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Characterisation of α_2-adrenoceptor subtypes involved in gastric emptying, gastric motility and gastric mucosal defence

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Abstract

The effect of clonidine on ethanol-induced gastric mucosal damage, gastric emptying and gastric motility was compared. The clonidine-induced gastroprotective effect (0.03–0.09 μmol/kg, s.c.) was antagonised by yohimbine (5 μmol/kg, s.c.), prazosin (0.23 μmol/kg; α_{2B}-adrenoceptor antagonist) and naloxone (1.3 μmol/kg, s.c.). Clonidine also inhibited the gastric emptying of liquid meal (0.75–3.75 μmol/kg, s.c.) and gastric motor activity (0.75 μmol/kg, i.v.) stimulated by 2-deoxy-D-glucose (300 mg/kg, i.v.). Inhibition of gastric emptying and motility was reversed by yohimbine (5 and 10 μmol/kg, s.c., respectively), but not by prazosin (0.23 μmol/kg, s.c.) or naloxone (1.3 μmol/kg, s.c.). Oxymetazoline–an α_{2A}-adrenoceptor agonist–inhibited both gastric emptying (0.67–6.8 μmol/kg, s.c.) and motility (0.185–3.4 μmol/kg, i.v.), whereas it failed to affect gastric mucosal lesions. The results indicate that in contrast to the gastroprotective effect, which is mediated by α_{2B}-adrenoceptor subtype, α_{2A}-adrenoceptor subtype may be responsible for inhibition of gastric emptying and motility. However, the site of action (central, peripheral, both) remains to be established.

Keywords: Gastric mucosal damage; Gastric emptying and motility; α_2-Adrenoceptor subtype

1. Introduction

Activation of pre-synaptic α_2 adrenergic receptors has been known to mediate several responses in gastrointestinal (GI) tract. For example clonidine–an α_2-adrenoceptor agonist–was shown to inhibit gastrointestinal transit (Asai et al., 1997a), and colonic motility (Umezawa et al., 2003). α_2-Adrenoceptors are likely to be involved also in regulation of gastric acid secretion (Blandizzi et al., 1988; Cheng et al., 1981; DiJoseph et al., 1987; Kunchandy et al., 1985; Müllner et al., 2001, 2002) and gastric mucosal protection against different types of mucosal damage (Gyires et al., 2000a,b,c). Activation of α_2-adrenoceptors can influence also gastric emptying; however, reports on the effect of α_2-adrenoceptor agonists on gastric emptying are inconclusive, since both inhibition and lack of effect have been described (Asai et al., 1997b; Ruwart et al., 1980; Sninsky et al., 1986; Tanila et al., 1993). The contribution of central α_2-adrenoceptors appears to be significant in the regulation of gastrointestinal functions, since intracerebroventricular administration of clonidine resulted in inhibition of small intestinal motility (Fargeas et al., 1986; Umezawa et al., 2003), decrease of gastric acid secretion (Müllner et al., 2001) and mucosal lesions induced by different irritants (Gyires et al., 2000a,b,c).

Different subtypes of α_2-adrenoceptors have been recognised and designated as α_{2A}, α_{2B}, α_{2C} and α_{2D}. α_{2C}- and α_{2D}-adrenoceptors show pharmacological profile very close to α_{2B}, and α_{2A} (Bylund, 1985, 1992), respectively. It was suggested that α_{2B}-adrenoceptor subtype may mediate the gastroprotective effect (Gyires et al., 2000b), while α_{2A}-like-adrenoceptor subtypes may play a role in the anti-secretory effect of α_2-adrenoceptor stimulants (Blandizzi et al., 1995; Müllner et al., 2001).

Correlation between gastric emptying, gastric motility and gastric ulcer formation has intensively been studied, however, data of the literature are contradictory. Stimulated gastric contractility has been proposed to be one of the aggressive factors in gastric ulcer formation (Okada et al., 1989; Okumura et al., 1995). Increased gastric motility was also assumed to be involved in the ulcerogenic effect of indomethacin (Takeuchi et

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al., 2002), and the protective effect of amylin against indomethacin-induced gastric lesions was supposed to be due to inhibition of increased gastric motility (Guidobono et al., 1997). Gastric mucosal damage induced by ethanol may be due to the contraction of the circular muscle, which can lead to mucosal compression and consequently mucosal necrosis and ulceration (Mersereau et al., 1988). Ethanol was reported to exert dual action on gastric motility depending on the concentration; ethanol at low concentration inhibits the amplitude and frequency of phasic contraction in canine antral smooth muscle (Sanders and Bauer, 1982), whereas high concentration causes tonic contraction of vascular smooth muscle in rats and of gastric smooth muscle in guinea pig (Werber et al., 1997; Zheng et al., 1997). Relaxation of circular muscles may protect the gastric mucosa through flattening the folds (Sanders and Bauer, 1982). Such an action has been postulated to play a role in the cytoprotective effect of prostaglandins (Jafri et al., 2001; Takeuchi and Nobuhara, 1985). Dopamine-induced mucosal protection against acidified ethanol was also supposed to be mediated by inhibition of gastric motor activity (Takeuchi et al., 1988).

