesis" led him to accept, though reluctantly, the importance of hormonal control of gastric secretion, and this was probably the major reason of his departure from gastro-intestinal physiology to research on conditioned reflexes (51).

The cephalic or anticipation phase is usually accompanied by increased appetitive behavior and by the plasma increment of various gut hormones related to the food intake. One of them is ghrelin, that was found to be released from the X/A cells of empty stomach similarly to motilin released from EC cells with which it shares close chemical homology and similar spectrum of biological action) at the time of sham-feeding, leading to some increase in gastric acid secretion and gastrin release as well as the alteration in gastric motor activity (33). Also alcohol, that releases ghrelin, increases gastric secretion while increasing the appetitive behavior, whereas gastric distention, leptin, peptide YY (PYY), oxyntomodulin, glucagone-like peptide-1 (GLP-1) and amylin released from intestinal or pancreatic endocrine cells show opposite effects.

Gastric phase of gastric secretion, that according to Edkins (6) is mediated by hormone gastrin, also appears to involve short and long vago-vagal reflexes initiated by the distention of the stomach by food and chemical irritation of gastric mucosal receptors by products of food digestion. However, both vagal nerves and gastrin appear to act mainly indirectly on gastric glands that is *via* releasing histamine from the ECL-cells located in close vicinity of oxyntic cells to stimulate their H₂-histamine receptors.

Discovery of histamine secretagogue activity and its relation to gastrin by L. Popielski (8), former pupil of Pavlov in St. Petersburg, appointed (due to Pavlov intervention) in 1904 to be chairman of the Department of Pharmacology in Lvov (Lemberg) in the eastern part of Galicia, initially supported neural reflex nature of the gastric acid control in agreement with the dogma of Pavlov's *nervism*. However, before his death in 1920, he obtained in 1916 the preparation of histamine that at that time was called beta-imidazolylethyl-amine or simply "Beta-1", discovered earlier by Berger in collaboration with H. Dale (52) who somehow missed the action of this amine on gastric secretion. Following accidental discovery of potent secretagogue effect of histamine, Popielski still persistently rejected "Edkin's gastrin hypothesis" of gastric secretion and believed that gastrin has no role in the stimulation of gastric acid secretion. Popielski using pure synthetic histamine found that this compound administered subcutaneously to dogs with a gastric fistula induced an abundant and dose-dependent gastric acid stimulation (8). This secretion was not affected by vagotomy or scopolamine, indicating that it acts directly on parietal cells independently of vagal nerves and cholinergic innervation (8). As stated by B.P. Babkin in his famous book "*Secretory Mechanisms of the Digestive Glands*" (51), it is a historical paradox that the secretagogue action of histamine was discovered by a man who spent practically his entire research career under the strong influence of Pavlov *nervism* in contesting the theory that the gastric digestive glands could be regulated by gastrin (8). His discovery related to extremely potent gastric secretagogue activity of histamine apparently was at variance with the concept of the hormonal regulation of gastric acid secretion (Edkin's hypothesis) until 1964, when R. Gregory and H. Tracy of Liverpool (7) isolated, purified, and finally synthesized gastrin, showing that gastrin is a peptide. With progress of gastrin purification and

chemical identification, attempts were made to “revitalize” the “Edkin's hypothesis” of gastrin. Uvnas and his group (10, 11) demonstrated in a series of experiments on sham-fed Pavlovian dogs provided with antral pouches, that gastrin release remains under vagal control, as sham-feeding effectively stimulated gastric secretion only in dogs with a preserved and innervated antral portion of the stomach. Following antrectomy, a background minute dose of gastrin (to mimic the amounts of hormone released physiologically), restored fully the sham-feeding-induced gastric secretion, indicating a potentiation between gastrin, histamine and vagus in the stimulation of gastric acid secretion (11). In the meantime, C.F.Code (9) collected, however, massive evidence for establishing that histamine, rather than gastrin, is the “final common chemostimulator” of oxyntic cells by showing that: 1. histamine, as shown by Popielski, acts directly on oxyntic cells to stimulate H^+ secretion; 2. histamine can be detected in large amounts in oxyntic mucosa, being released locally by ECL-cells, expressing active histidine decarboxylase to transform histidine into histamine; 3. histaminase, that destroys histamine, can not be detected in oxyntic mucosa; 4. histamine is released into the blood draining the stomach and can be detected in urine following acid secretion and; 5. histamine is released by stimulants of gastric secretion such as food or gastrin (9). Then, Code (9) described the pathway of histamine generation and metabolism, showing that it originates from the histidine by its decarboxylation by histidine decarboxylase (HDC) and by methylation of its side chain to yield methyl derivatives. These methylated histamine derivatives were found to be even more powerful gastric stimulants, but when converted to a 1-4methyl derivative by methylation at the imidazole ring, they lost stimulatory effectiveness. It is now well-established, that gastrin and acetylcholine act on ECL-cells in antral mucosa to release histamine which in turn stimulates *via* H_2 -receptors at the oxyntic cells. Three other histamine receptors have been identified (H_1 , H_3 and recently H_4). Gastrin was found to stimulate the production, storage, and release of histamine from ECL cells and to aid the proliferation of these cells. Several other substances such as cytokines (e.g. IL-1), aspirin, indomethacin, dexamethasone, lipopolysaccharide were found to stimulate HDC activity and to release histamine (53). Hypergastrinemic states such as observed in patients with *gastrinoma* or following prolonged treatment with potent antisecretory drugs such as H_2 -receptor antagonists or potent proton pump inhibitors (omeprazole, lansoprazole, pantoprazole etc) may develop ECL-cell hyperplasia and carcinoid tumors with excessive production and release of histamine (53).

