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Short communication

Functional evidence that gastroprotection can be induced by activation of central α_{2B}-adrenoceptor subtypes in the rat

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Abstract

Clonidine injected intracerebroventricularly (i.c.v.) (0.47 nmol/rat) exerted gastric mucosal protective effect against acidified ethanol. Evidence was obtained that the gastroprotective effect of clonidine was blocked by i.c.v. injected α_2-adrenoceptor antagonists yohimbine (non-subtype selective antagonist), prazosin and 2-[2-(4-(*O*-methoxyphenyl)piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2H,4H)-isoquinolindione (ARC-239) (representative $\alpha_{2B/2C}$-adrenoceptor blocking agents) and opioid receptor antagonists naloxone (a non-selective, moderately μ-opioid receptor preferring antagonist), naltrindole and naltriben δ-opioid receptor antagonists). The centrally injected naltrindole (0.5 nmol/rat) antagonised also the gastroprotective effect of clonidine — but not that of the δ-agonist [D-Ala^2, D-Leu^5]enkephalin — administered peripherally. The results suggest that central $\alpha_{2B/2C}$-adrenoceptor subtypes and opioid — particularly δ — receptors are likely to be involved in the gastric mucosal protective effect of clonidine. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Gastroprotection; Clonidine; α_{2B}-Adrenoceptor; Opioid receptor

1. Introduction

Presynaptic α_2-adrenoceptors mediate several responses in gastrointestinal tract. They are involved in the regulation of gastric acid secretion and activation of α_2-adrenoceptors may result in gastric protection against different types of mucosal damage (DiJoseph et al., 1987; Blandizzi et al., 1995; Gyires, 1997).

The antisecretory effect of α_2-adrenoceptor stimulants may be mediated by both peripheral and central α_2-adrenoceptors (Cheng et al., 1981; Pascaud et al., 1983). Recently, it was demonstrated that gastroprotection can also be initiated by the activation of central α_2-adrenoceptors (Gyires et al., to be published).

The aim of the present study was partly to analyse which α_2-adrenoceptor subtypes might be involved in the central gastroprotective effect of clonidine. Namely, different subtypes of α_2-adrenoceptors have been recognised, named α_{2A}, α_{2B}, α_{2C} and α_{2D}; α_{2C}- and α_{2D}-adrenoceptors show pharmacological profiles very close to those of α_{2B} and α_{2A} binding sites, respectively. α_{2A}-Receptors display high affinity to oxymethazoline and low affinity to prazosin and ARC-239, while converse selectivity for these drugs are shown by α_{2B}- and α_{2C}-receptor subtypes (Bylund, 1992; Bylund et al., 1988).

Our previous results showed that α_{2B}-adrenoceptor subtype is likely to be involved in the gastroprotective effect of α_2-adrenoceptor stimulants following peripheral administration (Gyires, 1997). On the other hand, it is well known that several responses induced by clonidine involve opioidergic mechanism (Farsang et al., 1980), therefore, the possible involvement of central opioid system in the mucosal defence induced by clonidine has also been analysed.

Gastric mucosal damage was induced by acidified ethanol (acid-independent ulcer model) in the rat. The effect of intracerebroventricularly (i.c.v.) injected antagonists of α_2-adrenoceptors (yohimbine, prazosin, ARC 239) and opioid receptors (naloxone, a non-selective, moderately μ-opioid receptor preferring antagonist; naltrindole, δ-opioid receptor antagonist; and naltriben, selective δ_2-opioid receptor antagonist) on gastroprotective effect of clonidine has been analysed.

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2. Materials and methods

2.1. Gastric mucosal lesions induced by acidified ethanol

Experiments were performed in male Wistar rats weighing 140–160 g (Charles River, Hungary). After 24 h of food deprivation, the animals were given 0.5 ml of acidified ethanol (98% ethanol in 200 mmol/l HCl) orally. An hour later, the animals were killed by overdose of ether, the stomachs were excised and examined for lesions. The compounds were injected orally (p.o.) 40 min, subcutaneously (s.c.) 30 min and i.c.v. 10 min before the oral administration of acidified ethanol. The i.c.v. injection to the lateral ventricle was performed according to Noble et al. (1967) in conscious rats. The site of i.c.v. injection was 2 mm from either side of the midline on the line drawn through the anterior base of the ears. The volume of i.c.v. injection was 0.01 ml. The antagonists were given i.c.v. 10 min before the central injection, and 10 and 20 min after the s.c. and oral administration of the substances respectively. The mucosal lesions were examined by an observer who was unaware of the treatment the rats received. The gross mucosal damage was assessed by calculating a lesion index based on the number and length of haemorrhagic mucosal necrosis, as described previously (Gyires, 1990).