On the other hand, delay in gastric emptying was also supposed to play an important role in the mechanism of gastric ulcer formation, and the gastroprotective effect of metoclopramide against aspirin-induced mucosal damage was thought to be mediated partly by acceleration of gastric emptying (Gupta et al., 1989).

Furthermore, Gutierrez-Cabano (1994) suggested that gastric contractile activity is unlikely to play a major role in the development or prevention of gastric lesions induced by necrotizing agents. Likewise, hypermotility is unlikely to serve as a major factor in stress ulceration, and the smooth muscle relaxing effect of atropine and verapamil may contribute only partly to their anti-ulcer effect (Koo et al., 1986).

The aim of the present study was to analyse

- which α_2-adrenoceptor subtype may mediate the inhibition of gastric emptying and gastric motility,
- whether inhibition of gastric motor activity and contraction may contribute to the gastroprotective effect of α_2-adrenoceptor agonists.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 150–170 g (ethanol-ulcer, gastric emptying) and 260–280 g (gastric motility) has been used. The rats were deprived of food for 24 h with free access to tap water. They were housed in wire mesh bottom cages to prevent coprophagy. The rats were kept on a 12 h light–dark cycle and under condition of controlled temperature.

2.2. Gastric mucosal damage induced by acidified ethanol

After 24 h food deprivation the animals were given orally 0.5 ml acidified ethanol (98% ethanol in 200 mmol/l HCl). One hour later the animals were overdosed by ether, the stomachs were excised, opened along the greater curvature, rinsed with saline, and examined for lesions. Total number of mucosal lesions was assessed in blinded manner by calculation of lesion index based on a 0–4 scoring system described previously (Gyires, 1990). The lesion index was calculated as the total number of lesions multiplied by the respective severity factor.

Clonidine and oxymetazoline were injected subcutaneously (s.c.), 30 min before ethanol. The antagonists were given s.c. 30 min before the agonists.

2.3. Measurement of gastric emptying

Gastric emptying was assessed by means of a phenol red content assay in conscious rats (Improta and Broccardo, 1994). Animals received 1.5 ml of 1.5% methylcellulose solution containing 0.5 mg/ml phenol red. After 60 min the rats were overdosed by ether, stomachs were removed, placed in 100 ml of 0.1 N NaOH solution and homogenized for 30 s. 5 ml of homogenate were added to 0.5 ml of 20% trichloroacetic acid solution. After centrifugation (30 min) the supernatant was added to 4 ml of 0.5 N NaOH. The absorbance of the samples was read spectrophotometrically at 560 nm.

Phenol red recovered from the stomach of rats killed immediately after the administration of methylcellulose meal served as standard stomach (A_{standard}).

The test compounds were given s.c. 30 min before the methylcellulose administration. Control groups were treated with saline.

Gastric emptying (GE%) was calculated according to the following formula:

$$\text{GE\%} = 1 - \frac{A_{\text{test60}} - A_{\text{control60}}}{A_{\text{standard}} - A_{\text{control60}}} \times 100$$

A_{test60}: Amount of phenol red extracted from the test stomach 60 min after methylcellulose-phenol red administration. $A_{\text{control60}}$: Amount of phenol red extracted from the control stomach 60 min after methylcellulose-phenol red administration.

2.4. Determination of gastric motility

The method of LeFebvre et al. (1992) was followed. Under urethane anaesthesia (1.25 g/kg) a tracheal cannula was inserted to ensure a clear airway, femoral vein was cannulated with a polyethylene tubing for intravenous (i.v.) administration of the drugs. An intragastric balloon (approximately 10 × 25–30 mm) created from thin latex rubber connected with plastic tubing was introduced into the stomach via mouth. The balloon was filled with 1.6–2 ml warm water (37 °C). This volume range was determined to be the level required to induce an intragastric pressure of 11–12 cmH_2O. The distal end of tubing was connected to a pressure transducer to monitor the intragastric balloon pressure. The exact location of the balloon was verified after each experiment. After 30-min equilibrium period 2-

deoxy-D-glucose (2-DG) was injected intravenously (300 mg/kg) into the femoral vein to enhance gastric contractions and gastric tone. The test compounds (clonidine and oxymetazoline) were given i.v. when the gastric motor activity (intragastric balloon pressure, frequency and amplitude of contractions) became stable (25–30 min after 2-DG administration). The antagonists (yohimbine, prazosin, naloxone) were injected 10–15 min after the agonists (clonidine, oxymetazoline) injection.

