JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2015, 66, 3, 319-329
www.jpp.krakow.pl

Review article

K. GYIRES, V.E. TOTH, Z.S. ZADORI

GASTRIC MUCOSAL PROTECTION: FROM THE PERIPHERY TO THE CENTRAL NERVOUS SYSTEM

Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Gastric mucosal integrity can be influenced both by peripheral and central mechanisms. In the periphery several protective factors play a role in gastric mucosal defense. Moreover, receptors located in the gastric mucosa (e.g. toll-like receptors, proteinase-activated receptors, α_2-adrenoceptors, opioid receptors) may also be involved in the regulation of gastric mucosal integrity. Activation of peripheral δ-opioid receptors by opioid peptides was shown to induce gastric mucosal defense. In contrast, the gastroprotective action mediated by α_2-adrenoceptors ($\alpha_{2B/C}$-subtypes) is likely to be initiated centrally. Namely, central nervous system (CNS) is also involved in the regulation of gastrointestinal functions; hypothalamus and dorsal vagal complex (DVC) have prominent role in this process. In DVC several receptors have been identified, among others, μ and δ-opioid-, α_2-adrenergic-, cannabinoid CB_1- and CB_2-, angiotensin II AT_1-, nociceptin NOP-, neurokinin NK1- and TRH-receptors. Activation of these receptors results in gastric mucosal protection, mainly in a vagal dependent manner. In addition, glutamate (together with GABA and norepinephrine) is involved in synaptic connections between nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMNV) neurons. AP-7, a selective NMDA receptor antagonist blocked the gastroprotective effect of opioid peptides, indicating that N-Methyl-D-aspartic acid (NMDA) might play a role in centrally induced gastroprotective effect. Moreover, interactions between neuropeptides may have also importance in centrally initiated gastric mucosal protection. Clarification of the role of neuropeptides in gastric mucosal defense may serve as a basis for the development of new strategies to enhance gastric mucosal resistance against injury.

Key words: *gastric mucosal protection, central nervous system, mediators, opioid receptors, α_2-adrenoceptors, centrally-induced gastroprotection, peripherally-induced gastroprotection, neuropeptides*

INTRODUCTION

Gastric mucosal integrity can be influenced both by peripheral and central mechanisms. In the last decades the peripheral mechanisms of gastric mucosal defense have been intensively studied, and many details have been clarified. Some of these results have served as a basis for the development of new strategies, therapeutic targets in the treatment of gastric mucosal lesions.

On the other hand, in the last 20 years, central regulation of gastric mucosal protection has been intensively studied as well, and convincing evidence was obtained on the role of central nervous system (CNS) in the regulation of gastric mucosal integrity. However, several mechanisms of the centrally induced gastric mucosal protection have not been clarified yet, and further studies are needed to determine the role of CNS under physiological and pathophysiological conditions in gastric mucosal homeostasis as well as to reveal how the central regulatory mechanisms can be utilized in human therapy.

GASTRIC MUCOSAL PROTECTION

Peripheral mechanisms of gastric mucosal protection: mediators, receptors

Gastric mucosal barrier to acid consists of several layers: the pre-epithelial mucus bicarbonate layer, an epithelial layer, and a post-epithelial layer which involves blood vessels, non-epithelial cells and enteric nerves. The latter two have basic role in generation of several substances which play a role in gastric mucosal integrity and gastric mucosal defense, e.g. bicarbonate, mucus, phospholipids, trefoil peptides and prostaglandins (PGs) (1-5). Prostaglandins of the E and I series are potent vasodilators, producing this effect in the stomach through EP_2/EP_4 and IP receptors (6, 7). Moreover, they reduce the permeability of the gastric epithelium (directly, or *via* enhancement of the effectiveness of surface-active phospholipids) (1, 6), thereby reducing acid back-diffusion. In addition, primary afferent sensory neurons innervate gastric mucosal and submucosal vessels, form a dense plexus at the mucosal base and regulate mucosal blood flow. Their stimulation results in the release of calcitonin gene-related peptide (CGRP) and substance (SP) CGRP partly directly, partly indirectly through the release of nitric oxide (NO) induces submucosal vasodilation (8-10). Namely, NO has a basic role in gastric mucosal defense by increasing gastric mucosal blood flow and microcirculation (8-10).

Somatostatin is also likely to be involved in gastric mucosal defense (11), it reduces the elevated level of SP, vasoactive intestinal polypeptide (VIP) and leukotriens in ethanol-induced gastric lesions (12), and also reduces stress-induced mucosal injury by inducing antioxidant, anti-inflammatory and anti-

apoptotic actions (13). Our recent finding showed that its mucosal level dramatically decreased following intragastric administration of absolute ethanol parallel with the development of the gastric mucosal lesions in the rat, while gastroprotective agents, such as endomorphin reversed the reduced level of somatostatin (3).

Furthermore, protein- and non-protein sulfhydryls (such as reduced glutathione, GSH) were also shown as endogenous protective compounds (14), and it was suggested that the maintenance of a critical level of non-protein sulfhydryls in the gastric mucosa besides nitric oxide is necessary for the gastroprotective action (14, 15).

Recently the potential role of hydrogen sulfide in gastric mucosal defense was raised: H_2S, similarly to NO, is an important mediator of gastric mucosal protection (16) and inhibition of endogenous H_2S synthesis increases the susceptibility of the mucosa to damage induced by non-steroidal anti-inflammatory drugs (17). On the other hand, exogenous H_2S donors can increase the resistance of the mucosa to injury (18). Several mechanisms are involved in the gastroprotective effect of H_2S, such as maintenance and/or elevation of gastric mucosal blood flow, stimulation of bicarbonate secretion, reduced proinflammatory cytokine expression/release, increased prostaglandin synthesis, reduced leukocyte-endothelial adherence, decreased reactive oxygen metabolite production and enhanced tissue repair (18, 19).

In addition, several other factors, e.g. antioxidant enzymes, heme oxygenase-1, matrix metalloproteinases and trefoil factor family (TFF) proteins take part in the complex mucosal protective system (20).

Antioxidant enzymes, such as the above mentioned GSH, or superoxide dismutase (SOD) and catalase are able to counteract oxidative stress caused by excessive production and/or decreased elimination of reactive oxygen species (ROS). A decrease of SOD activity and GSH concentration significantly contributes to cell damage (21). ROS can induce tissue damage by promoting lipid peroxidation and increasing the production of inflammatory mediators and proinflammatory cytokines (22-24). Antioxidant enzymes are able to neutralize ROS, for instance SOD converts superoxide radical anion ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2), which is thereafter converted to water and oxygen by catalase.

Heme oxygenase-1 (HO-1 or Hsp32), the inducible form of heme oxygenase, also exerts cytoprotective effect. The expression of this enzyme may be induced by oxidative stress, inflammatory cytokines or heavy metals (24). HO-1 catalyzes the oxidative degradation of the pro-oxidant heme to antioxidant and cytoprotective carbon monoxide and biliverdin (which is then converted to bilirubin by biliverdin reductase) (22, 25). The activity of HO-1 is also increased during the healing of gastric ulcers, which indicates its involvement in the mucosal repair processes (26).

Matrix metalloproteinases (MMPs) play a role both in the pathogenesis and in healing of peptic ulcers. MMPs are zinc-dependent endopeptidases that degrade extracellular matrix proteins and are essential for extracellular matrix remodeling and wound healing (27). They are synthesized and secreted by various gastric cells (fibroblasts, epithelial and inflammatory cells) (28, 29), and several animal studies have demonstrated that NSAIDs or ethanol increased the activity of MMP-1, MMP-3, MMP-9 and MMP-13, while decreased the expression of MMP-2 (30-32). Also a recent human study showed that the expression of MMP-9 correlates with the severity and recurrence of gastric ulcers (33).

Trefoil factor family (TFF) proteins (TFF1-3) are also able to enhance mucosal barrier functions by stabilizing the mucus gel and promoting epithelial restitution (34, 35). Although the protective role of these small protease-resistant proteins has been demonstrated in various ulcer models (36, 37), the exact molecular mechanism is still not clear. Recent reports indicate that activation of the C-X-C chemokine receptor type 4 (CXCR4) and the apical Na^+/H^+ exchanger-2 (NHE2) is required for TFF-induced mucosal repair (38, 39).

Activation of immune cells can also affect gastric mucosal integrity. Mast cells and macrophages resident within the lamina propria act as “alarm cells.” Sensing the presence of foreign substances, these cells are capable of liberating an array of inflammatory mediators and cytokines that can alter mucosal blood flow and enhance the recruitment of granulocytes into the affected region. For example mast cells can be activated by several factors (ischemia, bacteria, antigens, bile acids, *etc*.). As a result, they release histamine and platelet-activating factor, which can increase the epithelial and vascular endothelial permeability. In addition, stimulation of the expression of adhesion molecules and release of tumor necrosis factor α (TNF-α) from mast cells can also be observed. TNF-α further stimulates leukocyte-endothelial adhesive interactions. In the contrary, prostaglandins and nitric oxide can suppress the reactivity of mast cells, consequently, can counteract many of these effects (40 - 42).

