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Involvement of nitric oxide in the gastroprotective effect of ACEA, a selective cannabinoid CB_1 receptor agonist, on aspirin-induced gastric ulceration

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The involvement of nitric oxide in the gastroprotective effect of ACEA (arachidonyl-2-chloroethylamide), a selective cannabinoid CB_1 receptor agonist, on aspirin-induced gastric ulceration was studied in rats. ACEA (3 mg/kg i.p.) significantly reduced gastric ulcer formation. The gastroprotection of ACEA was attenuated by pretreatment with L-NAME (25 and 50 mg/kg i.p.), a nitric oxide synthase inhibitor. The combination of L-arginine (300 mg/kg i.v.), a precursor of nitric oxide with L-NAME (50 mg/kg i.p.) reversed the protective activity of ACEA (3 mg/kg i.p.). These results suggest that endogenous nitric oxide may be involved in the protective effect of ACEA.

1. Introduction

The gastrointestinal tract of many species, including humans, contains an endocannabinoid system were endocannabinoids (anandamide and 2-arachidonoylglycerol) are synthesized locally and act on CB_1 and CB_2 receptors, modulating a variety of functions (Coutts and Izzo 2004; Izzo and Camilleri 2008). CB_1 receptors are located in the enteric nervous system and in sensory terminals of vagal and spinal neurons and regulate neurotransmitters release, while CB_2 receptors are mostly distributed in the immune system with a role presently still difficult to establish (Massa and Monory 2006; Pertwee 2001). Within the gastrointestinal tract endocannabinoids appear to elicit an overall inhibitory effect on diverse gastrointestinal functions, e.g. secretion and motility. Experimental studies revealed that cannabinoids inhibit gastric acid secretion (Adami et al. 2002, 2004; Coruzzi et al. 1999), gastric emptying and contractility (Izzo et al. 1999a; Krowicki et al. 1999), intestinal secretion (Tyler et al. 2000) and motility (Izzo et al. 1999b; Jones and Wessinger 2005) as well as increase of gastric mucosal defense (Germanò et al. 2001). Though these effects are most likely mediated via a CB_1-dependent inhibition of cholinergic excitatory transmission (Pertwee 2001; Mancinelli et al. 2001), there is also recent evidence for a CB_2-mediated reduction of cholinergic transmission (Mulè et al. 2007). Furthermore functional data indicate that the effect of cannabinoids on certain gastrointestinal functions can at least in part be mediated by non-adrenergic non-cholinergic (NANC) transmitters, including nitric oxide (NO) (Kurjak et al. 2008).

In the gastrointestinal tract NO participates in the modulation of the smooth musculature tone, regulates acid and gastric mucus secretion, alkaline production, and is involved in the maintenance of mucosal blood flow (Dijkstra et al. 2004; Martin et al. 2001; Shibata et al. 2006). Under physiological conditions, NO acts as an endogenous mediator modulating both, the repairing and integrity of the tissues, and exhibits gastroprotective properties against different types of aggressive agents (Calatayud et al. 2001; Cho 2001; Elliott and Wallace 1998; Kalia et al. 2000).

In addition, it has been shown that NO secreted from endothelial cells or the sensory nerve endings is essential for gastroprotection evoked by many physiological factors including growth factors or gastrointestinal hormones, such as cholecystokinin (CCK), gastrin, leptin and ghrelin (Brzozowski et al. 2000; Konturek et al. 1992, 1995).

The functional coupling between cannabinoids and NO release from constitutive nitric oxide synthase (NOS) has not yet been shown in the gastrointestinal tract, but could be demonstrated in vascular endothelial cells (Stefano et al. 2003), tracheal smooth muscle (Nieri et al. 2003) and isolated corpus cavernosum (Ghasemi et al. 2006).

2. Investigations and results

We have recently shown that ACEA, a selective cannabinoid CB_1 receptor agonist, reduces aspirin (ASA)-induced gastric ulceration (Rutkowska and Fereniec-Gołębiowska 2006). The antiulcerative effect of ACEA could be related to its antisecretory effect because the activation of CB_1 receptors inhibits gastric acid secretion (Adami et al. 2002, 2004; Coruzzi et al. 1999). On the other hand, CB_1 agonists have been shown to be involved in the release of NO (Romano and Lograno 2006; Stefano et al. 2003) which is a crucial mediator of gastrointestinal mucosal defense (Calatayud et al. 2001; Cho 2001; Elliot and Wallace 1998). The object of this study was to clarify the interaction between the antiulcer effect of ACEA and NO.