2.4.1. Analysis of gastric motor activity

The frequency, amplitude of gastric contractions and intragastric pressure were examined in 6 min blocks. Intragastric pressure correlates well with fundic contractions (Ferreira et al., 2002), which are of tonic type and are likely to be responsible for generating intragastric balloon pressure (Hasler, 1995). Phasic (antral) contractions are superimposed on tonic contractions.

Gastric motor activity was characterised by gastric motility index (MI), which was calculated by dividing the sum of amplitudes of each phasic contraction by the number of contractions over 6 min and was expressed in cmH_2O.

$$\text{Motility index } (cmH_2O) = \frac{\sum \text{Amplitude } (cmH_2O)(6 \text{ min})}{\text{Number of contractions } (6 \text{ min})}.$$

Tonic intragastric pressure was determined from the bottom points of phasic pressure wave (Raybould et al., 1989; Shi et al., 2003). The mean of intragastric pressure (cmH_2O) over 6 min observation period was calculated as follows:

$$\frac{\sum \text{Lowest points of phasic contractions } (cmH_2O)(6 \text{ min})}{\text{Number of contractions } (6 \text{ min})}.$$

The frequency of phasic contractions over a 6 min period was determined and was converted to contractions per minute.

2.5. Drugs

Clonidine, 2-deoxy-D-glucose, naloxone, prazosin, yohimbine (all from Sigma Chemical Co., St. Louis, MO, USA), oxymetazoline (RBI Natick, USA). The drugs were dissolved in saline. Control animals received the drug solvent.

2.6. Statistical analysis

All data are presented as the means±S.E.M. Statistical analysis of the data was evaluated by means of analysis of variance (ANOVA) for repeated measures followed by Newmann–Keuls test for multiple comparison. A probability of $P<0.05$ was considered statistically significant.

3. Results

3.1. The effect of clonidine and oxymetazoline on ethanol-induced gastric mucosal damage

Clonidine inhibited the ethanol-induced gastric mucosal damage in the doses of 0.03–0.09 μmol/kg, s.c. (Fig. 1A). The mucosal protective effect of clonidine was reversed by yohimbine (5 μmol/kg, s.c.; non-selective antagonist of α_2-adrenoceptors), prazosin (0.23 μmol/kg, s.c.; antagonist of α_{2B}-adrenoceptor subtype in addition to its classic α_1-adrenoceptor blocking action) and naloxone (1.3 μmol/kg, s.c., opioid receptor antagonist) (Fig. 1B). Oxymetazoline proved to be ineffective in the doses of 0.042–1.68 μmol/kg, s.c. (Fig. 1C).