2.2. Drugs

Clonidine HCl, naloxone HCl, naltrindole HCl, prazosin HCl, yohimbine HCl (all from Sigma Chemical, St. Louis,

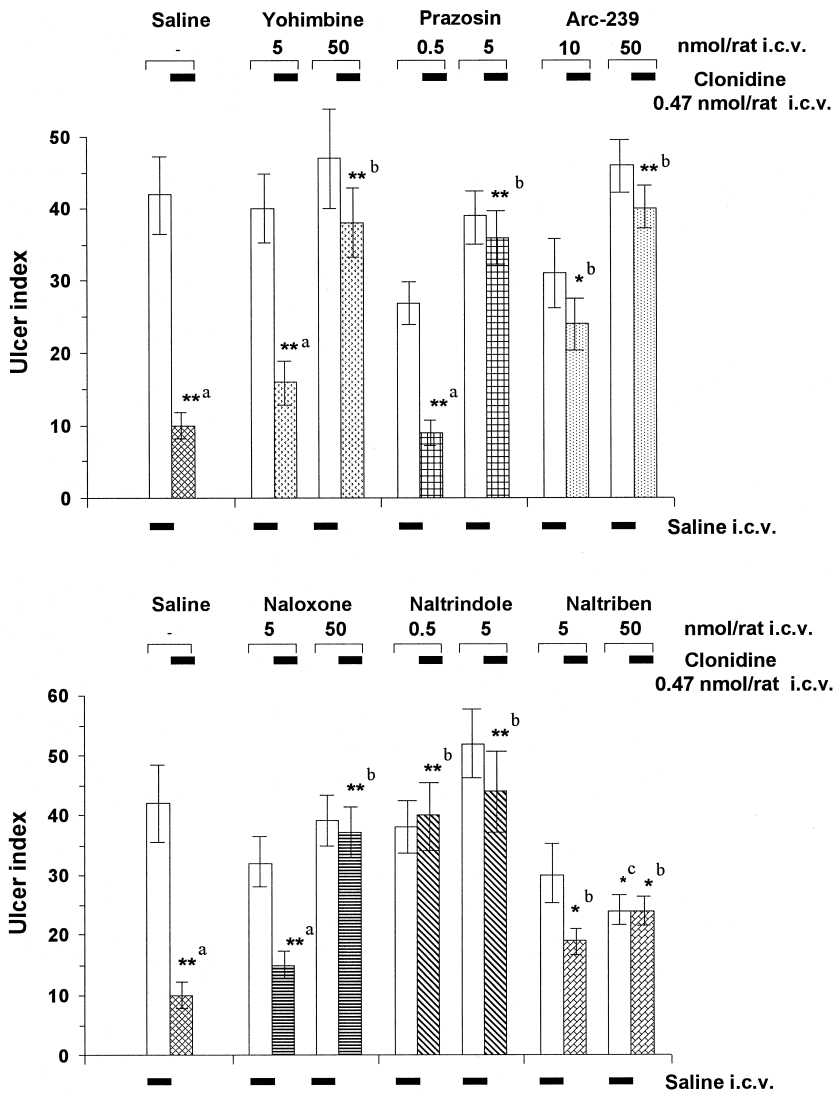

Fig. 1. The effects of i.c.v. injected pre-synaptic α_2-adrenoceptor blocking drugs yohimbine (5, 50 nmol/rat), prazosin (0.5, 5 nmol/rat) and ARC-239 (10, 50 nmol/rat) (upper panel) and opioid receptor antagonists naloxone (5, 50 nmol/rat), naltrindole (0.5, 5 nmol/rat) and naltriben (5, 50 nmol/rat) (lower panel) on the gastroprotective effect of clonidine (0.47 nmol/rat i.c.v.) against acidified ethanol induced lesions in the rat. Data are expressed as mean ± SE of 10 animals. $^{*}P < 0.05$; $^{**}P < 0.01$; (a) compared to respective control group (blank columns); (b) compared to saline–clonidine treated group; (c) compared to saline–saline treated group.

Mo, USA), 2-[2-(4-(*O*-methoxyphenyl)piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2H,4H)-isoquinolindione (ARC-239; Karl Thomae, Biberach/Riss Germany), [D-Ala^2, D-Leu^5]enkephalin (DADLE, synthesised by A. Magyar Eötvös University, Budapest), naltriben HCl (synthesised by L. Hosztafi, ICN Alkaloida, Tiszavasvári, Hungary).