Under chronic inflammatory conditions (e.g. *Helicobacter pylori* infection) inflammatory cells in lamina propria do not produce only proinflammatory cytokines, but also anti-inflammatory cytokines, such as interleukin 10 (IL-10), which thereafter suppresses the production of various proinflammatory molecules, e.g. IL-1, IL-2, IL-8, TNF-α and IFN-γ (43). Consequently, IL-10 has a counter-regulatory effect in mucosal inflammatory processes, which may reduce tissue damage caused by inflammation, but may also hamper the elimination of harmful stimulus by suppression of the immune response (43).

Several peripheral receptors have been described to be involved in gastric mucosal defense/injury.

A specific ionotropic receptor is the transient receptor potential vanilloid-1 (TRPV1) (44). In the gastrointestinal tract, TRPV1 can be identified in intrinsic enteric neurons, extrinsic sensory neurons, epithelial and endocrine cells (45, 46). TRPV1 receptor is activated by capsaicin (47-49). Capsaicin given orally was found to inhibit gastric mucosal lesions in different experimental ulcer models (50, 51) and in humans (52) by stimulating the nerve endings and efferent function of primary afferents, resulting in the release of CGRP.

Toll-like receptors (TLRs) play an essential role in the host microbial interaction by sensing conserved microbial structures (pathogen-associated molecular patterns, PAMPs). In humans 10 family members (TLR1-10) have been identified thus far, which recognize different bacterial or viral components, like peptidoglycan (TLR2), lipopolysaccharide (TLR4) or flagellin (TLR5), but some of them (e.g. TLR2 and TLR4) are also capable of responding to different endogenous molecules, released during inflammation or tissue damages (53, 54). Gastric epithelial cells express various TLRs (TLR2, 4, 5 and TLR9), whose activation (e.g. by *H. pylori*) induces inflammatory responses and may delay ulcer healing (55-57). Therefore, antagonists of TLRs may serve as novel therapeutic approaches for gastrointestinal ulcers.

Proteinase-activated receptors (PARs), particularly PAR1 and PAR2, are also important regulators of GI functions. These unique G-protein-coupled receptors are distributed throughout the GI tract, and their activation has been reported to increase gastric mucus secretion and mucosal blood flow, to reduce gastric acid secretion and to induce cytoprotection (58-60). Interestingly, capsaicin-sensitive sensory neurons are involved in PAR2-, but not in PAR1-induced gastroprotection - the latter one seems to be mainly mediated by PGs (59, 60).

Adenosine has a basic role in signaling processes and induces numerous physiological responses in all mammalian tissues. Four adenosine receptors have been identified, namely A_1, A_{2A}, A_{2B} and A_3. Activation of A_{2A} receptors elicits anti-inflammatory effects (61) and the selective A_{2A} receptor agonist ATL-146e has been shown to reduce gastric mucosal lesions induced by water-immersion stress, aspirin- and indomethacin (62-64).

Are peripheral opioid receptors and α_2-adrenoceptors involved in gastric mucosal protection?

The presence of μ- and δ-opioid receptors were demonstrated in gastric fundus, antrum and corpus, primarily located in the submucosal plexus, deep muscular plexus, and mucosa (65). We wondered if activation of these receptors can affect gastric mucosal defense. It was found that δ-opioid receptor selective peptides such as [D-Ala^2,D-Leu^5]-enkephalin (DADLE), [D-Pen^2,D-Pen^5]-enkephalin (DPDPE) and deltorphin II injected subcutaneously exerted a dose-dependent inhibition on the development of mucosal lesions induced by acidified ethanol, their ID_{50} values were 0.037 (0.02 – 0.057), 1.8 (1.3 – 2.52) and 3.5 (2.12 – 5.7) μmol/kg, respectively. Since opioid peptides cannot (or poorly) pass the blood-brain barrier, their mucosal protective effect is likely to be due to activation of peripheral opioid receptors. Because naltrindole, the selective δ-opioid receptor antagonist inhibited the gastroprotective effect of all above mentioned peptides, it was concluded that activation of δ-opioid receptors may mediate gastric mucosal protection (66). The mechanism of gastroprotective effect may be at least partly mediated by endogenous nitric oxide, as it was suggested also by previous findings (67).

Moreover, based on the well-known interaction between opioid receptors and α_2-adrenoceptors, the question was raised whether activation of α_2-adrenergic receptors can elicit gastric mucosal protection as well. We found that α_2-adrenoceptor stimulants, clonidine and rilmenidine injected either orally or subcutaneously (s.c.) exerted gastroprotective effect against ethanol-induced gastric lesions in a dose dependent manner, their ED_{50} values were 32 (12 – 84) and 25 (10 – 62.5) nmol/kg for clonidine; 25 (10 – 62.5) and 3.1 (0.5 – 20) nmol/kg for rilmenidine, following oral or s.c. administration, respectively (68). Pharmacological analysis with selective antagonists of the $\alpha_{2A/D}$ and $\alpha_{2B/C}$-adrenoceptor subtypes suggested that the $\alpha_{2B/2C}$-adrenoceptor subtypes are likely to mediate this mucosal protective effect, while the α_{2A}-one has no important role in it. We wondered if the distribution of α_2-adrenoceptor subtypes in gastric mucosa confirms the concept on the peculiar role of $\alpha_{2B/2C}$-adrenoceptor subtypes in gastric mucosal protection. However, though expression of all the three subtypes could be detected in gastric mucosa of the rat, the dominant subtype was the α_{2A}-one (68). Consequently, the findings on distribution of the adrenoceptor subtypes in gastric mucosa does not support the conclusion of pharmacological analysis, that the $\alpha_{2B/2C}$-adrenoceptor subtypes have a prominent role in mucosal defense (68). Moreover, ST 91 (2-[2,6-diethylphenylamino]-2-imidazoline), an $\alpha_{2B/2C}$-

