The progress in basic and clinical gastrology indicates that gastric mucosal integrity represents a balance between offensive and defensive factors. The main offensive factors appear to be gastric acid and pepsin under health conditions, while the non-steroidal anti-inflammatory drugs (NSAID) and *Helicobacter pylori* (*H. pylori*), infecting this mucosa, are currently considered the most important “aggressive” factors under pathological conditions. To the list of the aggressive factors, also stress, certain cytokines (TNF-α, IL-8, IL-11 and IL-18) and oxygen or nitrogen free radicals should be added. The aims of this review is the presentation of the involvement of aggressive and protective factors in the control of gastric acid secretion and appetite regulating hormones in maintaining gastric mucosal integrity and its protection against damaging factors.

**Key words:** gastric acid, mucosal damage, gastroprotection, vagal nerves, gastrin, ghrelin, leptin, melatonin

THE CONTROL OF GASTRIC ACID SECRETION UNDER NORMAL AND PATHOLOGICAL CONDITIONS

The stomach can be divided into two major areas; the oxyntic gland area, occupying about 80% of the gastric surface and the antral gland area, representing remaining 20% of surface. The most typical feature of the oxyntic mucosa is the presence of the parietal cells (*Fig. 1*), that secrete acid due to the activity of proton pumps located in the lining of intracellular secretory
cannaliculi opening at the luminal surface of these cells (1). The hallmark of the antral gland area are the G-cells, accumulated in higher number in the lesser curvature and producing, storing and releasing gastrin (2, 3), the major gastric hormone, releasing histamine from the enterochromaffin-lile cells (ECL-cell). Gastrin interacts at the level of intracellular signaling pathways with histamine and acetylcholine (Ach) in the stimulation of the parietal cells (4). In addition, the Gr-cells producing ghrelin (5-7), the D-cells, producing somatostatin (SST) (2, 3) and the entero-chromaffin (EC) cells some of which generate ANP (7, 8) and some others, called also entero-endocrine (EE) cells, produce melatonin (MT), the most ubiquitous and phylogenetically oldest signaling molecule (9, 10). SST inhibits acid secretion by acting in paracrine fashion directly on parietal cells as well as indirectly by inhibiting histamine release from the ECL cells that are in close vicinity parietal cells (Fig. 2). Histamine, released by the ECL-cells, mainly in response to gastrin, acts on the parietal cells through stimulation of their membrane-bound histamine-H$_2$ receptors coupled to activation of adenylate cyclase and generation of adenosine 3’,5’-cyclic monophosphate (cAMP), playing the role of the main intracellular “second messenger” in the stimulation of gastric acid secretion.

Fig. 1. Acid secretion induced by excitation of parietal cell receptors (H$_2$-R, M$_3$-R and CCK$_2$-R) by specific agonists (histamine, acetylcholine and gastrin) and activation by protein-kinases of the resting proton pumps with their fusion with membrane covering intracellular canaliculi and secreting H$^+$ into these canaliculi.
SST-producing D-cells are present in large number, especially in the mucosa of pyloric gland area, and here their hormonal product, SST, exerts a direct tonic restraint on the G-cells controlling gastrin secretion, thus indirectly affecting gastric acid secretion (11).

Besides luminal acid, several other substances affect SST secretion including ANP, secreted by EC-cells, representing the subtype of antral EE-cells, causing inhibition of the the gastrin secretion from the antral G-cells and, subsequently, enhancing gastric acid secretion (2). Other cells, called EE cells have been recently identified in the lining of the entire gastrointestinal tract (GIT) to generate melatonin (MT) from the L-tryptophan (Fig. 3)(9, 10). MT is an indol, that is the most ubiquitous and probably the oldest signaling molecule, serving predominantly as powerful antioxidant, directly scavenging oxygen (ROS - reactive oxygen species) or nitric free radicals (RNS - reactive nitrogen species) and also as an effective stimulator of the expression and activity of anti-oxidative enzymes protecting gastric mucosa against ROS and RNS generated in the mucosa under various oxidative stress conditions (9).

The hallmark of the oxyntic glands are the parietal cells that derive from progenitor or stem cells in the neck and *isthmus* of the gland and then they migrate downward to replace the damaged oxyntic and peptic cells and upwards...
to replace the desquamated and apoptotic surface epithelium cells. The turnover of the surface epithelial cells is several times shorter (2-3 weeks) when compared to those in the deeper portion of oxyntic glands.

Gastric mucosa is well-supplied with neural fibers of intramural neurons forming a neural network called the enteric nervous system (ENS) and forming two plexuses; one, myenteric plexus located between longitudinal and circular muscle layers to regulate the motility, and another or submucosal plexus situated just under the mucosa, that is mainly involved in the control of microcirculation, secretion and absorption (14-17). The ENS is often called the “little brain” because it contains over 100 million neurons operating within the GIT and acting, at least in part, independently of the CNS. It does, however, send numerous projections to CNS and receives from there efferent fibers, reaching the gastro-intestinal tract (GIT) through the extramural sympathetic and parasympathetic nerves synapsing on the ENS neurons, and thus forming the neural “brain-gut axis” (Fig. 4).

