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T. BRZOZOWSKI, P.C. KONTUREK*, Z. SLIWOWSKI, D. DROZDOWICZ, S. KWIECIEN, M. PAWLIK, R. PAJDO, S. J. KONTUREK, W.W. PAWLIK, E. G. HAHN*

NEURAL ASPECTS OF GHRELIN-INDUCED GASTROPROTECTION AGAINST MUCOSAL INJURY INDUCED BY NOXIOUS AGENTS

Department of Physiology, Jagiellonian University Medical College, Cracow, Poland;
*Department of Medicine I, University Erlangen-Nuremberg, Erlangen, Germany

Ghrelin, identified in oxyntic mucosa has been recently implicated in the control of food intake and growth hormone (GH) release but whether this hormone can influence the gastric secretion and gastric mucosal integrity have been little studied. We compared the effects of intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) administration of ghrelin on gastric secretion in rats equipped with gastric fistula (GF) and gastric lesions induced in rats by 75% ethanol and ischemia-reperfusion (I/R) with or without vagotomy or functional ablation of afferent sensory nerves by capsaicin. The number and the area of gastric lesions was measured by planimetry, the GBF was assessed by H_2-gas clearance method and blood was withdrawn for the determination of the plasma ghrelin and gastrin levels. Ghrelin (5-80 μg/kg i.p. or 600-5000 ng/rat i.c.v.) increased gastric acid secretion and attenuated gastric lesions induced by ethanol and I/R. These protective effects of ghrelin were accompanied by the significant rise in the gastric mucosal blood flow (GBF) and plasma ghrelin and gastrin levels. Ghrelin given i.p. or injected i.c.v. in standard doses 20 μg/kg or 5000 ng/kg, respectively, significantly attenuated the gastric mucosal damage and significantly raised the GBF. Ethanol applied i.g. in smaller concentrations (12.5% and 25%) produced a significant increase in plasma immunorective ghrelin levels and this effect was inhibited in rats receiving ethanol in higher concentrations (75% and 100%). Ghrelin-induced protection after its i.p. or i.c.v. administration and accompanying increase in the GBF were completely abolished by vagotomy and capsaicin-deactivation of sensory nerves. Concurrent treatment with CGRP added to ghrelin restored the gastroprotective and hyperemic effects of ghrelin applied i.p. or i.c.v. in rats with capsaicin denervation. We conclude that central and peripheral ghrelin exerts a potent protective and gastric secretory effects in rats exposed to ethanol and I/R, and that these actions involve vagal nerve integrity, partially depending upon afferent nerves and hyperemia mediated by sensory neuropeptides such as CGRP released from these nerves.

Key words: *ghrelin, gastroprotection, gastric mucosal blood flow, vagal nerve, sensory afferent nerves, calcitonin gene related peptide, ethanol, ischemia-reperfusion*

INTRODUCTION

Ghrelin is a novel 28-amino acid peptide with an n-octanoyl ester in the serine 3 that has recently been discovered in rat and human gastrointestinal tract, particularly gastric mucosa, as an endogenous ligand for growth hormone (GH) secretagogue receptor (GHS-R) (1, 2). Recent studies revealed that ghrelin is produced and stored in granules of the special neuroendocrine cells (A-like cells), located mainly in oxyntic mucosa but not in ECL, EC or D cells (3, 4). Ghrelin shows some similarity in structure and function to motilin and potently stimulates the release of GH, acting as a natural ligand for the GHRS which is the endogenous counter of the family of synthetic, peptidyl and nonpeptidyl GH secretagogues (5, 6).

Recent studies revealed that the functions of this novel hormone are related to the control of food intake and GH secretion. Ghrelin stimulates food intake and body weight gain exerting a modulating effect on energy expenditure (7, 8). The release of ghrelin may be influenced by the status of fasting and nutrient feeding because central and peripheral administration of this peptide to rats resulted in an increase in their feeding behaviour (8). Previous studies in humans revealed that gastrectomy produced dramatic fall in the plasma ghrelin levels, whereas fasting and *anorexia nervosa* were accompanied by elevated plasma ghrelin concentration supporting the notion that the gastrointestinal tract, primarily the stomach, is a major source of circulating ghrelin that could be considered as a starvation-related hormone (2, 9, 10).

The role of ghrelin in the mechanism of gastric mucosal defence and gastroprotection has been little investigated except for the report of Sibilia *et al*. (11) and our recent studies (12, 13). These studies showed that central (intracerebroventicular; i.c.v.) administration of ghrelin reduced the lesions induced by ethanol (11) and that intraperitoneal (i.p.) administration of ghrelin attenuates stress- and ethanol-induced gastric lesions (12, 13). Moreover, this gastroprotective effect of systemic ghrelin against ethanol- and stress-induced damage was attenuated by the blockade of NOS activity with L-NNA and by the functional ablation of sensory afferent nerves with capsaicin suggesting that NO and neuropeptides released from sensory nerves play an important role in this protection (12, 13). This is in agreement with the general hypothesis that prostanoids, NO and sensory neuropeptides cooperate in the mechanism of maintenance of gastric integrity (14). The question remains whether centrally applied ghrelin can influence gastric acid secretion, gastric lesions caused by ethanol applied in a different concentrations and gastric injury induced by stomach exposure to ischemia-reperfusion (I/R) and finally, whether this appetite hormone requires vagal and sensory nerve integrity in its action on the stomach.

