JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2009, 60, Suppl 7, 93-100
www.jpp.krakow.pl

N. SHUJAA[1], Z.S. ZADORI[1], A.Z. RONAI[1], I. BARNA[2], Z. MERGL[2], M.M. MOZES[3], K. GYIRES[1]

ANALYSIS OF THE EFFECT OF NEUROPEPTIDES AND CANNABINOIDS IN GASTRIC MUCOSAL DEFENSE INITIATED CENTRALLY IN THE RAT

[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [2]HAS Institute of Experimental Medicine, Budapest, Hungary; [3]Department of Pathophysiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Increasing number of evidence suggest that gastric mucosal protection can be induced also centrally. Several neuropeptides, such as TRH, amylin, adrenomedullin, enkephalin, β-endorphin, nociceptin, nocistatin, ghrelin or orexin given centrally induce gastroprotection and the dorsal vagal complex and vagal nerve may play prominent role in this centrally initiated effect. Since also cannabinoid receptors are present in the dorsal vagal complex, we aimed to study whether activation of central cannabinoid receptors result in gastric mucosal defense and whether there is an interaction between cannabinoids and endogenous opioids. Gastric mucosal damage was induced by 100% ethanol in rats. The cannabinoids were given intravenously (i.v.) or intracerebroventricularly (i.c.v.), while the antagonists were given i.c.v or intracisternally (i.c.). Gastric lesions were evaluated macroscopically 60 min later. Anandamide, methanandamide and WIN55,212-2 reduced ethanol-induced mucosal lesions after both peripheral (0.28-5.6 μmol/kg, 0.7-5.6 μmol/kg and 0.05-0.2 μmol/kg i.v., respectively) and central (2.9-115 nmol/rat, 0.27-70 nmol/rat and 1.9-38 nmol/rat i.c.v., respectively) administration. The gastroprotective effect of anandamide and methanandamide given i.c.v. or i.v.was reversed by the CB_1 receptor antagonist SR141716A (2.16 nmol i.c.v.). Naloxone (27.5 nmol i.c.v.) also antagonized the effect of i.c.v. or i.v. injected anandamide and WIN55,212-2, but less affected that of methanandamide. The gastroprotective effect of anandamide was diminished also by endomorphin-2 antiserum. In conclusion it was first demonstrated that activation of central CB_1 receptors results in gastroprotective effect. The effect is mediated at least partly by endogenous opioids.

Key words: *cannabinoid agonist,* cannabinoid *antagonist, naloxone, endomorphin-2 antiserum, intracerebroventricular, ethanol, damage* CB_1 receptor

INTRODUCTION

Acid and pepsin play a prominent role in the development of peptic ulcer disease and the treatment with antisecretory drugs (proton pump inhibitors, histamine H_2-receptor blocking drugs) results in excellent therapeutic effect. However, there are numerous data which suggest that factors relating to mucosal resistance to acid may be equally important. *E.g*. stress ulcer can develop in patients in intensive care units and mortality due to gastric bleeding associated with stress ulcer can reach, even exceed 50% (1-3). Moreover, bleeding complications and fatal events have not decreased even after the introduction of proton pump inhibitors (4). It is well known that in upper gastric ulcers the acid secretion is normal or decreased, indicating that decreased mucosal resistance may be responsible for the development of mucosal damage. In distal, antral and duodenal ulcers, where typically hypersecretion is observed, acid output is in normal range in about half of the patients, referring again to the basic role of gastric mucosal defensive processes. The same questions can be raised, that was raised by Sachs *et al*. (5) more than 30 years ago, namely how it can be explained that while the incidence of acid-related disorders in life is about 20% of the population, everybody secretes acid throughout the life. Though several additional factors have been identified which contribute to the development of mucosal lesions in a given individual, the impaired mucosal defensive mechanisms may play a crucial role in this process.

Mucosal defense can be initiated both peripherally and centrally. The peripheral mechanisms and mediators involved in mucosal protection have been well documented; both structural and functional elements have been described. Structural elements such as the adherent mucus $–HCO_3$ layer, are responsible for surface neutralization of acid, as well as forming a protective physical barrier against luminal acid and pepsin. The mucus-bicarbonate production is regulated by numerous factors, such as vagal nerve as well as hormons, like gastrin, cholecystokinin, ghrelin, leptin, calcitonin gene-related peptide (CGRP), melatonin prostaglandins (PG), nitric oxide (NO) and various growth factors (6, 7). Among the functional elements, gastric mucosal blood flow has critical role in gastric mucosal integrity. The role of capsaicin-sensitive afferent fibers in gastric mucosal defense has been analysed in detail (8) and recent paper showed the role of capsaicin-sensitive afferent fibers in stress-induced ulcer formation as well (9). Endogenous NO acting in concert with prostacyclin and sensory neuropeptides may have a basic role in the maintenance of gastric mucosal integrity by enhancing mucosal microcirculation (10).

In contrast with the peripheral mechanisms of mucosal protection, much less has been known about the central processes, mediators and brain area(s) that may play a role in the maintenance of gastric mucosal integrity and/or stimulation of mucosal defensive mechanisms. Dorsal vagal complex (nucleus tractus solitarii and dorsal motor nucleus of vagus) and vagal nerve play a prominent role in the regulation of gastric functions, like acid secretion and motility and as it was demonstrated recently, also in gastric mucosal defense (11-15). Several neuropeptides and their receptors were identified in dorsal vagal complex (DVC), like angiotensin II, β-endorphin, bombesin, CCK, corticotropin-releasing factor (CRF), dynorphin, enkephalins, galanin, neuropeptide Y (NPY), neurotensin, somatostatin, thyreotropin releasing hormone (TRH), vasopressin, vasoactive intestinal polypeptide (VIP) (16), amylin (17), endomorphins (18), nociceptin and nocistatin (19) or leptin (20). Neuropeptides can influence the activity of dorsal vagal complex also by neuronal projections. For instance, from the cerebral cortex and hypothalamus ghrelin-containing neurons project to the DVC (21), or similarly, orexigenic neurons project from the lateral hypothalamic area to the dorsal vagal complex (22). Neuropeptides can influence gastric acid secretion, gastrointestinal motility, and as it was demonstrated in the last decade, they can induce gastric mucosal protection given centrally. For example, in rats TRH injected intracisternally (i.c.) or directly into the dorsal motor nucleus of vagus in the dose below the threshold that stimulates acid secretion reduced mucosal lesions induced by ethanol (14). Intracisternal injection of peptide YY and adrenomedullin (13, 15) as well as i.c.v. administered amylin (23) decreased the ethanol-induced gastric mucosal lesions in the rat. Moreover, different opioid peptides (11, 12), ghrelin (24, 25), orexin (25), nociceptin (26) and nocistatin (27) also induced mucosal protection against ethanol-induced mucosal lesions following central administration.