The external autonomic nerves, such as vagal nerves, contain about 10% of efferent fibers that function mainly as pre-ganglionic cholinergic neurons synapsing with the neurons of ENS (Fig. 5). Postganglionic neurons of ENS utilize a variety of other neurotransmitters, such as gastrin releasing peptide (GRP),

Fig. 3. Functional aspects of gastric secretion, showing major stimulatory and inhibitory pathways regulating gastric acid secretion with focus on the antral natriuretic peptide (ANP) and melatonin (MT).
Fig. 4. Brain-gut axis and its components

Fig. 5. Overall scheme showing regulation of gastric acid secretion involving vagal nerves acting on gastric fundus and antrum through neural, hormonal or paracrine pathways in subjects without or with H. pylori infection and the presence of acid in gastric lumen [Adapted with permission from Schubert ML and Peura DA (8)].
vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP) and substance P (SP). These neurotransmitters may act directly on the parietal cell as is the case for Ach, or they regulate acid secretion indirectly by affecting the release of gastrin from G-cells, SST from D-cells, histamine from ECL cells, ghrelin from Gr cells, ANP and melatonin from the EC and EE cells. The majority (over 90%) of vagal fibers are, however, sensory afferents that are either intrinsic (confined to the gastric wall) or extrinsic, both containing and releasing at their terminals SP, CGRP, VIP or PACAP. There is a good body of evidence that luminal acid or \textit{H. pylori} adhering to the surface of epithelial cells activate the sensory nerves containing neuropeptides such as GRP, SP, PACAP or CGRP, which, in turn, activate higher autonomic centers or stimulate locally the GIT mucosa to secrete SST resulting in the inhibition of gastrin secretion and subsequently gastric acid secretion (17, 18).

There are three main stimulants for acid secretion, each acting through separate stimulatory receptors on the parietal cells and two main transduction pathways within these cells (19, 20) (see Fig 1). Ach released locally from the cholinergic neurons of ENS in the wall of the stomach activates M\textsubscript{3} receptors on oxyntic cells, leading to an increase in intracellular Ca\textsuperscript{2+} (20). Histamine, released from the ECL-cells (21) stimulates the H\textsubscript{2}-receptors of parietal cells to activate membrane-bound adenylate cyclase-cAMP system and increases the intracellular level of cAMP. Gastrin act via separate CCK\textsubscript{2}-receptors on parietal cells, to cause an increase in intracellular Ca\textsuperscript{2+} level (similarly as after muscarinic stimulation). This explains the relatively high efficacy of H\textsubscript{2} receptor antagonists in the inhibition of acid secretion because, not only do they interrupt the effect of histamine released from the ECL cells on parietal cells, but also they block the effect of gastrin, which acts predominantly by releasing histamine from ECL cells. Both intracellular messengers, cAMP and Ca\textsuperscript{2+}, stimulate the intracellular protein kinases that in turn activate quiescent (inactive) H\textsuperscript{+}, K\textsuperscript{+}-ATPases in cell cytoplasm, called also “proton pumps” (see Fig. 1), to translocate them into the membrane covering the intracellular cannaliculi, which secrete H\textsuperscript{+} in high concentrations (up to 170 mM). Such high concentration of H\textsuperscript{+}, in the cannaliculi might damage the parietal cells but here uniquely expressed cyclooxygenase-2 (COX-2) and generated PGE\textsubscript{2} serve as safeguard for parietal cells (22) through the cytoprotective local effect of this arachidonate metabolite on parietal cell in similar to that historically described by Andre Robert in late 70s (23). The most potent antisecretory agents are the proton pump inhibitors (PPI) which block active H\textsuperscript{+},K\textsuperscript{+}-ATPase or proton pumps, the final step in acid secretion and thus, they inhibit acid secretion induced by all three agonists.

SST and probably also PGE\textsubscript{2}, released due to constitutively expressed COX-1 in the gastric mucosa, appear to play a key role in the feed-back control of H\textsuperscript{+} secretion acting both in oxyntic and antral mucosa (24, 25). In the basal or interdigestive phase, it is SST that is responsible for maintaining acid at an economically low level, though its activity is partly reduced by ghrelin, which is
released at the highest rate when the stomach is empty and not-stimulated either by a meal or secretory stimuli acting via cephalic phase mechanisms (25-28). In the *antrum*, SST exerts a tonic restraint on gastrin secretion, while in the oxyntic mucosa, it inhibits acid secretion from the parietal cells by direct action on these cells as well as through the inhibitory effect on ECL-cells and histamine release, the major stimulatory pathway involved gastric stimulation of acid secretion. Concerning ghrelin involvement, Gr cells are present predominantly in the in oxyntic mucosa and ghrelin release in the empty stomach exerts its stimulatory influence on parietal cells probably by enhancing the release of gastrin from the antral G-cells (in fasted stomach) and by the stimulation of histamine release from the ECL-cells in the vicinity of the parietal cells (29).

There are two major mechanisms, whereby acid stimulates SST secretion in both the oxyntic and the antral mucosa (Figs 2 and 3) i.e. (a) by acting directly on the D cells to enhance SST release and (b) by release from the ECL-cells of histamine and the stimulatory effects of this via $H_3$ on the D-cells to activate the major feedback control of acid secretion of parietal cell (see Figs 2 and 3).

Postprandially, there is a robust acid secretion that is initiated by food intake and activation of vagal reflexes involving both extramural vagal nerves during cephalic phase of gastric secretion and intramural reflexes involving cholinergic neurons activated during the gastric phase, both resulting in the inhibition of SST release and direct stimulation of parietal cells by previously described ,the “gastrin-histamine team”. During the cephalic phase, the cholinergic neurons are initially activated via the thought, smell and taste of food activating the conditioned and unconditioned reflexes postulated over hundred years ago by Pavlov (30). During the gastric phase, this is accomplished mainly by the reflex activation of extramural and intramural cholinergic neurons by food-induced distention of gastric wall, leading directly to inhibition of SST-containing D cells via activation of $M_2$ and $M_4$ receptors on these cells. In the *antrum*, the inhibition of SST secretion leads to the enhanced release of gastrin (due to the reduction in local tonic inhibitory activity of SST), while in the oxyntic gland area, the inhibition of SST augments the “gastrin-histamine team”-induced stimulation of acid secretion from parietal cells. In addition, cholinergic neurons, acting via $M_3$ receptors, directly stimulate the G-cells to release gastrin and parietal cells to secrete acid. After ingestion of the food, they are further activated during the gastric phase by the food bolus distending the stomach, direct action on open-type G-cells of various luminal factors such as products of protein digestion and activating intramural and long vago-vagal reflexes releasing GRP from postsynaptic neurons that stimulates gastrin secretion and directly the parietal cells (Fig. 5).