This study was designed: 1) to determine the effect of i.p. and i.c.v. administration of ghrelin on the formation of gastric lesions induced by ischemia-reperfusion (I/R) and the gastric mucosal blood flow (GBF) and to compare these

effects with those in rats exposed to graded concentration of ethanol; 2) to determine the plasma levels of ghrelin of intact animals and in those exposed to ethanol applied in graded concentrations with or without the pretreatment with central or peripheral administration of ghrelin, and 3) to elucidate the involvement of vagal and sensory innervation as well as gastrin in gastroprotective action of ghrelin against I/R and ethanol damage.

MATERIAL AND METHODS

Male Wistar rats, weighing 180 - 220 g and fasted for 24 h, were used in our studies. All experimental procedures were approved by the Jagiellonian University Institutional Animal Care and Use Committee.

Gastric secretory studies

The effects of ghrelin (murine recombinant) purchased from Bachem AG, Bubendorf, Switzerland, on gastric acid secretion were examined in 50 conscious rats equipped about 1 month earlier with a Thomas-type gastric fistula (GF) as described previously (15). The animals were fasted overnight but had free access to water 24 h before the experiment and they were placed in individual Bollman-type cages to maintain the minimum restraint necessary. For the i.c.v. injection of vehicle (saline) or ghrelin, the GF rats underwent surgery 48 h before the secretory studies according to the method published elsewhere (16). 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 very 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 µl 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. At the day of secretory test, the GF 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 then vehicle or ghrelin was injected i.c.v. in various doses (0.1-5 µg/kg) in a volume of 5 µl using a 10 µl Hamilton microsyringe. Each dose of the peptide was administered on a separate test day. For the comparison, another group of rats was treated with ghrelin administered intraperitoneally (i.p.) and gastric secretion was examined as in tests with i.c.v. application of this hormone. The collection of gastric juice was continued for the final 2 h after all i.c.v. or i.p. injections and the volume and acid concentration of each collected sample of gastric juice were measured and acid outputs (expressed in term of micromoles of acid per 30 min) were determined as described before (16).

Production of gastric lesions and measurement of gastric blood flow (GBF)

Acute gastric lesions were produced by exposing 90 rats to an intragastric (i.g.) application of 1.5 ml of 12.5-100% ethanol by means of a metal orogastric tube as described in our previous studies (17, 18) or by exposing of their stomach to 30 min of ischemia (I) by clamping of celiac artery followed by 3 h of reperfusion (R) as originally described by Wada *et al* (19) and our group (17). Briefly, under pentobarbital anesthesia (50 mg/kg i.p.), the abdomen was opened, the celiac artery identified and clamped with a small device for 30 min followed by removal of the clamp to obtain reperfusion.

One hour after the ethanol application and at 3 h upon the termination of I/R, 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 H_2-gas clearance technique as described previously (15). The measurements were made in three areas of the oxyntic 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 saline and pinned open for macroscopic examination. The area of necrotic lesions in oxyntic mucosa following ethanol and those induced by I/R were determined by computerized planimetry (Morphomat, Carl Zeiss, FRG) by the person who did not know to which experimental group animals belonged.

Involvement of vagal and sensory nerves in gastroprotection induced by ghrelin

In subsequent studies four major series (A, B, C and D) of experiments were carried out. Series A was used to assess the effect of exogenous ghrelin given i.p. against the mucosal lesions induced by 75% ethanol (considered as a standard concentration) and I/R. Series B, C, and D were used to study the involvement of vagal and sensory innervation, in protection afforded by central (i.c.v.) or peripheral (i.p.) injection of ghrelin against I/R-induced mucosal damage. The standard dose of 20 μg/kg (i.p.) or 5000 ng/rat (i.c.v.) of ghrelin was used against graded concentrations of ethanol and I/R lesions and the rationale for choosing this dose was based on our preliminary observations showing that ghrelin at these dosages given i.p. or i.c.v. inhibited the area of ethanol-induced lesions by about 50% (13).

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 μl/rat i.c.v) followed 30 min later by 75% ethanol or I/R; 2) ghrelin (standard dose; 20 μg/kg i.p. or 5000 ng/rat i.c.v.) followed 30 min later by ethanol applied i.g. in graded concentrations ranging from 12.5% up to 100%, 3) ghrelin (600-5000 ng/rat i.c.v.) followed 30 min later by 75% ethanol; 4) ghrelin (5-80 μg/kg i.p.) followed 30 min later by I/R; 5) ghrelin (standard dose; 20 μg/kg i.p. or 5000 ng/rat i.c.v.) followed 30 min later by 75% ethanol and I/R.