As mentioned before, two types of gastrin/CCK-receptors have been identified including CCK_1 -R specific for CCK and CCK_2 -R recognizing both gastrin and CCK (54). The activation CCK_2 -R of parietal cells, similarly as M_3 -R, stimulate H^+ secretion through the rise of intracellular Ca^{2+} concentration, while histamine acts on these cells through the H_2 -R to activate the adenylate cyclase-cAMP system activating protein kinases and eventually Na^+,K^+ -ATPase or proton pump of parietal cells. Specific antagonists of CCK_2 -R such as S-0509 (see *Fig.9*) (48) are effective inhibitors of gastric acid secretion and useful tool in the examining acid-pepsin

secretion. CCK₂-R were also found to inhibit gastrin-induced gastric acid secretion in animals but no studies with that antagonist have been carried out in humans. Surprisingly, gastrin-deficient mice, have impaired basal and gastrin-stimulated H⁺ secretion, but their parietal cells *in vitro* respond normally to major secretagogues mediators such as gastrin, histamine or acetylcholine and possess intact intracellular mediators for these secretagogues such as the release of Ca²⁺ and adenylate-cAMP system mediating their effects on H⁺ secretion, suggesting that intracellular Ca²⁺-related signaling pathway is upregulated to compensate for the loss of gastrin effect *via* CCK-2-receptors (54). Furthermore, as gastrin is known to enhance histamine production in ECL cells, the deficiency of gastrin in these animals results in the decrease in histamine content in oxyntic mucosa.

As almost all species, including humans are infected by *H. pylori* that usually colonizes antral mucosa as depicted for the first time by Prof W. Jaworski in 1896 (55) and then confirmed by Nobel prize winners Marshall and Warren this year (see Fig. 3 and 4) this *H. pylori* infection causes the disturbance of the ratio of G-cells to D-cells and impairment of gastrin-somatostatin relation (see Fig. 6) resulting in hypergastrinemia with accompanied hyperchlorhydria and duodenal ulcerogenesis, the physiological analysis of gastric secretory data should consider the *H. pylori* status, particularly that the infected stomach is capable of producing a side chain α -methylated histamine that is a potent stimulant of the G-cells and gastrin release. With respect to oxyntic mucosa such *H. pylori* infection, that may result from prolong rise in the intragastric pH due to e.g. use of PPI or other gastric acid inhibitors, such infection results in gastric hypochlorhydria and atrophic gastritis accompanied with extremely high plasma gastrin due to removal of acid-mediated inhibition of somatostatin release that normally controls gastrin secretion. Thus, our knowledge of gastric acid secretory mechanisms, so tediously elaborated during last century, has been seriously challenged by spiral bacteria which also affects the integrity of gastric mucosa as well as, gastric pathology, including gastritis, gastro-duodenal ulcerogenesis and probably also cancerogenesis depends on the *H. pylori* status, as appreciated by Nobel prize, presented this year to discoverer of this bacteria and providing evidence that it contributes to gastric pathology (Fig. 10 and 11).

CONCLUDING REMARKS

a. The most potent stimulant of oxyntic cells appears to be histamine, whose gastric secretagogue activity was discovered approximately 80 years ago by Popielski and confirmed to be the major gastric acid secretagogue and final common chemostimulator of oxyntic cells.

b. Histamine is released from the ECL-cells, by food and other gastric secretagogues, especially gastrin and in the antral mucosa it stimulates D-cells by H₃-receptor to release somatostatin that by local paracrine action controls gastrin

