

P.C. KONTUREK<sup>1</sup>, T. BRZOZOWSKI<sup>2</sup>, R. PAJDO<sup>2</sup>, A. NIKIFORUK<sup>1</sup>, S. KWIECIEN<sup>2</sup>,  
I. HARSCH<sup>1</sup>, D. DROZDOWICZ<sup>2</sup>, E.G. HAHN<sup>1</sup>, S.J. KONTUREK<sup>2</sup>

## GHRELIN - A NEW GASTROPROTECTIVE FACTOR IN GASTRIC MUCOSA

<sup>1</sup>Department of Medicine I, University Erlangen-Nuremberg, Erlangen, Germany  
and <sup>2</sup>Department of Physiology, Jagiellonian University Medical College, Cracow, Poland

Ghrelin, a novel peptide expressed in the gastrointestinal tract, especially in the gastric mucosa, exerts several biological activities including the stimulation of appetite and food intake, the stimulation of intestinal motility and the release of growth hormone. The aim of this study was to examine the expression of ghrelin in gastric mucosa after its exposure to ethanol and its effects on gastric lesions induced by ethanol with and without pretreatment with indomethacin. Acute gastric lesions were induced by intragastric administration of 75% ethanol in rats pretreated with saline-vehicle or ghrelin injected intraperitoneally (i.p.) without or with i.p. pretreatment with indomethacin. At the end of experiments, the rats were anesthetized, the stomach was exposed to measure gastric blood flow (GBF), to determine the area of gastric lesions and to take biopsy samples from the oxyntic mucosa for determination of transcripts of ghrelin, tumor necrosis alpha (TNF- $\alpha$ ) and transforming growth factor alpha (TGF $\alpha$ ) using RT-PCR and to assess the generation of PGE<sub>2</sub> by RIA. Exposure of gastric mucosa to 75% ethanol resulted in numerous mucosal lesions of an area of about 115 mm<sup>2</sup> and in the increase of mucosal expression of TNF- $\alpha$ , PGE<sub>2</sub>, TGF $\alpha$  and ghrelin with concomitant decrease in GBF. Exogenous ghrelin reduced dose-dependently acute gastric lesions with simultaneous attenuation of GBF and a decrease in the expression of TNF- $\alpha$  but not TGF $\alpha$ . Pretreatment with indomethacin, which suppressed the generation of PGE<sub>2</sub> by about 85%, augmented ethanol-induced gastric lesions and eliminated the ghrelin-induced protection of mucosa against ethanol. We conclude that ghrelin, whose mucosal expression is enhanced after exposure to ethanol, exhibits a strong gastroprotection, at least in part, due to its anti-inflammatory action mediated by prostaglandins.

**Key words:** *ghrelin, gastroprotection, gastric mucosal blood flow, tumor necrosis factor alpha, prostaglandins*

## INTRODUCTION

Ghrelin, the endogenous ligand for growth hormone (GH) secretagogue receptors (GHS-R), has been recently identified in the rat and human stomach (1). Besides the stimulation of GH release, ghrelin exerts several important biological activities including transmission of appetite-related signals from digestive organs to the brain, regulation of food intake and energy balance, motilin-like stimulation of gastrointestinal motility and control of insulin secretion (2 - 5).

Ghrelin expression is not only restricted to the stomach but it has been shown to be present in the intestines, pancreas, kidney, liver, hypothalamus, pituitary and even immune cells (6, 7). The biological activity of ghrelin is mediated by a G-protein-coupled receptors (GHS-R) (8).

Both rat and human ghrelin peptides consist of 28 amino acids, differing only in two residues and are modified at ser3 residue by n-octanoic acid and this modification is essential for the biological activity of ghrelin. The precursors of rat and human ghrelin are both composed of 117 amino acids, while ghrelin is single chain, consisting of 28 amino acid residues.

The actions of ghrelin are mediated by specific receptors. Two types of receptors, referred to as GHS-R types 1a and 1b. Both receptors are widely distributed in central (brain, pituitary) and peripheral organs (8).

The majority of circulating ghrelin appears to be of gastric origin. In *situ* hybridization and immuno-histochemical analyzes demonstrated that ghrelin-containing cells are distinct endocrine cell-type (known as X/A-like cells) found in the mucosal epithelium of the oxyntic portion of the stomach (9). Following gastrectomy, the plasma concentration of ghrelin falls to about 35% of normal levels (10).

Sibilia *et al.* (11) reported for the first time that ghrelin protects gastric mucosa against acute damage induced by ethanol and this protection involves sensory nerves and endogenous NO release. However, the mechanism of gastroprotection is complex and involves a variety of factors (12, 13), particularly prostaglandins (PG), growth factors, especially transforming growth factor alpha (TGF $\alpha$ ), some gut hormones such as cholecystokinin and leptin and gastric blood flow (GBF) but so far these factors have not been considered in ghrelin-promoted gastroprotection and their role in this protection has not been clarified.