The involvement of vagal nerves in gastroprotection by i.p. and i.c.v. application of ghrelin (series C) was studied in rats with or without subdiaphragmatic vagotomy after cutting off vagal nerves as described previously (20). About 30 min before the experiment, rats were anesthetized with ether and abdomen was opened by small incision. Both branches of vagal nerves were identified at the level of diaphragm and finally cut off. The control rats were treated similarly except the vagi were only uncovered but left intact. Vagotomized and sham-operated rats received ghrelin (20 μg/kg i.p. or 5000 ng/kg i.c.v.) and 30 min later were exposed to 3h of I/R. Then rats were sacrificed and area of gastric lesions and GBF were measured in similar manner as mentioned above.

The role of sensory afferent nerves and neuropeptides such as CGRP released from sensitive afferent nerve endings in gastroprotection by ghrelin was tested by employing rats with capsaicin induced deactivation of these nerves (21, 22). For this purpose the animals were pretreated with capsaicin (Sigma Co., St. Louis, MO) injected s.c. for 3 consecutive days at a respective dose of 25, 50 and 50 mg/kg about 2 weeks before the experiment (23). All injections of capsaicin were performed under ether anesthesia to counteract the respiratory impairment associated with injection of this agent. All animals pretreated with capsaicin showed negative wiping movement test, confirming functional denervation of the capsaicin sensitive nerves. The experimental protocol included the following study groups, each consisting of 6-8 animals; 1) vehicle (saline 1 ml i.p.) followed 30 min later by I/R in rats with intact afferent nerves; 2) ghrelin (standard dose; 20 μg/kg i.p. or 5000 ng/kg i.c.v.) followed 30 min later by I/R in rats with intact sensory nerves; 3) vehicle (saline 1 ml i.p.) followed 30 min later by I/R in rats with capsaicin deactivated afferent nerves, and 4) ghrelin (20 μg/kg i.p or 5000 ng/kg i.c.v.) followed 30 min later by I/R in rats with capsaicin deactivated afferent nerves with or without the concurrent supplementation therapy with exogenous CGRP (10 μg/kg s.c.).

Determination of plasma ghrelin and gastrin levels by radioimmunoassay

At the termination of some experiments with ghrelin alone applied i.p. or i.c.v. and ghrelin injection followed 30 min later by ethanol, the rats were anesthetized with ether and the blood samples (about 3 ml) were taken from the *vena cava* for the measurement of plasma ghrelin and gastrin levels by RIA as described previously (3, 12). For comparison, intact rats fasted overnight and given i.p. only vehicle saline were also anaesthetized with ether and the blood samples were collected for the determination of control values of plasma ghrelin concentration. 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 ghrelin levels using RIA-kit for rat ghrelin from Bachem AG (Bubendorf, Switzerland) (12). Briefly, the RIA-ghrelin involved the competition of a rat ghrelin sample with ^{125}I-rat ghrelin tracer for binding to a specific rabbit antighrelin polyclonal antibody. The limit of assay sensitivity was 4 pg per tube; the intra-assay variation was less than 9% and the interassay variation less than 6%. The plasma gastrin level was measured using gastrin antiserum 4562 (a gift of Professor J. F. Rehfeld, Rigshospitalet, Copenhagen, Denmark). This antiserum recognizes the C-terminal amidated end of human and rat gastrin-17 as described before (23).

Statistical analysis

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

RESULTS

Effects of exogenous ghrelin applied i.p. or i.c.v. on gastric acid secretion

The effects of vehicle (saline) or ghrelin applied in the different doses ranging from 5 to 80 μg/kg (i.p.) or from 300 ng/rat up to 5000 ng/rat (i.c.v.) on gastric acid secretion from the GF in conscious rats are shown in *Fig. 1*. In control rats injected with vehicle i.p. or i.c.v, the basal acid output averaged 113 ± 11 μmol/30 min or 111 ± 15 μmol/30 min, respectively. Ghrelin given i.p. or i.c.v in graded doses ranging from 5 μg/kg to 80 μg/kg or 300 ng/rat up to 5000 ng/rat, respectively, raised dose-dependently gastric acid secretion with the highest output of gastric acid secretion attained at the dose of 80 μg/kg of this peptide applied i.p. or 5000 ng/kg injected i.c.v., and reaching about 50% of basal, pretreatment outputs (*Fig. 1*).

