

T. BRZOZOWSKI, P. C. KONTUREK*, R. PAJDO, S. KWIECIEŃ, A. PTAK,
Z. ŚLIWOWSKI, D. DROZDOWICZ, M. PAWLIK, S. J. KONTUREK, E. G. HAHN

BRAIN-GUT AXIS IN GASTROPROTECTION BY LEPTIN AND CHOLECYSTOKININ AGAINST ISCHEMIA-REPERFUSION INDUCED GASTRIC LESIONS

Department of Physiology, Jagiellonian University School of Medicine, Cracow, Poland and
*Department of Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany

Leptin, a product of *ob* gene controlling food intake, has recently been detected in the stomach and shown to be released by CCK and implicated in gastroprotection against various noxious agents but it is unknown whether centrally applied leptin influences ischemia-reperfusion (I/R)-induced gastric erosions that progress into deeper gastric ulcerations. In this study we compared the effects of leptin and CCK-8 applied intracerebroventricularly (i.c.v.) or intraperitoneally (i.p.) on gastric mucosal lesions induced by I/R and topical application of 75% ethanol. Several major series of Wistar rats were used to examine the effects of leptin and CCK applied centrally on gastroprotection against I/R and ethanol in rats with A) vagotomy by cutting of vagal nerves, B) suppression of NO-synthase with L-NNA (20 mg/kg i.p.), C) inactivation of sensory nerves by capsaicin (125 mg/kg s.c.) and D) inhibition of CGRP receptors with CGRP₈₋₃₇ (100 µg/kg i.p.) applied with or without the i.c.v. pretreatment with leptin or CCK-8. Rats were anesthetized 1 h after ethanol administration or at 3 h and 3 days upon the end of ischemia to measure the gastric blood flow (GBF) and then to determine the area of gastric lesions by planimetry. Blood was withdrawn for the measurement of plasma leptin and gastrin levels by radioimmunoassay (RIA). Leptin (0.1—20 µg/kg i.p.) dose-dependently attenuated gastric lesions induced by 75% ethanol and I/R; the dose reducing these lesions by 50% (ED₅₀) was 8 µg/kg and 6 µg/kg, respectively and this protective effect was similar to that obtained with CCK-8 applied in a standard dose of 10 µg/kg i.p. This protective effect of leptin was accompanied by a significant increase in GBF and plasma gastrin levels whereas CCK-8 increased plasma leptin levels but failed to affect plasma gastrin levels. Leptin and CCK-8 applied i.c.v. in a dose of 625 ng/rat reduced significantly the area of I/R induced gastric lesions and raised the GBF and plasma leptin levels with the extent similar to those achieved with peripheral administration of leptin or CCK-8 (10 µg/kg i.p.). The protective and hyperemic effects of centrally administered leptin or CCK-8 (625 ng/rat i.c.v.) were completely abolished by vagotomy and significantly attenuated by sensory denervation with capsaicin or by CGRP antagonist, CGRP₈₋₃₇. The pretreatment with L-NNA to inhibit NO-synthase activity attenuated significantly the protective and hyperemic effects of CCK but not those of leptin while capsaicin denervation counteracted leptin-induced protection and rise in the GBF but attenuated significantly those of CCK. We conclude that: 1) central leptin exerts a potent gastroprotective activity against I/R-induced gastric erosions that progress into deeper gastric lesions and this protection depends upon vagal activity and sensory nerves and involves hyperemia probably mediated by NO and 2) leptin mimics the gastroprotective effect of CCK and may be implicated in the protective and hyperemic actions of this peptide against mucosal damage evoked by I/R.

Key words: *leptin, cholecystokinin, gastroprotection, vagal nerves, sensory nerves, calcitonin gene related peptide, gastric blood flow, nitric oxide*

INTRODUCTION

Leptin is accepted as a key peripheral protein product of the *ob* gene acting on central leptin receptors (Ob-R) that control food intake and the energy expenditure (1, 2). Previous studies have documented that the expression and secretion of leptin is highly correlated with body fat mass and adipocyte size and that leptin is present in detectable amounts in the serum of experimental animals such as mice and rats as well as in humans (3—6). Leptin is believed to be secreted mainly by white adipocytes and the placenta but recent study revealed that leptin messenger RNA and leptin protein are also expressed in the rat gastric mucosa suggesting that stomach can be another important source of leptin (7, 8). Both, CCK and feeding were also shown to elevate significantly plasma concentration of leptin and this effect was associated with the fall of the leptin content in the gastric fundic mucosa, suggesting that the observed rise in plasma leptin may originate from this mucosa (7). Recently, we confirmed original observation by Bado and coworkers (7) that administration of CCK or peptone meal releasing CCK elevates the plasma level of leptin and found that leptin applied peripherally mimics the gastroprotective effects of CCK against ethanol injury suggesting that it may mediate the protective and hyperemic action of CCK on the stomach (8). Since both CCK and feeding were shown to be highly gastroprotective against ethanol-induced gastric damage (8), the question remains whether exogenous leptin applied centrally and that found in the stomach are as effective as peripherally administered leptin in protection against another type of gastric lesions such as caused by ischemia-reperfusion in the rat stomach.

The expression of leptin *Ob-R* in mouse hypothalamus, especially one of its splice variants such as *Ob-Rb*, suggests that this region of the brain is an important site of leptin action (9, 10). Isoforms of the leptin receptor, members of interleukin (IL)-6 cytokine receptor family were found in multiple tissues, including the brain (11, 13). Moreover, it was shown that a single intracerebroventricular (i.c.v.) injection of leptin reduces food intake at doses that have no effect when delivered peripherally (12, 13). It was suggested that all the effects of peripheral leptin can be reproduced by lower doses of leptin applied into the lateral cerebral ventricle (12). However, the role of this centrally applied peptide in gastric mucosal integrity and gastroprotection as well as the identity of the neural or humoral pathways that are activated by centrally applied leptin are not known.

Previous studies documented that the gastroprotective effects of CCK against the mucosal damage induced by ethanol could also be attributed to the

activation of specific CCK_A receptors and the increase in gastric blood flow (GBF) by this peptide (14, 16). Since nitric oxide (NO), a potent vasodilator derived from L-arginine in the gastric mucosa, and sensory afferent nerves were recently implicated in the mechanism of gastroprotection afforded by CCK and leptin applied peripherally (8, 14, 16), the question arises whether vagal innervation, NO and sensory afferents are also involved in the gastroprotection by leptin and CCK administered intracerebrally against gastric injury induced by ischemia-reperfusion.

