Gastric mucosal integrity and gastric motility

Pharmacological analysis of α_2 adrenoceptor subtypes involved in regulation of gastric motility in the rat

Ph.D dissertation

Katalin Fülöp

Semmelweis University, School of Ph.D. Studies

Supervisor: Klára Gyires, M.D., Ph.D., D.Sc.

President of examination board: Dr. Kornélia Tekes, Ph.D., D.Sc.
Members of examination board: Dr. Éva Szökő, Ph.D.
Dr. Tibor Zelles, Ph.D.

Chairman of final examination committee:
Members of final examination committee:

Department of Pharmacology and Pharmacoterapeutics
Semmelweis University, Faculty of Medicine
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SUMMARY

Gastric mucosal integrity and gastric motility - Pharmacological analysis of α_2 adrenoceptor subtypes involved in regulation of gastric motility in the rat

Potential correlation between influence of gastric motility, gastric acid secretion and gastric mucosal protection induced by α_2 adrenoceptor agonists has been studied.

Gastric emptying: Gastric emptying was inhibited by clonidine (ED_{30}:0.54 μmol/kg, s.c.) and the selective α_{2A} adrenoceptor agonist oxymetazoline (ED_{30}: 0.81 μmol/kg, s.c) given peripherally. The delaying effect of clonidine was inhibited by the α_2 antagonist yohimbine (12.82 μmol/kg, s.c.), but not the $\alpha_{2B/2C}$ subtype selective antagonist prazosin (1.19 μmol/kg, s.c.) and ARC-239 (0.68 μmol/kg, s.c.). The opioid antagonist naloxone (2.75 μmol/kg, s.c.). failed to affect delaying effect of clonidine. Gastric emptying was also inhibited by i.c.v. clonidine (ED_{30}: 29.84 nmol/rat, i.c.v.) and oxymetazoline (ED_{30}:7.93 nmol/rat, i.c.v.). Gastric emptying was also delayed by i.c.v. morphine (ED_{30}:118 nmol/rat), the μ opioid receptor agonist DAGO (ED_{30}: 5.7 nmol/rat) and the δ opioid receptor agonist deltorphin II (56.7 nmol/rat). **Gastric motility**: The 2-deoxy-D-glucose stimulated gastric motility was inhibited by clonidine in the dose of 0.75 μmol/kg i.v., and the effect was antagonized by yohimbine, but not by prazosin and naloxone. The inhibitory effect of oxymetazoline (0.185-3.4 μmol/kg, i.v.) was only partially reversed by yohimbine. **Gastric acid secretion**: The antisecretory effect of i.c.v. clonidine was blocked by yohimbine, naloxone but not by prazosin. **Gastroprotection:** The protective effect of i.c.v. clonidine on ethanol induced damage was blocked by yohimbine, prazosin, and naloxone. The protective doses of clonidine (ED_{50}: 0.14 nmol/rat, i.c.v.), DAGO (ED_{50}: 0.0068 nmol/rat, i.c.v.) proved to be much lower than the gastric emptying delaying and the antisecretory ones**. In conclusion**, i**./** $\alpha_{2B/2C}$ subtypes may mediate the gastroprotection, while $\alpha_{2A/2D}$ subtypes may account for the antisecretory and gastric motility and emptying inhibitory effect of α_2 adrenoceptor agonists. ii./Opioid component may be involved in their gastroprotective and antisecretory effects, but not in the inhibitory effect of gastric motility and gastric emptying. iii./The protective doses of opioid and α_2 adrenergic receptor stimulants are much lower, than gastric emptying and gastric motility inhibitory ones. *Consequently,* no correlation is likely to be exist between the gastroprotective effect and affection of gastric motor activity and gastric acid secretion induced by α_2 adrenoceptor stimulants.

ÖSSZEFOGLALÁS

A gyomornyálkahártya-integritás és gyomormotilitás - α_2 adrenoceptor szubtípusok gyomormotilitás szabályozásban játszott szerepének farmakológiai analízise patkányon

Kísérleteinkben azt vizsgáltuk, hogy a gyomor motoros aktivitásának és a gyomorszekréciónak a változásai kapcsolatban állnak-e az α_2 adrenoceptor agonisták gyomorvédő hatásával. **Gyomorürülés:** A klonidin (ED_{30}: 0,54 μmol/kg, s.c.) és az oxymetazolin (ED_{30}: 0,81 μmol/kg, s.c) késleltette a gyomorürülést perifériás adás során. A klonidin hatását az α_2 antagonista yohimbin (12,82 μmol/kg, s.c.) gátolta, míg a $\alpha_{2B/2C}$ antagonista prazosin (1,19 μmol/kg, s.c.) és ARC-239 (0,68 μmol/kg, s.c.) nem. Hasonlóan, a nitrogén monoxid szintáz inhibitor, N^G-nitro-L-arginin (91.3 μmol/kg, i.v.) és az opioid antagonista naloxon (2,75 μmol/kg, s.c.) sem befolyásolta a hatást. A klonidin (ED_{30}: 9,84 nmol/patkány, i.c.v.) és az oxymetazolin (ED_{30}: 7,93 nmol/patkány, i.c.v.) centrális adagolás során is késleltette a gyomorürülést. Az i.c.v. adott μ opioid receptor agonisták, így a morfin (ED_{30}: 118 nmol/patkány, i.c.v.) és a DAGO (ED_{30}: 15,7 nmol/patkány, i.c.v.) ill. a δ opioid receptor agonista deltorphin II (56,7 nmol/patkány, i.c.v.) szintén gátolták a gyomorürülést. **Gyomormotilitás:** A 2-deoxi-D-glukóz stimulálta gyomormotilitást a klonidin (0,75 μmol/kg, i.v.) gátolta, melyet a yohimbin antagonizált, míg a $\alpha_{2B/2C}$ antagonista prazosin nem befolyásolt. Az oxymetazolin (0,185-3,4 μmol/kg, i.v.) gátló hatását a yohimbin csak részben függesztette fel. **Gyomorsav-szekréció**: A klonidin által centrálisan indukált szekréciógátlást a yohimbin és a naloxon antagonizálta. **Gyomornyálkahártya-védelem**: A klonidin centrálisan indukált protektív hatását a yohimbin, prazosin és naloxon is gátolta. A klonidin (ED_{50}: 0.14 nmol/patkány, i.c.v.) és a DAGO (ED_{50}: 0.0068 nmol/patkány, i.c.v.) gasztroprotektív dózisa sokkal kisebb a gyomorsav-szekréciót és ürülést gátló dózisoknál. **Következtetés:** i./Valószínűleg $\alpha_{2B/2C}$ szubtípusok vesznek részt a gyomorvédő hatás, míg $\alpha_{2A/2D}$ szubtípusok a szekréció- és gyomorürülés-gátló hatás szabályozásában. ii./Az α_2 adrenoceptor stimulánsok gyomorvédő és szekréciógátló mechanizmusában opioid elemek is szerepelnek, míg a gyomorürülést gátlóban nem. iii./Az α_2 adrenoceptor agonisták gyomorvédő dózisa sokkal kisebb a gyomorürülést befolyásolónál. *Mindezek alapján* az α_2 és opioid receptor agonisták protektív dózisai nem befolyásolják a gyomorürülést, továbbá a gyomorsav-szekréció és gyomormotilitás gátlása nem játszik szerepet a klonidin gyomorvédő hatásában.

ABBREVIATIONS

2-DG	2-deoxy-D-glucose
ANOVA	analysis of variance
CGRP	calcitonin-gene-related peptide
CNS	central nervous system
DMV	dorsal motor nucleus of vagus
DVC	dorsal vagal complex
ENS	enteric nervous system
GABA	γ-aminobutyric acid
GI	gastrointestinal
i.c.	intracisternal
i.c.v.	intracerebroventricular
i.p.	intraperitoneal
i.v.	intravenous
L-NNA:	N^G-nitro-L-arginine
n:	number of animals
NANC	non-adrenergic non-cholinergic
NO	nitric oxide
NSAID	non-steroid anti-inflamatory drugs
NTS	nucleus of solitary tract
p.o.	per os
PG	prostaglandin
PVN	paraventricular nucleus of the hypothalamus
s.c.	subcutaneous
S.E.M.	standard error of mean
TRH	thyrotropin-releasing hormone
VIP	vasoactive intestinal peptide

1 INTRODUCTION

1.1 Overview of factors involved in gastric mucosal integrity

The maintenance of gastric mucosal integrity has been considered to be ensured by the fine balance between defensive mechanisms and aggressive factors. The unstirred layer of the mucus, bicarbonate, the mucosal microcirculation and the cell renewal all protect the gastric mucosa against aggressive factors such as pepsine, gastric acid, different chemicals and bacterial invasion. The disruption of mucosal integrity may lead to development of peptic ulcer disease. Peptic ulcers develop mainly in the duodenum, in the antral part of the stomach and in the oesophagus. Duodenal ulcers are considered to be associated with gastric acid hypersecretion while the gastric ulcers are rather characterized by hyposecretion. Thus the pathogenic mechanisms of lesion formation are different in these ulcer types. Nevertheless in both cases the disruption of fine balance between agressive and defensive processes can be responsible for ulcerogenesis.

Medication of gastroduodenal ulcers is mainly based on decreasing the deleterious action of aggressive factors (for instance by the neutralization of luminal acid, inhibition of gastric acid secretion, eradication of Helicobacter pylori) and protecting of the mucosa.

The surveillance system of digestive tract involves barriers (mucus gel layer, epithelial cells) and different mechanisms which are coordinated by the nervous system (including the enteric nervous system (ENS) and central nervous system (CNS), the endocrine system and the immune system[1]. Moreover these systems are cooperating with each other in the regulation of defensive mechanism.

1.1.1 Structural elements of gastric mucosal defense:

One of the most important elements in the gastrointestinal tract is the mucus gel layer covering the gastric mucosa. This layer has gel nature and viscous property and behaves as a barrier. The physiological functions of this barrier are to impede the diffusion of gastric acid, bacteria and different macromolecules such as bacterial toxins to the epithelial cells [2-4]. In addition, the gel layer also contains surface active phospholipids with hydrophobic properties to make the surface impermeable for luminal acid [4].

The tightly connected epithelial cells form another structural element. The epithelium has the ability to renew itself continuously. The older cells are extruded into the lumen and replaced by younger cells without breaking the barrier [5]. Study demonstrated that the gastric epithelium of the rat by itself was capable of resisting to acid challenge in a strongly acidified environment of pH 0.8 for more than 80 min without any detectable injury [6]. The secreted bicarbonate also defends the mucosa by neutralizing the acid diffusing back to the mucosal surface [4].

1.1.2 Functional components and mediators of gastric mucosal defense:

Sufficient mucosal blood flow is essential for maintenance of gastric mucosal integrity [7]. It takes part in the neutralization of noxious chemicals and in the delivery of oxygen and nutrients to the epithelial cells. It also plays a critical role in the disposal of H^+s diffusing from the lumen or from the parietal cells [7-9].

Several mediators, including prostaglandins (PG), nitric oxide (NO) and calcitonin-gene-related peptide (CGRP), are involved in the regulation of the physiological defensive mechanisms [7].

Continuous PG production is necessary for the maintenance of mucosal integrity. PGs influence gastrointestinal functions differently. They uniformly inhibit the gastric acid secretion [10] but they have variable effects on non-parietal secretion, including mucus and bicarbonate production. For instance PGE_2 stimulates it whereas PGI_2 does not [11]. In addition, they take part in the maintenance of sufficient mucosal blood flow thereby preventing ishemia which in turn may lead to necrosis [5;12]. Several studies proposed the involvement of PGs in the mediation of gastric contractility. Thus, endogenous PGs were shown to facilitate tonic contractions of proximal stomach and to decrease the amplitude of antral phasic contractions [13]. Milenove and coworkers [14] demonstrated that PGE_2 had a dual action on the gastrointestinal smooth muscle, relaxed the circular muscle and contracted the longitudinal smooth muscle [14]. Inhibition of PG synthesis by NSAIDs results in increased susceptibility of gastrointestinal mucosa toward noxious factors [5]. Certain prostaglandins (e.g. PGE_2, PGI_2) were found to defend the gastric mucosa against noxious events even in a low dose that did not influence the gastric acid secretion. This secretion-independent defensive mechanism was defined as cytoprotection [15]. However, as the results of Lacy and coworkers

suggested, histologically the protection was not complete, because more than 95% of the superficial epithelial cells was shown to be destroyed by absolute ethanol, while the deep layer of the mucosa was protected [16].

NO has been considered to be another essential mediator of the mucosal defensive mechanisms. NO was reported to be involved in the elevation of mucosal microcirculation [17], in the increase of mucus and bicarbonate secretion, in ulcer healing and in the inhibition of gastric acid secretion [5;18]. In addition, NO was demonstrated to be involved in the spontaneous relaxant motor activity of the rat antrum [19], and to be one of the major inhibitory neurotransmitters in pylorus [20;21]. NO was proposed to have cytoprotective effect on rat gastric mucosa as well [22]. However, deleterious effects of NO were also reported. Thus it was suggested that upregulation of inducible NO synthase by bacterial endotoxin and overproduction of NO in gastrointestinal mucosa might induce an enhanced mucosal permeability leading to intestinal damage [23]. Similarly higher dose of nitroprusside induced extensive mucosal injury which might be due to the overproduction of NO or its metabolite, peroxynitrate [24]. These findings also indicate that an adequate balance is required in the regulatory mechanisms to preserve the mucosal integrity.

Capsaicin sensitive sensory afferents serve as a rapid alarm system, and are among the most important elements of gastroduodenal defense. The capsaicin-sensitive afferents have both vagal and spinal origin. They monitor the luminal content and become quickly activated by chemical stimuli resulting in release of different mediators. A well-known mechanism regulated by capsaicin sensitive sensory afferents is the acid back-diffusion model. According to this model the luminal acid diffusing into the mucosa stimulates the sensory afferent terminals, resulting in CGRP release which in turn induces NO formation in the endothelium. NO relaxes the vascular smooth muscle cells thereby elevating the mucosal blood flow. The increased microcirculation facilitates the delivery of bicarbonate to the epithelium therefore neutralizing the acid [7;9;25].

Mucosal immune system is a further component of the gastrointestinal surveillance system. It also plays a dominant role in the rapid alarm activation of mucosal defense, including chemochine secreting epithelial cells, mucosa-associated lymphoid tissues

(lymphocythes, macrophages, mast cells) and antigen-presenting M cells which are specialized mucosal cells [26].

1.1.3 Gastrointestinal motility - gastric mucosal integrity:

Gastrointestinal motility (GI) seems to be also a critical factor in the maintenance of mucosal integrity. Gastric contractions characterized by high amplitudes may induce microvascular disturbances in specific sites of the mucosa probably by abnormal compression of the gastric wall, thereby leading to insufficient mucosal blood flow, increased vascular permeability and cellular damage [27-32]. Several of the above mentioned mediators were reported to affect the GI motility, such as NO, PGs.

An important role of PGs in mediation of GI motility can be raised because they were reported to influence the gastric smooth muscle contractility [13;14]. Mucosal endogenous PG deficiency induced by indomethacin was shown to be associated with gastric hypermotility [31;33-35]. It was reported however, that gastric erosions did not develop at a lower PG level (which did not influence either the gastric motility or the gastric acid secretion) in the absence of other risk factors [36], while severe gastric damage developed in the presence of another deleterious factor such as luminal acid, hypermotility or chemical ablation of capsaicin sensitive afferents [36;37]. 2-deoxy-D-glucose (2-DG), which enhances the gastric motility by a central vagal stimulation, caused by itself non-haemorrhagic lesions on gastric mucosa, but these lesions became haemorrhagic under PG-deficient conditions induced by a low dose of indomethacin [30].

Kunikata and coworkers proposed that the spasmodic nature of intestinal contractions might cause an interruption of the unstirred mucus gel layer therefore making the mucosa vulnerable to the bacterial invasion or different irritants [38]. This assumption was based on the experiments that thiaton, a spasmolitic drug, proved to be protective in the indomethacin induced intestinal damage associated with hypermotility [38].

Previous studies described both deleterious and beneficial effects of gastric emptying. Thus the delay of that caused by large doses of morphine was proposed to aggravate the ethanol-induced gastric lesions [94]. Beside the deleterious effect of the delayed gastric emptying, beneficial effect of its elevation was also reported.

Metoclopramide, which is widely used as antiemetic and prokinetic drug, was found to produce gastroprotective effect on aspirin-induced ulcer-model in rats [39]. Among others the protective role of enhancement of gastric emptying was also raised by authors in this protective process [39].

Ethanol is a widely used agent for experimental ulcer induction. With regard to pathogenic mechanism of ethanol induced lesion formation the question can be raised how it can affect the gastric contractility. It seemed to have a concentration dependent, dual effect on GI contractile activity, since high concentration of ethanol was found to cause tonic contraction of vascular smooth muscle in rats, of gastric smooth muscle in guinea pigs and of circular smooth muscle strips prepared from feline fundus [40;41]. On the other hand, at low concentrations ethanol was found to inhibit the amplitudes and frequency of phasic contractions in canine antral smooth muscle [42;43].

1.1.4 CNS and gastric mucosal integrity:

In the last decades intensive research has been focused on the role of CNS in the maintenance of gastric mucosal integrity. Several areas of the brain have been shown to be involved in the regulation of GI functions like the dorsal vagal complex (DVC) located in the lower medulla or certain nucleui of the hypothalamus.

The vagal activation has been shown to influence several visceral functions such as gastric acid secretion, gastric motility, gastric emptying, intestinal transit time, bicarbonate sectretion, release of hormones such as insuline and metabolism in the liver [44-46]. Stomach is supposed to have the largest representation in the DVC compared to other subdiafragmatic structures. In rats the abdominal vagal branches have been estimated to include afferents in 80-90% terminating primally in the nucleus of the solitary tract (NTS) and their cell bodies residing primally in the nodose ganglion. Cell bodies of the vagal efferent are localized mainly in the dorsal motor vagus (DMV) and send axons to the visceral organs [45;47]. The DMV receives strong input from the NTS, as well as projections from the paraventricular nuclei of the hypothalamus (PVN), which is also considered to have a prominent role in the mediation of GI functions. The electrical stimulation of the PVN was shown to increase the gastric lesion formation induced by stress, while electrolytic lesion of the PVN decreased it [48]. Furthermore the elevation of thyrotropin-releasing hormone (TRH) level in these nuclei of the

hypothalamus was reported to be followed by acid hypersecretion and lesion formation in the stomach [45].

In addition, a host of transmitters and brain-gut peptides were shown in the NTS and DMV, such as glutamate, GABA (γ-aminobutyric acid), norepinephrine, acetylcholine as well as opioids, TRH, nociceptin [45;49-53], which were shown to play a role in the regulation of gastric functions. Thus the medullary TRH was assumed to be involved in the control of the gastric functions by stimulation of DVC, or TRH microinjected into the DVC was found to elevate the intragastric and pyloric motility [54].

Besides TRH other brain-gut peptides also induced gastric mucosal defense, like adrenomedullin, amylin, acceptingness, neuropeptide Y, CCK, and nociceptin [51;55-58]. Central injection of amylin, a pancreatic polypeptide, was demonstrated to induce gastroprotection in both indomethacin and ethanol ulcer models in rats [56]. Both peripherally and centrally injected nociceptin proved to be gastroprotective on ethanol induced gastric lesion in rats [57]. i.c.v. administered adrenomedullin was demonstrated to induce mucosal protective processes on reserpine-induced gastric lesions [55]. Central application of amino acid neurotransmitter GABA was also reported to be protective on stress induced ulcerogenesis [59].

Data of the literature suggest that different intensity of vagal outflow might influence gastric functions differently. Thus the stable analogue of TRH, the RX77368, injected intracisternally in a low dose, which did not influence the gastric acid secretion, elicited vagally mediated gastroprotection against gastric injuries caused by 60% ethanol. On the other hand, a higher dose of the compound RX77368 might cause a strong elevation of vagal discharge, thereby resulting in the enhancement of gastric acid secretion in rats [50;51;60;61].

How can the centrally initiated effect be conveyed to the periphery? Attaché and coworkers demonstrated [51] that TRH or its stable analogue RX77368 injected into the cisterna magna in a subdirectory dose evoked gastroprotection in rats. They suggested that the centrally induced cytoprotective effect might be a result of the central activation of a cholinergic vagal pathway which might stimulate the peripheral production of PGE_2. In addition, the centrally induced processes might also stimulate the efferent function of capsaicin sensitive afferents on the periphery. As a consequence CGRP and NO release

might lead to the elevation of mucosal microcirculation leading in turn to the increase of the mucosal resistance [50;51]. However, the gastroprotection appears to involve very complex mechanisms. For example, amylin was demonstrated to be gastroprotective after central administration in both indomethacin and ethanol ulcer models in rats. This protective process seemed to be NO-independent in the former and NO-dependent in the latter ulcer model [56]. The different mechanism involved in the gastroprotective effect of amylin may also be due to the different pathomechanism of mucosal damage in the two ulcer models. It should also be kept in mind that the indomethacin ulcer model was considered to be acid-dependent [62], while the ethanol ulcer model was supposed to be acid-independent. Consequently, the ethanol ulcer model can be used for studying the cytoprotective/ gastroprotective action [62;63].

