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GASTROPROTECTION AND CONTROL OF FOOD INTAKE BY LEPTIN. COMPARISON WITH CHOLECYSTOKININ AND PROSTAGLANDINS

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Circulating peptide leptin which is the product of the *ob* gene is known to provide feedback information on the size of fat stores to central OB-receptors that control food intake. Recently, leptin messenger RNA and leptin protein have been detected in gastric epithelium and leptin was found to be released by CCK into circulation but the physiological role of this gastric leptin remains unknown. As CCK has been reported to protect gastric mucosa against various noxious agents, we designed the study to determine the influence of leptin and CCK on the gastroprotection and the control of food intake and to compare them with classic gastroprotective substance, prostaglandin E_2 , in rats with acute gastric mucosal lesions induced by topical application of 75% ethanol. Four series of Wistar rats (A, B, C and D) were used to determine; A) the effects of various doses of leptin (0.1–10 $\mu\text{g}/\text{kg}$) given intraperitoneally (i.p.) on ethanol-induced gastric lesions, gastric blood flow (GBF) and plasma levels of immunoreactive leptin; B) the effects of various doses of CCK-8 (0.1–10 $\mu\text{g}/\text{kg}$ i.p.) on ethanol-induced gastric lesions, GBF and plasma levels of leptin; C) the effects of various doses of PGE_2 (12.5–100 $\mu\text{g}/\text{kg}$) given intragastrically (i.g.) on ethanol-induced gastric lesions and GBF and D) the influence of leptin, CCK and PGE_2 on the intake of liquid meal in rats. Rats were anesthetized with ether 1 h after i.g. administration of 75% ethanol to measure the GBF using H_2 -gas clearance technique and blood samples were withdrawn for the measurement of plasma leptin levels by radioimmunoassay (RIA). Food intake was assessed in separate group of rats fasted 18 h and then fed with liquid caloric meal. Leptin, CCK and PGE_2 reduced dose-dependently gastric lesions induced by 75% ethanol, the dose reducing these lesions by 50% (ED_{50}) being, respectively, 1 $\mu\text{g}/\text{kg}$, 5 $\mu\text{g}/\text{kg}$ and 20 $\mu\text{g}/\text{kg}$. The protective effects of leptin, CCK-8 and PGE_2 were accompanied by significant attenuation of the fall of the GBF caused by ethanol. Leptin and CCK reduced also dose-dependently the food intake while PGE_2 was not effective. Leptin and CCK resulted a dose-dependent increment in the plasma leptin levels. We conclude that: 1) exogenous leptin and CCK, causing similar increments in plasma immunoreactive leptin levels, protect dose-dependently gastric mucosa against the damage provoked by 75% ethanol; 2) Leptin and CCK afford similar gastroprotective activity to that attained with PGE_2 but unlike PGE_2 were highly effective in the reduction in food intake and 3) the protective effects of leptin, CCK and PGE_2 were accompanied by significant increase of GBF suggesting that the protection afforded by these substances are mediated, at least in part, by gastric hyperemia.

Key words: leptin, cholecystokinin, prostaglandin E_2 , gastroprotection, gastric blood flow

INTRODUCTION

Leptin is recognized as a key peripheral protein product of the *ob* gene acting on central hypothalamic OB-receptors that control food intake and the energy expenditure (1). Recent studies showed that immunoreactive leptin can be found in detectable amounts in the plasma of experimental animals such as mice and rats as well as in humans (2—6). Leptin is secreted by adipocytes and the placenta but recent study revealed that leptin messenger RNA and leptin protein are present in the rat gastric epithelium suggesting that stomach can be an important source of leptin (4, 5). Moreover, CCK-8 was found to increase the plasma levels of leptin and that this effect was accompanied by the fall of the content of immunoreactive leptin in the gastric epithelium. It was proposed that leptin acts centrally to inhibit the neuropeptide Y (NPY), which is a peptide involved in the stimulation of the appetite (7). Leptin acts synergistically with CCK in the control of satiety and plasma leptin levels may be elevated after administration of exogenous CCK (3, 8, 9).