As shown in the Fig., animals receiving oral ASA (200 mg/kg) presented had gastric lesions with a total

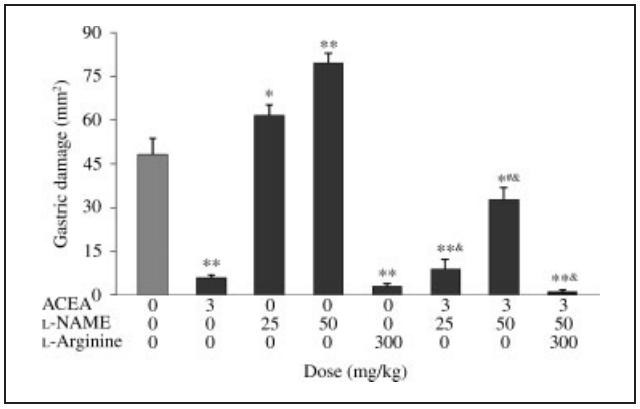

Fig: Effects of ACEA on gastric lesions induced by aspirin in L-NAME and L-NAME + L-arginine-pretreated rats. Data are presented as the means ± SEM from 5–6 animals * $P < 0.01$ and ** $P < 0.001$ compared to vehicle control; # $P < 0.001$ compared to ACEA; & $P < 0.001$ compared to L-NAME

score of 48.2 ± 5.62 mm^2 (mean ± SEM). Pretreatment with ACEA (3 mg/kg i.p.) significantly inhibited gastric ulcers formation to 5.8 ± 0.91 mm^2 ($p < 0.001$ compared with control group). L-Arginine (300 mg/kg i.v.), a NO precursor significantly decreased aspirin-induced gastric lesions to 3.0 ± 0.89 mm^2 ($p < 0.001$), while L-NAME (25 and 50 mg/kg i.p.), a nitric oxide synthase inhibitor significantly increased these lesions to 61.7 ± 3.48 mm^2 ($p < 0.01$) and to 79.5 ± 3.18 mm^2 ($p < 0.001$), respectively. L-NAME administered at 50 mg/kg i.p. significantly reduced the antiulcer effect of ACEA ($p < 0.001$ compared with ACEA group). L-NAME at 25 mg/kg i.p. also reduced the antiulcer effect of ACEA but not significantly. The combination of L-arginine (300 mg/kg i.v.) with L-NAME (50 mg/kg i.p.) reversed the protective activity of ACEA (3 mg/kg i.p.).

3. Discussion

The present study confirms our previous results showing that ACEA, a selective CB_1 receptor agonist, protects gastric mucosa against acute gastric injury induced by administration of ASA (Rutkowska and Fereniec-Gołębiowska 2006) and shows for the first time that NO may be involved in the protective effect of ACEA.

We demonstrated that pretreatment with L-NAME, a NO synthase inhibitor attenuated the gastoprotective effect of ACEA, which was reversed by addition of L-arginine, a NO precursor.

The antiulcerative effect of ACEA could be related to its antisecretory effect because cannabinoid agonists reduce acid secretion via activation of CB_1 receptors (Adami et al. 2002, 2004; Coruzzi et al. 1999).

Gastric acid plays an essential role in the development of gastric ulcers and inhibition of gastric acid secretion is a main mechanism of gastroprotection evoked by "classical" anti-ulcer drugs such as proton pump inhibitors (Bergmann et al. 1992) and H_2-receptor antagonists (Burgess et al. 1995). On the other hand, Dembiński et al. (2006) have found that the endocannabinoid anandamide, acting via a CB_1 receptor, protects gastric mucosa against lesions evoked by water immersion and restrain stress (WRS) and this gastroprotective effect has been associated with an increase in gastric mucosal blood flow and mucosal DNA synthesis.

Gastric mucosal blood flow has a vital role in gastric mucosal protection. A high blood flow is considered good protection against injury, as it dilutes, neutralizes, and removes hazardous substances that have penetrated the gastric mucosal barrier (Sørbye and Saves 1994).

