Analysis of the mechanisms involved in the regulation of gastric mucosal integrity and gastrointestinal motility

Ph.D. Doctoral Thesis

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INTRODUCTION

Treatment of peptic ulcers can raise difficulties even nowadays. In a significant percent of gastric ulcers the acid secretion is in normal range or even decreased and reduced mucosal defense may have a crucial role in the pathogenesis. In these cases treatment with antisecretory drugs often fails to result in a satisfactory therapeutic effect. Consequently, augmentation of endogenous defensive mechanisms is another possible approach of anti-ulcer therapy, but the number of such medicaments is very limited. Thus, the investigation of mucosal protective factors is particularly important. Mucosal integrity is maintained by several peripheral and - recently intensively studied - central factors. It was raised that local mediators (e.g. prostaglandins, NO, H₂S or CGRP released from sensory afferents) may act in concert in regulation and maintenance of mucosal integrity through stimulation of mucus and bicarbonate secretion, increasing mucosal blood flow, decreasing gastric acid secretion and inhibiting the release of inflammatory mediators. In the last decades it turned out, that central nervous system (CNS) is also involved in regulation of mucosal integrity through influencing the activity of various peripheral factors. Several centrally (intracisternally, intracerebroventricularly or into different nuclei) injected neuropeptides exert gastroprotection in both acid-dependent and/or acid-independent ulcer models, like amylin, bombesin, adrenomedullin or PYY. The two most important brain regions involved in central gastroprotection are hypothalamus and dorsal vagal complex (DVC), but other structures (e.g. amygdala, nucleus accumbens, locus coeruleus) are also have a significant role.

Endogenous opioid system - Endomorphins

The endogenous opioid system regulates several central and peripheral functions and also influences many gastrointestinal processes. Opioid receptors have been identified in the GI tract as well as in the hypothalamus and DVC, moreover, endogenous opioids (enkephalins, dynorphins) are localized in enteral neurons and chromaffin cells. Opioids delay gastric emptying, inhibit gastrointestinal motility, decrease gastric acid secretion and induce gastroprotection after both central and peripheral administration in different (acid-dependent and independent) ulcer models. Among the three opioid receptor subtypes µ-opioid receptors have the most prominent role in the GI tract, however, the gastrointestinal effects of their endogenous
ligands, the endorphins, discovered in 1997 were barely investigated and mostly in vitro experiments were performed.
The first group of questions I aimed to study in my doctoral work was the investigation of the gastroprotective effect of centrally (i.c.v.) injected endorphins in the rat.

**Nociceptin and nocistatin**

Nociceptin (N/OFQ), the endogenous ligand of the NOP receptor (formerly ORL-1, opioid receptor-like) is an opioid-related peptide, which was discovered independently by two laboratories in 1995. Several effects of N/OFQ have been described in the past years, like analgesia after intrathecal administration, bradycardic and hypotensive effect, stimulation of feeding, inhibition of gastrointestinal motility and also gastroprotection. All of these effects are opioid-like, and despite of the original concept that N/OFQ does not bind to opioid receptors, some of the above mentioned effects were significantly decreased after pretreatment with an opioid receptor antagonist.

In contrast, N/OFQ after supraspinal administration induced hyperalgesia (it is the origin of its name) and decreased the analgesic effect of several opioid agonists (e.g. morphine, endomorphin-1, DAMGO, DPDPE, deltorphin II). A possible mechanism is the inhibition of certain neurons originating from periaqueductal grey, which neurons are normally activated by opioids. In this regard N/OFQ is an anti-opioid peptide. The above data suggest a close interaction between N/OFQ and the opioid system.

The next group of questions I aimed to study in my doctoral work was the analysis of the role of opioid system in the gastroprotection induced by N/OFQ (and NST).