The aim of the present study was: 1) to examine the expression of ghrelin in the gastric mucosa by means of RT-PCR and Western blot after exposure to 75% ethanol; 2) to study the effect of pretreatment with exogenous ghrelin on acute gastric lesions induced by ethanol and accompanying changes in the GBF; 3) to assess the effect of ghrelin pretreatment on the mucosal gene expression of major growth factor such as TGF $\alpha$  (13) and major proinflammatory cytokine, tumor necrosis factor alpha (TNF- $\alpha$ ) (14 and 4) to determine the role of PG and GBF in the gastroprotective activity afforded by ghrelin.

## 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.

*Gastroprotection studies and measurement of gastric blood flow and generation of prostaglandin E<sub>2</sub>*

Acute gastric lesions were induced in rats by intragastric (i.g.) application of 75% ethanol as described previously (12). Briefly, 1.5 ml of 75% ethanol was administered intragastrically (i.g.) to fasted rats by means of a metal orogastric tube. In studies on gastroprotection induced by exogenous ghrelin given i.p., following groups of rats were used: 1) vehicle (1 ml saline i.p.) followed 30 min later by 75% ethanol; 2) ghrelin (2.5-80 µg/kg i.p.) followed 30 min later by 75% ethanol and 3) ghrelin (20 µg/kg i.p.) given alone or in combination with indomethacin (5 mg/kg i.p.) followed 30 min later by 75% ethanol.

At the end of experiments (60 min after ethanol exposure), the animals were lightly anesthetized with ether, their abdomen was opened by the midline incision and the stomach exposed for the measurement of GBF by the means of H<sub>2</sub>-gas clearance method. For this purpose double electrodes of an electrolytic regional blood flowmeter (Biotechnical Science, Model RGF-2, Osaka, Japan) were inserted into the gastric mucosa. One of these electrodes was used for the local generation of gaseous H<sub>2</sub> and another for the measurement of tissue H<sub>2</sub>. With this method, the H<sub>2</sub> generated locally is carried out by the blood flow, while the polarographic current detector shows the decreasing H<sub>2</sub>. The tissue H<sub>2</sub> clearance curve was used to calculate an absolute flow rate (ml/100g/min) in the oxyntic area as described previously (12). The measurement of GBF were made in three areas of the gastric mucosa and the mean values of measurements were calculated and expressed as percent changes from the values recorded in the vehicle (saline) treated animals.

After measurement of the GBF, the stomach was removed, rinsed with water and the area of acute gastric lesions in the oxyntic mucosa was determined by computerized planimetry by a person who did not know to which experimental group the animals belonged.

Prostaglandin generation E<sub>2</sub> was measured in duplicate using RIA kits (New England Nuclear, Munich, Germany). The capability of the mucosa to generate prostaglandin E<sub>2</sub> was expressed in nanograms per gram of wet tissue weight.

*Determination of transcripts for ghrelin, TNF-α, and TGFα in gastric mucosa by RT-PCR*

Immediately after the measurement of the surface of gastric mucosal lesions, the mucosal specimens (about 200 mg) were scraped off on ice using a slide glass and immediately snap frozen in liquid nitrogen and stored at -80°C until analysis. Total RNA was extracted from mucosal samples using a guanidium isothiocyanate/phenol chloroform single step extraction kit from Stratagene (Heidelberg, Germany) based on the method described by Chomczynski and Sacchi (15). Following precipitation, RNA was resuspended in RNase-free TE buffer and its concentration was estimated by absorbance at 260 nm wavelength. Furthermore, the quality of each RNA sample was determined by running the agarose-formaldehyde electrophoresis. RNA samples were stored at -80°C until analysis.

Single stranded cDNA was generated from 5 µg of total cellular RNA using Moloney murine leukemia virus reverse transcriptase (MMLV-RT) (Stratagene, Heidelberg, Germany) and oligo-(dT)-primers (Stratagene, Heidelberg, Germany). Briefly, 5 µg of total RNA was uncoiled by

heating (65°C for 5 min) and then reverse transcribed into complementary DNA (cDNA) in a 50 µl reaction mixture that contained 50 U MMLV-RT, 0.3 µg oligo-(dT)-primer, 1 µl RNase Block Ribonuclease Inhibitor (40U/µl), 2 µl of a 100 mM/l mixture of deoxyadenosine triphosphate (dATP), deoxythymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP), 10 mM/l Tris-HCl (pH=8.3), 50 mM KCl, 5 mM MgCl<sub>2</sub>). The resultant cDNA (2 µl) was amplified in a 50 µl reaction volume containing 2 U Taq polymerase, dNTP (200 µM each) (Pharmacia, Germany), 1.5 mM MgCl<sub>2</sub>, 5 µl 10x polymerase chain reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH=8.3) and specific primers for GAPDH or ghrelin used at final concentration of 1 mM (all reagents from Takara, Shiga, Japan). The mixture was overlaid with 25 µl of mineral oil to prevent evaporation. The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT) and the incubation and thermal cycling conditions were as followed: denaturation at 94°C for 1 min, annealing at 60°C for 45 sec and extension 72°C for 2 min. The number of cycles was 33 for ghrelin, TNF-α and 30 for TGFα and 28 for β-actin. The nucleotide sequence of the primers are demonstrated in the *Table 1*. All primer sequences were based on the sequences of the published cDNAs and were synthesized by GIBCO BRL/Life Technologies (Eggenstein, Germany) (1, 13 14). The primer for GAPDH were purchased by Clontech (Heidelberg, Germany)