This study was designed: 1) to compare the effect of central (i.c.v.) and parenteral (i.p.) administration of leptin and CCK on gastric mucosal lesions induced by ischemia-reperfusion (I/R) and accompanying changes in the gastric blood flow (GBF) and plasma leptin and gastrin levels and 2) to assess the involvement of vagal, sensory innervation, NO-arginine pathway and neuropeptides released from sensory nerve endings such as calcitonin gene related peptide (CGRP) in gastroprotection against I/R damage induced by leptin and CCK-8.

MATERIAL AND METHODS

Male Wistar rats, weighing 180—220 g and fasted for 24 h, were used in studies on gastroprotection. Studies were approved by the Ethic Committee for the Animal Research of Jagiellonian University.

Gastric lesions induced by ischemia-reperfusion and ethanol

Ischemia-reperfusion (I/R) erosions were produced in 120 rats by the method originally proposed by Wada *et al* (15). Briefly, under pentobarbital anesthesia (50 mg/kg i.p.), the celiac artery was clamped with a small device for 30 min and this was followed by removal of the clamp to obtain I/R state as described above. Erosions were determined already after 60 min of reperfusion (time 0) and these erosions were further examined at 3 h after the termination of I/R in rats treated with vehicle (saline-control), leptin (10 µg/kg i.p.) and CCK-8 (10 µg/kg i.p.) applied in a total volume of 1 ml. Leptin (murine recombinant) or CCK-8 (both purchased from Sigma Co. Ltd., MO, USA) were applied 30 min before I/R and this treatment was repeated at day 1 after the termination of I/R. In separate groups of rats, the longer observation periods after the end of I/R up to day 3 after I/R have been used to check whether leptin and CCK-8 could affect mucosal recovery from the lesions induced by I/R. In another group of rats, we wanted to compare whether leptin and CCK are effective against gastric lesions induced by necrotizing irritant such as ethanol. In this model, the acute gastric lesions were induced by an intragastric (i.g.) application of

75% ethanol in a volume of 1.5 ml by means of a metal orogastric tube as reported before (16).

For the i.c.v. injection of vehicle, leptin or CCK, the rats underwent surgery 48 h before the clamping of the celiac artery according to the method described previously (17, 18). Briefly, under light ether anesthesia, an incision was made along with the mid-line of the skull, the skull bones were cleaned of connective tissue and the point of intersection between the sagittal and coronary sutures was visualized. The point at the distance of approximately 2.5 mm from either sagittal and coronary suture was defined and in this place a small hole in the skull was made, using needle with a sharp end. The hole was made by rotary movement of the needle and the wound of the head was closed by a clip. The effectiveness of i.c.v. administration was verified by injecting 10 μ l of dye (0.1% toluidine blue). The visualization of dye on the walls of lateral ventricle indicated the exact location of i.c.v. injection (18). At the day of gastric lesions induction vehicle, leptin or CCK-8 were injected i.c.v. in various doses (6.25—1300 ng/rat) in a volume of 5 μ l using a 10 μ l Hamilton microsyringe. For the comparison, a standard dose (10 μ g/kg) of leptin or CCK was administered intraperitoneally (i.p.) and the area of gastric lesions and GBF were determined as in experiments with i.c.v. application of the peptides.

Determination of gastric blood flow and plasma gastrin concentrations

To evaluate the effect of I/R or vehicle on gastric blood flow, the groups of animals were anesthetized with ether and the abdomen was opened by midline incision. The stomach was exposed to assess the blood flow using H₂-gas clearance technique as described previously (19). Briefly, the gastric blood flow was measured in the intact gastric mucosa and then immediately after 30 min ischemia and following at 3 h and then at day 3 after the end of I/R using double electrodes of electrolytic regional blood flowmeter (Biotechnical Science, Model RBF-2, Osaka, Japan) inserted through the serosa into the mucosa. One for these electrodes was used for the local generation in the mucosa of H₂ and another for measurement in tissue of this H₂. With this method, the H₂ generated locally is carried out by flow of blood, while the polarographic current detector reads out decreasing tissue H₂. The clearance curve of tissue H₂ was used to calculate an absolute blood flow rate (ml/min/100 g) in the oxyntic gland area as described previously (16, 19). The measurement was made in three areas of the gastric oxyntic mucosa and the mean values of these measurements were calculated and expressed as percent changes from those recorded in vehicle-treated control animals not exposed to I/R.

Immediately after measurement of blood flow, a venous blood sample was withdrawn from the vena cava into the EDTA containing vials and used either

for the determination of plasma gastrin by RIA as described previously (19). The stomach was removed, rinsed with water and pinned open for macroscopic examination. The area of necrotic lesions in oxyntic mucosa was determined by computerized planimetry (Morphomat, Carl Zeiss, FRG) (19).

Involvement of vagal and sensory nerves and NO-arginine system in gastroprotection afforded by central leptin and CCK

In subsequent studies four series (A, B, C and D) of experiments were carried out to determine the involvement of vagal and sensory nerves as well as NO in the gastroprotective effects of leptin and CCK-8 applied centrally or peripherally.

Series A was used to compare the effects of leptin or CCK-8 applied i.c.v. with those achieved with a standard dose of leptin or CCK given peripherally (i.p.) against the mucosal lesions induced by 75% ethanol and 30 min of ischemia followed by 3 h of reperfusion (I/R).

Series B, C and D were used to study the involvement of vagal nerves, sensory innervation and CGRP receptor antagonism, respectively, in the protection afforded by i.c.v. administration of leptin or CCK-8 against ischemia-reperfusion induced gastric damage.

The following groups of rats in series A, each consisting of 6—8 animals, were used 1) vehicle (saline 1 ml i.p. or 5 μ l i.c.v.) followed 30 min later by 75% ethanol or I/R; 2) leptin (6.25—1300 ng/rat i.c.v.) followed 30 min later by ethanol and I/R; 3) leptin (10 μ g/kg i.p.) followed 30 min later by ethanol or I/R; 4) CCK-8 (6.25—1300 ng/rat i.c.v.) followed 30 min later by ethanol and I/R; 5) CCK-8 (10 μ g/kg i.p.) followed 30 min later by ethanol and I/R.

The involvement of vagal nerves (series B) in gastroprotection by leptin or CCK-8 was studied in rats with intact vagal nerves or vagotomy performed by cutting off subdiaphragmatically these vagal nerves (8, 20). The role of sensory afferent nerves in gastroprotection by leptin or CCK-8 (series C) was tested in rats with capsaicin induced deactivation of these nerves. For this purpose the animals were pretreated with capsaicin (Sigma Co., St. Louis, MO) injected s.c. for 3 consecutive days at a dose of 25, 50 and 50 mg/kg about 2 weeks before the experiment as described elsewhere (14, 21, 22).