CCK is the principal physiological “enterogastrone” involved in the control of gastric secretion, gastric emptying, gastrointestinal motility and food intake (10) but its role in gastric mucosal integrity has been only recently recognized (11—14). It has been reported that this peptide attenuates the gastric lesions induced by strong mucosal irritants such as ethanol and exerts its physiological effects *via* type A of CCK receptors, that have been also involved in the action of this peptide as an enterogastrone on gastric mucosa (15—18). Previous studies documented that the gastroprotective effect of CCK against the mucosal damage induced by ethanol could be attributed to the increase in gastric blood flow (GBF) evoked by this peptide (12—14). 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 proposed that CCK-A receptors on sensory nerves are involved in CCK-induced gastroprotection (13, 14, 19, 20). As CCK was found to increase the gastric mucosal expression and release of leptin it prompted our present study to determine the involvement of leptin in gastroprotection afforded by CCK and to compare this protection to that afforded by classic “cytoprotective” substance such as PGE₂ (21).

This study was designed: 1) to determine the effects of exogenous leptin and CCK on the gastric lesions induced by 75% ethanol, food intake and the gastric blood flow and 2) to compare the gastroprotective effects of leptin with those attained with classic gastroprotective substance such as PGE₂ and 3) to assess the plasma levels of leptin in tests with pretreatment with exogenous leptin and CCK followed by the exposure to 75% ethanol.

MATERIAL AND METHODS

Male Wistar rats, weighing 180–220 g, were fasted for 24 h with only water available at all times and used in studies with food intake and in tests with gastroprotection.

Feeding experiments

Special group of 10 rats used for studies with feeding. Liquid meal (rat Liquid Diet, Polfa, Poland) consisting of cow milk with addition of 5% sucrose and containing about 0.82 kcal/ml was used in all experiments with feeding. Sixty minutes prior feeding, the fasted animals were placed in Bollman-type individual cages. Liquid meal was then presented in calibrated glass cylinders for measurement of food intake. Based on our previous experiments with feeding, the feeding period was limited only to 30 min and the food consumed over the first 15 min was used to express data because during this period both the maximum rate of food intake was observed and the maximum effect of CCK or leptin occurred (22). In tests with hormonal peptides, leptin or CCK (both purchased from Sigma Co, MO, USA) was administered i.p. in various doses ranging from 0.1 to 100 µg/kg, each dose being injected in separate test day in saline (1 ml). This dose range was selected because it caused significant protection of gastric mucosa from ethanol damage.

Gastroprotection studies and measurement of gastric blood flow (GBF)

Acute gastric lesions were induced by an intragastric (i.g.) application of 75% ethanol similarly to the method described previously (23, 24). Briefly, 75% ethanol in a volume of 1.5 ml was administered i.g. to rats by means of a metal orogastric tube. After 60 min, the animals were lightly anesthetized with ether, their abdomen was opened by the midline incision and stomach exposed for the measurement of GBF by means of H₂-gas clearance technique as described previously (25). For this purpose double electrodes of 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 H₂ and another for the measurement of tissue H₂. With this method, the H₂ generated locally was carried out by blood flow, while the polarographic current detector read out decreasing tissue H₂. The tissue H₂ clearance curve was used to calculate an absolute flow rate (ml/100g/min) in the oxyntic area as described previously (25). The measurements were made in three areas of the mucosa and the mean values of the measurements were calculated and expressed as percent changes of those recorded in the vehicle (saline) treated animals. After the GBF measurement, 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) (12, 13) by the person who did not know to which experimental group animals belonged.

Experimental groups of rats

In subsequent studies three major series (A, B and C) of experiments were carried out. Series A, B and C was used to compare the effects of exogenous leptin (0.1–100 µg/kg i.p.), CCK (1–100 µg/kg i.p.) and PGE₂ (12.5–100 µg/kg i.g.) on acute gastric mucosal lesions induced by 75% ethanol.