Polymerase chain reaction products were detected by electrophoresis on a 1,5% agarose gel containing ethidium bromide. Location of a predicted products was confirmed by using 100-bp ladder (Takara, Shiga, Japan) as a standard size marker. The gel was then photographed under UV transillumination. The intensity of PCR products was measured using video image analysis system (Kodak Digital Science) as described earlier (12). The signal for each transcript was standardized against that of the β-actin mRNA from each sample and the results were expressed as transcript /β-actin mRNA ratio.

### *Protein extraction and analysis of ghrelin expression in the gastric mucosa by Western blot*

Shock frozen tissue from rat stomach was homogenized in lysis buffer (100 mmol Tris-HCl, pH 7.4, 15% glycerol, 2mmol EDTA, 2% SDS, 100 mmol DDT) by the addition of 1:20 dilution of aprotinin and 1:50 dilution of 100 mmol PMSF. Insoluble material was removed by centrifugation

*Table 1.* Specification of primers used in RT-PCR analyses.

PRIMER	SEQUENCE	PCR product bp	Anneal. Temp
β-actin	5'-TTG TAA CCA ACT GGG ACG ATA TGG-3' 3'-GAT CTT GAT CTT CAT GGT GCT AGG-5'	764	60
Ghrelin	5'-TTG AGC CCA GAG CAC CAG AAA-3' 3'-AGT TGC AGA GGA GGC AGA AGC T-5'	394	64
TNF-α	5'-TAC TGA ACT TCG GGG TGA TTG GTC C-3' 3'-CAG CCT TGT CCC TTG AAG AGA ACC-5'	296	63
TGFα	5'-ATG GTC CCC GCG GCC GGA CA-3' 5'-GAC CAC TGT CTC AGA GTG GCA GCA GGC AGT CCT TCC TTT-3'	476	66

at 12000g for 15 min. Approximately 100 µg of cellular protein extract were loaded into a well, separated electrophoretically through a 13.5% SDS-polyacrylamide gel and transferred onto Sequi-Blot™ PVDF membrane (BioRad, USA) by electroblotting. Skim fast milk powder (5%w/v) in TBS/Tween-20 buffer (137 mmol NaCl, 20mmol Tris-HCl, pH7.4, 0.1% Tween-20) was used to block filters for at least 1 hr at room temperature. 1:500 dilution of specific primary goat polyclonal antibody against ghrelin (c-18) (Santa Cruz, USA) or 1:1000 dilution of rabbit polyclonal anti-β-actin (Sigma Aldrich, Germany) antibody was added to the membrane, followed by an anti-goat or anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (1:2000, Santa Cruz, USA). Incubation of primary antibody was followed by 3 washes with TBS-Tween-20 buffer for 10 min. Incubation of the secondary antibody was followed by 4 washes for 10 min. Non-isotopic visualization of immunocomplexes was achieved by chemiluminescence using BM Chemiluminescence Blotting Substrate (Boehringer, Mannheim, Germany). Thereafter, the developed membrane was exposed to an X-ray film (Kodak, Wiesbaden, Germany). Comparisons between different treatment groups were made by determining the leptin/β-actin ratio of the immunoreactive area by densitometry.

### *Statistical analysis*

The results are expressed as means ± SEM. Statistical analysis was performed by using an analysis of variance and nonpaired Student's t test. Differences with  $p < 0.05$  were considered as significant.

## RESULTS

### *Effect of exogenous ghrelin on the ethanol-induced gastric lesions and the gastric blood flow and gastric mucosal expression of ghrelin*

Intragastric (i.g.) application of 75% ethanol, given to fasted rats in a volume of 1.5 ml, induced acute erosions with a mean area of about  $115 \pm 13$  mm<sup>2</sup> (Fig. 1). The development of acute gastric lesions were accompanied by a significant decrease in GBF ( $59 \pm 4$  % of control value). As shown on Fig. 1, the pretreatment with ghrelin given i.p. in graded doses reduced dose-dependently the area of mucosal injury induced by ethanol. This protective effect of ghrelin was accompanied by a significant and dose-dependent rise in GBF.

Fig. 2 demonstrates the expression of ghrelin at the level of mRNA and protein in intact gastric mucosa and in that pretreated with vehicle (saline) and exposed to 75% ethanol. At both mRNA and protein levels (Western blot analysis), the exposure of gastric mucosa to ethanol was accompanied by a significant upregulation of mucosal ghrelin expression as compared with those traced in the intact gastric mucosa.

