Peptidergic and cholinergic neurons and mediators in peptone-induced gastroprotection: role of cyclooxygenase-2

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Ehrlich, Karlheinz, Susanne Plate, Thomas Stroff, Britta Gretzer, Michael Respondek, and Brigitta M. Peskar. Peptidergic and cholinergic neurons and mediators in peptone-induced gastroprotection: role of cyclooxygenase-2. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G955–G964, 1998.—This study investigates the neural pathways, mediators, and cyclooxygenase isoenzymes involved in the gastroprotection conferred by peptone in rats. Intragastric perfusion with 8% peptone protected against gross and histological damage induced by subsequent perfusion with 50% ethanol. The gastroprotective effect of peptone was near maximally inhibited by gastrin immunoneutralization, inactivation of capsaicin-sensitive afferent neurons, calcitonin gene-related peptide (CGRP), or somatostatin receptors, and by the nitric oxide (NO) synthase inhibitor L-ARGININE [2-(cyclohexyloxy)-4-nitrophenyl]methanesulfonyl fluoride (L-NS-398 and L-745,337 dose dependently (50% inhibitory dose, 0.2, 0.8, and 1.5 mg/kg, respectively) attenuated the peptone-induced protection. Dexamethasone was ineffective. These results indicate that protective effects of peptone involve endogenous gastrin and possibly somatostatin and are mediated by capsaicin-sensitive afferent fibers, cholinergic, and bombesin/GRP neurons. CGRP, NO, and prostaglandins participate as essential mediators. The study provides evidence that prostaglandins derived from a constitutive cyclooxygenase-2 contribute to mucosal defense in the presence of ulcerogens and thus participate in homeostatic functions of the stomach.

Partly digested proteins, such as peptone, are potent stimulants of gastrin secretion (4). The gastric response to peptone is mediated by intramural cholinergic and bombesin/gastrin-releasing peptide (GRP) neurons (27). It has been shown (15) that intraluminal peptone protects the gastric mucosa against a subsequent ethanol challenge. The protective effect was inhibited by a gastrin (CCK-B) receptor antagonist, suggesting that endogenous gastrin is involved as an essential mediator (15).

In rats, stimulation of the vagus nerve was found to confer protection against ethanol-induced gastric mucosal damage (35). Vagally induced protection is associated with increased gastric formation of prostaglandin E2 and is abolished by blockade of prostaglandin generation (35).

Prostaglandins are biosynthesized from the precursor fatty acid arachidonic acid by the enzyme cyclooxygenase (COX). Two isoforms of cyclooxygenase exist: the constitutive COX-1, which is ubiquitously expressed (for review, see Ref. 3) and COX-2, which shows 61% homology with COX-1 and is expressed in certain cell types, such as macrophages, fibroblasts, and endothelial cells, on induction by growth factors and extracellular stimuli associated with cell activation (for review, see Ref. 21). It has been suggested that prostaglandins synthesized by the constitutive COX-1 are mainly involved in the maintenance of essential physiological functions, whereas prostaglandins synthesized via the inducible COX-2 participate in pathophysiological reactions, particularly those related to inflammation and cell growth (33). Recently, selective inhibitors of COX-2 have been developed. In contrast to nonselective COX inhibitors, such as indomethacin, selective COX-2 inhibitors have been found to lack ulcerogenic activity in the stomach (2, 6, 20). From these findings, it was suggested that COX-1-derived, but not COX-2-derived, prostaglandins participate in the maintenance of gastric mucosal integrity (2, 6, 20, 33).

The present study was designed to examine the hormonal and neural pathways through which peptone in the stomach increases the resistance of the mucosa to injury and to characterize the mediators involved. The results provide further evidence that the protective response to peptone involves endogenous gastrin and possibly somatostatin and is mediated by capsaicin-sensitive afferent fibers, cholinergic nerves, and bombesin/GRP neurons. CGRP, NO, and prostaglandins are involved as essential mediators of the protective effect. The selective COX-2 inhibitors NS-398 [N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (6) and L-745,337 [5-methanesulfonamido-6-(2,4-difluorothio-phenyl)-1-indanone (2)] counteract the protection conferred by peptone, suggesting that COX-2-derived prostaglandins contribute to protective effects and thus have a function in gastrointestinal homeostasis.
**MATERIALS AND METHODS**

**Experimental Protocol**

Male Wistar rats (weighing 180–220 g) were deprived of food for 24 h with free access to tap water. Rats were kept on a 12:12-h light-dark cycle and under conditions of controlled temperature (22 ± 1°C). All experimental protocols were approved by the Animal Care Committee of Ruhr-University of Bochum. For gastric perfusion experiments anesthesia was induced by injection of pentobarbital (50 mg/kg ip).