1.1.5 Alpha$_2$ adrenoceptors and gastric mucosal integrity:

The involvement of α_2 adrenoceptors in gastric mucosal integrity was also suggested on the basis of the results that these receptors are also located in the main regulatory systems of GI tract. Thus α_2 adrenoceptors were shown in the ENS where they were thought to be localized mainly presynaptically in the excitatory nicotinic synapses of the enteritis as well as of the mucosa plexus [64]. However, they were assumed not to be present on the smooth muscle cells of GI tract [64]. In addition, α_2 adrenoceptors localized on enterochromaffi cells of the digestive tract were proposed to account for inhibition of 5-HT release [65].

α_2 adrenoceptors were shown in the CNS as well. Autoradiographic studies showed the presence of α_2 adrenoceptors in the NTS and in the DMV [53;66] which are known to mediate several GI functions such as gastric acid secretion, gastric motility, gastric emptying, intestinal transit time and bicarbonate secretion [45]. Besides a very high density of α_2 adrenoceptors was observed in the locus coeruleus, in the dorsomedial nucleus of hypothalamus and in some ventricular areas as well [53;66].

The α_2 adrenoceptors were subdivided into different subtypes. To date four α_2 adrenoceptor subtypes have been recognized, such as the α_{2A}, α_{2B}, α_{2C} and α_{2D} [67-69]. The fourth adrenoceptor subtype was accepted later to be a variant of α_{2A} adrenoceptor subtype in certain species [70].

Both postsynaptic and presynaptic localization of α_2 adrenoceptors were also reported. Postsynaptic $\alpha_{2A/D}$ subtype was assumed to be responsible for the enhancement of regional cerebral blood flow in the prefrontal cortex, while presynaptic α_2 adrenoceptors seemed to modulate the release of Ach in the myenteric plexus. [58;75].

α_2 adrenoceptors are known to regulate different physiological functions. Subtype selectivity has also been demonstrated in several α_2 adrenoceptor-mediated actions. For example α_2 adrenoceptor subtypes take part in the control of vascular tone, $\alpha_{2A/D}$ and α_{2B} adrenoceptors mostly appear to control the arterial vasoconstriction, the α_{2C} subtype seems to account for venous vasoconstriction, while presynaptic $\alpha_{2A/D}$ are also mediating central analgesic, sedative and hypothermic responses induced by dexmetomidine in mice [71]. Some studies suggested that α_{2C} subtype might take part in the regulation of motor behavior and memory processes [72;73]. Reports suggested that stimulation of central α_2 ($\alpha_{2A/D}$ and $\alpha_{2B/C}$) was involved in producing fever in response to bacterial LPS [74].

In the last decades attempts have been made to develop different ligands selective for the subtypes of α_2 adrenoceptors. The $\alpha_{2A/D}$ is known to have selective agonists such as oxymetazoline or guanfacine. ST-91 was described as an $\alpha_{2\text{-NON-A}}$ agonist and possibly a selective agonist of α_{2B} adrenoceptors [75-77]. Selective antagonists of $\alpha_{2A/D}$ adrenoceptors are BRL44408 and BRL48962 [78;79]. ARC-239 and prazosin (besides their α_1 adrenoceptor blocking property) are selective for the α_{2B} adrenoceptor subtype [80]. Rauwolscine and MK912 are selective antagonists of the α_{2C} adrenoceptor subtype[81].

1.1.6 Interactions between the endogenous opioid and the α_2 adrenergic system

Several studies suggested the interactions between opioid and α adrenergic systems. The α_2 adrenoceptor agonist prototype clonidine is known to suppress opioid withdrawal symptoms [82-84]. In addition, the antinociceptive and antihypertensive effects of clonidine were antagonized by naloxone as well [85;86].

The hypothesis of the interaction between α_2 adrenergic system and opioid system is also supported by the observation that the α_2 adrenoceptors are co-distributed with μ and δ opioid receptors in the dorsal vagal complex [51;66]. Furthermore the presence of

interaction between α_2 adrenoceptors and opioid receptors was assumed in the guinea pig myenteric plexus [87].

1.1.7 Opioid system and gastric mucosal integrity

Endogenous opioid system is also known to play a role in the regulation of gastric functions. Constipation caused by morphine is considered to be related to a decrease in gut motility and gut secretion and to an elevation of intestinal fluid absorption [88-90].

In addition, previous studies indicated the involvement of endogenous opioid peptides in maintenance of gastric mucosal integrity [91-93]. However, data of literature are quite contradictory, since after the administration of opioid agonists both lesion-aggravating [91;94] and gastroprotective effects [92;93;95] were observed on different experimental ulcer models, and both effects were abolished by the administration of opioid antagonists suggesting that the action was mediated by opioid receptors.

Three major types of opioid receptors have been described and cloned, namely, μ-[96], δ-[97;98] and κ-[99] which have been subdivided into further subtypes. All of these belong to G-protein coupled receptor superfamily. The cellular mechanisms of their action are the inhibition of cyclic AMP accumulation, the inhibition of Ca^{2+} channels, and the activation of K^+ channels. They bind their endogenous opioid ligands and opiate alkaloids with different affinity and selectivity [100;101].

The distribution of opioid receptor types shows a specific pattern in the CNS and in the ENS. Species differences were also found in their localization [102]. In the CNS both μ- and δ-opioid receptors were observed in the NTS, vagal nerve fibres and in the substantia gelatinosa of the spinal cord. In contrast, in the thalamus and hypothalamus the μ-opioid receptors showed a very high density and the concentration of δ-opioid receptors was found to be scarce [49]. In the ENS functional and pharmacological studies suggested that the effects of opioids on the intestinal motility were mainly mediated by μ and κ opioid receptor types [89;103]. In rats μ-opioid receptors were observed on the myenteric and submucosal plexus, on fibers projecting to the muscle, vasculature, mucosa and on the interstitial cells of Cajal [90;104]. Opioid peptides and alkaloids are known to decrease the intestinal transit in mammalian species by changing the coordinated reflex motor activity in segmentation of intestine [89;105]. They seemed to affect the fine coordination of intestinal motility and propulsive activity by decreasing

the release of inhibitory neurotransmitters such as vasoactive intestinal peptide (VIP) and NO thereby suppressing the descending relaxation processes. Furthermore, they also inhibited the electrically evoked Ach release on the cholinergic excitatory neurons [105]. Several findings suggested that endogenous opioids and opiates might act on enteric neurons by modulating the release of transmitters rather than having a direct effect on the GI smooth muscle [102].

The endogenous ligands of opioid receptors, the endogenous mammalian opioid peptides, such as enkephalins, endorphins, dynorphins have been pharmacologically well studied. Enkephalins preferentially bind to δ-opioid receptors, dynorphins to κ-opioid receptors whereas endorphin do not discriminate between δ- and μ-opioid receptors [106]. Recently endomorphins (endomorphin-1 and endomorphin-2) have been isolated from mammalian brain which show high affinity and selectivity toward μ- opioid receptors [107;108]. Endomorphins have been proposed to be the forth mammalian endogenous opioid peptides after enkephalins, endorphins and dynorphins.

Selective agonists and antagonists for opioid receptors have been synthetized. Thus DAGO ({D-Ala^2, MePhe^4, Gly^5-ol}-enkephalin) is one of the most potent synthetic enkephalin analogues which has high selectivity for μ opioid receptors [109]. Deltorphin II and DADLE ({D-Ala^2, D-Leu^5}-enkephalin) are selective agonists for the δ-opioid receptors. The selective antagonist for κ-opioid receptors is the nor-binaltorphimine (BNI) , and for δ-opioid receptors is the naltrindol. Naloxone and naltrexone are non-selective antagonists for opioid receptors [88].

2 AIMS OF THE STUDY:

As it was discussed in the introduction, the gastric mucosal protection involves several mechanisms. It has been shown that influence of gastric motor activity might affect the gastric mucosal integrity.

The main purpose of the present experimental series was

i./to compare the effects of α_2 adrenoceptor agonists (e.g. potency, efficacy, receptors/receptor subtypes involved in the actions, potential mechanisms) in gastric ulcer model and gastric motility models,

ii./to analyse whether there may be correlations between the alteration of gastric motor activity and gastric mucosal protective processes. The experiments were completed with the examination of gastric acid secretion as well as with experiments focusing on the effect of μ and δ opioid receptor agonists on gastric mucosal damage and gastric emptying.

In order to answer the questions raised above we aimed to study:

- the role of α_2 adrenoceptors and opioid receptors in gastric mucosal defense as well as to reveal a potential interaction between α_2 adrenergic and opioid systems in gastric mucosal protection,
- the role of α_2 adrenoceptors in regulation of gastric acid secretion, as well as to explore a potential interaction between α_2 -adrenergic and opioid systems in the inhibition of gastric acid secretion,
- the role of α_2 adrenoceptors and opioid receptors in the regulation of gastric emptying after both central and peripheral administrations by analysing

  a./how α_2 adrenoceptor agonists affect gastric emptying,

  b./which α_2 adrenoceptor subtype(s) may be involved in the regulation of gastric emptying,

  c./whether NO plays a role in the in the delay of gastric emptying induced by stimulation of α_2 adrenoceptors,

  d./whether the vagal nerve has any role in the delay of gastric emptying evoked by the stimulation of central α_2 adrenoceptors,

e./whether there is an interaction between α_2 adrenergic and opioid systems in influencing gastric emptying,

f./how μ and δ opioid receptor stimulants influence the gastric emptying.

- the role of α_2 -adrenoceptors in the regulation of gastric motor activity by analysing

  a./how α_2 adrenoceptor agonists affect the basal and stimulated gastric motility (gastric tone and gastric contraction),

  b./which α_2 adrenoceptor subtype(s) may be involved in the regulation of gastric motility,

  e./whether there is an interaction between α_2 adrenergic and opioid systems in the regulation of gastric motor activity.

For measuring gastric motor activity we applied two methods: the phenol red method to determine gastric emptying and miniature intragastric balloons to study gastric motility. The protective effect of α_2 adrenoceptor stimulants were studied on (the acid-independent) ethanol induced lesion formation. We measured the gastric acid secretion on pylorus ligated rats.

3 MATERIALS AND METHODS

3.1.1 Animals:

Male Wistar rats weighing 150-170 g (gastric emptying) and 260-280 g (gastric motility) were used. Animals were housed under a standard 12/12 h light-dark cycle, in a temperature controlled room (22 ± 2°C) and 24 hours before experimentation food was deprived, but free tap water was available. For avoiding coprophagy, animals were placed in wire mesh bottom cages. Experiments were carried out in accordance with the ethical guidelines set by the Ethical Board of Semmelweis University, Budapest, Hungary based on the Declaration of Helsinki (EC Directive 86/609/EEC). The animals were humanely killed before removing stomachs for determination of gastric emptying, gastroprotection and gastric acid secretion and after studying gastric motor activity.

3.1.2 Intracerebroventricular injections (i.c.v.):

Intracerebroventricular injection was performed in conscious rats as described by Noble and coworkers [110]. Briefly, animals were gently fixed and injections were made with microsyringe bearing 27 gauge needle with stops at 4 mm from the needle tip at a point 1.5 mm caudal and 1.5 mm lateral from bregma. The volume of the i.c.v. injection was 10µl.

3.1.3 Intracisternal injection (i.c.):

Injections were performed in concious rats using a Hamilton microsyringe. A silicone–ring was fixed 7 ml from the tip of needle to control the proper depth of the injection. The head of the animals were gently bent, and the needle was inserted in the midline into the cleft between occiput and atlas at an 40° angle relative to the plane of occipital bone. The bone structures bordering the narrow gap guide the needle. The volume of the i.c. injection was 5 µl.

3.1.4 Bilateral cervical vagotomy:

The cervical section of the vagus nerves was exposed under ether anaesthesia, and bilateral cervical vagotomy was performed. In the sham operated control rats the vagus

was similarly exposed but the vagal trunk were not sectioned. The incisions were closed and all animals were allowed 2 h recovery from operation.

3.1.5 Ethanol-induced gastric lesions:

In order to study the gastroprotection, gastric lesions were produced by acidified ethanol. Rats were fasted for 24 hours before experiments but free access to tap water was allowed. Studies were carried out using 7-10 rats per group. 0.5 ml of acidified ethanol (98% ethanol in 200 mmol/ml HCl) was given to the animals orally by using a stainless feeding tube. One hour later the animals were sacrificed by overdosing aether. Thereafter stomach was removed, opened along the greater curvature and rinsed with saline. Then the lesions were examined, the total number of mucosal lesions was carried out in blinded manner by calculation of Ulcer Index basing on a previously described method [92]. Briefly, a 0 to 4 scoring system was established. Consequently, the lesions were systematized according to their severity. Thus in the cases of the small petechies, haemorrhagies 1 score was given and the 2, 3, 4 mm long lesions received 2, 3, 4 score, respectively. The numbers of different severity lesions were multiplied by the respective severity factors and finally the sum of them was taken as Ulcer Index. Agonists were injected s.c. and i.c.v., 20 and 10 min before ethanol, respectively. Antagonists were administered 20 and 10 min before s.c. and i.c.v. agonists, respectively. The percentual inhibition of mucosal damage was calculated according to the following formula:
100 - lesion index in treated group / lesion index in control group x100.

3.1.6 Measurement of gastric acid secretion:

Experiments were performed according to the method described by Shay and coworkers [111] in conscious rats. A group of seven rats was allocated for each experiment. Through an abdominal midline incision stomachs were exposed under light aether anaesthesia and the pylori were ligated. Four hours later animals were killed by overdosing aether, the cardias were quickly clamped, stomachs were removed and the gastric juices were collected. These were centrifuged to remove residues, thereafter the gastric juice volumes were measured and the samples were titrated against 0.1N NaOH up to pH 7 to determine gastric acid concentration. For the measurement of acidity a TTT85 titrator (Radiometer, Copenhagen) was used. Results were expressed as volume

in ml and total acid output in μEq/4h. Agonists were administered i.c.v. 10 min after the pylorus ligation.

3.1.7 Determination of gastric emptying:

Experiments were performed in concious rats according to the phenol red content assay described previously [112]. In brief, 1.5 ml of the test meal containing a non-absorbable marker (0.5 mg/ml phenol-red in 1.5% methylcellulose solution) was given orally by use of a stainless feeding tube. Sixty min later animals were sacrificed by overdosing eather. The abdominal cavity was opened, the pyloric and cardial ends of stomach were quickly clamped, thereafter stomachs were removed and immersed in 100 ml of NaOH solution. Samples were homogenized for 30 s then proteins in 5 ml of homogenates were precipitated with 0.5 ml of 20% trichloacetic acid solution. After centrifugation the pH of supernatants was adjusted to alkaline with 4 ml of 0.5 N NaOH and the absorbance of the sample was read at a wavelength of 560 nm (A_{test60}).

On each experimental day there were two groups of rats which served as controls and received the test meal, as well. One of these were killed immediately while the other only after 60 min, stomachs were removed and the same procedure was carried out as described above. The first group served as a standard control and the absorbance of the amount of phenol red recovered from their stomachs showed the 0 % emptying (A_{stand}). The second one served as the 60 min control (100% emptying)($A_{control60}$)**.**

Percent gastric emptying rate of each group was calculated according to the following formula: $100 - (A_{test60} - A_{control60}) / (A_{stand} - A_{control60})x100$

Drug administration: For determination of the central effects of agonists, test compounds were administered i.c.v. or i.c. 10 min before test meal. S.c. and i.v. agonists were injected 20 and 15 min before test meal, respectively. By the same route, antagonists were administered 10 and 20 min before i.c.v. and s.c., respectively. In some cases antagonists were injected intravenously 15 min before the agonists. Control groups were treated with saline. A group of five rats was allocated for each experiment.

3.1.8 Determination of gastric motility:

The gastric motility was determined by the previously described balloon-method [113]. According to this technique it becomes possible to study separately the antral

contractions and the fundic tone. Studies were carried out using 3-6 rats per group. The experiments were performed under urethane (1.25 g/kg i.p.) anaesthesia. A tracheal tube was inserted to ensure open airway, thereafter a miniature rubber balloon was leaned into the stomach via mouth and inflated with warm (~37°C) saline (1.6-2 ml) to a pressure of approximately 12 cmH_2O. The other end of the tube was connected to a pressure transducer to monitor the intragastric pressure. A cannula was implanted into the femoral vein for administration of drugs. Animals were allowed to get stabilized for 30 min. Since gastric motor activity was strongly inhibited under urethane anaestesia, 2-DG (300 mg/kg i.v.) was injected to stimulate gastric contractions and to increase gastric tone. 25-30 min after the 2-DG injection, when amplitudes of contractions and intragastric pressure had become stable, the test substances were administered and the antagonists were injected 10-15 min later. Compounds were dissolved in saline, and the control groups were treated with the solvent.

We chose the intragastric pressure and the mean of amplitudes as parameters to characterise the gastric motor activity. The tonic intragastric pressure was determined by the previously described method of Shi and coworkers [167]. The mean of the intragastric pressure was calculated as follows: sum of the lowest points of the phasic contractions (cmH_2O) for 6 min divided by the number of contractions for 6 min. The intragastric pressure was expressed in cmH_2O.

The means of amplitudes (Motility index) were expressed in cmH_2O and were calculated by dividing the sum of amplitudes by the number of contractions both for a six-minute period.

3.1.9 Preparation of drugs:

Compounds used were: the α_2 adrenoceptor agonist clonidine hydrocloride (Sigma Chemical, St. Louis, Mo, USA), the α_2 adrenoceptor antagonist yohimbine hydrocloride (Sigma Chemical, St. Louis, Mo, USA), the selective α_{2B} adrenoceptor antagonist prazosin hydrocloride (Sigma Chemical, St. Louis, Mo, USA), the non-selective opioid receptor antagonist naloxone hydrocloride (Sigma Chemical, St. Louis, Mo, USA), the NO synthase inhibitor N^G-nitro-L-arginine, the central vagus stimulant 2-deoxy-D-glucose (Sigma Chemical, St. Louis, Mo, USA), the selective α_{2A} adrenoceptor agonist oxymetazoline (RBI Natick, USA), the selective α_{2B} adrenoceptor antagonist ARC-239

dihydrocloride (Karl Thomae, Biberach/Riss, Germany), the selective δ opioid receptor agonist deltorphin ll (synthetized by G. Tóth, Biological Research Centre of Hungarian Academy of Sciences, Szeged, Hungary), the selective μ opioid receptor agonist DAGO (Synthetized by A. Magyar, Eötvös University, Budapest, Hungary), the μ opioid receptor agonist morphine hydrocloride (Biogal, Hungary), urethane (Sigma Chemical, St. Louis, Mo, USA) and aether. Compounds were dissolved in saline in a volume of 0.5 ml per 100g body weight for i.p., s.c., i.v. administration. In the case of i.c. and i.c.v. injection volumes were 5 μl and 10 μl, respectively.

3.1.10 Statistical analysis:

All values were expressed as means ± S.E.M.. In the cases of measurement of gastric emptying and ethanol induced lesion formation data were evaluated by analysis of variance (ANOVA) and Newman-Keuls post hoc test was used for multiple comparisons. In the case of the measurement of gastric acid secretion differences were evaluated by Student's t-test or ANOVA followed by Tukey test for multiple comparison. Differences were considered significant at $p<0.05$.

4 RESULTS

4.1 GASTROPROTECTION-Experiments on ethanol ulcers

4.1.1 Studies of the effects of α_2 adrenoceptor stimulants on the ethanol-induced lesion formation in rats

4.1.1.1 The effect of i.c.v. and p.o. injected clonidine on the ethanol-induced gastric lesion in rats

The α_2 adrenoceptor stimulant clonidine significantly inhibited the ethanol-induced lesion formation after both p.o. (11.75-375.9 nmol/kg) and i.c.v. (0.17-3.76 nmol/rat) application. The peripheral and central effects were dose-dependent. The ED_{50} values were 26.7 nmol/kg for p.o. and 0.14 nmol/rat for i.c.v. administration (Fig. 1). The maximal effect was reached after the doses of 93.98 nmol/kg p.o. and 0.24 nmol/rat i.c.v.. However, the gastroprotective effect of p.o administered clonidine was decreased when higher dose was given (375.9 nmol/kg).