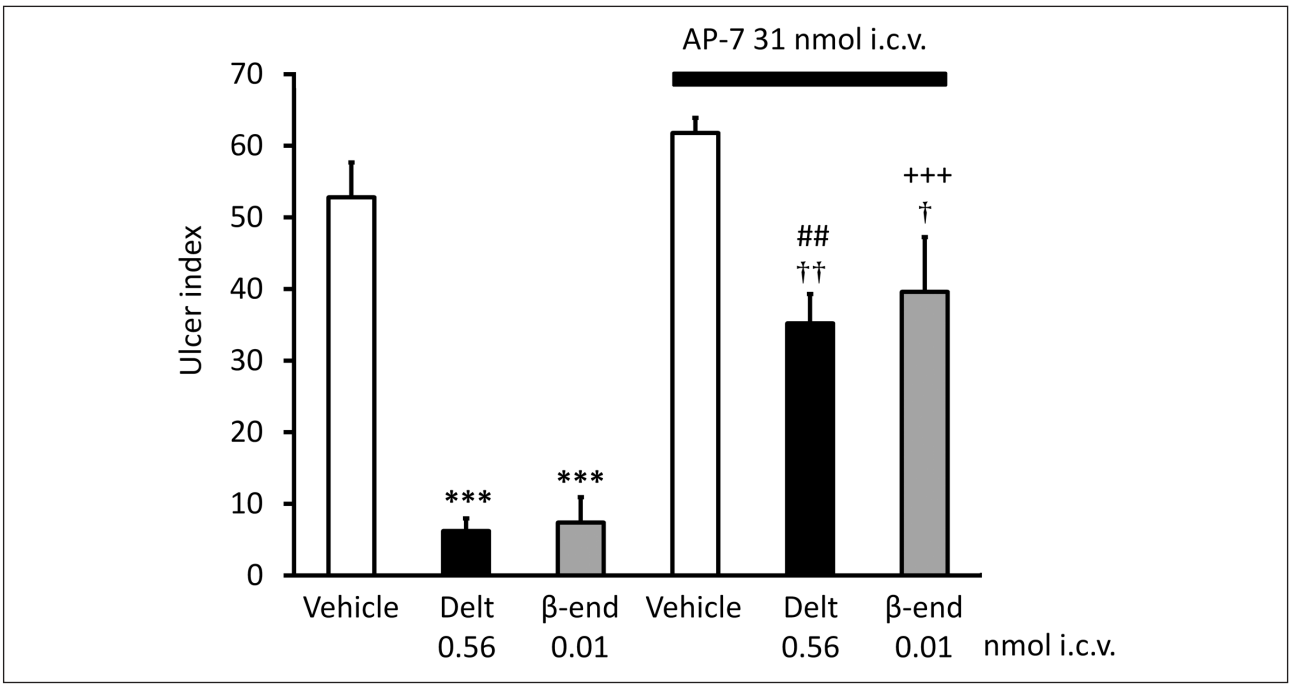

Fig. 1. The effect of AP-7 (DL-2-Amino-7-phosphonoheptanoic acid, 31 nmol/rat i.c.v.) on the gastroprotective effect of deltorphin II (Delt, 0.56 nmol/rat i.c.v.) and β-endorphin (β-end, 0.01 nmol/rat i.c.v.). Gastric mucosal injury was induced by acidified ethanol (2 ml concentrated HCl + 98 ml absolute ethanol), which was injected orally after 24 hours food deprivation in a volume of 0.5 ml/animal. The ulcer index was determined by evaluating the mucosal lesions macroscopically one hour after the ethanol challenge. Opioids were given intracerebroventricularly (i.c.v.) 10 minutes before ethanol in a volume of 10 μl in conscious rats. AP-7 was injected 10 minutes before the opioids. Opioids were dissolved in physiologic saline. AP-7 was dissolved in 1 molar equivalent of NaOH, and then diluted with saline. Control animals received the drug solvents.
Each column represents mean ± S.E.M., n = 5; ***$P < 0.001$ compared with vehicle-treated group (column 1); $^{\#\#}P < 0.01$ compared with deltorphin II-treated group (column 2); $^{+++}P < 0.001$ compared with β-endorphin-treated group (column 3); $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with AP-7 + vehicle-treated group (column 4) (ANOVA, Newman-Keuls post hoc test).

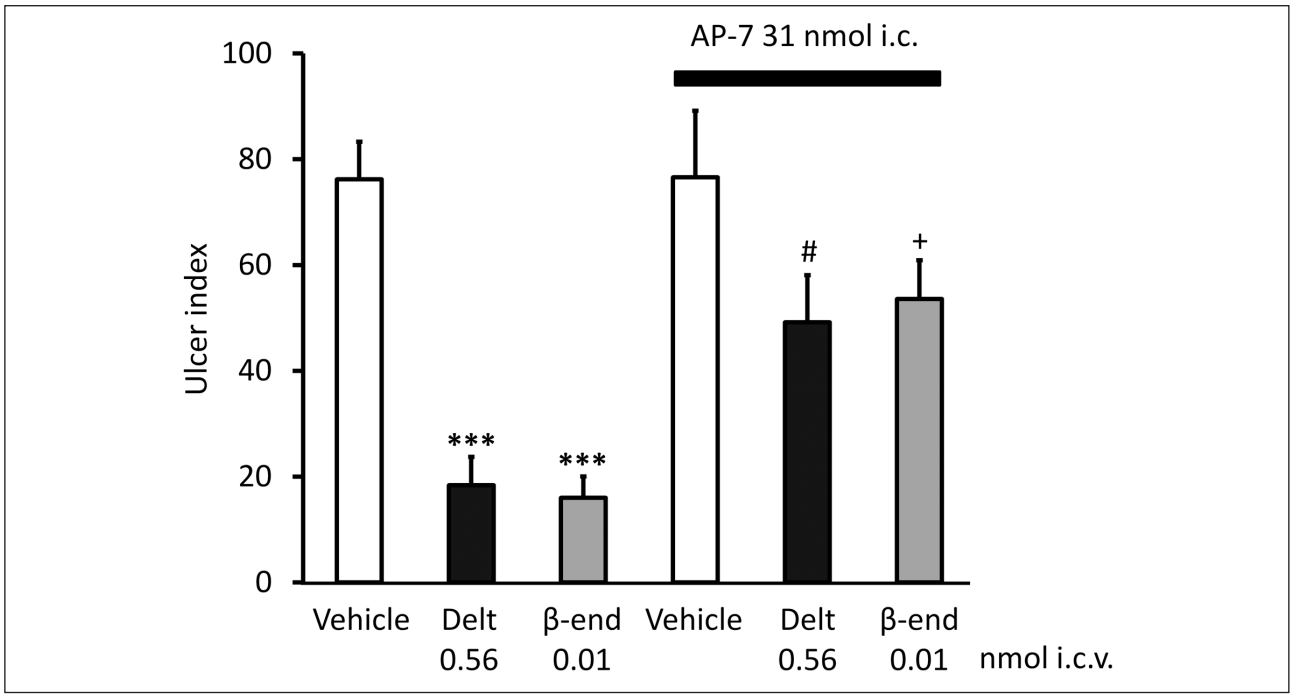

Fig. 2. The effect of AP-7 (31 nmol/rat i.c.) on the gastroprotective effect of deltorphin II (Delt, 0.56 nmol/rat i.c.v.) and β-endorphin (β-end, 0.01 nmol/rat i.c.v.). Gastric mucosal injury was induced by acidified ethanol. Opioids were given intracerebroventricularly (i.c.v.) 10 minutes before ethanol in a volume of 10 µl in conscious rats. AP-7 was injected intracisternally (i.c.) 10 minutes before the opioids in a volume of 5 µl. Opioids were dissolved in physiologic saline. AP-7 was dissolved in 1 molar equivalent of NaOH, and then diluted with saline. Control animals received the drug solvents.
Each column represents mean ± S.E.M., n = 5; ***P < 0.001 compared with vehicle-treated group (column 1); $^{\#}$P < 0.05 compared with deltorphin II-treated group (column 2); $^{+}$P < 0.05 compared with β-endorphin-treated group (column 3) (ANOVA, Newman-Keuls post hoc test).

adrenoceptor subtype preferring, peripherally acting adrenoceptor stimulant exerted only a slight, non-significant inhibition of gastric mucosal lesions in the rat (68). In addition, the gastroprotective effect of rilmenidine given s.c. was antagonised by the intracerebroventricularly (i.c.v.) injected α_2-adrenoceptor antagonist yohimbine (68). These findings suggest that the site of gastroprotective action of α_2-adrenoceptor stimulants is not likely to be in the periphery, but rather in the CNS.

Centrally induced gastroprotection

1. Central opioid receptors and a2-adrenoceptors in gastric mucosal protection

The above results prompted us to analyze the role of central α_2-adrenoceptors in gastroprotection. It was found that both clonidine and rilmenidine exerted gastroprotective effect following i.c.v. administration, their ED_{50} values are 200 (90 – 400) and 10 (1 – 10) pmol, respectively. In addition, ST 91, the above mentioned $\alpha_{2B/2C}$-adrenoceptor subtype preferring agonist, which passes poorly the blood-brain barrier and failed to significantly affect the gastric mucosal lesions following peripheral administration, proved to be effective following i.c.v. administration. The centrally initiated gastroprotective effect of clonidine (470 pmol), rilmenidine (45 pmol) and ST-91 (33 nmol) was antagonized by the non-selective α_2-adrenoceptor antagonist yohimbine, as well as by the $\alpha_{2B/2C}$ adrenoceptor preferring antagonists prazosin and ARC 239, indicating that $\alpha_{2B/2C}$-like adrenoceptor subtypes may mediate the action (68). The same conclusion could be drawn from the results of our subsequent study carried out in genetically engineered mice (69). In addition, naloxone also reversed the mucosal protective effect of clonidine, rilmenidine and ST-91 suggesting an opioid component in their action (68, 70). Therefore, we examined the effect of opioid peptides injected i.c.v. and intracisternally (i.c.) on ethanol-induced experimental ulcer formation. The results showed that DADLE, DPDPE and deltorphin II (selective δ-opioid receptor agonists), DAGO ([D-Ala^2,Phe^4,Gly^5-ol]-enkephalin, a selective µ-opioid receptor agonist) and β-endorphin (ligand of both receptor types) produced a dose-dependent inhibition of acidified ethanol-induced gastric mucosal damage. The ED_{50} values for β-endorphin, DAGO, DADLE, deltorphin II, and DPDPE were 3.5 (1.6 – 7.35), 6.8 (2.26 – 20.4), 75 (36 – 144), 120 (40 – 360), and 1100 (458 – 26409) pmol/rat, respectively, following i.c.v. administration, and 0.8 (0.62 – 1.024), 9.0 (2.4 – 33), 45 (16 – 126), 0.25 (0.08 – 0.775) and 7 (1.66 – 29.4) pmol/rat following i.c. injection (71).

The above results confirmed the pivotal role of CNS in regulation of gastric mucosal integrity. In the last two decades increasing number of evidence suggest that central administration of different neuropeptides, neurotransmitters and neuromodulators (either i.c.v, i.c., or directly into specific brain nuclei, e.g. the dorsal motor nucleus of vagus /DMNV) results in gastric mucosal protection (3, 10, 72, 73).

Different brain areas have been suggested to be involved in the centrally induced gastroprotection. Among them, the hypothalamus and particularly the dorsal vagal complex (DVC) (including DMNV, nucleus of the solitary tract /NTS/ and area postrema) seem to have a prominent role. Vagal dependent mechanism of gastroprotection was demonstrated e.g. for thyreotropine-releasing hormone (TRH), adrenomedullin, peptide YY (74-77), clonidine (78), opioid peptides (71), nociceptin, nocistatin (79), and angiotensin II (Ang II) (80).