Protective effect of peptone. Rats were anesthetized and tracheotomized, and the abdomen was opened by a midline incision. The gastric lumen was perfused at a rate of 0.9 ml/min via a needle injected into the stomach lumen through the forestomach. The perfusate was collected through a cannula (3 mm diameter) inserted from the duodenum through the pylorus. Perfusions were performed using isotonic peptone solution (8% peptone dissolved in H2O, 300 mosmol/l) for 20 min followed by perfusion with 8% peptone dissolved in 50% ethanol for 15 min. Controls were perfused with saline for 20 min followed by 50% ethanol in saline for 15 min. After the ethanol-perfusion period, rats were killed by cervical dislocation. The stomach was removed, and gross mucosal damage was assessed in a blinded manner by calculation of a lesion index using a 0–3 scoring system based on the number and length of lesions, as described previously (31).

For histological study, a strip of the stomach wall parallel to the limiting ridge was processed using routine methods, stained with hematoxylin and eosin, and examined under a light microscope. Six rats per group were studied. One full-length portion of tissue across the entire corpus was cut from each stomach (section length, 3.2 ± 0.07 cm, n = 48). The sections were evaluated in a randomized blinded fashion by a histologist who was unaware of the experimental protocol. The following two grades of histological injury were assessed. Grade 1 was classified as superficial damage confined to the surface epithelium, and grade 2 was deep damage extending beyond the surface epithelium into the region of the pits and glands. For each stomach strip the length of mucosal areas showing superficial and deep damage was determined and expressed as a percentage of the total section length studied.

Functional ablation of afferent neurons. Rats were treated with a subcutaneous injection of capsaicin (25 and 50 mg/kg on day 1, 50 mg/kg twice on day 2). Capsaicin was dissolved in 10% ethanol, 10% Tween 80, and 80% saline (vol/vol). Control animals received equal volumes (2 ml/kg) of vehicle. All injections were performed under ether anesthesia, and to counteract the respiratory impairment associated with the administration of capsaicin, the rats were pretreated with terbutaline (0.2 mg/kg), atropine (0.2 mg/kg), and theophylline (20 mg/kg) before capsaicin injection. Rats were used for experiments 14 days after this treatment. On the day before the experiments, inactivation of afferent neurons was ascertained by evaluating the reduction of the protective wiping movements in response to intraocular instillation of a 0.1 mg/ml solution of capsaicin.

Effect of atropine and methyl atropine. Rats were treated with atropine (1 mg/kg iv) 10 min before the intragastric perfusion with 8% peptone. After a 20-min perfusion period, the lumen was perfused with 8% peptone in 50% ethanol for a further 15 min. Controls received the vehicle (0.5 ml/kg of saline). To differentiate between a central and peripheral mechanism of the atropine effect, experiments were also performed with methyl atropine (1 mg/kg iv), which does not cross the blood-brain barrier.

Effect of anti-CGRP antibodies. Globulin aliquots were prepared by ammonium sulfate precipitation from 2 ml of a polyclonal anti-rat CGRP antiserum raised in a rabbit or from 2 ml of serum obtained from nonimmunized rabbits. Details of the immunization procedure, preparation of globulin fractions, and the characterization of the antiserum have been described previously (16). Globulin aliquots were injected intravenously 10 min before starting the intragastric peptone perfusion.

Effect of human CGRP-(8–37). Groups of rats received the CGRP1 receptor antagonist human (h) CGRP-(8–37) (500 pmol·kg⁻¹·min⁻¹ for 40 min) or vehicle (0.1% BSA in saline) by intravenous infusion at a rate of 20 μl/min. Infusions of hCGRP-(8–37) or vehicle were started 5 min before the intragastric peptone perfusion and were continued until the end of the experiment.

Effect of inhibition of NO biosynthesis. Groups of rats were pretreated 10 min before intragastric peptone perfusion with intravenous injection of the NO biosynthesis inhibitor Nω-nitro-l-arginine methyl ester (L-NAME; 10 mg/kg) or its inactive enantiomer D-NAME (10 mg/kg). Additional groups of rats received L-arginine or D-arginine (each 400 mg/kg iv) immediately before L-NAME.

Effect of antigastrin antibodies. Globulin aliquots were prepared by ammonium sulfate precipitation from 2 ml of a polyclonal anti-rat gastrin-17 antiserum raised in a rabbit. The immunization procedure and preparation of globulin fractions were identical to those described previously for the anti-CGRP antibodies (16). Globulin aliquots were injected intravenously 10 min before starting the intragastric peptone perfusion. Previous experiments have shown that injection of the antigastrin antibodies abolished the protective effect of intravenously infused gastrin-17 (data not shown).