### *Effect of pretreatment with ghrelin on gastric mucosal mRNA expression of TNF-α and TGFα*

In gastric oxyntic mucosal samples taken from vehicle-treated rats without ethanol only a weak signal for TNF-α mRNA was observed, while in the gastric

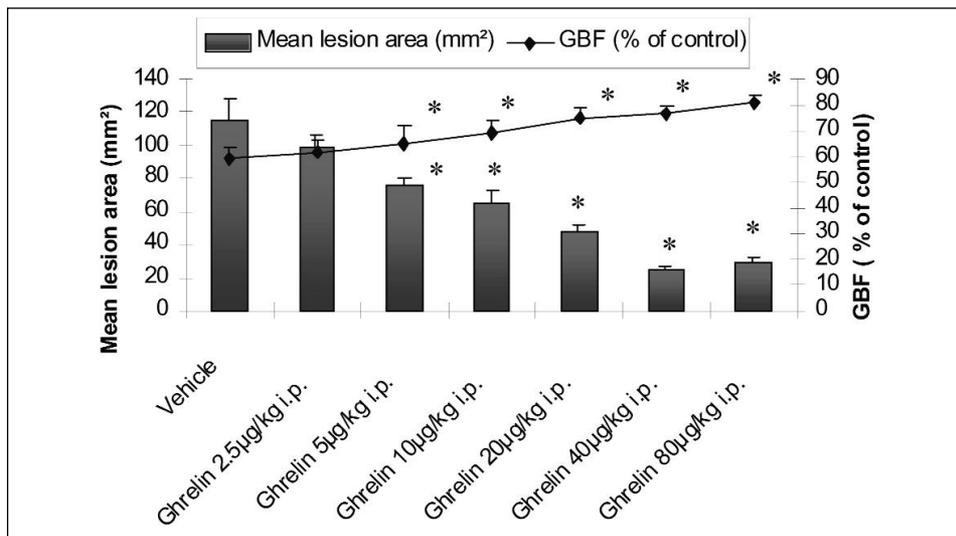


Fig 1. The mean area of ethanol-induced gastric mucosal lesions and gastric mucosal blood flow in rats treated with vehicle (saline) or with various doses of ghrelin (5-80µg/kg i.p.). Means ± SEM of 6-8 rats. Asterisk indicates a significant change as compared to the vehicle control values.

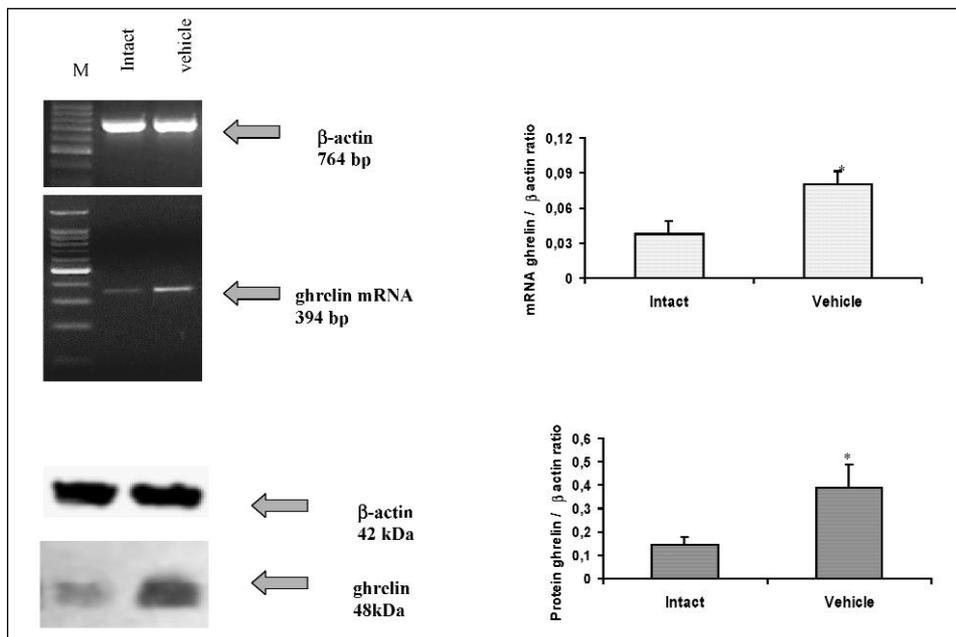
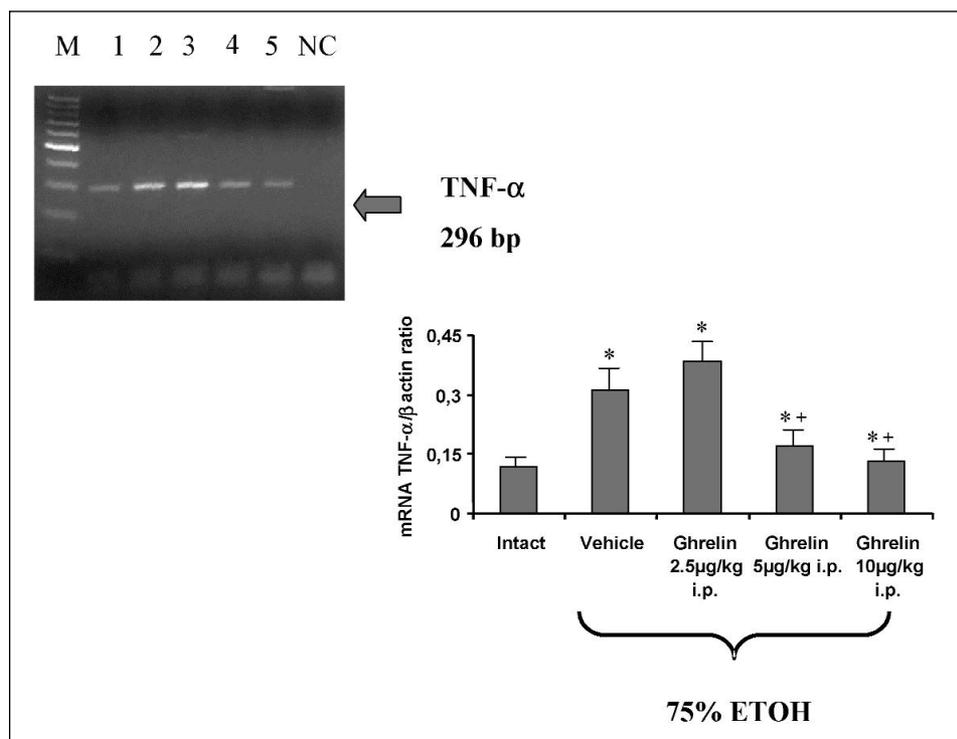