Effects of exogenous ghrelin on ethanol- and I/R-induced lesions, the alterations in the GBF and plasma ghrelin and gastrin levels

The results of i.c.v. administration of ghrelin on the mean number of gastric lesions induced by ethanol and accompanying changes in the GBF and plasma ghrelin levels are presented in *Fig. 2*. Such pretreatment with ghrelin applied i.c.v. reduced dose-dependently the number of gastric lesions evoked by ethanol with the threshold reduction occurring at a dose of 2500 ng/rat and with the ID_{50}

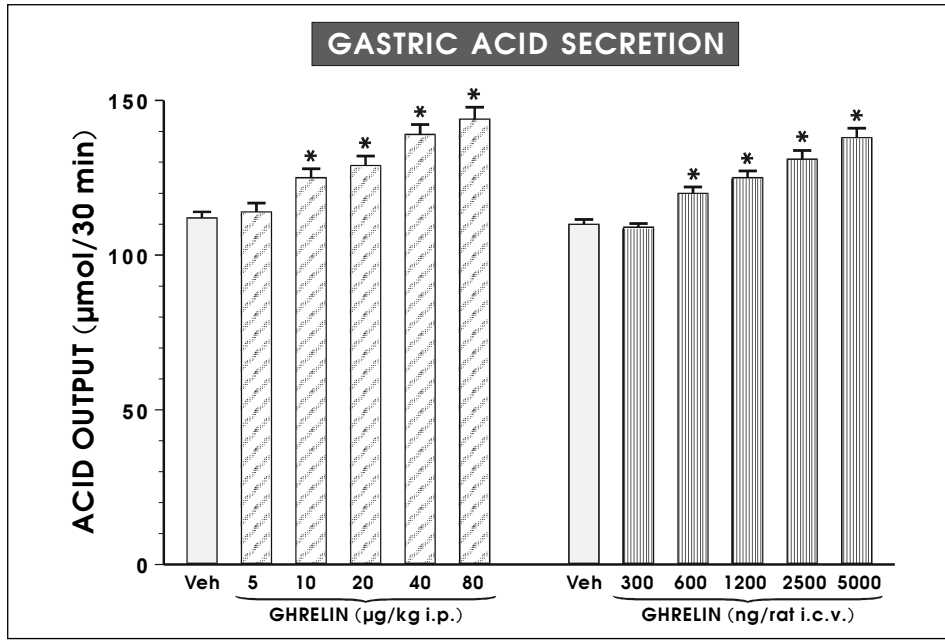

Fig. 1. Gastric acid secretion in conscious rats equipped with gastric fistula and injected with graded doses of ghrelin applied i.p. (5-80 µg/kg) or i.c.v. (300 – 5 000 ng/rat i.c.v.). Mean ± SEM of 6-8 rats. Asterisk indicates significant change as compared to the value recorded in the vehicle-treated control rats.

averaging about 1600 ng/rat of ghrelin. The protective effects of ghrelin were accompanied by a significant and dose-dependent rise in the GBF and plasma ghrelin levels starting from the dose of 1200 ng/rat of ghrelin applied i.c.v. The maximal reduction of the ethanol-induced gastric lesions after central ghrelin administered i.c.v. at the dose of 5000 ng/rat was comparable with those achieved with peripheral ghrelin applied i.p. in a standard dose of 20 µg/kg (*Fig. 2*).

As shown in *Fig. 3*, ethanol applied i.g. in different concentrations ranging from 12.5% up to 100% resulted in a concentration-dependent increase in the area of gastric lesions and this effect was accompanied by the significant fall in the GBF. Pretreatment with ghrelin (20 µg/kg i.p. or 5000 ng/rat i.c.v.) significantly decreased the area of gastric lesions induced by ethanol applied in graded concentrations. This attenuation of lesions induced by ethanol was accompanied by the reversal of the fall in the GBF evoked by increasing concentrations (12.5 % - 100%) of this topical damaging agent. Plasma ghrelin levels were significantly raised in animals exposed to ethanol applied i.g. in a concentrations of 12.5% and 25% as compared to the basal immunoreactive ghrelin increments in vehicle-treated control rats without ethanol administration (*Fig. 4*). With increasing concentrations of ethanol (50%-100%), a significant decrease in the