Effect of blockade of bombesin/GRP receptors. Groups of rats were treated with intravenous injections of the selective gastrin (CCK-B) receptor antagonist CAM-1028 [(2-[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-(1.7.7-trimethyl-bicyclo(2.2.1)hept-2-yl)-oxy(carbonyl)amino]propylamino)-1-phenylethyl] amino-4-oxo-15-1α,2β-[5*(5*4*5*)]-butanoate-N-methyl-D-glucamine (0.3–3 mg/kg) or of the CCK-A receptor antagonist CAM-1481 [N-(α-methyl-N-[tricyclic(3.3.1.1.3)7dodec-2-yl-oxycarbonyl]-L-tryptophyl)-D-3-(phenylmethyl)-β-alanine] (3 mg/kg) 10 min before starting the intragastric peptone perfusion. Previous studies have shown that CAM-1028, but not CAM-1481, abolished the protective effect of intravenous infusions of gastrin-17 (data not shown).

Effect of blockade of bombesin/GRP receptors. Groups of rats were treated with intravenous injections of the selective bombesin/GRP receptor antagonist D-22213 [RC-3095, D-Tp4,Leu4,Leu4(CH2)5NH]Leu4-bombesin-(6–14)] and b (25). Injections (180–720 nmol/kg) were administered 10 min before injection of bombesin (6 nmol/kg iv) or 1 min before and 15 min after starting the intragastric peptone perfusion. These studies showed that a single injection of D-22213 at 540 nmol/kg abolished the protective effect of bombesin. The effect was, however, extremely short-lived, and suppression of bombesin-induced protection lasting for a period of 40 min (as necessary to counteract the effect of a 35-min intragastric peptone perfusion) was only achieved when D-22213 was injected twice during this period. Additional groups of rats were treated with intravenous injection of D-22213 (360 and 720 nmol/kg) 5 min before and 10 min after starting an intravenous infusion of gastrin-17 (50 pmol·kg⁻¹·min⁻¹ for 20 min). Fifteen minutes after starting the gastrin perfusion, 1 ml of 70% ethanol was instilled orally, and mucosal damage was assessed 5 min later.
Effect of blockade of somatostatin receptors. Groups of rats were treated with the somatostatin receptor antagonist cyclo(7-aminooctanoyl-Phe-o-Trp-Lys-Thr[Ala]) (5) (0.1–3 nmol/kg iv) 10 min before starting the intragastric perfusion with 8% peptone. Additional groups of rats received the somatostatin receptor antagonist (3 and 10 nmol/kg iv) 10 min before an intravenous infusion of gastrin-17 (50 pmol·kg⁻¹·min⁻¹ for 20 min). Ethanol (1 ml, 70%) was instilled orally 15 min after starting the gastrin infusion, and mucosal damage was assessed 5 min later.

Effect of inhibition of COX-1 and COX-2. To assess the role of prostaglandins generated via the COX-1 and COX-2 pathways, groups of rats were treated orally with the dual COX-1 and COX-2 inhibitor indomethacin (0.4–20 mg/kg), the selective COX-2 inhibitors NS-398 (0.04–3 mg/kg) and L-745,337 (0.7–5 mg/kg), or dexamethasone (3 mg/kg). COX inhibitors were administered 60 min before the peptone perfusion started and dexamethasone was administered 24 and 2 h before the peptone perfusion. Controls received the vehicle (0.25% methylcellulose, 2.5 ml/kg).

To exclude unspecific damage-modifying effects, all pretreatments used in the study were examined in rats treated with intragastric ethanol perfusion in the absence of peptone.

Measurement of Gastric Mucosal Blood Flow

After induction of anesthesia, rats were tracheotomized and the stomach was exposed by a midline incision. A plastic cannula (3.5 mm diameter) was inserted in the forestomach and tied in place to allow free access to the gastric lumen for the measurement of blood flow by laser-Doppler flowmetry. A Teflon-coated laser optic probe (standard probe, 0.25 mm fiber separation; PeriFlux PF 409, Perimed) was inserted into the gastric lumen via the forestomach cannula and allowed to rest on the corpus mucosa. After a resting period of 60 min, gastric mucosal blood flow was recorded continuously with a laser-Doppler flow monitor (diode laser of wavelength 780 nm; PeriFlux 4001 Master, Perimed). The jugular vein was cannulated for continuous infusion of saline (1.5 ml/h) to avoid dehydration. Then the gastric lumen was perfused with 8% peptone in H₂O for 20 min followed by perfusion with 8% peptone in 50% ethanol for 15 min, and gastric mucosal blood flow was monitored until the end of the experiment. Controls were perfused with saline instead of peptone. To exclude an interference of the intragastric peptone solution with the measurement of luminal 8% peptone.

Measurement of Systemic Arterial Blood Pressure

In anesthetized rats, the carotid artery was cannulated for measurement of mean systemic arterial blood pressure (Iso- tec pressure transducer; Hugo Sachs, Hugstetten, Germany). After a resting period of 60 min, the stomachs were perfused with 8% peptone in H₂O for 20 min followed by perfusion with 8% peptone in 50% ethanol for 15 min, and mean arterial blood pressure was monitored until the end of the experiment. Controls received intragastric perfusions with saline instead of peptone.