Fig. 2. Gastric mucosal mRNA and protein expression of ghrelin in intact rats and in rats exposed to 75% ethanol (left panel) and semiquantitative determination of the ratios of ghrelin mRNA over β-actin mRNA and ghrelin protein over β-actin protein (right panel) on intact gastric mucosa and in that pretreated with vehicle-saline and then 75% ethanol. Asterisk indicates a significant change as compared with the value obtained in intact gastric mucosa.

mucosa of rats pretreated with vehicle and then exposed to 75% ethanol, the distinct upregulation of mRNA for TNF- $\alpha$  was observed. The expression of mRNA for  $\beta$ -actin was well preserved in the mucosal samples taken from rats treated with vehicle or ghrelin with or without the exposure to 75% ethanol (not shown). *Fig. 3* demonstrates that the pretreatment with graded doses of ghrelin leads to a significant decrease in gastric mucosal mRNA expression of TNF- $\alpha$  and this decrease occurs at the dose range (5 and 10  $\mu$ g/kg i.p.) that was shown before (see *Fig. 1*) to attenuate significantly the gastric mucosa against the damage caused by 75% ethanol.

*Fig. 4* shows the effect of ghrelin pretreatment on the gastric mucosal expression of TGF $\alpha$ . The signal for TGF $\alpha$  mRNA was detected in the intact gastric mucosa and this was further significantly enhanced in gastric mucosa exposed to 75% ethanol. Ghrelin applied i.p. in graded doses did not influence



*Fig. 3.* Determination of  $\beta$ -actin mRNA and TNF- $\alpha$  by RT-PCR and the ratio of TNF- $\alpha$  mRNA over  $\beta$ -actin mRNA in the intact gastric mucosa (lane 1) and in that treated with vehicle plus ethanol (lane 2), and ghrelin given i.p. in dose of 2.5  $\mu$ g/kg (lane 3), 5  $\mu$ g/kg (lane 4) and 10  $\mu$ g/kg (lane 5) prior to the exposure to ethanol. M-DNA size marker; NC-negative control. Mean  $\pm$  SEM of 4-6 rats. Asterisk indicates a significant change as compared with value in intact muosa. Cross indicates a significant decrease as compared with value obtained in rats exposed to ethanol alone with pretreatment with ghrelin.