The important role of the endocannabinoid system in the gastrointestinal (GI) tract under physiological and pathophysiological conditions has been demonstrated recently. Cannabinoid type 1 (CB_1) receptors are present in neurons of the enteric nervous system and in sensory terminals of vagal and spinal neurons, moreover, CB_1 receptors are also identified in the dorsal vagal complex: in nucleus tractus solitarii (NTS) (28), in dorsal motor nucleus of vagus (29) and prominently, in area postrema (29). Beside the DVC, CB_1 receptors were described also in the paraventricular nuclei (PVN) of the hypothalamus (30), and projection from PVN to the dorsal vagal complex is well documented (31, 32). Cannabinoids given both peripherally and centrally affect numerous gastrointestinal functions; it was shown that cannabinoids inhibited gastric motility in the rat through activation of CB_1 receptors given peripherally (33, 34). Similarly, cannabinoid agonists inhibited gastrointestinal transit in the mice after both central and peripheral administration (35). CB_1 receptor agonists given i.v. decreased the gastric acid secretion induced by indirect acting secretagogues, such as 2-deoxy-D-glucose (36, 37), but did not change acid output to histamine. This latter finding indicates that CB_1 receptors are not located on parietal cells, but rather on vagal pathways. Since intracerebroventricularly injected cannabinoid agonists were ineffective in preventing the pentagastrin stimulated gastric acid secretion, the CB_1 receptor-mediated inhibition of gastric acid secretion in the rat may be located mainly peripherally (38).

Cannabinoids have been shown to decrease the formation of experimental gastric ulcers as well. Tetrahydrocannabinol, for example, reduced mucosal damage induced by pylorus ligation (39) and Cannabis sativa was effective against restraint-induced gastric ulcerations (40). Furthermore, anandamide reduced the gastric ulceration induced by water immersion and restrain stress (41) and WIN55,212-2 produced anti-ulcer effect in the cold/restraint stress model (42). Moreover, the selective cannabinoid CB_1 receptor agonist, ACEA (arachidonyl-2-chloroethylamide) significantly reduced gastric ulcer formation induced by aspirin (43). These ulcer models are acid dependent models, consequently, the gastric mucosal protective effect of cannabinoids may be related to their antisecretory effect. Since cytoprotective (gastroprotective) effect, originally described by Andre Robert (44) was demonstrated in chemically or physically induced acute gastric ulcer models and the protective effect was unrelated to inhibition of acid secretion, the question was raised whether cannabinoids can inhibit gastric mucosal lesions in acid-independent ulcer models as well.

Therefore the aims of the present study were to determine: i) whether cannabinoids can influence the formation of gastric mucosal lesions induced by ethanol, which is an acid-independent ulcer model; ii) whether central components are involved in the gastroprotective effect of cannabinoids, and finally; iii) whether interaction between cannabinoid and opioid system can be demonstrated in the gastroprotection as well.

MATERIALS AND METHODS

Animals

Experiments were carried out on male Wistar rats (Charles River) weighing 150-170 g received from the breeding colony of Semmelweis University. The animals were kept in a 12-hour light/dark cycle and under condition of controlled temperature. They were maintained on standard rat laboratory chow and tap water ad libitum. All procedures conformed to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. The study was approved by the Animal Ethics Committee of Semmelweis University, Budapest (permission number: 1810/003/2004).

Gastric mucosal damage induced by acidified ethanol

After twenty four hours 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 killed by overdose of 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 (45). The lesion index was calculated as the total number of lesions multiplied by the respective severity factor. The percentual inhibition of mucosal damage was calculated as follows:

$$100 - \left[\frac{\text{lesion index in treated group}}{\text{lesion index in control group}} \times 100 \right]$$

Drugs were injected either intravenously (i.v.), intracerebroventricularly (i.c.v.) or intracisternally (i.c.) in a volume of 5 ml/kg, 10 µl and 5 µl, respectively. The i.c.v. injection to the lateral ventricle was performed according to Noble *et al*. (46) in conscious rats, the intracisternal injection was carried out as described previously (11) based on the method of Ueda *et al*. (47). The cannabinoid receptor agonists (anandamide, methanandamide, WIN55,212-2 and ACEA) were injected 10 min before the ethanol challenge. The different antagonists (the cannabinoid CB_1 receptor antagonist SR141716A, the non-selective opioid receptor antagonist naloxone, the δ-opioid receptor selective naltrindole and the κ-opioid receptor selective norbinaltorphimine (norBNI)) were injected i.c.v., the endomorphin-2 antiserum i.c. 10 min before the administration of the cannabinoid receptor agonists.

Materials

Anandamide, methanandamide, arachidonyl-2-chloroethylamide (ACEA) and (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate (WIN 55 212-2) were purchased from Tocris Bioscience. N-(piperidine-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride (SR141716A) (NIDA) was a generous gift of T. Freund. Naloxone, naltrindole and norbinaltorphimine (NorBNI) were purchased from Sigma. The property of antiserum to endomorphin-2 was described previously (48, 49). The antiserum was used at a 20-fold final dilution. The same dilution of non-reactive rabbit serum was used as control. Anandamide, methanandamide and ACEA were dissolved in ethanol, and stock solutions were diluted with saline. WIN55,212-2 and SR141716A were dissolved in DMSO and then diluted with saline. All the other drugs were dissolved in saline. Animals in the control groups received the drug solvents.

RESULTS

The effect of anandamide, methanandamide and WIN55,212-2 given i.v. and i.c.v. on gastric mucosal damage induced by ethanol

As *Fig. 1*. demonstrates, anandamide (0.28-5.6 μmol/kg), methanandamide (0.7-5.6 μmol/kg) and WIN55,212-2 (0.05-0.2 μmol/kg) inhibited the ethanol-induced gastric mucosal lesions in a dose-dependent manner given i.v. Similarly, gastroprotective effect was induced by anandamide (2.9-115 nmol/rat), methanandamide (0.27-70 nmol/rat) and WIN55,212-2 (1.9-38 nmol/rat) when they were injected i.c.v. WIN55,212-2 in the dose of 1.9 nmol/rat induced still a very pronounced (80%) inhibition of gastric mucosal lesions, experiments are in progress to determine the threshold dose (*Fig. 2*).

The effect of SR141716A given i.c.v on the gastroprotective effect of anandamide and methanandamide given either i.c.v. or i.v.

