Gastric mucosal integrity and gastric motility

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

Ph. D. thesis

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1 INTRODUCTION

Maintenance of gastric mucosal integrity depends on several factors, e.g. mucosal microcirculation, mucosal barrier, production of gastric mucus, and other 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. Previous studies described both deleterious and beneficial effects of gastric emptying. Thus its elevation was assumed to be gastroprotective on aspirin-induced gastric damage while its delay caused by large doses of morphine was proposed to aggravate the ethanol-induced gastric lesions.

According to the literature brain-gut peptides - like TRH, adrenomedullin, amylin, neuropeptide Y - produced gastric mucosal defense given centrally. Moreover, opioid peptides are also likely to be involved in maintenance of gastric mucosal integrity and in regulation of various gastric functions such as gastric motor activity and gastric acid secretion. Furthermore, α_2-adrenoceptors were also reported to influence gastric mucosal integrity and different gastric functions.

2 AIMS OF THE STUDY

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:

1. 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,
2. 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,
3. 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.

4. 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.

3 MATERIALS AND METHODS

3.1 Animals:

Male Wistar rats weighing 150-170 g (gastric emptying, gastroprotection, gastric acid secretion) and 260-280 g (gastric motility) were used. 24 hours before experiments food was deprived, but free tap water was available.

3.2 Ethanol-induced gastric lesions:

Gastric lesions were produced by acidified ethanol. Rats (5-7 rats per group) were fasted for 24 hours before experiments but free access to tap water was allowed. 0.5 ml of acidified ethanol (98% ethanol in 200 mmol/l HCl) was given to the animals orally. One hour later the animals were sacrificed by overdosing aether. Then the gastric lesions were examined, the total number of mucosal lesions was carried out in blinded manner by calculation of Ulcer Index based on a previously described method. 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. Compounds were injected s.c. and i.c.v., 20 and 10 min before ethanol, respectively. The percentual inhibition of mucosal damage was calculated according to the following formula:

100 – (ulcer index in treated group / ulcer index in control group x100).

3.3 Measurement of gastric acid secretion:

Experiments were performed according to the method described by Shay and coworkers in conscious rats (7 rats/group). The pylori were ligated under light aether anaesthesia. 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 and s.c. 20 min after the pylorus ligation.

3.4 Determination of gastric emptying:

Experiments were performed in concious rats (5 rats per group) according to the phenol red content assay described previously. 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 aether. 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. When agonists were given peripherally they were injected s.c. and i.v. 20 and 15 min before test meal, respectively. Antagonists were administered i.c.v. and s.c., 10 and 20 min prior to the administration of agonist, respectively. Control groups were treated with saline.

3.5 Determination of gastric motility:

The gastric motility was determined by the previously described balloon-method. According to this technique it becomes possible to study separately the fundic and antral movements. The registered intragastric pressure mainly originates from the fundic tone while the phasic contractions characterize the movements of the antrum. Studies were carried out using 3-6 rats per group. Experiments were performed under urethane anaesthesia (1.25 g/kg i.p.).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. Gastric motor activity was stimulated by 2-DG (300 mg/kg i.v.). The increase of gastric contractions and gastric tone become stable 25-30 min after the 2-DG injection; the test substances were administered after the stabilisation of increased gastric motor activity, and the antagonists were injected 10-15 min later – when the agonists reached their peak effects. 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 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.

3.6 Bilateral cervical vagotomy:

The cervical section of the vagus nerves was exposed under ether anaestesia, 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.

3.7 Drugs:

α_2 adrenoceptor agonist	clonidine hydrocloride,
α_{2A} adrenoceptor agonist	oxymetazoline,
non-selective α_2 adrenoceptor antagonist	yohimbine hydrocloride,
α_{2B} adrenoceptor antagonist	prazosin hydrocloride,
α_{2B} adrenoceptor antagonist	2-[2-(4-(O-methoxyphenyl)piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2H,4H)-isoquinolindione dihydrocloride (ARC-239),
opioid receptor agonist	morphine hydrocloride,
selective μ opioid receptor agonist	[D-Ala^2,MePhe^4, Gly^5-ol]-enkephalin (DAGO),
selective δ opioid receptor agonist	deltorphin II,
non-selective opioid receptor antagonist	naloxone hydrocloride,
NO synthase inhibitor	N^G-nitro-L-arginine (L-NNA),
central vagal stimulant	2-deoxy-D-glucose (2-DG),
short acting anaesthetic	aether,
long acting anaesthetic	urethane.

Compounds were dissolved in saline in a volume of 5 ml/kg for imp., s.c., i.v. administration. In gastric motility experiments the volume of iv. injection was 2 ml/kg body weight. In the case of i.c. and i.c.v. injection volumes were 5 μl and 10 μl, respectively. Control groups were treated with the solvent.

3.8 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 measurement of gastric acid secretion differences were evaluated by Student's t-test or ANOVA followed by Tukey test for multiple comparisons. Differences were considered significant at $p<0.05$.

4 RESULTS

4.1. The effect of α_2 adrenoceptor agonist and opioids on gastric emptying

According to our results gastric emptying was inhibited by both the non-selective α_2 adrenoceptor agonist clonidine and the selective α_{2A} adrenoceptor agonist oxymetazoline after peripheral administration showing a potential role of α_2 adrenoceptors in the regulation of gastric emptying. The ED_{30} values were 0.54 μmol/kg for s.c. injected clonidine and 0.81 μmol/kg for the s.c. administered oxymetazoline. The α_2 antagonist yohimbine (12.82 μmol/kg, s.c.), but not the $\alpha_{2B/2C}$ adrenoceptor antagonist prazosin (1.19 μmol/kg, s.c.) and ARC-239 (0.68 μmol/kg, s.c.) inhibited the gastric emptying delaying effect of clonidine (3.76 μmol/kg, s.c.) indicating a dominant role of the α_{2A} adrenoceptor subtype in the mediation of gastric emptying.