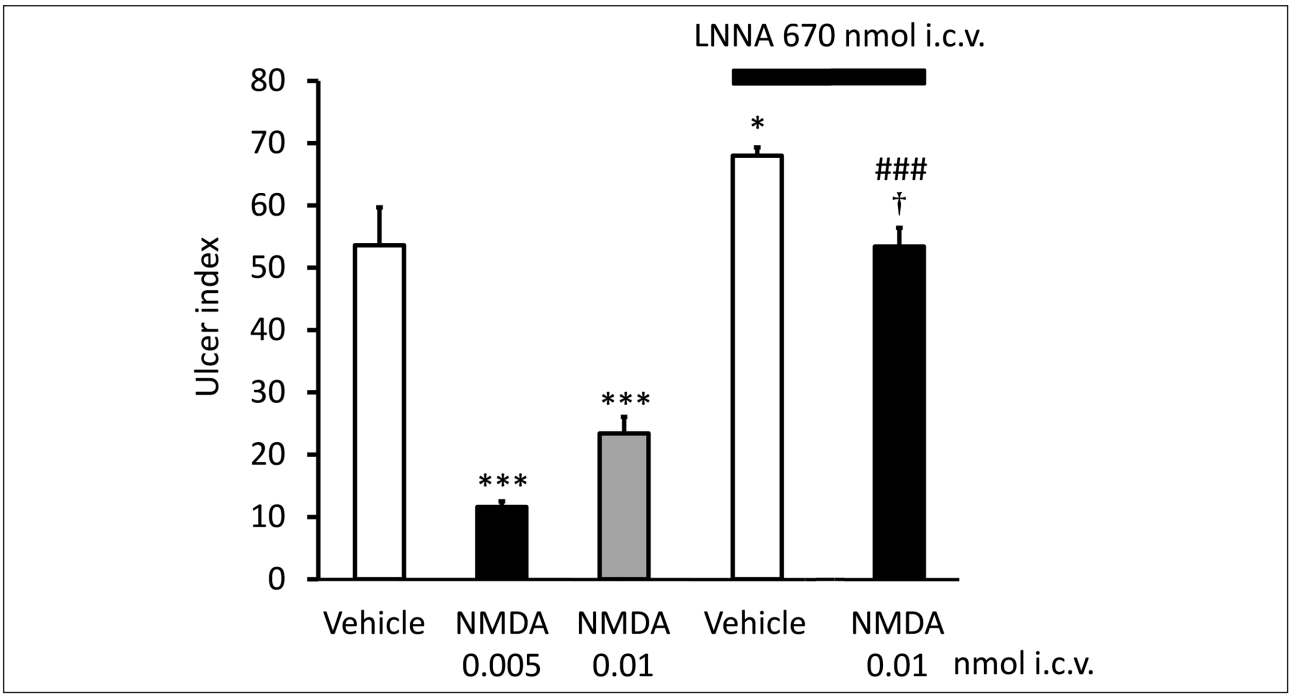

Fig. 3. The inhibitory effect of N^G-nitro-L-arginine (LNNA, 670 nmol i.c.v.) on the gastroprotective effect of NMDA. Gastric mucosal injury was induced by acidified ethanol. NMDA (0.005 and 0.01 nmol/rat) was given intracerebroventricularly (i.c.v.) 10 minutes before ethanol in a volume of 10 µl in conscious rats. LNNA was injected 10 minutes before NMDA. Both NMDA and LNNA were dissolved in saline.
Each column represents mean ± S.E.M., n = 5; *P < 0.05, ***P < 0.001 compared with vehicle-treated group (column 1); ###P < 0.001 compared with NMDA (0.01 nmol)-treated group (column 3); †P < 0.05 compared with LNNA + vehicle-treated group (column 4) (ANOVA, Newman-Keuls post hoc test).

2. Potential role of excitatory amino acids and nitric oxide in centrally-induced gastroprotection

Signals from sensory receptors in the gastrointestinal tract *via* primary afferents terminate in the NTS where they are integrated and transmitted to parasympathetic preganglionic neurons of the DMNV. Principally glutamate, GABA and norepinephrine are involved in synaptic connections between NTS and DMNV neurons and convey sensory signals to vagal efferent impulses (81). While less is known about catecholaminergic transmission between the NTS and DMNV, many studies have demonstrated that electrical stimulation of various NTS subnuclei elicits glutamatergic excitatory and GABAergic inhibitory currents in DMNV neurons (82-85).

The vagal afferent-vagal efferent reflex (vagovagal reflex) plays a crucial role in upper gastrointestinal reflexes, and in regulation of gastric motor activity (81). Activation of both non-NMDA (kainate) and NMDA receptors in the DMNV *in vivo* increases gastric contractility, and this effect was blocked by the appropriate antagonists (86).

Activation of vagal efferent cholinergic nerves may affect gastric mucosal integrity e.g. through stimulation of gastric motor activity (87, 88). On the other hand, cholinergic activation was shown to stimulate the release of gastric mucosal PGs and NO, as well as the effector function of capsaicin-sensitive afferent fibers containing CGRP resulting in enhanced mucosal microcirculation, and consequently gastric mucosal protection (51, 89-91).

Based on these data the question was raised if glutamatergic/GABAergic system may be involved in centrally initiated gastroprotection. As described above, central δ-opioid receptors may mediate gastric mucosal protection (71). The site of action is most probable in the brainstem, since δ-opioid receptor agonists proved to be more effective and potent given i.c. than i.c.v. injection. Since δ-opioid receptors were identified in the NTS, but not in the DMNV (92), (while µ-opioid receptor was shown in both DMNV and NTS (93)), NTS was supposed to be the site of action of δ-opioid receptor stimulants. We wondered if glutamatergic pathway would play a role in conveying the opioid-receptor induced action to the DMNV. As *Figs. 1* and *2* show, AP-7 (DL-2-amino-7-phosphonoheptanoic acid), a competitive antagonist of the NMDA receptors blocked the gastroprotective effect of the δ-opioid receptor selective ligand deltorphin II and that of β-endorphin given both i.c.v. and i.c. These findings suggest that glutamate through NMDA receptors might play a role in the δ-opioid receptor-induced gastroprotective action. If NMDA receptors are involved indeed in mediation of the effect of opioid peptides, NMDA itself should induce mucosal protective effect as well. Accordingly, as *Fig. 3* shows, NMDA injected i.c.v. exerted mucosal protective action in the doses of 5 and 10 pmol.

In accordance with this finding, L-glutamate injected into the lateral hypothalamus was shown to increase defensive mechanisms (e.g. mucosal blood flow) (94). Moreover, the injection of kainate (a specific agonist for the kainate receptor, that mimics the effect of glutamate) into the raphe pallidus exerted gastroprotective action as well (95).

Furthermore, co-localization of neuronal nitric oxide synthase and NMDA receptor subunit 1 in NTS was described, which provides anatomical support for the hypothesis that NMDA receptor activation can affect NTS-controlled functions *via* actions on neurons that synthesize nitric oxide (NO) (96). The NO production after NMDA receptor activation in the NTS was markedly reduced by prior i.c. injection of L-NAME (N^G-nitro-L-arginine methyl ester), an NO synthase inhibitor,