Pretreatment with the selective CB_1 receptor antagonist SR141716A (2.16 nmol/rat i.c.v.) reversed the gastroprotective

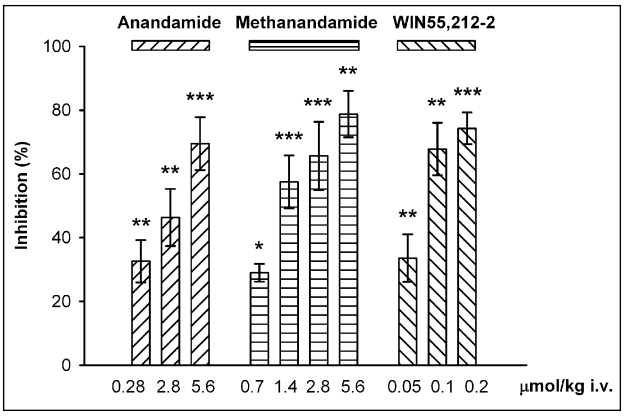

Fig. 1. The inhibitory effect of different non-selective cannabinoid receptor agonists (anandamide, methanandamide and WIN55,212-2) on gastric mucosal injury induced by ethanol in the rat. The compounds were injected intravenously (i.v.) 15 min before the ethanol challenge. Each column represents mean±S.E.M., n=5. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared with the respective control groups (ANOVA, Newman–Keuls post hoc test).

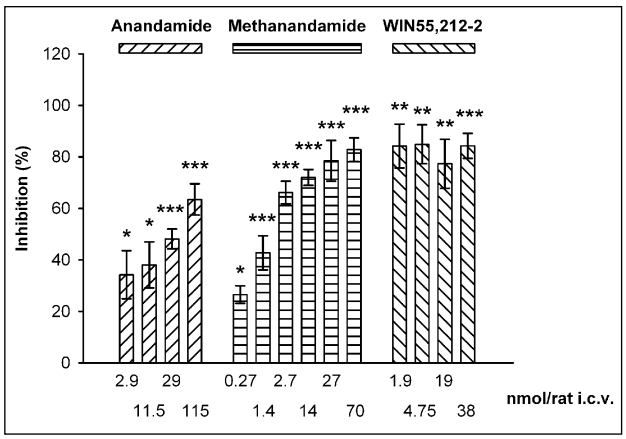

Fig. 2. The inhibitory effect of different non-selective cannabinoid receptor agonists (anandamide, methanandamide and WIN55,212-2) on gastric mucosal injury induced by ethanol in the rat. The compounds were injected intracerebroventricularly (i.c.v.) 10 min before the ethanol challenge. Each column represents mean±S.E.M., n=5. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared with the respective control groups (ANOVA, Newman–Keuls post hoc test).

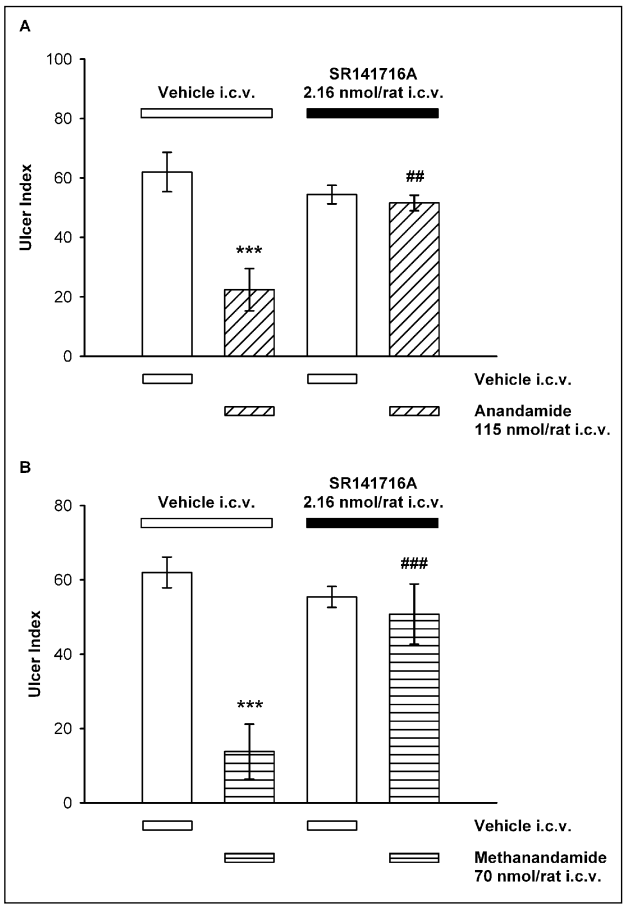

Fig. 3. The effect of the CB_1 cannabinoid receptor antagonist SR141716A (2.16 nmol/rat i.c.v.) on the gastroprotective effect of anandamide (115 nmol/rat i.c.v.) and methanandamide (70 nmol/rat i.c.v.) on gastric mucosal injury induced by ethanol in the rat. Each column represents mean±S.E.M., n=5. $^{***}p<0.001$ compared with vehicle-treated group (column 1); $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared with vehicle + CB receptor agonist (anandamide or methanandamide)-treated group (column 2) (ANOVA, Newman–Keuls post hoc test).

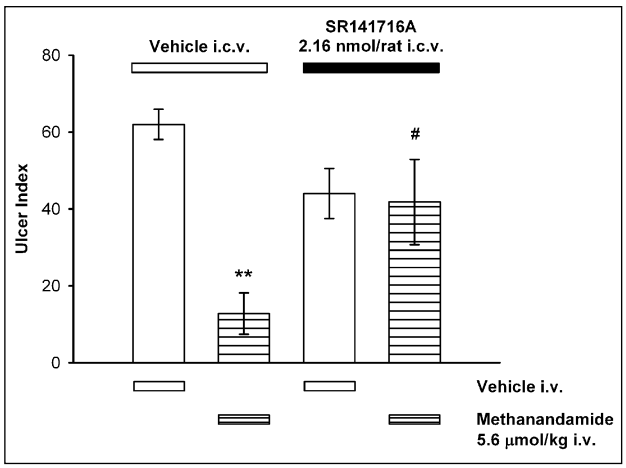

Fig. 4. The effect of the CB_1 cannabinoid receptor antagonist SR141716A (2.16 nmol/rat i.c.v.) on the gastroprotective effect of methanandamide (5.6 μmol/kg i.v.) on gastric mucosal injury induced by ethanol in the rat. Each column represents mean±S.E.M., n=5. $^{**}p<0.01$ compared with vehicle-treated group (column 1); $^{\#}p<0.01$ compared with vehicle + methanandamide-treated group (column 2) (ANOVA, Newman–Keuls post hoc test).

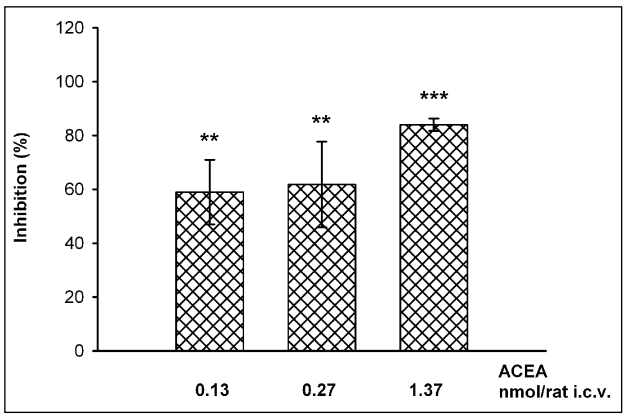

Fig. 5. The inhibitory effect of the selective cannabinoid CB_1 receptor agonist ACEA on gastric mucosal injury induced by ethanol in the rat. The compound was injected intracerebroventricularly (i.c.v.) 10 min before the ethanol challenge. Each column represents mean±S.E.M., n=5. $^{**}p<0.01$, $^{***}p<0.001$ compared with the control group (ANOVA, Newman–Keuls post hoc test).