The precursor protein of N/OFQ, preproN/OFQ also contains the sequence of another peptide, which was discovered in 1998 and designated nocistatin (NST). Despite the fact that NST does not bind to the NOP receptor, it antagonizes several N/OFQ-induced effects, like its hyperalgesic and orexigenic effect or the impaired learning and memory. In contrast with the original assumption, however, NST is more than a simple functional antagonist of N/OFQ. For instance, NST failed to antagonize some N/OFQ-induced effects (e.g. the cardiovascular effects or stimulated acid secretion), moreover, NST increased the facilitation of flexor reflex elicited by low dose of N/OFQ. It was also recently demonstrated that NST is a biologically active peptide just like N/OFQ, since it inhibited GABAergic and glycineergic neurotransmission in the spinal cord dorsal horn and the $K^+$-
induced \[^1\text{H}\]5-HT release from mouse and rat cortical synaptosomes. The effects of NST in the GI tract have not been investigated yet. In one section of my doctoral work I was analysing the gastroprotective effect of NST and the interaction between NST and N/OFQ.

**α₂- and imidazoline-receptors**

Beside peptic ulcers another group of gastroenterological diseases, the functional gastrointestinal disorders (FGID, like functional dyspepsia or irritable bowel syndrome) also represent a serious clinical problem. These syndroms are characterized by motility disorders and visceral hypersensitivity, and patients are usually treated with prokinetics or fundic relaxants, however, in several studies these drugs showed no benefit over placebo. α₂-adrenoceptor agonists can relax the proximal stomach and reduce the sensation and discomfort associated with gastric and colorectal distension, so represent a new pharmacological tool in the therapy of FGIDs.

The role of α₂-adrenoceptors in regulation of gastrointestinal functions has been well documented. α₂-receptor agonists can influence gastric emptying and acid secretion, gastrointestinal motility and fluid transport after peripheral and/or central administration. Moreover, they also exert gastroprotective effect. Among the three α₂-receptor subtypes (α₂A, α₂B and α₂C) the α₂A-one mediate most of the effects. Oxymetazoline, a selective α₂A-receptor agonist is frequently used for the analysis of α₂-receptor mediated functions, however, our preliminary results suggested that besides the activation of presynaptic α₂A-receptors it may have other effects as well. One part of my experiments focus on the analysis of these mechanisms.

The imidazoline hypothesis, which raises the possibility that central sympathoinhibition caused by α₂-receptor agonists is actually mediated by central imidazoline receptors was introduced in 1984. This suggestion was supported by observations that clonidine and its chemical relatives bind to specific imidazoline-binding sites (IBS), in addition to α₂-adrenoceptors. The role of imidazolin receptors has also been proposed in gastrointestinal effects of α₂-agonists, however, data of the literature is rather contradictory, which may be due to the fact that imidazoline receptor agonists also possess reasonable affinity for α₂-adrenoceptors.

In the last part of my experiments I investigated the role of imidazoline receptors in the regulation of gastrointestinal motility.
AIMS

The aims of the study were to clarify:

1. Do endomorphins exert gastroprotective effect similar to other µ-opioid receptor agonists?
   - Is the endogenous endomorphin system involved in maintenance of mucosal integrity?
   - Which peripheral protective factors mediate the endomorphin-induced gastroprotection?

2. Can NST also induce gastroprotection similar to N/OFQ, and if so, is it independent from the effect of N/OFQ?
   - What kind of interaction exists between these two peptides in this experimental model?
   - Do opioid receptors and endogenous opioid system have any role in the gastroprotective effect of N/OFQ and NST?
   - Which peripheral protective factors mediate the N/OFQ- and NST-induced gastroprotection?

3. Are there any other mechanisms involved in the inhibitory effect of oxymetazolin on gastric motility beside activation of presynaptic α2A-adrenoceptors?
   - Do imidazoline receptors take part in the regulation of gastrointestinal motility?

METHODS

Animals

For ethanol ulcer experiments and measurement of gastric motility male Wistar rats (140-170 g and 250-350 g, respectively), for in vitro analysis of intestinal motility guinea-pigs from either sex (TRIK strain, 350-450 g) were used. Before the in vivo experiments animals were deprived of food for 24 hours. They were housed in wire mesh bottom cages to prevent coprophagy.

Administration of drugs
Compounds were given intracerebroventricularly (i.c.v., 10 µl/rat), intracisternally (i.c., 5 µl/rat), intravenously (i.v., in ethanol ulcer experiments: 0.5 ml/100 g, in vivo measurement of gastric motility: 0.1-0.2 ml/100 g), orally (0.5 ml/100 g) or intraperitoneally (i.p., 0.5 ml/100 g).