The following treatments were applied in rats of series A; 1) vehicle (1 ml of saline i.p.) followed 30 min later by 75% ethanol and 2) leptin (0.1–100 µg/kg i.p.) followed 30 min later by 75% ethanol. In series B, vehicle or CCK-8 (1–100 µg/kg i.p.) was administered and this was followed 30 min later by 75% ethanol. In series C, PGE₂ (12.5–100 µg/kg i.g.) was used to study the effects of this agent on ethanol-induced gastric lesions. At the termination of some experiments with i.p. administration of leptin and CCK-8 or with i.g. PGE₂, 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 (3,5). For comparison, intact rats fasted overnight and given only vehicle saline i.p. were also anaesthetized 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 minutes 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) (5). 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 was less than 9%.

Statistical analysis

Results are expressed as means \pm SEM. Statistical analysis was done using nonparametric Mann-Whitney and Friedman two-way analysis of variance. Differences with $p < 0.05$ were considered as significant.

RESULTS

Effects of exogenous leptin, CCK-8 and PGE₂ on food intake

Cumulative intake of liquid meal during normal feeding of this meal is shown on Fig. 1. Rats ate vigorously during the first 15 min-period but then

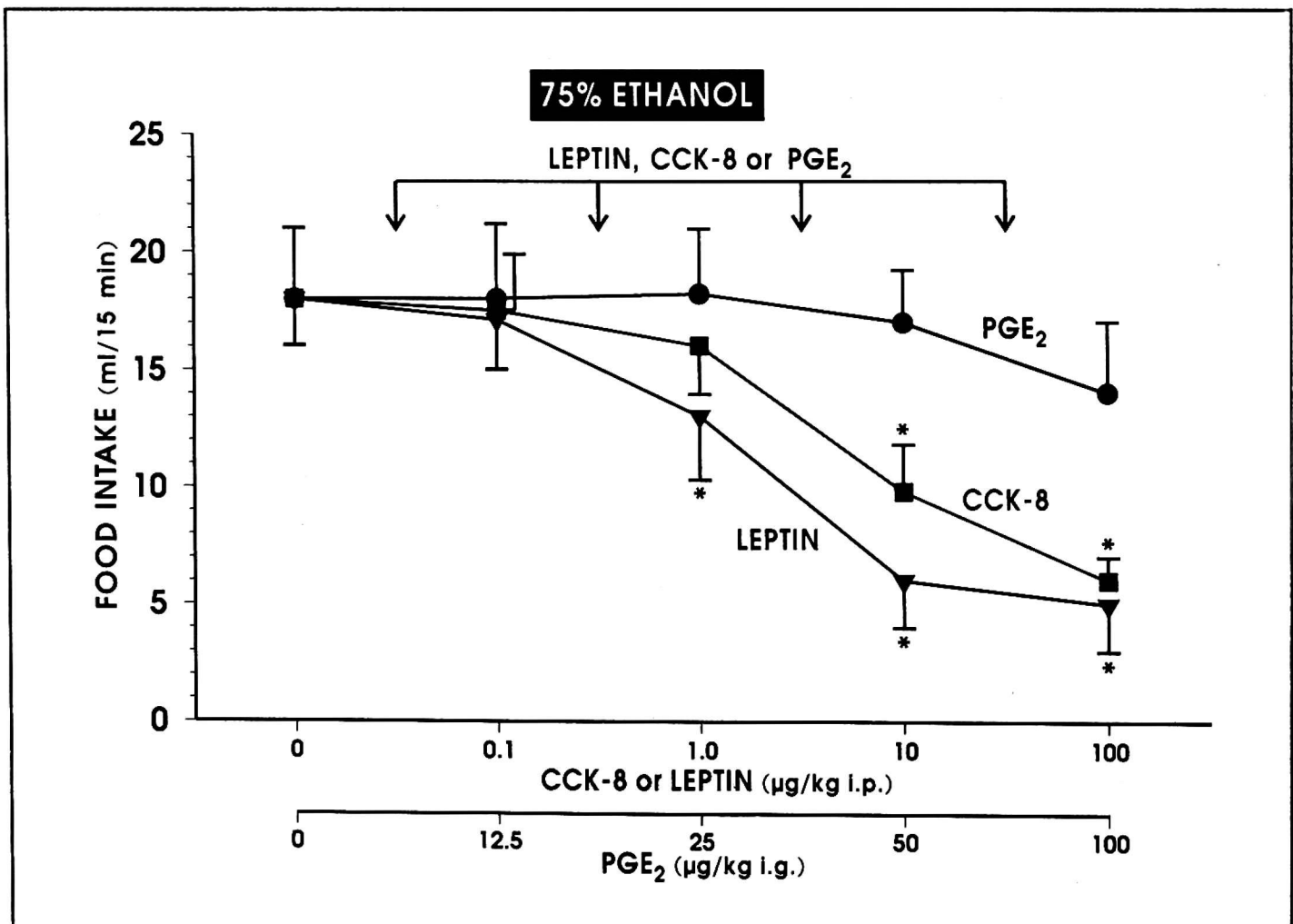


Fig. 1. Food intake (ml/15 min) in control tests with vehicle saline administration and following administration of varying doses of leptin (i.p.), CCK (i.p.) or PGE₂ (i.g.). Means \pm SEM of 10 tests on 10 rats. Asterisk indicates significant change as compared to vehicle control values.

the rate of food intake was much smaller during the remainder of the feeding period. CCK or leptin injected i.p. in varying doses reduced dose-dependently the food intake in rats the threshold dose of CCK which significantly reduced the food intake was 1 $\mu\text{g}/\text{kg}$ and that of leptin was also 1 $\mu\text{g}/\text{kg}$. With the highest dose of CCK and leptin the food intake was only about 40 and 35%, respectively.

Effect of exogenous leptin, CCK-8 and PGE_2 on the ethanol induced lesions and the GBF and plasma leptin levels

As shown on Fig. 2, 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 $\mu\text{g}/\text{kg}$ and with the ID_{50} averaging about 10 $\mu\text{g}/\text{kg}$ of peptide. The pretreatment with CCK-8 administered i.p.

