ELSEVIER

European Journal of Pharmacology 374 (1999) 263–276

ejp

www.elsevier.nl/locate/ejphar

Leptin in gastroprotection induced by cholecystokinin or by a meal. Role of vagal and sensory nerves and nitric oxide

Tomasz Brzozowski [a], Peter Ch. Konturek [b,*], Stanisław J. Konturek [a], Robert Pajdo [a], Aleksandra Duda [a], Piotr Pierzchalski [a], Władysław Bielański [a], Eckhart G. Hahn [b]

[a] *Department of Physiology, Jagiellonian University School of Medicine, ul. Grzegorzecka 16, 31-531 Cracow, Poland*
[b] *Department of Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany*

Received 4 February 1999; received in revised form 21 April 1999; accepted 26 April 1999

Abstract

Leptin, detected recently in the stomach, is a product of the *ob* gene released by cholecystokinin (CCK) and plays an important role in the control of food intake but its influence on gastroprotection against the damage caused by noxious agents has not been studied. This study was designed to compare the effects of leptin and cholecystokinin-8 (CCK-8) on gastric mucosal lesions induced by topical application of 75% ethanol or acidified aspirin. Four series of Wistar rats (A, B, C and D) were used to determine the effects of: (A) suppression of prostaglandin biosynthesis by indomethacin (5 mg/kg i.p.); (B) inhibition of nitric oxide (NO)-synthase by nitro-L-arginine methyl ester (L-NAME) (5 mg/kg i.v.); (C) blockade of sensory nerves by capsaicin (125 mg/kg s.c.) and (D) bilateral vagotomy, on the gastric lesions induced by intragastric (i.g.) application of ethanol with or without pretreatment with CCK-8, a known gastroprotective substance or leptin. CCK-8 (1–100 μg/kg i.p.) and leptin (0.1–50 μg/kg i.p.) dose dependently attenuated gastric lesions induced by 75% ethanol; the dose reducing these lesions by 50% being about 10 μg/kg and 8 μg/kg, respectively. The protective effects of CCK-8 and leptin were accompanied by a significant rise in gastric blood flow (GBF) and luminal NO concentration. Leptin was also effective to attenuate aspirin-induced damage and the accompanying fall in the GBF, whereas CCK-8 dose dependently worsened aspirin damage and failed to influence GBF. CCK (1–100 μg/kg i.p.), given in graded doses, produced a dose-dependent increase in the plasma leptin level and a rise of the expression of *ob* messenger RNA (mRNA) in gastric mucosa, the maximum being reached at a dose of 100 μg/kg. Pretreatment with CCK-8 (10 μg/kg i.p.) or with 8% peptone, that is known to stimulate CCK release, also produced a significant rise in plasma leptin levels and up-regulation of *ob* mRNA while reducing significantly the gastric lesions induced by 75% ethanol to the same extent as that induced by exogenous leptin (10 μg/kg i.p.). Indomethacin, which suppressed prostaglandin generation by ~90%, failed to influence leptin- or CCK-8-induced protection against ethanol, whereas L-NAME attenuated significantly CCK-8- and leptin-induced protection and hyperemia but addition to L-NAME of L-arginine, but not D-arginine, restored the protective and hyperemic effects of both hormones. The *ob* mRNA was detected as a weak signal in the intact gastric mucosa and in that exposed to ethanol alone but this was further enhanced after treatment with graded doses of CCK-8 or peptone meal applied prior to ethanol. We conclude that: (1) exogenous leptin or that released endogenously by CCK or meal exerts a potent gastroprotective action depending upon vagal activity, and involving hyperemia probably mediated by NO and sensory nerves but unrelated to endogenous prostaglandins; (2) leptin mimics the gastroprotective effect of CCK and probably mediates the protective and hyperemic actions of CCK in the rat stomach.

Keywords: Leptin; Cholecystokinin; Peptone meal; Gastroprotection; Gastric blood flow; Gastric secretion; Sensory nerve; Nitric oxide (NO); Ethanol; Reverse transcriptase-polymerase chain reaction (RT-PCR)

1. Introduction

Leptin is accepted as a key peripheral protein product of the *ob* gene acting on central receptors for leptin (OB receptor) that control food intake and energy expenditure (Friedman and Halaas, 1998). Recent studies revealed the presence of leptin in the serum of experimental animals such as mice and rats as well as of humans (Shalev et al., 1997; Wang et al., 1997; Bado et al., 1998; Barbier et al., 1998; Houseknecht et al., 1998). Leptin is secreted by adipocytes and the placenta but recent study revealed that leptin messenger RNA (mRNA) and leptin protein are also

* Corresponding author. Tel.: +48-12-211006; Fax: +48-12-211578

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00314-3

present in the rat gastric epithelium, suggesting that the stomach might be an important source of this circulating hormone (Bado et al., 1998; Houseknecht et al., 1998). Moreover, Bado et al. (1998) demonstrated that feeding or cholecystokinin-8 (CCK-8) greatly increased the concentration of leptin in the plasma and this effect was associated with a fall of the leptin content in the gastric epithelium with a concomitant decrease in gastric mucosal leptin immunoreactivity. The physiological significance of leptin in the stomach and its contribution to gastric mucosal integrity and gastroprotection remain unknown. It was proposed that leptin acts centrally to inhibit neuropeptide Y, which is a peptide involved in the stimulation of appetite (Hakansson et al., 1998). Recent evidence indicates that leptin can act synergistically with CCK in the control of satiety and that plasma leptin levels are elevated after administration of exogenous CCK or following injection of cytokines such as interleukin-1 and tumor necrosis factor-α (TNF-α) (Barrachina et al., 1997; Matson et al., 1997; Barbier et al., 1998).

CCK is one of the principal physiological enterogastrones involved in the control of gastric secretion, gastric emptying, motility and food intake but its role in gastric mucosal integrity has only recently been recognized. It has been reported that CCK attenuates the gastric lesions induced by strong irritants such as ethanol (Stroff et al., 1994; Konturek et al., 1995a,b; Mercer et al., 1996). CCK exerts its physiological actions via specific CCK_A receptors, that were proposed to explain the mechanism of action of this enterogastrone on gastric mucosa (Silverman et al., 1987; Lloyd et al., 1992a,b; Corwin and Smith, 1993). The gastroprotective effect of CCK against the mucosal damage induced by ethanol has been attributed to the activation of specific CCK_A receptors and to the increase in gastric blood flow (GBF) evoked by this peptide (Konturek et al., 1995a,b; Mercer et al., 1996). Since the CCK-induced protection against ethanol damage was reversed by the pretreatment with CCK_A but not CCK_B receptor antagonists and by the denervation of afferent nerves with capsaicin, it was assumed that CCK_A receptors on sensory nerves are involved in CCK-induced gastroprotection (Evangelista and Maggi, 1991; Konturek et al., 1995b; Mercer et al., 1996). Moreover, peptone meal, that is known to raise plasma gastrin and CCK levels, also attenuated gastric lesions induced by strong irritants such as ethanol (Konturek et al., 1995a) but the contribution of leptin, that was shown to act synergistically with CCK in the regulation of food intake (Matson et al., 1997), to the mechanism of protection afforded by CCK, has not been studied. It is also not yet known whether leptin gene expression and the increase in the plasma concentration of this hormone contribute to the mechanism of CCK-induced gastroprotection against the damage induced by topical irritants.

Endogenous CCK was shown to influence gastric acid secretion (Konturek et al., 1992) but the effect of leptin on secretory activity of the stomach remains to be tested. Since both feeding and CCK can induce leptin release (Bado et al., 1998) the question arises as to whether this product of the *ob* gene may contribute to gastroprotective effect of CCK. However, no study has yet been undertaken to determine whether exogenous leptin or that released endogenously, for instance by CCK, can influence both the damage induced by corrosive substances such as ethanol and the GBF.

A potent vasodilator such as nitric oxide (NO), derived from L-arginine in the gastric mucosa, has recently been implicated in the mechanism of gastric mucosal defense and the gastroprotection afforded by certain hormones, including CCK (Stroff et al., 1994; Konturek et al., 1995a,b; Heinemann et al., 1996) but the involvement of NO in the gastroprotection induced by leptin has not been studied.

This study was designed: (1) to compare the effect of exogenous leptin and that released endogenously (by CCK or meal) with the effect of CCK-8 and peptone meal on the gastric lesions induced by 75% ethanol and GBF; (2) to examine the involvement of NO, sensory nerves, and endogenous prostaglandins in leptin- and CCK-8-induced gastroprotection against ethanol damage and (3) to determine the plasma levels of leptin and the expression of leptin mRNA in intact gastric mucosa and in that treated with CCK-8 with and without exposure to 75% ethanol.

2. Material and methods

Male Wistar rats, weighing 180–220 g and fasted for 24 h, were used in gastric secretory tests and in studies on gastroprotection. All experimental procedures were approved by the Jagiellonian University Institutional Animal Care and Use Committee.

2.1. Gastric secretory studies

The effects of leptin or CCK-8 (Sigma, St. Louis, MO, USA) on gastric acid secretion were examined in 20 conscious rats equipped about 1 month earlier with a gastric fistula as described previously (Brzozowski et al., 1996). The animals were fasted overnight but had free access to water for 24 h before the experiment and they were placed at 10 a.m. in individual Bollman type cages to maintain the minimum restraint necessary. The gastric fistula was opened and the stomach was rinsed gently with about 5 ml of tap water at 37°C. The basal gastric secretion was collected for 60 min and leptin was injected i.p. in various doses ranging from 0.1 to 50 μg/kg, each dose being administered on a separate test day. In control tests, vehicle (1 ml of saline, s.c.) or CCK-8 was given i.p. in graded doses starting from 1 to 100 μg/kg dissolved in the same volume (1 ml) as that used in tests with leptin. The collection of gastric juice was continued for the final 60 min. The volume and acid concentration of each sample

of gastric juice were measured and acid outputs (expressed in terms of mmol of acid per 30 min) and pepsin output (expressed in terms of mg per 30 min) were determined as described before (Brzozowski et al., 1996).