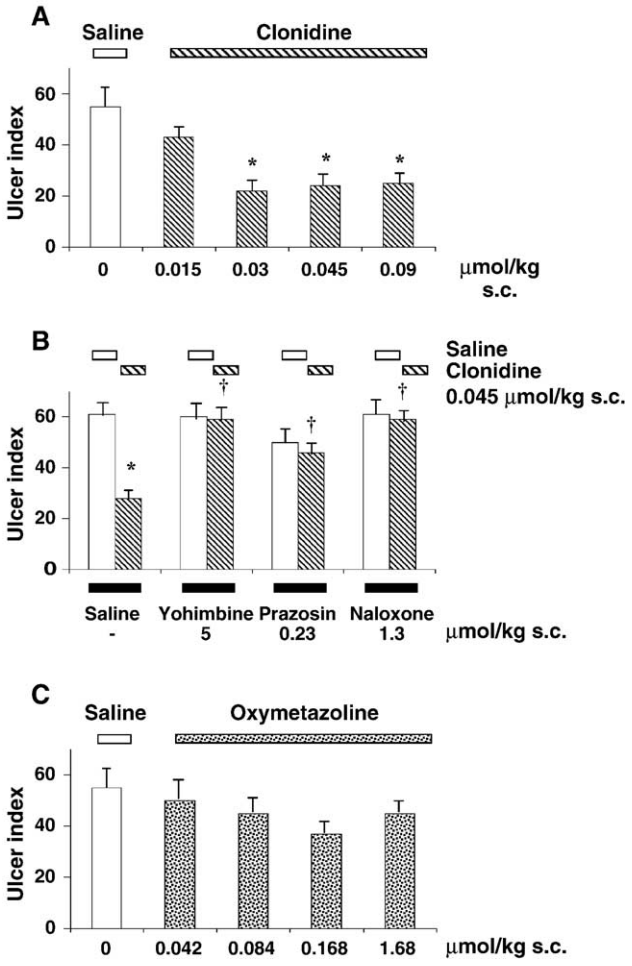

Fig. 1. A: The effect of clonidine on gastric mucosal damage induced by acidified absolute ethanol in the rat. B: The effect of yohimbine (5 μmol/kg, s.c.), prazosin (0.23 μmol/kg, s.c.) and naloxone (1.3 μmol/kg, s.c.) on the gastroprotective effect of clonidine (0.045 μmol/kg, s.c.). C: The effect of oxymetazoline on gastric mucosal damage induced by acidified absolute ethanol in the rat. Values are expressed as means±S.E.M. of 5 rats. *$P<0.05$, compared to column 1; †$P<0.05$, compared to column 2 (ANOVA for repeated measures followed by Newmann–Keuls test for multiple comparison).

3.2. The effect of clonidine and oxymetazoline on gastric emptying

Clonidine (0.19–3.7 μmol/kg, s.c.) exerted a dose-dependent inhibition on gastric emptying (Fig. 2A). The effect of clonidine (3.7 μmol/kg, s.c.) was reversed by yohimbine (5 μmol/kg, s.c.), but not by prazosin (0.23 μmol/kg) or naloxone (1.3 μmol/kg, s.c.) (Fig. 2B). Oxymetazoline also induced a dose-dependent inhibition of gastric emptying (0.17–6.8 μmol/kg, s.c.) (Fig. 2C). Yohimbine (5 μmol/kg, s.c.) reduced the

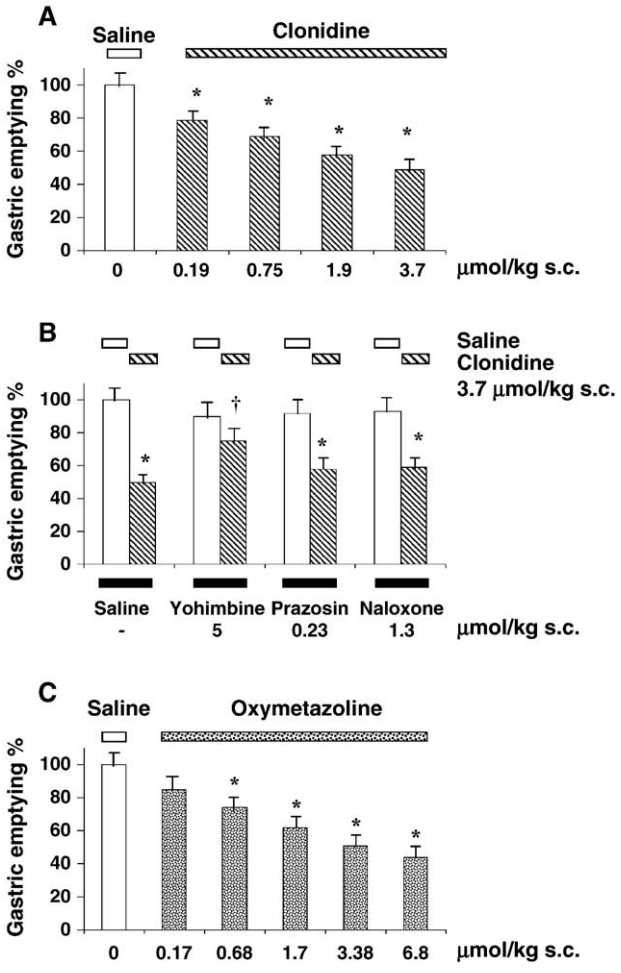

Fig. 2. A: The effect of clonidine on gastric emptying using the phenol red assay in the rat. B: The effect of yohimbine (5 μmol/kg, s.c.), prazosin (0.23 μmol/kg, s.c.) and naloxone (1.3 μmol/kg, s.c.) on the clonidine-induced (3.7 μmol/kg, s.c.) inhibition of gastric emptying. C: The effect of oxymetazoline on gastric emptying using the phenol red assay in the rat. Dates are presented in percent as means±S.E.M. of 5 rats. *$P<0.05$, compared to the respective control group; †$P<0.05$, compared to column 2 (ANOVA for repeated measures followed by Newmann–Keuls test for multiple comparison).

inhibitory effect of oxymetazoline on gastric emptying (not shown).