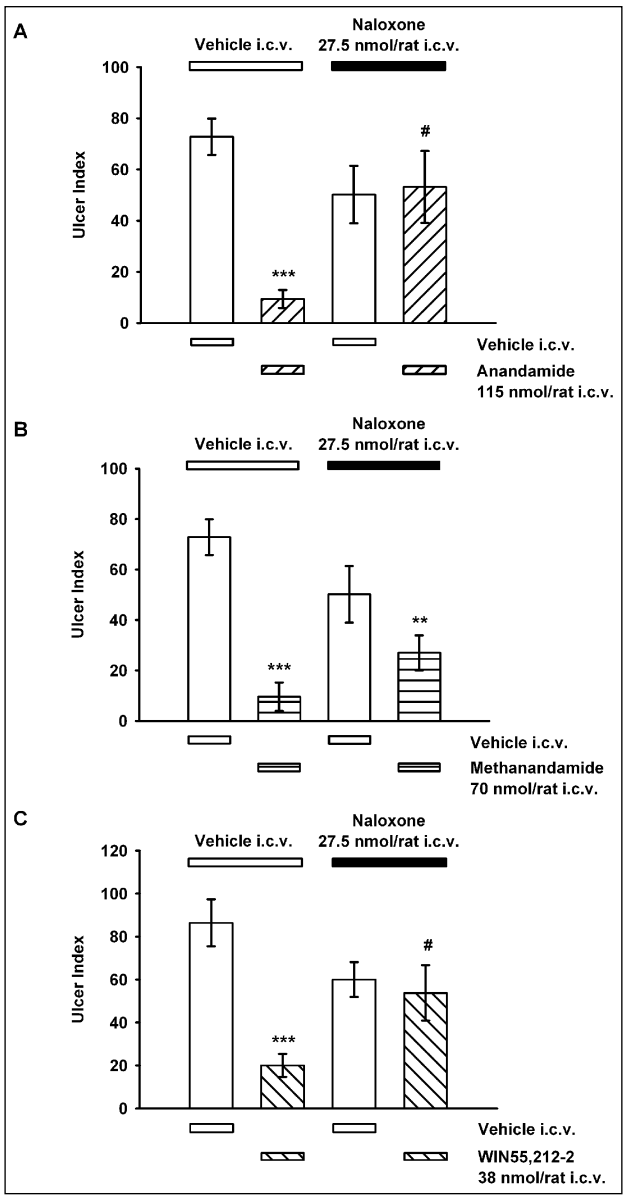

Fig. 6. The effect of naloxone (27.5 nmol/rat i.c.v.) on the gastroprotective effect of anandamide, methanandamide and WIN55,212-2 (115, 70 and 38 nmol/rat i.c.v., respectively) on gastric mucosal injury induced by ethanol in the rat. Each column represents mean±S.E.M., n=5. $^{**}p<0.01$, $^{***}p<0.001$ compared with vehicle-treated group (column 1); $^{\#}p<0.05$ compared with vehicle + CB receptor agonist-treated group (column 2) (ANOVA, Newman–Keuls post hoc test).

effect of both anandamide (115 nmol/rat i.c.v.) and methanandamide (70 nmol /rat i.c.v.) (*Fig. 3*). Similarly, centrally injected SR141716A also antagonized the gastroprotective effect of methanandamide administered peripherally (5.6 μmol/kg i.v.) (*Fig. 4*).

The effect of ACEA on gastric mucosal damage given i.c.v.

Since the previous results suggested the potential involvement of central CB_1 receptors in the gastroprotective effect of cannabinoids, the effect of selective CB_1 receptor agonist ACEA was studied against ethanol-induced ulcer formation. It was found that ACEA in the doses of 0.13-1.37 nmol/rat induced 60-80% inhibition of the mucosal lesions (*Fig. 5*).

The effect of naloxone given i.c.v. on the gastroprotective effect of anandamide, methanandamide and WIN55,212-2 injected either i.c.v or i.v.

The gastroprotective effect of centrally (i.c.v.) injected anandamide (115 nmol/rat) and WIN55,212-2 (38 nmol/rat) was reversed by naloxone (27.5 nmol/rat i.c.v.), however the mucosal protective effect of methanandamide (70 nmol/rat i.c.v.) was less affected(*Fig. 6*). Similar results were obtained when the effect of centrally injected naloxone was examined on the protective effect of anandamide (5.6 μmol/kg), methanandamide (5.6 μmol/kg) and WIN55,212-2 (0.2 μmol/kg) given intravenously; naloxone antagonized the mucosal protective effect of anandamide and WIN55,212-2,