2.2. Gastroprotection studies and measurement of GBF

Acute gastric lesions were induced by intragastric (i.g.) application of 75% ethanol or acidified aspirin, similarly to the method described previously by our group for ethanol or aspirin as damaging agent (Brzozowski et al., 1992, 1997a). Briefly, 75% ethanol in a volume of 1.5 ml was administered i.g. to rats by means of a metal orogastric tube. Aspirin in a dose of 100 mg/kg was dispersed in 0.2 N HCl, also administered by orogastric tube. After 60 min, the animals were lightly anesthetized with ether, their abdomen was opened by a midline incision and stomach was exposed for the measurement of GBF by means of the hydrogen (H_2)-gas clearance technique described previously (Konturek et al., 1997). For this purpose double electrodes of an electrolytic regional blood flowmeter (Biotechnical Science, Model RBF-2, Osaka, Japan) were inserted into the gastric mucosa. One of these electrodes was used for the local generation of gaseous H_2 and another for the measurement of tissue H_2. With this method, the H_2 generated locally is carried out by the blood flow, while the polarographic current detector shows the decreasing tissue H_2. The tissue H_2 clearance curve was used to calculate an absolute flow rate (ml/100 g per min) in the oxyntic area as described previously (Konturek et al., 1997). The measurements were made in three areas of the mucosa and the mean values of the measurements were calculated and expressed as percent changes from the values recorded in the vehicle (saline) treated animals. After measurement of the GBF, the stomach was removed, rinsed with water and pinned open for macroscopic examination. The area of necrotic lesions in the oxyntic mucosa was determined by computerized planimetry (Morphomat, Carl Zeiss) (Konturek et al., 1995a,b) by a person who did not know to which experimental group the animals belonged.

2.3. Experimental groups of rats

In subsequent studies, two major series (A and B) of experiments were carried out. Series A was used to compare the effects of exogenous CCK-8 with those of leptin given i.p. on the mucosal lesions induced by 75% ethanol. In addition, series B was used to study the effect of endogenous prostaglandins, NO, vagal and sensory nerves on the protection afforded by leptin or CCK-8 against ethanol-induced mucosal damage.

The following groups of rats in series A were used (1) vehicle (1 ml of saline i.p.) followed 30 min later by 75% ethanol; (2) leptin (0.1–50 μg/kg i.p.) followed 30 min later by 75% ethanol; (3) CCK-8 (1–100 μg/kg i.p.) followed 30 min later by 75% ethanol; (4) CCK-8 (10 μg/kg i.p.) or leptin (10 μg/kg i.p.) followed 30 min later by 75% ethanol. In series B, the role of endogenous prostaglandins in the protection induced by leptin or CCK-8 was examined in two ways; (1) by the reversal of the gastroprotective activity of leptin or CCK by indomethacin and (2) by direct measurement of the mucosal generation of prostaglandin E_2 by radioimmunoassay (RIA) in tests with or without indomethacin. The following groups of rats were used; (1) vehicle (saline 1 ml i.p) followed 90 min later by 75% ethanol; (2) vehicle followed 60 min later by leptin or CCK-8 (both applied in a dose of 10 μg/kg i.p.) and then 30 min later by 75% ethanol; (3) indomethacin (5 mg/kg i.p.) followed 60 min later by vehicle (saline i.p.) and finally 30 min later by 75% ethanol; (4) indomethacin (5 mg/kg i.p.) followed 60 min later by leptin or CCK (10 μg/kg i.p.) and finally 30 min later by 75% ethanol.

At the termination of each experiment with vehicle, leptin or CCK-8 without or with pretreatment with indomethacin, mucosal biopsy samples of the oxyntic gland area (about 200 mg) were taken immediately after the animals had been killed by cervical dislocation to determine the mucosal generation of prostaglandin E_2 by specific RIA as described previously (Brzozowski et al., 1996). The mucosal sample was placed in preweighed Eppendorf vials and 1 ml of Tris buffer (50 mM/l, pH 9.5) was added to each vial. The samples were finely minced (for about 15 s) with scissors, washed and centrifuged for 10 s, and the pellet was resuspended in 1 ml of Tris. Each sample was then incubated on a vortex mixer for 1 min and centrifuged for 15 s. The pellet was weighed and the supernatant was transferred to a second Eppendorf vial containing indomethacin (10 mM) and kept at −20°C until RIA. Prostaglandin E_2 was measured in duplicate using RIA kits (New England Nuclear, Munich, Germany). The ability of the mucosa to generate prostaglandin E_2 was expressed in ng/g wet tissue weight.

2.4. Involvement of NO, vagal and sensory nerves in gastroprotection induced by leptin and CCK-8

The implication of NO in the gastroprotection induced by leptin and CCK-8 was determined in three ways: (1) with N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO-synthase (Whittle et al., 1990) injected i.v. without or with addition of L-arginine (both purchased from Sigma), the substrate of NO-synthase (Brzozowski et al., 1997b); (2) by indirect measurement of the NO synthase product i.e., NO in gastric lumen (Green et al., 1981); and (3) by addition to L-NAME of L-arginine, a substrate for NO-synthase or of D-arginine, which is not a substrate for NO-synthase. The following groups of rats were used; (1) vehicle (saline 1 ml i.v.) followed 60 min later by 75% ethanol; (2) vehicle (saline i.v.) followed 30 min later by leptin (10 μg/kg i.p.) or CCK-8 (10 μg/kg i.p.) and then 30 min later by 75% ethanol; (3) L-NAME (5 mg/kg i.v.) followed 15 min later by vehicle (saline s.c.)

and then 30 min later by 75% ethanol; (4) L-NAME (5 mg/kg i.v.) followed 15 min later by leptin (10 μg/kg i.p.) or CCK-8 (10 μg/kg i.p.) and then 30 min later by 75% ethanol; (5) L-arginine or D-arginine (300 mg/kg i.v.) followed 15 min later by L-NAME (5 mg/kg i.v.) and then 15 min later by leptin (10 μg/kg i.p) or CCK-8 (10 μg/kg i.p.) and finally 30 min later by 75% ethanol. The lumen concentration of NO was quantified indirectly as nitrate (NO_3^-) and nitrite (NO_2^-) levels in the gastric contents, using the nitrate/nitrite kit purchased from Cayman Lab., MI, USA as described in detail recently (Konturek et al., 1998). This method is based on the Griess reaction and generation of chromophore absorbing at 595 nm, according to the original procedure (Green et al., 1981). Since NO released by epithelial cells into the gastric lumen is quickly transformed into NO_3^- and NO_2^- (Moncada et al., 1991), we measured photometrically the sum of both these products of NO-synthase as an index of NO production by the enzyme in the gastric mucosa. For this purpose, the gastric contents were aspirated just before removal of the stomach following the i.g. injection of 1 ml of saline to wash out the lumen contents. After centrifugation for 10 min at 3000 rpm, the samples were mixed with Griess reagent from the commercially available kit. In all tests including those with vehicle (control), leptin and CCK-8, GBF was measured in the oxyntic mucosa of each group of animals as mentioned before and was expressed as a percent of the control value recorded in vehicle-treated gastric mucosa.

The involvement of vagal nerves in the gastroprotection by leptin or CCK-8 was studied in rats with or without vagotomy after cutting off vagal nerves as described previously (Lloyd et al., 1993). About 30 min before the experiment rats were anesthetized with ether and the abdomen was opened by a small incision. Both branches of the vagal nerves were identified and transected and finally cut off. The control rats were treated similarly except that the vagi were only uncovered but left intact. Vagotomized and sham-operated rats received leptin (10 μg/kg i.p.) or CCK-8 (10 μg/kg i.p.) and 30 min later 75% ethanol was introduced. One hour later the rats were killed and the area of gastric lesions and GBF were measured as mentioned above.

The role of sensory afferent nerves in the gastroprotection by leptin or CCK-8 was tested in rats with capsaicin-induced deactivation of these nerves. For this purpose the animals were pretreated with capsaicin (Sigma) injected s.c. for 3 consecutive days at a dose of 25, 50 and 50 mg/kg about 2 weeks before the experiment (Brzozowski et al., 1996). All injections of capsaicin were performed under ether anesthesia to counteract the respiratory impairment associated with injection of this agent. To check the effectiveness of the capsaicin denervation, a drop of 0.1 mg/ml solution of capsaicin was instilled into the eye of each rat and the protective wiping movements were counted as previously described (Whittle et al., 1990). Control rats received a vehicle injection. All animals pretreated with capsaicin showed a negative wiping movement test, confirming functional denervation of the capsaicin sensitive nerves. The following groups were used; (1) vehicle (saline 1 ml i.p.) followed 30 min later by 75% ethanol in rats with intact afferent nerves; (2) leptin (10 μg/kg i.p.) and CCK-8 (10 μg/kg i.p.) followed 30 min later by 75% ethanol in rats with intact sensory nerves; (3) vehicle (saline 1 ml s.c.) followed 30 min later by 75% ethanol in rats with capsaicin deactivated afferent nerves; (4) leptin (10 μkg i.p) or CCK-8 (10 μg/kg i.p.) followed 30 min later by 75% ethanol in rats with capsaicin deactivated afferent nerves.