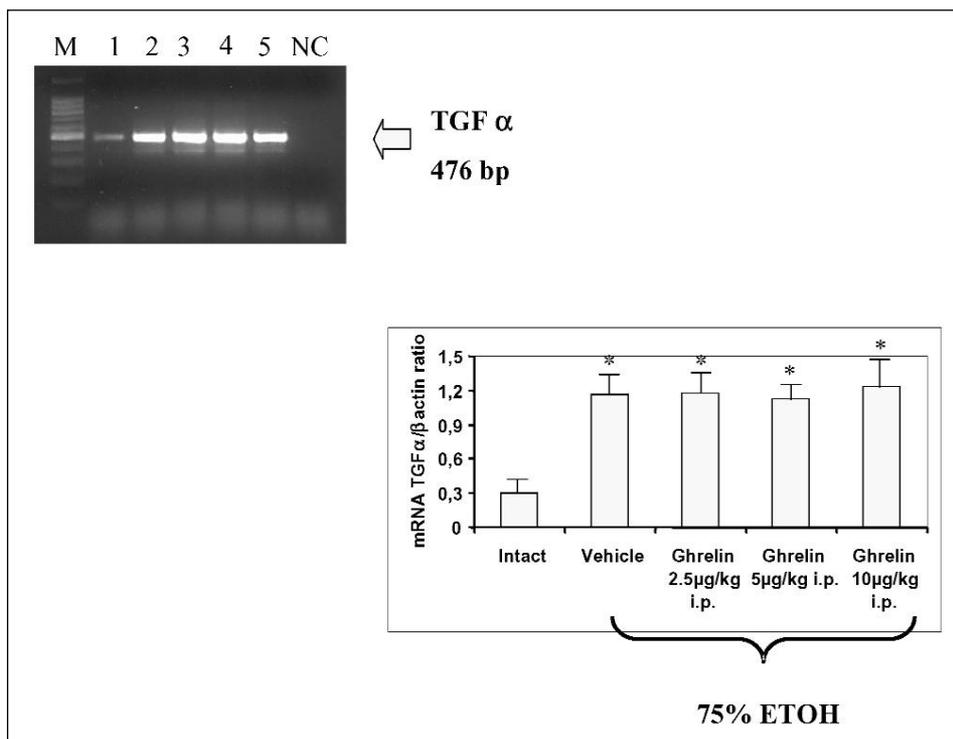


Fig. 4. Determination of  $\beta$ -actin mRNA for TGF $\alpha$  by RT-PCR and the ratio of TGF $\alpha$  mRNA over  $\beta$ -actin mRNA in the intact gastric mucosa (lane 1) and that treated with vehicle prior ethanol (lane 2), and ghrelin given i.p. in dose of 2,5  $\mu$ g/kg (lane 3), 5  $\mu$ g/kg (lane 4) and 10  $\mu$ g/kg (lane 5) prior to the exposure to ethanol. M-DNA size marker; NC-negative control. Mean  $\pm$  SEM of 4-6 rats. Asterisk indicates a significant change as compared with intact control values.

significantly the expression of TGF $\alpha$  mRNA which remained at the comparable level as in rats exposed to ethanol alone without ghrelin.

*Effect of prostaglandin suppression with indomethacin on the ghrelin-induced gastroprotection against gastric mucosal damage and changes in the gastric mucosal blood flow*

As demonstrated in the Fig. 5 pretreatment with ghrelin (10  $\mu$ g/kg) resulted in a similar attenuation in the area of ethanol-induced gastric lesions and similar in the gastric blood flow as shown in the Fig. 1.

Pretreatment with indomethacin given i.p. at a dose of 5 mg/kg aggravated by itself gastric lesions induced by 75% ethanol and resulted in a significant fall GBF as compared to that in vehicle-treated rats. The gastroprotective effect of ghrelin against ethanol damage was accompanied by a significant rise in the generation of mucosal PGE<sub>2</sub>. Following pretreatment with indomethacin a

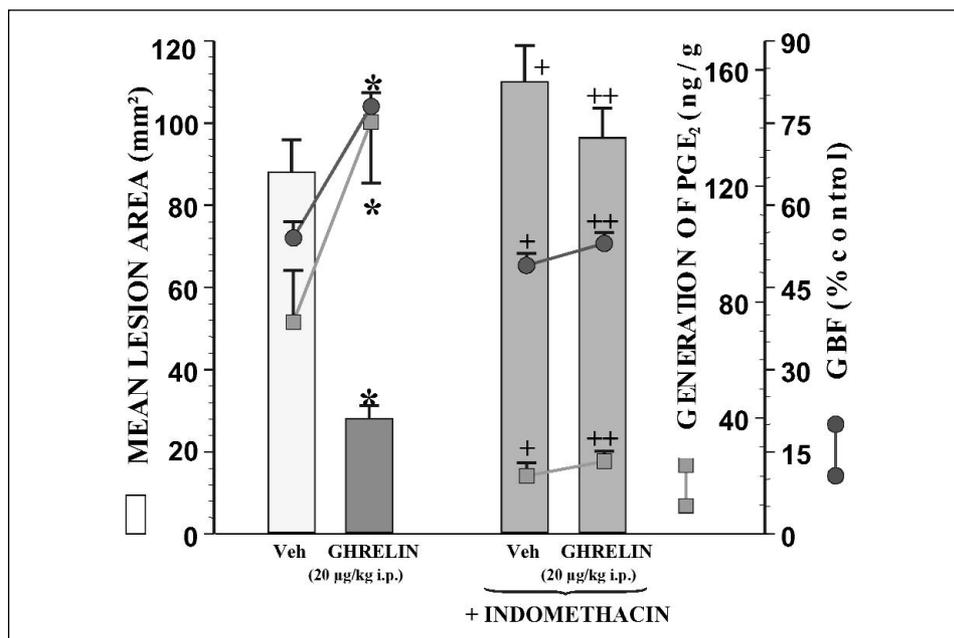


Fig. 5. The mean area of gastric lesions induced by ethanol and accompanying changes in the GBF in rats pretreated with vehicle (saline) or ghrelin (10µg/kg i.p.) with or without administration of indomethacin (5 mg/kg i.p.). Mean  $\pm$  SEM of 6-8 rats. Asterisk indicates a significant decrease as compared to the value obtained in vehicle-treated rats. Cross indicates a significant increase as compared to the values in vehicle-treated rats. Double cross indicates a significant change as compared to the respective value obtained in rats without pretreatment with indomethacin.