Ethanol ulcer model

Gastroprotection induced by different drugs was analyzed by an acid-independent model, where mucosal protection is not due to decreased acid secretion. After 24 h food deprivation male Wistar rats (140 - 170 g) were given orally 0.5 ml acidified ethanol (98% ethanol in 200 mmol/l HCl). One hour later the animals were killed by overdose of ether, stomachs were opened along the greater curvature, rinsed with saline, and examined for lesions. Total number of mucosal lesions was assessed in blinded manner by calculation of lesion index based on a 0–4 scoring system. The ulcer index was calculated as the total number of lesions multiplied by the respective severity factor. The percentual inhibition of mucosal damage was calculated as follows: 100 - [(Ulcer index in treated group / Ulcer index in control group) x 100]. Agonists (endomorphins, N/OFQ, NST) were given i.c.v. or i.c. 10 minutes before the ethanol challenge, while antagonists were given 10 minutes (i.c.v. and i.c.), 20 minutes (i.p.) or 1 hour (p.o. and i.v.) before the injection of agonists. (Only exception was the irreversible μ-opioid receptor antagonist β-funaltrexamine, which was injected 24 hours before the experiment.) Bilateral cervical vagotomy was carried out 2 hours before the experiment.

In vivo measurement of gastric motor activity

Male Wistar rats weighing 250–350 g were used. After 24 h food deprivation animals were anesthetized with urethane (1.25 g/kg i.p.), then an intragastric balloon (approximately 10 mm x 30 mm) created from thin latex rubber connected with plastic tubing was introduced into the stomach via mouth. The balloon was filled with 2 ml warm water (37°C) to set the basal intragastric pressure to 10 ± 0.5 cmH₂O. The exact location of the balloon was verified after each experiment. A 15-30 min equilibrium period was registered before every experiments. The effect of α2-adrenoceptor agonists was studied both on basal and stimulated motility. The gastric motor activity was stimulated either by insulin (5 IU/rat, i.v.), through activation of the vagal nerve or by carbachol (0.14 µmol/kg i.v.) + hexamethonium (37 µmol/kg), through selective stimulation of peripheral muscarinic receptors. α2-receptor agonists were given i.v. when the
stimulated gastric motor activity became stable (30–60 min after the injection of insulin and 10-15 min following the administration of carbachol + hexamethonium). The antagonists were injected 10 min after the administration of α₂-adrenoceptor agonists. During the computer analysis of the experiments three parameters of gastric motor activity were determined: mean amplitude of gastric contractions, sum of amplitudes (calculated by multiplying the mean height of contractions with the number of contractions in a 5 min period) and intragastric tonic pressure. Pressure values were expressed in cmH₂O.

**In vitro measurement of intestinal motility**

The small intestine (jejunum and ileum) of adult guinea-pigs (TRIK strain, either sex, 350 ± 450 g body weight) was isolated and divided into eight segments, each being approximately 10 cm long. The segments were set up in organ baths containing 30 ml of Tyrode solution at 37°C. The composition of the Tyrode solution was (mM): NaCl 136.9, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.0, NaHCO₃ 11.9, NaH₂PO₄ 0.4 and glucose 5.6. After a 30 min equilibrium period prewarmed Tyrode solution was continuously infused into the lumen of the segments at a rate of 0.5 ml/min in order to elicit propulsive peristalsis. After basal peristaltic activity had been recorded for a 30 min period, the drugs to be tested were added to the bath in a cumulative manner at 15 min intervals to the serosal surface of the intestinal segments, at volumes not exceeding 300 µl. Antagonists were given 15 min before the first concentration of the agonists. During the evaluation two parameters were determined; the intraluminal pressure (IP), which correlates with the emptying capacity of the segments and the peristaltic pressure threshold (PPT), which is the intraluminal pressure at which a peristaltic wave is triggered. Pressure values were expressed in cmH₂O. Concentration-response curves were fitted to mean pressure values using the following equation (Hill-equation, 4 parameter): P = P<sub>min</sub> + [(P<sub>max</sub> - P<sub>min</sub>) x X<sup>alt</sup> / IC<sub>50</sub> + X<sup>alt</sup>], where P is the pressure value, X is the concentration of the agonist in nmol, nH is the Hill coefficient and IC<sub>50</sub> is the concentration that 50% of the maximal inhibition.