At the termination of some experiments with i.p. administration of leptin or CCK-8 followed 30 min later by 75% ethanol, the rats were anesthetized with ether and blood samples (about 3 ml) were taken from the vena cava for the measurement of plasma leptin by RIA as described previously (Bado et al., 1998; Barbier et al., 1998). For comparison, intact rats fasted overnight and given only vehicle saline i.p. were also anaesthetized with ether and blood samples were collected for the determination of control values of leptin in plasma. The blood samples collected in heparin-coated polypropylene tubes were centrifuged at 3000 rpm for 20 min at 4°C, and the supernatant clear plasma was then stored at −80°C until measurement of plasma leptin using the RIA-kit for rat leptin from Linco Research (St. Charles, MO, USA) (Bado et al., 1998). Briefly, this RIA involved the competition of a rat leptin sample with ^{125}I-rat leptin tracer for binding to a specific rabbit antileptin polyclonal antibody. The limit of assay sensitivity was 0.05 ng/ml; the intra-assay variation was less than 7% and the interassay variation was less than 9%.

2.5. Reverse transcriptase-polymerase chain reaction (RT-PCR) for detection of mRNA for leptin

The stomachs were removed from rats treated with vehicle (control) and those treated with graded doses of CCK-8 with or without i.g. application of 75% ethanol for the determination of leptin mRNA expression by RT-PCR with specific primers. Gastric mucosal specimens were scraped off from oxyntic mucosa using a glass slide and were immediately snap frozen in liquid nitrogen and stored at −80°C until analysis. Total RNA was extracted from mucosal samples with a guanidinium isothiocyanate/phenol chloroform method, using a kit from Stratagene® (Heidelberg, Germany). The total RNAconcentration in each sample was determined by 1% agarose–formaldehyde gel electrophoresis and ethidium bromide staining. Aliquoted RNA samples were stored at −80°C until analysis. Single stranded complementary DNA (cDNA) was generated from 5 μg of total cellular RNA using StrataScript reverse transcriptase and oligo-(dT)-primers (Stratagene®). Briefly, 5 μg of total RNA was uncoiled by heating (65°C for 5 min) and then reversed by transcribing

into cDNA in a 50 μl reaction mixture that contained 50 U of Moloney murine leukemia virus reverse transcriptase (MMLV-RT), 0.3 mg oligo-(dT)-primer, 1 ml RNase Block Ribonuclease Inhibitor (40 U/μl), 2 ml of a 100 mmol/l mixture of deoxyadenosine triphosphate (dATP), deoxyribothymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP), 5 ml 10 × RT buffer (10 mmol/l Tris–HCl, pH = 8.3, 50 mM KCl, 5 mM $MgCl_2$). The resultant cDNA (2 μl) was amplified in a 50 μl reaction volume containing 0.3 ml (2.5 U) Taq polymerase, 200 mM (each) dNTP (Pharmacia, Germany), 1.5 mM/l $MgCl_2$, 5 ml 10 × polymerase chain reaction buffer (50 mM KCl, 10 mM Tris–HCl, pH = 8.3) and primers used at a final concentration of 0.5 mM. The mixture was overlaid with 25 μl of mineral oil to prevent evaporation. The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT) in the area dedicated for performing the PCR reaction. The nucleotide sequences of the primers for leptin and β-actin were based on the published cDNA encoding leptin and β-actin, respectively (Nudel et al., 1983; Bado et al., 1998). The sense primer for leptin was CTG CTC AAA GCC ACC ACC TCT G and the antisense primer was CCT GTG GCT TTG GTC CTA TCT G. The sense primer for β-actin was TTG TAA CCA ACT GGG ACG ATA TGG and for antisense, GAT CTT GAT CTT CAT GGT GCT AGG. The primers were synthesized by GIBCO BRL/Life Technologies, Eggenstein, Germany.

Polymerase chain reaction products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Location of a predicted product was confirmed by using DNA 100-bp ladder (GIBCO, Eggenstein, Germany) as a standard size marker.

The intensity of bands was quantified using densitometry (LKB Ultrascan, Pharmacia, Sweden) as we described in detail previously (Konturek et al., 1997). The signal for leptin mRNA was standardized against the β-actin signal for each sample and the results were expressed as leptin mRNA/β-actin mRNA ratio.

2.6. Statistical analysis

The results are expressed as means ± S.E.M. Statistical analysis was done using the non-parametric Mann–Whitney test and Friedman two-way analysis of variance. Differences with $P < 0.05$ were considered as significant.

3. Results

3.1. Effects of exogenous leptin and CCK on gastric secretion

The effects of vehicle or leptin applied i.p. in graded doses ranging from 0.1 to 50 μg/kg or CCK-8 injected s.c. in doses ranging from 1 to 100 μg/kg, on gastric acid and pepsin secretions from the gastric fistula in conscious rats are shown in Table 1. In control rats vehicle-treated, basal acid output averaged 121 ± 12 μmol/30 min and pepsin output reached the value of 0.93 ± 0.12 mg/30 min. Leptin given i.p. at concentrations of 0.1 μg/kg or higher failed to affect significantly gastric acid and pepsin secretions (Table 1). In contrast, CCK-8 was ineffective when injected i.p. in small doses of 0.1 or 1 μg/kg but when administered in a higher dose of 10 μg/kg, it significantly increased gastric acid and pepsin outputs by about 12% and 11%, respectively, as compared to vehicle-control animals. With increasing doses of CCK to 100 μg/kg i.p., a further significant rise in the gastric acid and pepsin outputs was observed as compared to those recorded in vehicle-treated rats or injected with 1 μg/kg of this peptide (Table 1).

3.2. Effect of exogenous leptin, CCK-8 and peptone meal on the ethanol induced lesions and the GBF and plasma leptin levels

As shown in Fig. 1, the pretreatment with leptin given i.p. reduced dose dependently the area of gastric lesions caused by 75% ethanol with the threshold reduction occurring at a dose of 1 μg/kg and with the ID_{50} averaging about 10 μg/kg of leptin (Fig. 1). The pretreatment with CCK-8 administered i.p. in graded doses ranging from 1 μg/kg up to 100 μg/kg also attenuated significantly the area of lesions induced by 75% ethanol with the ID_{50} averaging 8 μg/kg (Fig. 2). The protective effect of leptin and CCK-8 was accompanied by a significant and dose-dependent rise in GBF and plasma leptin levels. The GBF in the intact gastric mucosa averaged 48 ± 7 ml/min per

Table 1
Gastric acid and pepsin secretion in conscious rats equipped with a gastric fistula and injected i.p. with graded doses of leptin (0.1–50 μg/kg) or CCK-8 (0.1–100 μg/kg)

Type of test	Acid output (μM/30 min)	Pepsin output (mg/30 min)
Vehicle (control)	121 ± 12	0.93 ± 0.12
Leptin (μg / kg i.p.)		
0.1	116 ± 9	0.91 ± 0.09
1	120 ± 7	0.87 ± 0.06
10	110 ± 4	0.92 ± 0.07
50	102 ± 6[a]	0.80 ± 0.08[a]
CCK (μg / kg i.p.)		
0.1	126 ± 4	0.91 ± 0.05
1	132 ± 5	0.94 ± 0.06
10	144 ± 8[a]	1.05 ± 0.08[a]
100	146 ± 6[a,b]	1.12 ± 0.09[a,b]

Mean ± S.E.M. of 6–8 rats.
[a] Indicates significant change as compared to the value recorded in the vehicle-treated control rats.
[b] Indicates significant change as compared to the value obtained in test with CCK applied in a dose of 1 μg/kg.

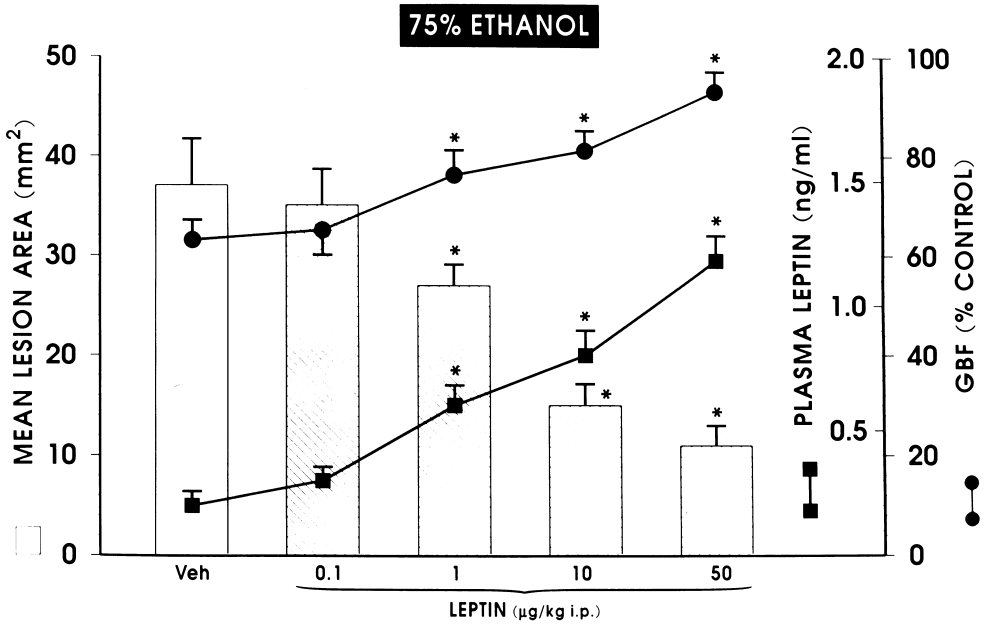

Fig. 1. The area of ethanol-induced gastric lesions, GBF and plasma immunoreactivity of leptin in rats treated with vehicle (saline) or with various doses of leptin (0.1–50 μg/kg i.p.). Means ± S.E.M. for 6–8 rats. Asterisk indicates a significant change as compared to the vehicle control values.