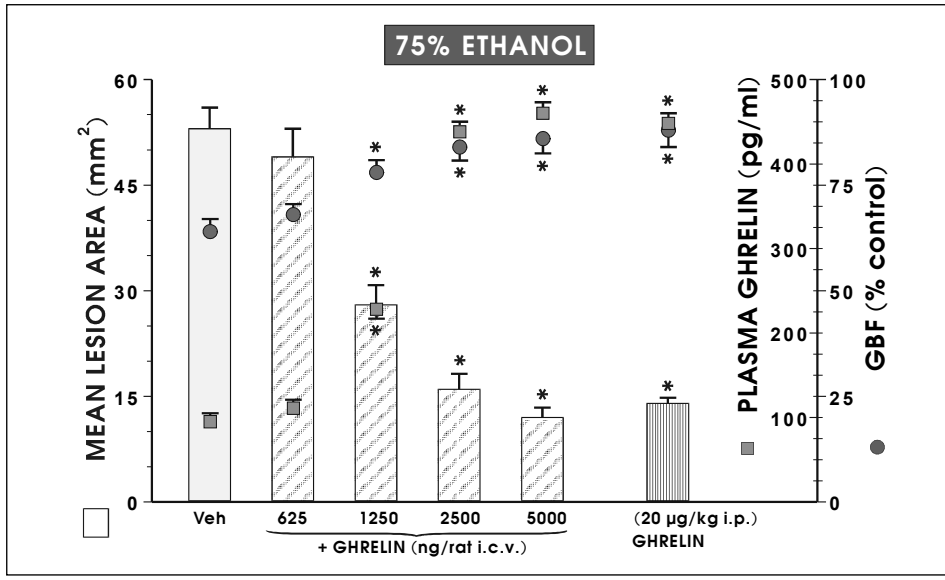

Fig. 2. The mean area of gastric lesions induced by 75% ethanol, the alterations in the gastric blood flow (GBF) and plasma immunoreactivity of ghrelin in rats treated with vehicle (saline) or with various doses of ghrelin (600 - 5 000 ng/rat i.c.v.) or with ghrelin applied in a standard dose of 20 µg/kg i.p. Means ± SEM of 6-8 rats. Asterisk indicates a significant change as compared to the vehicle-control values.

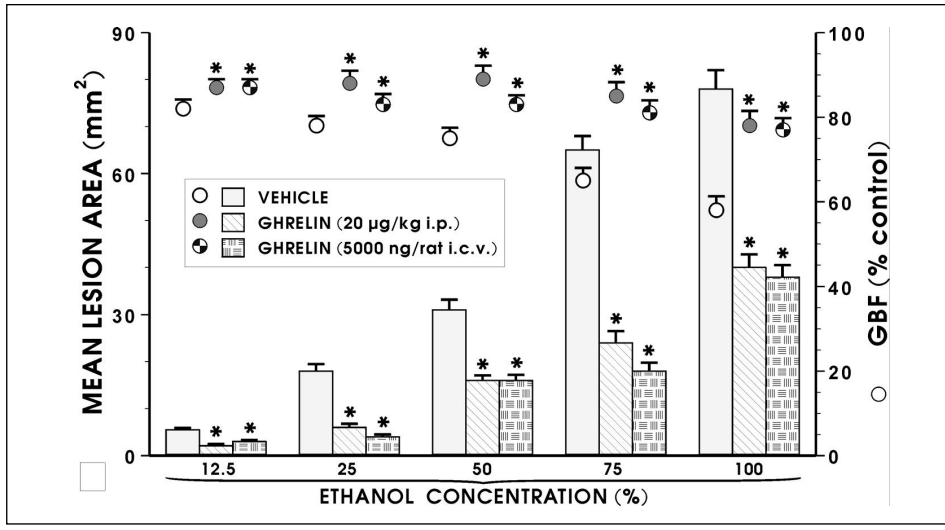

Fig. 3. The mean area of gastric lesions induced by ethanol applied i.g. in various concentrations ranging from 12.5 % up to 100% and accompanying changes in the gastric blood flow (GBF) in rats pretreated with vehicle (saline) or ghrelin applied i.p. in a standard dose of 20 µg/kg i.p. or injected i.c.v. in a standard dose of 5000 ng/rat. Means ± SEM of 6-8 rats. Asterisk indicates a significant change as compared to control values obtained at each concentration of ethanol without ghrelin pretreatment.

plasma ghrelin concentration was observed as compared with those detected in rats treated with ethanol given in lower concentrations (12.5% and 25%), however these plasma ghrelin levels in rats with ethanol applied in highre concentrations (50% - 100%) were still significantly elevated over the values recorded in vehicle-control animals.

Effect of vagotomy on ghrelin afforded gastroprotection against I/R-induced gastric damage and the alterations in the GBF

Fig. 5 shows the effect of i.c.v. or i.g. administration of ghrelin on the area of gastric lesions induced by I/R in vagotomized rats and, for comparison, in those with intact vagal nerves without vagotomy (sham-operated). Pretreatment with ghrelin applied in the standard dose of 20 μg/kg i.p. or 5000 ng/rat i.c.v., which produced over 50% reduction in the area of I/R lesions followed by a significant rise in the GBF as compared to respective values obtained in vehicle-controls. Vagotomy failed to affect significantly I/R-induced lesions and the fall in GBF but significantly attenuated the protective and hyperemic activity of ghrelin (*Fig. 5*).

Ghrelin-pretreated animals exhibited a marked increase in plasma gastrin levels (49 ± 4 pM/L in vehicle control animals exposed to vehicle plus I/R *vs* 77