Following food intake, the acid secretion starts to increase and this secretion is accomplished both by removing the restraint exerted by SST on gastrin and histamine release, and by the action of “gastrin-histamine-Ach” team stimulating the parietal cells. Postprandial cholinergic (Ach) stimulation during the cephalic
phase requires preserved vagal pre- and post-synaptic nerves and intact antrum with copious release of gastrin to stimulate gastric acid secretion, confirming well-known vagal-gastrin interaction in the cephalic and gastric phase stimulation of acid secretion (31, 32).

With an exit of acidified food from the stomach to duodenum, gastric acid secretion gradually declines due to increased SST secretion activated by gastric acid passing through the antral mucosa and acting directly on the antral D cells to stimulate SST release (33). This leads eventually to the restoration of basal secretory state. In addition, with decreased gastric distention, the activation of VIP neurons that stimulate SST release, helps the restoration of the basal state of acid secretion in enterogastrone-like manner (35-37) thought the term enterogastrone-like action has been originally referred to the inhibition of gastric acid secretion by fat (not acid) digestion products in the intestine. Thus, with exit of gastric acid from the stomach, the luminal gastric pH declines and acid itself stimulates SST (enterogastrone-like substance) release from both the antrum and the fundus of the stomach.

It should be emphasized that both luminal acid and the presence of H. pylori modulate acid secretion by affecting SST release and subsequent alteration of gastrin release and acid secretion (8). These effects were confirmed in a luminally perfused mouse stomach (Fig. 6A). The mouse stomach perfused with different concentrations of acid showed that gastric luminal effluent collected at 5 min intervals exhibited gradual decline in gastrin release accompanied by the rise in SST release as measured by radioimmunoassay. As shown on this figure, the baseline pH in the stomach was about 4, and as acid was added to luminal perfusate, the secretion of SST was stimulated. These studies were confirmed and extended by Harty’s group (38). He applied a rat antral sleeve method, using various agonists and antagonists and demonstrated that acid activates sensory CGRP neurons that stimulate SST secretion and hence inhibit gastrin release. These pathways explain also how PPI induce hypergastrinemia. PPI decrease acid secretion, in part, by a reduction in acid activation of CGRP neurons resulting in the decrease of SST secretion. Decreased SST secretion, by removing the restraint exerted by this hormone on gastrin secretion, leads to an increase in gastrin release. The same event occurs in patients with atrophic gastritis involving oxyntic gland area. With a more profound decrease in acid secretion, plasma SST level showed marked increase, while was gastrin secretion was pronounced resulting in hypergastrinemia. In susceptible patients, the marked hypergastrinemia can lead to hyperplasia of ECL cells, followed by dysplasia, and then eventually neoplastic transformation into carcinoid tumors (39).

**H. PYLORI AND GASTRIC SECRETION**

It is well recognized that H. pylori infection involving the antral mucosa results in gastric acid hypersecretion and may cause peptic ulcer formation,
particularly in duodenum where islets of gastric metaplastic mucosa appear in infected patients. Only about 10-15% of patients chronically infected with *H. pylori* show antral predominant gastritis (Fig. 7), leading to gastric acid hypersecretion (40). In these patients, *H. pylori* induces a decrease in SST secretion in antral gland area, leading to an increase in gastrin and subsequently to a rise in acid secretion. The precise mechanism by which *H. pylori* infecting the antral mucosa decreases SST secretion is not known but it is likely to involve cytokines released from the inflammatory infiltrate. When levels of acid and pepsin overwhelm the individual’s mucosal defense mechanisms, a duodenal ulcer may develop, particularly in the area where gastric metaplastic islets appear in the duodenum.

Most patients, whoever, who exhibit chronic *H. pylori* infection of the oxyntic gland area secrete less than normal amounts of acid, so they have, therefore, gastric acid hypossecretion (40). This is due to atrophy of the parietal cells caused by; (1) gastritis, (2) cytokines released from the inflammatory infiltrate such as interleukin-1beta (IL-1β), and (3) cytotoxic factors released by *H. pylori* that

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**Fig. 6.** Plasma SST and gastrin levels collected from the mouse stomach perfused with various concentrations of acid (A), with broth containing *H. pylori* before and after administration of tetradotoxin (B), with broth containing *H. pylori* before and after activation of sensory nerves with capsaicin (C) and with broth containing *H. pylori* before and after administration of blocker of receptors for CGRP (D) (unpublished observations).
inhibit transcription of the H⁺,K⁺-ATPase as demonstrated by Saha et al (41). Among these cytotoxic factors produced by H. pylori are; ammonia released by this germ that typically shows high urease activity and the action on mucosal cells of cytotoxins produced by the germ such as CagA, VagA, LPS and others. It is of interest that eg, CagA, the most noxious H. pylori produced factors, enter the mucosal cells through special type IV secretory mechanism, resulting in the damage of mucosal cytoskeleton and intracellular activation of NFkB leading to the abundant production of pro-inflammatory cytokines such as IL-8. It is known from the pre-H. pylori area that the acute infection involving entire gastric mucosa (pangastritis) induces epidemic hypochlorhydria. This pathology may be related to H. pylori infection, occurring immediately after gastric infection with this germ expressing CagA and VacA, also involves an increase in SST and a decrease in gastrin secretion, leading to a decrease in acid secretion. Studies on rat antral mucosa, using an Ussing chamber to maintain the polarity of the luminal and serosal surfaces of the mucosa showed that exposure of the luminal aspect of mucosa to H. pylori cultured from patients with duodenal ulcer results in germ concentration-dependent increase in SST secretion and decrease in gastrin secretion (8). These changes in SST and gastrin secretion appears to be reversible and the removal of the germ from the mucosa caused a prompt reversible increase in SST secretion and decrease in gastrin secretion (see Fig. 6). Both responses