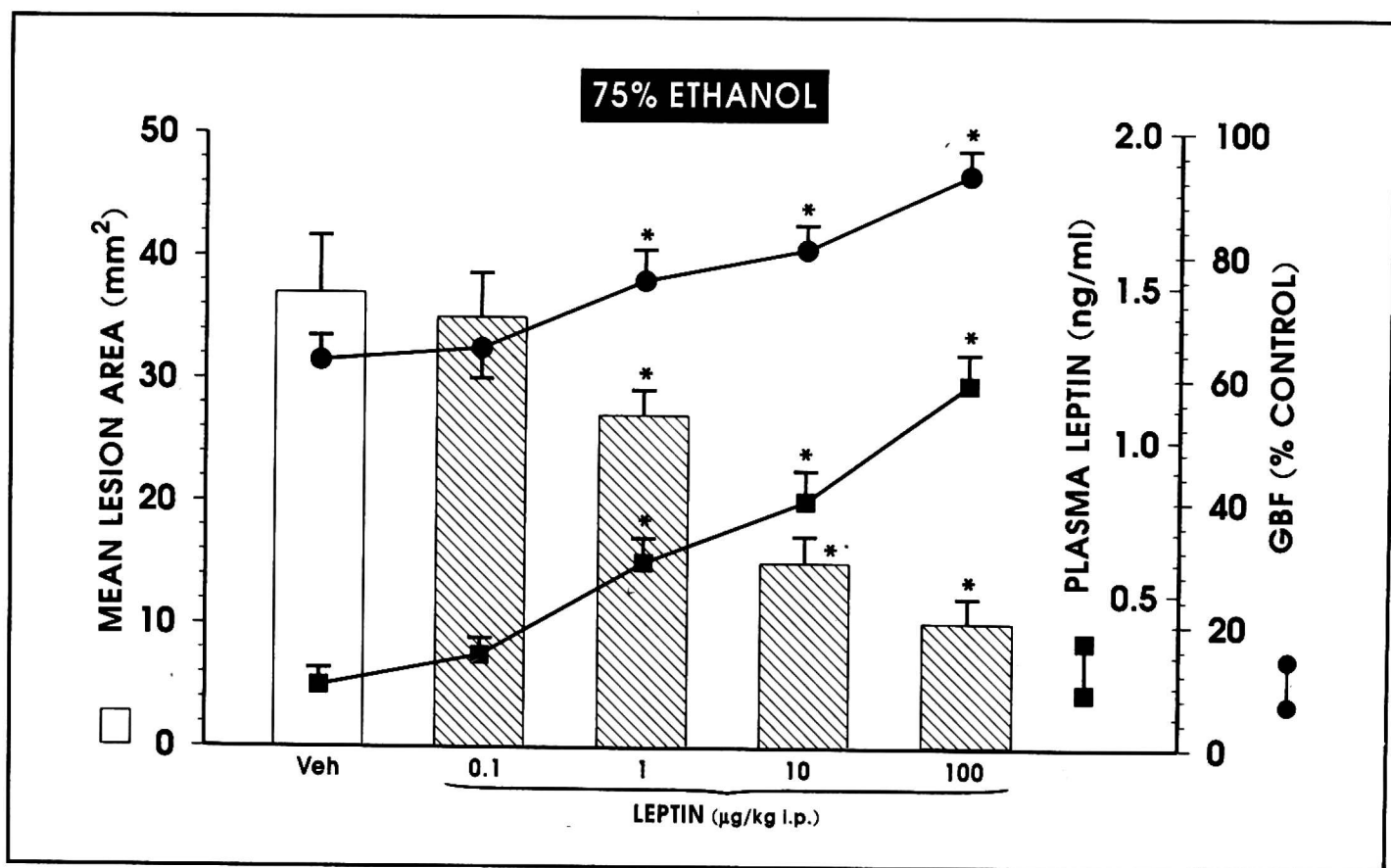


Fig. 2. The area of ethanol-induced gastric lesions, gastric blood flow (GBF) and plasma immunoreactivity of leptin in rats treated with vehicle (saline) or with various doses of leptin (0.1–100 $\mu\text{g}/\text{kg}$ i.p.). Means \pm SEM of 6–8 rats. Asterisk indicates a significant change as compared to the vehicle control values.

in graded doses ranging from 1 $\mu\text{g}/\text{kg}$ up to 100 $\mu\text{g}/\text{kg}$ attenuated significantly the area of lesions induced by 75% ethanol with the ID_{50} averaging 8 $\mu\text{g}/\text{kg}$ (Fig. 3). The protective effect of leptin and CCK-8 were 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/100 g (taken as