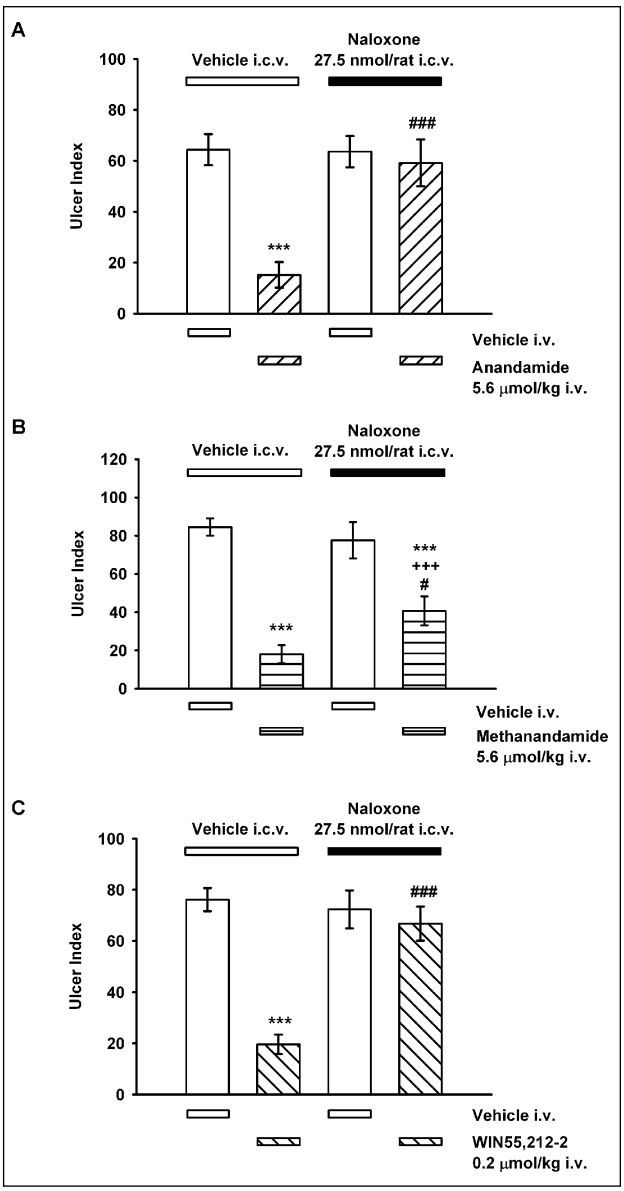

Fig. 7. The effect of naloxone (27.5 nmol/rat i.c.v.) on the gastroprotective effect of anandamide, methanandamide and WIN55,212-2 (5.6, 5.6 and 0.2 μmol/kg i.v., respectively) on gastric mucosal injury induced by ethanol in the rat. Each column represents mean±S.E.M., n=5. $^{***}p<0.001$ compared with vehicle-treated group (column 1); $^{\#}p<0.05$, $^{\#\#\#}p<0.001$ compared with vehicle + CB receptor agonist-treated group (column 2); $^{+++}p<0.001$ compared with naloxone-treated group (column 3) (ANOVA, Newman–Keuls post hoc test).

and decreased the protective effect of methanandamide in a significant manner. However, methanandamide exerted gastroprotective effect also following the pretreatment with naloxone (*Fig. 7*).

The effect of naltrindole and norbinaltorphimine given i.c.v. on the gastroprotective effect of anandamide injected i.c.v.

Both the δ-opioid receptor antagonist naltrindole (5 nmol/rat i.c.v.) and the κ-opioid receptor antagonist norbinaltorphimine (norBNI) (14 nmol/rat i.c.v.) counteracted the mucosal protective effect of anandamide (115 nmol/rat i.c.v.) (*Fig. 8*).

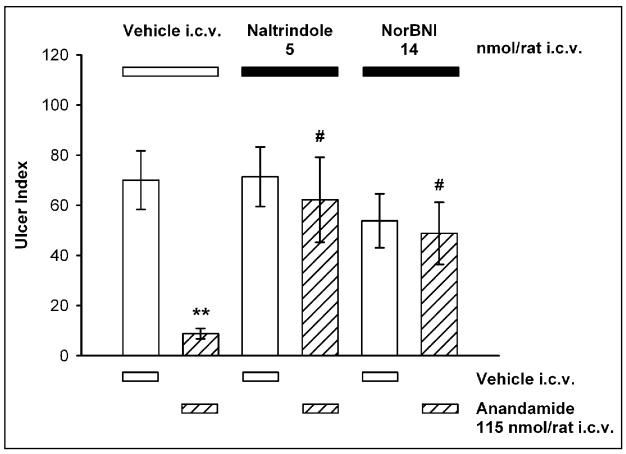

Fig. 8. The effect of naltrindole and norbinaltorphimine (norBNI) (5 and 14 nmol/rat i.c.v., respectively) on the gastroprotective effect of anandamide (115 nmol/rat i.c.v.) on gastric mucosal injury induced by ethanol in the rat. Each column represents mean±S.E.M., n=5. $^{**}p<0.01$ compared with vehicle-treated group (column 1); $^{\#}p<0.05$ compared with vehicle + anandamide-treated group (column 2) (ANOVA, Newman–Keuls post hoc test).

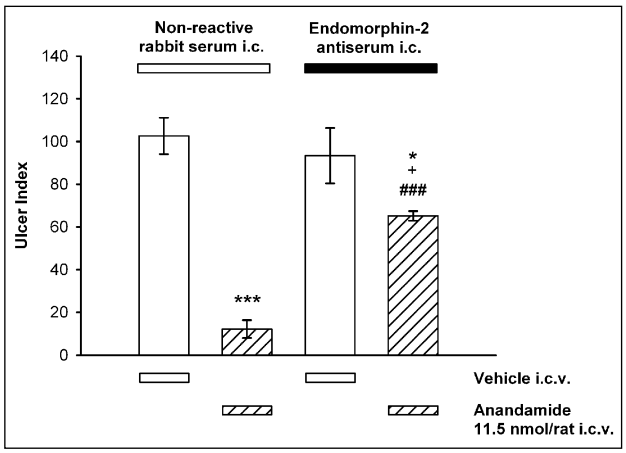

Fig. 9. The effect of endomorphin-2 antiserum (i.c.) on the gastroprotective effect of anandamide (11.5 nmol/rat i.c.v.) on gastric mucosal injury induced by ethanol in the rat. Each column represents mean±S.E.M., n=5. $^{*}p<0.05$, $^{***}p<0.001$ compared with vehicle-treated group (column 1); $^{\#\#\#}p<0.001$ compared with anandamide-treated group (column 2); $^{+}p<0.05$ compared with endomorphin-2-antiserum-treated group (column 3) (ANOVA, Newman–Keuls post hoc test).

The effect of endomorphin-2 antiserum injected i.c. on the gastroprotective effect of anandamide given i.c.v.

Data are shown on *Fig. 9*. The endomorphin-2 antiserum did not affect the formation of ethanol-induced mucosal lesions, however decreased in a significant manner the protective effect of anandamide (11.5 nmol/rat i.c.v.).

DISCUSSION

The present results demonstrate first that the endocannabinoid anandamide, its stable analogue methanandamide and the synthetic, non-selective cannabinoid derivative WIN55,212-2

inhibit the gastric mucosal lesions induced by ethanol given both centrally (i.c.v.) and peripherally (i.v.). Since the centrally induced protective effect of the cannabinoid agonists was reversed by centrally injected SR141716A, a selective cannabinoid CB_1 receptor antagonist, it can be concluded that central CB_1 receptors may mediate the gastric mucosal protective effect. Moreover, centrally injected SR141716A was also able to reverse the mucosal protection induced by the agonists given peripherally, confirming the primary role of centrally located CB_1 receptors in the gastroprotective effect of cannabinoids. This assumption is further supported by the findings that the effective dose range of cannabinoids (anandamide, methanandamide) against ethanol-induced lesions given i.c.v. are much lower than that injected i.v.