Assessment of Eicosanoid Formation

Groups of rats were perfused with saline or 8% peptone for 20 min. Then the stomach was removed and opened, and mucosal fragments from the corpus region were excised, blotted, and weighed. Two aliquots of 40 mg of tissue were incubated in oxygenated Tyrode solution for 10 min at 37°C. After incubation, the medium was removed and analyzed for the content of prostaglandin E₂ and 6-ketoprostaglandin F₁α using RIA. Additional groups of rats were treated intravenously with atropine or orally with indomethacin (20 mg/kg), NS-398 (3 mg/kg), or L-745,337 (5 mg/kg). Controls received the corresponding vehicles. Sixty minutes later, the stomach was removed and mucosal fragments were incubated as described above.
pretreatment values compared with 5% increase in saline-perfused rats). During the ethanol-perfusion period, gastric mucosal blood flow increased by 28% in peptone-pretreated rats and by 36% in saline-pretreated rats (P > 0.05 each vs. pretreatment values) (Table 1). The presence of peptone in the gastric lumen did not interfere with the measurement of gastric mucosal blood flow. Thus perfusion of the gastric lumen with capsaicin (320 μM) for 20 min increased gastric mucosal blood flow over basal values by 34 ± 8% in the absence of luminal peptone and by 30 ± 11% in the presence of 8% peptone (n = 4/group).

**Effect of Peptone on Systemic Arterial Blood Pressure**

The resting mean arterial blood pressure was 112 ± 5mmHg (n = 10). After 20 min perfusion with peptone or saline, mean arterial pressure was 108 ± 12 and 115 ± 5mmHg, respectively. After a subsequent 15-min perfusion with 50% ethanol in peptone or 50% ethanol in saline the mean arterial blood pressure was 97 ± 13 and 98 ± 11mmHg, respectively. Differences among pretreatment values compared with 5% increase in saline-perfused rats. During the ethanol-perfusion period, gastric mucosal blood flow increased by 28% in peptone-pretreated rats and by 36% in saline-pretreated rats (P > 0.05 each vs. pretreatment values) (Table 1). The presence of peptone in the gastric lumen did not interfere with the measurement of gastric mucosal blood flow. Thus perfusion of the gastric lumen with capsaicin (320 μM) for 20 min increased gastric mucosal blood flow over basal values by 34 ± 8% in the absence of luminal peptone and by 30 ± 11% in the presence of 8% peptone (n = 4/group).

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against rat gastrin-17 significantly ($P < 0.001$) attenuated the protective effect of peptone (lesion index 25 ± 2.5, $n = 8$). Globulin aliquots prepared from nonimmunized rabbits had no effect (see Fig. 2).

**Effect of Blockade of Gastrin Receptors**

Pretreatment with the selective gastrin (CCK-B) receptor antagonist CAM-1028 dose dependently counteracted the protective effect of peptone [50% inhibitory dose (ID$_{50}$), 0.5 mg/kg]. CAM-1028 at a dose of 1 mg/kg abolished the protection. In contrast, the selective CCK-A receptor antagonist CAM-1481 at a dose of 3 mg/kg was ineffective (lesion index 10 ± 1 vs. 9 ± 1, rats treated with CAM-1481 vs. treated with peptone only, respectively; $n = 6$ for each group). Results are shown in Fig. 4.

**Effect of Blockade of Bombesin/GRP Receptors**

Pretreatment with the selective bombesin/GRP receptor antagonist D-22213 administered as a single intravenous injection dose dependently (ID$_{50}$, 320 nmol/kg) inhibited the protective effect of bombesin (6 nmol/kg iv) and at a dose of 540 nmol/kg abolished the protection. Similarly, the protective effect of intragastric perfusion with peptone was dose dependently inhibited by D-22213 (ID$_{50}$, 438 nmol/kg), although due to the extremely short half-life of biological activity, D-22213 had to be injected twice to antagonize the protective effect of peptone. At the dose of 720 nmol/kg, however, D-22213 completely blocked the protective activity of intragastric peptone. D-22213 (720 nmol/kg, injected twice) partially attenuated the protective activity of gastrin-17 (50 pmol·kg$^{-1}$·min$^{-1}$) (Fig. 5).

**Effect of Blockade of Somatostatin Receptors**

Intravenous injection of the somatostatin receptor antagonist cyclo(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr-[BZL]) (0.1–3 nmol/kg iv) counteracted the protective effect of intragastric perfusion with 8% peptone in a dose-dependent manner (ID$_{50}$, 0.7 nmol/kg). The highest dose studied abolished the peptone-evoked protection. In contrast, the same somatostatin receptor antagonist up to a dose of 10 nmol/kg had no significant effect on the protective activity of gastrin-17 (50 pmol·kg$^{-1}$·min$^{-1}$) (Fig. 6).