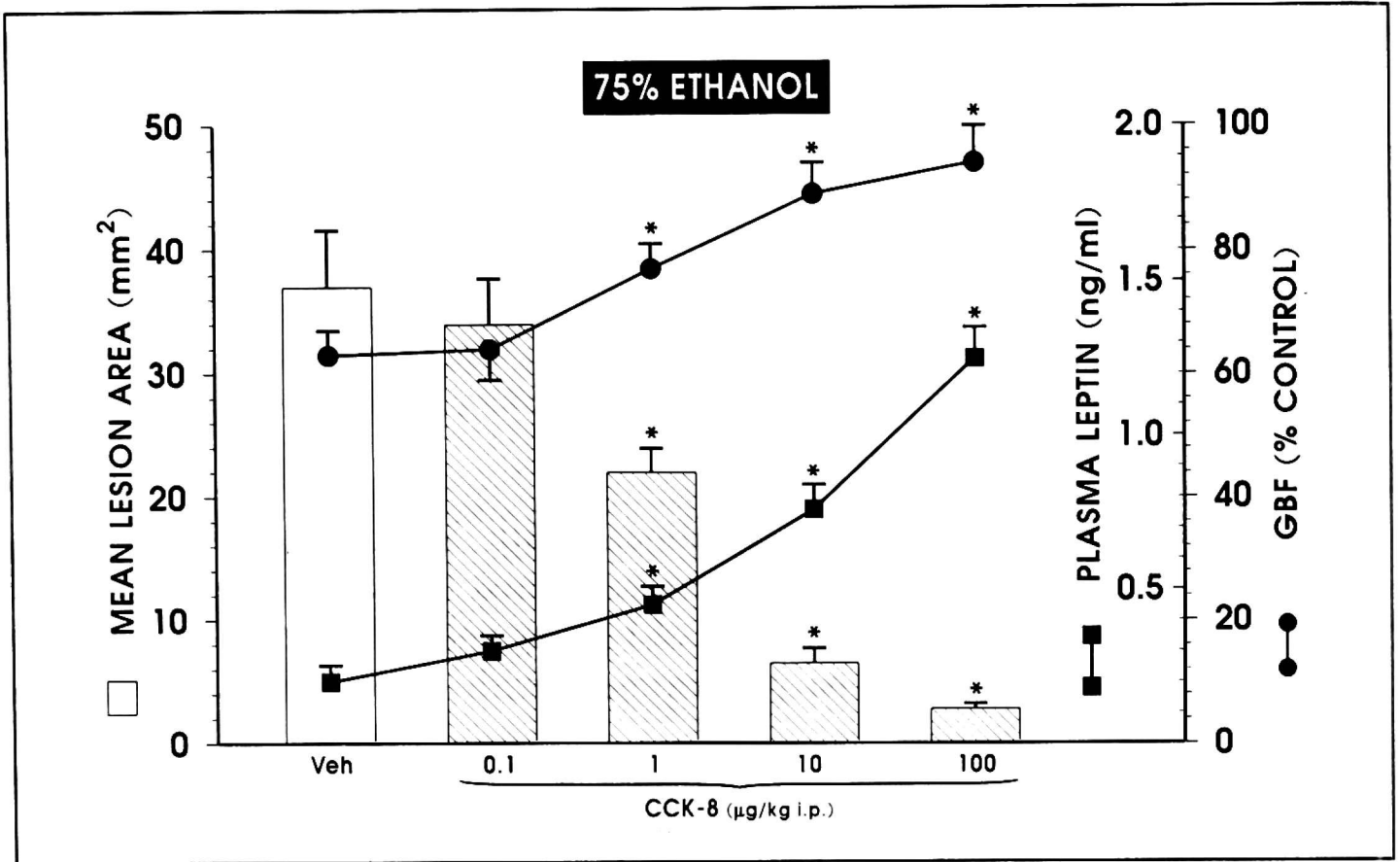


Fig. 3. The area of ethanol-induced gastric lesions, gastric blood flow (GBF) and plasma immunoreactivity of leptin in rats treated with vehicle (saline) or with various doses of CCK (1–100 µg/kg i.p.). Means \pm SEM of 6–8 rats. Asterisk indicates a significant change as compared to the vehicle control values.