There are only few data in the literature on the role of central cannabinoid receptors in gastrointestinal functions. *E.g.* WIN55,212-2 given centrally was more effective in inhibition of intestinal motility, when administrated peripherally, which may suggest central site of action. However, SR141716A given intracerebroventricularly failed to antagonize the effect of WIN55,212-2 injected intraperitoneally, indicating the primary role of peripheral CB_1 receptors in the inhibition of upper intestinal motility (50). Others showed that WIN55,212-2 (2-239 nmol/mouse) and cannabinol (24-4027 nmol/mouse) decreased, while the CB_1 antagonist SR141716A (2-539 nmol/mouse) increased transit in mice and the ED_{50} values were lower when administered i.c.v., than when administered i.p., suggesting the involvement of a central CB_1 receptors in the action. However, since hexamethonium failed to affect the action of cannabinoid agonists on intestinal transit, the role of peripheral components in the effect has also been raised (35). Adami *et al*. (38) found that the synthetic cannabinoid receptor agonists WIN55,212-2 (50 and 100 μg/kg) and HU-210 (25, 50 and 100 μg/kg) were ineffective either on the basal secretion or on the pentagastrin-stimulated acid output after i.c.v. administration, but in constrast, intravenously both WIN55,212-2 (100 and 1000 μg/kg) and HU-210 (10-100 μg/kg) significantly inhibited pentagastrin-induced acid secretion, and SR141716A antagonized this effect, indicating that CB_1 receptors mediating inhibition of gastric acid secretion in the rat are mainly peripherally located. Moreover, it was found that anandamide and WIN55,212-2, when administered peripherally to partially satiated animals, elicited significant and prolonged hyperphagia. In contrast, central injections of these cannabinoids had no effect on feeding, except at the highest dose (10 μg), which resulted already in motor impairment (51). They conluded that cannabinoid agents can affect food intake predominantly by engaging peripheral CB_1 receptors localized to capsaicin-sensitive sensory terminals.

Several lines of evidence suggest that opioid and cannabinoid receptors can functionally interact. Very recent data show that the antihyperalgesic action of anandamide against carrageenan-induced hyperalgesia was reversed by the opioid receptor antagonist naloxone, indicating that its antinociceptive effect may involve at least partly the opioid system (52). Another recent paper showed that AM404, an endocannabinoid transport inhibitor, potentiated antinociception induced by cholestasis, which is associated with increased activity of the endogenous opioid system that results in analgesia. These results suggest a possible interaction between opioid and cannabinoid systems in this experimental model (53).

The interactions may be direct, such as through receptor heteromerization, or indirect, such as through signaling cross-talk that includes agonist-mediated release and/or synthesis of endogenous ligands that can activate downstream receptors (54). Data of the literature suggest possibility of an indirect interaction between opioids and cannabinoids; activation of cannabinoid receptors may induce the release of opioid peptides. *E.g.* intrathecal administration of anandamide, delta9-tetrahydrocannabinol (THC) and (-)-3-[2-hydroxy-4-(1,1-dimethyheptyl)ptyl)phenyl]-4-(3-hydr oxypropyl)-cicloexan-1-ol (CP55,940) induced spinal antinociception accompanied by differential kappa-opioid receptor involvement and dynorphin A peptide release (55). Others showed that while delta9-tetrahydrocannabinol releases dynorphin A and leucin-enkephalin (56, 57), anandamide failed to induce the release of dynorphin A (56).

Our present findings demonstrated first a cannabinoid-opioid interaction in centrally-induced gastric mucosal protection. Opioid peptides can induce gastric mucosal protection given both peripherally (58) and centrally (11, 12). It was shown that naloxone given centrally antagonized the gastroprotective effect of anandamide and WIN55,212-2 injected i.c.v., the effect of methanandamide was only slightly affected. Since the centrally injected naloxone also inhibited the mucosal protective effect of intravenously injected anandamide, methanandamide and WIN55,212-2, the interaction may be located primarily in the CNS. Our findings confirmed that the interaction between cannabinoid and opioid system is likely to be indirect, namely endomorphin-2 antiserum reduced the protective effect of anandamide in a significant manner suggesting that anandamide may induce the release of endomorphin-2. Endomorphin-2 and endomorphin-1 are μ-opioid receptor selective endogenous opioids (59), however, endomorphin-2 can induce the release of other endogenous opioids like dynorphine (60) and [Met5]enkephalin (61). This may explain partly that both the κ-opioid receptor antagonist norBNI and the δ-opioid receptor antagonist naltrindole reduced the gastroprotective effect of anandamide. However, it also can be raised that anandamide itself induce the release of dynorphine or enkephalin, though the data of the literature is contradictory in this respect (55, 56).

The precise site of action of the centrally-initiated gastroprotection has not been clarified. The dorsal vagal complex is supposed to play an important role in centrally induced gastroprotection as it is suggested by the data of the literature (13-15) and our previous findings (11, 12). It may be speculated that the site of action of the cannabinoid-induced gastroprotection and the interaction between cannabinoid-opioid system in gastric mucosal defense is the dorsal vagal complex, since: i) cannabinoid CB_1 receptors are located in this area (28, 29), ii) different opioid peptides were identified in the DVC, *e.g.* expression of preproenkephalin and preprodynorphin messenger RNA was described in this region (62), β-endorphin is synthetized in the nucleus tractus solitarii (besides arcuate nucleus, from where endorphin-containing fibers project to the NTS (63)) and also endomorphin-1 and endomorphin-2 has been found in this area (18), iii) endomorphin-2 antiserum given intracisternally decreased the mucosal protective effect of anandamide that was given. i.c.v. The above data on co-localisation of ligands and receptors of cannabinoid and opioid system may serve a basis for a potential interaction between this two systems. It may be hypothetised that activation of CB_1 receptors in DVC (or hypothalamus) directly or indirectly through the release of endogenous opioids (or by both mechanisms) initiates a chain of events which results in gastric protection against mucosal injury. Previous studies suggested that gastroprotection can be induced by low level of central vagal stimulation, and the consequent release of NO, PG and CGRP (64, 65). Experiments are in progress on the role of vagal nerve in the gastroprotective effect of cannabinoids.

In conclusion, it was first demonstrated that cannabinoids induce gastric mucosal protection against ethanol-induced lesions by activation of central CB_1 receptors. The gastroprotective effect may be mediated at least partly by endogenous opioids, since naloxone as well as endomorphin-2 antiserum decreased the

protective effect anandamide. Further experiments are needed to clarify the mechanism of the gastroprotective action of cannabinoids in the periphery.

Acknowledgements: The authors wish to thank Mrs. I. Szalai and Mrs. I. Wachtl for their technical assistance.

The work was supported by ETT 529/2006 from the Scientific Health Council and National Office for Research and Technology (NKTH), Hungary, provided *via* Szentagothai Knowledge Centre.

Conflict of interests: None declared.