Fig. 7. Effects of H. pylori the gastric corpus and antrum.
were blocked by the neurotoxin, tetrodotoxin, indicating that neural pathways were involved. Both responses were also blocked by capsaicin, inactivating involvement of sensory pathways. Both pathways were blocked by the blocker receptor antagonist CGRP$_{8-37}$ confirming that CGRP sensory neurons were involved. Thus, *H. pylori*, similar to intraluminal acid, activates sensory CGRP neurons that stimulate SST secretion, thus inhibiting gastrin release and acid secretion. This pathway may explain, at least in part, how *H. pylori* can effectively colonize the stomach. When *H. pylori* is ingested, it induces a hypochlorhydria that allows itself to inhabit the stomach. The activation of the neural pathways also explains how relatively small number of *H. pylori* can have such a profound effect on acid secretion because the effect of these bugs is augmented via these neural networks.

In summary, the key regulator of acid secretion is the SST producing or D-cell. During the interdigestive period, acid secretion is maintained at low level by the action of SST. In the antrum, SST tonically inhibits gastrin secretion, while in the oxyntic gland area, SST tonically restrains the parietal cell directly as well as indirectly by inhibiting histamine secretion from ECL cells. After ingestion of a meal, a marked acid secretion appears due to the activation of cholinergic neurons that decrease SST secretion in both the antral and oxyntic portion of the stomach, leading to a remarkable increase in gastrin release from the G cells as well as to the stimulation of histamine secretion from the ECL cells. There is an effective feedback mechanism, whereby acid stimulates SST and that in turn inhibits gastrin and acid secretion. In states of hypochlorhydria, induced either by atrophic gastritis or by antisecretory agents, a decrease in acid leads to a decrease in SST and, thus, to hypergastrinemia. Among patients with chronic *H. pylori* infection, about 15% of cases of this infection is limited to the antral mucosa and either due to the germ itself or the cytokines produced by inflammatory infiltrate, these patients manifest markedly decreased SST secretion and thus increased gastrin and acid secretion. In states of hypochlorhydria, which is probably due to activation of CGRP neurons stimulating the SST that in turn decrease gastrin release and acid secretion. This is one mechanism that permits *H. pylori* to colonize within the noxious environment of the stomach.

**GASTRIC MUCOSAL PROTECTION AND ITS MECHANISMS**

Gastric acid and pepsin secretions produced by oxyntic glands in response to their complex neuro-hormonal stimulation gain an access to the stomach lumen, but its back-diffusion through a continuous thick mucus gel adherent to reach the mucosal surface is relatively slow. This adherent mucus gel forms an “unstirred layer” that acts as physical barrier against luminal pepsin from
reaching and damaging of the underlying gastro-duodenal epithelium (42). As surface mucosa epithelium secretes continuously $\text{HCO}_3^-$ into this unstirred layer, its major function is to neutralize back-diffusing acid into the mucus gel layer and to maintain a near neutral pH at the mucosal surface interface. The source of the $\text{HCO}_3^-$ secretion by mucosal cells is an energy-dependent metabolic process in these surface epithelial cells but some of $\text{HCO}_3^-$ released ion to the blood draining the oxyntic gland area during its active acid secretion creates the “alkaline tide”. Bicarbonate exiting from acid-secreting parietal cells may also alkalinize the blood that perfused surface epithelium to be up taken by these cells and then secreted into the unstirred layer of mucus gel. The role of this adherent mucus gel layer is not only to support surface neutralization of acid but also to form a protective physical barrier against luminal pepsin attempting to reach and damage the underlying epithelium. The mechanisms regulating mucus-alkaline secretion appear to be controlled by various factors including vagal-cholinergic stimulation and neuro-hormonal or numerous humoral factors such as gastrin, CCK, ghrelin, leptin, CGRP, MT, PG, NO, various growth factors such as epidermal growth factor (EGF), transforming growth factors (TGF) and others (42). These factors were found not only to alter mucus/alkaline secretion but, as described for the first time by A. Robert (43), to induce gastroprotection (cytoprotection) against numerous topical necrotizing agents such as ethanol, aspirin or even boiling water. Most recognized gastroprotective or cytoprotective agents initially