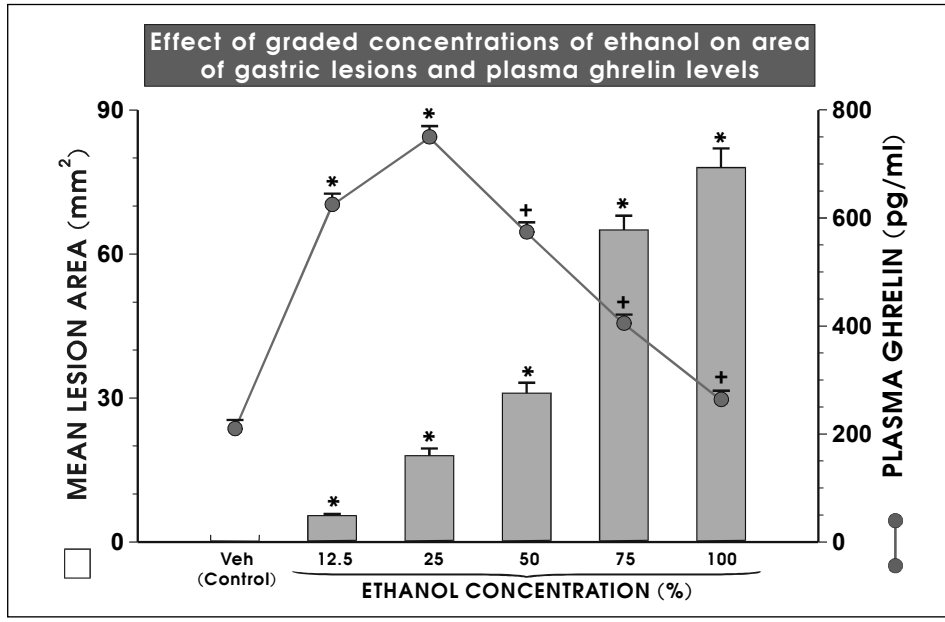

Fig. 4. The mean area of ethanol-induced gastric damage and the plasma immunoreactive ghrelin levels in rats exposed to different concentrations of ethanol ranging from 12.5% up to 100%. Mean ± SEM of 6-8 rats. Asterisk indicates a significant change compared to the value obtained in vehicle-treated rats. Cross indicates a significant change as compared to the values obtained with ethanol given to rat at the concentration of 25%.

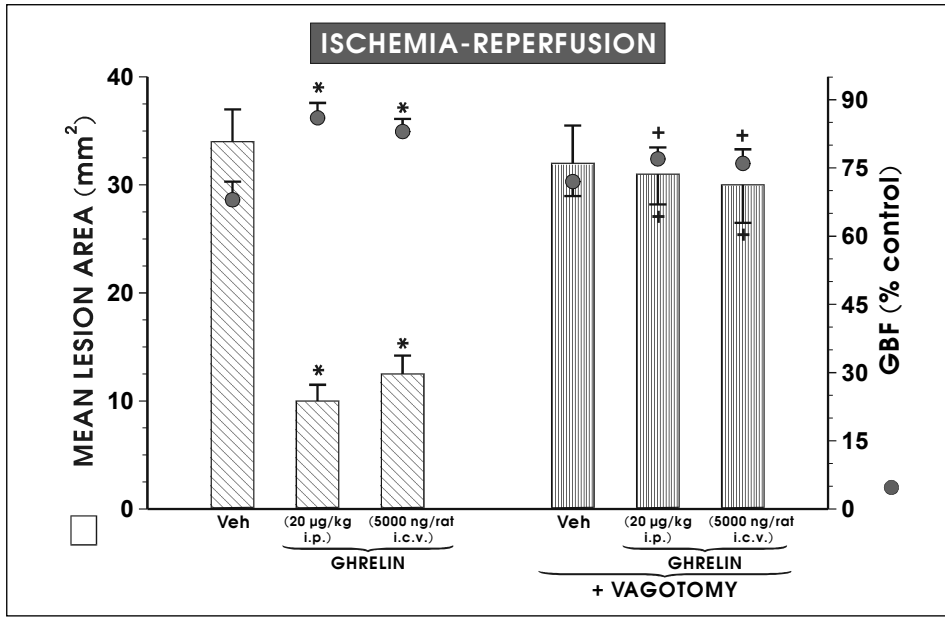

Fig. 5. The area of I/R-induced gastric lesions and the alterations in the GBF in rats with intact vagal nerves and those with vagotomy with or without pretreatment with vehicle (control) and ghrelin (20 µg/kg i.p. or 5000 ng/rat i.c.v.). Mean ± SEM of 6-8 rats. Asterisk indicates a significant change compared to the value in vehicle-treated rats. Cross indicates a significant change compared to the value obtained in rats without vagotomy.

± 6 pM/L or 86 ± 9 in rats pretreated with i.p. or i.c.v. ghrelin, respectively, and then exposed to I/R).

Effect of deactivation of sensory nerves by capsaicin on ghrelin-induced gastroprotection and alterations in the GBF

Deactivation of primary afferent nerves by parenteral pretreatment with neurotoxic dose of capsaicin (about 2 weeks before the experiment) significantly aggravated the number of I/R-induced gastric lesions as compared to vehicle-treated rats and also significantly reduced the GBF when compared to that in animals with intact sensory nerves (*Fig. 6*). In rats with capsaicin deactivation of afferent nerves, the protective activity of ghrelin applied in a standard dose of 20 µg/kg (i.p.) or 5000 ng/rat (i.c.v.), and accompanying rise in the GBF were significantly reduced as compared with those in rats with intact sensory nerves. The concurrent treatment with CGRP (10 µg/kg s.c.) added to ghrelin injected i.p. or i.c.v. in rats with capsaicin-denervation restored the protection and accompanying rise in the GBF with the extent similar to that observed in ghrelin-treated rats with intact sensory nerves.