The drugs were dissolved in saline. Control animals received the drug solvent.

2.3. Statistical analysis

All data are presented as the means ± SEM. 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 central injection of yohimbine, prazosin, ARC-239, naloxone, naltrindole and naltriben on the gastroprotective effect of clonidine-injected i.c.v.

Clonidine in the dose of 470 pmol/rat i.c.v. inhibited the gastric mucosal lesion induced by acidified ethanol by 80%. Yohimbine (50 nmol/rat), prazosin (5 nmol/rat) and ARC-239 (10, 50 nmol/rat) antagonised the gastroprotective effect of clonidine however lower doses (5 and 0.5 nmol/rat) of yohimbine and prazosin, respectively, failed to exert significant inhibitory effect on clonidine-induced protective effect (Fig. 1).

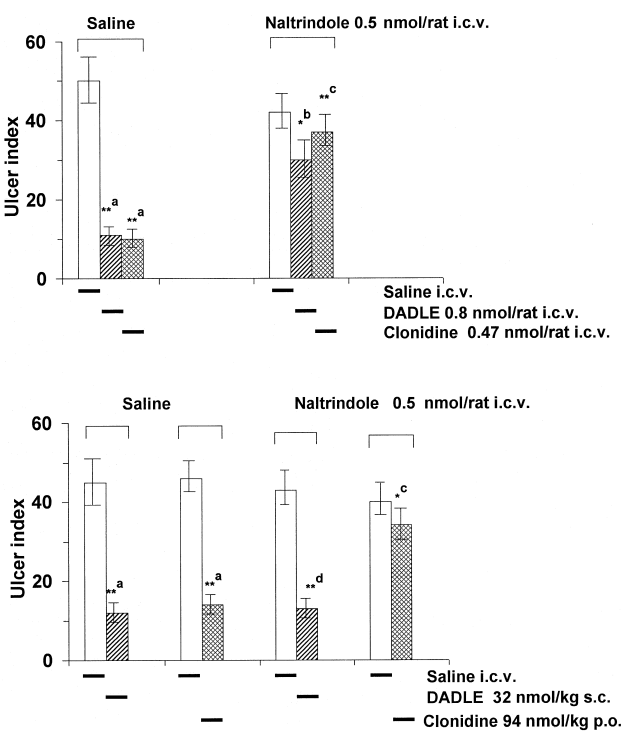

Fig. 2. The effect of the i.c.v. injected naltrindole (0.5 nmol/rat) on the gastric mucosal protective effect of DADLE and clonidine against acidified ethanol induced lesions administered either i.c.v. (upper panel) or peripherally (s.c. and p.o, respectively) (lower panel). Data are expressed as mean ± SE of 10 animals. $^{*}P < 0.05$; $^{**}P < 0.01$; (a) compared to saline–saline treated group; (b) compared to saline–DADLE treated group; (c) compared to saline–clonidine treated group; (d) compared to saline–naltrindole treated group.

The opioid antagonists naloxone in higher dose (50 nmol/rat) and naltrindole (5, 0.5 nmol/rat) had similar action, inhibited the mucosal protective effect of clonidine. Naltriben also decreased the gastroprotective effect of clonidine, although the evaluation of interaction was complicated by the fact that naltriben per se decreased the ethanol-induced gastric damage.

3.2. The effect of i.c.v. injection of naltrindole on the gastroprotective effect of DADLE and clonidine-injected either centrally or peripherally (subcutaneously or orally)

Both DADLE (0.8 nmol/rat i.c.v.) and clonidine (0.47 nmol/rat i.c.v.) inhibited the ethanol-induced gastric mucosal lesions by more than 80%. The doses of clonidine and DADLE were selected on the basis of previous dose–response studies. The gastroprotective effect of both substances was antagonised by naltrindole (0.5 nmol/rat i.c.v.). However, when clonidine (94 nmol/kg p.o.) and DADLE (32 nmol/kg s.c.) were injected peripherally, naltrindole antagonised the effect of clonidine but not that of DADLE (Fig. 2).

4. Discussion

Investigations on gastric mucosal protective mechanisms are focused mainly on the local mucosal processes. However, recently involvement of central components in gastric mucosal protection has also been raised (Taché et al., 1994, 1998; Guidobono et al., 1998; Yang et al., 1999). In our present experimental series, it was demonstrated that gastroprotection (or cytoprotection) could be initiated by the activation of central α_2-adrenoceptors.