100 g (taken as a 100%), and this value was not significantly affected following i.p. application of vehicle (saline). When 75% ethanol was applied i.g. to vehicle-pretreated rats, a significant reduction in GBF by about 30% was recorded. With graded concentrations of leptin or CCK-8 administered before 75% ethanol, the area of gastric lesions was significantly reduced and a significant increase in the GBF starting with 1 μg/kg of leptin or CCK-8 was recorded (Fig. 2).

Fig. 3 shows the effect of pretreatment with 8% peptone meal applied i.g. in a volume of 1 ml on the area of gastric lesions induced by 75% ethanol and accompanying changes in GBF and plasma leptin levels. Pretreatment with 8% peptone significantly reduced the area of gastric lesions and the fall in GBF caused by 75% ethanol to an extent similar to that observed after the administration of exogenous leptin or CCK applied i.p. in a dose of 10 μg/kg. The plasma levels of immunoreactive leptin in the test

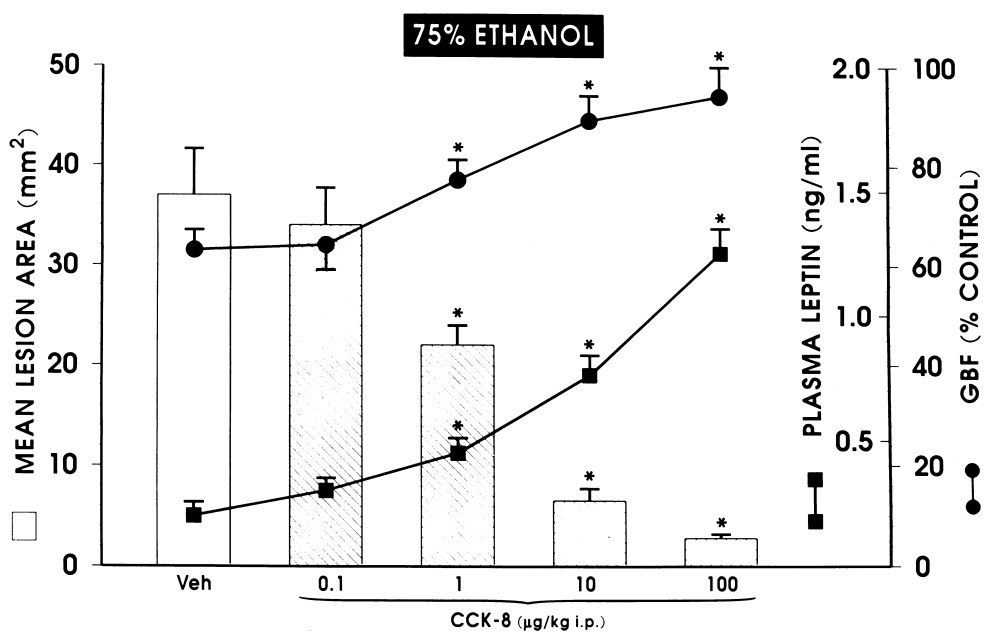

Fig. 2. The area of ethanol-induced gastric lesions, GBF and plasma immunoreactivity of leptin in rats treated with vehicle (saline) or with various doses of CCK (0.1–100 μg/kg i.p.). Means ± S.E.M. for 6–8 rats. Asterisk indicates a significant change as compared to the vehicle control values.

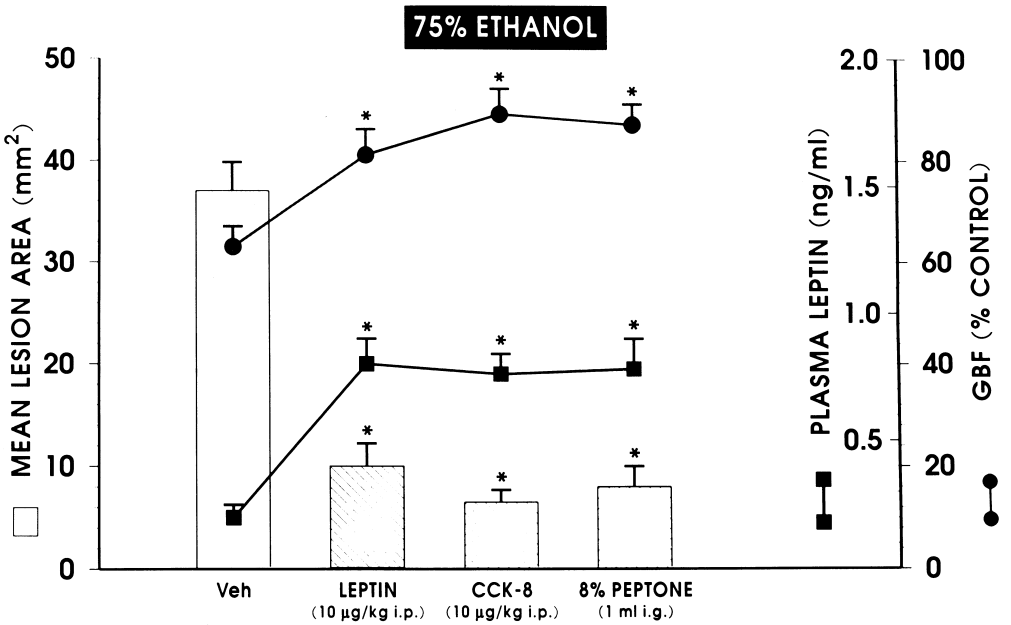

Fig. 3. Mean area of gastric lesions induced by 75% ethanol, changes in the GBF and the plasma leptin concentration in rats treated with vehicle (saline), leptin (10 μg/kg i.p.), CCK-8 (10 μg/kg i.p.) and 8% peptone meal (1 ml i.g.). Mean ± S.E.M. for 6–8 rats. Asterisk indicates a significant change as compared to the value obtained in vehicle-control animals without ethanol treatment.

with peptone meal were significantly elevated as compared to those in the respective control rats treated with vehicle (Fig. 3). In further studies, leptin was used i.p. in a standard dose of 10 μg/kg that caused over 80% reduction in the area of ethanol-induced lesions and an increase in plasma leptin to the level observed after the administration of exogenous CCK-8 (Fig. 3).

3.3. Effect of the suppression of prostaglandin biosynthesis on gastroprotective effects of leptin and CCK-8

As shown in Fig. 4, the administration of indomethacin (5 mg/kg i.p.) 90 min before i.g. application of 75% ethanol tended to increase the mean area of lesions induced by this topical irritant but this increase was not

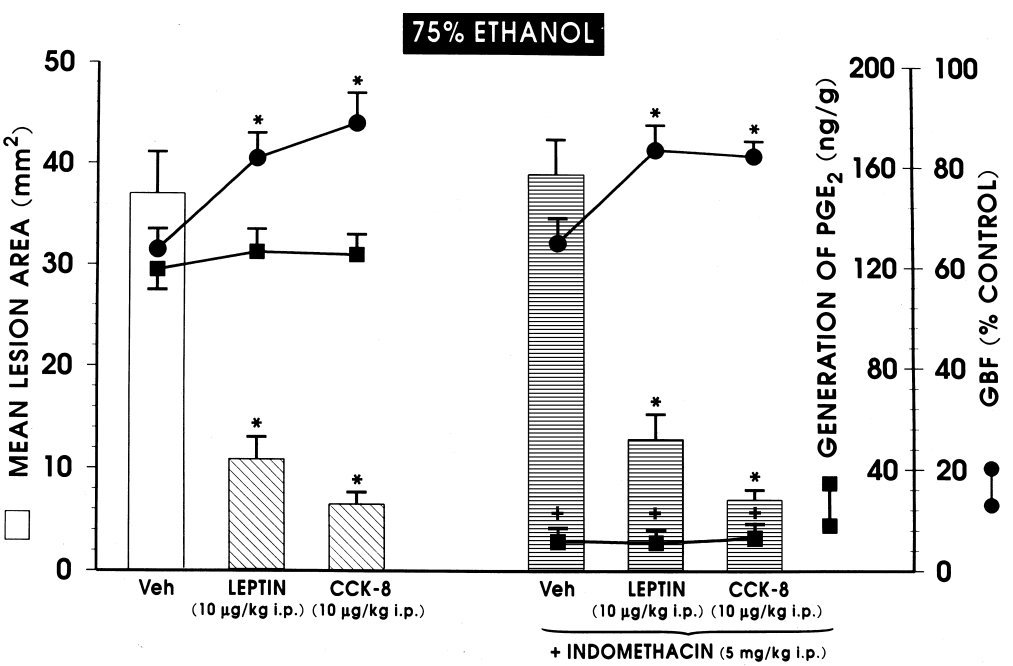

Fig. 4. Mean area of gastric lesions induced by 75% ethanol, changes in the GBF and the generation of prostaglandin E_2 in the gastric mucosa of rats treated with vehicle, leptin (10 μg/kg i.p.) and CCK (10 μg/kg i.p.) with or without pretreatment with indomethacin (5 mg/kg i.p.). Mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared to the value obtained in vehicle-control animals. Cross indicates a significant change as compared to the value obtained in rats without pretreatment with indomethacin.