3.3. The effect of clonidine and oxymetazoline on 2-deoxy-D-glucose-stimulated gastric contraction and motility

Baseline values for intragastric balloon pressure was 11–12 cmH_2O. Neither clonidine (0.75–3.75 μmol/kg, i.v.) nor yohimbine (10 μmol/kg, i.v.) influenced the basal intragastric balloon pressure and the spontaneous gastric motor activity. I. v. injection of 2-deoxy-D-glucose (2-DG) (300 mg/kg) resulted in a rapid increase of intragastric balloon pressure (21.2±2.6 cmH_2O) and gastric motor activity. In most experiments after the rapid increase, a slight decrease of intragastric balloon pressure was observed, and the intragastric balloon pressure was stabilised at this lower value (16±0.6 cmH_2O). Phasic contractions were superimposed on the intragastric balloon pressure. Clonidine failed to influence the 2-DG-stimulated gastric motor activity in the doses of 0.19–0.38 μmol/kg. However, both the increased intragastric balloon pressure, and the stimulated gastric contractions were inhibited by 0.75 μmol/kg, i.v. dose of clonidine (Figs. 3 and 4). The inhibitory effect of clonidine on stimulated gastric motor activity was reduced by yohimbine, but was not affected either by prazosin or naloxone (Fig. 4, Table 1). Oxymetazoline also decreased the 2-DG-induced increased intragastric pressure and gastric contractility in the doses of 0.185–3.4 μmol/kg, i.v. (Table 2, Fig. 5). The inhibitory effect of oxymetazoline however, was only partially antagonised by yohimbine (10 μmol/kg, i.v.); it reversed the reduced phasic activity, but failed to affect the reduced intragastric balloon pressure (Fig. 5, Table 2).

4. Discussion

α_2-Adrenoceptors have physiological role in the regulation of gastrointestinal function. Activation of α_2-adrenoceptors has been reported to inhibit gastric acid secretion (Blandizzi et al., 1988; Cheng et al., 1981; DiJoseph et al., 1987; Kunchandy et

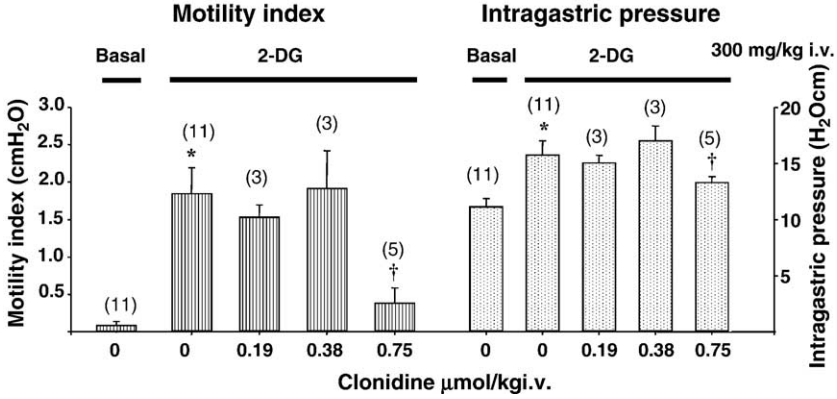

Fig. 3. The effect of different doses of clonidine on 2-deoxy-D-glucose (2-DG) (300 mg/kg)-induced gastric contraction (motility index) and intragastric balloon pressure recorded with latex balloon inserted orally in the stomach. Numbers in brackets at the top of the columns mean the number of experiments. Gastric motor activity was determined under basal condition and over 6 min periods before and after the administration of clonidine. *$P<0.05$, compared to basal values; †$P<0.05$, compared to 2-DG-treated group (ANOVA for repeated measures followed by Newmann–Keuls test for multiple comparison).

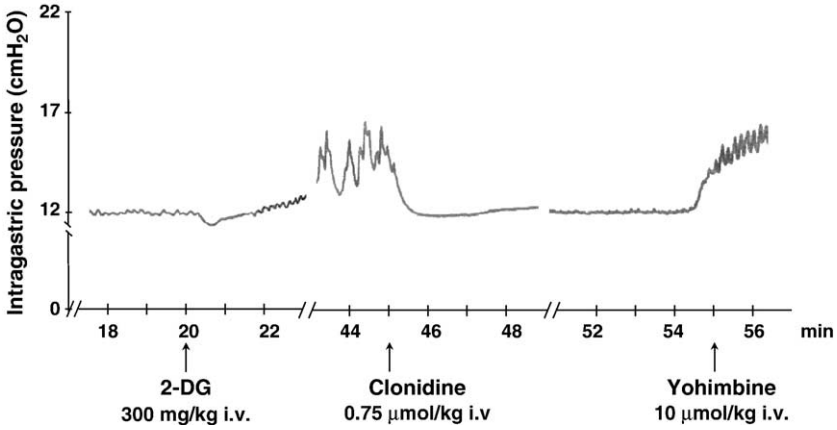

Fig. 4. Representive gastric contractility traces illustrating the effect of clonidine (0.75 μmol/kg, i.v.) on gastric contraction induced by 2-deoxy-D-glucose (2-DG) (300 mg/kg, i.v.). The contractions were recorded with latex balloon inserted orally in the stomach. Clonidine was given 25 min after 2-DG administration. Yohimbine (10 μmol/kg, i.v.) was administered 10 min after the injection of clonidine.