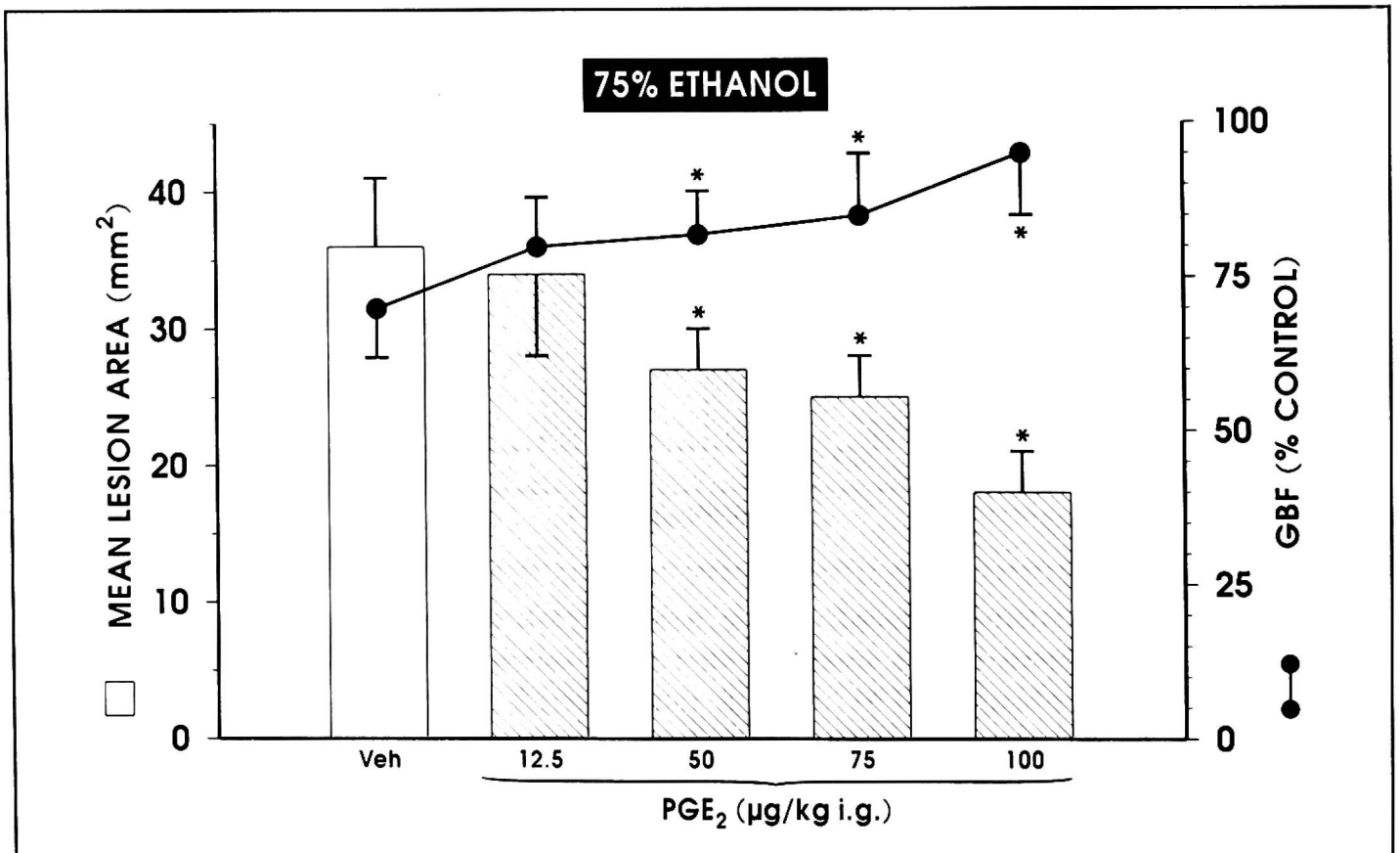


Fig. 4. Mean area of gastric lesions induced by 75% ethanol and the changes in the GBF in the gastric mucosa of rats treated with vehicle, or various doses of PGE₂ (12.5–100 µg/kg i.g.). Mean \pm SEM of 6–8 rats. Asterisk indicates a significant change as compared to the value obtained in vehicle-control animals.

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, the significant reduction in GBF by about 30% was recorded. With graded doses of leptin or CCK8 administered before 100% ethanol, the area of gastric lesions was significantly attenuated and a significant increase in the GBF starting with 1 $\mu\text{g}/\text{kg}$ of leptin or CCK8 was recorded (*Fig. 3*).

Fig. 4 shows the effects of i.g. administration of PGE_2 on the area of gastric lesions induced by 75% ethanol and the GBF following various doses of PGE_2 . PGE_2 caused significant and dose-dependent reduction in the ethanol-induced lesion area starting at the dose of 50 $\mu\text{g}/\text{kg}$ and this was accompanied by gradual and significant increase in GBF reaching at the dose of 100 $\mu\text{g}/\text{kg}$ the level not significantly different from that recorded in intact rats not exposed to 75% ethanol.