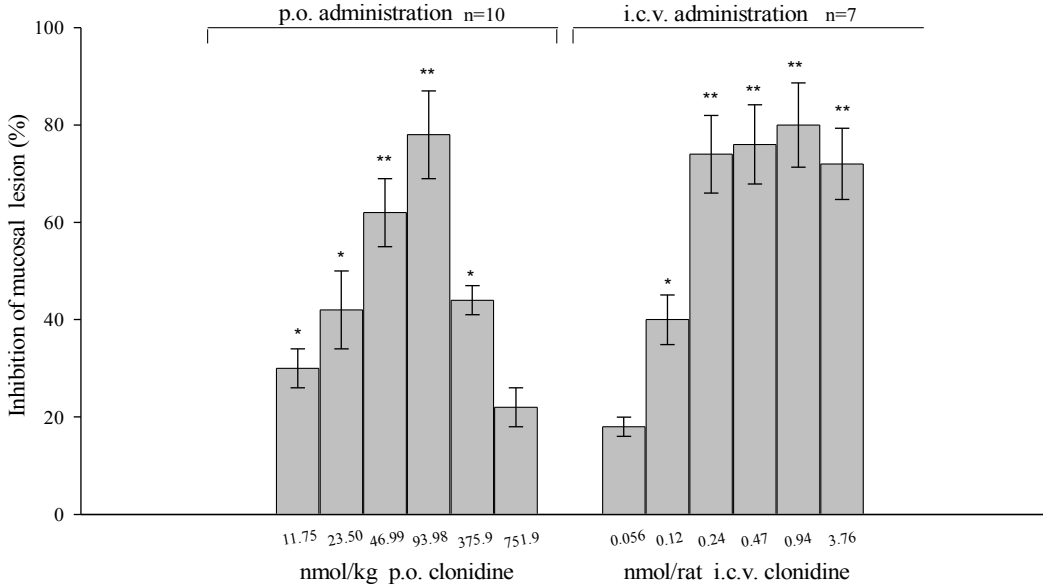

Fig. 1. The effect of clonidine given p.o. and i.c.v. on the ethanol induced gastric lesions in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. *p<0.05, **p<0.01 compared to the control groups (0 % inhibition)

4.1.1.2 The effect of centrally administered yohimbine and prazosin on the gastric mucosal protective action of i.c.v. injected clonidine in rats

In the following experiments we examined the effects of different α_2 adrenoceptor antagonists on the gastroprotective action induced by centrally administered clonidine. Clonidine was injected i.c.v. in a dose of 0.47 nmol/rat. We decided to use this dose of clonidine after the previous dose-response studies. As presented in Fig. 2., clonidine decreased the ethanol-induced lesion formation in a significant manner. The non-selective α_2 adrenoceptor antagonist yohimbine (50 nmol/rat, i.c.v.) and the selective α_{2B} adrenoceptor antagonist prazosin (5 nmol/rat, i.c.v.) did not influence the ethanol-induced lesion formation. However both yohimbine and prazosin inhibited the gastric mucosal protective action of clonidine and their effects were significant.

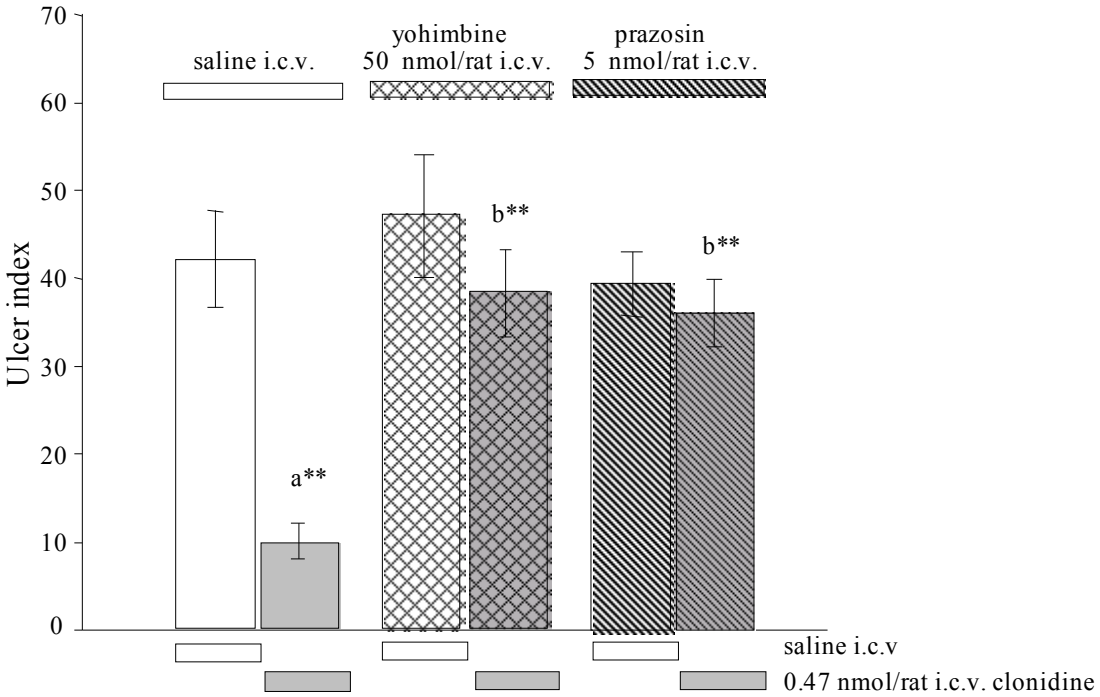

Fig. 2. The effect of yohimbine (50 nmol/rat, i.c.v.) and prazosin (5 nmol/rat, i.c.v.) on the gastroprotective action of clonidine (0.47 nmol/rat i.c.v.) on ethanol induced gastric lesions in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=10, **p<0.01, a - compared to the control groups , b - compared to the group treated by clonidine

4.1.2 Studies of the interaction between central opioid and α_2 adrenergic systems on ethanol-induced lesion formation in rats

4.1.2.1 The effect of centrally administered naloxone on the gastric mucosal protective action of i.c.v. injected clonidine in rats

In the next experiments we studied whether there was interaction between the central α_2 adrenergic and opioid systems. Therefore we examined if an opioid antagonist affected the gastric mucosal protective action of i.c.v. administered clonidine. As Fig. 3. shows the centrally injected clonidine significantly reduced the lesion formation induced by ethanol in a dose of 0.47 nmol/rat i.c.v.. The opioid antagonist naloxone - injected i.c.v. in a dose of 50 nmol/rat - did not influence the ethanol-induced mucosal lesion formation, but abolished the gastroprotective effect of clonidine.

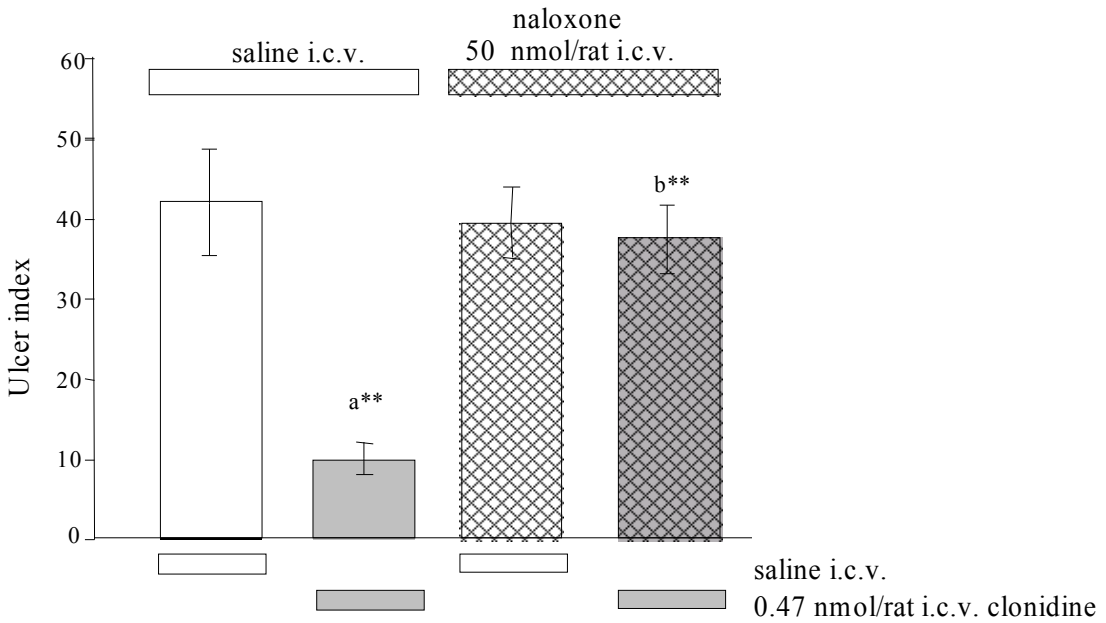

Fig. 3. The effect of the centrally injected naloxone (50 nmol/rat,i.c.v.) on the mucosal protective action of clonidine (0.47 nmol/rat i.c.v.) on ethanol induced gastric lesions in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=10, **p<0.01, a - compared to the control group, b - compared to the group treated by clonidine

4.1.3 Study of the role of central opioid receptors in ethanol-induced lesion formation in the rat

4.1.3.1 The effect of centrally administered DAGO and deltorphine II on ethanol-induced gastric lesions in rats

In order to examine the role of central μ and δ opioid receptors we chose DAGO as selective μ, and deltorphine II as selective δ opioid receptor agonist. The compounds were given i.c.v.. As Fig. 4. represents, the centrally injected DAGO dose-dependently inhibited the gastric mucosal lesion formation induced by ethanol. Similarly, the δ opioid receptor selective agonist deltorphine II diminished the ethanol-induced gastric erosions in a dose-dependent fashion. The ED_{50} values were 0.0068 nmol/rat i.c.v. for DAGO and 0.12 nmol/rat i.c.v. for deltorphine II.

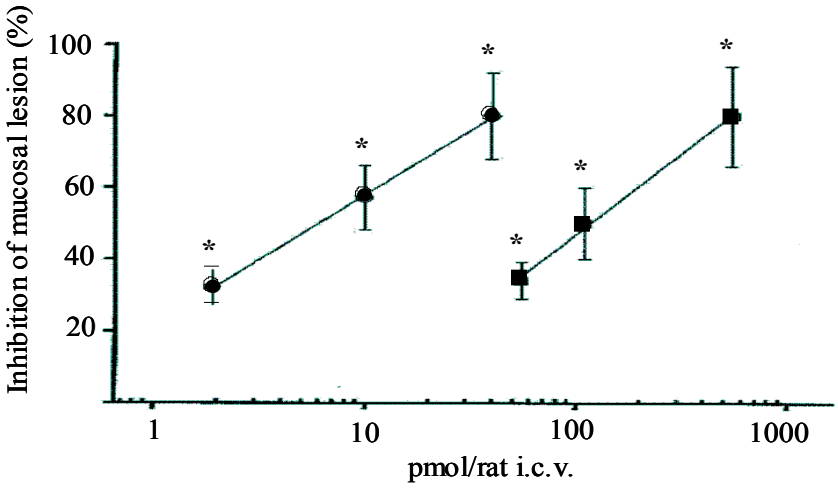

Fig. 4. Effect of i.c.v. injected DAGO (O) and deltorphine II (□) on ethanol-induced gastric lesions in rats. Each point represent the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=7 *p<0.05 compared to the control groups (0 % inhibition)

4.2 GASTRIC ACID SECRETION-Experiments on pylorus-ligated rats

4.2.1 Studies of the role of α_2 adrenoceptors in the regulation of gastric acid secretion in rats

4.2.1.1 The effect of i.c.v. injected clonidine and oxymetazoline on pylorus-ligated rats

In the following experimental series we studied the effects of various α_2 adrenoceptor stimulants on the gastric acid secretion in pylorus-ligated rats. The compounds were administered i.c.v., 10 min after pylorus ligation. As Fig. 5. shows, the non-selective α_2 adrenoceptor agonist clonidine in a dose range of 11.25-47 nmol/rat i.c.v. inhibited the gastric acid secretion in a dose-dependent fashion. The effects were significant above the dose of 23.5 nmol/rat. The ED_{50} value was 20 nmol/rat for i.c.v. clonidine. Similarly, the α_{2A} subtype selective oxymetazoline dose-dependently inhibited the gastric acid secretion (Fig. 5.). The effects were significant in the i.c.v. dose range of 8.4-67.5 nmol/rat. The ED_{50} value was 7.5 nmol/rat for i.c.v. oxymetazoline.

4.2.1.2 The effect of centrally administered yohimbine on gastric antisecretory action of i.c.v. injected clonidine and oxymetazoline in pylorus-ligated rats

The aim of these experiments was to confirm the receptorial mechanisms of the antisecretory actions of clonidine and α_{2A} subtype selective oxymetazoline. Therefore we examined the effect of non-selective α_2 adrenoceptor antagonist yohimbine on the antisecretory action of these compounds. As Fig. 6. represents, the i.c.v. injected yohimbine in a dose of 51.2 nmol/rat by itself did not influence the gastric acid secretion in pylorus ligated rats. Both the i.c.v. administered clonidine (23.5 nmol/rat i.c.v.) and oxymetazoline (16.8 nmol/rat i.c.v.) significantly inhibited the gastric acid secretion. The effect of clonidine was antagonized by yohimbine. The action of oxymetazoline was also inhibited by the same dose of yohimbine and the effect was significant (Fig. 6.).

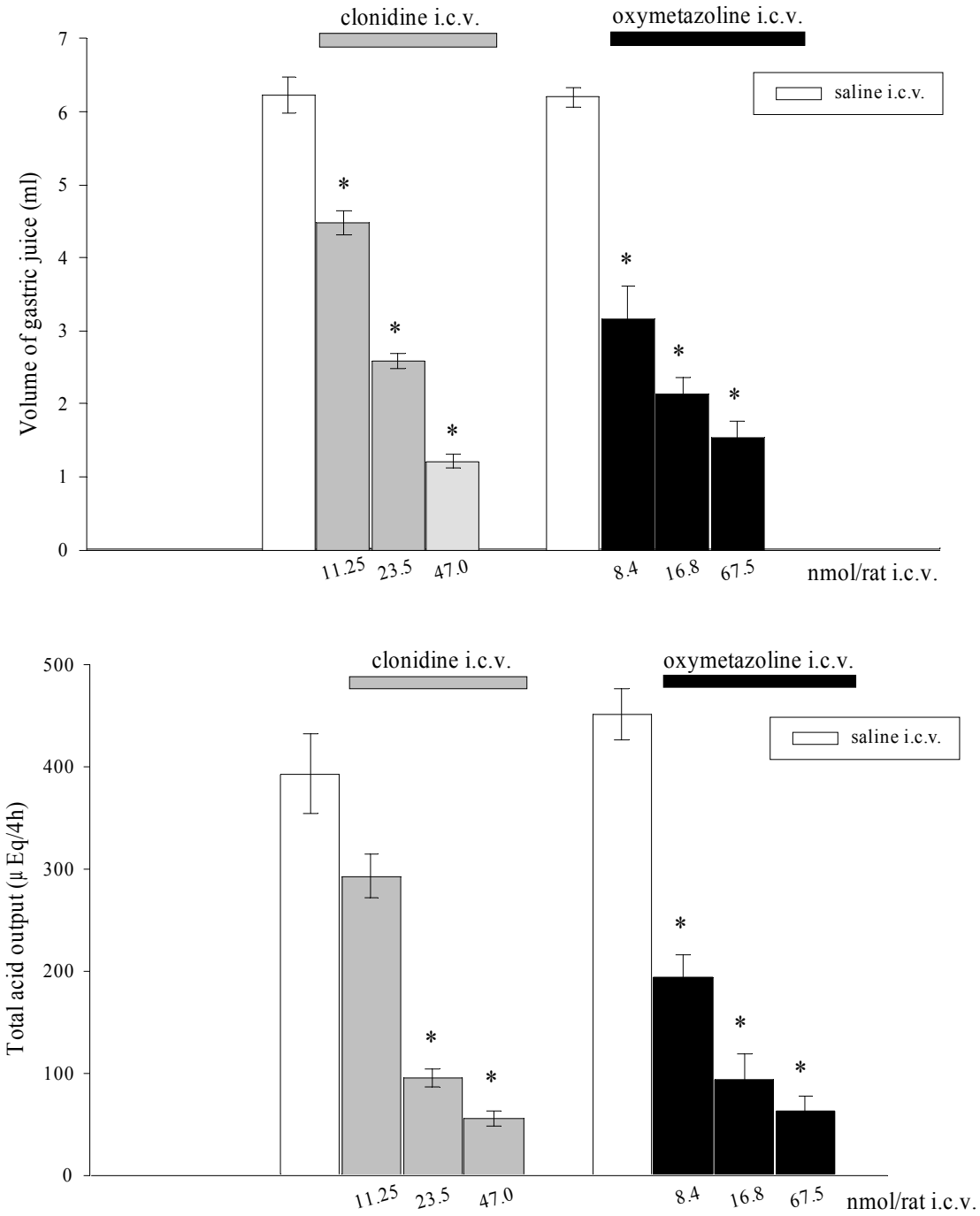

Fig. 5. The effect of i.c.v. injected clonidine and oxymetazoline on the different parameters of gastric secretion such as the volume of gastric juice (above) and Total Acid Output (below) in pylorus ligated rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Tukey post hoc test was used for multiple comparisons. * $p < 0.05$ compared to the control groups

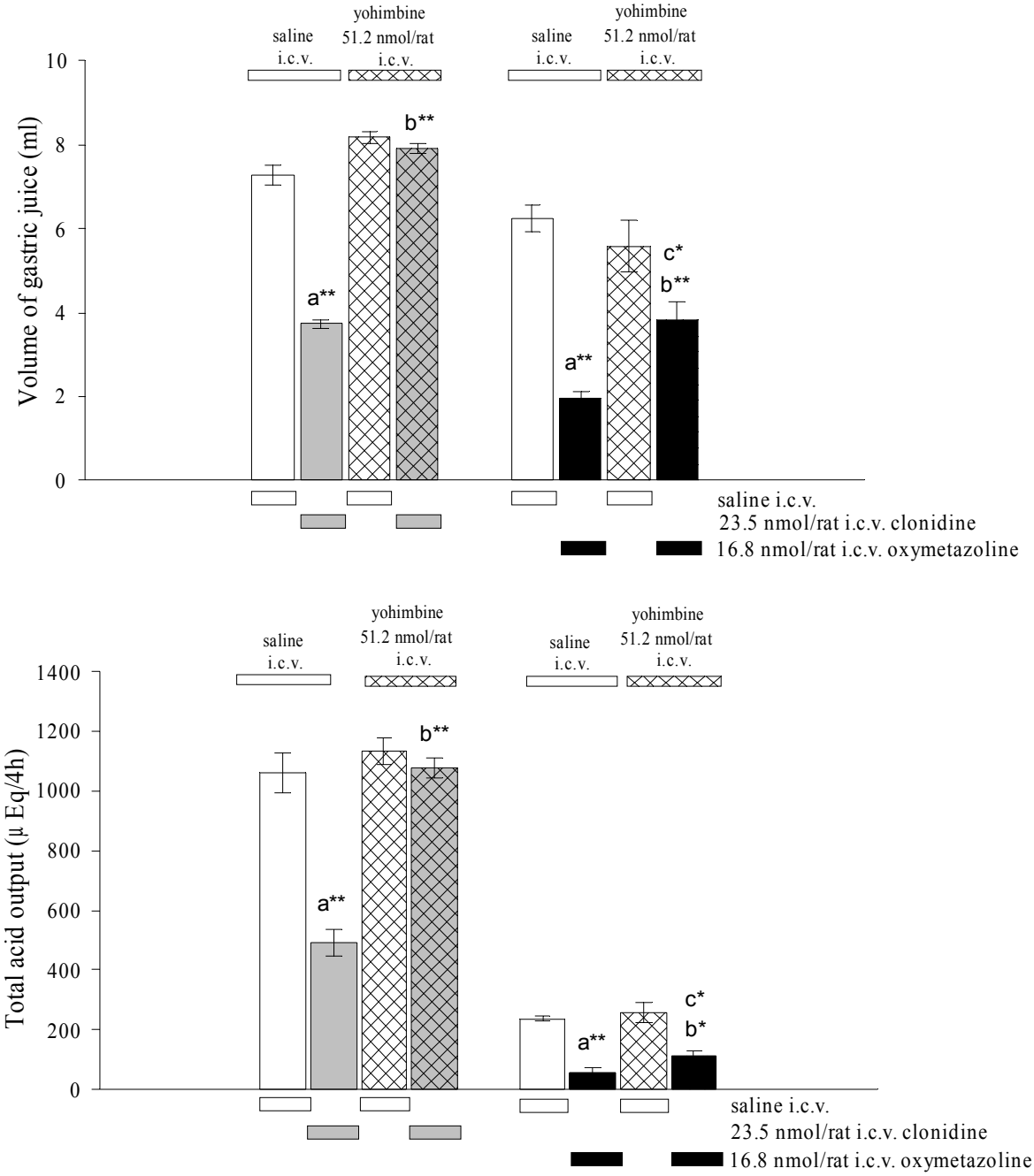

Fig. 6. The effect of centrally administered yohimbine (51.2 nmol/rat i.c.v.) on the gastric antisecretory action of i.c.v. injected clonidine (23.5 nmol/rat i.c.v.) and oxymetazoline (16.8 nmol/rat i.c.v.) in pylorus ligated rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of Student's t-test. n=7, * $p<0.05$, ** $p<0.01$ a: compared to the control groups, b: compared to the clonidine/oxymetazoline treated group, c: compared to the yohimbine treated group