al., 1985), gastric motility and gastrointestinal transit (Asai et al., 1997a). These responses are thought to be mediated through activation of pre-synaptic α_2-adrenoceptors located on the vagus and inhibition of acetylcholine release. Activation of α_2-adrenoceptors results also in decrease of different types of gastric mucosal damage, both in acid dependent and acid independent ulcer models (DiJoseph et al., 1987; Kunchandy et al., 1985).

Most of the evidence for multiple α_2-adrenoceptor subtypes has come from radioligand binding assay. However, there was a rapid accumulation of experimental data on the functional role of different α_2-adrenoceptor subtypes. For example, $\alpha_{2A/D}$- and α_{2B}-adrenoceptor subtypes exert more control over arterial contraction, whereas α_{2C}-adrenoceptors are primarily responsible for venous contraction. Furthermore, α_{2A}-, $\alpha_{2B/2C}$-adrenoceptor subtypes were found to be involved in producing fever in response to bacterial lipopolysaccharide (Bencsics et al., 1995). α_2-Adrenoceptors are involved also in nociception and α_2 non-A subclasses of adrenoceptors were suggested to mediate spinal anti-nociceptive effect (Bencsics et al., 1995; Takano and Yaksh, 1992). On the other hand, decrease of α_{2A}-adrenoceptor immunoreactivity following nerve injury may result in an attenuation of the influence of descending inhibitory noradrenergic input into spinal cord resulting in increased excitatory transmitter release (Bencsics et al., 1995; Stone et al., 1999). Graham et al. (2000) suggested that combination of α_{2A}- and α_{2C}-adrenoceptor agonist may provide a unique and highly effective drug combination for the treatment of pain. Activation of α_{2A}-adrenoceptors plays an important role in mediating the antinociceptive effect of clonidine in the medullary dorsal horn (Wang et al., 2002). Also α_2-adrenoceptor subtype may mediate the antinociceptive effect of amitriptiline and imipramine (Ghelardini et al., 2000). Our previous results suggested that central α_{2B}-adrenoceptors may mediate gastric mucosal protection, since prazosin, antagonist of α_{2B}-adrenoceptor subtype in addition to its classic α_1-adrenoceptor blocking action (Bylund, 1988; Ruffulo et al., 1993; MacKinnon et al., 1994) and ARC-239 (prefers α_2-adrenergic receptor to α_1 more than prazosin; Bylund et al., 1988) given i.c.v. reversed the protective effect of centrally injected clonidine (Gyires et al., 2000b). In the periphery, α_{2A}-adrenoceptor subtype was supposed to be involved in the modulation of vagally stimulated as well as in basal gastric acid secretion in the rat (Blandizzi et al., 1995). Peripheral α_{2B}-adrenoceptors

Table 1
The effectof yohimbine (10 μmol/kg, i.v.), prazosin (0.23 μmol/kg, i.v.) and naloxone (1.3 μmol/kg, i.v.) on the inhibitory effect of clonidine (0.75 μmol/kg, i.v.) on gastric motor activity stimulated by 2-deoxy-D-glucose (2-DG) (300 mg/kg, i.v.) in the rat

Compound	*n*	Motility index mean±S.E.M. (cmH_2O)	Intragastric pressure mean±S.E.M. (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]	14.0±1.1[a]

n: number of rats, a: $P<0.05$, compared to 2-DG treated group; b: $P<0.05$, compared to 2-DG+clonidine treated group.

Table 2
The effect of oxymetazoline on 2-deoxy-D-glucose (2-DG)-stimulated gastric motor activity in the rat

Compound	*n*	Motility index mean±S.E.M (cmH_2O)	Intragastric pressure mean±S.E.M. (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)+oxymetazoline			
0.185 μmol/kg, i.v.	3	1.7±0.16[b]	14.1±3
1.85 μmol/kg, i.v.	4	0.2±0.01[b]	10.8±2[b]
3.4 μmol/kg, i.v.	5	0.1±0.001[b]	11.1±2[b]
2-DG+oxymetazoline 3.4 μmol/kg, i.v.+yohimbine 10.0 μmol/kg, i.v.	3	1.7±0.1[c]	11.2±1.1

n: number of rats, a: $P<0.05$, compared to basal value (first line); b: $P<0.05$, compared to 2-DG-group; c: $P<0.05$, compared to 2-DG+oxymetazoline (3.4 μmol/kg)-group.