It is known that vasorelaxation to cannabinoids occurs via a variety of mechanisms (Randall et al. 2004). In some vessels the cannabinoid agonists can produce endothelium-dependent relaxation via release of NO from endothelium (Romano and Lograno 2001; Stefano et al. 2003).

NO participates in the gastric defense mechanism by increasing gastric mucosal blood flow and mucus secretion and by inhibiting of gastric acid secretion (Calatayud et al. 2001; Cho 2001; Elliott and Wallace 1998). In addition, the protective action of NO may result from its antiinflammatory activity, especially from the inhibition of cytokine-induced endothelial activation (De Caterina et al. 1995). It has been shown that the inhibition of NO synthesis induced acute mucosal damage and this effect was inhibited by concurrent administration of L-arginine (Whittle et al. 1990) and this is in agreement with the results of our study that L-NAME and L-arginine increases and decreases aspirin-induced gastric damage, respectively. In the present study, treatment with L-NAME markedly attenuated the antiulcer effect of ACEA, which was reversed by addition of L-arginine. These findings suggest that NO is involved in the protective effect of ACEA.

It is tempting to speculate that agonistic activity of ACEA on a cannabinoid CB_1 receptor causes releasing of NO, which acts as a gastroprotective agent.

We conclude that the increasing level of NO by ACEA might be one of the contributory factors in its protective effect on gastric mucosa. Further investigations such as direct measurement of NO release or activity of nitric oxide synthase may require confirming the interaction between antiulcer effect of the cannabinoid CB_1 receptor agonists and NO.

4. Experimental

4.1. Animals

The studies were carried out on male and female Wistar rats weighing 160–240 g (purchased from a licensed breeder). The animals were kept in a colony room at a temperature 20 ± 2 °C under 12/12 h light/dark cycle (lights on at 7 a.m.), with food and water freely available. The experimental procedures were approved by the Local Ethics Committee and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

4.2. Drugs and chemicals

ACEA (arachidonyl-2-chloroethylamide, ethanol solution 5 mg/ml; Tocris); aspirin (acetylsalicylic acid, ASA; Sigma); L-arginine hydrochloride (Merck); N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME; Sigma); cremophor EL (Sigma). ACEA was diluted with cremophor : saline (1 : 14), L-arginine and L-NAME were dissolved in isotonic saline, immediately before use.

4.3. Aspirin-induced gastric ulcers

Overnight fasted rats, with acces to water *ad libitum*, were divided into 8 groups as following manner:

- Group I (control) received (subcutaneously, s.c.) saline and 15 min after (intraperitoneally, i.p.) a mixture of ethanol : Cremophor : saline (5 : 1: 14).
- Group II received (s.c.) saline and 15 min after (i.p.) ACEA at a dose of 3 mg/kg.
- Group III received (s.c.) L-NAME at a dose of 25 mg/kg and 15 min after (i.p.) a mixture of ethanol : Cremophor : saline (5 : 1 : 14).
- Group IV received (s.c.) L-NAME at a dose of 50 mg/kg and 15 min after (i.p.) a mixture of ethanol : Cremophor : saline (5 : 1 : 14).
- Group V received (intravenously, i.v.) L-arginine at a dose of 300 mg/kg and 15 min after (i.p.) a mixture of ethanol : Cremophor : saline (5 : 1 : 14).
- Group VI received (s.c.) L-NAME at a dose of 25 mg/kg and 15 min after (i.p.) ACEA at a dose of 3 mg/kg.
- Group VII received (s.c.) L-NAME at a dose of 50 mg/kg and 15 min after (i.p.) ACEA at a dose of 3 mg/kg.
- Group VIII received (i.v.) L-arginine (300 mg/kg), 10 min after (s.c.) L-NAME (50 mg/kg) and 15 min after (i.p.) ACEA (3 mg/kg).

Injection volumes were 4 ml/kg.

One hour after last injection all groups were treated orally (p.o.) with 200 mg/kg ASA (in 10 ml of distilled water per kg of rat body weight). 3 h later, the rats were euthanized by cervical dislocation under thiopental anesthesia. The stomach was removed, rinsed with saline, opened along the greater curvate and examined for lesions. The area (mm^2) of lesions was measured, summed per stomach, and used as a lesions score.

4.4. Statistics

The data were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls test as a post-hoc. The accepted level of significance was $p < 0.05$.

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