In tests with calcitonin gene related peptide (CGRP) antagonist, CGRP₈₋₃₇ (18) (series D) rats were injected with CGRP₈₋₃₇ (100 μ g/kg i.p.) 30 min before i.c.v. or i.p. administration of standard dose of leptin, CCK or vehicle followed 30 min later by standard I/R. In control experiments, vehicle saline was administered (1 ml i.p.) 30 min before i.c.v. or i.p. leptin or CCK given in the same doses as in tests with CGRP₈₋₃₇ and this was followed 30 min later by I/R.

At the termination of some experiments with i.c.v. or i.p. administration of leptin or CCK-8 followed 30 min later by I/R, the rats were anesthetized with

ether and the blood samples (about 3 ml) were taken from the vena cava for the measurement of plasma leptin by RIA as described previously (7, 8, 18). For comparison, intact rats fasted overnight and given i. p. only vehicle saline were also anesthetized with ether and the 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 RIA-kit for rat leptin from Linco Research Inc. (St. Charles, Missouri, USA). Briefly, this RIA involved the competition of a rat leptin sample with ¹²⁵I-rat leptin tracer for binding to a specific rabbit antileptin polyclonal antibody. The limit of assay sensitivity was 0.5 ng/ml; the intra-assay variation was less than 7% and the interassay variation less than 9%.

Statistical analysis

Results are expressed as means ± SEM. Statistical analysis was done using analysis on variance and two way ANOVA test when appropriate. Differences with $p < 0.05$ were considered as significant.

RESULTS

Effects of leptin and CCK-8 on I/R-induced lesions and the GBF and plasma gastrin and leptin levels

The results of i.p. administration of graded doses of leptin ranging from 0.1 µg/kg up to 20 µg/kg on the area of gastric lesions induced by 30 min ischemia followed by 3 h of reperfusion (I/R) and accompanying changes in the GBF and plasma leptin levels are presented in *Fig. 1*. Such pretreatment reduced dose-dependently the area of gastric lesions caused by I/R with the threshold reduction occurring at a dose of 1 µg/kg and with the ID₅₀ averaging about 8 µg/kg of leptin. The magnitude of inhibition by leptin (10 µg/kg i.p.) of I/R damage was similar to that achieved with CCK-8 applied i.p. in a dose of 10 µg/kg. These protective effects of leptin or CCK-8 were accompanied by a significant and dose-dependent rise in the GBF and plasma gastrin levels (*Fig. 1*).

As shown in *Table 1* and *2*, the i.g. application of 75% ethanol in rats pretreated with vehicle resulted in a widespread denudation of mucosal surface and deep necrosis averaging about 46 and 23% of mucosal strip length. In contrast, rats pretreated with leptin or CCK-8 applied i.p. showed a significant reduction in the area of denuded surface and deep necrosis as compared to those pretreated with vehicle. In further studies, leptin was used i.p. or i.c.v. in a

standard dose of 10 $\mu\text{g}/\text{kg}$ or 625 ng/rat , respectively, that caused over 50% reduction in the area of ethanol-induced lesions and that raised the plasma leptin to the level observed after the administration of exogenous CCK-8 (Table 1 and 2).

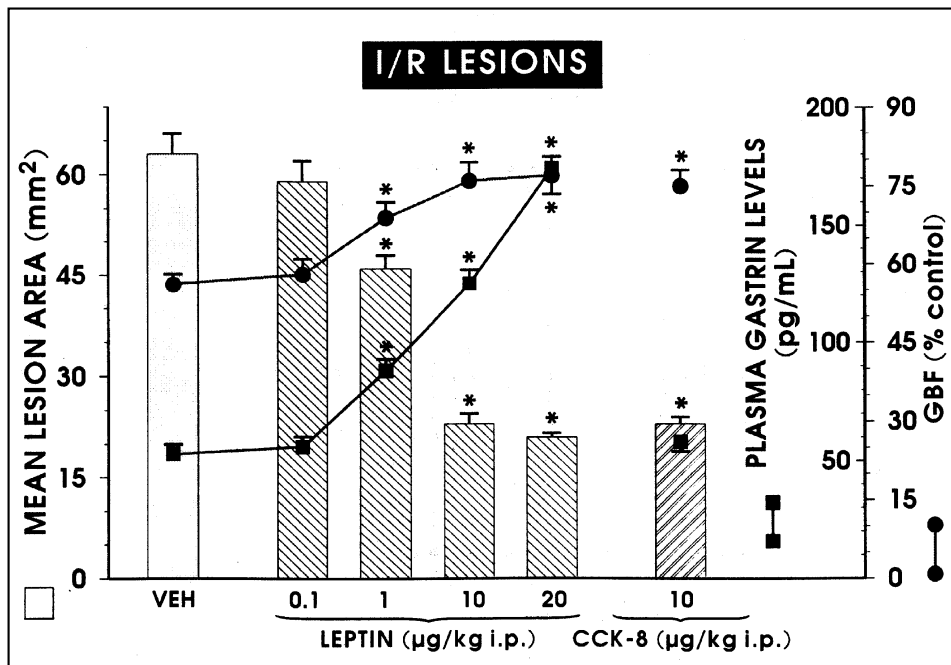


Fig. 1. The area of ischemia-reperfusion (I/R)-induced gastric lesions, gastric blood flow (GBF) and plasma immunoreactivity of gastrin in rats treated with vehicle (Veh; saline) or with various doses of leptin (0.1–20 $\mu\text{g}/\text{kg}$ i.p.) and CCK applied in a dose of 10 $\mu\text{g}/\text{kg}$ i.p. Means \pm SEM of 6–8 rats. Asterisk indicates a significant change as compared to the vehicle control values.

Table 1. Mean area of gastric lesions, the GBF and plasma leptin and gastrin levels in rats exposed to 75% ethanol with or without the pretreatment with leptin or CCK-8 applied alone i.p. in a dose of 10 $\mu\text{g}/\text{kg}$ or injected i.c.v. in a dose of 625 ng/rat . Mean \pm S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared to the value obtained in vehicle-treated gastric mucosa.