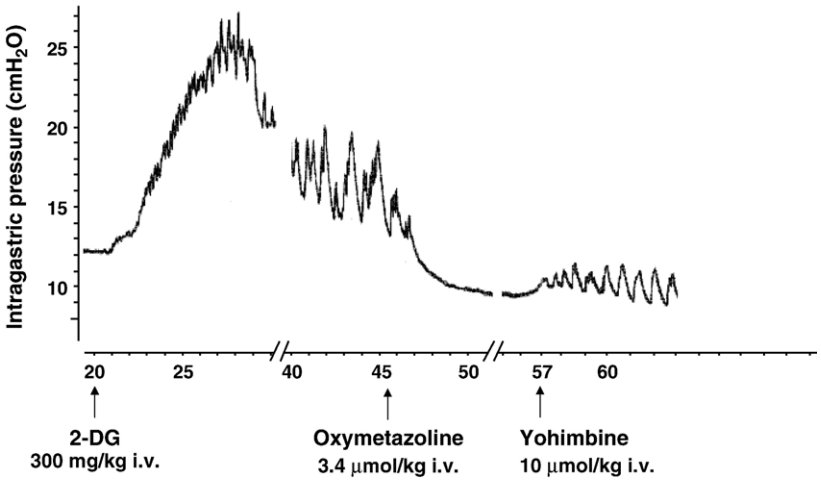

Fig. 5. Representive gastric contractility traces illustrating the effect of oxymetazoline (3.4 μmol/kg, i.v.) on gastric contractions induced by 2-deoxy-D-glucose (2-DG) (300 mg/kg, i.v.). The contractions were recorded with latex balloon inserted orally in the stomach. Oxymetazoline was given 25 min after 2-DG administration. Yohimbine (10 μmol/kg, i.v.) was administered 10 min after the injection of oxymetazoline.

may be responsible for the increased free water clearance, while α_{2A}-adrenoceptor subtypes may mediate the enhanced osmolar clearance induced by clonidine (Intengan and Smyth, 1996).

Our present data confirmed the involvement of $\alpha_{2B/2C}$-adrenoceptor subtypes in gastric mucosal protection, since (i) prazosin (s.c.) antagonised the gastroprotective effect of clonidine, (ii) the α_{2A}-adrenoceptor selective agonist oxymetazoline proved to be ineffective against the ethanol-induced gastric mucosal damage.

Furthermore, subtype selectivity was analyzed also in α_2-adrenoceptor-induced inhibition of gastric emptying. Our results showed that clonidine (0.9–3–7 μmol/kg, s.c.) inhibited gastric emptying. Our findings are in agreement with results of Asai et al. (1997a), who found that though the inhibitory effect of clonidine on gastric emptying was statistically significant, the degree of inhibition was not very pronounced. Others (Cooper and McRitchie, 1985) reported that clonidine induced a strong inhibition on gastric emptying in contrast with the findings of Ruwart et al. (1980), who found that clonidine failed to affect gastric emptying. The discrepancy between studies might be due to the differences in the volume of test liquids used (5 ml vs. 0.25 ml). Our data suggest that the inhibition of gastric emptying induced by activation of α_2-adrenoceptors is likely to be mediated by α_{2A}-adrenoceptor subtype, since prazosin failed to affect the inhibitory effect of clonidine on gastric emptying in the same dose range that has been used for verification of α_{2B}-adrenoceptor subtype-mediated effects of clonidine (Intengan and Smyth, 1996), and that antagonised the gastroprotective effect of clonidine (see above). In addition, the α_{2A}-adrenoceptor subtype selective agonist oxymetazoline exerted a pronounced inhibition on gastric emptying.

Though the rate of gastric emptying is supposed to linearly correlate with intragastric pressure, Tanaka et al. (1998) found no parallelism between gastric emptying and gastric motor activity. They observed that cisaprid increased gastric motor activity, whereas delayed gastric emptying. Our results suggested that clonidine in the doses that delayed gastric emptying in conscious rats did not influence the basal tone and motor activity of the stomach in urethan anesthetised animals (it also should be kept in mind that urethan itself may inhibit gastric motor activity by increasing blood glucose level as it was suggested by Takeuchi et al., 1994 and Ferreira et al., 2001). On the other hand, clonidine inhibited the 2-DG-induced increased gastric motor activity and gastric tone in urethan anesthetised rats (0.75 μmol/kg, i.v.). 2-DG stimulates gastric motor activity by its central vagal stimulant effect. It acts in the brain, especially in the hypothalamus to increase vagal tone (Kadekaro et al., 1975). Moreover, 2-DG was suggested to exert its vagal stimulatory action through activating medullary thyreotrop releasing hormone (TRH) release (Okumura et al., 1995) and there is neuroanatomical, electrophysiological and functional evidence that TRH plays a physiological role in vagal activation. Others showed that 2-DG acts as a metabolic blocker of glucose utilization into the cells (Horton et al., 1973) and stimulates the glycoprivic receptors in the lateral hypothalamic area (Tache, 1988).