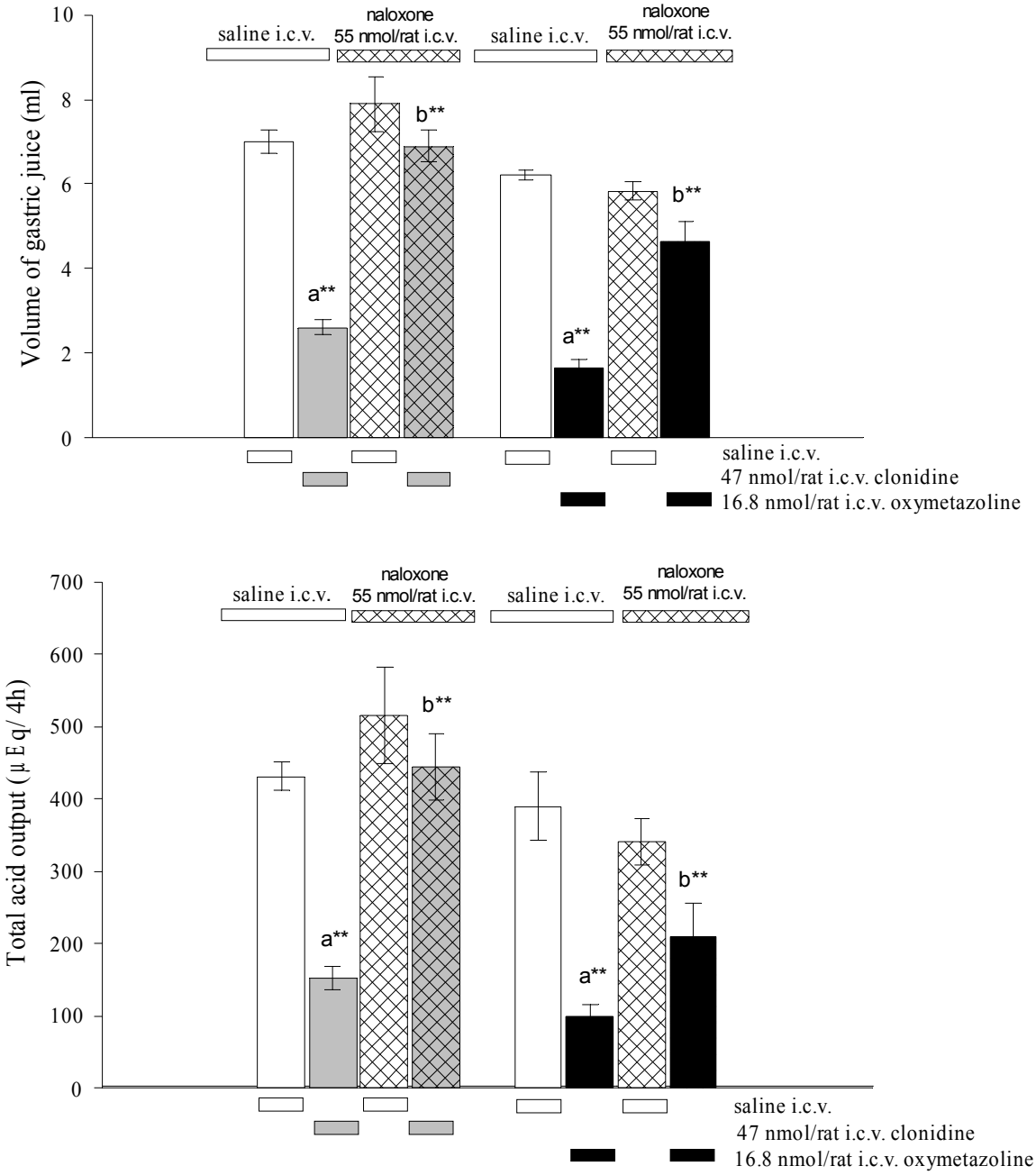

Fig. 7. The effect of centrally administered naloxone (55 nmol/rat i.c.v.) on the gastric antisecretory action of i.c.v. injected clonidine (47 nmol/rat i.c.v.) and oxymetazoline (16.8 nmol/rat i.c.v.) in pylorus ligated rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of Student's t-test. n=7, ** $p<0.01$; a: compared to control group, b: compared to the clonidine/oxymetazoline treated group

4.2.2 Studies of the interaction between central opioid and α_2 adrenergic systems in the antisecretory action in pylorus-ligated rats

4.2.2.1 The effect of i.c.v. administered naloxone on the gastric antisecretory action of centrally injected clonidine and oxymetazoline in pylorus-ligated rats

In order to explore the interaction between α_2 adrenergic and opioid systems, we examined the effect of non-selective opioid receptor antagonist naloxone on the antisecretory action of clonidine (47 nmol/rat i.c.v.) and oxymetazoline (16.8 nmol/rat i.c.v.). As Fig. 7. shows, the i.c.v. injected naloxone in a dose of 50 nmol/rat by itself did not affect the gastric acid secretion, but antagonized the antisecretory effect of both clonidine and oxymetazoline.

4.3 GASTRIC EMPTYING-Experiments on gastric emptying of phenol red solution

4.3.1 Studies of the effects of α_2 adrenoceptor stimulants on gastric emptying in rats

4.3.1.1 The inhibitory effects of clonidine and oxymetazoline on gastric emptying after systemic administration

In the following experiments we studied the effect of α_2 adrenoceptor stimulants on the gastric emptying of phenol red solution. The compounds were administered subcutaneously 20 min before the test meal. As Fig 8. represents, the peripherally injected clonidine dose-dependently (0.19-3.76 μmol/kg, s.c.) inhibited the gastric emptying. The effect proved to be significant above the s.c. dose of 0.75 μmol/kg. Similarly, the selective α_{2A} adrenoceptor agonist oxymetazoline dose-dependently (0.17-6.8 μmol/kg, s.c.) delayed the gastric emptying (Fig. 9.). The effects were found to be significant above the s.c. dose of 0.68 μmol/kg. The maximal inhibition evoked by s.c. administered clonidine in a dose of 3.76 μmol/kg was 50.6 ± 6.21%, while the maximal

inhibition observed following s.c. injected oxymetazoline in the dose of 3.38 μmol/kg was 51.58±8.27%. Therefore to characterize the potency of the compounds the ED_{30} (and not ED_{50}) values were calculated. ED_{30} values were 0.54 μmol/kg for clonidine and 0.81 μmol/kg for oxymetazoline (Fig. 9.).

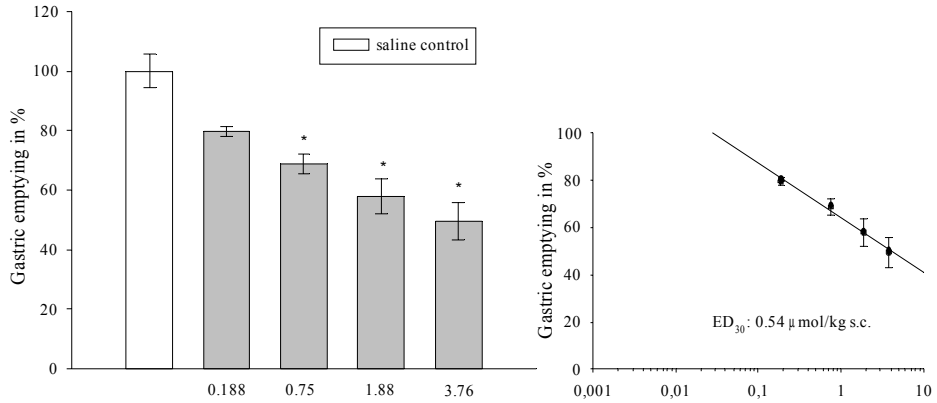

Fig. 8. The effect of s.c. injected clonidine on the gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05 compared to the control group

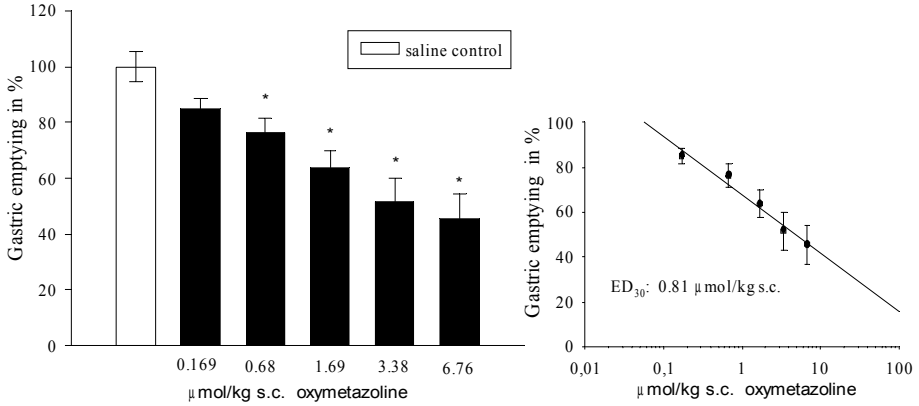

Fig. 9. The effect of s.c. injected oxymetazoline on the gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05 compared to the control group

4.3.1.2 The inhibitory effect of s.c yohimbine on the gastric emptying delaying action of s.c. clonidine and oxymetazoline

In these experiments we examined the effect of the α_2 adrenoceptor antagonist yohimbine on the gastric delaying action of clonidine and oxymetazoline. Yohimbine (12.82 μmol/kg s.c.) alone did not influence the gastric emptying of test meal. Systemic

administration of clonidine (3.76 µmol/kg s.c.) and oxymetazoline (3.38 µmol/kg s.c.) produced a significant inhibition on gastric emptying. The gastric emptying delaying action of clonidine was partially but significantly inhibited by s.c. yohimbine (Fig. 10.). Moreover, yohimbine antagonized the inhibitory action of oxymetazoline (Fig. 11.).

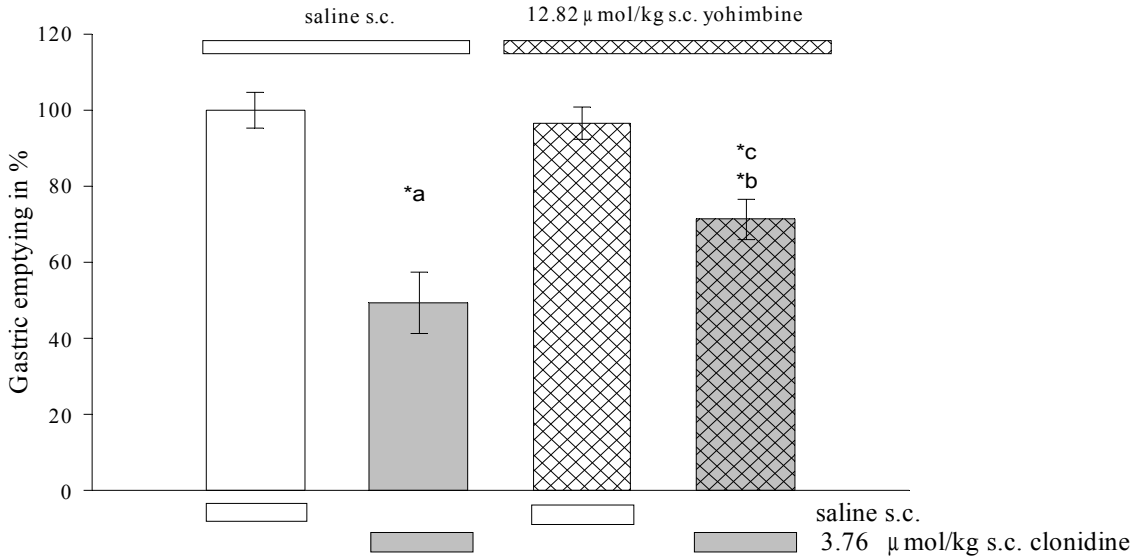

Fig. 10. The effect of yohimbine (12.82 µmol/kg s.c.) on the delay of gastric emptying induced by s.c. clonidine (3.76 µmol/kg s.c.) in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc tests was used for multiple comparisons. n=5, *p<0.05;a - a: compared to control group, b - compared to the clonidine treated group, c - compared to the yohimbine treated group

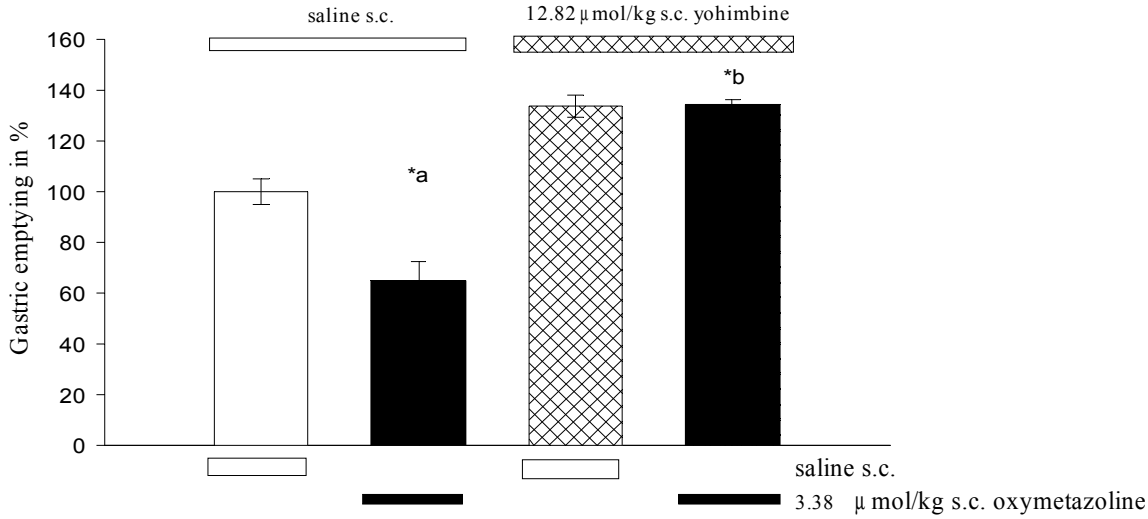

Fig. 11. The effect of yohimbine (12.82 µmol/kg s.c.) on the delay of gastric emptying induced by oxymetazoline (3.38 µmol/kg s.c.) in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5; *p<0.05; a: compared to control group, b - compared to the clonidine treated group,

4.3.1.3 The effect of prazosin and ARC-239 on gastric emptying delaying action of clonidine and oxymetazoline.

The aim of these studies was to analyze the role of various α_2 adrenoceptor subtypes in the regulation of gastric emptying. In each of these experiments (Fig. 12.-14.) the s.c. administered clonidine (3.76 μmol/kg s.c.) significantly inhibited the gastric emptying of test meal. As Fig. 12. represents, the s.c. injected α_{2B} adrenoceptor antagonist prazosin in a dose of 1.19 μmol/kg alone did not affect the gastric emptying and neither did it reverse the inhibitory effect of clonidine. Similarly, the α_{2B} adrenoceptor antagonist, ARC-239 given in a dose of 0.68 μmol/kg s.c. (Fig. 13.) did not affect the gastric emptying and failed to influence the gastric emptying delaying action of clonidine. Neither did ARC-239 in the same s.c. dose influence the inhibitory response of the α_{2A} adrenoceptor agonist oxymetazoline (3.38 μmol/kg, s.c.) as Fig. 14. shows.

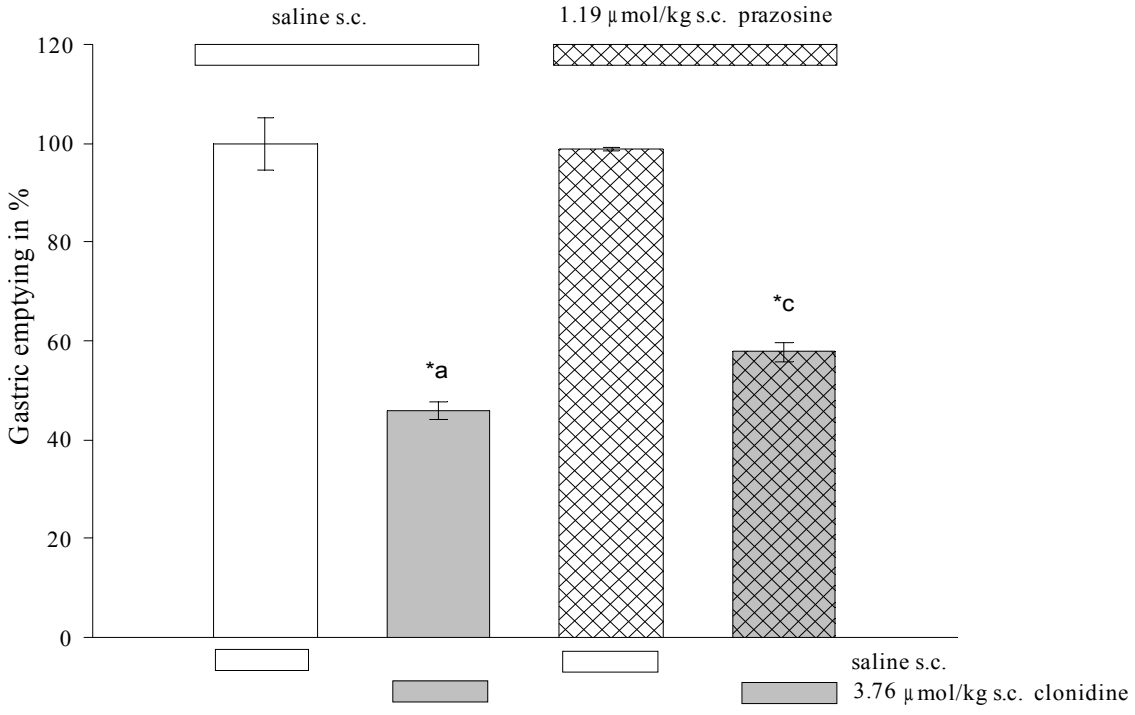

Fig. 12. The effect of prazosin (1.19 μmol/kg s.c.) on the delay of gastric emptying induced by clonidine (3.76 μmol/kg s.c.) in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05;a- compared to control group, c - compared to the prazosin treated group

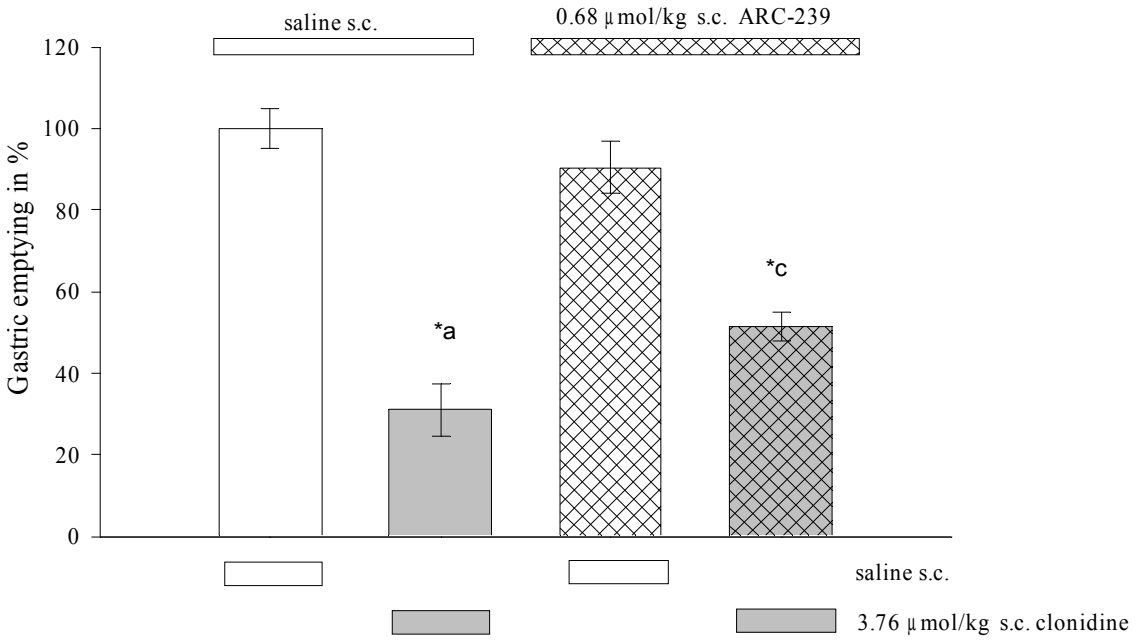

Fig. 13 . The effect of ARC-239 (0.68 µmol/kg s.c.) on the delay of gastric emptying induced by clonidine (3.76 µmol/kg s.c.) in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05; a- compared to control group, c - compared to the ARC-239 treated group

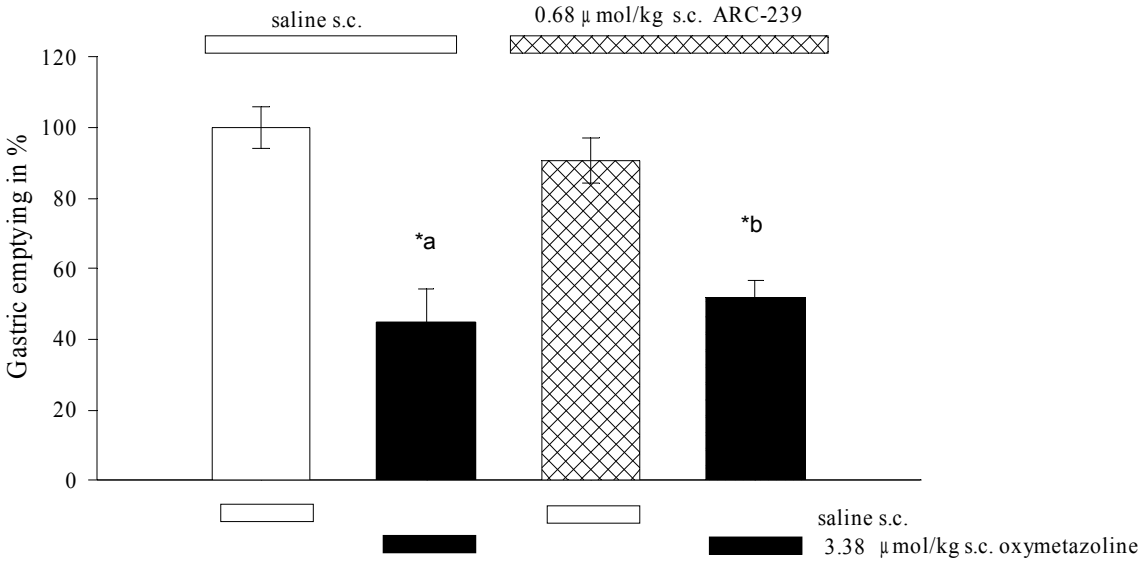

Fig. 14. The effect of ARC-239 (0.68 µmol/kg s.c.) on the delay of gastric emptying induced by oxymetazoline (3.38 µmol/kg s.c.) in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05; a- compared to control group, c - compared to the ARC-239 treated group

4.3.1.4 The effect of N^G-nitro-L-arginine on the delay of gastric emptying induced by peripherally injected clonidine

In the next experiment we aimed to explore if NO played a role in the gastric emptying delaying action of clonidine. Fig.15. shows that NO synthase inhibitor L-NNA itself significantly reduced the gastric emptying of phenol red solution after i.v. administration (91.3-182 μmol/kg i.v.) in a dose-dependent fashion. For further experiments we chose the dose of 91.3 μmol/kg i.v. of L-NNA to study the involvement of NO in the gastric emptying delaying effect of clonidine. Fig. 16. displays that L-NNA itself slightly decreased the gastric emptying and failed to influence the inhibitory effect of peripherally given clonidine (3.76 μmol/kg s.c.).