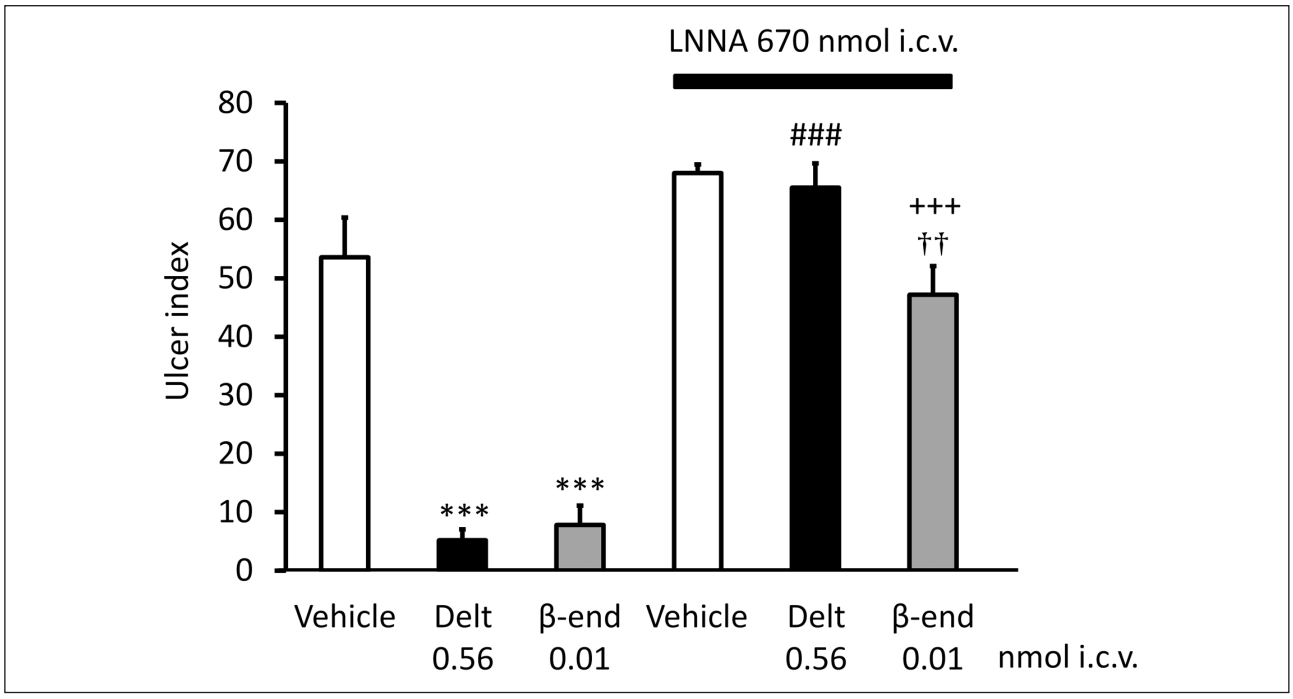

Fig. 4. The inhibitory effect of N^G-nitro-L-arginine (LNNA, 670 nmol i.c.v.) on the gastroprotective effect of deltorphin II (Delt, 0.56 nmol/rat i.c.v.) and β-endorphin (β-end, 0.01 nmol/rat i.c.v.). Gastric mucosal injury was induced by acidified ethanol. Opioids were given intracerebroventricularly (i.c.v.) 10 minutes before ethanol in a volume of 10 µl in conscious rats. LNNA was injected 10 minutes before the opioids. Opioids and LNNA were dissolved in physiologic saline.
Each column represents mean ± S.E.M., n = 5; ***P < 0.001 compared with vehicle-treated group (column 1); ###P < 0.001 compared with deltorphin II-treated group (column 2); +++P < 0.001 compared with β-endorphin-treated group (column 3); ††P < 0.01 compared with LNNA + vehicle-treated group (column 4) (ANOVA, Newman-Keuls post hoc test).

suggesting that the increase in NO level after NMDA receptor activation is caused by activation of NO synthase in the NTS (97). Further studies confirmed that NO has prominent role in NMDA-mediated actions (97, 98).

Based on these data we wondered if NO may also be involved in the centrally-induced effect of opioid peptides. As *Figs. 3* and *4* demonstrate, the NO synthase inhibitor N^G-nitro-L-arginine (LNNA, 670 nmol i.c.v.) blocked the gastroprotective effect of NMDA, as well as that of deltorphin II and β-endorphin, and the effect was reversed by L-arginine, but not by D-arginine (not shown) indicating that NO is likely to mediate the gastroprotective effect of both NMDA and opioid peptides.

On the basis of these findings it might be speculated that opioid peptide-induced gastroprotecive effect is mediated, at least partly by an NMDA-NO pathway. To answer the question, whether this chain of events - according to our original hypothesis - occurs within the DVC, and activation of δ- (µ)-opioid receptor results in activation of DMNV through NMDA-NO pathway, further studies are needed.

3. Gastroprotective effect of endogenous substances: neuropeptides and non-neuropeptides

The role of CNS in mucosal injury/protection has been raised already in the 19th century. However, systematic analysis of the mechanism of centrally initiated gastric mucosal protection started only about 20 years ago.

As depicted above, DVC has a prominent role in the regulation of gastrointestinal functions. Different receptor populations have been identified in the DVC, such as μ- and δ-opioid receptors (92, 93), α_2-adrenoceptors (99, 100), cannabinoid CB_1- and CB_2 receptors (101-104), angiotensin II AT_1 receptor (105, 106), nociceptin *NOP* receptor (107) and tachykinin *NK1, NK2* and *NK3*-receptors (108-111) (*Table 2*).

Our research in the last decade focused on the mechanism of centrally initiated gastric mucosal protection. As a first step we examined which of the receptors localized in the DVC may have a role in gastric mucosal defense/injury. Our results showed that activation of opioid-, (10, 71) α_2-, (68-70, 78) nociceptin- (79) cannabinoid CB_1 (111), neurokinin NK1-, NK2-, NK3-receptors (112) and angiotensin II AT_1-receptors (80) initiated a chain of events resulting in stimulation of mucosal protective processes. Further studies are needed to clarify whether gastroprotection can be induced also by elevation of the endogenous level of neuropeptides, e.g. *via* increase of the endogenous opioid levels by opiorphin or its synthetic analogue, inhibitors of enkephalin-inactivating peptidases (113).

The mechanism of centrally initiated mucosal protection is under an intensive analysis. Our results suggest a vagal dependent mechanism of centrally induced gastroprotective action for α_2 stimulants (78), opioid peptides (71), angiotensin II (80) as well as nociceptin and nocistatin (79), in accordance with the results of Polidori *et al.* (114).

However, sympathetic nervous system may also be involved in the gastroprotective effect of opioid peptides and α_2-adrenoceptor agonists, since their effect markedly decreased following i.c.v. administration of the catecholaminergic neurotoxine, 6-hydroxydopamine, that reduced the noradrenaline concentration in a significant manner in the NTS (73). In the periphery, the decreased gastric mucosal level of CGRP and partly somatostatin due to ethanol administration was restored by i.c.v. administration of endomorphins (3), substance P (112) and cannabinoids (80).

Table 1. Some local mechanisms involved in mucosal defense or injury.

Compound / Receptor	**Mechanism**	**References**
Bicarbonate, mucus, phospholipids	- form an unstirred layer on the mucosal surface, which acts as a physical barrier against luminal pepsin - this layer retains secreted bicarbonate to maintain a neutral pH at the epithelial cells	1, 2, 3, 4, 5
Prostaglandins	- increase bicarbonate and mucus secretion - reduce the permeability of the gastric epithelium and the back-diffusion of acid - enhance mucosal blood flow - inhibit acid secretion and motility - inhibit inflammatory mediator release from mast cells	4, 5, 6, 7
Calcitonin gene-related peptide (CGRP) and nitric oxide (NO)	- increase mucin synthesis - induce submucosal vasodilation and enhance mucosal blood flow - inhibit acid secretion - induce anti-inflammatory effect	8, 9, 10, 11
Somatostatin	- reduces the elevated level of substance P, VIP and leukotriens - has antioxidant, anti-inflammatory and anti-apoptotic actions	12, 13
Protein- and non-protein sulfhydryls	- antioxidant or reactive metabolite-eliminating effects, counteract oxidative stress	14, 15
Hydrogen sulfide (H_2S)	- increases mucosal blood flow - stimulates bicarbonate secretion - reduces proinflammatory cytokine production and leukocyte-endothelial adherence - increases prostaglandin synthesis - decreases reactive oxygen metabolite production - enhances tissue repair	18, 19
Heme oxygenase-1 (HO-1)	- counteract oxidative stress, catalyzes the oxidative degradation of the pro-oxidant heme to antioxidant and cytoprotective CO and biliverdin - promotes tissue repair	22, 25, 26
Matrix metalloproteinases (MMPs)	- involved both in the pathogenesis and healing of peptic ulcers	30, 31, 32, 33
Trefoil factor family (TFF) proteins (TFF1-3)	- enhance mucosal barrier functions by stabilizing the mucus gel and promoting epithelial restitution	34, 35
TRPV1 receptors	- their activation on sensory neurons and epithelial cells stimulates the efferent function of afferent nerve endings and releases CGRP / NO, which is manifested in gastric mucosal protection	50, 51, 52
Toll-like receptors (TLRs)	- play an essential role in the host microbial interaction by sensing conserved microbial structures - induce inflammatory responses and may delay ulcer healing	53, 54, 55, 56, 57
Proteinase-activated receptors (PARs)	- increase mucus secretion - enhance mucosal blood flow - inhibit gastric acid secretion	58, 59, 60
Adenosine A_{2A} receptors	- their activation reduces the elevated proinflammatory cytokine level in gastric mucosa following NSAID-induced lesions	62, 63, 64

In our further experiments we aim to analyse the interactions between different neuropeptides and other neurotransmitters/neuromodulators in gastroprotection. Some data of the literature suggest interaction of neuropeptides in gastric mucosal defense. For example TRH-enkephalin interaction was observed in the amygdaloid complex during gastric stress ulcer formation in rats (115), or stress induced the release of CRF, that stimulated the release of β-endorphin and somatostatin, and reduced that of TRH (116, 117). Our results suggest that endogenous opioids seem to be involved in the gastroprotective process of α_2-adrenoceptor agonists (68, 78), nociceptin, nocistatin (79) and cannabinoids (111). The production of the endocannabinoid 2-arachydonoylglycerol (2-AG) and transactivation of the CB_1 receptors may also contribute to the gastric mucosal protective mechanism of Ang II (80).