The inhibitory effect of clonidine on the 2-DG-induced increased intragastric pressure and motor activity was reversed by yohimbine indicating that the effect was mediated by α_2-adrenoceptors. In contrast, prazosin failed to affect the inhibitory action of clonidine suggesting the involvement of non-B-adrenoceptor subtype. Since the α_{2A} subtype selective-adrenoceptor agonist oxymetazoline also exerted a pronounced inhibition on gastric contractions and tone induced by 2-DG, it may be concluded that α_{2A}-like-adrenoceptor subtypes may mediate the inhibition of gastric motor activity induced by activation of α_2-adrenoceptors. However, since oxymetazoline-induced inhibition of gastric motor function was only weakly affected by yohimbine, beside activation of alpha-2-adrenoceptors other mechanism(s) may also be involved in the inhibitory effect of oxymetazoline. This assumption is supported by the findings of Patil and Ishikawa (2004) who demonstrated atropine-like effect of oxymetazoline.

The above data suggest that the inhibitory effect of clonidine on 2-DG induced increased gastric motor activity may be due to the activation of pre-synaptic α_2-adrenoceptors (particularly α_{2A}-adrenoceptor subtype) located on the vagus resulting in reduction of acetylcholine release from cholinergic nerve terminal. However, further studies are needed to clarify the potential role of central α_2-adrenoceptors in inhibition of increased gastric motor activity induced by central vagal activation.

Several lines of evidence suggest an opioid link in α_2-adrenoceptor mediated responses. For example, clonidine blocks acute opiate withdrawal syndrome (Gold et al., 1978); the antihypertensive effects of clonidine or α-methyldopa were inhibited by naloxone or naltrexone and involvement of α-endorphin and dynorphin in the central hypotensive effect was suggested (Farsang and Kunos, 1979; Van Giersbergen et al., 1989). Also, the analgesic action of clonidine is likely to be due to the release of enkephalin-like substances (Nakamura and Ferreira, 1988). Our previous results suggest the involvement of opioid component in the centrally initiated gastroprotective effect of clonidine, since naloxone and β-endorphine anti-serum (injected intracisternally) reversed the gastroprotective effect of clonidine (given intracerebroventricularly) (Gyires et al., 2000a). Similarly, the anti-secretory effect of clonidine may also involve opioid component (Müllner et al., 2001), since naloxone exerted antagonistic effect. We wondered if opioid system may play a role also in the clonidine-induced inhibition of gastric emptying and gastric motor activity. Though Asai and Power (1999) reported that naloxone inhibited gastric emptying of liquids, particularly in higher doses (1 mg/kg, i.p.), we found that naloxone (0.5 mg/kg, s.c.) did not affect either the gastric emptying or the inhibitory action of clonidine on gastric emptying and increased gastric motility, indicating that opioid system may not be involved in the clonidine-induced inhibition of gastric motor functions.

It can be concluded that inhibition of gastric motor activity is not likely to contribute to the gastroprotective effect, since

(i) the mucosal protective dose of clonidine is much lower than the doses needed to decrease gastric motor activity (0.03–0.06 vs. 0.75–1.5 μmol/kg),
(ii) different-adrenoceptor subtypes are likely to mediate the gastroprotective effect and the inhibitory action on gastric motor activity and emptying,
(iii) endogenous opioids may contribute to the mucosal protective effect, but not to the inhibitory effect of clonidine on gastric motor activity and emptying.

In summary, our present results suggest involvement of different α_2-adrenoceptor subtypes in inhibition of gastric mucosal lesion and gastric motor functions; while α_{2B}-adrenoceptor subtypes may mediate mucosal defence, α_{2A}-adrenoceptor subtypes may be responsible for the inhibition of both gastric emptying and increased gastric motor activity. While previous studies suggested the dominant role of central α_2 (α_{2B}) adrenoceptors in the gastroprotective effect (Gyires et al., 2000b), further experiments are needed to elucidate the role of central adrenoceptors (and subtypes) in regulation of gastric emptying and motility.

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