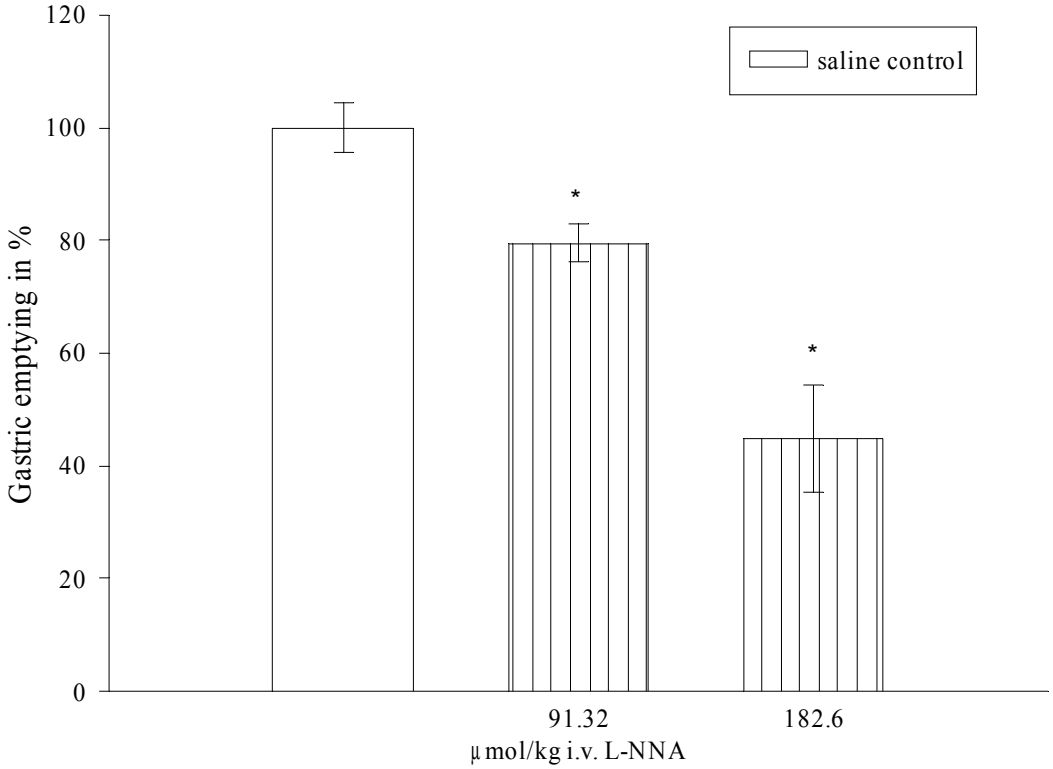

Fig. 15. The effect of L-NNA given i.v. on the gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05, compared to control group

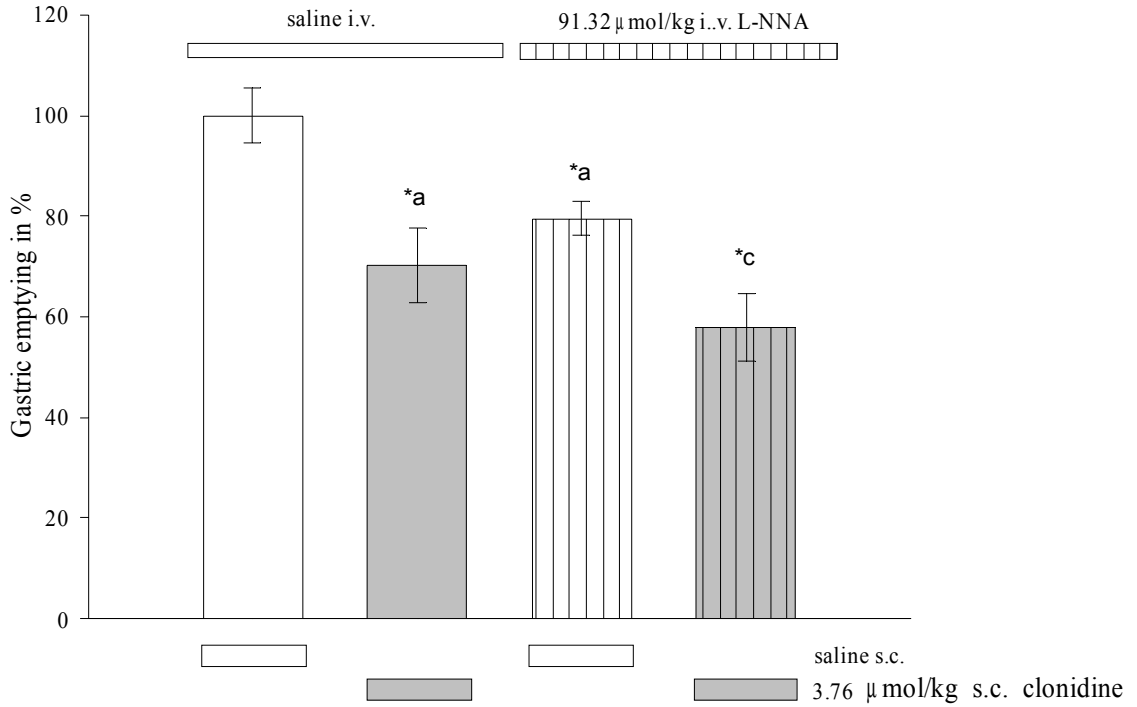

Fig. 16. The effect of L-NNA (91.32 µmol/kg i.v.) on the delay of gastric emptying induced by clonidine (3.76 µmol/kg s.c.) in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5,*p<0.05; a - compared to control group, c - compared to the L-NNA treated group

4.3.2 Studies of the interaction between opioid and α_2 adrenergic systems in the regulation of gastric emptying in rats

4.3.2.1 The effect of naloxone on the delay of gastric emptying induced by peripherally injected clonidine

Thereafter we wondered if there was an opioid component in the mechanism of gastric emptying delaying action of clonidine. Thus we examined the effect of s.c. given, non-selective opioid receptor antagonist naloxone on the gastric delaying action of clonidine. As Fig. 17. shows, the s.c. injected clonidine in a dose of 3.76 µmol/kg significantly inhibited the gastric emptying of the test meal. The s.c. injected naloxone in a dose of 2.75 µmol/kg did not affect the gastric emptying of phenol red solution by itself and failed to influence the effect of clonidine.

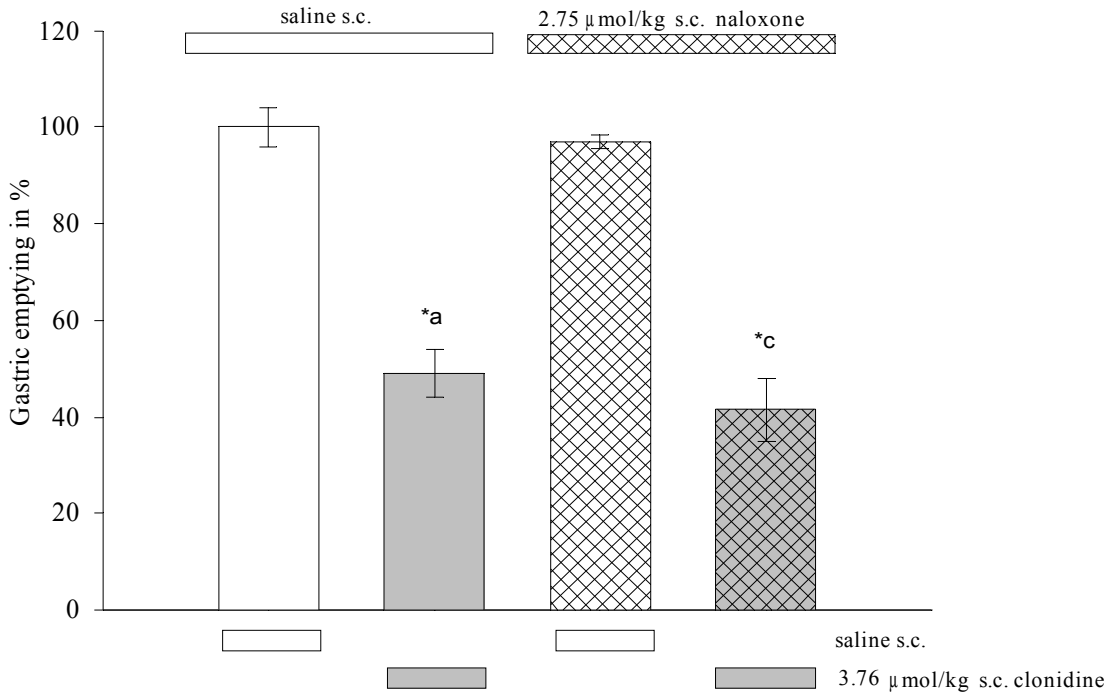

Fig. 17. The effect of naloxone (2.75 µmol/kg s.c.) on the delay of gastric emptying induced by clonidine in a dose of 3.76 µmol/kg s.c. in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5,*p<0.05, a- compared to control group, c - compared to naloxone treated group

4.3.3 Studies of the involvement of central α_2 adrenoceptors in the mediation of gastric emptying in the rat

4.3.3.1 The effect of centrally administered clonidine and oxymetazoline on gastric emptying of phenol red solution

We examined whether inhibition of gastric emptying induced by α_2 adrenoceptor agonists can also be initiated centrally. In these experiments clonidine and oxymetazoline were injected i.c.v., 10 min before the phenol red solution. As Fig. 18.-19. show, both clonidine and oxymetazoline given i.c.v. dose-dependently decreased the gastric emptying. The effects of clonidine and oxymetazoline were significant above the doses of 18.18 nmol/rat i.c.v., and 4.22 nmol/rat i.c.v. respectively. In both cases the maximal inhibition was approximately 40%, therefore to characterize the potency of

these compounds ED_{30} values were calculated. These values were 29.84 nmol/rat for clonidine and 7.93 nmol/rat for oxymetazoline. Fig. 20. represents the effects of clonidine (37.6 nmol/rat) and oxymetazoline (33.78 nmol/rat) after both i.c. and i.c.v. administrations. According to these results clonidine and oxymetazoline tended to be more effective following i.c. than following i.c.v. administration, however the differences in the effects were not significant and examination of additional doses are needed for the final conclusion on the i.c.v. and i.c. effectiveness.

Tabl. 1. shows the ED_{30} values of α_2 adrenoceptor agonist for gastric delaying effects. In order to make our i.c.v. and s.c. results comparable with each other, data were converted to nmol/kg by calculating with rats weighing 150g. The ratios of ED_{30} values of gastric emptying show that clonidine and oxymethazoline may equipotently act on gastric emptying after both peripheral and central administration.

Tabl. 1. Comparisons of peripheral and central effective doses of clonidine and oxymethazoline on gastric emptying in rats. To make i.c.v. values comparable to s.c. ones, data were converted to nmol/kg by calculating with rats weighing 150g.

clonidine (nmol/kg)			oxymetazoline (nmol/kg)		
peripheral(s.c.)	central(i.c.v.)	$ED_{30\,periph}/ED_{30\,i.c.v}$	peripheral(s.c.)	central(i.c.v.)	$ED_{30\,periph}/ED_{30\,i.c.v}$
540	198.9	2.71	810	52.87	15.32

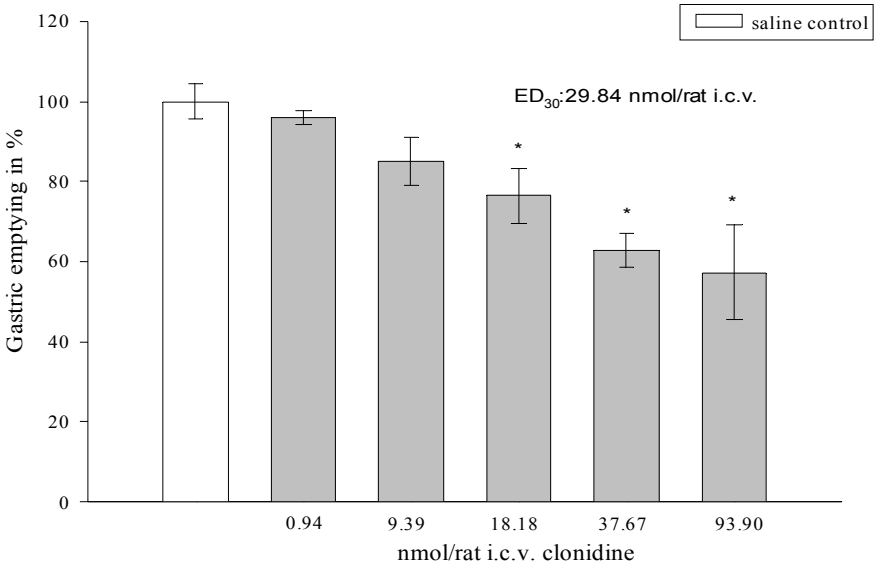

Fig. 18. The effect of clonidine given i.c.v. on the gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *$p<0.05$ compared to control group

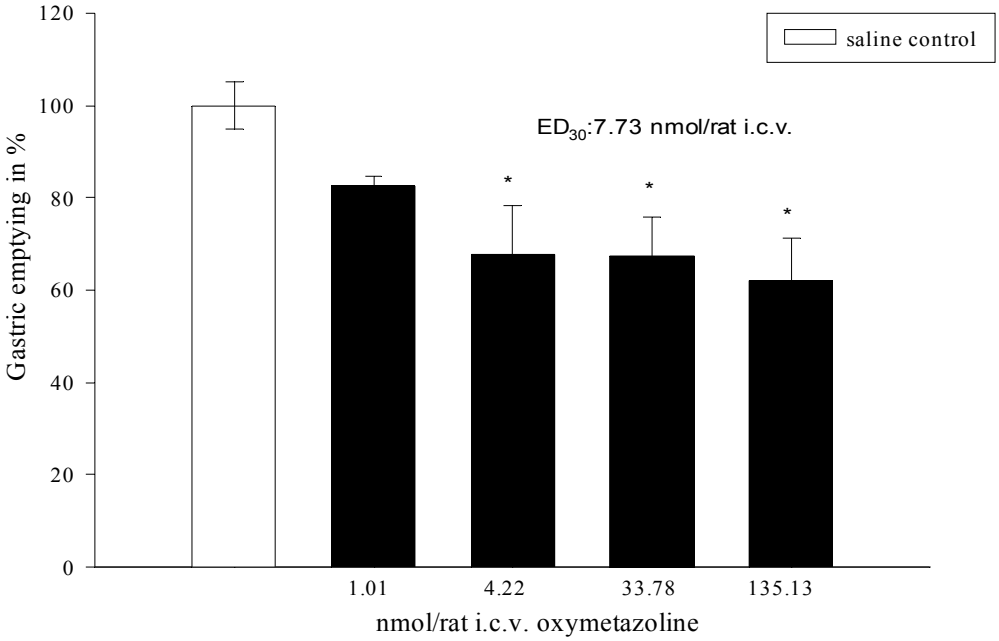

Fig. 19. The effect of oxymetazoline given i.c.v. on the gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05 compared to control group

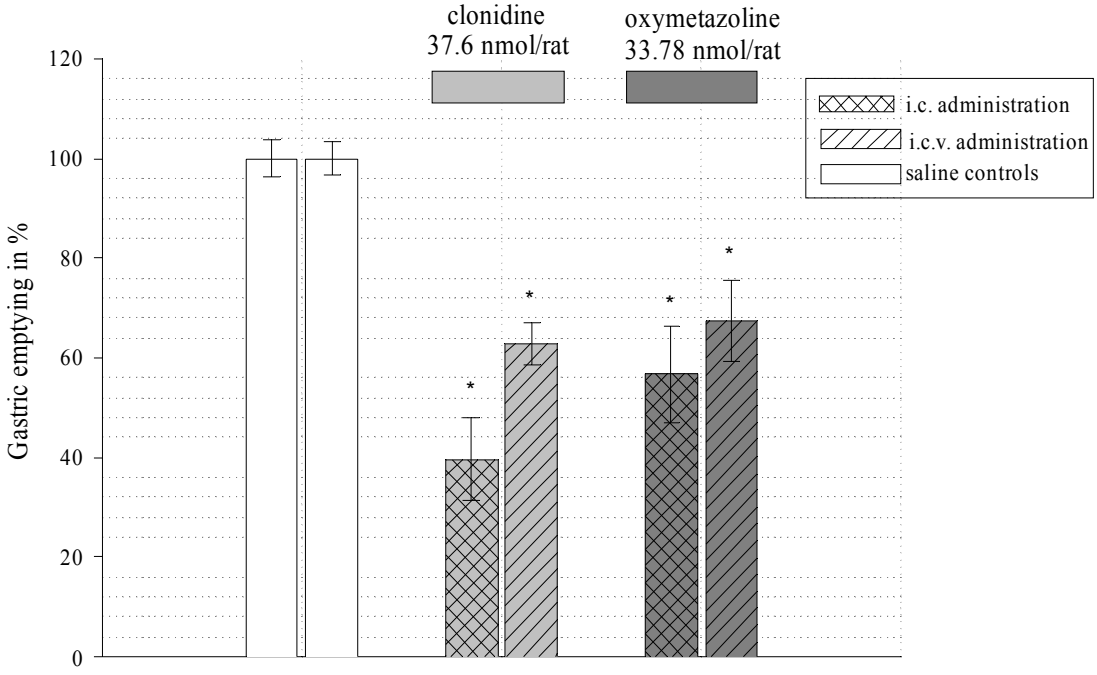

Fig. 20. Comparison of the effects of clonidine (37.6 nmol/rat) and oxymetazoline (33.78 nmol/rat) on the gastric emptying after i.c.v. and i.c. administration in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05 compared to control group

4.3.3.2 The effect of bilateral cervical vagotomy on the gastric emptying delaying effect of clonidine

In order to determine whether the centrally induced gastric emptying delaying action of α_2 adrenoceptor agonists depends on the integrity of vagal nerve, we examined the effect of clonidine after bilateral cervical vagotomy. Fig. 21. represents that i.c.v. injected clonidine in a dose of 37.6 nmol/rat significantly delayed the gastric emptying of phenol red solution. The bilateral cervical vagotomy failed to significantly affect the gastric emptying and did not block the inhibitory effect of centrally injected clonidine on gastric emptying of test meal.