**Effect of Inhibition of COX-1 and COX-2**

Indomethacin (0.4–20 mg/kg), which inhibits both COX-1 and COX-2 dose dependently, antagonized the protective effect of intragastric peptone perfusion (ID$_{50}$, 4.2 mg/kg). Indomethacin at 10 mg/kg reduced the peptone-induced protection by 66% (lesion index 22 ± 2 vs. 10 ± 1, with $n = 7$) vs. without indomethacin ($n = 6$), respectively; $P < 0.001$). Likewise, the selective COX-2 inhibitors NS-398 (0.04–3 mg/kg) and L-745,337 (0.7–5 mg/kg) dose dependently inhibited the protective effect of intraluminal peptone (ID$_{50}$, 0.8 and 1.5 mg/kg).

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**Fig. 3.** Effect of treatment with $N^\alpha$-nitro-L-arginine methyl ester (L-NAME) (10 mg/kg) alone or plus 400 mg/kg of either L-arginine (L-Arg) or d-arginine (d-Arg) or with d-NNAME (10 mg/kg) alone 10 min before peptone perfusion. Results show gross mucosal damage calculated as lesion index after 50% ethanol perfusion and are means ± SE of 6 experiments in each group. *$P < 0.05$, **$P < 0.001$ vs. saline-perfused control rats; ***$P < 0.001$ vs. rats perfused with peptone in the absence of pretreatments.

**Fig. 4.** Effects of antigastrin antibodies, CAM-1028 (gastrin/CCK-B receptor antagonist), and CAM-1481 (CCK-A receptor antagonist) on protection conferred by peptone against mucosal damage induced by intragastric perfusion with 50% ethanol. Rats received intravenous injections of globulin fractions prepared from serum of a rabbit immunized against rabbit gastrin-17 or of the CCK receptor antagonists 10 min before peptone perfusion. Controls were perfused with saline instead of peptone. Bars are means ± SE of 6–8 rats. *$P < 0.001$ vs. control; ***$P < 0.001$ vs. rats perfused with peptone in the absence of pretreatments.

**Fig. 5.** Effect of the bombesin/gastrin-releasing peptide receptor antagonist D-22213 on protection conferred by intravenous injection of bombesin (6 nmol/kg) (▲), intragastric perfusion with peptone (●), and intravenous infusion of gastrin-17 (50 pmol·kg$^{-1}$·min$^{-1}$) (●). D-22213 was injected intravenously 10 min before administration of bombesin, 1 min before and 15 min after starting peptone perfusion, and 5 min before and 10 min after starting gastrin-17 infusion. Values are means ± SE of 4–6 experiments.
mg/kg, respectively). The maximum inhibition of peptone-evoked protection was 55% with NS-398, reached at a dose of 1 mg/kg, and 73% with L-745,337, reached at a dose of 2 mg/kg. Increasing the doses of indomethacin or of the selective COX-2 inhibitors did not augment the inhibitory effect. Dexamethasone did not affect peptone protection (Fig. 7). None of the drugs significantly affected ethanol-induced gastric mucosal damage in the absence of peptone.

**Histological Injury**

Treatment with peptone prevented the development of grade 2 deep histological mucosal injury extending beyond the surface epithelium into the region of the pits and glands in rats with intact afferent neurons but not in rats with functional ablation of these nerves. In rats with intact afferent neurons, histological damage of the mucosa was significantly higher in rats treated before peptone with anti-CGRP antibodies, L-NAME (10 mg/kg iv), D-22213 (twice 720 nmol/kg iv), NS-398 (1 mg/kg po), or L-745,337 (2 mg/kg po) before intragastric peptone perfusion were compared with rats perfused with peptone alone. Controls were perfused with saline. Afferent denervation was induced by pretreatment with capsaicin 2 wk before experiments were performed. Values are means ± SE of 6 experiments per group. *P < 0.001 vs. control; •P < 0.02, •P < 0.01, •P < 0.002 vs. rats perfused with peptone alone; P < 0.001 vs. peptone in rats with intact afferent neurons.

**Eicosanoid Formation and Effect of COX Inhibitors and Atropine**

Perfusion of the gastric lumen with 8% peptone for 20 min did not increase mucosal synthesizing capacity for prostaglandin E2 and 6-ketoprostaglandin F1α. Pretreatment with indomethacin (20 mg/kg) suppressed ex vivo release of prostaglandin E2 and 6-ketoprostaglandin F1α from gastric mucosal tissues by 93% and 96%, respectively. In contrast, pretreatment with NS-398 (3 mg/kg) and L-745,337 (5 mg/kg) did not inhibit gastric mucosal prostaglandin synthesizing capacity (Table 2). Likewise, pretreatment with atropine (1 mg/kg) did not reduce mucosal formation of prostaglandin E2 (1,485 ± 320 vs. 1,195 ± 230 pg·mg⁻¹·10 min⁻¹ in controls) and 6-ketoprostaglandin F1α (730 ± 90 vs. 690 ± 95 pg·mg⁻¹·10 min⁻¹ in controls, n = 6/group). Qualitatively identical results were obtained when the effects of COX inhibitors and atropine were assessed using methanol extraction of gastric mucosal fragments to determine prostaglandin tissue levels instead of synthesizing capacity (data not shown).