Type of test	Mean lesion area (mm ²)	GBF (% control)	Plasma leptin (ng/ml)	Plasma gastrin pM
Vehicle	63 \pm 4	67 \pm 4	0.38 \pm 0.04	45 \pm 6
Leptin (10 $\mu\text{g}/\text{kg}$ i.p.)	29 \pm 3*	81 \pm 5*	2.62 \pm 0.08*	89 \pm 8*
CCK-8 (10 $\mu\text{g}/\text{kg}$ i.p.)	18 \pm 2*	83 \pm 4*	1.68 \pm 0.08*	52 \pm 5
Leptin (625 ng/rat i.c.v.)	22 \pm 4*	73 \pm 6*	2.95 \pm 0.10*	82 \pm 6*
CCK (625 ng/rat i.c.v.)	26 \pm 5*	79 \pm 5*	2.19 \pm 0.18*	44 \pm 5

The i.c.v. pretreatment with leptin and CCK-8 applied in a dose of 625 ng/rat attenuated significantly the area of lesions induced by I/R in the manner similar to that exhibited by leptin and CCK-8 (10 μ g/kg i.p.) (Fig. 2). Leptin in the standard dose of 10 μ g/kg given i.p. or 625 ng/rat applied i.c.v., which

Table 2. Quantitative histology in the gastric mucosa of rats exposed to 75% ethanol with or without the pretreatment with vehicle (saline), leptin or CCK-8 applied i.p. and i.c.v. in standard doses of 10 μ g/kg and 625 ng/rat. Results are expressed as percentage of the mucosal strip length. Mean \pm S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared to the value obtained in vehicle-pretreated gastric mucosa.

Type of test	Denuded surface	Deep necrosis
Vehicle	48 \pm 7	25.6 \pm 2.5
Leptin (10 μ g/kg i.p.)	21 \pm 3*	9.1 \pm 0.5*
CCK-8 (10 μ g/kg i.p.)	17 \pm 2*	8.4 \pm 0.4*
Leptin (625 ng/rat i.c.v.)	28 \pm 4*	9.5 \pm 0.6*
CCK (625 ng/rat i.c.v.)	22 \pm 5*	6.9 \pm 0.8*

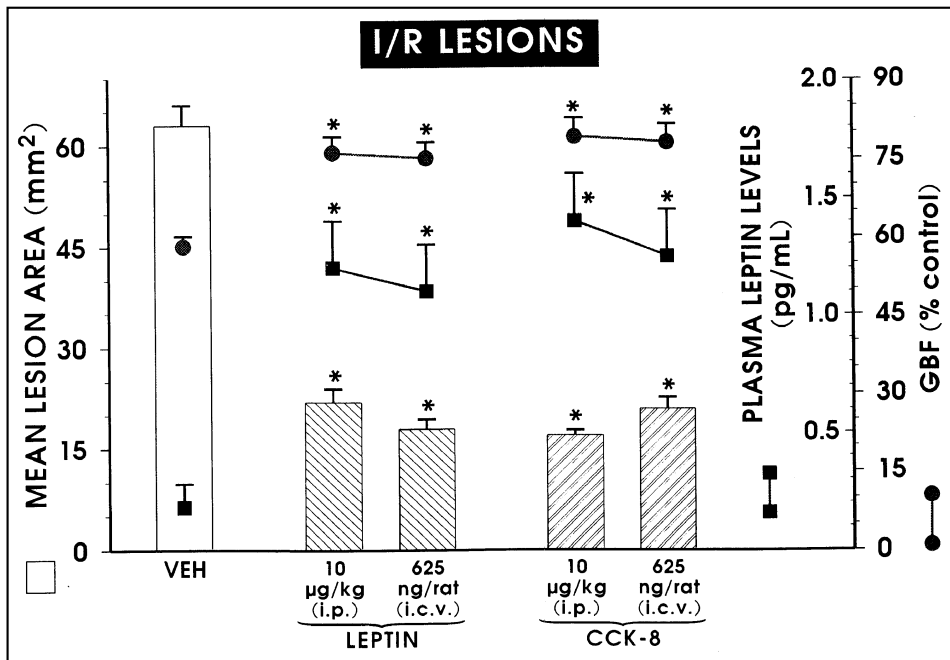


Fig. 2. The area of I/R-induced gastric lesions, gastric blood flow (GBF) and plasma immunoreactivity of leptin in rats treated with vehicle (saline) or with leptin and CCK (10 μ g/kg i.p. or 625 ng/rat i.c.v.). Means \pm SEM of 6–8 rats. Asterisk indicates a significant change as compared to the vehicle control values.

produced a significant decline in I/R lesions and a rise in the GBF, elevated significantly plasma leptin levels as compared to the respective values in vehicle-control animals. CCK-8 applied in standard doses (10 $\mu\text{g}/\text{kg}$ i.p. or 625 ng/rat i.c.v.) also resulted in the protection against I/R lesions and the rise in the GBF as well as in plasma leptin levels similar to those obtained with leptin applied either i.p. or i.c.v. (Fig. 2).

The area of gastric erosions induced by 30 min ischemia followed by 3 h or 3 days of reperfusion and accompanying changes in the GBF and plasma gastrin levels in rats treated with vehicle or leptin applied i.c.v. in a dose of 625 ng/rat are presented in Fig. 3. The area of these erosions in vehicle-control rats averaged $78 \pm 4 \text{ mm}^2$ at 3 h after the end of ischemia and this was significantly reduced by leptin administered i.c.v. (Fig. 3). After 24 h these erosions progressed into deeper lesions (data not shown) whose area reached the maximum at day 3. The area of these deeper lesions remained significantly smaller in rats treated leptin (625 ng/kg i.c.v.) than that measured in vehicle-treated controls (Fig. 3).