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**Fig. 8.** Gastric mucosal barrier and its components.
examined by Robert were native or stable analogs of PGE, but then they were found to be useful only in the prevention of mucosal damage but failed to enhance of healing of acute or chronic gastric lesions and ulcerations, unless applied in high pharmacological dose that inhibit gastric acid secretion, indicating that this anti-ulcer effects of stable PGE analogs represent merely their gastric acid inhibitory, but not proposed cytoprotective activity (44), but the activation of gastric mucosal protective mechanisms such as stimulation of mucus/alkaline secretion, increased mucosal blood flow, enhanced mucosal hydrophobicity, increased gastric mucosal content of sulphydryls, and facilitation of repair process of this mucosa may also be included (23). It is of interest that these stable PG analogs such as misporostol were found to be of particular usefulness in the treatment of gastro-duodenal mucosal damage caused by therapeutic application of potent inhibitors of enzymes producing endogenous PG (COX-1 and NSAID) that are known to induce mucosal damage and ulcerations (44), particularly when used sporadically. In contrast, the repeated administration of these agents, especially aspirin (ASA) was shown to induce increased tolerance of gastric mucosa to these aggressive agents possibly due to the mucosal production of gastroprotective lipoxins as originally proposed by Wallace or melatonin as recently proposed by Konturek et al (45). The great hope related to the possible clinical use of PGE stable analogs in the prevention and therapy of gastric damage evoked by various irritants declined with the discovery of very potent antisecretory agents such as H2-receptor antagonists and PPI. However, endogenous PG became of interests as the major mediators of gastroprotection induced by numerous anti-ulcer drugs (44). In addition, the mucosa adaptation to various gastric irritants including NSAID, ethanol or stress discovered in both, humans and experimental animals, appears to be mediated by PG, NO or neuropeptides such as CGRP released in response to such irritation (46). The role of PG, NO and afferent nerves with their neuropeptides such as CGRP, was proved to be of crucial importance under conditions such as ischemia. Following short-term ischemia, various organs including stomach become resistant to injury by a variety of irritants and this phenomenon, called ischemic preconditioning, was found to be mediated by PG, afferent nerves CGRP and NO (46) (Fig. 9).

Recently, various scavengers of free radicals have been suggested to be involved in the pathogenesis of mucosal lesions induced by stress conditions (47). Most of mucosal lesions such as ulcers and or inflammatory changes accompanying the use of NSAID or H. pylori infection were found to be accompanied by the excessive generation of reactive oxygen (ROS) or nitrogen species (NOS) and decreased activity of antioxidative enzymes such as catalase, superoxide dismutase (SOD) or glutathione peroxidase (GPx). Scavengers of ROS or NOS of endogenous origin such as melatonin or exogenous origin including vitamin C, tocopherol, flavonoids and melatonin or its precursor L-tryptophan, were found to be highly effective in the prevention and treatment of gastroduodenal mucosal lesions induced by various mucosal irritants (47).
Further studies are needed to assess whether the scavengers of reactive oxygen or nitrogen species might be beneficial in the therapy of gastroduodenal lesions and ulcerations.

In summary, surface epithelium lining the gastric mucosa produces mucus/alkaline secretion that serves an important role as one of several lines in mucosal protection such as mucosal cell regeneration and mucosal blood flow, all playing the role in the maintenance of mucosal integrity.

HORMONES INVOLVED IN THE REGULATION OF FOOD INTAKE CONTRIBUTE TO MUCOSAL DEFENSE

The appetite affecting hormones such as CCK, leptin, ghrelin, orexins and cannabinoids, were considered for the long time to not only food intake, but also gastric mucosal integrity (48). Recent evidence indicates that appetite regulating hormones such as CCK that is known to serve as the peripheral signal for the hypothalamus to control appetite behavior, could be also implicated in gastroprotection and ulcer healing. For instance, Bado et al (49) and our group (48) were first to find out that the administration of exogenous leptin that was accompanied by a significant increment in plasma levels of this peptide, exhibited dose-dependent gastroprotective activity against the ethanol-induced lesions.
similar to that obtained with exogenous CCK. It is of interest that this protective effect of leptin occurred without any alteration in gastric acid secretion by this peptide. It was, therefore, proposed that leptin might be considered as a truly gastroprotective peptide because its protective activity occurred at a dose level that did not affect gastric acid secretory activity, as originally proposed by Robert (43). The cooperation of leptin with other gut-originating peptides such as CCK in gastroprotection is unknown despite the existence of a functional synergistic interaction between these two gut peptides in the suppression of food intake (50-51). As the protective effect of CCK was accompanied by the elevated plasma leptin level and decreased leptin contents in the gastric mucosa, the enhanced resistance of gastric mucosa against the noxious effect of mucosal irritants such as ethanol strongly suggests that leptin may be involved in the mechanism of CCK-induced gastroprotection (52). The physiological importance of leptin in maintenance of gastric integrity was further supported by the fact that peptone meal, that is known to stimulate the release of CCK and to raise its plasma levels, also significantly elevated the concentrations of leptin in the plasma, while attenuating significantly ethanol-induced gastric lesions (53). Furthermore, the fact that ob mRNA was overexpressed in the stomach of CCK-treated rats invited the speculation that the increments in plasma leptin levels following treatment with CCK originates mainly from the gastric mucosa and plays an important role in CCK-evoked gastroprotection against ethanol lesions due to local activation of leptin gene and enhancement of the release of gastric leptin into the circulation (53). We and others have demonstrated that the mechanism of the gastric acid stimulatory effects of exogenous CCK and those evoked by endogenous CCK released in response to peptone meal are similar and depend upon the activation of type A CCK receptors (52-54).