significant increase in gastric lesions provoked by ethanol was observed and indomethacin application, which significantly suppressed (by about 85%) the gastric mucosal generation of PGE<sub>2</sub>, greatly attenuated the reduction in gastric mucosal lesions and accompanying rise in GBF induced by ghrelin.

## DISCUSSION

The present study demonstrates that ghrelin is a potent and important gastroprotective factor in the gastric mucosa. Similarly to the previously published results by Sibilía *et al* (11), we observed that exogenous ghrelin administered intraperitoneally dose-dependently attenuates the gastric mucosal lesions induced by ethanol and we found that this gastroprotective effect is accompanied by the significant rise in GBF.

Our study showed for the first time that the exposure of rat stomach to ethanol leads to a significant increase in the gastric mucosal expression of ghrelin both at mRNA and protein levels indicating that ghrelin, besides its recognized role in the

control of appetite, energy balance and gastric motility (1 - 4), appears to be an important gastroprotective factor expressed locally in the gastric mucosa in response to mucosal injury.

The mechanisms by which ghrelin affords its gastroprotective activity against the mucosal damage induced by ethanol is still poorly understood. According to the Sabilia *et al.* (11), the beneficial effect of ghrelin on acute gastric injury is mediated by endogenous NO release and requires the integrity of the sensory nerves. In the present study, we demonstrated for the first time that the treatment with ghrelin caused a dose dependent decrease in TNF- $\alpha$  mRNA expression indicating an important antiinflammatory effect of this peptide (14). This mechanism seems to be of importance since the upregulation of TNF- $\alpha$  expression represents a central pathophysiological event in the acute mucosal injury by ethanol (14). It is possible that ghrelin acts on inflammatory cells *via* ghrelin receptors (GHS-R) leading to the suppression of TNF- $\alpha$  production (6). Further studies are needed to assess the mediation of GHS-R receptors in the gastric mucosa in gastropotection afforded by ghrelin.

Since certain growth factors, particularly TGF $\alpha$ , are involved in the maintenance of gastric mucosal integrity and protection against injury (13, 16, 17), an attempt was made to determine whether ghrelin influences the gastric mucosal mRNA expression of TGF $\alpha$ . In agreement with previous studies (16, 17), we showed a significant upregulation of TGF $\alpha$  mRNA in the gastric mucosa injured by ethanol. However, the pretreatment with ghrelin did not influence the overexpression of mRNA for TGF $\alpha$  in ethanol-injured mucosa. Since the overexpression of TGF $\alpha$  persisted in ethanol-treated rats during the administration of ghrelin it is not excluded that this growth factor also contributes to the gastroprotective activity of ghrelin, but its effect could not be demonstrated because of existing strong overexpression of TGF $\alpha$  by ethanol. Highly elevated expression of TGF $\alpha$  might mask the stimulatory effect of ghrelin in ethanol treated gastric mucosa. Perhaps larger doses of ghrelin or lower concentrations of ethanol could be more useful to demonstrate the rise expression of TGF $\alpha$  by ghrelin in ethanol-injured mucosa.

Another important endogenous substances involved in the gastric mucosal defense are PG that were first endogenous agents recognized as "cytoprotective" factors (18, 19). To investigate whether these classic cytoprotective arachidonate metabolites are involved in ghrelin-induced gastroprotection and accompanying hyperemia, the rats were pretreated with indomethacin to suppress the cyclooxygenase (COX) activity (19) and then, ghrelin was applied in a dose that was shown to afford gastroprotection against ethanol damage. Prostaglandin suppression (by about 85%) by indomethacin was found by itself to aggravate the ethanol-induced lesions but, when indomethacin was combined with ghrelin a significant attenuation of both, ghrelin-induced gastroprotection and accompanying gastric hyperemia were observed in our experiments. This observation indicates that the beneficial effects of ghrelin on the gastric mucosa,

namely gastroprotection and hyperemia are mediated, at least in part, by PG generated in the gastric mucosa by this peptide.