**DISCUSSION**

Perfusion of the gastric lumen with peptone markedly reduced damage caused by subsequent exposure of the mucosa to ethanol, confirming previous observations (15). Because the 8% solution of peptone used in the study is isotonic, the protective effect cannot be attributed to hyperosmolality. The protection conferred by peptone depended on intact afferent neurons and was not observed in rats pretreated with a high neurotoxic dose of capsaicin to induce inactivation of afferent neurons.
neurons. Capsaicin-sensitive afferent neurons repre-
se an important line of mucosal defense. The gastric
mucosa is densely innervated with such neurons, par-
cularly in the vicinity of mucosal and submucosal
arterial vessels that control blood flow to the mucosa
(9). Activation of afferent neurons by intragastric admin-
istration of low concentrated capsaicin or agents that
weaken the gastric mucosal barrier, thus leading to
acid back-diffusion, was found to confer protection
against various noxious agents (9). Stimulation of
afferent nerves results in release of CGRP, the most
abundant neuropeptide contained in these neurons,
which then promotes NO generation. NO seems to be
the exclusive mediator of CGRP-induced protection
because inhibition of NO biosynthesis completely blocks
the effect (16, 23). In contrast, CGRP and NO do not
participate in afferent nerve-independent gastroprotec-
tion elicited by sulfhydryl-donating or -blocking agents,
metals, or sodium salicylate (28). In vitro incubation
experiments of rat antrum mucosa have shown that
intraluminal peptone stimulates release of CGRP, indi-
cating activation of afferent neurons (19). In our study,
CGRP immunoneutralization or CGRP receptor block-
ade suppressed the protective effect of intraluminal
peptone. Furthermore, pretreatment with the NO syn-
thase inhibitor l-NAME abolished the protective activ-
ity of peptone, confirming previous observations (15).
Simultaneous administration of l-arginine, which acts
as a substrate for NO synthase, reversed the effect of
l-NAME. This indicates that the attenuation of peptone-
induced protection by l-NAME is due to inhibition of
NO biosynthesis and not to properties of l-NAME to act
as a muscarinic antagonist in the cholinergic transmis-
sion (1). Taken together, these findings provide evi-
dence for a crucial role of primary afferent neurons in
mediating the protective activity of peptone. This pro-
posal is in keeping with a study demonstrating that
gastric acid secretory responses induced by peptone
involve capsaicin-sensitive afferent neurons (26).

Activation of afferent neurons by intragastric capsa-
icin or acid back-diffusion elicits an increase in gastric
mucosal blood flow (9). This hyperemic reaction has
been suggested to underlie afferent nerve-mediated protection (9). Both CGRP and NO are potent vasodilat-
ing mediators that contribute to the gastric mucosal
hyperemia evoked by afferent nerve stimulation (10,
34). In our study, peptone perfusion of the gastric
lumen under experimental conditions that near-maxi-
mally reduced ethanol-induced mucosal damage did
not influence mean arterial blood pressure and had
only minor effects on gastric mucosal blood flow. Thus
gastric mucosal blood flow was increased by 13% during
peptone perfusion in the absence of ethanol. Perfusion
of the gastric lumen with ethanol elevated gastric
mucosal blood flow by 28% in peptone-treated rats and
36% in saline-treated rats. In both groups, the effect
did not reach the level of statistical significance. From
these findings it seems unlikely that the protection
evoked by intragastric peptone is the result of in-
creased gastric mucosal blood flow. Afferent nerve-
mediated protective effects are not necessarily associ-
ated with an increase in gastric mucosal blood flow.
Thus we have recently shown that analogs of the	

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<th>Treatment</th>
<th>Prostaglandin E2 ( \text{pg/mg} )</th>
<th>6-Ketoprostaglandin F12 ( \text{pg/mg} )</th>
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<tr>
<td>Saline</td>
<td>601 ± 191</td>
<td>943 ± 57</td>
</tr>
<tr>
<td>Peptone</td>
<td>588 ± 212</td>
<td>1,281 ± 613</td>
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<tr>
<td>Vehicle</td>
<td>705 ± 103</td>
<td>1,084 ± 134</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>49 ± 7*</td>
<td>39 ± 8*</td>
</tr>
<tr>
<td>NS-398</td>
<td>682 ± 121</td>
<td>1,002 ± 122</td>
</tr>
<tr>
<td>L-745,337</td>
<td>669 ± 98</td>
<td>907 ± 98</td>
</tr>
</tbody>
</table>
| Values are means ± SE of 6 experiments/group. For gastric perfusion experiments rats were perfused intragastrically with saline or peptone (8%) for 20 min. Then the stomachs were removed, and mucosal fragments from the corpus region were excised and incubated in Tyrode solution for 10 min at 37°C. Release of prostaglandins into incubation medium was determined using RIA. For oral bolus administration experiments rats were treated orally with indomethacin (20 mg/kg), NS-398 (3 mg/kg), or L-745,337 (5 mg/kg). Controls received vehicle (0.25% methylcellulose, 2.5 ml/kg). Sixty minutes later, the stomachs were removed and mucosal fragments were incubated for 10 min at 37°C to determine release of prostaglandins into the medium. COX, cyclooxygenase. *P < 0.001 vs. vehicle-treated control rats.
induced protection by a CCK-B receptor antagonist was also reported by Konturek et al. (15).