Table 2
Mean area of aspirin-induced gastric lesions and GBF in rats pretreated with vehicle (Veh) or leptin applied i.p. in graded doses starting from 0.1 μg/kg to 50 μg/kg or in those pretreated with CCK-8 applied in i.p. in graded doses ranging from 0.1 μg/kg to 100 μg/kg

Type of test	Mean lesion area (mm^2)	GBF (% control)
Veh + acidified aspirin	56 ± 6	48 ± 7
Leptin (μg/kg i.p.) + acidified aspirin		
0.1	59 ± 4	46 ± 4
1	49 ± 6	56 ± 5
10	37 ± 5[a]	66 ± 6[a]
50	15 ± 2[a]	72 ± 4[a]
CCK-8 (μg/kg i.p.) + acidified aspirin		
0.1	59 ± 8	46 ± 3
1	67 ± 5	44 ± 6
10	69 ± 4[a]	40 ± 3
100	75 ± 5[a]	39 ± 2[a]

Results are means ± S.E.M. for 6–8 rats.
[a]Indicates significant change as compared to the value obtained in rats treated with vehicle (control).

statistically significant. The generation of prostaglandin E_2 in the vehicle-control gastric mucosa averaged 126 ± 18 ng/g of wet tissue weight and this was not significantly influenced by exposure of gastric mucosa to 75% ethanol (Fig. 4). Leptin or CCK applied i.p. in a standard dose of 10 μg/kg before 75% ethanol failed to influence significantly the generation of prostaglandin E_2 in the gastric mucosa as compared to that measured in rats with ethanol alone. The mucosal content of prostaglandin E_2 was decreased in all rats pretreated with indomethacin but despite this decrease the leptin- or CCK-afforded protection was observed in rats injected with indomethacin (Fig. 4).

Acidified aspirin given i.g. in a dose of 100 mg/kg, that was shown previously to induce total suppression of the mucosal prostaglandin E_2 content (Konturek et al., 1995b), produced gastric lesions with a mean area of 56 ± 6 mm^2 (Table 2). This damage was accompanied by about 50% reduction in the GBF as compared to the value recorded in intact mucosa. The pretreatment with CCK-8 applied in a standard dose of 10 μg/kg i.p. failed to affect significantly either the formation of gastric lesions or accompanying fall in GBF. With increasing the dose of CCK-8, a further significant augmentation of these lesions was observed and this effect was accompanied by a significant fall in the GBF. Leptin administered in a small dose of 0.1 μg/kg i.p. failed to affect either the area of aspirin-induced gastric lesions or the accompanying decrease in GBF. In contrast, higher doses, starting from 1 μg/kg of leptin attenuated significantly these lesions and significantly increased the GBF to above the value attained in aspirin-treated rats without leptin administration (Table 2).

3.4. Effect of suppression of NO-synthase, vagotomy and deactivation of sensory nerves by capsaicin on leptin- and CCK-8-induced gastroprotection

Table 3 shows the effects of L-NAME (5 mg/kg i.v.) on gastroprotection induced by leptin or CCK-8 (10 μg/kg i.p.) and accompanying changes in GBF. L-NAME alone did not induce mucosal lesions (data not shown) but significantly increased the area of ethanol-induced gastric lesions and produced a significantly greater fall in GBF than that measured in rats treated with ethanol alone. When L-NAME was injected prior to leptin, the area of lesions caused by ethanol was significantly increased and the GBF was significantly attenuated as compared to those in rats treated with leptin without the addition of L-NAME. A similar reversal of the protective effects of CCK-8 (10 μg/kg i.p.) against ethanol damage was observed when L-NAME was administered prior to CCK-8. Addition to L-NAME of L-arginine (300 mg/kg i.v.), but not D-arginine

Table 3
Mean area of gastric lesions induced by 75% ethanol, the GBF and generation of NO in rats treated with vehicle, leptin (10 μg/kg i.p.) or CCK-8 (10 ug/kg i.p.) without or with pretreatment with L-NAME (5 mg/kg i.v.) or combination of L-NAME plus L-arginine (300 mg/kg i.v.) or L-NAME plus D-arginine (300 mg/kg i.v.)

Type of test	Mean lesion area (mm^2)	GBF (% control)	Generation of NO (μM/l)
Vehicle	36 ± 4	61 ± 5	5.8 ± 0.8
Leptin	10 ± 3[a]	82 ± 5[a]	9.5 ± 1.4[a]
CCK-8	7 ± 1.8[a]	85 ± 6[a]	9.8 ± 1.9[a]
L-NAME	45 ± 3[b]	54 ± 3[b]	4.1 ± 0.3[b]
L-NAME + Leptin	34 ± 6[b]	58 ± 3[b]	4.3 ± 0.5[b]
L-NAME + CCK-8	36 ± 4[b]	54 ± 5[b]	4.6 ± 0.6[b]
L-Arginine + L-NAME + Leptin	12 ± 4[c]	80 ± 6[c]	8.7 ± 1.6[c]
L-Arginine + L-NAME + CCK-8	11 ± 3[c]	78 ± 4[c]	9.2 ± 2.1[c]
D-Arginine + L-NAME + Leptin	35 ± 4	62 ± 5	4.8 ± 0.7
D-Arginine + L-NAME + CCK-8	33 ± 5	64 ± 6	4.3 ± 0.6

Mean ± S.E.M. for 6–8 rats.
[a]Significant change as compared to the value obtained in vehicle-treated gastric mucosa.
[b]Significant change compared to the value obtained in rats without pretreatment with L-NAME.
[c]Significant change as compared to the value obtained in L-NAME-treated rats without addition of L-arginine.

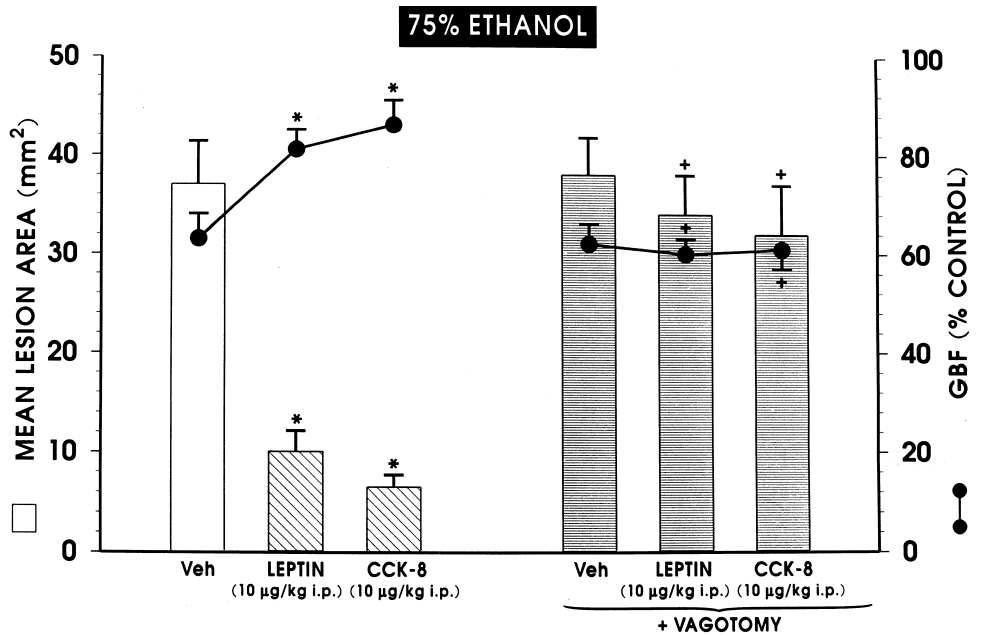

Fig. 5. Mean area of gastric lesions induced by 100% ethanol and GBF in rats with intact vagal nerves and those with vagotomy with or without pretreatment with vehicle (control), CCK-8 (10 μg/kg i.p.) or leptin (10 μg/kg i.p.). Mean ± S.E.M. for 6–8 rats. Asterisk indicates a significant change compared to the value in vehicle-treated rats. Cross indicates a significant change compared to the value obtained in rats without vagotomy.