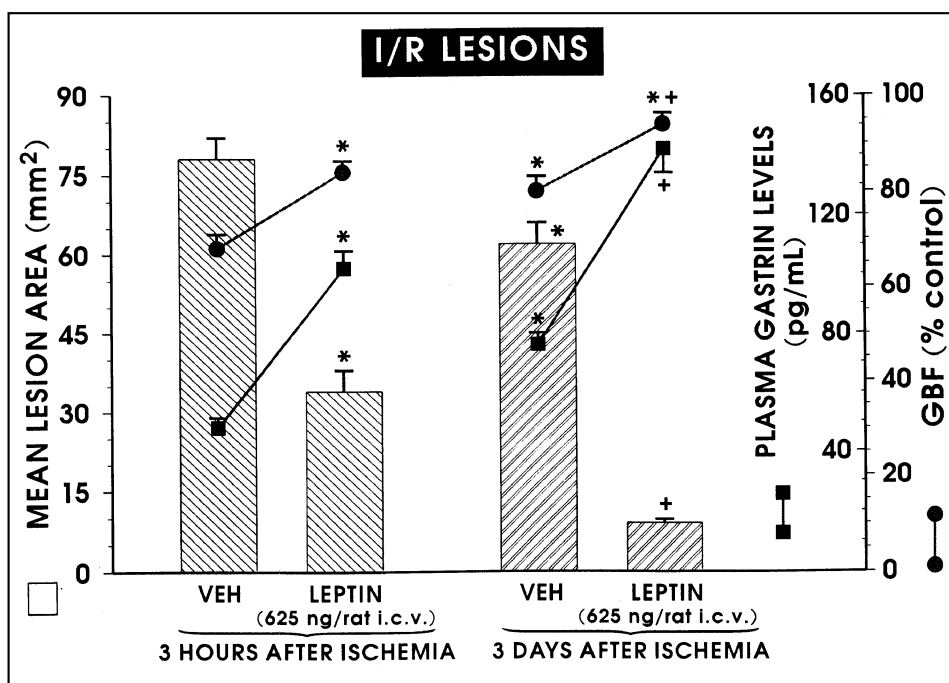


Fig. 3. Mean area of gastric lesions induced by I/R and accompanying changes in the GBF and plasma gastrin levels in rats treated with vehicle (saline) and leptin (625 ng/rat i.c.v.) observed at 3 h and 3 days upon the termination of ischemia. Mean \pm SEM of 6–8 rats. Asterisk indicates a significant change as compared to the value obtained in vehicle-control rats. Cross indicates a significant change as compared to the value obtained

The GBF in intact mucosa of vehicle-treated rats averaged 48 ± 6 ml/min-100 g (taken as 100%) and clamping of the celiac artery caused an immediate and almost complete stopping of GBF and this data has not been included for the sake of clarity. As shown in *Figs. 2* and *3*, three hours after removal of the clamp, the GBF was reduced by about 30% as compared to the value recorded in vehicle-treated controls. In rats treated with leptin applied i.c.v., the increase of the GBF was significantly higher than in vehicle-controls at 3 h and 3 days upon the end of ischemia (*Fig 3*).

Effect of vagotomy and deactivation of sensory nerves with capsaicin on the leptin and CCK-induced gastroprotection and GBF

Figs. 4 and *5* show the effect of leptin and CCK-8 applied either i.c.v. or i.p. on gastric lesions induced by I/R in vagotomized and capsaicin-deactivated rats as compared to those with intact vagal and sensory nerves. Vagotomy

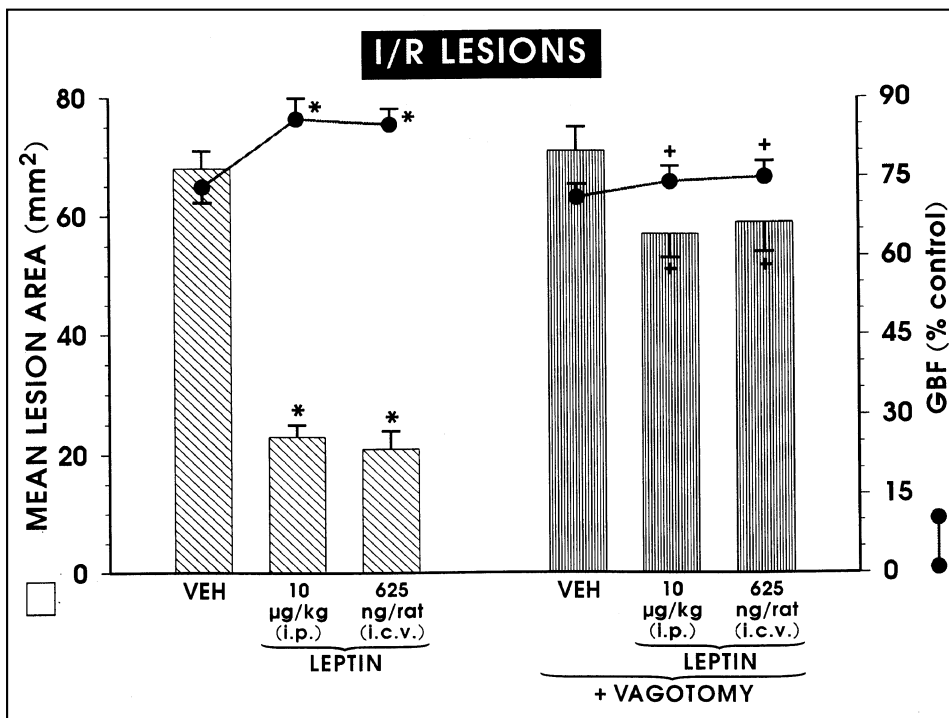


Fig. 4. Mean area of gastric lesions induced by I/R and GBF in rats with intact vagal nerves and those with vagotomy with or without pretreatment with vehicle (control), leptin (10 µg/kg i.p. and 625 ng/kg i.c.v.) and CCK-8 (10 µg/kg i.p. and 625 ng/rat i.c.v.). Mean \pm SEM of 6–8 rats. Asterisk indicates 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.

which by itself failed to affect significantly ethanol-induced lesions and the accompanying fall in GBF, abolished almost completely the protective and hyperemic activity of leptin injected i.c.v. or i.p. (Fig. 4).

Deactivation of primary afferent nerves with parenteral pretreatment with neurotoxic dose of capsaicin (about 2 weeks before the experiment) augmented significantly the area of I/R-induced gastric lesions while attenuating significantly the GBF as compared to vehicle-treated rats with intact sensory nerves (Fig. 5). In rats with capsaicin deactivation of afferent nerves, the protective activity of leptin applied i.c.v. and i.p. accompanying rise in the GBF were partly reduced reaching the value still significantly lower to that obtained in respective capsaicin-denervated rats treated with vehicle. The protection and the rise in GBF caused by CCK-8 (10 $\mu\text{g}/\text{kg}$ i.p. and 625 ng/kg i.c.v.) in rats with intact sensory nerves were almost completely abolished in capsaicin-denervated animals exposed to I/R.