DISCUSSION

This study demonstrates for the first time that administration of exogenous leptin that was accompanied by a significant increment in plasma levels of this peptide exhibited dose-dependent gastroprotective activity against the ethanol-induced lesions and this protection was similar to that obtained with exogenous CCK. Since the protective effects of leptin occurred at the dose levels which did not affect gastric acid secretion (unpublished data), this study indicates that leptin is truly gastroprotective substance because its protective activity appears to be independent on gastric acid secretory activity. We confirm our and other observations (11—14) that pretreatment with CCK8 applied in the doses that had mild stimulatory effect on gastric acid secretion, dose-dependently attenuate mucosal lesions induced by ethanol and this effect, like that observed with leptin, was accompanied by a significant and dose-dependent elevation of GBF. As the protective effect of CCK was accompanied by elevated plasma leptin level and decreased leptin contents in the gastric mucosa, the enhanced resistance of gastric mucosa against the noxious effect of ethanol strongly suggests 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 possibly also plasma leptin level (5), attenuated significantly ethanol-induced gastric lesions similarly as exogenous CCK (12). The common feature of both leptin and CCK is an attenuation of the post-ethanol fall in GBF that might originate from the excessive release of nitric oxide (NO) as proposed previously (11—13) suggesting that the protective and hyperemic effects of both these hormonal peptides

may be mediated by NO. Further studies are required to explain the involvement of NO in gastroprotective and hyperemic effects of leptin.

In contrast to NO, PG do not appear to contribute to the observed gastroprotection and the reduction in food intake by leptin or CCK. Unlike CCK or leptin, endogenous PGE₂ applied intragastrically in doses that protected gastric mucosa against ethanol damage failed to affect the food intake. Furthermore, our preliminary results (data not published) showed that the suppression of cyclooxygenase (COX) by indomethacin failed to influence the protective and hyperemic effects of exogenous leptin or CCK8, confirming that endogenous PG may not be involved in these effects.

It has been shown previously that CCK is one of the major physiological enterogastrone-like substances modulating the secretory function of the stomach such as inhibition of gastric secretion by gastric distension or intraduodenal fat (10, 17, 20). 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 type A CCK receptors (12, 13, 16, 17). The role of CCK-A receptors in the action of CCK was further supported by observation that the blockade of CCK-A receptor with its highly selective antagonist, MK-329, 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 (18). All the above observations were obtained, however, using anesthetized animals, showing relatively negligible gastric secretory activity due to its inhibition by anesthetics as observed by Garner's group (26). Our study (unpublished results) on gastric secretion performed on conscious rats without anesthetics shows that exogenous CCK is rather weak stimulant of gastric secretion whereas leptin failed to affect this secretion, again indicating some differences at least with respect to their gastric secretory effects between exogenous CCK and leptin. The small but significant difference in the gastric secretory action of these peptides does not necessarily disagree with previous finding indicating the existence of a functional synergistic interaction between CCK and leptin in the suppression of food intake by these peptides (8, 9). This synergistic interaction of CCK and leptin probably involved central receptors for satiety signals.

Another attempt in this study was related to the role of endogenous leptin in the mechanism of protective action of CCK against the lesions provoked by 75% ethanol.

In the present study we confirmed our previous observation that CCK exhibits dose-dependent protection against ethanol-induced damage being accompanied by an increase in GBF (12,13) and reaching nearly 90% at higher dose of this hormonal peptide. We found that this CCK-induced protection was accompanied by dose-dependent increment in the plasma leptin level

similar to that observed after administration of exogenous leptin producing the rate of gastroprotection similar to that afforded by CCK.

Our observation that plasma leptin release is significantly increased in CCK-treated rats invites the speculation that the rise in plasma leptin increments following treatment with CCK originates mainly from the gastric mucosa and plays an important role in CCK-evoked gastroprotection against ethanol lesions due possibly to local activation of leptin gene and enhancing the release of gastric leptin into the circulation but whether leptin and CCK protects directly the mucosal cells requires appropriate experimental evidence.

In summary, this results provides for the first time an evidence that leptin originating from the stomach is as effective as CCK in the protection of 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 (data not published) followed by an elevated plasma leptin concentration well correlated with CCK-induced protection indicating that leptin release is an important component of CCK-related control of gastric mucosal integrity and circulation. Further studies are needed to clarify whether the protective and hyperemic effects of leptin appear to depend upon the vagal and afferent sensory nerves and NO but they appear to be unrelated to endogenous PG and gastric secretion.

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