Table 2. Examples for the involvement of central receptors in gastroprotection

Receptor	**Localization in the DVC (reference)**	**Factors involved in the gastroprotective effect (reference)**
μ- and δ-opioid receptors	area postrema, NTS and DMNV (92, 93)	vagal nerve, nitric oxide, prostaglandins, CGRP, somatostatin (3, 71)
α_{2B}- and α_{2C}-adrenoceptors	NTS and DMNV (99, 100)	the endogenous opioid system (activation of opioid receptors and release of β-endorphin), vagal nerve, nitric oxide, prostaglandins (68, 69, 70, 78)
CB_1 cannabinoid receptor	NTS and DMNV (101, 102, 103)	the endogenous opioid system (activation of opioid receptors and release of endomorphin-2), CGRP, vagal nerve, muscarinic receptors (80, 111)
AT_1 angiotensin receptor	area postrema, NTS and DMNV (105, 106)	the endogenous cannabinoid system (transactivation of CB_1 receptors), CGRP, vagal nerve, muscarinic receptors (80)
NOP receptor	area postrema, NTS and DMNV (107)	the endogenous opioid system (activation of opioid receptors), vagal nerve, CGRP, nitric oxide, sympathetic nerves (79, 114)
NK1-, NK2- and NK3 receptors	area postrema, NTS and DMNV (108, 109, 110)	the endogenous opioid system (activation of μ-opioid receptors and release of endomorphin-2), muscarinic receptors, prostaglandins, nitric oxide, CGRP (112)

Abbreviations: NTS: nucleus of solitary tract, DMNV: dorsal motor nucleus of vagus, DVC: dorsal vagal complex

The role of CNS in gastric mucosal homeostasis has been well documented in the last 15 – 20 years. Analysis of the central regulation of gastric functions, identification of endogenous substances and their receptors that may influence the central and peripheral mechanisms of gastric mucosal defense, clarification of the interaction of neuropeptides (brain-gut peptides) with each other and with other endogenous substances, all may serve as a basis for better understanding of the complex mechanism of the maintenance of gastric mucosal integrity as well for the development of new strategies to enhance gastric mucosal resistance against injury.

Conflict of interests: None declared.

REFERENCE

1. Wallace JL. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? *Physiol Rev* 2008; 88: 1547-1565.
2. Laine L, Takeuchi K, Tarnawski A. Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology* 2008; 135: 41-60.
3. Gyires K, Nemeth J, Zadori ZS. Gastric mucosal protection and central nervous system. *Curr Pharm Des* 2013; 19: 34-39.
4. deFoneska A, Kaunitz JD. Gastroduodenal mucosal defense. *Curr Opin Gastroenterol* 2010; 26: 604-610.
5. Allen A, Flemstrom G. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *Am J Physiol Cell Physiol* 2005; 288: 1-19.
6. Takeuchi K. Gastric cytoprotection by prostaglandin E_2 and prostacyclin: relationship to EP1 and IP receptors. *J Physiol Pharmacol* 2014; 65: 3-14.
7. Kotani T, Kobata A, Nakamura E, Amagase K, Takeuchi K. Roles of cyclooxygenase-2 and prostacyclin/IP receptors in mucosal defense against ischemia/reperfusion injury in mouse stomach. *J Pharmacol Exp Ther* 2006; 316: 547-555.
8. Li DS, Raybould HE, Quintero E, Guth PH. Calcitonin gene-related peptide mediates the gastric hyperemic response to acid back-diffusion. *Gastroenterology* 1992; 102: 1124-1128.
9. Holzer P. Neural emergency system in the stomach. *Gastroenterology* 1998; 114: 823-839.
10. Gyires K. Neuropeptides and gastric mucosal homeostasis. *Curr Top Med Chem* 2004; 4: 63-73.
11. Holzer P. Role of visceral afferent neurons in mucosal inflammation and defense. *Curr Opin Pharmacol* 2007; 7: 563-569.
12. Karmeli F, Eliakim R, Okon E, Rachmilewitz D. Somatostatin effectively prevents ethanol- and NSAID-induced gastric mucosal damage in rats. *Dig Dis Sci* 1994; 39: 617-625.
13. Nassar NN, Schaalan MF, Zaki HF, Abdallah DM. Octreotide ameliorates gastric lesions in chronically mild stressed rats. *World J Gastroenterol* 2011; 17: 1135-1142.
14. Szabo S, Nagy L, Plebani M. Glutathione, protein sulfhydryls and cysteine proteases in gastric mucosal injury and protection. *Clin Chim Acta* 1992; 206: 95-105.
15. Ali AT. The role of nitric oxide and sulphydryls in gastric mucosal protection induced by sodium cromoglycate in rats. *J Pharm Pharmacol* 1995; 47: 739-743.

16. Wallace JL. Physiological and pathophysiological roles of hydrogen sulfide in the gastrointestinal tract. *Antioxid Redox Signal* 2010; 12: 1125-1133.
17. Fiorucci S, Antonelli E, Distrutti E, *et al.* Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. *Gastroenterology* 2005; 129: 1210-1224.
18. Wallace JL, Caliendo G, Santagada V, Cirino G. Markedly reduced toxicity of a hydrogen sulphide-releasing derivative of naproxen (ATB-346). *Br J Pharmacol* 2010; 159: 1236-1246.
19. Wallace JL. Hydrogen sulfide: a rescue molecule for mucosal defence and repair. *Dig Dis Sci* 2012; 57: 1432-1434.
20. Al Jiboury H, Kaunitz JD. Gastroduodenal mucosal defense. *Curr Opin Gastroenterol* 2012; 28: 594-601.
21. Kwiecien S, Jasnos K, Magierowski M, *et al.* Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress - induced gastric injury. *J Physiol Pharmacol* 2014; 65: 613-622.
22. Bindu S, Mazumder S, Dey S, *et al.* Nonsteroidal anti-inflammatory drug induces proinflammatory damage in gastric mucosa through NF-kappaB activation and neutrophil infiltration: anti-inflammatory role of heme oxygenase-1 against nonsteroidal anti-inflammatory drug. *Free Radic Biol Med* 2013; 65: 456-467.
23. Davies GR, Simmonds NJ, Stevens TR, Grandison A, Blake DR, Rampton DS. Mucosal reactive oxygen metabolite production in duodenal ulcer disease. *Gut* 1992; 33: 1467-1472.
24. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 1997; 37: 517-554.
25. Llesuy SF, Tomaro ML. Heme oxygenase and oxidative stress. Evidence of involvement of bilirubin as physiological protector against oxidative damage. *Biochim Biophys Acta* 1994; 1223: 9-14.
26. Guo JS, Cho CH, Wang WP, Shen XZ, Cheng CL, Koo MW. Expression and activities of three inducible enzymes in the healing of gastric ulcers in rats. *World J Gastroenterol* 2003; 9: 1767-1771.
27. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003; 92: 827-839.
28. Shahin M, Konturek JW, Pohle T, Schuppan D, Herbst H, Domschke W. Remodeling of extracellular matrix in gastric ulceration. *Microsc Res Tech* 2001; 53: 396-408.
29. Park SH, Hong H, Han YM, *et al.* Nonsteroidal anti-inflammatory drugs (NSAID) sparing effects of glucosamine hydrochloride through N-glycosylation inhibition; strategy to rescue stomach from NSAID damage. *J Physiol Pharmacol* 2013; 64: 157-165.
30. Pradeepkumar Singh L, Vivek Sharma A, Swarnakar S. Upregulation of collagenase-1 and -3 in indomethacin-induced gastric ulcer in diabetic rats: role of melatonin. *J Pineal Res* 2011; 51: 61-74.
31. Kim SJ, Park YS, Paik HD, Chang HI. Effect of anthocyanins on expression of matrix metalloproteinase-2 in naproxen-induced gastric ulcers. *Br J Nutr* 2011; 106: 1792-1801.
32. Ganguly K, Swarnakar S. Induction of matrix metalloproteinase-9 and -3 in nonsteroidal anti-inflammatory drug-induced acute gastric ulcers in mice: regulation by melatonin. *J Pineal Res* 2009; 47: 43-55.
33. Li SL, Zhao JR, Ren XY, Xie JP, Ma QZ, Rong QH. Increased expression of matrix metalloproteinase-9 associated with gastric ulcer recurrence. *World J Gastroenterol* 2013; 19: 4590-4595.
34. Hoffmann W. Trefoil factors TFF (trefoil factor family) peptide-triggered signals promoting mucosal restitution. *Cell Mol Life Sci* 2005; 62: 2932-2938.
35. Hoffmann W. Trefoil factor family (TFF) peptides: regulators of mucosal regeneration and repair, and more. *Peptides* 2004; 25: 727-730.
36. Konturek PC, Brzozowski T, Konturek SJ, *et al.* Role of spasmolytic polypeptide in healing of stress-induced gastric lesions in rats. *Regul Pept* 1997; 68: 71-79.
37. Farrell JJ, Taupin D, Koh TJ, *et al.* TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. *J Clin Invest* 2002; 109: 193-204.
38. Xue L, Aihara E, Wang TC, Montrose MH. Trefoil factor 2 requires Na/H exchanger 2 activity to enhance mouse gastric epithelial repair. *J Biol Chem* 2011; 286: 38375-38382.
39. Dubeykovskaya Z, Dubeykovskiy A, Solal-Cohen J, Wang TC. Secreted trefoil factor 2 activates the CXCR4 receptor in epithelial and lymphocytic cancer cell lines. *J Biol Chem* 2009; 284: 3650-3662.
40. Kubes P, Kanwar S, Niu XF, Gaboury JP. Nitric oxide synthesis inhibition induces leukocyte adhesion via superoxide and mast cells. *FASEB J* 1993; 7: 1293-1299.
41. Kanwar S, Wallace JL, Befus D, Kubes P. Nitric oxide synthesis inhibition increases epithelial permeability via mast cells. *Am J Physiol* 1994; 266: 222-229.
42. Wallace JL, Granger DN. The cellular and molecular basis of gastric mucosal defense. *FASEB J* 1996; 10: 731-740.
43. Bodger K, Wyatt JI, Heatley RV. Gastric mucosal secretion of interleukin-10: relations to histopathology, Helicobacter pylori status, and tumour necrosis factor-alpha secretion. *Gut* 1997; 40: 739-744.
44. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997; 389: 816-824.
45. Ward SM, Bayguinov J, Won KJ, Grundy D, Berthoud HR. Distribution of the vanilloid receptor (VR1) in the gastrointestinal tract. *J Comp Neurol* 2003; 465: 121-135.
46. Zhao H, Simasko SM. Role of transient receptor potential channels in cholecystokinin-induced activation of cultured vagal afferent neurons. *Endocrinology* 2010; 151: 5237-5246.
47. Szolcsanyi J. Forty years in capsaicin research for sensory pharmacology and physiology. *Neuropeptides* 2004; 38: 377-384.
48. Szallasi A, Blumberg PM. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol Rev* 1999; 51: 159-212.
49. Holzer P. Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. *Pharmacol Ther* 2011; 131: 142-170.
50. Szolcsanyi J, Bartho L. Capsaicin-sensitive afferents and their role in gastroprotection: an update. *J Physiol (Paris)* 2001; 95: 181-188.
51. Holzer P, Lippe IT. Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa. *Neuroscience* 1988; 27: 981-987.
52. Mozsik G, Szolcsanyi J, Domotor A. Capsaicin research as a new tool to approach of the human gastrointestinal physiology, pathology and pharmacology. *Inflammopharmacology* 2007; 15: 232-245.
53. Miyake K. Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. *Semin Immunol* 2007; 19: 3-10.
54. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. *Int Rev Immunol* 2011; 30: 16-34.
55. Smith MF, Jr., Mitchell A, Li G, *et al.* Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for

Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells. *J Biol Chem* 2003; 278: 32552-32560.
56. Nadatani Y, Watanabe T, Tanigawa T, *et al.* High-mobility group Box 1 inhibits gastric ulcer healing through Toll-like receptor 4 and receptor for advanced glycation end products. *PLoS One* 2013; 8: e80130.
57. Lagunes-Servin H, Torres J, Maldonado-Bernal C, *et al.* Toll-like receptors and cytokines are upregulated during Helicobacter pylori infection in children. *Helicobacter* 2013; 18: 423-432.
58. Nishikawa H, Kawai K, Nishimura S, *et al.* Suppression by protease-activated receptor-2 activation of gastric acid secretion in rats. *Eur J Pharmacol* 2002; 447: 87-90.
59. Kawabata A, Nishikawa H, Saitoh H, *et al.* A protective role of protease-activated receptor 1 in rat gastric mucosa. *Gastroenterology* 2004; 126: 208-219.
60. Kawabata A, Kinoshita M, Nishikawa H, *et al.* The protease-activated receptor-2 agonist induces gastric mucus secretion and mucosal cytoprotection. *J Clin Invest* 2001; 107: 1443-1450.
61. Cronstein BN. Adenosine, an endogenous anti-inflammatory agent. *J Appl Physiol* 1994; 76: 5-13.
62. Odashima M, Otaka M, Jin M, *et al.* Selective adenosine A receptor agonist, ATL-146e, attenuates stress-induced gastric lesions in rats. *J Gastroenterol Hepatol* 2005; 20: 275-280.
63. Odashima M, Otaka M, Jin M, *et al.* Attenuation of gastric mucosal inflammation induced by aspirin through activation of A2A adenosine receptor in rats. *World J Gastroenterol* 2006; 12: 568-573.
64. Koizumi S, Odashima M, Otaka M, *et al.* Attenuation of gastric mucosal inflammation induced by indomethacin through activation of the A2A adenosine receptor in rats. *J Gastroenterol* 2009; 44: 419-425.
65. Nishimura E, Buchan AM, McIntosh CH. Autoradiographic localization of opioid receptors in the rat stomach. *Neurosci Lett* 1984; 50: 73-78.
66. Gyires K, Ronai AZ, Toth G, Darula Z, Furst S. Analysis of the role of delta opioid receptors in gastroprotection in the rat. *Life Sci* 1997; 60: 1337-1347.
67. Gyires K. The role of endogenous nitric oxide in the gastroprotective action of morphine. *Eur J Pharmacol* 1994; 255: 33-37.
68. Gyires K, Zadori ZS, Shujaa N, Minorics R, Falkay G, Matyus P. Analysis of the role of central and peripheral alpha2-adrenoceptor subtypes in gastric mucosal defense in the rat. *Neurochem Int* 2007; 51: 289-296.
69. Zadori ZS, Shujaa N, Brancati SB, Hein L, Gyires K. Both alpha2B- and alpha2C-adrenoceptor subtypes are involved in the mediation of centrally induced gastroprotection in mice. *Eur J Pharmacol* 2011; 669: 115-120.
70. Fulop K, Zadori Z, Ronai AZ, Gyires K. Characterisation of alpha2-adrenoceptor subtypes involved in gastric emptying, gastric motility and gastric mucosal defence. *Eur J Pharmacol* 2005; 528: 150-157.
71. Gyires K, Ronai AZ. Supraspinal delta- and mu-opioid receptors mediate gastric mucosal protection in the rat. *J Pharmacol Exp Ther* 2001; 297: 1010-1015.
72. Tache Y, Adelson D, Yang H. TRH/TRH-R1 receptor signaling in the brain medulla as a pathway of vagally mediated gut responses during the cephalic phase. *Curr Pharm Des* 2014; 20: 2725-2730.
73. Gyires K. Analysis of the Effect of Different Neuropeptides in Gastric Mucosal Defense Initiated Centrally. In: Cell/Tissue Injury and Cytoprotection/Organoprotection in the Gastrointestinal Tract: Mechanisms, Prevention and Treatment, Filaretova LP, Takeuchi K (eds). Basel, Karger, 2012, vol 30, pp. 161-169.
74. Yang H, Kawakubo K, Tache Y. Intracisternal PYY increases gastric mucosal resistance: role of cholinergic, CGRP, and NO pathways. *Am J Physiol* 1999; 277: G555-G562.
75. Tache Y, Yoneda M. Central action of TRH to induce vagally mediated gastric cytoprotection and ulcer formation in rats. *J Clin Gastroenterol* 1993; 17: 58-63.
76. Tache Y. Brainstem neuropeptides and vagal protection of the gastric mucosal against injury: role of prostaglandins, nitric oxide and calcitonin-gene related peptide in capsaicin afferents. *Curr Med Chem* 2012; 19: 35-42.
77. Kaneko H, Mitsuma T, Nagai H, *et al.* Central action of adrenomedullin to prevent ethanol-induced gastric injury through vagal pathways in rats. *Am J Physiol* 1998; 274: 1783-1788.
78. Gyires K, Ronai AZ, Mullner K, Furst S. Intracerebroventricular injection of clonidine releases beta-endorphin to induce mucosal protection in the rat. *Neuropharmacology* 2000; 39: 961-968.
79. Zadori ZS, Shujaa N, Koles L, Kiraly KP, Tekes K, Gyires K. Nocistatin and nociceptin given centrally induce opioid-mediated gastric mucosal protection. *Peptides* 2008; 29: 2257-2265.
80. Gyires K, Ronai AZ, Zadori ZS, *et al.* Angiotensin II-induced activation of central AT receptors exerts endocannabinoid-mediated gastroprotective effect in rats. *Mol Cell Endocrinol* 2014; 382: 971-978.
81. Browning KN, Travagli RA. Short-term receptor trafficking in the dorsal vagal complex: an overview. *Auton Neurosci* 2006; 126-127: 2-8.
82. Willis A, Mihalevich M, Neff RA, Mendelowitz D. Three types of postsynaptic glutamatergic receptors are activated in DMNX neurons upon stimulation of NTS. *Am J Physiol* 1996; 271: 1614-1619.
83. Travagli RA, Gillis RA, Rossiter CD, Vicini S. Glutamate and GABA-mediated synaptic currents in neurons of the rat dorsal motor nucleus of the vagus. *Am J Physiol* 1991; 260: G531-G536.
84. Davis SF, Derbenev AV, Williams KW, Glatzer NR, Smith BN. Excitatory and inhibitory local circuit input to the rat dorsal motor nucleus of the vagus originating from the nucleus tractus solitarius. *Brain Res* 2004; 1017: 208-217.
85. Browning KN, Travagli RA. Neuropeptide Y and peptide YY inhibit excitatory synaptic transmission in the rat dorsal motor nucleus of the vagus. *J Physiol* 2003; 549: 775-785.
86. Sivarao DV, Krowicki ZK, Abrahams TP, Hornby PJ. Vagally-regulated gastric motor activity: evidence for kainate and NMDA receptor mediation. *Eur J Pharmacol* 1999; 368: 173-182.
87. Takeuchi K, Nishiwaki H, Okabe S. Effects of dopamine on gastric mucosal lesions induced by ethanol in rats. Possible involvement of antigastric motor activity mediated with alpha 2-adrenoceptors. *Dig Dis Sci* 1988; 33: 1560-1568.
88. Takeuchi K, Niida H, Matsumoto J, Ueshima K, Okabe S. Gastric motility changes in capsaicin-induced cytoprotection in the rat stomach. *Jpn J Pharmacol* 1991; 55: 147-155.
89. Kiraly A, Suto G, Tache Y. Role of nitric oxide in the gastric cytoprotection induced by central vagal stimulation. *Eur J Pharmacol* 1993; 240: 299-301.
90. Kiraly A, Suto G, Livingston EH, Guth PH, St Pierre S, Tache Y. Central vagal activation by TRH induces gastric hyperemia: role of CGRP in capsaicin-sensitive afferents in rats. *Am J Physiol* 1994; 267: G1041-G1049.
91. Kato K, Matsuno Y, Matsuo Y, *et al.* Role of mucosal prostaglandins in vagally-mediated adaptive cytoprotection in the rat. *Gastroenterol Jpn* 1992; 27: 1-8.
92. Mansour A, Fox CA, Burke S, *et al.* Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. *J Comp Neurol* 1994; 350: 412-438.