**Compounds**

The following drugs were used: atropine, carbamoylcholine chloride (carbachol), CGRP<sub>8-37</sub>, clonidine, hexamethonium, indomethacin, naloxone, naltrindole, N<sup>ω</sup>-nitro-L-arginine (L-NNA), nociceptin/OFQ (N/OFQ), norbinaltorphimine (norBNI), prazosin propranolol, ritlenidine, urethane,
yohimbine (Sigma), 1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one (J-113397), β-funaltrexamine, BRL 44408, nocistatin (Tocris Bioscience), oxymetazoline (RBI Natick), human insulin (rDNS, Actrapid Penfill, Novo Nordisk), endomorphin-1 and endomorphin-2 (Biological Research Centre of Hungarian Academy of Sciences, Szeged), endomorphin-1 és -2 antiszérumok (István Barna, Hungarian Academy of Sciences), diprotin A (HAS/ELTE Research Group of Peptide Chemistry, Budapest, Hungary). All drugs were dissolved in saline with the exception of J-113397 and CGRP₈₋₃₇ (dissolved in DMSO), diprotin A (dissolved in Tween) and indomethacin (suspended in 1% methylcellulose and given orally). The control animals received the drug solvent.

Statistical analysis

Statistical analysis of the data was evaluated by means of analysis of variance (ANOVA) followed by Newman–Keuls test for multiple comparisons, or Student’s t-test (paired or unpaired). A probability value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Analysis of the gastroprotective effect of endomorphins

Both endomorphin-1 (0.03 - 20 pmol) and endomorphin-2 (0.03 - 3 pmol) exerted a dose-dependent gastroprotective effect after i.c.v. administration. This effect was induced by very low (picomolar) doses, which is good correlation with the previous reports that gastroprotection can be induced by much lower doses of opioids than those needed for antinociception. (Analgetic doses of endomorphins are in nanomolar range.) Pretreatment with intrecisternal injection of endomorphin-antiserum abolished the effect of endomorphins, which implies that endomorphins elicit their effect in the brain stem, very likely through activation of the DVC. Literature data related to the analgesic potency of endomorphins is quite contradictory. According to several reports endomorphins induce analgesia with similar potency, while others reported that endomorphin-1 is more potent analgetic. With respect to their gastroprotective effect endomorphin-1 turned out to be slightly more potent than endomorphin-2, which can be due to the differences observed between the two peptides (like different central distribution or activation of disparate µ-receptor subtypes).
Dose-response curves of both endomorphins proved to be bell-shaped, since gastroprotection highly reduced after administration of greater doses. Further studies are needed to clarify the underlying mechanism, however, it can be speculated that endomorphins in higher doses activate such endogenous receptors/systems, which can counteract the opioid-induced mucosal protection. It is also possible that inhibition of gastric motility and consequent delayed emptying of ethanol is the reason of this phenomenon. The effect of both endomorphins was antagonized by the non-selective opioid receptor antagonist naloxone, which indicates that their effect is mediated by opioid-receptors. Though endomorphins possess high selectivity for the \( \mu \)-receptor, the involvement of \( \delta \)- and \( \kappa \)-receptors also cannot be ruled out, especially considering that some effects of endomorphin-2 proved to be mediated by enkephalins and dynorphins. Therefore, further experiments are needed to elucidate the role of other opioid receptors in this mucosal protective effect.

Diprotin A (0.5-1 \( \mu \)mol), an inhibitor of dipeptidyl peptidase IV (DPP IV), a key enzyme in the degradation of endomorphins, dose-dependently inhibited the formation of ethanol-induced mucosal lesions. Pretreatment with naloxone antagonized this effect. These results suggest the involvement of the endogenous endomorphin system in the maintenance of mucosal integrity.