In previous studies (27) using the vascularly perfused rat stomach, it was shown that peptone stimulates gastrin secretion by activating intramural cholinergic and noncholinergic bombesin/GRP neurons. This was concluded from the fact that the peptone-evoked gastrin release was fully blocked by TTX and partially inhibited by atropine or a selective bombesin/GRP receptor antagonist (27). The gastrin response to peptone was abolished by a combination of the bombesin/GRP receptor antagonist with atropine. This observation was taken as evidence that activation of cholinergic and bombesin/GRP neurons accounts for the gastrin response to peptone (27). The present study shows that both atropine and the selective bombesin/GRP receptor antagonist D-22213 antagonize the protective effect of peptone. The effect of atropine was mimicked by methyl atropine, which does not cross the blood-brain barrier, indicating that it is mediated by a peripheral mechanism. Whereas atropine partially inhibited the peptone-induced protection, antagonism of bombesin/GRP receptors completely counteracted the effect. Endogenous bombesin/GRP may have a more important function in peptone-evoked gastrin release in the intact rat compared with the isolated in vitro perfused stomach. In addition, activation of bombesin/GRP receptors could contribute to the protective effect of endogenous gastrin. This contention is in keeping with previous findings that bombesin exerts potent gastroprotective effects (30) and with results of the present study that blockade of bombesin/GRP receptors partially attenuates the protection induced by gastrin-17.

Pretreatment with the somatostatin receptor antagonist cyclo(7-aminohexanoyl-Phe-o-Trp-Lys-Thr[BZL]) counteracted the protective effect of peptone in a dose-dependent manner. In contrast, the somatostatin receptor antagonist did not affect the protection induced by gastrin-17, indicating that gastrin does not exert its protective effect through release of endogenous somatostatin. Exogenously administered CGRP and endogenous CGRP released on stimulation of primary afferent neurons were found to promote secretion of somatostatin from the isolated perfused rat stomach (11). Whereas numerous studies have shown that in the rat stomach bombesin/GRP is a potent stimulator for the release of somatostatin (see Ref. 8), both stimulation (17) and inhibition (27) of somatostatin release have been described for peptone. Furthermore, in our study we failed to demonstrate that exogenous somatostatin exerts protection (data not shown), although Karmeli et al. (14) have described such an effect.

It is well established that the vagus nerve plays an important role in gastric mucosal defense. Thus central vagal stimulation induced by intracisternal injection of RX-77368, the stable analog of thyrotropin-releasing hormone, prevented ethanol-induced gastric lesions in rats by a muscarinic-mediated mechanism (35). The protective effect of vagal stimulation was associated with increased formation of prostaglandin E$_2$ in the stomach and abolished by pretreatment with indomethacin and atropine, suggesting that vagal activation may play a role in the control of gastric mucosal integrity against injury through cholinergic prostaglandin release (35).

Biosynthesis of prostaglandins is rate limited by the initial conversion of arachidonic acid to prostaglandin G and prostaglandin H by the COX enzyme. Two major isoforms of COX have been described, the constitutive COX-1 and the inducible COX-2 (3, 21). High levels of COX-1 protein and mRNA are detected in most normal tissues (13, 22). Whereas COX-2 protein is lower or even undetectable in most normal tissues, the COX-2 isomerase can be induced rapidly in vitro by cytokines, growth factors, mitogens, and endotoxin. It can also be induced in vivo by proinflammatory stimuli in a variety of animal models of inflammation, in synovial tissues from patients with rheumatoid arthritis, and in human and animal tumors (for review, see Ref. 21). From these findings it has been proposed that prostaglandins generated via the COX-1 pathway are essential for physiological functions such as platelet homeostasis and maintenance of renal hemodynamics and gastrointestinal mucosal integrity, whereas prostaglandins generated by the COX-2 isomerase have a role in pathophysiological events, in particular inflammation, pain, pyrexia, and tumor growth (21, 33). In contrast to nonselective nonsteroidal anti-inflammatory drugs that block both COX-1 and COX-2, selective COX-2 inhibitors do not produce gastric lesions in rats (2, 6, 20); it has thus been suggested that COX-1-derived, but not COX-2-derived, prostaglandins are involved in gastric mucosal defense reactions against injury (33).