(300 mg/kg. i.v.), restored the gastroprotective and hyperemic effects of leptin or CCK-8 in rats with ethanol-induced gastric lesions. Fig. 5 shows the effect of leptin and CCK-8 on gastric lesions induced by 100% ethanol in vagotomized rats and in rats with intact vagal nerves. Vagotomy failed to affect significantly ethanol lesions and GBF but attenuated significantly the protective and hyperemic activity of leptin and CCK-8. The reduction of ethanol lesions and accompanying increase in the GBF induced by leptin were almost completely abolished in

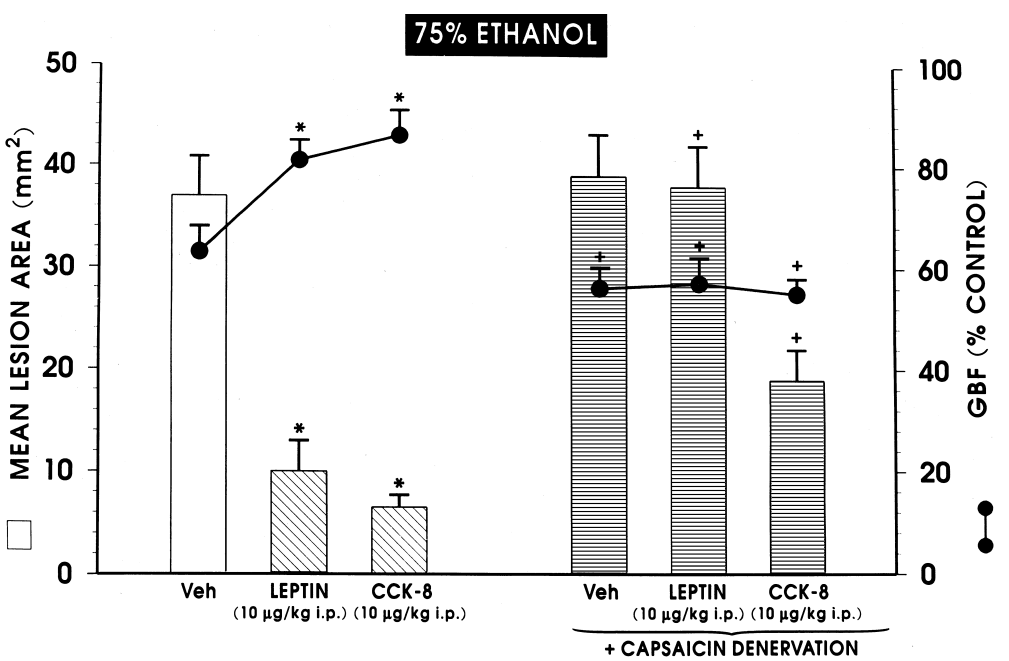

Fig. 6. Mean area of ethanol-induced gastric lesions and changes in GBF in gastric mucosa of rats with intact sensory nerves or those with capsaicin-deactivated sensory nerves with or without pretreatment with vehicle, leptin (10 μg/kg i.p.) or CCK-8 (10 μg/kg i.p.). Mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change compared to the value obtained in rats with intact sensory nerves pretreated with vehicle. Cross indicates a significant change as compared to the value obtained in rats without capsaicin denervation.

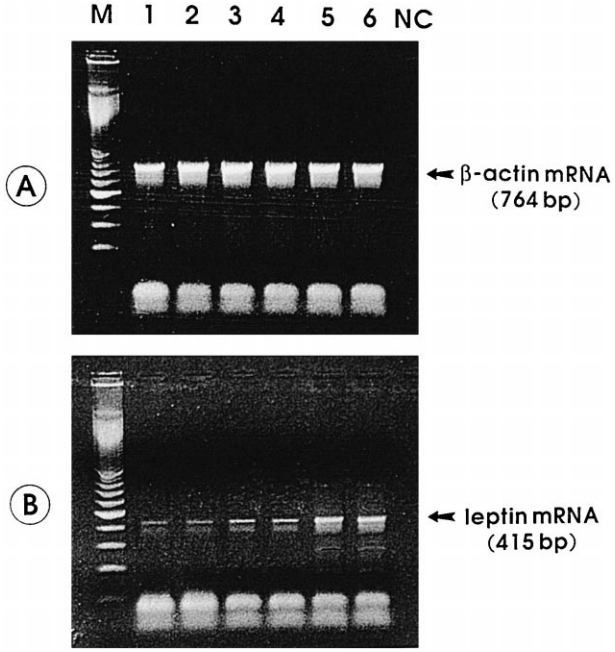

Fig. 7. Determination of (A) β-actin mRNA and (B) *ob* mRNA by RT-PCR in the intact gastric mucosa (lane 1) and in those exposed to ethanol alone (lane 2) and treated with CCK-8 given i.p. in graded doses of 1 μg/kg (lane 3), 10 μg/kg (lane 4), 100 μg/kg (lane 5), or with 8% peptone meal (lane 6). M—DNA size marker; NC—negative control.

vagotomized animals as compared to sham-operated controls with intact vagal nerves. The protection induced by CCK-8 against ethanol damage and the accompanying rise in GBF were also significantly attenuated in rats that underwent vagotomy. Deactivation of primary afferent nerves with parenteral pretreatment with capsaicin (about 2 weeks before the experiment) failed to affect significantly the area of ethanol lesions as compared to that in vehicle-treated rats but significantly reduced the GBF when compared to that in animals with intact sensory nerves (Fig. 6). Pretreatment of capsaicin-denervated rats with leptin or CCK-8 attenuated significantly the area of ethanol lesions and completely abolished the increase in GBF induced by both peptides as compared to those in rats with intact sensory nerves.

Fig. 7 shows the expression of *ob* mRNA measured with the RT-PCR technique in vehicle treated gastric mucosa and in that exposed to 75% ethanol without or with i.p. application of CCK-8 (1–100 μg/kg) or 8% peptone meal applied i.g. in a volume of 1 ml. The expression of *ob* mRNA was detected in vehicle-treated gastric mucosa and in those exposed to ethanol without or with the pretreatment with CCK-8 or 8% peptone meal. As a positive control, the rat β-actin was assayed in all mucosal specimens to verify the efficiency of cDNA synthesis from extracted RNA (Fig. 7A and B). The ratio of leptin mRNA to β-actin failed to show a significant change in ethanol-treated animals as compared to the value obtained in vehicle-treated gastric mucosa. In contrast, the ratio of leptin mRNA to β-actin showed a significant rise starting from the dose of 1 μg/kg of CCK and was further significantly enhanced by CCK-8 at the dose of 100 μg/kg or by 8% peptone meal (Fig. 8).

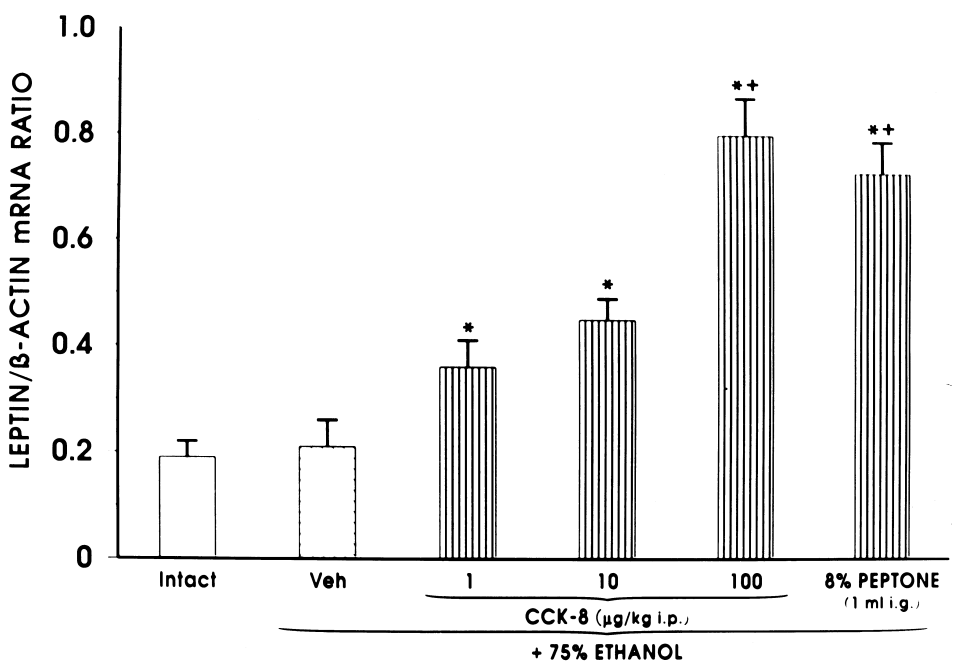

Fig. 8. The ratio of leptin mRNA over β-actin mRNA in experiments, as in Fig. 7. Asterisk indicates a significant change as compared to the value obtained in vehicle (control) gastric mucosa. Cross indicates a significant change as compared to the value obtained in gastric mucosa of rats pretreated with CCK-8 applied in the doses of 1 and 10 μg/kg.

4. Discussion

This study demonstrated for the first time that administration of exogenous leptin, that was accompanied by a significant increase in plasma levels of this peptide, showed a dose-dependent gastroprotective activity against the ethanol-induced lesions similar to that obtained with exogenous CCK. As this protection provided by leptin occurred without alterations in gastric acid secretion, our results indicate that leptin is a truly gastroprotective substance (Konturek et al., 1995b). We confirmed our and other previous observations (Lloyd et al., 1992a,b; Pendley et al., 1993; Stroff et al., 1994; Konturek et al., 1995a,b; Mercer et al., 1996) that pretreatment with CCK-8 applied in doses that have only a mild stimulatory effect on gastric acid secretion, also dose dependently attenuates mucosal lesions induced by ethanol and that this effect, like that observed with leptin, was accompanied by a significant and dose-dependent rise in GBF. As CCK, that caused an elevation of plasma leptin level the study present and a decrease of leptin contents in the gastric mucosa (Bado et al., 1998), enhanced the resistance of gastric mucosa to the noxious effect of ethanol it is reasonable to assume that leptin may be involved in the mechanism of CCK-induced gastroprotection.