In the next series of my experiments the peripheral factors involved in the gastroprotective effect of endomorphins were elucidated. Assuming that the same factors mediate the gastroprotective effect of both endomorphins, these studies were carried out only with endomorphin-2.

First I analyzed, how the central effect of endomorphins is conveyed to the periphery. The vagal nerve is one of the most important connections between the CNS and the stomach and has a dual role in the regulation of mucosal integrity. On the one hand, an increased activity of vagal efferents can induce ulcerogenic effect through the release of histamine, the increased acid secretion and enhanced motility. On the other hand, however, vagal activation increases mucosal defense, since Ach can induce the release of NO and PGs. It has also been demonstrated, that vagal nerve mediates the gastroprotective effect of several centrally injected neuropeptides. In my experiments neither the blockade of muscarinic Ach-receptors with atropine, nor the pretreatment with ganglion-blocking agent hexamethonium influenced the effect of endomorphin-2, which suggests that postganglionic cholinergic receptors may not be involved in the endomorphin-induced gastroprotection. However, we cannot completely exclude the role of the vagal nerve, since preganglionic neurons containing nitric oxide synthase have also been described, which project directly to the fundus and their
The gastroprotective effect of endomorphin-2 was also inhibited by the CGRP\textsubscript{1}-receptor antagonist CGRP\textsubscript{8-37}, which suggests the involvement of capsaicin sensitive extrinsic afferents. Though endomorphin-induced CGRP-release has not been described yet, co-localization of endomorphin-2 and CGRP in spinal afferents and in the NTS has been reported. Beside CGRP and NO also the PGs have an essential role in the maintenance of mucosal integrity. As my results show, pretreatment with indomethacin and the consequent inhibition of PG formation significantly decreased the protective effect of endomorphin-2, which indicates that PGs are also involved in these mechanisms. However, since the protective effect was not completely antagonized, PGs may only have a secondary role and the two most important mediators are CGRP and NO.

It is well established that activation of β-adrenoceptors and the subsequent elevation of mucosal blood flow enhances mucosal defense. Therefore, I also investigated the role of sympathetic nervous system and the β-adrenoceptors in the endomorphin-induced mucosal protection. The inability of propranolol to reduce the protective effect of endomorphin-2 suggests that β-receptors are not involved in it.