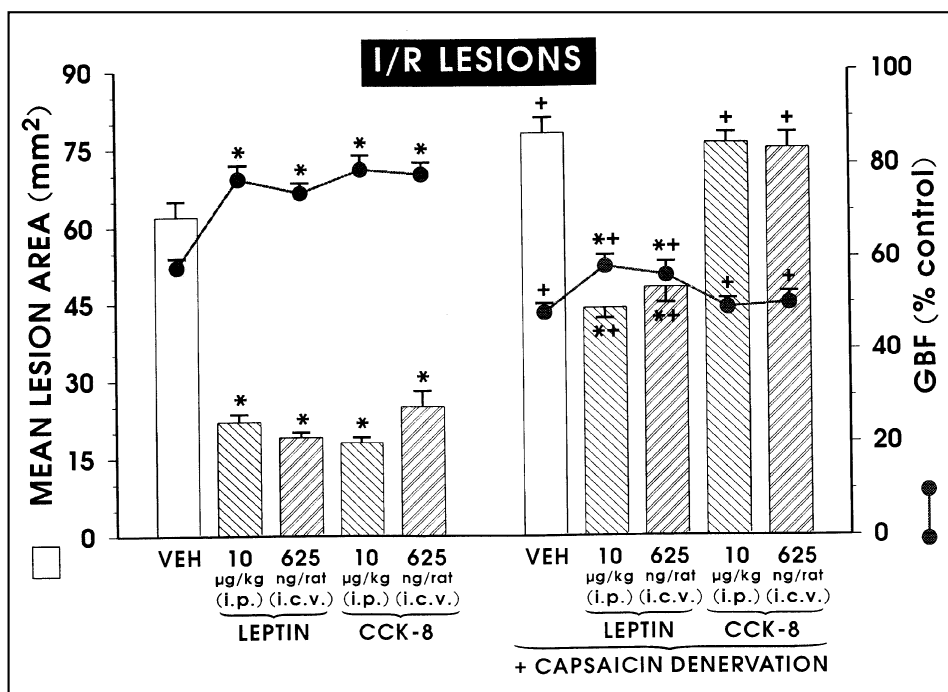


Fig. 5. Mean area of I/R-induced acute gastric lesions and changes in GBF in gastric mucosa of rats with intact and capsaicin-induced sensory denervation and treated with vehicle (control), leptin (10 $\mu\text{g}/\text{kg}$ i.p. and 625 ng/rat i.c.v.) and CCK-8 (10 $\mu\text{g}/\text{kg}$ i.p. and 625 ng/rat i.c.v.). Mean \pm SEM of 6–8 rats. Asterisk indicates a significant change compared to the value obtained in vehicle-control gastric mucosa. Cross indicates a significant change as compared to the value obtained in rats without sensory denervation.

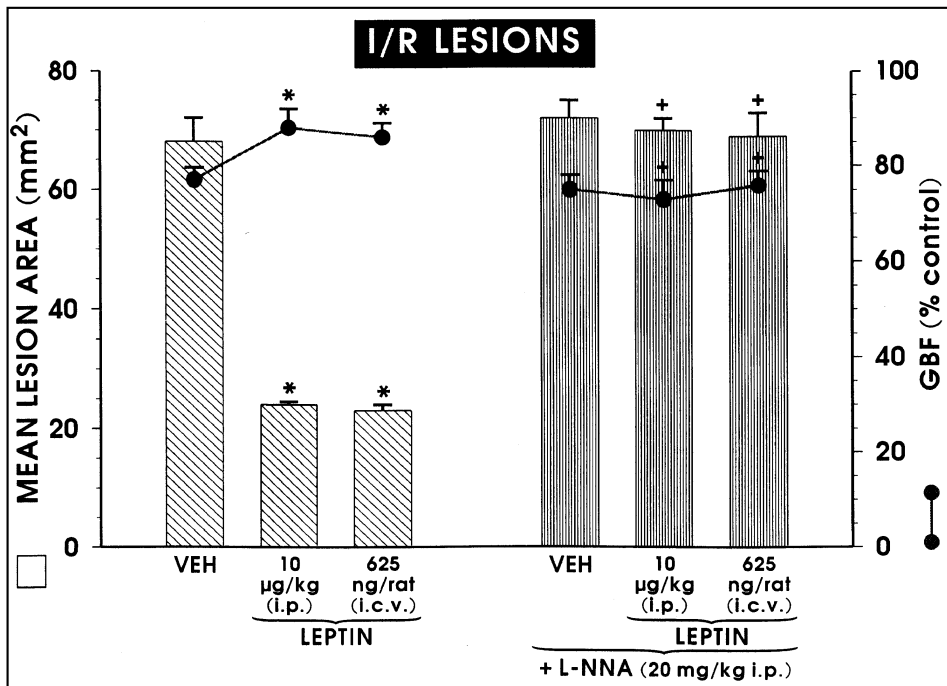


Fig. 6. Mean area of I/R-induced gastric lesions and changes in GBF in gastric mucosa of rats treated with vehicle (control) and leptin (10 µg/kg i.p. and 625 ng/rat i.c.v.) with or without the pretreatment with L-NNA (20 mg/kg i.p.). Mean ± SEM of 6–8 rats. Asterisk indicates a significant change compared to the value obtained in vehicle-control gastric mucosa. Cross indicates a significant change as compared to the value obtained in rats without the pretreatment with L-NNA.

Effect of suppression of NO-synthase and blockade of CGRP with CGRP₈₋₃₇ on leptin- and CCK-8 afforded gastroprotection

The results with the effect of L-NNA (20 mg/kg i.p.) on gastroprotection induced by leptin given i.p. in a dose of 10 µg/kg i.p. or applied i.c.v. in a dose of 625 ng/rat and accompanying changes in the GBF are presented in Fig. 6. L-NNA alone did not induce mucosal lesions (data not shown) and failed to affect significantly both, the area of I/R-induced gastric lesions and the accompanying fall in the GBF as compared to those in rats treated with ethanol alone. When L-NNA was injected prior to leptin (2.5 µg/kg i.c.v. or 10 µg/kg i.p.), the area of I/R-induced lesions was significantly increased and the GBF and was significantly attenuated as compared to those obtained in rats treated with leptin alone (Fig. 6).