In the light of several subclasses of α_2-adrenoceptors, it was appropriate to consider whether discriminable pharmacology might exist for centrally induced gastroprotection. Namely, in contrast with binding studies, the evidences from functional investigations have been less convincing, and there are only few in vivo studies, which demonstrate alpha-adrenoceptor subtype-mediated pharmacological actions. For example, α_{2A}- and $\alpha_{2B/2C}$-adrenoceptor subtypes were found to be involved in producing fever in response to bacterial lipopolysaccharide (Bencsics et al., 1995) and α_2 non-A subclasses of receptors was suggested to be involved in spinal anti-nociceptive effect (Takano and Yaksh, 1992). Moreover, α_{2A}-adrenoceptor subtype was supposed to be involved in the modulation of acid secretion induced by vagal stimulation in the rat (Blandizzi et al., 1995), and our data showed that the

cytoprotective/gastroprotective effect of peripherally administered clonidine in different ulcer models is likely to be mediated by α_{2B}-adrenoceptor subtype (Gyires, 1997). Clonidine was shown to exert a dose-dependent inhibition injected i.c.v. on gastric mucosal damage induced by acidified ethanol (Gyires et al., to be published). Our present observation suggests that the clonidine-induced gastroprotection may be due to the activation of central α_2-, particularly α_{2B}-adrenoceptors. Namely, the gastroprotective effect of clonidine-given i.c.v. was antagonised in a dose-dependent manner by yohimbine (non-subtype selective α_2-adrenoceptor antagonist), prazosin (α_{2B}-adrenoceptor antagonist in addition to its classic action as an α_1-adrenoceptor antagonist) and ARC-239 (prefers α_2-adrenergic receptor to α_1 more than prazosin) (Bylund et al., 1988).

Moreover, the effect of clonidine could be inhibited not only by blocking α_2-adrenoceptors, but also by centrally injected opioid antagonists. Naloxone, the non-selective, moderately μ-opioid receptor preferring antagonist, naltrindole, the δ-opioid receptor antagonist and naltriben, the selective δ_2-opioid receptor antagonist inhibited the gastroprotective effect of clonidine, indicating that central opioid — particularly δ — receptors are involved in the mucosal protective effect of clonidine. In our previous experiments, it was shown that 0.5 nmol/rat of naltrindole failed to inhibit the gastroprotective effect of morphine (10 nmol/rat i.c.v.) but blocked that of deltorphin II — a selective δ receptor agonist — indicating that the dose of naltrindole applied is really selective for δ-opioid receptors. Naltrindole (5 nmol/rat i.c.v.) aggravated the ethanol-induced lesions, which might suggest the involvement of central δ receptors in maintaining the gastric mucosal integrity. Our previous results indicated also that peripheral δ-receptors might have a role in gastric mucosal protective mechanism (Gyires et al., 1997). The inhibitory effect of naltriben was lower, compared to that of naltrindole. The weaker antagonist effect and the slight agonist (protective) effect of naltriben might be explained by its κ agonist property (Stewart et al., 1994). Namely, κ-opioid receptor activation may mediate mucosal defence (Gyires, 1990). On the other hand, it may also be speculated that central δ_2-opioid receptor subtype has only minor role in the mucosal protective processes, therefore, the selective δ_2-opioid receptor antagonist naltriben inhibited, to a lesser extent, the gastroprotective effect of clonidine than naltrindole, which blocks both δ_1- and δ_2-opioid receptors.

Further evidence for the central origin of the gastroprotective action was obtained from the experiments when the effect of centrally injected naltrindole was examined on gastroprotection induced by orally administered clonidine. Naltrindole (0.5 nmol/rat i.c.v.) blocked the mucosal protective effect of clonidine (94 nmol/kg p.o.), but failed to influence that of the δ-opioid receptor agonist DADLE (32 nmol/kg s.c.). DADLE, if at all, passes poorly the blood brain barrier, therefore, its gastroprotective action upon s.c. administration is due only to peripheral mechanism. Since naltrindole injected i.c.v. failed to influence the effect of DADLE given s.c. (but blocked that of injected i.c.v.), it may be concluded that naltrindole in this dose has no peripheral action at all. Consequently, the gastroprotective effect of peripherally (orally) administered clonidine is also due to central mechanism.

In conclusion, the present data suggest that clonidine activating central α_2 — especially $\alpha_{2B/2C}$ — adrenoceptor subtype exerts gastric mucosal protection. Clonidine, through central α_{2B}-adrenoceptor subtype, initiates a chain of events, which involves — as a first step — the endogenous opioid system. Experiments are in progress to elucidate the mechanism(s), which transduce the centrally initiated effect to the periphery.

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