The common feature of both, leptin and CCK, appears to be a significant rise in the release of NO into gastric lumen suggesting that the protective and hyperemic effects of both these hormonal peptides are mediated by NO (54). This is corroborative with the observation that the gastroprotective effects of leptin and CCK can be blocked by pretreatment with the NOS suppression with L-NAME and restored by addition to L-NAME of L-arginine, a NOS substrate, but not D-arginine, which not a substrate of NOS (53). Thus, NO seems to play a crucial role in both the mucosal hyperemic and gastroprotective effects of CCK-8 and leptin. The NO could originate from cNOS expression and activity because mRNA for cNOS was upregulated in the gastric mucosa of leptin- and CCK-treated rats. Interestingly, prostaglandins do not appear to contribute to the observed gastroprotection by leptin and CCK because the suppression of cyclooxygenase (COX) by indomethacin failed to influence the protective and hyperemic effects of exogenous leptin or CCK-8 (56). In clear contrast to the effect of COX suppression, leptin-induced gastroprotection and accompanying hyperemia were almost completely abolished in vagotomized rats suggesting that vagal nerves are necessary for the protective activity of this peptide (58, 59).
Indeed, previous observations revealed that the vasodilator response to low dose CCK-8 was inhibited by acute bilateral subdiaphragmatic vagotomy and atropine (55). Moreover, it was postulated that peptides of the gastrin/CCK family could participate in the mechanism of gastric mucosal integrity through the activation of sensory nerves releasing a variety of vasodilatory mediators such as CGRP and tachykinins (56). Furthermore, the gastroprotective action of CCK-8 on the gastric mucosa involves activation of CCK-A receptors localized on vagal capsaicin-sensitive sensory fibers because the functional ablation of afferent sensory neurons with capsaicin greatly reduced hyperemic activity of both leptin and CCK-8 but it became more effective against the protection afforded by CCK-8 than by leptin. This finding indicates that sensory nerves are essential for microcirculatory response, but appear to be of less importance in the mediation of protective of leptin. Since the protective and hyperemic effects of leptin and CCK were significantly attenuated by vagotomy and capsaicin deactivation of sensory afferent nerves, it is reasonable to assume that vago-vagal reflexes possibly involved in the release of NO and sensory neuropeptides from these nerves could contribute to the gastroprotection and the hyperemia observed after administration of leptin and CCK-8 (53).

It is well known, that aspirin, a classic NSAID, which possesses anti-inflammatory activity, can damage gastric mucosa when ingested in larger amounts (44-46). It is of interest that in contrast to CCK, leptin attenuated the damage induced by ASA. Leptin administration which remained gastroprotective against these ASA-induced gastric damage did not influence gastric acid secretion, while CCK which enhanced gastric acid secretion failed to protect gastric mucosa against ASA lesions. This indicates that leptin is superior to CCK in protection against gastric lesions induced by ASA, that requires gastric luminal acid to damage this mucosa. Thus, it seems likely that CCK by increasing gastric acid secretion could contribute to the enhanced formation of ASA-induced damage, while leptin that did not raise this secretion was effective against both, ethanol- and ASA-induced damage.

Another gut-brain originated appetite hormone, ghrelin describe as a 28-amino acid peptide, has been discovered in rat and human gastrointestinal tract, particularly in oxyntic mucosa of empty stomach, as an endogenous ligand for growth hormone secretagogue receptor (GHS-R) (57, 58). In contrast to leptin which inhibits appetite, ghrelin stimulates food intake and body weight gain exerting a modulating effect on energy expenditure by acting peripherally through afferent nerves and centrally on hypothalamic feeding centers. This peptide was also shown to enhance the gastric motility and gastric secretion. Recent studies revealed that ghrelin is produced by the special Gr (A/X) cells that are located mainly in oxyntic mucosa and which differ from other endocrine cells such as ECL, EC or D cells (58). Ghrelin has no relevant homology with any known gastrointestinal peptides but potently stimulates the release of GH and acts as a natural ligand for the GHS. Physiological actions of ghrelin are mediated by two
receptor subtypes GHS-R1a and GHS-R1b, the latter being devoid of high-affinity ligand binding and signal transduction activity. Administration of ghrelin causes weight gain via appetite stimulation and reduction in fat oxidation. Previous studies in humans revealed that gastrectomy produced dramatic fall in the plasma ghrelin levels, whereas fasting and anorexia nervosa were accompanied by the elevated plasma ghrelin concentration supporting the notion that the gastrointestinal tract, primarily the stomach, is a major source of circulating ghrelin (57) that could be considered as a starvation-related hormone.

Orexin-A (OX-A) is a novel 33 amino acid peptide originally purified from rat brain, in which an N-terminal pyroglutamyl residue and an amidated C terminus were discovered (59). Family of orexins also include orexin-B, which possesses an amidated C terminus and shares about 46% of homology of orexin-A (60, 61). Both, orexin-A and orexin-B act through two subtypes of receptors named OX-1R and OX-2R (61). It was previously reported that icv administration or direct injection of orexin-A into the lateral hypothalamus (LHA) results in an increase in the food intake in rodents in a dose-dependent manner (61). Immunohistochemical studies revealed that orexin-positive neurons are present in the lateral hypothalamic area. This is corroborative with the observation that an orexin-A antibody, injected intracisternally, inhibits both, gastric secretion and food intake stimulated in fasted rats, suggesting that brain orexin has a physiologically relevant action on feeding behavior (62, 63).

Recently, neurons and endocrine cells in the gut were reported to display orexin-A-like immunoreactivity (63). It was documented that orexigenic and anorectic neuropeptides found in the hypothalamus including neuropeptide Y (NPY), ghrelin and galanin are present within the gut of rodents and humans (63). Besides brain, the mRNA for preproorexin-A was also detected in the stomach, pancreas and ileum of humans and experimental animals (64). Since these neurons also displayed the leptin immunoreactivity it was proposed that orexin cells in the gut may be able to integrate the signal from adipose tissue along with the nutrient signal from the mucosa. With all these information, orexins are members of the growing list of peptides that coexist and act in both the CNS and ENS supporting and extending the concept of brain-gut axis involvement in the control of feeding and energy homeostasis.