Analysis of the gastroprotective effect of nociceptin and nocistatin

In the second section of my work I analyzed the effect of N/OFQ and NST on the ethanol-induced ulcers. Much less data have been reported about NST than N/OFQ, and in most papers NST was used as a functional antagonist of N/OFQ. However, in contrast to the original concept, several recent data suggest that NST is a biologically active peptide per se. My findings confirm the latter hypothesis and demonstrate that NST after i.c.v. injection induces gastroprotection with almost the same potency and efficacy as N/OFQ. The dose-response curves (similarly to endomorphins) proved to be bell-shaped, since administration of higher doses (2 and 5 nmol) resulted in a decreased protective effect. Bell-shaped dose-response relationship was also observed by other effects of these peptides, for instance by the N/OFQ-induced allodynia or the inhibitory effect of N/OFQ- and NST on the \textsuperscript{[3H]}5-HT release. Further studies are needed to
elucidate the reason, why their protective effect declines by higher doses. Since inhibitory effect of N/OFQ on the gastric motility has been reported, the delayed emptying of ethanol can be one possible explanation. The gastric mucosal protective effect of N/OFQ is likely to be mediated by the NOP receptor, because J-113397, a selective competitive antagonist of this receptor abolished the N/OFQ-induced protection. Since NST induces the same effect as N/OFQ, the question was raised whether NST acts via the N/OFQ-system (either by direct activation of the NOP receptor or through stimulation of the release of N/OFQ), or it acts independently. Since J-113397 failed to influence the effect of NST, it can be concluded, that NST exerts the mucosal protective effect irrespective of N/OFQ-system, through activation of its own (and so far unidentified) receptor. As mentioned above, NST was described originally as a functional antagonist of N/OFQ. It inhibited e.g. the N/OFQ-induced hyperalgesia, allodynia or stimulated food-intake. In contrast, the lack of effect or even potentiation has also been described which indicates that the interaction between NST and N/OFQ is far more complex. The present data suggest the existence of different types of interaction between NST and N/OFQ with respect to their gastroprotective effect. Additive interaction in the gastroprotection was observed when NST and N/OFQ were given in low doses. In contrast, when higher doses were used, the gastroprotective effect of N/OFQ was reduced. The mechanism of the antagonism has not been clarified yet. Reduction of the pharmacological actions after combination of a full and partial agonist is a well-known phenomenon, however, this possibility can be excluded since N/OFQ and NST act at different receptors. Taking into consideration the bell-shaped dose–response relationship, it might be raised, that after combination the doses of the two peptides are added and reach the dose range where the protective effect already diminishes. I also investigated the role of the endogenous opioid system in the N/OFQ- and NST induced gastroprotection, since on the one hand it has been demonstrated, that opioids mediate the protective effect of various systems (α2-adrenoceptors, NMDA, cannabinoids), and on the other hand, several lines of evidence suggest an interaction between the opioid- and N/OFQ system. It was found that the gastroprotective effect of both N/OFQ and NST was decreased or abolished by the nonselective opioid receptor antagonist naloxone, the µ-opioid receptor antagonist β-funaltrexamine, the δ-opioid receptor antagonist naltrindole and the κ-opioid receptor antagonist norbinaltorphimine, suggesting the involvement of central µ-, δ- and κ-opioid receptors in mediating the mucosal protective effect of N/OFQ and NST.
One possible explanation, that N/OFQ and NST may activate directly these opioid receptors, since (in contrast to the original finding) it was reported that N/OFQ has a low to moderate affinity to µ- and κ-opioid receptors. It should be kept in mind that the gastroprotective dose range of opioid peptides given i.c.v. or i.c. was far below the doses that were needed to induce analgesic action. Consequently, it might be speculated that moderate activation of opioid receptors by N/OFQ may be sufficient to initiate a chain of events resulting in gastroprotective action. Another possibility that N/OFQ and NST may increase the release of endogenous opioid peptides. This assumption is supported by the finding that N/OFQ can modulate the stimulated release of [Met\textsuperscript{5}]enkephalin from the guinea pig myenteric plexus.

The gastroprotective effect of both N/OFQ and NST is likely to be vagal-dependent and mediated by NO and PGs in the periphery. Literature data indicate that capsaicin sensitive afferent fibers and CGRP are also involved in the N/OFQ-induced mucosal protection. Such a role of CGRP in the effect of NST remains to be established.

Analysis of the effect of α\textsubscript{2}-adrenoceptors and imidazoline receptors on the gastrointestinal motility

The selective α\textsubscript{2A}-adrenoceptor agonist oxymetazoline (0.2 - 3.4 μmol i.v.) exerted a strong inhibitory effect on the gastric motility of the rat: each measured parameters (the mean of amplitudes, the sum of amplitudes and the intragastric tonic pressure) was decreased significantly. However, this inhibitory effect differed from that of clonidine in several aspects. First of all, clonidine (1.9 and 3.8 μmol/kg i.v.) failed to affect the basal motor activity, while oxymetazoline inhibited it. Secondly, the inhibitory effect of clonidine on the insulin-induced gastric motility was reversed by the non-selective α\textsubscript{2}-receptor antagonist yohimbine and the selective α\textsubscript{2A}-adrenoceptor antagonist BRL 44408, while both antagonists failed to influence the effect of oxymetazoline. Furthermore, oxymetazoline also inhibited the carbachol + hexamethonium-stimulated gastric motility, while clonidine had no effect on it. Thus, it may be concluded, that clonidine exerts its effect solely through activation of presynaptic α\textsubscript{2A}-adrenoceptors and the inhibition of Ach-release from myenteric nerves. In contrast, beside activation of presynaptic α\textsubscript{2A}-adrenoceptors postsynaptic components may also contribute to the oxymetazoline-induced inhibition of gastric motor function. What kind of postsynaptic receptors can mediate the effect of oxymetazoline? One alternative is that postsynaptic inhibitory α\textsubscript{1}-receptors. Oxymetazoline beside α\textsubscript{2A}-adrenoceptors also binds to α\textsubscript{1A}-receptors, and
inhibitory α₁-receptors have been described in gastric fundus. However, this mechanism is implausible since prazosin failed to reverse the effect of oxymetazoline. Another possibility is the direct inhibition of muscarinic receptors, because recently atropine-like properties of oxymetazoline have been demonstrated in human ciliary muscles and in guinea-pig ileum. A further alternative that 5HT-receptors mediate the inhibitory action of oxymetazoline. Several 5HT-receptor subtypes have been identified in the GI tract (5HT₁, 5HT₂, 5HT₃, 5HT₄, 5HT₇) on neurons and smooth muscles, and the relaxant effect of 5HT₁-receptors has been well established (5HT₁-agonists improve the gastric accommodation and represent a new therapeutical approach in the treatment of FGIDs). It has been reported that oxymetazoline binds to 5HT₁A, 5HT₁B, 5HT₁C and 5HT₁D-receptors, which may contribute to its inhibitory effect. However, recent findings suggest that among 5HT₁-subtypes the 5HT₁D is the one, which stimulates the release of NO from nitricergic myenteric neurons and responsible for the relaxant effect. It still remains to be established, whether oxymetazoline can also bind to this subtype. Finally, the role of imidazoline receptors can also be supposed in the effect of oxymetazoline, but it should be borne in mind that clonidine binds to imidazoline receptors as well. Moreover, our preliminary data suggests that idazoxan (a mixed α₂/I₁-antagonist) also failed to inhibit the effect of oxymetazoline.