The physiological importance of leptin in maintaining gastric integrity is supported by the fact that peptone meal, that is known to increase the release of CCK and to raise its plasma levels, also significantly elevated the concentrations of leptin in the plasma, while attenuating significantly ethanol-induced gastric lesions. These effects were similar to those achieved with exogenous CCK, resulting in similar increases in plasma leptin levels. The common feature of both leptin and CCK action is a significant rise in gastric lumen NO production, suggesting that the protective and hyperemic effects of both these hormonal peptides are mediated by gastric production of NO. This is confirmed by fact that the effects of leptin and CCK can be blocked by pretreatment with the L-NAME suppression of NO-synthase and reversed by addition of L-arginine, a NO-synthase substrate, to L-NAME. The excessive gastric NO production by leptin and CCK may derive from an over-expression of constitutive NO-synthase or up-regulation of inducible NO-synthase mRNA in gastric mucosa but the type of NO-synthase involved in this protection requires further studies. In contrast to NO, prostaglandins do not appear to contribute to the gastroprotection by leptin and CCK. Suppression of cyclooxygenase by indomethacin failed to influence the protective and hyperemic effects of exogenous leptin or CCK-8, confirming that endogenous prostaglandins are not involved in these effects. Since the protective and hyperemic effects of CCK were significantly attenuated by vagotomy and capsaicin deactivation of sensory afferent nerves, it is likely that the vago-vagal reflexes, possibly involving the release of NO and neuropeptides from these nerves, could contribute to the protection and the hyperemia observed after administration of CCK-8.

The gastroprotective and hyperemic effects of leptin applied intraperitoneally were also reduced by vagotomy and by functional ablation with capsaicin of sensory afferent nerves but it is not clear whether these effects originated predominantly from the local action of leptin in the stomach or also involved the central nervous system. A central, rather than peripheral, action of leptin on gastroprotection is evidenced by the relatively smaller effects of capsaicin denervation on the gastroprotection afforded by leptin than on that obtained with CCK. Further studies on the distribution of the receptors for leptin and CCK are needed to answer the question concerning the exact localization of the action of these receptors. It may be important to mention at this point that a functional synergistic interaction between CCK and leptin has been established, and it has been proposed that this interaction leads to early suppression of food intake which, like the gastroprotective activity described here, probably involves CCK_A and capsaicin-sensitive afferent fibers (Barrachina et al., 1997).

It has been shown previously that CCK is one of the major physiological enterogastrone-like substances mediating the secretory function of the stomach, such as an inhibition of gastric secretion by i.g. distension or intraduodenal fat (Corwin and Smith, 1993; Brand and Schmidt, 1995; Feinle et al., 1996; Brzozowski et al., 1998). We and others have demonstrated that the mechanism of the gastric acid stimulatory effects of exogenous and endogenous CCK are similar and depend upon the activation of CCK_A receptors (Corwin and Smith, 1993; Konturek et al., 1995a,b). A role of CCK_A receptors in the action of CCK was further supported by the observation that their blockade with highly selective antagonist, devazepine, completely reversed the CCK-induced inhibition of gastric acid secretion and that the immunoneutralization of endogenous somatostatin by administration of somatostatin monoclonal antibody abolished this inhibition (Lloyd et al., 1992a,b). All the above observations were, however, made in anesthetized animals, showing a relatively slight gastric secretory activity due to the inhibition by anesthetics as observed by Bastaki et al. (1995). Our present study on gastric secretion performed on conscious rats without anesthetics showed that exogenous CCK is a rather weak stimulant of gastric secretion, whereas leptin fails to affect this secretion, again indicating some differences, at least with respect to gastric secretory effects of exogenous CCK vs. leptin. The small but significant difference in the gastric secretory action of these peptides does not necessarily militate against the existence of a functional synergistic interaction between CCK and leptin in the suppression of food intake by these peptides (Barrachina et al., 1997; Matson et al., 1997). This synergistic interaction of CCK and leptin probably involves central gastric, rather than local, receptors for satiety signals (Banks et al., 1996; Barrachina et al., 1997).

Since aspirin- but not ethanol-induced gastric damage depends upon gastric acidity it is reasonable to assume that CCK, by enhancing gastric acid secretory activity, contributes to the enhanced formation of aspirin-induced damage due to increased acid secretion, while leptin, despite the failure to affect this secretion, was effective against both aspirin- and ethanol-induced damage. This indicates that leptin has a wider spectrum of gastroprotective activity than CCK and this protection extends to the damage induced by both acid-independent (ethanol) and acid-dependent mucosal irritants (aspirin).

This study attempted to assess the role of endogenous leptin in the mechanism of the protective action of CCK against the lesions provoked by 75% ethanol. We confirmed our previous observations that CCK provides dose-dependent protection against ethanol-induced damage and this protection is accompanied by an attenuation in the fall of GBF caused by ethanol (Konturek et al., 1995a,b). We found that this CCK-induced protection was accompanied by a dose-dependent increase in the plasma leptin level within the range of that observed after administration of exogenous leptin with a gastroprotection similar to that by CCK.

Previous studies demonstrated that the plasma leptin concentration is increased in early stages of intestinal inflammation, suggesting that proinflammatory cytokines could be responsible for leptin overexpression (Grunfeld et al., 1996; Sarraf et al., 1997; Barbier et al., 1998). This acute release of cytokines in the peripheral blood might be induced by endotoxins such as lipopolysaccharides that were also shown to produce a significant rise in the plasma leptin increases (Barbier et al., 1998). It has been speculated that peripherally generated inflammatory mediators and cytokines activate the hypothalamic–pituitary–adrenal axis, leading to an increase in circulating glucocorticosteroids which could then directly stimulate *ob* gene expression and the plasma leptin concentration (De Vos et al., 1995). Our present observation that *ob* mRNA is overexpressed in the stomach of CCK-treated rats invites the speculation that the rise in plasma leptin increases following treatment with CCK originates mainly from the gastric mucosa and plays an important role in the CCK-evoked gastroprotection against ethanol lesions due to local activation of the leptin gene and enhancement of the release of gastric leptin into the circulation. Whether leptin and CCK protect the mucosal cells directly may also be considered but this requires appropriate experimental evidence.

It was proposed that the inhibitory effect of CCK in the control of important physiological functions such as inhibition of gastric secretion or gastric emptying requires an intact vagal pathway because vagotomy abolished these functions (Schwortz et al., 1993). In our study, vagotomy also significantly attenuated the leptin- and CCK-afforded gastroprotection and the accompanying rise in the GBF, suggesting that vagal nerves are, indeed, an important pathway in the mediation of the protective action of these hormones. This suggestion is supported by our observation that leptin-induced gastroprotection and accompanying hyperemia were almost completely abolished in vagotomized rats, suggesting that intact vagal nerves are prerequisites for the protective activity of this peptide. Our results are consistent with the previous observation that the vasodilator response to low-dose CCK-8 was inhibited by acute bilateral subdiaphragmatic vagotomy and atropine (Heinemann et al., 1996). It was proposed, therefore, that the vasodilator effect of CCK-8 may be mediated by vago-vagal reflexes involving acetylcholine and NO as vasodilator messengers (Heinemann et al., 1996). We believe that the release of these mediators could be a reasonable explanation for the protective and hyperemic effects of this enterogastrone as well as leptin, observed in our present study.

It is of interest that the protective and hyperemic actions of CCK and leptin against ethanol damage were accompanied by a rise in the production of NO by gastric mucosa. L-NAME used in this study to achieve a potent but not specific suppression of NO-synthase activity, prevented the protective and hyperemic effects of both peptides and completely reversed the increase in luminal NO release produced by leptin and CCK-8. The co-administration of the substrate for NO-synthase activity L-arginine (Whittle et al., 1990; Brzozowski et al., 1997a,b), but not D-arginine, restored the protection, gastric hyperemia and luminal release of NO induced by leptin and CCK in animals pretreated with L-NAME. This supports our notion that NO plays a crucial role in both the mucosal hyperemic and gastroprotective effects afforded by CCK-8 and leptin.

Previous studies have demonstrated that prostaglandins and NO cooperate with sensory nerves to the mechanism of gastric mucosal integrity and cytoprotection (Whittle et al., 1990). It was postulated that peptides of the gastrin/CCK family participate in the mechanism of gastric mucosal integrity through the activation of sensory nerves releasing a variety of vasodilatator mediators such as calcitonin gene releasing peptide (CGRP) and tachykinins (Evangelista and Maggi, 1991; Holzer et al., 1991). Moreover, it was proposed that the protective action of CCK-8 on the gastric mucosa involves activation of CCK_A receptors localized on vagal capsaicin-sensitive sensory fibers (Evangelista and Maggi, 1991). In our study, the functional ablation of afferent sensory neurons with capsaicin greatly reduced the hyperemic activity of both leptin and CCK-8 but was more effective against the protection afforded by CCK-8 than that by leptin. This finding indicates that sensory nerves are essential for the microcirculatory response but appear to be of less importance in the mediation of protective effects induced by leptin. This observation is in keeping with the recent findings that the protective effect of CCK-8 was only modestly modified by capsaicin-induced deactivation of sensory nerves, thus limiting the role of sensory nerves in

this protection (Stroff et al., 1994; Konturek et al., 1995a). On the contrary, the protective and hyperemic effects of leptin were completely abolished in capsaicin-denervated animals, indicating that sensory afferent nerves are of crucial importance for the gastroprotective activity of this peptide.

In summary, these results provide the first evidence that leptin, probably originating from the stomach, is as effective as CCK to protect the gastric mucosa against the lesions induced by ethanol and that this peptide may contribute to the gastroprotective action of CCK. Furthermore, the overexpression of *ob* mRNA followed by an elevated plasma leptin concentration correlated well with CCK-induced protection, indicating that leptin release is an important component of the CCK control of gastric mucosal integrity and circulation. These protective and hyperemic effects of leptin appear to be dependent upon the vagal and afferent sensory nerves and NO, but appear to be unrelated to endogenous prostaglandin and gastric secretion.

Acknowledgements

The authors are grateful to Ms. Kinga Ogonowska for her invaluable editorial assistance in the preparation of this manuscript.