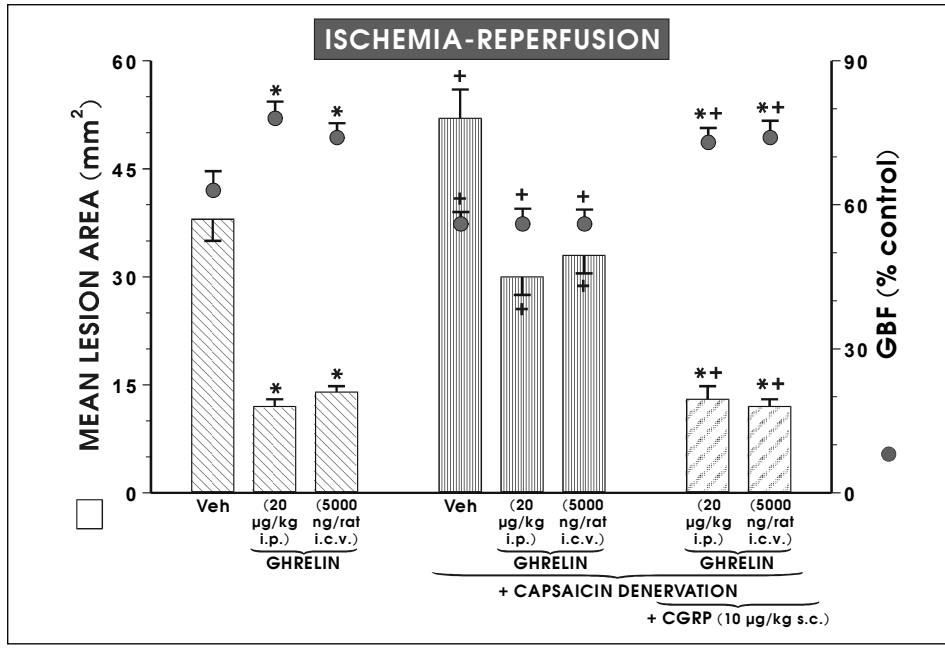

Fig. 6. The area of I/R-induced gastric lesions and the alterations in the GBF in rats with intact sensory afferent nerves and those with capsaicin sensory denervation with or without pretreatment with vehicle (control) and ghrelin applied i.p. in a dose of 20 µg/kg or administered i.c.v. in a dose of 5000 ng/rat without or with the combination with exogenous CGRP (10 µg/kg s.c.). Mean ± 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 capsaicin. Cross and asterisk indicate a significant change as compared to the values obtained in capsaicin-denervated animals.

DISCUSSION

The present study demonstrates that ghrelin applied centrally or peripherally exhibits comparable gastroprotective activity against the mucosal damage induced by corrosive substance such as ethanol and those caused by I/R. This is in keeping with previous observations of other investigators (11) and our group (12, 13) suggesting that exogenous ghrelin could be considered as an important protective factor for the gastric mucosa. We found that ghrelin attenuated the gastric lesions induced by ethanol in various concentrations and those induced by I/R while producing an increase in gastric mucosal blood flow and the plasma level of this hormone. The results of secretory studies revealed that ghrelin applied i.c.v. and i.p. in doses that were gastroprotective against I/R and ethanol injury significantly raised gastric acid secretion in conscious rats provided with the GF suggesting that alterations in gastric secretion do not play any significant role in the gastroprotective activity of this peptide and therefore ghrelin can be considered as truly cytoprotective substance. As expected, ghrelin-induced protection after its

central administration was accompanied by a significant and dose dependent rise in the plasma ghrelin concentrations and marked attenuation of the fall in the GBF caused by ethanol suggesting that ghrelin-evoked gastric hyperemia could be an important mechanism of the protective effect of this peptide in the rat stomach. However, no studies have yet been demonstrated that ghrelin exerts direct effects on vasculature. Thus, it is not excluded that exogenous ghrelin or that produced in the stomach, was capable of reaching the central nervous system *via* the blood stream or the ghrelin signal was conveyed to the brain by triggering response in the vagal afferent fibers. Indeed, we have shown herain that plasma increments of ghrelin increased dramatically in rats subjected to ethanol given in the lower concentrations e.g. 12.5% and 25% that produced gastric damage with return of this plasma ghrelin level back to control values in those receiving absolute ethanol that caused a profound increase in the area of haemorrhagic erosions. This finding requires further studies to explore the relationship between plasma ghrelin levels and lower concentrations of ethanol in the stomach but it seems likely that ghrelin may not only limit of the extent of ethanol damage but could be involved in the phenomenon of adaptive cytoprotection mediated by mild irritants including ethanol applied in lower concentrations (5%-20%).

