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ACEA (arachidonyl-2-chloroethylamide), the selective cannabinoid CB_1 receptor agonist, protects against aspirin-induced gastric ulceration

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The effect of a selective cannabinoid CB_1 receptor agonist, ACEA (arachidonyl-2-chloroethylamide) in an aspirin-induced ulcer model was studied in rats. ACEA (1.25–5 mg/kg i.p.) significantly reduced gastric ulcer formation to 24, 21 and 0.6% respectively. These results confirm the cytoprotective effect of CB_1 receptor agonists and suggest that the endocannabinoid system might be the target for a novel class of anti-ulcer drugs.

1. Introduction

Cannabis and cannabinoids exert many of their biological functions through interaction with at least two receptor subtypes, the cannabinoid CB_1 and CB_2 receptors (Childers and Breivogel 1998; Sugiura and Waku 2000; Pertwee and Ross 2002). Both receptors activate similar transduction mechanisms, including inhibition of adenylate cyclase and N-type Ca^{2+}-channels (Childres and Deadwyler 1996; Nocerino et al. 2000; Lutz 2002; Grotenhermen 2003). The CB_1 receptor, which accounts for the psychoactive effects of cannabis, is not only abundant in the brain but is also present in the peripheral neurons (Pertwee 1998). In the peripheral nervous system, CB_1 receptors are located presynaptically or prejunctionally and their activation can produce a suppression of neurotransmitter release (Nocerino et al. 2000). The CB_2 receptor is mainly located in immune cells, and has been implicated in the immunomodulating effects of cannabinoids (Kaminski et al. 1992).

CB_1 receptors are expressed in peripheral neurons of the myenteric plexus in different animal species (Pertwee 2001; Pinto et al. 2002; Coutts and Izzo 2004), furthermore CB_1 receptor immunoreactivity has been detected on cholinergic neurones innervating smooth muscle, mucosa and submucosal vessels of the rat stomach (Adami et al. 2002). In humans, the mRNA for the CB_1 receptor has been found in stomach and colon (Shire et al. 1995).

Activation of CB_1 receptors inhibits gastric acid secretion (Adami et al. 2002, 2004), gastric emptying and contractility (Izzo et al. 1999a; Krowicki et al. 1999; Landi et al. 2002), intestinal secretion (Tyler et al. 2000; Izzo et al. 2003) and motility (Izzo et al. 1999b; Landi et al. 2002; Jones and Wessinger 2005) as well as increases of gastric mucosal defense (Germanò et al. 2001).

2. Investigations and results

It has recently been shown that the non-selective cannabinoid receptor agonist, the aminoalkylindole WIN 55, 212–2 reduced stress – induced gastric ulceration (Germanò et al. 2001). In the present study we investigated the possible cytoprotective action of a selective CB_1 receptor agonist ACEA (arachidonyl-2-chloroethylamide) against aspirin (ASA) – induced gastric ulcers.

ASA (200 mg/kg p.o.) caused lesions in the gastric mucosa within 3 h; the lesion score was 53.86 ± 4.97 mm^2 (mean ± SEM). ACEA at doses of 1.25–5 mg/kg i.p. significantly inhibited gastric ulcers formation to 24, 21 and 0.6% respectively and this effect was dose-dependent. Ranitidine (60 mg/kg p.o.), used as a reference drug, reduced gastric ulceration to 5.6% (Fig.).

3. Discussion

Previous studies have revealed that the cannabinoid receptor agonist WIN 55, 212–2 reduced stress-induced gastric ulcers. This protective effect of WIN 55, 212–2 was coun-

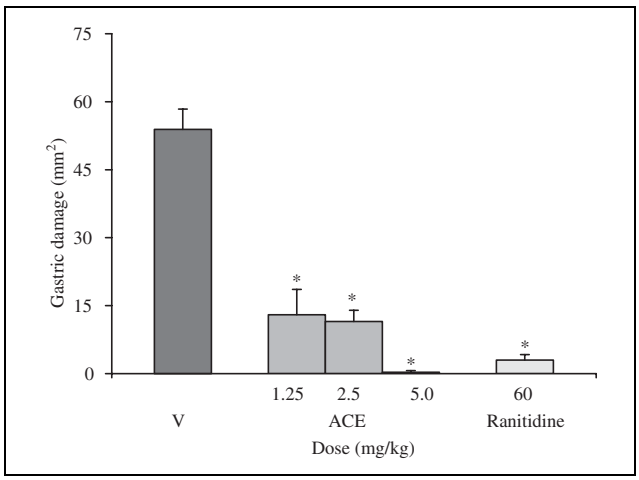

Fig.: Effect of ACEA on aspirin-induced gastric ulcers. Data were presented as the means ± SEM from 6–8 animals. * Significant difference from control vehicle (V), at $P < 0.001$

teracted by the cannabinoid CB_1 receptor antagonist SR 141716A but not by the cannabinoid CB_2 receptor antagonist SR 144528, thus indicating the involvement of CB_1 receptors (Germanò et al. 2001).

The present results show that the selective CB_1 receptor agonist ACEA prevented the occurrence of gastric lesions induced by non-steroidal anti-inflammatory drug ASA. The anti-ulcer activity of ACEA might be related to its antisecretory effect. It has recently been shown that cannabinoid agonists, by a mechanism that involves the CB_1 receptor, reduced *in vivo* acid secretion induced by cholinergically mediated secretagogues such as 2-deoxy-D-glucose and pentagastrin, but not induced by histamine, which activates H_2 receptors on the parietal cells (Coruzzi et al. 1999; Adami et al. 2002). The antisecretory effect of these agents was observed only after peripheral administration and was reduced by cervical vagotomy or ganglionic blocade (Adami et al. 2002, 2004). These findings have lead to the suggestion that cannnabinoid agonists act through a peripheral mechanism, by activation of CB_1 receptors located on vagal efferent pathways to the gastric mucosa (Coutts and Izzo 2004).

In conclusion, our findings confirm the gastroprotective effect of CB_1 agonists and suggest that the gut endocannabinoid system may prove to be a promising target for the development of new anti-ulcers drugs.

4. Experimental

4.1. Animals

The studies were carried out on male Wistar rats weighing 150–200 g (purchased from a licensed breeder). The animals were kept in a room at a temperature $20 \pm 2°$ under 12/12 h light/dark cycle (lights on at 7 a.m.) for at least 7 days before the experiment and had free access to food and water. All animal procedures met the guidelines of the European Communities Directive 86/609/EEC regulating animal research and were approved by the Local Ethics Committee.

4.2. Drugs and chemicals

ACEA (arachidonyl-2-chloroethylamide, ethanol solution 5 mg/ml; Tocris); ranitidine (Sigma); aspirin (acetylsalicylic acid, ASA; Sigma); cremophor EL (Sigma). ACEA was diluted with cremophor: saline (1 : 14) and ranitidine was disolved in distilled water.

4.3. Aspirin-induced gastric ulcers

Overnight fasted rats, with acces to water *ad libitum*, were treated orally (p.o.) with 200 mg/kg ASA (in 10 ml of distilled water per kg of rat body weight) and euthanized by cervical dislocation 3 h later. 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.

One hour before ASA, different groups of animals were treated intraperitoneally (i.p.) with graded doses of ACEA (1.25–5 mg/kg), ranitidine (60 mg/kg, p.o.) or vehicle (ethanol: cremophor: saline; 5 : 1 : 14; i.p.), in a volume of 4 ml/kg.

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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