As shown in Fig. 7, the pretreatment with CGRP antagonist, CGRP₈₋₃₇ (100 µg/kg i.p.), which by itself had no significant influence on the area of gastric

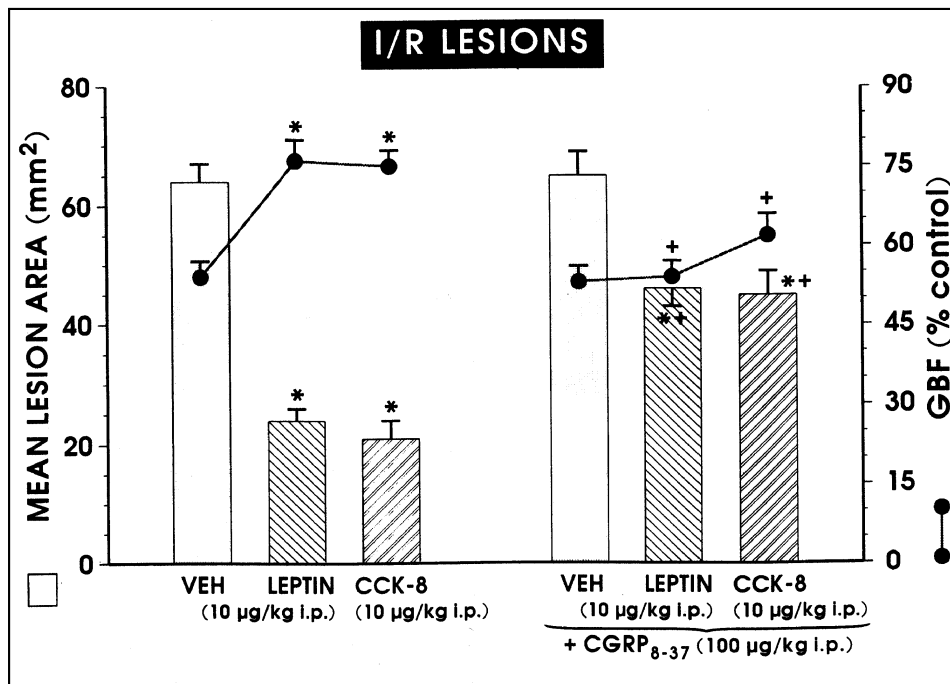


Fig. 7. Mean area of I/R-induced gastric lesions and changes in GBF in gastric mucosa of rats treated with vehicle (control), leptin (10 µg/kg i.p. and 2.5 µg/kg i.c.v.) and CCK-8 (10 µg/kg i.p. and 625 ng/rat i.c.v.) with or without the pretreatment with CGRP₈₋₃₇. Mean ± SEM of 6–8 rats. Asterisk indicates a significant change compared to the value obtained in vehicle-control gastric mucosa. Cross indicates a significant change as compared to the value obtained in rats without CGRP₈₋₃₇.

lesions and the GBF in ethanol treated gastric mucosa, attenuated significantly the protection and hyperemia attained with standard doses of leptin (10 µg/kg i.p.) and CCK (10 µg/kg i.p.) against gastric lesions evoked by I/R.

DISCUSSION

This study demonstrates that central and peripheral leptin and CCK exhibit a potent gastroprotective activity against ischemia-reperfusion erosions progressing into deeper gastric lesions and this appears to depend upon vagal activity and sensory nerves and may involve gastric hyperemia probably mediated by NO and neuropeptides such as CGRP, released from sensory afferent fibers. Furthermore, our present study suggests that central and peripheral leptin can mimic the gastroprotective effect of CCK against ischemia-reperfusion injury and may be implicated in the protective and hyperemic actions of this enterogastrone in the rat stomach.

This study confirms and further extends our previous original observation (18) that exogenous leptin administered centrally exhibits dose-dependent protection of the stomach against the ethanol-induced lesions with the extent similar to that obtained with exogenous CCK resulting in similar rise of plasma leptin concentration. Our results show for the first time that leptin applied i.c.v. in the doses that were gastroprotective against ethanol injury is also effective in attenuation of the gastric lesions induced by I/R. We confirmed our and other previous observations (8, 14, 16, 22) that pretreatment with CCK-8, applied in the doses that were recently shown to exert only mild stimulatory effect on gastric acid secretion, also attenuated mucosal lesions induced by I/R and that this effect, like that observed with leptin, was accompanied by a significant attenuation of the fall in the gastric blood flow provoked by I/R. Since the protective effect of CCK was accompanied by elevated plasma leptin level, the protection and hyperemia against deleterious effect of I/R might be mediated by leptin released by this enterogastrone.

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 inhibition of gastric secretion by intragastric distention or intraduodenal fat (19, 20, 23, 24). We and others have demonstrated that the mechanism of the gastric acid stimulatory effects of exogenous and endogenous CCK is similar and depends upon the activation of CCK_A receptors (16,19—24). In agreement with these studies we have shown recently (18) that exogenous CCK is rather weak stimulant of gastric secretion whereas leptin at higher doses inhibited this secretion, indicating some differences, between CCK and leptin, at least, with respect to gastric secretory effects. In the present study, peripheral CCK failed to influence plasma gastrin levels, whereas systemic administration of leptin raised significantly the plasma level of this hormone, again, suggesting that these peptides differ in their biological activity. Since trophic and gastroprotective actions of gastrin are well documented (26), we believe that such increase in the plasma gastrin levels after leptin administered either i.p. or i.c.v. could be explanatory for gastroprotective activity of this peptide against the lesions evoked by I/R but could not serve as a suitable explanation for the protection observed after CCK. This difference in the gastric secretory and plasma gastrin responses to these two peptides does not necessarily militates against previous finding demonstrating the existence of a functional synergistic interaction between CCK and leptin on the suppression of food intake by these peptides (10, 27). This synergism between CCK and leptin probably involves central rather than peripheral gastric receptors for satiety signals (13, 28) but this issue requires further study.

This study attempted to compare the effects of exogenous and endogenous leptin applied intracerebroventricularly or peripherally with those exhibited by CCK on gastric lesions induced by I/R and 75% ethanol and accompanying

changes in the GBF. Firstly, we confirmed our previous observations that CCK given peripherally exerts a potent protection against ethanol-induced damage being accompanied by an increase in GBF (8, 14, 16, 18) and then we extended this observation by showing that this peptide applied i.c.v. also affords such gastroprotection. The pretreatment with CCK-8 administered i.c.v. in smaller dose 625 ng/rat than those applied peripherally, reduced significantly the area of I/R- and ethanol-induced lesions and this was accompanied by a significant attenuation of the fall in GBF caused by I/R and ethanol and by the rise in plasma leptin levels as measured by RIA. The major finding of this study is demonstration for the first time in the experimental model of injury induced by I/R that this protection afforded by i.c.v. CCK is accompanied by an increment in plasma leptin levels within the limits similar to those observed after parenteral administration of exogenous leptin producing comparable degree of gastroprotection to that afforded by CCK.