The imidazoline receptors started to receive more attention in the 80’s, when the imidazoline hypothesis was introduced. According to this theory clonidine (and its chemical relatives) exert their antihypertensive effect via stimulation of imidazoline receptors instead of α₂-adrenoceptors. The role of imidazoline (especially I₁) receptors has also been suggested in the regulation of GI functions. However, most I₁ agonists possess appreciable affinity for α₂-adrenoceptors as well, which renders the investigation of imidazoline receptor-mediated functions more difficult. I analyzed the role of imidazoline receptors in guinea-pig ileum. The results suggest that beside α₂-adrenoceptors I₁ receptors also play role in the regulation of gastrointestinal motility, since rilmenidine, an I₁ agonist dose-dependently inhibited the peristaltic activity of the small intestine. Moreover, the inhibitory effect of clonidine (which binds with high affinity to I₁ and with moderate affinity to I₂ receptors) was significantly reduced by I₁ receptor antagonist efaroxan. However, further studies are needed to elucidate the precise role of imidazoline receptors.
THE PRINCIPAL FINDINGS OF THE THESIS

1. Both endomorphin-1 and endomorphin-2 induce gastroprotection after i.c.v. administration with similar potency and efficacy, similarly to other µ-receptor agonists.

2. The endogenous endomorphin system may take part in the maintenance of mucosal integrity.

3. The classic mucosal protective factors (CGRP, NO and PGs) may be involved in mediating the effect of centrally injected endomorphin-2 in the periphery. The role of the vagal nerve and the sympathetic nervous system cannot be excluded, however, ganglionic nicotinic receptors, muscarinic receptors and β-adrenoceptors may not be involved in the endomorphin-induced mucosal protection.

4. Beside N/OFQ also centrally injected NST can induce gastroprotection, with almost the same potency and efficacy. This effect is independent from the N/OFQ-system and may be mediated by the (still unidentified) receptor of NST.

5. Pretreatment with higher dose of NST resulted in decrease of the protective effect of N/OFQ, though both peptides exerted a pronounced gastroprotective action per se. In contrast, addition of their effects was observed, when lower doses were applied.

6. The gastroprotective effect of both N/OFQ and NST is likely to be mediated by the central opioid system and µ-, δ- and κ-opioid receptors may be involved in this action.

7. The effect of both peptides is vagus-dependent, and may be mediated by NO and PGs in the periphery.

8. The inhibitory effect of oxymetazoline in the gastric motility consists of two components, because beside the activation of presynaptic α2A-receptors postsynaptic mechanisms may also be involved in it.

9. Beside α2-adrenoceptors also I1 receptors may regulate the gastrointestinal motility.
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