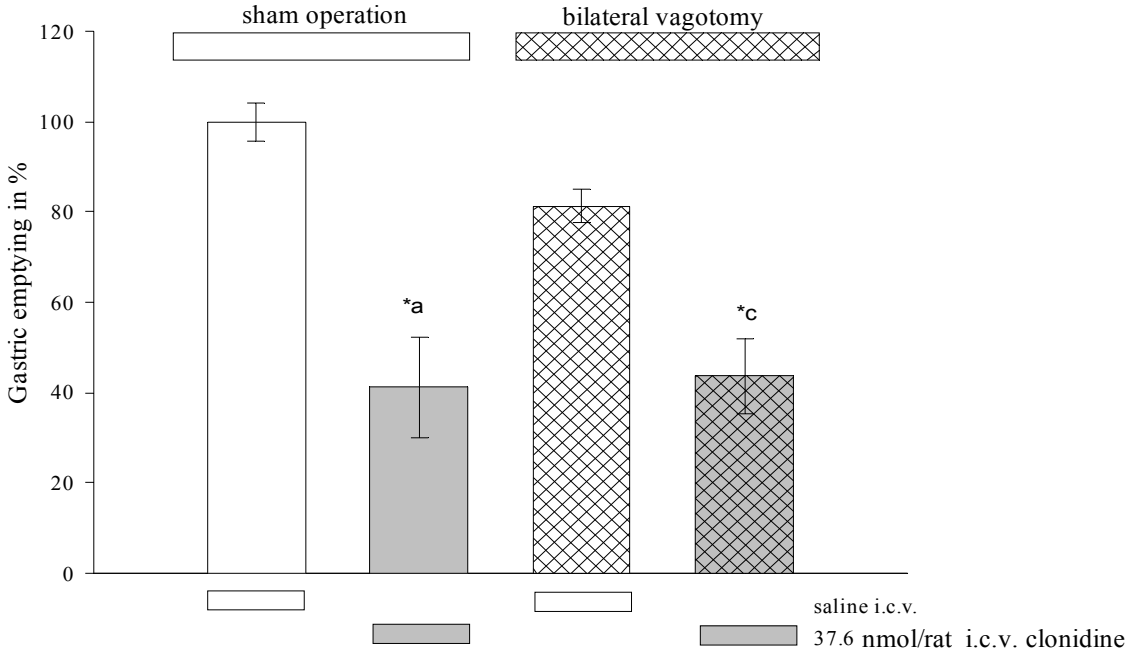

Fig. 21. The effect of bilateral cervical vagotomy on the delay of gastric emptying induced by centrally administered clonidine (37.6 nmol/rat i.c.v.) in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05; a - compared to control group, c - compared to vagotomized group

4.3.4 Studies of the involvement of opioid receptors in regulation of gastric emptying in rats

4.3.4.1 The effect of peripherally administered morphine on the gastric emptying of phenol red solution

In the following studies we examined the effects of μ opioid receptor agonist morphine on the gastric emptying (Fig. 22.). Morphine in the s.c. doses of 26.4-105.5 μmol/kg dose-dependently inhibited the gastric emptying of phenol red solution and its effects were significant.

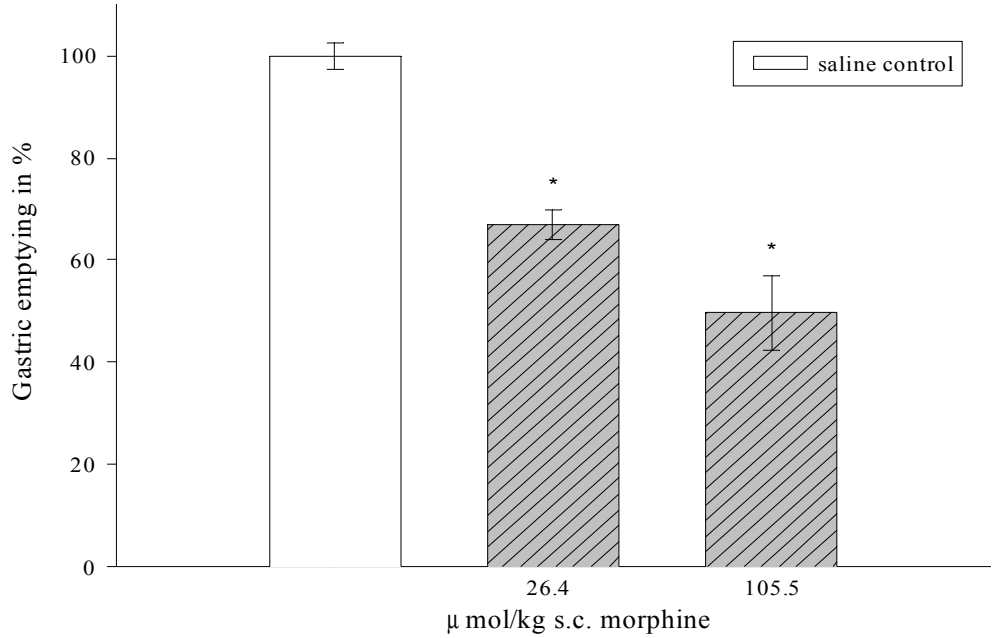

Fig. 22. The effect of s.c. administered morphine on gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5,*p<0.05 compared to control group

4.3.4.2 The effect of centrally given morphine and DAGO on the gastric emptying of phenol red solution

We also examined the effects of centrally injected μ opioid receptor agonists such as morphine and DAGO. Fig. 23. shows the effect of i.c.v. administered morphine. According to these results i.c.v. injected morphine dose-dependently delayed the gastric emptying of phenol red solution in the dose range of 52.9-529 nmol/rat. At these doses

the inhibitory actions proved to be significant. Similarly, the i.c.v. administered DAGO (Fig. 24.) dose-dependently decreased the gastric emptying in the dose range of 9.75-58.5 nmol/rat and the effects were found to be significant above the dose of 19.5 nmol/rat. The maximal inhibition evoked by the compounds was 45.13±8.81 % for morphine (529 nmol/rat) and 52.05±6.12% for DAGO (58.5 nmol/rat). ED_{30} values were 118 nmol/rat for morphine and 15.7 nmol/rat for DAGO.

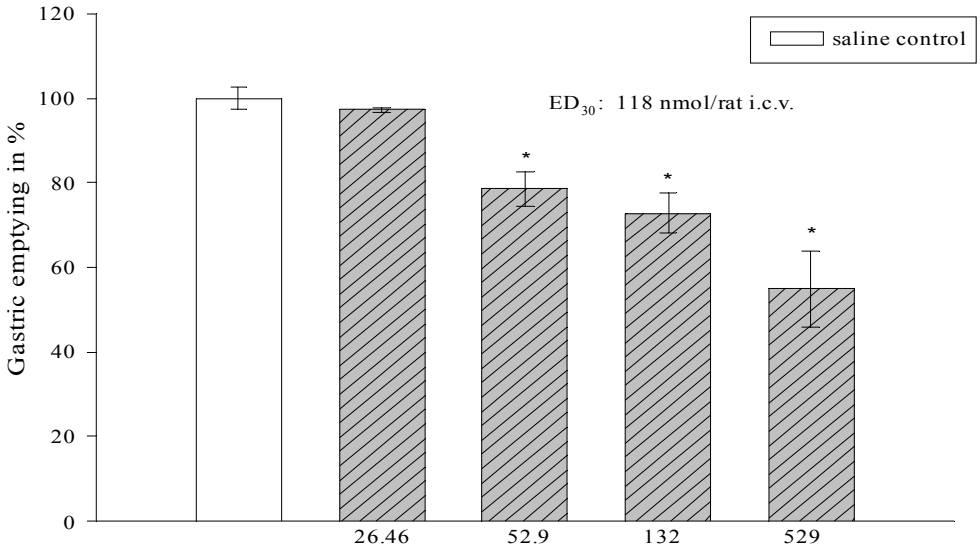

Fig. 23. The effect of morphine given i.c.v. on the gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M. and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5, *p<0.05 compared to compared to control group

4.3.4.3 The effect of s.c. naloxone on the gastric emptying delaying action of i.c.v. morphine and DAGO

In order to confirm the receptorial mechanism in the gastric emptying delaying action of morphine and DAGO, we examined the effect of the non-selective opioid receptor agonist naloxone on the inhibition of both compounds (Fig. 25.-26.). The s.c. admistered naloxone in a dose of 2.75 μmol/kg did not influence the gastric emptying of test meal, but antagonized the gastric emptying delaying effects of both morphine (529 nmol/rat i.c.v.) (Fig. 25.) and DAGO (19.5 nmol/rat i.c.v.) (Fig. 26.).

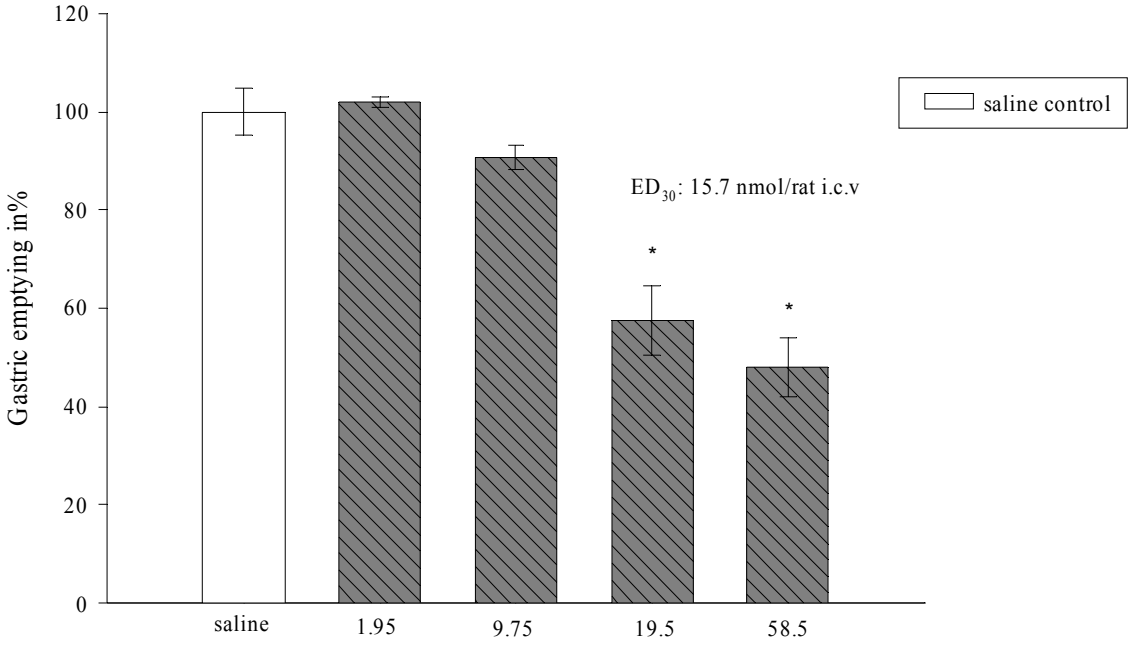

Fig. 24. The effect of DAGO given i.c.v. on the gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M. and were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5,*p<0.05 compared to control group

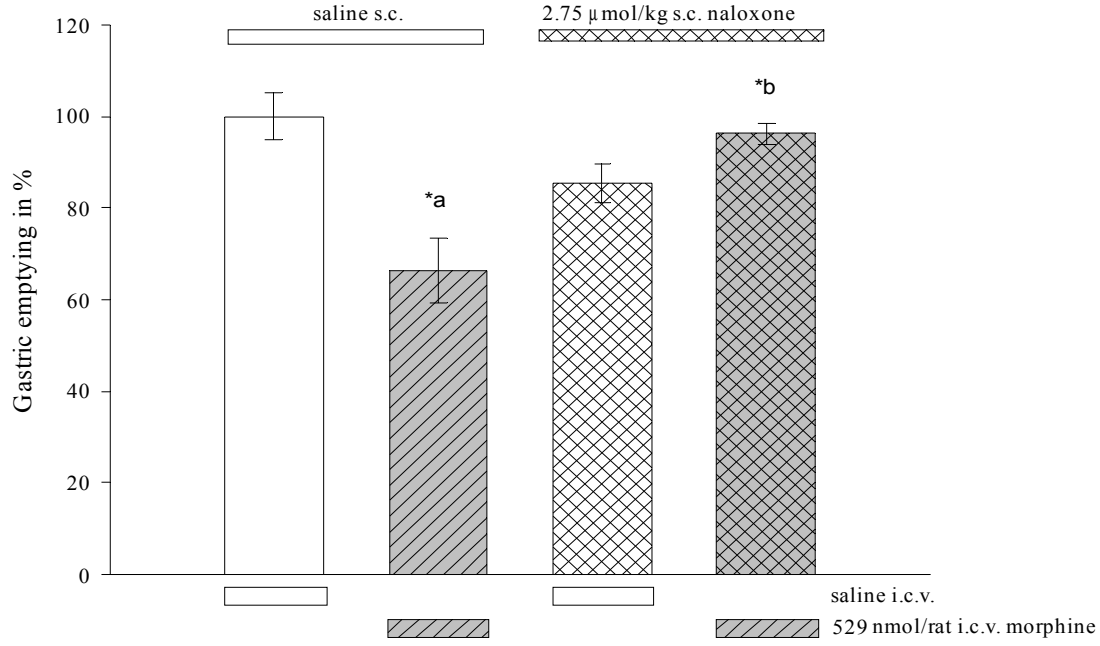

Fig. 25. The effect of naloxone (2.75 µmol/kg s.c.) on the delay of gastric emptying induced by i.c.v. injected morphine in a dose of 529 nmol/rat in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5,*p<0.05, a- compared to control group, b - compared to the morphine treated group

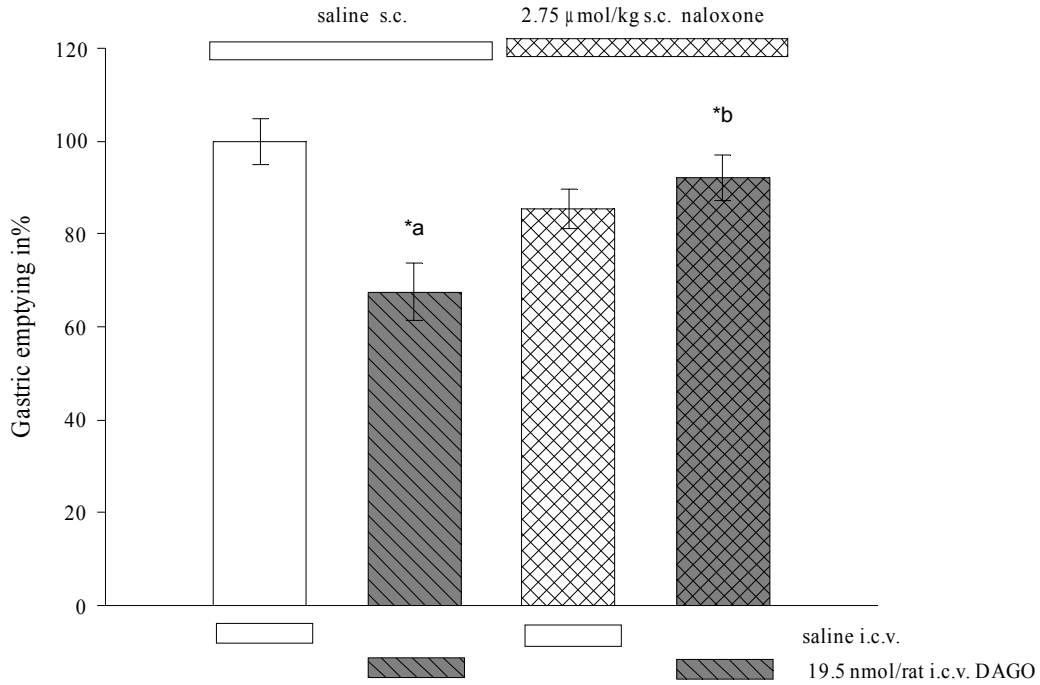

Fig. 26. The effect of naloxone (2.75 µmol/kg s.c.) on the delay of gastric emptying induced by i.c.v. injected DAGO in a dose of 19.5 nmol/rat in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. n=5,*p<0.05, a- compared to control group, b - compared to DAGO treated group

4.3.4.4 The effect of centrally given deltorphine II on the gastric emptying of phenol red solution

Our following question was whether the central δ opioid receptors were involved in the regulation of gastric emptying. As Fig. 27. shows, the selective δ opioid receptor agonist deltorphine II in the dose of 5.67 nmol/rat did not influence the gastric emptying after i.c.v. administration. However, the i.c.v. injected deltorphine II in the dose of 56.7 nmol/rat significantly inhibited the gastric emptying of the test meal. The inhibition in % was 32.65%.

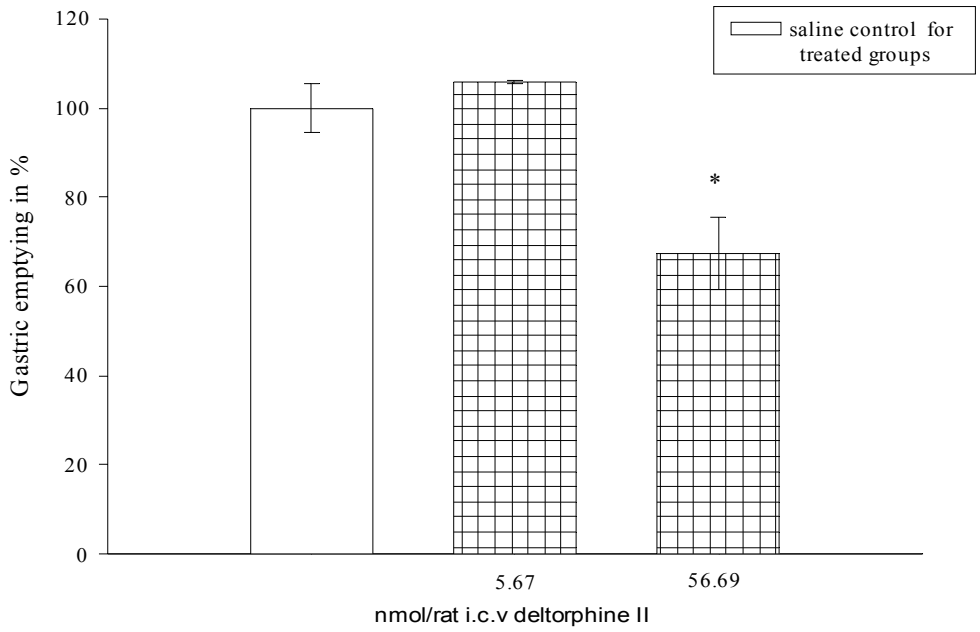

Fig. 27. The effect of deltorphine II given i.c.v. on the gastric emptying of phenol red solution in rats. Each bar represents the means ± S.E.M and data were analyzed by the means of ANOVA. n=5, * $p<0.05$ compared to control group

4.4 GASTRIC MOTILITY - Experiments by using intragastric balloon:

4.4.1 Studies of the role of α_2 adrenoceptors in regulation of gastric motility in rats

4.4.1.1 The effect of peripherally administered clonidine on the basal gastric motor activity

In the subsequent experimental series we studied two characteristic components of gastric motility such as the fundic tone (Intragastric pressure) and the antral phasic contractions (Motlity Index). Experiments were performed under urethane anaesthesia (1.25 g/kg i.p.) which strongly suppressed the gastric motor activity. Fig. 28. shows that the i.v. given clonidine in the dose of 0.75 μmol/kg failed to influence the basal gastric tone and gastric contractility. Therefore in further experiments the gastric functions were stimulated by 2-DG (300 mg/kg i.v.). The gastric tone was adjusted to 11-12 cmH_2O.

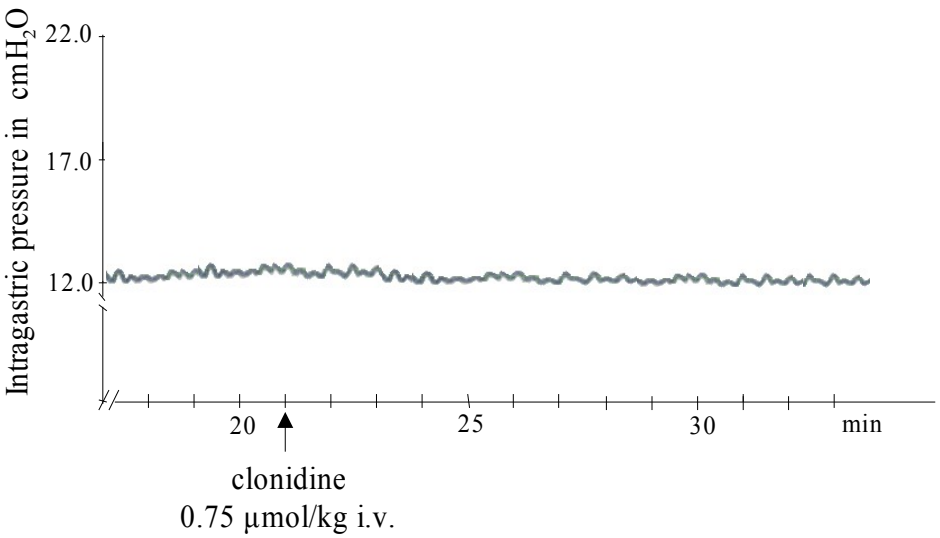

Fig. 28. Representative gastric contractility traces illustrating the lack of effect of clonidine (0.75 µmol/kg i.v.) on the basal gastric motor activity under urethane anaesthesia (1.25 g/kg i.p.) in rats.