REFERENCES

1. Cook DJ, Fuller HD, Guyatt GH, *et al.* Risk factors for gastrointestinal bleeding in critically ill patients. Canadian Critical Care Trials Group. *N Engl J Med* 1994; 330: 377-381.
2. Faisy C, Guerot E, Diehl JL, Iftimovici E, Fagon JY. Clinically significant gastrointestinal bleeding in critically ill patients with and without stress-ulcer prophylaxis. *Intensive Care Med* 2003; 29: 1306-1313.
3. Yacyshyn BR, Thomson AB. Critical review of acid suppression in nonvariceal, acute, uppergastrointestinal bleeding. *Dig Dis* 2000; 18: 117-128.
4. Conrad SA. Acute upper gastrointestinal bleeding in critically ill patients: causes and treatment modalities. *Crit Care Med* 2002; 30: S365-S368.
5. Sachs G, Chang HH, Rabon E, Schackman R, Lewin M, Saccomani G. A nonelectrogenic H+ pump in plasma membranes of hog stomach. *J Biol Chem* 1976; 251: 7690-7698.
6. Konturek SJ, Konturek PC, Pawlik T, Sliwowski Z, Ochmanski W, Hahn EG. Duodenal mucosal protection by bicarbonate secretion and its mechanisms. *J Physiol Pharmacol* 2004; 55(Suppl 2): 5-17.
7. Konturek SJ, Konturek PC, Brzozowski T, Bubenik GA. Role of melatonin in upper gastrointestinal tract. *J Physiol Pharmacol* 2007; 58(Suppl 6): 23-52.
8. Holzer P. Neural emergency system in the stomach. *Gastroenterology* 1998; 114: 823-839.
9. Kwiecien S, Pawlik MW, Sliwowski Z, *et al*. Involvement of sensory afferent fibers and lipid peroxidation in the pathogenesis of stress-induced gastric mucosa damage. *J Physiol Pharmacol* 2007; 58(Suppl 3): 149-162.
10. Whittle BJ, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. *Br J Pharmacol* 1990; 99: 607-611.
11. Gyires K, Ronai AZ, Mullner K, Furst S. Intracerebroventricular injection of clonidine releases beta-endorphin to induce mucosal protection in the rat. *Neuropharmacology* 2000; 39: 961-968.
12. Gyires K, Ronai AZ. Supraspinal delta- and mu-opioid receptors mediate gastric mucosal protection in the rat. *J Pharmacol Exp Ther* 2001; 297: 1010-1015.
13. Kaneko H, Mitsuma T, Nagai H, *et al.* Central action of adrenomedullin to prevent ethanol-induced gastric injury through vagal pathways in rats. *Am J Physiol* 1998; 274: R1783-R1788.
14. Tache Y, Yoneda M, Kato K, Kiraly A, Suto G, Kaneko H. Intracisternal thyrotropin-releasing hormone-induced vagally mediated gastric protection against ethanol lesions: central and peripheral mechanisms. *J Gastroenterol Hepatol* 1994; 9(Suppl 1): S29-S35.
15. Yang H, Kawakubo K, Tache Y. Intracisternal PYY increases gastric mucosal resistance: role of cholinergic, CGRP, and NO pathways. *Am J Physiol* 1999; 277: G555-G562.
16. Diz DI, Barnes KL, Ferrario CM. Functional characteristics of neuropeptides in the dorsal medulla oblongata and vagus nerve. *Fed Proc* 1987; 46: 30-35.
17. Paxinos G, Chai SY, Christopoulos G, *et al.* In vitro autoradiographic localization of calcitonin and amylin binding sites in monkey brain. *J Chem Neuroanat* 2004; 27: 217-236.
18. Martin-Schild S, Gerall AA, Kastin AJ, Zadina JE. Differential distribution of endomorphin 1- and endomorphin 2-like immunoreactivities in the CNS of the rodent. *J Comp Neurol* 1999; 405: 450-471.
19. Boom A, Mollereau C, Meunier JC, *et al.* Distribution of the nociceptin and nocistatin precursor transcript in the mouse central nervous system. *Neuroscience* 1999; 91: 991-1007.
20. Boghossian S, Lecklin A, Dube MG, Kalra PS, Kalra SP. Increased leptin expression in the dorsal vagal complex suppresses adiposity without affecting energy intake and metabolic hormones. *Obesity (Silver Spring)* 2006; 14: 1003-1009.
21. Hou Z, Miao Y, Gao L, Pan H, Zhu S. Ghrelin-containing neuron in cerebral cortex and hypothalamus linked with the DVC of brainstem in rat. *Regul Pept* 2006; 134: 126-131.
22. Date Y, Ueta Y, Yamashita H, *et al.* Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci USA* 1999; 96: 748-753.
23. Guidobono F, Pagani F, Ticozzi C, Sibilia V, Netti C. Investigation on the mechanisms involved in the central protective effect of amylin on gastric ulcers in rats. *Br J Pharmacol* 1998; 125: 23-28.
24. Brzozowski T, Konturek PC, Sliwowski Z, *et al.* Neural aspects of ghrelin-induced gastroprotection against mucosal injury induced by noxious agents. *J Physiol Pharmacol* 2006; 57(Suppl 6): 63-76.
25. Konturek SJ, Brzozowski T, Konturek PC, *et al.* Brain-gut and appetite regulating hormones in the control of gastric secretion and mucosal protection. *J Physiol Pharmacol* 2008; 59(Suppl 2): 7-31.
26. Morini G, De Caro G, Guerrini R, Massi M, Polidori C. Nociceptin/orphanin FQ prevents ethanol-induced gastric lesions in the rat. *Regul Pept* 2005; 124: 203-207.
27. Zadori ZS, Shujaa N, Koles L, Kiraly KP, Tekes K, Gyires K. Nocistatin and nociceptin given centrally induce opioid-mediated gastric mucosal protection. *Peptides* 2008; 29: 2257-2265.
28. Partosoedarso ER, Abrahams TP, Scullion RT, Moerschbaecher JM, Hornby PJ. Cannabinoid 1 receptor in the dorsal vagal complex modulates lower oesophageal sphincter relaxation in ferrets. *J Physiol* 2003; 550: 149-158.
29. Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb Exp Pharmacol* 2005; 299-325.
30. Castelli MP, Piras AP, Melis T, *et al.* Cannabinoid CB1 receptors in the paraventricular nucleus and central control of penile erection: immunocytochemistry, autoradiography and behavioral studies. *Neuroscience* 2007; 147: 197-206.
31. Grijalva CV, Novin D. The role of the hypothalamus and dorsal vagal complex in gastrointestinal function and pathophysiology. *Ann NY Acad Sci* 1990; 597: 207-222.
32. Swanson LW, Kuypers HG. A direct projection from the ventromedial nucleus and retrochiasmatic area of the hypothalamus to the medulla and spinal cord of the rat. *Neurosci Lett* 1980; 17: 307-312.
33. Izzo AA, Mascolo N, Capasso R, Germano MP, De Pasquale R, Capasso F. Inhibitory effect of cannabinoid agonists on