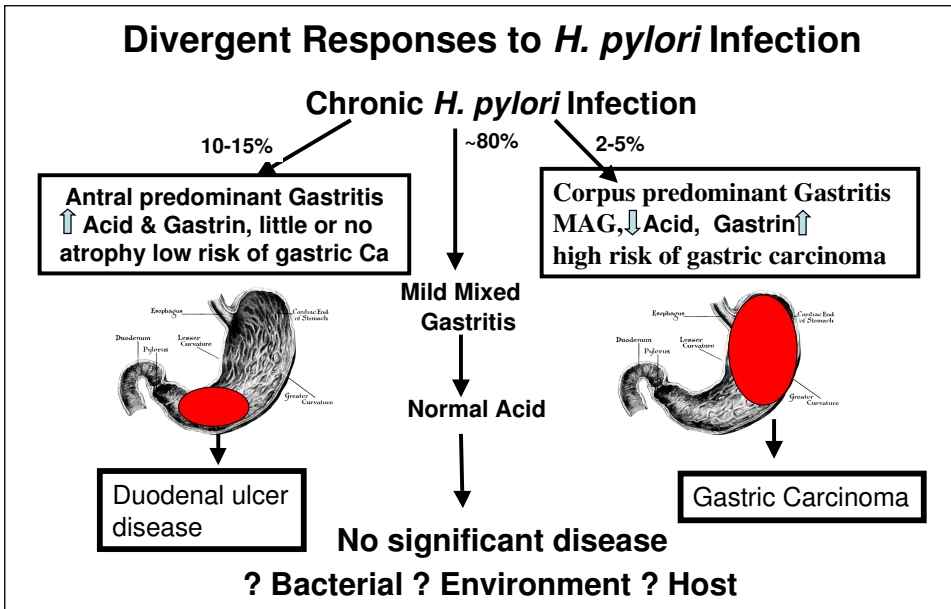


Fig. 10. *H. pylori* infection results in gastritis in all subjects but in some of them (15%) causes gastric, duodenal ulcers (DU) or gastric cancer (Ca).

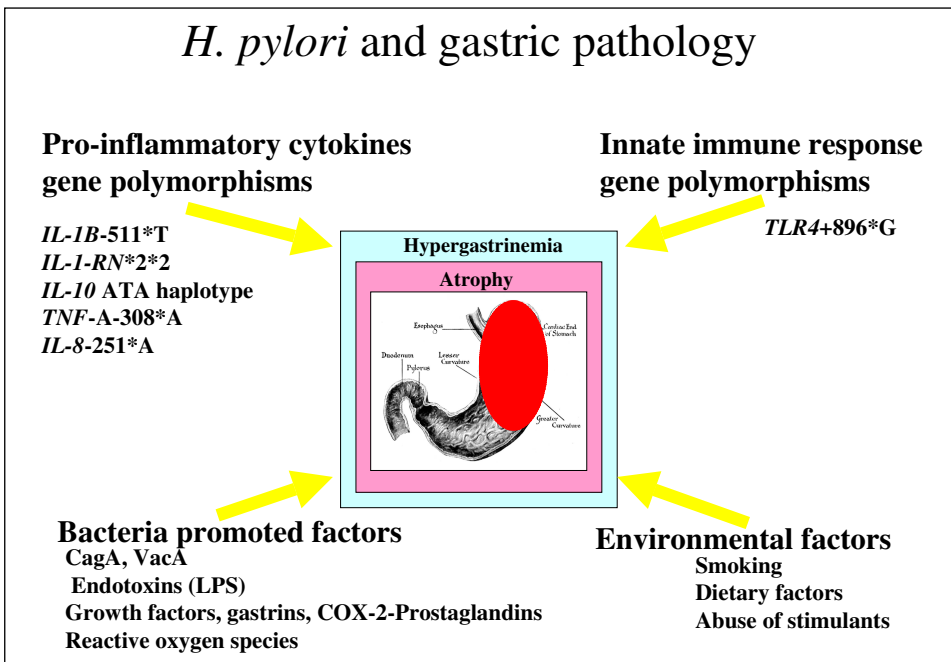


Fig. 11. *H. pylori* infection may be also responsible for gastric cancerogenesis that follows expression and action on gastric mucosa of cytotoxins (CagA and VacA), proinflammatory cytokines and gastrins.

release and H⁺ secretion by oxyntic cells as well as inhibits the D-cells to limit the inhibition of gastric acid secretion.

c. Duodenal but not gastric ulcer, which usually results from the *H. pylori* infection, is accompanied by hyperchlorhydria and this is regularly associated with excessive release of histamine from the ECL-cells due to increased concentrations of gastrin, as well as hypergastrinemia induced by the action on N α -methyl histamine, a product of *H. pylori* infected stomachs.

d. Neuro-hormonal pharmacology of the stomach and duodenum succeeded in discovery and clinical use of potent inhibitors of gastric acid secretion such as H₂-R antagonists and PPI that abolish all modes of gastric acid secretory stimulation but, unfortunately, result in hypergastrinemia that may help in spreading of *H. pylori* infection towards the proximal part of the stomach with subsequent fundic atrophy and cancerogenesis. The eradication of *H. pylori* abolishes hyperchlorhydria and hypergastrinemia and is successful in treatment of peptic ulcer and probably also in prevention of the *H. pylori*-related gastric cancerogenesis.

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