4.4.1.2 The effect of peripherally administered clonidine on the gastric motor activity stimulated by 2-DG

In these experiments we studied the effect of the α_2 adrenoceptor agonist clonidine on the stimulated gastric motor activity by 2-DG (300 mg/kg i.v.). After administration of 2-DG both the fundic tone and antral phasic contractions were elevated. Compounds were injected 15-20 min after 2-DG. As Fig. 29. displays, the i.v. given clonidine in the doses of 0.19 and 0.38 failed to influence the stimulated gastric motor functions, however, in the dose of 0.75 µmol/kg significantly decreased the fundic tone and the amplitudes of antral phasic contractions.

4.4.1.3 The effect of i.v. yohimbine on the 2-DG stimulated gastric motor inhibitory action of clonidine

In order to confirm the receptorial mechanism of the action induced by clonidine, we examined the effect of the α_2 adrenoceptor antagonist yohimbine on gastric motor inhibition induced by this compound. Fig. 30. shows the antagonism evoked by yohimbine. As it can be seen, clonidine in the i.v. dose of 0.75 µmol/kg suppressed both the amplitudes of antral phasic contractions and the fundic tone. Both parameters of the gastric motor activity were reversed by i.v. injected yohimbine in the dose of 10

μmol/kg. According to our data presented in Tabl. 2. the effect of yohimbine on the gastric motor inhibitory action of clonidine proved to be significant.

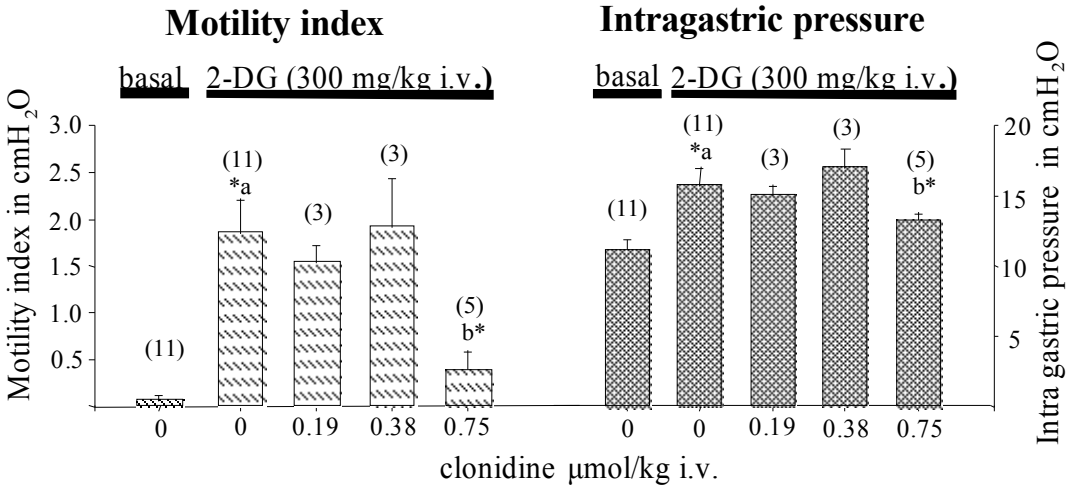

Fig. 29. The effect of different dose of clonidine on Motility Index and Intragastric pressure. The gastric motor activity was stimulated by 2-DG (300 mg/kg i.v.). Clonidine was given 20-25min after the administration of 2-DG.The numbers in brackets above the bars show the number of animals used in experiments. Each bar represents the means±S.E.M., data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. *p<0.05, a- compared to 1st column; b- compared to the 2nd column

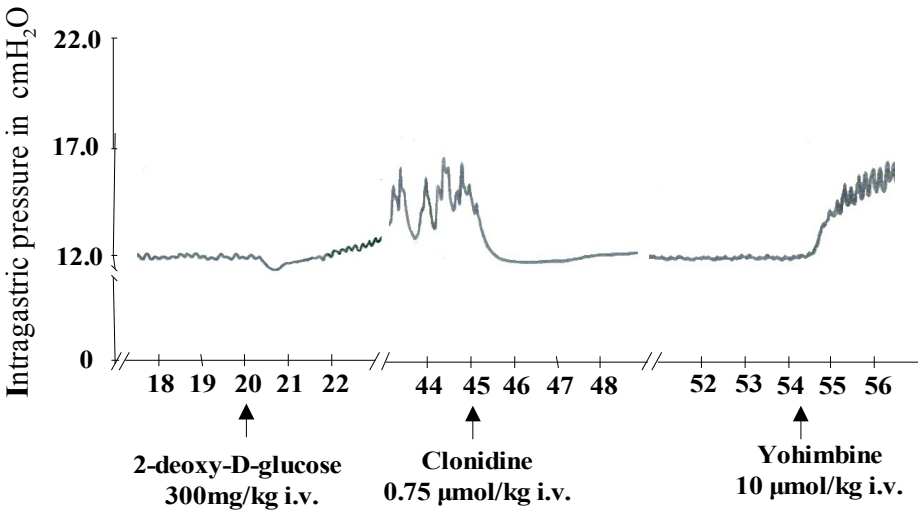

Fig. 30. Representative gastric contractility traces illustrating the effect of yohimbine (10 μmol/kg i.v.) on the inhibitory action of clonidine (0.75 μmol/kg i.v.) on the gastric motor activity stimulated by 2-DG (300 mg/kg i.v.). Clonidine was given 25 min after the administration of 2-DG, yohimbine was administered 10 min after the injection of clonidine.

Table 2. The effect of yohimbine (10 µmol/kg i.v.), prazosin (0.23 µmol/kg i.v.) and naloxone (1.3 µmol/kg i.v.), on the inhibitory action of clonidine (0.75 µmol/kg i.v.) on the stimulated gastric motor activity by 2-DG (300 mg/kg i.v.). Table shows the Motility Index and Intragastric pressure. Clonidine was given 20-25 min after the administration of 2-DG. Each bar represents the means ± S.E.M., and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. *$p<0.05$, a- compared to the group treated by 2 -DG; b- compared to the groups treated by 2-DG+clonidine, n= number of animals.

Compound	n	Moltility index cmH_2O	Intragastric pressure cmH_2O
-	12	0.15 ± 0.01	12.1 ± 0.2
2-DG	12	1.3 ± 0.1	17.1 ± 1
2-DG + clonidine	12	0.5 ± 0.06[a]	13.1 ± 0.8[a]
2-DG + clonidine + yohimbine	6	2.1 ± 0.1[b]	15.8 ± 1.0[b]
2-DG + clonidine + prazosin	3	0.7 ± 0.07[a]	13.8 ± 0.9[a]
2-DG + clonidine + naloxone	3	0.8 ± 0.08[a]	14.1 ± 1.1[a]

4.4.1.4 The effect of i.v. prazosin on the 2-DG stimulated gastric motor inhibitory action of clonidine

To determine the α_2 adrenoceptor subtype(s) involved in the mediation of gastric motor functions, we studied the effect of the selective α_{2B} adrenoceptor antagonist prazosin on the gastric motor inhibitory action of clonidine. As Tabl. 2. shows, i.v. injected prazosin in the dose of 0.23 µmol/kg failed to influence the inhibitory effect of clonidine (0.75 µmol/kg i.v.) on the amplitudes of phasic contractions and on the gastric tone.

4.4.1.5 The effect of peripherally administered oxymetazoline on the gastric motor activity stimulated by 2-DG

We also studied the effect of the α_{2A} adrenoceptor selective agonist oxymetazoline on the stimulated gastric motor functions. As Tabl. 3. shows the i.v. administered oxymetazoline in the dose range of 0.185-3.4 µmol/kg dose-dependently inhibited the gastric tone and the amplitudes of phasic contractions. The effect was significant above the i.v. dose of 1.85 µmol/kg.

4.4.1.6 The effect of i.v. yohimbine on the 2-DG stimulated gastric motor inhibitory action of oxymetazoline

In order to confirm the receptorial mechanism of the inhibitory action induced by i.v. oxymetazoline, we examined the effect of the α_2 adrenoceptor antagonist yohimbine on the action of this compound. As Fig. 31. shows, the i.v. injected yohimbine in a dose of 10 μmol/kg failed to affect the inhibitory action induced by oxymetazoline (3.4 μmol/kg i.v.) on gastric tone, however, the phasic contractions of the antrum returned. According to our data presented in Tabl. 3. the effect of yohimbine on the antral contractility suppression evoked by oxymetazoline proved to be significant.

Tabl. 3. The effect of different doses of oxymetazoline on the gastric motor activity stimulated by 2-DG (300 mg/kg i.v.), and the effect of yohimbine (10 μmol/kg i.v.) on the inhibitory action of oxymetazoline (3.4 μmol/kg i.v.). Table shows the Motility Index and Intragastric pressure. Oxymetazoline was given 20-25 min after administration of 2-DG. Each bar represents the means ± S.E.M., and data were analyzed by the means of ANOVA. Newman-Keuls post hoc test was used for multiple comparisons. *p<0.05, a- compared to the basal value; b- compared to the group treated by 2-DG; c- compared to the group treated by 2-DG+oxymetazoline (3.4 μmol/kg i.v.), n= number of animals.

Compound	n	Moltility index cmH_2O	Intragastric pressure cmH_2O
-	12	0.8 ± 0.07	12 ± 0.2
2-DG (300 mg/kg i.v.)	12	2.5 ± 0.2[a]	17.5 ± 1[a]
2-DG (300 mg/kg i.v.) + oxymetazoline in a dose of:			
0.185 μ mol/kg i.v.	3	1.7 ± 0.16[b]	14.1 ± 3
1.85 μ mol/kg i.v.	4	0.2 ± 0.01[b]	10.8 ± 2[b]
3.4 μ mol/kg i.v.	5	0.1 ± 0.001[b]	11.1 ± 2[b]
2-DG + oxymetazoline (3.4 μ mol/kg i.v.) + yohimbine (10.0 μ mol/kg i.v.)	3	1.7 ± 0.1[c]	11.2 ± 1.1

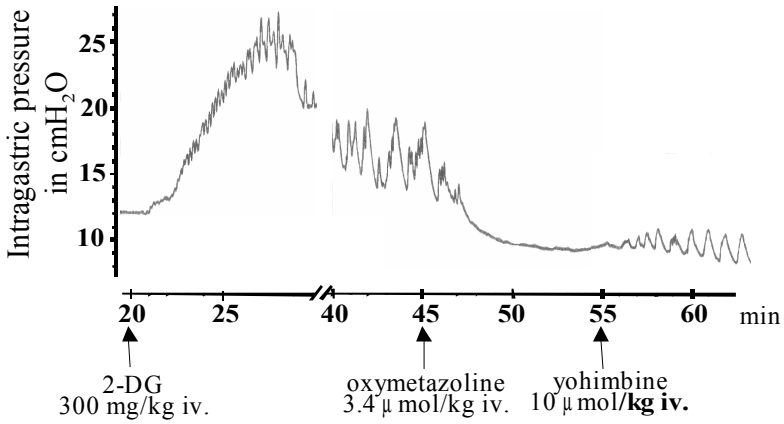

Fig. 31. Representative gastric contractility traces illustrating the effect of yohimbine (10 µmol/kg i.v.) on the inhibitory action of oxymetazoline (3.4 µmol/kg i.v.) on the gastric motor activity stimulated by 2-DG (300 mg/kg i.v.). Oxymetazoline was given 25 min after the administration of 2-DG, yohimbine was administered 10 min after the injection of oxymetazoline. Yohimbine failed to reverse the inhibitory action of oxymetazoline on the basal tone of the stomach but the phasic gastric contractions returned.

4.4.2 Studies of the interaction between opioid and α_2 adrenergic systems in the regulation of gastric motility in rats

4.4.2.1 The effect of i.v. naloxone on the 2-DG stimulated gastric motor inhibitory action of clonidine

In the subsequent experiments we studied whether there was interaction between the α_2 adrenergic and opioid systems. Thus we examined the effect of the non-selective opioid receptor antagonist naloxone on the gastric motor function inhibitory action of clonidine. The i.v. injected naloxone in a dose of 1.3 µmol/kg did not affect the inhibition evoked by clonidine (0.75 µmol/kg i.v.) on the amplitudes of phasic contractions and gastric tone (Tabl. 2.).

5 DISCUSSION

Maintenance of gastric mucosal integrity depends on several factors, e.g. mucosal microcirculation, mucosal barrier, production of gastric mucus, and mucosal protective elements. Gastric motility may also be one of the factors, which affects the integrity of gastric mucosa. Gastric contractions characterized by high amplitudes may induce microvascular disturbances in specific sites of the mucosa probably by abnormal compression of the gastric wall, thereby leading to increased vascular permeability and cellular damage [27-32]. Previous studies described both deleterious and beneficial effects of gastric emptying. Thus its elevation was assumed to be gastroprotective on aspirin-induced gastric damage [39] while its delay caused by large doses of morphine was proposed to aggravate the ethanol-induced gastric lesions [94]. Based on these reports the main purpose of our present experimental series was to study the correlations between the gastric motor activity changes (gastric emptying and gastric motility) and mucosal protective processes induced by α_2 adrenoceptor agonists and opioid peptides. Our studies were completed with the examination of the relationship between the antisecretory and gastroprotective action of α_2 adrenoceptor agonists.

5.1.1 The effect of α_2 adrenoceptor stimulants on gastric emptying of phenol red solution.

The effects of α_2 adrenoceptor agonists on gastric emptying were examined by using the phenol red method described previously [112]. It has been reported that there are differences between the gastric emptying of solid and liquid meal in the regulatory mechanisms. The test meal used by us is viscous, rather liquid which is known to empty from the stomach in an exponential manner. In human, in healthy individuals, the half-emptying time for non-nutrient liquids is usually less than 20 min, which is in agreement with our observations in rats (not published data). Therefore this method is mainly suitable for studying inhibitory effects on gastric emptying.

There are several factors, determining the gastric emptying rate of liquids. The most important ones are the gastro-duodenal pressure gradient and the tone of the pyloric sphincter [114]. The pressure gradient necessary to pass the gastric content into duodenum is ensured on the one hand by the adequate fundic tone, on the other hand by the duodenal relaxation. Antral contractions are not considered to play a dominant role

in the gastric emptying of liquid meal because no correlation has been found between the antral motility and gastric emptying of liquid meal [115]. In addition, there are also vagal reflexes which may affect the gastric emptying rate. Thus distension of either the stomach wall or the duodenal wall may lead to gastric relaxation, thereby delaying the gastric emptying [114;116;117]. As a consequence, gastric emptying is delayed if the fundus is relaxed and/or the pyloro-duodenal resistency is increased. The latter is possible in cases of elevated pyloric and/or duodenal contractility and in the case of delay of intestinal transit. These different movements in GI tract are synchronized by the nervous system in time and space, and any disturbance in this coordination may lead to impairment of gastric emptying. Thus the disruption in the antro-pyloro-duodenal coordination or simultaneous increase of GI contractility has been suggested being responsible for the delay of gastric emptying induced by high doses of cisapride, which is a widely used prokinetic drug in clinic [118].

The first purpose of our experiments was to study the effect of α_2 adrenoceptor agonists on gastric emptying. In our experimental conditions clonidine, the α_2 adrenoceptor agonist, dose-dependently inhibited the gastric emptying of phenol red solution after both peripheral and central administrations. The non-selective α_2 adrenoceptor antagonist yohimbine reversed the delay of gastric emptying caused by s.c. clonidine indicating that the effect was likely to be mediated by α_2 adrenoceptors. However, the inhibition of the effect was not complete, which raised the involvement of other potential mechanisms in α_2 adrenoceptor mediated delay of gastric emptying. Furthermore, increasing the dose of clonidine above 93.9 nmol/rat i.c.v and 3.76 μmol/kg s.c. did not result in further inhibition of gastric emptying. The maximal inhibition evoked by s.c. administered clonidine in a dose of 3.76 μmol/kg was 50.6 ± 6.21%, while the maximal inhibition observed following i.c.v. injected clonidine in a dose of 93.9 nmol/rat was 43.75±11.82%. Therefore to characterize the potency of the compounds the ED_{30} (and not ED_{50}) values were calculated (Tabl. 4.). These values were 29.84 nmol/rat for i.c.v. and 0.54 μmol/kg for s.c. administration. It is known that peripherally administered clonidine may act on both peripheral and central sites since it crosses the blood-brain barrier. Therefore we compared the central and peripheral effective doses of clonidine on gastric emptying The ratio of these values ($ED_{30,}$ s.c./

ED_{30} i.c.v.) was 2.71, suggesting that clonidine might equipotently act at peripheral and central sites.

Next question to be answered was: which α_2 adrenoceptor subtype might be involved in mediation of gastric emptying. To date four α_2 adrenoceptor subtypes have been recognized, such as the α_{2A}, α_{2B}, α_{2C} and α_{2D} [67-69], however the last one is considered to be a variant of α_{2A} adrenoceptors [70]. Our data suggested the potential involvement of α_{2A} adrenoceptor subtype in the regulatory mechanism of gastric emptying. Indirect pieces of evidence were provided by our results that α_{2B} subtype selective antagonists prazosin and ARC-239 failed to influence the effect of clonidine on gastric emptying. In addition, the selective α_{2A} adrenoceptor agonist oxymetazoline also exerted an inhibitory action, the effect proved to be dose-dependent. In the case of oxymetazoline ED_{30} values were 810 nmol/kg for s.c. and 7.73 nmol/rat for i.c.v. administration. The ED_{30} value for s.c administration was 15.32 times higher than that for i.c.v. injection. Consequently, both central and peripheral α_{2A} adrenoceptors might be involved in mediation of the delay of gastric emptying.

Tabl. 4. Comparisons of peripheral and central effective doses of clonidine and oxymetazoline on different gastric functions such as gastroprotection and gastric emptying in rats. Data originate from our previous study [164] and from the present study. To make i.c.v. values comparable to s.c. ones data were converted to nmol/kg by calculating with rats weighing 150g.

	clonidine (nmol/kg)			oxymetazoline (nmol/kg)		
	peripheral(s.c.)	central(i.c.v.)	$ED_{50\ periph}/\ ED_{50\ i.c.v}$	peripheral(s.c.)	central(i.c.v.)	$ED_{50\ periph}/\ ED_{50\ i.c.v}$
$ED_{50\ gastroprotection}$	47.96 [164]	0.93	51.74	>1680 [164]	ineffective [164]	-
$ED_{30\ gastric\ emptying}$	540	198.9	2.71	810	52.87	15.32

It is well-known that clonidine and oxymetazoline also have an α_1 adrenoceptor agonist activity. However the role of α_1 adrenoceptors in the mediation of gastric emptying can be ruled out, because prazosin, which is also an antagonist of the α_1 adrenoceptors, has failed to antagonize the effect of clonidine.

Several publications suggested potential peripheral and central mechanisms involved in α_2 adrenoceptors-induced action. In the periphery it was demonstrated that α_2 adrenoceptor stimulants had different influence on motor activity in the stomach than in the intestine. Namely, it was found that the intestinal transit was strongly inhibited, whereas only slight action on gastric emptying was described [119]. In vitro study

indicated that the stimulation of α_2 adrenoceptors localized in the myenteric plexus decreased the fundic tone induced by the field stimulation of cholinergic neurones [64]. The α_2 adrenoceptor agonists were reported either not to influence or slightly stimulate the basal gastric tone. On the other hand, they were shown to decrease both the antral and pyloric contractions which might lead to gastroduodenal reflux [119]. Accordingly, the relatively slight inhibition of gastric emptying by α_2 adrenoceptor stimulants in our experiments might be explained by their strong inhibitory action on the intestinal transit and/or by reduction of pyloric contractions. The former might result in the elevation of intestinal resistency and with the latter this might lead to gastroduodenal reflux.

One of the major transmitters involved in the gastric relaxation processes seems to be the NO. NO synthases were found to be distributed in the whole stomach with different density. Most of them were localized in the myenteric plexus but they were also present in the submucosal plexus. The density of NO synthase was found to be scarce in fundus, more powerful in the antrum and very intensive in the pyloric region [120]. The presence of NO synthase indicates an important role of NO in the regulation of gastric motility. Study demonstrated that the vagal inhibitory efferent pathway of gastric accommodation reflex involved nicotine synapses and resulted in NO release from the rat myenteric plexus [121]. Moreover it was shown that pyloric relaxation was also predominantly under the control of NO release from the myenteric plexus [122]. However in our experiments L-NNA, an inhibitor of NO synthase, failed to influence the delay of gastric emptying induced by clonidine suggesting that NO was not involved in the effect of clonidine. Noteworthy, that L-NNA itself inhibited the gastric emptying of phenol red solution probably by increasing the pyloro-duodenal contractility. This assumption is supported by the observations of Ishiguchi and coworkers [21] who also found that L-NAME significantly inhibited the gastric emptying in rats and the effects of L-NAME on the basal contractions of pylorus and duodenum were significantly higher than on that of the antrum [21].