Little is known about the factors that might affect ghrelin and orexin-A release in the stomach and whether these peptides sharing similar effects on food intake, can contribute the mechanism of gastric mucosa integrity and if so, to what extent these peptides could affect, for instance, gastric lesions induced by stress. Such stress damage of gastric mucosa appears as acute gastric mucosal lesions occurring as complications in severely ill patients after burns, sepsis, major surgery or trauma to the central nervous system. Critically ill patients are at increased risk of developing stress-related gastric mucosal lesions and gastrointestinal bleedings. Previous human and animal studies documented that stress-induced bleeding erosions are accompanied by a marked decrease in the
gastric blood flow, a substantial decrease in the gastric mucosal PG content and NO release, an inhibition of gastric mucus and bicarbonate secretion and the increased formation of free oxygen metabolites appearing as a consequence of gastric ischemia and inflammation (66). Numerous studies demonstrated the usefulness of cold-restraint stress and proved it as a clinically relevant experimental model for studying of pathomechanism of acute gastric damage and protection (65). It had been shown that gastric acid plays an important role in the development of stress-induced gastric damage and is the most common endogenous factor responsible for the destruction of epithelial cells.

The studies on the role of ghrelin and orexin-A in the mechanism of gastric mucosal defense and gastroprotection has revealed that peripheral administration of ghrelin and orexin-A attenuated the formation of lesions induced by ethanol and cold stress (66, 67). It is of interest that centrally or peripherally applied ghrelin and orexin-A influenced gastric acid secretion, while attenuating stress-induced lesions and this effect appears to be mediated by the vagal innervation and the expression of CGRP mRNA, an important neuropeptide released from sensory nerve endings (78). Both these events are considered as important components of the brain-gut axis involved in the gastroprotective effects of not only CCK and leptin as mentioned in this review but also by ghrelin and orexin-A, especially against formation of stress-induced gastric lesions.

On the other hand, the *H. pylori* infection of the human gastric mucosa, which is now considered to play a role as the causal factor in the pathogenesis of gastritis and peptic ulcer, was found to attenuate the mucosal expression and release of ghrelin and to reduce appetite. This was confirmed in *H. pylori*-infected gastric mucosa of Mongolian gerbils, which is now widely accepted as an appropriate model to study the mechanism of *H. pylori* infection under experimental conditions (69). It is of interest, that after successful cure of *H. pylori* in healthy asymptomatic subjects the plasma ghrelin concentration increased, which in turn led to enhanced appetite and weight gain (69, 70). This imply that increase in ghrelin in *H. pylori* cured gastric mucosa may contribute to the increasing obesity seen in Western populations, where the prevalence of *H. pylori* is low.

Recently, endogenous PG have been implicated in the control of food intake and appetite but the possibility that these cytoprotective arachidonate metabolites could also play an important role in the gastroprotective actions of ghrelin and orexin-A has not been explored. The question remains whether both, ghrelin and orexin-A contribute to gastroprotection against gastric lesions caused not only by artificial irritants such as ethanol, but also against those caused by vascular disturbances resulting from stress or ischemia-reperfusion that lead to severe microbleeding erosions and the fall in the microcirculation. Arachidonate metabolites were believed to act as the classic mediators of cytoprotection but as mentioned above, in contrast to NO, endogenous PG do not appear to contribute to the observed gastroprotection by peptides such as leptin and CCK against ethanol-induced gastric lesions. Therefore, it was important to check whether endogenous PGs as
well as the expression of key enzymes of PG biosynthesis, namely, cyclooxygenase (COX-1) and COX-2 are involved in the possible gastroprotective activity of ghrelin and orexin-A against acute stress-induced gastric erosions. Moreover, the questions arose whether the suppression of COX by non-selective COX inhibitor, indomethacin, or rofecoxib, the highly selective COX-2 inhibitor, could influence the gastroprotective and hyperemic activity of ghrelin and OX-A.

The role of ghrelin in the mechanism of gastric mucosal defense and gastroprotection has been little investigated except for the report of Sibilia et al. (71) and our group (67, 68). Indeed, it has been documented that ghrelin-induced protection and hyperemia is accompanied by the enhancement in the mucosal PGE₂ generation. Ghrelin and orexin-A applied centrally exhibited comparable gastroprotective activity as that observed upon their peripheral administrations against the mucosal damage induced by corrosive substance such as ethanol and non-topical ulcerogen such as stress (Figs 9 and 10). This is supported by observation that ghrelin and orexin-A attenuated the gastric lesions induced by ethanol in various concentrations and those provoked by stress while producing an increase in gastric mucosal blood flow and the plasma level of these hormones. Both, indomethacin and rofecoxib greatly attenuated the protective and

![Fig. 10. Mean lesion area and gastric blood flow in rats treated with vehicle, ghrelin or orexin in rats pretreated with capsaicin alone or in combination with CGRP. Asterisk indicates a significant change as compared to vehicle-control. Asterisk and cross indicate a significant change as compared to rats without capsaicin denervation. Hashes indicates a significant change as compared to the values obtained in capsaicin denervated rats without supplementation with CGRP.](image-url)
hyperemic effects of not only ghrelin but also OX-A, indicating that endogenous PG possibly derived from COX-1- and COX-2-PG pathway may mediate beneficial effects of these hormones on the gastric mucosa of the stomach.