References

Bado, A., Levasseur, S., Attoub, S., Kermorgant, S., Laigneau, J.P., Bortoluzzi, M.N., Moizo, L., Lehy, T., Millo, M.G., Le Marchand-Brustel, Y., Lewin, M.J.M., 1998. The stomach is a source of leptin. Nature 394, 790–793.

Banks, W.A., Kastin, A.J., Huang, W., Jaspan, J.B., Maness, L.M., 1996. Leptin enters the brain by a saturable system independent of insulin. Peptides 17, 305–311.

Barbier, M., Cherbut, C., Aube, A.C., Blottiere, H.M., Galmiche, S., 1998. Elevated plasma leptin concentrations in early stages of experimental intestinal inflammation in rats. Gut 43, 783–790.

Barrachina, M.D., Martinez, V., Wang, L., Wei, J.Y., Tache, Y., 1997. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. Proc. Natl. Acad. Sci. U.S.A. 94, 10455–10460.

Bastaki, S.M., Waton, G., Garner, A., 1995. Effects of anaesthetic agents on basal and histamine-stimulated acid secretion in the fistula rat. Eur. J. Gastroenterol. Hepatol. 7, 1199–1202.

Brand, S.J., Schmidt, W.E., 1995. Gastrointestinal hormones. In: Yamada, Tadataka (Ed.), Textbook of Gastroenterology, Second edn., JB Lippincott, Philadelphia.

Brzozowski, T., Drozdowicz, D., Majka, J., Konturek, S.J., 1992. Studies on gastroprotection induced by capsaicin and papaverine. J. Physiol. Pharmacol. 43, 309–322.

Brzozowski, T., Konturek, S.J., Sliwowski, Z., Pytko-Polonczyk, J., Szlachcic, A., Drozdowicz, D., 1996. Role of capsaicin-sensitive sensory nerves in gastroprotection against acid independent and acid-dependent ulcerogens. Digestion 57, 424–432.

Brzozowski, T., Konturek, S.J., Śliwowski, Z., Pajdo, R., Drozdowicz, D., Stachura, J., 1997a. Role of β-adrenoceptors in gastric mucosal integrity and gastroprotection induced by epidermal growth factor. Digestion 58, 319–331.

Brzozowski, T., Konturek, S.J., Sliwowski, Z., Drozdowicz, D., Zaczek, M., Kedra, D., 1997b. Role of L-arginine, a substrate for nitric oxide-synthase, in gastroprotection and ulcer healing. J. Gastroenterol. 32, 442–452.

Brzozowski, T., Konturek, P.Ch., Konturek, S.J., Kwiecien̊, S., Pajdo, R., Brzozowska, I., Hahn, E.G., 1998. Involvement of endogenous cholecystokinin and somatostatin in gastroprotection induced by intraduodenal fat. J. Clin. Gastroenterol. 27, S125–S137, (Suppl. 1).

Corwin, R.L., Smith, G.P., 1993. Different effects of CCK A antagonists on gastric-acid response to CCK and pentagastrin. Peptides 14, 253–257.

De Vos, P., Saladin, R., Auwerx, J., 1995. Induction of *ob* gene expression by corticosteroids is accomplished by body weight loss and reduced food intake. J. Biol. Chem. 270, 15958–15961.

Evangelista, S., Maggi, C.A., 1991. Protection induced by cholecystokinin-8 (CCK-8) in ethanol-induced gastric lesions is mediated via vagal capsaicin-sensitive fibers and CCK receptors. Br. J. Pharmacol. 102, 119–122.

Feinle, C., D'Amato, M., Read, N.W., 1996. Cholecystokinin A receptors modulate gastric sensory and motor responses to gastric distension and duodenal lipid. Gastroenterology 110, 1379–1385.

Friedman, J.M., Halaas, J.L., 1998. Leptin and the regulation of body weight in mammals. Nature 395, 763–770.

Green, L., Tannenbaum, S.R., Goldman, P., 1981. Nitrate synthesis in the germ-free and conventional rat. Science 212, 56–58.

Grunfeld, C., Zhao, C., Fuller, J., 1996. Endotoxin and cytokines induce expression of leptin, the *ob* gene product in hamsters. J. Clin. Invest. 97, 2152–2157.

Hakansson, M.L., Brown, H., Ghilardi, N., Skoda, R.C., Meister, B., 1998. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J. Neurosci. 18, 559–572.

Heinemann, A., Jocic, M., Peskar, B.M., Holzer, P., 1996. CCK-evoked hyperemia in rat gastric mucosa in involves neural mechanisms and nitric oxide. Am. J. Physiol. 270, 253–258.

Holzer, P., Livingston, E.H., Saria, A., Guth, P.H., 1991. Sensory nerves mediate protective vasodilatation in the rat gastric mucosa. Am. J. Physiol. 260, G363–G370.

Houseknecht, K.L., Baile, C.A., Matteri, R.L., Superlock, M.E., 1998. The biology of leptin: a review. J. Anim. Sci. 76, 1405–1420.

Konturek, S.J., Bilski, J., Cieszkowski, M., 1992. Role of cholecystokinin in the intestinal fat- and acid-induced inhibition of gastric secretion. Regul. Pept. 42, 97–109.

Konturek, S.J., Brzozowski, T., Pytko-Polonczyk, J., Drozdowicz, D., 1995a. Exogenous and endogenous cholecystokinin protects gastric mucosa against the damage caused by ethanol in rats. Eur. J. Pharmacol. 273, 57–62.

Konturek, S.J., Brzozowski, T., Pytko-Polonczyk, J., Drozdowicz, D., 1995b. Comparison of cholecystokinin, pentagastrin and duodenal oleate in gastroprotection in rats. Scand. J. Gastroenterol. 30, 620–630.

Konturek, P.Ch., Brzozowski, T., Konturek, S.J., Elia, G., Wright, N., Sliwowski, Z., Thim, L., Hahn, E.G., 1997. Role of spasmolitic polypeptide in healing of stress-induced gastric lesions in rats. Regul. Pept. 68, 71–79.

Konturek, P.Ch., Brzozowski, T., Śliwowski, Z., Pajdo, R., Stachura, J., Hahn, E.G., Konturek, S.J., 1998. Involvement of nitric oxide and prostaglandins in gastroprotection induced by bacterial lipopolisaccharide. Scand. J. Gastroenterol. 33, 691–700.

Lloyd, K.C.K., Maxwell, V., Kovacs, T.O., Miller, J., Walsh, I.H., 1992a. Cholecystokinin receptor antagonist MK329 blocks intestinal fat induced inhibition of meal stimulated gastric acid secretion. Gastroenterology 102, 131–138.

Lloyd, K.C., Raybould, H.E., Walsh, I.H., 1992b. Cholecystokinin inhibits gastric acid secretion through type A cholecystokinin receptors and somatostatin in rats. Am. J. Physiol. 263, 287–292.

Lloyd, K.C.K., Holzer, H.H., Zittel, T.T., Raybould, H.E., 1993. Duode-

nal lipid inhibits gastric acid secretion by vagal, capsaicin-sensitive afferent pathways in rats. Am. J. Physiol. 264, G659–G663.

Matson, C.A., Wiater, M.F., Kuijper, J.L., Weigle, D.S., 1997. Synergy between leptin and cholecystokinin (CCK) to control daily caloric intake. Peptides 18, 1275–1278.

Mercer, D.W., Klemm, K., Cross, I.M., Smith, G.S., Cashman, M., Miller, T.A., 1996. Cholecystokinin induced protection against gastric injury is independent of endogenous somatostatin. Am. J. Physiol. 27, 692–700.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Nudel, U., Zakut, R., Shani, M., Neuman, S., Levy, S., Vaffe, D., 1983. The nucleotide sequence of the rat cytoplasmic beta-actin gene. Nucl. Acid Res. 11, 1759–1771.

Pendley, C.E., Fitzpatrick, L.R., Ewing, R.W., Molino, B.F., Martin, G.E., 1993. The gastrin/cholecystokinin-B receptor antagonist L365,260 reduces basal acid secretion and prevents gastrointestinal damage induced by aspirin, ethanol and cysteamine in the rat. J. Pharmacol. Exp. Ther. 265, 1348–1354.

Sarraf, P., Frederich, R.C., Turner, E.M., 1997. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J. Exp. Med. 185, 171–175.

Schwortz, G.J., Berkow, G., McHuhg, P.R., Moran, T.H., 1993. Gastric branch vagotomy blocks nutrient and cholecystokinin-induced suppression of gastric emptying. Am. J. Physiol. 264, 630–637.

Shalev, A., Vosmeer, S., Keller, U., 1997. Absence of short-term effects of glucagon-like peptide-1 and hyperglycemia on plasma leptin levels in man. Metabolism 46, 723–725.

Silverman, M.A., Greenberg, R.E., Bank, S., 1987. Cholecystokinin receptor antagonists: a review. Am. J. Gastroenterol. 82, 703–707.

Stroff, T., Lambrecht, N., Peskar, B.M., 1994. Nitric oxide as mediator of the gastroprotection by cholecystokinin-8 and pentagastrin. Eur. J. Pharmacol. 260, R1–R2.

Wang, Y.H., Tache, Y., Sheibel, A.B., Go, V.L.W., Wei, J.Y., 1997. Two types of leptin-responsive gastric vagal afferent terminals: an in vitro single-unit study in rats. Am. J. Physiol. 273, 833–837.

Whittle, B.J.R., Lopez-Belmonte, J., Moncada, S., 1990. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. Br. J. Pharmacol. 99, 607–611.