It has been shown that ghrelin, primarily produced in the empty stomach, but also in the hypothalamus (24) is an orexigenic peptide and affects both feeding at the levels of hypothalamus (*arcuate nuclei)* to stimulate neuropeptide Y (NPY) and Agouti-related peptide (AgPP)-related neurons and to release GH partly due to an activation of gastric vagal afferents transmitting visceral sensory signals to the brain (25). The observation that ghrelin stimulates gastric acid secretion is in keeping with the findings of Masuda *et al*. (26) and Date *et al*. (27) who also demonstrated an increase in the gastric secretion after parenteral administration of ghrelin. The mechanism by which ghrelin increases gastric acid secretion after its peripheral as well as central administration could be related to elevation of plasma gastrin concentration, since we found a considerable increase in this hormone level following ghrelin application. Gastrin could contribute, at least in part, to the secretory as well as protective and hyperemic effects of ghrelin but its effect on histamine release should also be considered as suggested before (28). It is not excluded that ghrelin, which is known for the release of GH, acts *via* GH release to protect gastric mucosa as it was shown to enhance the healing of gastric ulcers, while increasing plasma gastrin level (29).

Our previous studies have demonstrated that gastrin is released after exogenous ghrelin administration and that ghrelin mRNA was upregulated in the gastric mucosa exposed to WRS (12,13). Ghrelin-induced gastroprotection was followed by an increase in the plasma ghrelin level indicating that endogenous ghrelin might act as local protective substance to limit the extent of gastric damage provoked by WRS and, thus, could act as a mucosal integrity peptide similar to leptin, another gastric hormone involved in the control of food intake though acting in opposite direction than ghrelin (16, 30). Our present study

revealed that the exposure to ethanol applied in lower concentrations such as 12.5% and 25% results in an increase in the plasma immunoreactive ghrelin levels which peaked at the concenration of 50% but then it was decreased though still reaching the level significantly higher than that recorded in the vehicle-control animals not exposed to ethanol.

Since the mechanism of gastric mucosal defense against damage include neuropeptides such as CGRP released from afferent sensory nerves, we tested the hypothesis that ghrelin-induced gastroprotection involving NO could originate from the activation by this peptide of afferent sensory neurons. In our present study, the deactivation of primary afferent nerves, using large neurotoxic dose of capsaicin about 2 weeks before the experiment, aggravated the I/R-induced damage as compared to rats without capsaicin denervation and significantly reduced the GBF when compared to that in animals with intact sensory nerves. The major finding of this study is the demonstration for the first time that such capsaicin-induced deactivation of sensory nerves significantly attenuated the protective activity of ghrelin applied i.c.v. and i.p. and completely abolished the rise in GBF induced by this peptide. Moreover, the replacement therapy with exogenous CGRP, the major neuropeptide released from sensory afferent nerves, restored the protective and hyperemic activity of central anal periphenal ghrelin against the lesions induced by I/R indicating that this sensory nerve neuropeptide is essential for microcirculatory response and of crucial importance for the gastroprotective activity of this hormone.

Our study does not exclude the possibility that centrally applied ghrelin enhances the central parasympathetic outflow to the stomach and that also vagal efferent nerves are involved in gastroprotection afforded by this peptide applied centrally and peripherally. It was proposed for instance that the inhibitory effects of ghrelin on gastric acid secretion requires intact vagal pathway because vagotomy abolished the increase in gastric secretion induced by this peptide (27). In our study, vagotomy significantly attenuated the ghrelin afforded gastroprotection and the accompanying rise in the GBF after its central and peripheral administration indicating that, indeed, vagal pathway plays an important role in the mediation of the protective and hyperemic effects of this peptide against lesions evoked by I/R. Our results could be interpreted that ghrelin induced protection against damage induced by I/R might be due to the stimulation of vagal cholinergic pathways that are involved in the recruitment of CGRP from afferent sensory nerves.

In summary, these results demonstrate that administration of exogenous ghrelin applied i.c.v or i.p. that is accompanied by a significant increment in plasma hormone levels, exhibits dose-dependent gastroprotection against the intragestric ethanol administered in graded concentrations and attenuates gastric lesions caused by exposure of the rat stomach to I/R. Smaller concentrations of ethanol which resulted in a moderate gastric mucosal damage, produced a significant rise in the plasma immunoreactive ghrelin levels, suggesting that this release of ghrelin, which possibly derives from the gastric mucosa, can exert inhibitory

influence on the extent of ethanol damage and may contribute to the mechanism of adaptive cytoprotection originally ascribed to prostaglandins (31). Furthermore, an evidence was provided that these protective and hyperemic effects of centrally and peripherally applied ghrelin may affect vagal nerves, the principal component of brain-gut axis and seems to involve the cooperation between endogenous NO (12) and sensory nerves releasing neuropeptides such as CGRP.

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Author's address: Prof. Dr Tomasz Brzozowski, Department of Physiology, Jagiellonian University Medical College, 16 Grzegorzecka Str. 31-531 Cracow, Poland. Phone: (4812) 619-96-31; e-mail:mpbrzozo@cyf-kr.edu.pl