93. Mansour A, Fox CA, Burke S, Akil H, Watson SJ. Immunohistochemical localization of the cloned mu opioid receptor in the rat CNS. *J Chem Neuroanat* 1995; 8: 283-305.
94. Namiki T, Egawa M, Tominaga S, Inoue S, Takamura Y. Effects of GABA and L-glutamate on the gastric acid secretion and gastric defensive mechanisms in rat lateral hypothalamus. *J Auton Nerv Syst* 1993; 44: 217-223.
95. Kaneko H, Kaunitz J, Tache Y. Vagal mechanisms underlying gastric protection induced by chemical activation of raphe pallidus in rats. *Am J Physiol* 1998; 275: 1056-1062.
96. Lin LH, Talman WT. N-methyl-D-aspartate receptors on neurons that synthesize nitric oxide in rat nucleus tractus solitarii. *Neuroscience* 2000; 100: 581-588.
97. Matsuo I, Hirooka Y, Hironaga K, *et al.* Glutamate release via NO production evoked by NMDA in the NTS enhances hypotension and bradycardia in vivo. *Am J Physiol Regul Integr Comp Physiol* 2001; 280: R1285-R1291.
98. Kiss JP, Vizi ES. Nitric oxide: a novel link between synaptic and nonsynaptic transmission. *Trends Neurosci* 2001; 24: 211-215.
99. Tavares A, Handy DE, Bogdanova NN, Rosene DL, Gavras H. Localization of alpha 2A- and alpha 2B-adrenergic receptor subtypes in brain. *Hypertension* 1996; 27: 449-455.
100. Rosin DL, Talley EM, Lee A, *et al.* Distribution of alpha 2C-adrenergic receptor-like immunoreactivity in the rat central nervous system. *J Comp Neurol* 1996; 372: 135-165.
101. Van Sickle MD, Duncan M, Kingsley PJ, *et al*. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 2005; 310: 329-332.
102. Partosoedarso ER, Abrahams TP, Scullion RT, Moerschbaecher JM, Hornby PJ. Cannabinoid 1 receptor in the dorsal vagal complex modulates lower oesophageal sphincter relaxation in ferrets. *J Physiol* 2003; 550: 149-158.
103. Castelli MP, Piras AP, Melis T, *et al.* Cannabinoid CB1 receptors in the paraventricular nucleus and central control of penile erection: immunocytochemistry, autoradiography and behavioral studies. *Neuroscience* 2007; 147: 197-206.
104. Accorsi-Mendonca D, Almado CE, Dagostin AL, Machado BH, Leao RM. Inhibition of spontaneous neurotransmission in the nucleus of solitary tract of the rat by the cannabinoid agonist WIN 55212-2 is not via CB1 or CB2 receptors. *Brain Res* 2008; 1200: 1-9.
105. Lenkei Z, Palkovits M, Corvol P, Llorens-Cortes C. Distribution of angiotensin type-1 receptor messenger RNA expression in the adult rat brain. *Neuroscience* 1998; 82: 827-841.
106. Diz DI, Barnes KL, Ferrario CM. Hypotensive actions of microinjections of angiotensin II into the dorsal motor nucleus of the vagus. *J Hypertens Suppl* 1984; 2: 53-56.
107. Neal CR, Jr., Mansour A, Reinscheid R, *et al.* Opioid receptor-like (ORL1) receptor distribution in the rat central nervous system: comparison of ORL1 receptor mRNA expression with (125)I-[(14)Tyr]-orphanin FQ binding. *J Comp Neurol* 1999; 412: 563-605.
108. Polidori C, Massi M, Guerrini R, Grandi D, Lupo D, Morini G. Peripheral mechanisms involved in gastric mucosal protection by intracerebroventricular and intraperitoneal nociceptin in rats. *Endocrinology* 2005; 146: 3861-3867.
109. Mazzone SB, Geraghty DP. Characterization and regulation of tachykinin receptors in the nucleus tractus solitarius. *Clin Exp Pharmacol Physiol* 2000; 27: 939-942.
110. Lewis MW, Travagli RA. Effects of substance P on identified neurons of the rat dorsal motor nucleus of the vagus. *Am J Physiol Gastrointest Liver Physiol* 2001; 281: G164-G172.
111. Dixon MK, Nathan NA, Hornby PJ. Immunocytochemical distribution of neurokinin 1 receptor in rat dorsal vagal complex. *Peptides* 1998; 19: 913-923.
112. Shujaa N, Zadori ZS, Ronai AZ, *et al.* Analysis of the effect of neuropeptides and cannabinoids in gastric mucosal defense initiated centrally in the rat. *J Physiol Pharmacol* 2009; 60 (Suppl.7): 93-100.
113. Brancati SB, Zadori ZS, Nemeth J, Gyires K. Substance P induces gastric mucosal protection at supraspinal level via increasing the level of endomorphin-2 in rats. *Brain Res Bull* 2013; 91: 38-45.
114. Benyhe Z, Toth G, Wollemann M, *et al.* Effects of synthetic analogues of human opiorphin on rat brain opioid receptors. *J Physiol Pharmacol* 2014; 65: 525-530.
115. Ray A, Henke PG. TRH-enkephalin interactions in the amygdaloid complex during gastric stress ulcer formation in rats. *Regul Pept* 1991; 35: 11-17.
116. Nikolarakis KE, Almeida OF, Herz A. Stimulation of hypothalamic beta-endorphin and dynorphin release by corticotropin-releasing factor (in vitro). *Brain Res* 1986; 399: 152-155.
117. Mizoguchi H, Watanabe H, Hayashi T, *et al.* Possible involvement of dynorphin A-(1-17) release via mu1-opioid receptors in spinal antinociception by endomorphin-2. *J Pharmacol Exp Ther* 2006; 317: 362-368.

Received: February 17, 2014
Accepted: February 5, 2015

Author's address: Prof. Klara Gyires, Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Nagyvarad ter 4., 1089 Budapest, Hungary. E-mail: gyires.klara@med.semmelweis-univ.hu