As mentioned previously, the gastroprotective effects of ghrelin was accompanied by a significant rise in the GBF, which is known to play an essential role in the mechanism of gastric mucosal defense (19). This ghrelin-induced hyperemia could be probably attributed to the direct vasodilatory effect of this peptide (20, 21). This notion is supported by the fact that specific ghrelin receptors are expressed in blood vessels (21) and they could mediate the vasodilatory action of this hormone. Furthermore, the increased release of NO, a potent vasodilator could contribute to the ghrelin-induced gastroprotection and hyperemia as suggested previously (11, 21, 22) but this requires further studies to provide evidence that ghrelin, indeed, increases the gene and protein expression of NO synthase.

We conclude that ghrelin represents a novel important, local protective factor in the gastric mucosa. The beneficial effect of this peptide on the gastric mucosa could be due to its anti-inflammatory properties and is probably mediated, at least in part, by PG. The increased expression TGF $\alpha$  in ethanol-treated rats was not affected by ghrelin but this does not exclude the possible contribution of this "mucosa integrity factor" (13, 19) to the gastroprotection afforded by ghrelin and this requires further study.

#### REFERENCES

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; 402: 656-660.
2. Wren AM, Small CJ, Ward HL, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000; 141: 4325-4328.
3. Asakawa A, Inui A, Kaga T, et al. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; 120: 337- 345.
4. Konturek SJ, Pepera J, Zabielski K, et al. Brain-gut axis in pancreatic secretion and appetite control. *J Physiol Pharmacol* 2003; 54: 293-317.
5. Peeters TL. Central and peripheral mechanisms by which ghrelin regulates motility. *J. Physiol Pharmacol* 2003; 54 (suppl 4): 95-104.
6. Wang G, Lee H-M, Englander E, Greeley GH Jr. Ghrelin-not just another stomach hormone. *Reg Pept* 2002; 105: 75-81.
7. Hattori N, Saito T, Yagyu T, Jiang BH, Kitagawa K, Inagaki C. GH receptor, GH secretagogue receptor, and ghrelin expression in human T cells, B cells, and neutrophils. *J Clin Endocrinol Metab* 2001; 86: 4284-4291.
8. Gnanapavan S, Kola B, Bustin SA, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab* 2001; 87: 2988-2990.
9. Date Y, Kojima M, Hosoda H, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2002; 141: 4255-4261.

10. Ariyasu H, Takaya K, Tagami T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001; 86: 4753-4758.
11. Sibilina V, Rindi G, Pagani F, et al. Ghrelin protects against ethanol-induced gastric ulcers in rats: studies on the mechanism of action. *Endocrinology* 2003; 144: 353-359.
12. Brzozowski T, Konturek PC, Konturek SJ. Central leptin and cholecystokinin in gastroprotection against ethanol-induced damage. *Digestion* 2003; 62: 126-14020.
13. Konturek PC, Brzozowski T, Konturek SJ et al. Expression of epidermal growth factor and transforming growth factor alpha during ulcer healing. Time sequence study. *Scand J Gastroenterol* 1997; 32: 6-15.
14. Fiorucci S, Antonelli A, Migliorati G, et al. TNFalpha processing enzyme inhibitors prevent aspirin-induced TNFalpha release and protect against gastric mucosal injury in rats. *Aliment Pharmacol Ther* 1998; 12: 1139-1153.
15. Chromczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem* 1987; 162: 156-159
16. Konturek PC. Physiological, immunohistochemical and molecular aspects of gastric adaptation to stress, aspirin and to H.pylori-derived gastrottoxins. *J Physiol Pharmacol* 1997; 48: 3-42.
17. Coffey RJ, Romano M, Goldenring J. Roles for transforming growth factor alpha in the stomach. *J Clin Gastroenterol* 1995; 21 (Suppl 1): S36-S39.
18. Robert A. Cytoprotection by prostaglandins. *Gastroenterology* 1979; 77: 761-767.
19. Brzozowski T. Experimental production of peptic ulcer, gastric damage and cancer models and their use in pathophysiological studies and pharmacological treatment - Polish achievements. *J Physiol Pharmacol* 2003; 54 (Suppl): 99-126.
20. Okumura H, Nagaya N, Enomoto M, Nakagawa E, Oya H, Kangawa K. Vasodilatory effect of ghrelin, an endogenous peptide from the stomach. *J Cardiovasc Pharmacol* 2002; 39: 779-783.
21. Wiley KE, Davenport AP. Comparison of vasodilators in human internal mammary artery: ghrelin is a potent physiological antagonist of endothelin-1. *Br J Pharmacol* 2002; 136: 1146-1152.
22. Amagase K, Yokota M, Tsukimi Y, Okabe S. Characterization of "unhealed gastric ulcers" produced with chronic exposure of acetic acid ulcers to indomethacin in rats. *J Physiol Pharmacol* 2003; 54: 349-360.

Received: February 2, 2004

Accepted: May 7, 2004

Author's address: Peter Konturek, MD, First Department of Medicine, University Erlangen-Nuremberg, 91054 Erlangen, Ulmenweg 18, Germany. Tel.: +499131-8533371, fax.: +499131-8535209. E-mail: [pkonturek@aol.com](mailto:pkonturek@aol.com)