It is of interest that both CCK and leptin evoked gastric mucosal protection as reflected by the reduction in gross and microscopic injury, and hyperemia when applied centrally with the extent similar to that observed after peripheral administration of these peptides. This protective and hyperemic effects were mimicked by significantly smaller doses of either peptide given centrally than those employed peripherally, suggesting that both peptides may interact on the gastroprotection against damage provoked by strong irritant such as ethanol and that evoked by the exposure of gastric mucosa to I/R. The fact that following i.c.v. application of CCK there was a dose-dependent increase in plasma leptin suggests that following intracerebral administration, CCK rapidly leaks out into the systemic circulation to reach CCK receptors located in the periphery and to activate the release of leptin. This notion is supported by study performed with radiolabelled CCK-8 that was found to diffuse largely from cerebrospinal fluid into the systemic circulation after central injection (29). In another report CCK-8 given by i.c.v. route attenuated food intake by diffusing from the brain to abdominal organs (29). This does not exclude the possibility that the central parasympathetic outflow to the stomach is enhanced by centrally applied leptin and CCK-8 and that vagal efferent nerves are involved in gastroprotection against I/R afforded by these peptides. It was proposed before that the inhibitory effects of CCK in the control of certain physiological functions such as gastric emptying, gallbladder contraction or pancreatic enzyme secretion require intact vagal pathway because vagotomy abolished these functions of CCK (30—32). In our study, vagotomy also significantly attenuated both leptin- and CCK-afforded gastroprotection against I/R and the accompanying rise in the GBF indicating that vagal pathway, indeed plays an important role in the mediation of the protective action of these hormones. Our results are also in keeping with previous observations that the vasodilator response to low dose of CCK-8 was inhibited by acute bilateral

subdiaphragmatic vagotomy and atropine (22, 24, 25). Furthermore, the protection against damage induced by ethanol was observed after the stimulation of vagal cholinergic pathways that involved recruitment of endogenous prostaglandins and CGRP from afferent sensory nerves (33).

The implication of NO in gastroprotection induced by leptin and CCK-8 was observed in our previous report (8) by showing that an inhibitor of NOS, N^G-nitro-L-arginine methyl ester (L-NAME) (34) injected i.v. prior to leptin or CCK markedly attenuated the gastroprotection afforded by these peptides and luminal NO release and that the addition of L-arginine, the substrate for NO-synthase (34—36) restored the protective activity of leptin or CCK-8 and their stimulatory action on gastric mucosal release of NO. The notion that NO contributes to the protective activity of leptin and CCK was supported by our finding in I/R model that the common feature of both leptin and CCK was the lack of protection against I/R exhibited by these peptides in animals subjected to the pretreatment with NO-synthase inhibitor, L-NNA.

Since the mechanism of gastric mucosal defense includes NO that could be released from vascular endothelium, sensory nerves or gastric epithelial cells (34), we tested the hypothesis that leptin and CCK-8 induced gastroprotection involves NO generation that originates from the activation of afferent sensory neurons by these peptides. Previous studies demonstrated that prostaglandins cooperate with NO and sensory nerves in the mechanism of gastric mucosal integrity and cytoprotection (35). It was proposed that peptides of gastrin/CCK family participate in preserving gastric mucosal integrity through the activation of sensory nerves releasing a variety of sensory neuropeptides such as calcitonin gene releasing peptide (CGRP) and tachykinins (21, 35—37). Moreover, the protective action of CCK-8 on the gastric mucosa was found to involve the activation of CCK-A receptors localized on vagal capsaicin-sensitive sensory fibers (23). In our present study, the deactivation of primary afferent nerves, using large neurotoxic dose of capsaicin about two weeks before the experiment (37—41), augmented significantly the area of I/R lesions as compared to vehicle-treated rats and significantly reduced the GBF when compared to that in animals with intact sensory nerves. Such capsaicin-induced deactivation of sensory nerves also attenuated significantly the protective activity of leptin and to a somewhat greater extent of CCK-8, and completely abolished the rise in GBF induced by both peptide. This finding indicates that sensory nerves are essential for microcirculatory response and to a lesser extent for the protective effects induced by leptin but not by CCK-8. This observation disagree with the recent finding that the protective effect of CCK-8 was only modestly modified by capsaicin-induced deactivation of sensory nerves in ethanol model, thus, showing the limited role of sensory nerves in this CCK-induced protection as reported previously (14, 19). In contrast, both the protective and hyperemic effects of CCK-8 were completely abolished in

capsaicin-denervated animals indicating that sensory afferent nerves are of crucial importance for the gastroprotective activity of this peptide against I/R-induced gastric damage. Thus, is it reasonably to assume that the release of NO and other neurotransmitters from vagal nerves could contribute strongly to the protection and the hyperemia observed after administration of CCK. One of such neurotransmitter could be CGRP because in our study the CCK- and leptin-induced protection and accompanying hyperemia were attenuated by the CGRP antagonist, CGRP₈₋₃₇ that was shown before to inhibit protective effect of exogenous gastrin as well as that released endogenously by peptone meal (38) and to counteract the protective and hyperemic effect of leptin against lesions evoked by ethanol (18).

In summary, these results demonstrate that administration of exogenous leptin either centrally or peripherally, that is accompanied by a significant increment in its plasma levels and marked increase in plasma gastrin response, exhibits a potent gastroprotective activity against the I/R and ethanol-induced gastric lesions that is similar to that obtained with CCK (42, 43). An evidence was provided that exogenous leptin applied centrally and peripherally or that released by CCK from the stomach is as effective as CCK in the protection of the gastric mucosa against the injury induced by ethanol and could prevent the progression of I/R erosions into deeper gastric lesions suggesting that leptin may mediate the gastroprotective action of CCK as proposed recently (44). Our recent studies revealed that the protective effect of centrally applied CCK-8 does not appear to depend on an interaction with the brain structures but rather involves the activation of peripheral CCK_A receptors (18) possibly located in the stomach at the terminals of vagal afferent nerves. Our previous observations (8, 44) that overexpression of *ob* mRNA combined with an elevated plasma leptin and gastrin concentrations (43) was well correlated with CCK-induced protection and the maintenance of gastric circulation indicating that leptin release is an important component of CCK regulation of gastric mucosal integrity. The data presented here shows that these protective and hyperemic effects of leptin may involve CGRP released from sensory nerves endings and production of NO due to the upregulation of NO-synthase in gastric wall.

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R e c e i v e d: October 5, 2001

A c c e p t e d: October 18, 2001

Author's address: Prof. dr S. J. Konturek, Department of Physiology Jagiellonian University School of Medicine, 16 Grzegorzeczka Str., 31-531 Cracow, Poland, Phone: (+4812) 4211006, FAX: (+4812) 4211578.

E-mail: mpkontur@cyf-kr.edu.pl