We also aimed to explore if NO played a role in the gastric emptying delay induced by clonidine, therefore we examine how the NO synthase inhibitor N^G-nitro-L-arginine (91.3 μmol/kg i.v.) affected its inhibitory action. This compound itself slightly decreased the gastric emptying and failed to influence the effect of clonidine (3.76 μmol/kg, s.c.).

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 (2.75 μmol/kg, s.c.) on the gastric emptying delaying action of clonidine (3.76 μmol/kg, s.c.). As our results show naloxone failed to affect it.

Furthermore we also studied the central effects of α_2 adrenoceptor agonists. Gastric emptying was dose-dependently inhibited by the centrally given clonidine and oxymetazoline. The ED_{30} values were 29.84 nmol/rat for i.c.v. injected clonidine and 7.93 nmol/rat for the i.c.v. administered oxymetazoline. In order to determine how the centrally induced gastric emptying delaying action of clonidine conveys to the periphery, we examined the role of vagal nerve in the effect. Bilateral cervical vagotomy did not influence the inhibitory effect of i.c.v. clonidine (37.6 nmol/rat, i.c.v.) .

In another experimental series we investigated the role of central opioid receptors in the mediation of gastric emptying. The i.c.v. injected, μ opioid receptor agonist

morphine and DAGO dose-dependently delayed the gastric emptying of our test meal. The ED_{30} values were 118 nmol/rat for i.c.v. injected morphine and 15.7 nmol/rat for the i.c.v. administered DAGO. The effects of morphine (529 nmol/rat, i.c.v.) and DAGO (19.5 nmol/rat, i.c.v.) were antagonized by naloxone in an s.c. dose of 2.75 μmol/kg. In our experiments the i.c.v. given δ opioid receptor agonist deltorphin II also inhibited the gastric emptying in a dose of 56.7 nmol/rat.

4.2. The effect of α_2 adrenoceptor agonist on gastric motor activity

In a further set of experiments we studied the gastric motility by the use of an intragastric balloon technique. Using this method it is possible to separate two different movements of the stomach: the fundic tonic contraction and antral phasic contractions. As our results show clonidine in the dose of 0.75 μmol/kg i.v. inhibited the stimulated gastric motility such as the gastric tone and the gastric contractions. The inhibitory effect was antagonized by yohimbine (10 μmol/kg, i.v.), but not by the $\alpha_{2B/2C}$ antagonist prazosin (0.23 μmol/kg, i.v.), suggesting the potential role of α_{2A} adrenoceptor subtype in the regulation of gastric motility. The selective α_{2A} adrenoceptor agonist oxymetazoline (0.185-3.4 μmol/kg, i.v.) also inhibited the stimulated gastric motor activity, offering additional evidence for the involvement of α_{2A}-adrenoceptor subtype in the action. However, the effect of oxymetazoline (3.4 μmol/kg, i.v.) was only partially reversed by yohimbine (10 μmol/kg, i.v.). Namely, after administration of yohimbine the reduced gastric phasic contractions were reversed, but the gastric tone was not affected. We also studied the involvement of an opioid component in the mechanism of clonidine induced inhibition on gastric motility, however, naloxone (1.3 μmol/kg, i.v.) failed to influence the gastric emptying inhibitory action of clonidine.

4.3. The effect of α_2 adrenoceptor agonist on gastric acid secretion

In our experiments both clonidine and oxymetazoline decreased the gastric acid secretion after i.c.v. administration. The ED_{30} values were 20 nmol/rat for i.c.v. injected clonidine and 7.5 nmol/rat for the i.c.v. administered oxymetazoline. The centrally induced antisecretory effect of clonidine (23.5 nmol/rat i.c.v.) and oxymetazoline (16.8 nmol/rat i.c.v.) were blocked by yohimbine (51.2 nmol/rat, i.c.v.). Naloxone in the dose

of 50 nmol/rat i.c.v. reduced the antisecretory effect of clonidine (47 nmol/rat i.c.v.) and oxymetazoline (16.8 nmol/rat i.c.v.).

4.4. The effect of α_2 adrenoceptor agonist and opioids on gastric mucosal damage induced by ethanol

We examined the effect of the α_2 adrenoceptor agonist clonidine on the gastric mucosal lesions and it was found that the i.c.v. given clonidine reduced the gastric lesion formation on ethanol ulcer model. The ED_{50} value was 0.14 nmol/rat, i.c.v.. The gastroprotective effect of centrally injected clonidine (0.47 nmol/rat, i.c.v.) on ethanol induced damage was blocked by yohimbine (50 nmol/rat, i.c.v.) and prazosin (5 nmol/rat, i.c.v.). Naloxone (50 nmol/rat, i.c.v.) also reversed the gastroprotection induced by central clonidine, suggesting the presence of an opioid component in the gastroprotective mechanism.

The i.c.v. injected DAGO and deltorphine II also proved to be gastroprotective on ethanol-induced gastric lesions. The ED_{50} values were 0.0068 nmol/rat for i.c.v. injected DAGO and 0.120 nmol/rat for i.c.v. administered deltorphine II.

5 CONCLUSIONS

5.1. 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.

5.1.1. 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 higher 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.

5.1.2. 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.

5.2. 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.

RELEVANT PUBLICATIONS

Papers:

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