gastric emptying in the rat. *Naunyn Schmiedebergs Arch Pharmacol* 1999; 360: 221-223.
34. Krowicki ZK, Moerschbaecher JM, Winsauer PJ, Digavalli SV, Hornby PJ. Delta9-tetrahydrocannabinol inhibits gastric motility in the rat through cannabinoid CB_1 receptors. *Eur J Pharmacol* 1999; 371: 187-196.
35. Izzo AA, Pinto L, Borrelli F, Capasso R, Mascolo N, Capasso F. Central and peripheral cannabinoid modulation of gastrointestinal transit in physiological states or during the diarrhoea induced by croton oil. *Br J Pharmacol* 2000; 129: 1627-1632.
36. Adami M, Frati P, Bertini S, *et al.* Gastric antisecretory role and immunohistochemical localization of cannabinoid receptors in the rat stomach. *Br J Pharmacol* 2002; 135: 1598-1606.
37. Coruzzi G, Adami M, Coppelli G, Frati P, Soldani G. Inhibitory effect of the cannabinoid receptor agonist WIN 55,212-2 on pentagastrin-induced gastric acid secretion in the anaesthetized rat. *Naunyn Schmiedebergs Arch Pharmacol* 1999; 360: 715-718.
38. Adami M, Zamfirova R, Sotirov E, *et al.* Gastric antisecretory effects of synthetic cannabinoids after central or peripheral administration in the rat. *Brain Res Bull* 2004; 64: 357-361.
39. Sofia RD, Diamantis W, Harrison JE, Melton J. Evaluation of antiulcer activity of delta9-tetrahydrocannabinol in the Shay rat test. *Pharmacology* 1978; 17: 173-177.
40. De Souza H, Trajano E, de Carvalho FV, Palermo NJ. Effects of acute and long-term cannabis treatment of restraint-induced gastric ulceration in rats. *Jpn J Pharmacol* 1978; 28: 507-510.
41. Dembinski A, Warzecha Z, Ceranowicz P, *et al.* Cannabinoids in acute gastric damage and pancreatitis. *J Physiol Pharmacol* 2006; 57(Suppl 5): 137-154.
42. Germano MP, D'Angelo V, Mondello MR, *et al.* Cannabinoid CB1-mediated inhibition of stress-induced gastric ulcers in rats. *Naunyn Schmiedebergs Arch Pharmacol.* 2001; 363: 241-244.
43. Rutkowska M, Fereniec-Goltbiewska L. ACEA (arachidonyl-2-chloroethylamide), the selective cannabinoid CB_1 receptor agonist, protects against aspirin-induced gastric ulceration. *Pharmazie* 2006; 61: 341-342.
44. Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. *Gastroenterology* 1979; 77: 433-443.
45. Gyires K. Morphine inhibits the ethanol-induced gastric damage in rats. *Arch Int Pharmacodyn Ther* 1990; 306: 170-181.
46. Noble EP, Wurtman RJ, Axelrod J. A simple and rapid method for injecting H3-norepinephrine into the lateral ventricle of the rat brain. *Life Sci* 1967; 6: 281-291.
47. Ueda H, Amano H, Shiomi H, Takagi H. Comparison of the analgesic effects of various opioid peptides by a newly devised intracisternal injection technique in conscious mice. *Eur J Pharmacol* 1979; 56: 265-268.
48. Ronai AZ, Kiraly K, Szebeni A, *et al.* Immunoreactive endomorphin 2 is generated extracellularly in rat isolated L4,5 dorsal root ganglia by DPP-IV. *Regul Pept* 2009; 157: 1-2.
49. Szemenyei E, Barna I, Mergl Z, *et al.* Detection of a novel immunoreactive endomorphin 2-like peptide in rat brain extracts. *Regul Pept* 2008; 148: 54-61.
50. Landi M, Croci T, Rinaldi-Carmona M, Maffrand JP, Le Fur G, Manara L. Modulation of gastric emptying and gastrointestinal transit in rats through intestinal cannabinoid CB(1) receptors. *Eur J Pharmacol* 2002; 450: 77-83.
51. Gomez R, Navarro M, Ferrer B, *et al.* A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *J Neurosci* 2002; 22: 9612-9617.
52. Reis GM, Pacheco D, Perez AC, Klein A, Ramos MA, Duarte ID. Opioid receptor and NO/cGMP pathway as a mechanism of peripheral antinociceptive action of the cannabinoid receptor agonist anandamide. *Life Sci* 2009; 85: 351-356.
53. Hasanein P. The endocannabinoid transport inhibitor AM404 modulates nociception in cholestasis. *Neurosci Lett* 2009; 462: 230-234.
54. Bushlin I, Rozenfeld R, Devi LA. Cannabinoid-opioid interactions during neuropathic pain and analgesia. *Curr Opin Pharmacol* 2009; Oct 24: epub ahead of print.
55. Mason DJ, Jr., Lowe J, Welch SP. Cannabinoid modulation of dynorphin A: correlation to cannabinoid-induced antinociception. *Eur J Pharmacol* 1999; 378: 237-248.
56. Welch SP, Eads M. Synergistic interactions of endogenous opioids and cannabinoid systems. *Brain Res* 1999; 848: 183-190.
57. Welch SP. Interaction of the cannabinoid and opioid systems in the modulation of nociception. *Int Rev Psychiatry* 2009; 21: 143-151.
58. Gyires K, Ronai AZ, Toth G, Darula Z, Furst S. Analysis of the role of delta opioid receptors in gastroprotection in the rat. *Life Sci* 1997; 60: 1337-1347.
59. Zadina JE, Hackler L, Ge LJ, Kastin AJ. A potent and selective endogenous agonist for the mu-opiate receptor. *Nature* 1997; 386: 499-502.
60. Tseng LF, Narita M, Suganuma C, *et al.* Differential antinociceptive effects of endomorphin-1 and endomorphin-2 in the mouse. *J Pharmacol Exp Ther* 2000; 292: 576-583.
61. Ohsawa M, Shiraki M, Mizoguchi H, *et al.* Release of [Met5]enkephalin from the spinal cord by intraventricularly administered endomorphin-2, but not endomorphin-1 in the anesthetized rat. *Neurosci Lett* 2001; 316: 1-4.
62. Rutherfurd SD, Gundlach AL. Opioid peptide gene expression in the nucleus tractus solitarius of rat brain and increases induced by unilateral cervical vagotomy: implications for role of opioid neurons in respiratory control mechanisms. *Neuroscience* 1993; 57: 797-810.
63. Palkovits M, Eskay RL. Distribution and possible origin of beta-endorphin and ACTH in discrete brainstem nuclei of rats. *Neuropeptides* 1987; 9: 123-137.
64. Kato K, Yang H, Tache Y. Role of peripheral capsaicin-sensitive neurons and CGRP in central vagally mediated gastroprotective effect of TRH. *Am J Physiol* 1994; 266: R1610-R1614.
65. Kiraly A, Suto G, Tache Y. Role of nitric oxide in the gastric cytoprotection induced by central vagal stimulation. *Eur J Pharmacol* 1993; 240: 299-301.

Received: October 15, 2009
Accepted: December 11, 2009

Author's address: Dr. Klara Gyires, Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, 1089 Nagyvarad ter 4., Budapest, Hungary; E-mail: gyirkla@net.sote.hu