It has been shown that both, central ghrelin and orexin-A, are orexigenic peptides and affects feeding at the levels of hypothalamus (arcuate nuclei) and, as in case of ghrelin, the GH secretion partly due to an activation of gastric vagal afferents transmitting visceral sensory signals to the brain. The observation that ghrelin and orexin-A stimulate gastric acid secretion is in keeping with the original observation that an increase in the gastric secretion was achieved after parenteral but not topical administration of these peptides. The mechanism by which ghrelin and orexin-A increase gastric acid secretion after its peripheral as well as central administrations could be related to the elevation of plasma gastrin concentration, since a considerable increases in these hormone plasma levels was detected following ghrelin or orexin-A application. Gastrin itself could contribute, at least in part, to the secretory as well as protective and hyperemic effects of ghrelin and orexin-A but their direct or indirect effects on histamine release should also be considered. Existing evidence indicates that parenteral administration of histamine and agonists of histamine $H_1$, $H_2$ and $H_3$ was shown to afford gastroprotection against ethanol damage, predominantly due of enhancement in the protective mucus secretion and an increase in the gastric blood flow (72, 73), and the decrease in ethanol absorption and the formation of histodilutional barrier. Indeed, Suzuki al. (69) reported that acid secretory activity of ghrelin could be mediated by a potent acid secretagogue, histamine, but whether histamine could be implicated in gastroprotective action of ghrelin, an orexigenic peptide of gastric mucosa origin, has been little studied. Recent evidence accumulated at least, with the respect to ghrelin, indicates that ghrelin-induced gastroprotection was accompanied by the significant increase in plasma histamine level and an overexpression of histamine mRNA in the gastric mucosa. Moreover, the blockade of histamine $H_2$-receptors by ranitidine and an inhibition of histidine decarboxylase activity, a major enzyme involved in histamine biosynthesis by $\alpha$-FMH, attenuated ghrelin-induced gastroprotection against ethanol damage suggesting that gastroprotective action of ghrelin is mediated, at least in part, by the activation of HDC/histamine system in the gastric mucosa.

In addition, ghrelin and orexin-A significantly increased the expression of mRNA for CGRP, the peptide that is known to enhance the gastric mucosal blood flow. The mechanism by which ghrelin and orexin-A improve this gastric mucosal blood flow could involve a direct effect of these peptides on blood vessels due to their potent vasodilatory activity or indirect effect mediated by vasodilatory neuropeptides originating from afferent sensory nerves. The deactivation of rat primary afferent nerves, using large neurotoxic dose of capsaicin about 2 weeks before the stress experiment, aggravated stress-induced gastric damage as compared to rats without capsaicin denervation and significantly reduced the GBF when compared to that in animals with intact
sensory nerves (70). Such a capsaicin-induced deactivation of sensory nerves significantly attenuated the gastroprotective activity of central and peripheral ghrelin and orexin-A and completely abolished the rise in GBF induced by these peptides. Moreover, the replacement therapy with exogenous CGRP, the major neuropeptide released from sensory afferent nerves, restored the protective and hyperemic activity of ghrelin and orexin-A against the stress-induced gastric lesions (68). In addition, the expression of mRNA for CGRP, the major neuropeptide released from sensory afferent nerves, was enhanced in ghrelin-treated animals indicating that this major sensory nerve neuropeptide is essential for microcirculatory response and of crucial importance for the gastroprotective activity of this hormone.

The beneficial effects of ghrelin and orexin-A on the protection of gastric mucosa against acute injury could be related to the suppression of free oxygen metabolites and anti-inflammatory properties of this peptide. Recent studies indicate that ghrelin could be considered as an important mediator in the regulation of inflammation due to its anti-inflammatory activity (71). Ghrelin caused a significant reduction in the mRNA expression of proinflammatory cytokine, TNF-α, confirming that anti-inflammatory effect of ghrelin contributes to the gastroprotection and hyperemia exhibited by this peptide against stress- and ischemia-reperfusion-induced damage. Further support for the potent anti-inflammatory effects of ghrelin comes from the fact that ghrelin inhibited the activation of NFκB, which is considered as a critical signaling molecule in inflammation and pathogenesis of stress damage (72). Previous studies revealed that transcription factor NFκB activation is involved in acute injury of stomach and that activation of NFκB induces gene programs leading to transcription of factors that promote inflammation. Therefore, it is assumed that a potential mechanism whereby ghrelin could modulate inflammatory response is blocking of activation of NFκB and this was confirmed by observation that ghrelin inhibits an activation of NFκB in human endothelial cells in vitro.

In summary, we conclude that in addition to gastric secretory stimuli such as CCK, gastrin and histamine, also hormones such as leptin, ghrelin and orexin-A, besides their recognized function in the regulation of appetite, energy homeostasis, fat and carbohydrate metabolism and gastrointestinal motility, may contribute to gastroprotection due to their local, expression in the gastric mucosa in response to mucosal injury. This notion is supported by recent observations that mRNAs for ghrelin and OX-A are upregulated in the gastric mucosa exposed to stress, possibly playing the role in limitation of stress damage. Furthermore, both ghrelin and orexin-A may act through the activation of the capsaicin-sensitive sensory nerves in the gastric mucosa that are responsible for the increased production of CGRP in gastric mucosa exposed to stress. The contribution of CGRP to the CCK-, leptin-, ghrelin- and OX-A-induced gastroprotection is further supported by the observation that the functional ablation of sensory nerves by capsaicin attenuated significantly the protective activity of these hormones and
completely abolished the rise in GBF induced by these peptides. The addition of exogenous CGRP to leptin, ghrelin and OX-A in rats with deactivated sensory nerves restored the protection and accompanying rise in the GBF with the extent similar to that observed in those treated with these peptides in rats with intact sensory nerves. Therefore it is reasonable to conclude that sensory nerves and their neuropeptides such as CGRP, are essential common mechanism for microcirculatory response and gastroprotective activity of appetite hormones.

Conflict of interest statement: None declared.

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