In the CNS the primary site involved in the regulation of gastric functions is the dorsal vagal complex which receives input through the vagal sensory afferents from the stomach and sends efferents to modulate gastric functions just like gastric emptying, motility. The cell bodies of afferents are localized in the nodose ganglion and they are terminated on the NTS, while the cell bodies of efferents can be mainly found in the

DMV. There are synaptic connections between NTS and DMV. This area is considered to be a key element of vago-vagal reflex circuits such as receptive, adaptive relaxation. Studies proposed a hypothesis for the vagal mechanisms of gastric motility regulation. DMV might control the gastric functions through excitatory and inhibitory pathways. The excitatory pathway was supposed to comprise preganglionic cholinergic neurons and postganglionic cholinergic neurons. Two inhibitory pathways seemed to exist, one consisting of preganglionic cholinergic neurons and postganglionic non-adrenergic, non-cholinergic neurons and the other being a nitrergic preganglionic neuron synapsing onto a postganglionic non-adrenergic, non-cholinergic neuron [123;124]. According to this assumption the inhibition of gastric motor functions may be possible through either the activation of the inhibitory pathways or inhibition the of activity from an excitatory cholinergic DMV pathway.

Several compounds injected into the DVC affected the gastric motility. Namely, i.c. injection of TRH evoked vagal-mediated stimulation of gastric emptying of phenol red solution [125]. Nicotine microinjected into the medial NTS also decreased the antral motility and gastric tone [126]. This study also provided evidence for a separate regulation of the antral phasic contractions and gastric tone [126]. To be more specific, small dose of nicotine microinjected into the medial NTS decreased the fundic tone but higher dose was required to affect the antral phasic contractions. In addition, the inhibition on fundic tone was abolished by the α_2 antagonist yohimbine, while the antral phasic contractions were blocked by $GABA_A$ antagonist bicuculline [126].

Nagata and coworkers [127] suggested the involvement of central adrenergic system in the regulation of gastric motor activity. Namely, it was found that norepinephrine injected i.c. significantly inhibited the gastric motility [127]. This effect was antagonized by yohimbine but not by prazosin which may indicate that α_1 adrenoceptors are not involved in the action. Moreover, since the subtypes of α_2 adrenoceptors have been described the lack of effect may also suggest that non-$\alpha_{2B/C}$ adrenoceptor subtype is involved in the regulation of gastric motor activity [127]. I.c. clonidine was shown to decrease the gastric motility as well. Accordingly, the α_2 adrenoceptor antagonists were demonstrated to have a central stimulatory action on gastric motility, probably by blocking the effect of the endogenous norepinephrine [127]. The involvement of central α_2 adrenoceptors in the mediation of gastric functions was further supported by the facts

that α_2 adrenoceptors were found in high density in the DMC [66], and norepinephrine released from A2 neurons in the DMV was found to stimulate postsynaptic α_2 adrenoceptors which resulted in inhibition of the neurons of DMV [128].

In order to determine whether α_2 adrenoceptor agonists act in a lower brain stem or in a higher area, we examined their effects after i.c. and i.c.v. administration on gastric emptying. According to these results the α_2 adrenoceptor stimulants tended to be more effective after i.c. than i.c.v. injection, but the differences were not significant.

One of our questions was whether the centrally induced delay of gastric emptying was mediated by an inhibitory vagal pathway. Data of the literature provided evidence that the vagus mediated fundic and pyloric relaxation processes by activating inhibitory neural pathways in the ENS. Fundic tone and pyloric contractility might be determinant factors of the gastric emptying of liquids.

Regarding the fundic tone, the gastric accommodation reflex was reported to use non-adrenergic, non-cholinergic (NANC) transmitters, possibly NO, to mediate relaxation [121]. Furthermore, it was demonstrated that vagal stimulation produced a two-phasic response on vascularly isolated, perfused rat stomach. A rapid relaxation was followed by a prolonged relaxation. The former was blocked by a NO synthase inhibitor while the latter was reduced by a VIP antagonist [129].

Concerning the pyloric contractility, the vagal stimulation induced pyloric relaxation was significantly reduced by L-NAME, a NO synthase inhibitor [21]. Although Cruz and coworkers [130] also confirmed the presence of an inhibitory DMV pathway to the rat gastric smooth muscle, this pathway, however, did not seem to use NO as a neurotransmitter. Moreover, they did not find any evidence for the existence of a nitrergic–NANC pathway. Besides they also indicated the presence of an excitatory cholinergic-cholinergic DMV pathway in rats [130].

Our data showed that the delay of gastric emptying induced by centrally injected clonidine did not appear to be a vagally mediated inhibitory process since bilateral cervical vagotomy did not abolish the effect. Bilateral cervical vagotomy itself did not influence the gastric emptying of phenol red solution. Similarly, studies suggested that the vagotomy did not inhibit rather accelerated the gastric emptying of fluid [131, 132]. It was explained by the absence of gastric relaxation reflexes induced by the distension of oesophagus, stomach and duodenum, which normally delayed the gastric emptying of

liquid. In addition, other non-vagal systems such as splanchic nerves and hormonal systems might compensate the absence of vagal control [133]. To explain the lack of effect of bilateral cervical vagotomy on the delay of gastric emptying induced by centrally injected clonidine, further experiments are needed. It might be speculated that from the brain stem there are numerous projections to other brain areas (e.g. hypothalamus). Descending pathways from these areas - through spinal efferents - may influence the activity of GI tract by a vagal-independent mechanism. However, the precise mechanism of vagal-independent pathway in clonidine-induced centrally initiated delay of gastric emptying is to be analysed in the future.

5.1.2 The effect of α_2 adrenoceptor stimulants on the gastric motility stimulated by 2-DG

In another series of our experiments we studied the effect of α_2 adrenoceptor stimulants on gastric motility by the use of a miniature balloon. Using this method it is possible to separate two different movements of the stomach: the fundic tonic contraction and antral phasic contractions. The registered intragastric pressure mainly originates from the fundic tone while the phasic contractions characterize the movements of the antrum.

We studied the effect of α_2 agonists both on basal and stimulated gastric motor activity. The gastric motility was stimulated by 2-DG. This compound is reported to be a glucose analogue that interferes with glycolysis, causes glycoprivation thereby stimulating glucose-sensitive neurons in the brain stem which in turn activate the vagal outflow and stimulate the gastric functions [18;134-136]. In addition, study indicated that medullary TRH was involved in the vagally mediated 2-DG stimulation of gastric motor function [137].

In our experiments we found that clonidine did not influence the basal gastric motor activity, however, both clonidine and oxymethazoline decreased the stimulated gastric tone and antral contractions after i.v. administration. The effect of clonidine was reversed by yohimbine but not by prazosin suggesting that - in contrast with gastroprotection, where α_{2B} adrenoceptors were likely to mediate the protective action [138] - a non-α_{2B} subtype might be involved in the regulation of gastric motility. The α_{2A} subtype selective oxymetazoline also inhibited both gastric tone and gastric contractility

stimulated by 2-DG. Yohimbine antagonized only the reduction of gastric contractions, but failed to influence the inhibitory effect on gastric tone. This raises the possibility that inhibition of gastric activity by oxymethazoline is only partially mediated by α_2-adrenoceptors and other mechanisms may also be involved as our previous studies also suggested [132, 166]. This assumption is supported by the findings of Patil and coworkers who demonstrated an atropine-like effect of oxymetazoline [139].

The role of blood glucose level can also be raised in the modulation of gastric motility because our experiments were carried out under urethane anaesthesia, which decreased gastric motor activity. The inhibitory effect of urethane was suggested relating to its hyperglycaemic action [140]. Moreover, it is known that clonidine itself may affect the blood glucose level, since α_2 adrenoceptors can be found on the pancreatic β cells [141] and their stimulation results in inhibition of insuline release [142;143]. It was also reported that clonidine produced dose-dependent hyperglycaemic response in fed rats, and this action was proposed to be mediated by central α_2 adrenoceptors [144]. Takeuchi and coworkers demonstrated that hypermotility caused by indomethacin was associated with a reduction in blood glucose concentration [145]. Administration of glucose, consequently the elevation of blood glucose level, abolished hypermotility response induced by indomethacin. Others suggested the possibility that indomethacin might sensitize gastric contractility through glycoprivic receptors by inducing hypoglycemia [145]. Moreover, gastric emptying was delayed in normal rats with acute elevation in blood glucose level [146], and microinjection of glucose into the rat DMV was shown to decrease gastric motility and intragastric pressure [147]. Based on this data the blood glucose level also seems to modulate the gastric motor activity.

5.1.3 The effect of α_2 adrenoceptor stimulants on gastric acid secretion in pylorus-ligated rats.

Previous studies indicated the role of α_2 adrenoceptors in the mediation of gastric acid secretion. For instance α_2 adrenoceptor agonists decreased the gastric acid secretion after peripheral administration in pylorus ligated rats [63]. Data of the literature suggested that the antisecretory effect of these compounds might be mediated by both peripheral and central α_2 adrenoceptors [161,162]. Blandizzi and coworkers [163] proposed that α_{2A} adrenoceptor subtype might be responsible for the antisecretory actions

of α_2 adrenoceptors which is in agreement with our present results, since besides the i.c.v. injected clonidine the selective α_{2A} adrenoceptor agonist oxymetazoline also decreased the gastric acid secretion in a dose-dependent manner. The ED_{50} value was 20 nmol/rat for antisecretory action of i.c.v. injected clonidine and this was far higher (133 times higher) than the gastroprotective one (ED_{50}:0.14 nmol/rat, i.c.v.). The receptorial mechanisms were confirmed by the result that yohimbine antagonized the antisecretory action of clonidine and oxymetazoline.

5.1.4 Study of the interaction between α_2 adrenergic system and opioid system in the regulation of gastric emptying, gastric motility and gastric acid secretion

Several studies suggested that there were interactions between opioid and α_2 adrenergic systems. For example, clonidine is known to suppress opioid withdrawal symptoms in rats, and opioid antagonists inhibited the central α_2 adrenoceptor induced gastroprotection and antisectretory action [82;157]. Moreover the antinociceptive and antihypertensive effects of clonidine and α-methyl-DOPA were antagonized by naloxone, as well [86;158]. In addition, the gastroprotection induced by i.c.v. injected clonidine was proposed to be mediated by the release of β-endorphine [159]. Our present results confirmed the interaction between the two systems in the regulation of both gastroprotective and antisecretory actions induced by α_2 adrenoceptor stimulants. We wondered whether opioid component might be involved also in the mechanism of gastric emptying and gastric motility. In order to answer this question, first we examined the effect of different opioid receptor stimulants on gastric emptying and the effect of opioid receptor antagonist on gastric emptying delaying action of clonidine.

The effect of opioids on gastric emptying and gastric motility has intensively been studied. Both peripheral and central mechanisms may be responsible for the inhibitory effect of peripherally administered morphine on gastric emptying since this compound is known to cross the blood-brain barrier. Peripherally administered morphine was found to strongly inhibit both the gastric emptying and the intestinal transit in rats [119]. Opioid peptides and alkaloids were demonstrated to decrease the intestinal transit in mammalian species by changing the coordinated reflex motor activity into segmentation of intestine [89;105]. In the ENS functional and pharmacological studies on guinea pigs were proposed that the effects of opioids on intestinal motility were mainly mediated by μ and

κ opioid receptor types [89;103]. However, a central site of action was also suggested by studies. For example in sheep the inhibition of gastric contractions evoked by s.c. administered morphine was not antagonized by methyl-naloxone, a peripheral antagonist, while naloxone prevented it [148]. Several studies suggested that activation of central μ opioid receptors affected gastric motor activity and inhibited the small intestinal transit [149-151]. Improta and coworkers [112] found that stimulation of central μ opioid receptors inhibited the gastric emptying in rats and they concluded that δ opioid system was not involved in the regulation of this function [112]. It was also suggested that central inhibition of GI transit might be due to activation of central μ but not δ or κ opioid receptors [152].

Firstly we studied the effect of opioid receptor stimulants on gastric emptying. Morphine given both peripherally (26.4-105.5 μmol/kg s.c.) and centrally (26.46-529 nmol/rat i.c.v.) inhibited the gastric emptying. Similarly, the selective μ opioid receptor agonist DAGO delayed the gastric emptying of phenol red solution after i.c.v. administration suggesting the involvement of central μ opioid receptors in inhibition of gastric emptying. The effective doses (ED_{30}:104.4 nmol/kg i.c.v.) were far higher than the doses inducing mucosal protective processes (ED_{50}:0.045 nmol/kg i.c.v.) indicating that the protective doses did not affect the gastric emptying. Receptorial mechanism was confirmed by the fact that naloxone, a non-selective antagonist of the opioid receptors, reversed the inhibitory effect.

Our data suggest that inhibition of gastric emptying may also be mediated by central δ opioid receptors, since deltorphine II, a selective ligand of δ opioid receptors, significantly inhibited the gastric emptying. On the other hand, it was reported that the highly selective δ opioid receptor agonist SNC 80 inhibited the GI motility via a central mechanism, which was reversed by a μ opioid receptor antagonist [153]. Therefore authors suggested that a μ-δ opioid receptor interaction might be responsible for the inhibitory action of SNC 80. Consequently, further experiments are needed to analyse the involvement of central δ opioid receptors in the mediation of gastric emptying.

Several data indicate that the effects of morphine on the GI motor activity were different from that of α_2 agonists. Namely, morphine was found to inhibit the gastric emptying and intestinal transit in a similar degree [119]. Opioids were reported to inhibit both basal and stimulated gastric motility and strongly increased the pyloric contractions

[119;154-156]. In contrast, the α_2 adrenoceptor stimulants were found to strongly inhibit the intestinal transit whereas only slightly affected the gastric emptying. They did not influence or just slightly stimulated the basal gastric tone and they also decreased not only the antral but the pyloric contractions [119].

Finally, our present experiments failed to confirm an interaction between the α_2 adrenergic and opioid systems in the regulation of gastric emptying, since naloxone, a non-selective opioid receptor antagonist, failed to influence the effect of clonidine. This result is in agreement with the observation of Asai and coworkers, who found no significant relationship between the two systems [119]. Consequently, opioids and α_2 agonists affect GI motility and by different regulatory mechanisms.

Tabl. 5. Comparisons between the gastroprotective and gastric emptying delaying doses of clonidine, oxymethazoline and DAGO. Data originate from a previous study [164] and from the present study. To make i.c.v. values comparable to s.c. ones data were converted to nmol/kg calculating with rats weighing 150g.

	clonidine (nmol/kg)		oxymetazoline (nmol/kg)		DAGO (nmol/kg)
	peripheral(s.c.)	central(i.c.v.)	peripheral(s.c.)	central(i.c.v.)	central(i.c.v.)
$ED_{50\ gastroprotection}$	47.96 [164]	0.93	>1680 [164]	ineffective[164]	0.045
$ED_{30\ gastric\ emptying}$	540	198.9	810	52.87	104.4
$ED_{30\ gastric\ empt.}$ / $ED_{50\ gastroprot.}$	11.26	212.4	< 0.48		2303

Tabl. 6. Comparisons between the adrenergic regulatory mechanisms of gastric motor functions, gastroprotection, and gastric acid secretion in rats. Results originate from previous studies [138, 163] and from the present study.

	Gastroprotection	Gastric acid secretion	Gastric emptying	Gastric motility
α_2 adrenoceptor subtype	α_{2B} [138]	α_{2A} [163]	α_{2A}	α_{2A}
opioid component	+	+	–	–

5.1.5 Is there any correlation between the inhibition of gastric motility, gastric acid secretion and gastric mucosal protective effect of clonidine and DAGO?

The main purpose of this study was to determine if changes of gastric motility might have any role in the gastroprotective action induced by α_2 adrenoceptor stimulants and if their protective doses influenced the gastric emptying. Our present data and previous results based on the data of the literature are summarized in the Tables 4.-6.. In order to make i.c.v. values comparable to s.c. ones data were converted to nmol/kg calculating with rats weighing 150 g. According to these results we found several

differences between the effects of α_2 adrenoceptor agonists on gastric emptying, gastric motility and gastric acid secretion.

Firstly analysing the data, it can be observed that the gastroprotective doses of s.c. and i.c.v. injected α_2 adrenoceptor stimulants are far below the doses needed to affect gastric emptying (Tabl. 5.). In addition, the gastric motor activity was found not to be influenced by their i.v. doses lower than 380 nmol/kg (Fig. 29), whereas their gastroprotective doses were found to be in the range of 30-90 nmol/kg s.c. [164]. These results indicate that the gastroprotective doses of α_2 adrenoceptor stimulants do not inhibit the gastric emptying and gastric motility. Thus the inhibition of the gastric contractions may not account for their gastroprotective effect.

In the present experiments it can be observed that the gastroprotective effect of p.o. administered clonidine on ethanol ulcer has been decreased above the dose of 0.38 μmol/kg. This dose is just in the range that already influences the gastric motor activity (0.19-3.76 μmol/kg s.c.). This coincidence raises the possibility that larger doses of clonidine may inhibit the emptying of acidified ethanol therefore the gastric mucosa may longer be exposed to deleterious effects of ethanol which may lead to the decrease of protective effect.

Further difference is that NO, which is known to be an important mediator in the gastric relaxation processes, is not likely to be involved in the mechanism of the delay of gastric emptying induced by clonidine. On the contrary NO was found to play a role in the mediation of gastroprotection induced by centrally injected clonidine [160].

The peripherally administered clonidine may act on both peripheral and central sites since clonidine is known to cross the blood-brain barrier. Thus we compared the central and peripheral effective doses of clonidine as well as oxymetazoline on gastric emptying (Tabl. 4.). Based on this comparison clonidine and oxymethazoline were found to equipotently act on peripheral and central sites, whereas in the mucosal protective processes a dominant role of central α_{2B} adrenoceptors was suggested [138].

We also observed other differences between the mechanism of gastroprotection and the regulatory mechanism of gastric motility and emptying. Firstly, in the gastroprotection the α_{2B} subtype was proposed to be involved [138] while in the mediation of gastric motility and emptying the α_{2A} subtype seemed to play a role. Secondly, the gastroprotection proved to be vagal-dependent, whereas the centrally

induced inhibition of gastric emptying was not possible to abolish by bilateral cervical vagotomy [159]. In addition, in the mechanism of gastroprotection induced by the α_2 adrenoceptor agonist clonidine, interaction was found between α_2 adrenergic and opioid systems but the effect of clonidine on gastric motility and emptying did not appear to involve opioid component (Tabl. 6.).

According to earlier [159] and present experiments it was found that i.c.v. injected clonidine and oxymetazoline influenced the gastric emptying (ED_{30}: 29.84 nmol/rat i.c.v) and the gastric acid secretion (ED_{50}: 20 nmol/rat i.c.v) at a near similar dose range. In both gastric functions the α_{2A} subtypes might be involved. Therefore similarly to gastric motor decreasing effects, the inhibition of gastric acid secretion can not account for the gastroprotective action of the α_2 adrenoceptor stimulant clonidine either, since different α_2 adrenoceptor subtypes may mediate the effects and the antisecretory doses of clonidine are lower than the gastroprotective ones.

Based on our data it can be concluded that the gastroprotection induced by clonidine does not relate to the changes in gastric motor activity and gastric acid secretion, because the protective dose of clonidine has affected neither of them. Although the reduction of mucosal protective effect of clonidine at larger doses can be partly due to the delay of gastric emptying. Furthermore differences have been found in the regulatory mechanisms of these gastric functions.

Regarding the role of endogenous opioids in the mediation of gastric emptying our results show that the gastroprotective doses of the μ opioid recetor agonists do not affect the gastric emptying (Tabl. 5.).

6 CONCLUSIONS

α_2 adrenoceptors:

Our present study indicates the involvement of α_2 adrenergic system in the inhibition of gastric motor functions (gastric emptying and gastric motility), gastric mucosal damage and gastric acid secretion.

Based on our results it can be concluded that no correlation is likely to be between the gastroprotective effect and the inhibitory action on gastric motility and gastric acid secretion of clonidine, because

- the gastroprotective dose of clonidine is much lower than the doses needed to suppress gastric contractility, gastric emptying and gastric acid secretion, consequently, clonidine in gastroprotective doses does not influence other gastrointestinal functions. Delay of gastric emptying induced by higher doses of clonidine might contribute to the decreased gastroprotective effect of clonidine observed in this dose range,
- different α_2 adrenoceptor subtypes may mediate the gastroprotection and inhibition of gastric motility/gastric acid secretion, namely, α_{2B} adrenoceptor subtype may be responsible for the mucosal protective mechanisms, while α_{2A} adrenoceptor subtype may mediate the inhibition of gastric motility and gastric acid secretion,
- the mucosal protective processes may involve endogenous opioids but the inhibition of gastric motility induced by clonidine may not.

It can also be concluded, that

- NO is not likely to be involved in the mechanism of the inhibition of gastric emptying induced by α_2 adrenoceptor stimulation,
- a vagus-independent mechanism may mediate the inhibition of gastric emptying induced by activation of central α_2 adrenoceptors.

Opioid receptors:

- Inhibition of gastric emptying can be induced by activation of central opioid receptors; both μ and δ receptors are likely to be involved in the inhibitory action,
- The gastroprotective doses of μ opioid receptor stimulants may not influence the gastric emptying.

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RELEVANT PUBLICATIONS:

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