NS-398 (6, 20) and L-745,337 (2) are selective inhibitors of the COX-2 isomerase. Studies performed in our laboratory have revealed that, whereas indomethacin produced severe gastric mucosal lesions, neither NS-398 (unpublished results) nor L-745,337 (26a) caused damage to the gastric mucosa, confirming previous reports (2, 6, 20). However, NS-398 and L-745,337 dose dependently antagonized the protective effect of peptone. The ID$_{50}$ was 0.8 and 1.5 mg/kg for NS-398 and L-745,337, respectively. The maximal effective doses reduced the protective activity of peptone by 55% and 73%, respectively. The effects of the selective COX-2 inhibitors were comparable to those of the nonselective COX inhibitor indomethacin (ID$_{50}$, 4.2 mg/kg; maximal inhibition of protection, 66%). Prostaglandins are not involved in the increase in mucosal resistance after afferent nerve stimulation (16). Furthermore, atropine inhibited the protective effect of peptone to an extent comparable to that of the nonselective and selective inhibitors of prostaglandin formation. From these findings it is tempting to speculate that prostaglandins contribute to the cholinergic part of peptone-evoked protection. However, the attenuating effects of atropine and inhibition of prostaglandin formation were additive (data not shown), suggesting that peptone-induced protective effects in addition to stimulation of cholinergic neurons are prostaglandin dependent.

Dexamethasone did not affect the protection elicited by peptone. This finding is compatible with the concept...
that a constitutive, not an inducible, COX-2 enzyme generates the prostaglandins essential for peptone-evoked protection. This proposal is further supported by the finding that COX-2-sensitive protective effects of intragastric peptone occur within 20 min, a time period that seems rather short for effective enzyme induction.

Gastric mucosal formation of prostaglandin E2 or 6-ketoprostaglandin F1α was not increased to a measurable extent by perfusion of the gastric lumen with peptone and was not reduced by pretreatment with atropine. Whereas treatment with indomethacin substantially inhibited gastric mucosal formation of both prostaglandins, NS-398 and L-745,337 at doses that maximally blocked peptone-induced protection did not reduce prostaglandin formation in the gastric mucosa. The lack of measurable inhibitory activity on gastric prostaglandin generation indicates that NS-398 and L-745,337 do not counteract the peptone-induced protective effect of 20% ethanol. Similar to the findings described in this study, we could demonstrate neither measurable increases in mucosal production of prostaglandin E2 and 6-ketoprostaglandin F1α after instillation of the mild irritant nor reduction of prostaglandin generation after treatment with selective COX-2 inhibitors (7). The failure to demonstrate inhibition of gastric prostaglandin formation by selective COX-2 inhibitors may be due to the fact that COX-2-derived prostaglandins represent only a minority of the gastric prostaglandin pool. With the background of large amounts of prostaglandins generated via the COX-1 isoenzyme it may be difficult to demonstrate changes in the small COX-2-catalyzed prostaglandin compartment. The finding that NS-398 and L-745,337 did not affect dimercaprol-evoked protection (data not shown), which is not prostaglandin mediated and is insensitive to indomethacin, supports the hypothesis that the inhibitory effect of these agents on peptone-induced protection is indeed due to suppression of COX-2-derived prostaglandins. However, at present, we cannot completely rule out the possibility that effects of NS-398 and L-745,337 not related to inhibition of COX-2 interfere with the protective properties of peptone.

Constitutively expressed levels of COX-2 in addition to COX-1 are present in normal unstimulated tissues of various species. Thus the occurrence of COX-2 mRNA and COX-2 protein in small but possibly strategic areas in normal rat gastric mucosa with particularly strong expression of COX-2 in the endothelia of gastric mucosal microvessels has recently been described (32). Immunoreactivity for COX-2 has been found in the surface mucous cells in both the fundic and pyloric regions of the rat stomach (12). Using immunoblot analysis, expression of low but detectable levels of COX-2 protein could be demonstrated in microsomes prepared from gastrointestinal tissues of normal rats (13). Finally, in the human stomach approximately equivalent levels of COX-1 and COX-2 mRNA were present when RT-PCR was applied to quantitate the COX isoforms (22).

In conclusion, the results show that a complex system involving several neural pathways and chemical mediators underlies the gastroprotective effect of peptone. The findings of this and previous studies (4,11, 16, 27, 31, 35) suggest the following interactions of the systems involved. Peptone in the gastric lumen promotes the release of gastrin by stimulating intramural cholinergic and noncholinergic, bombesin/GRP-containing neurons. Gastrin then activates primary afferent neurons, thus inducing release of CGRP. CGRP in turn stimulates biosynthesis of NO, which acts as the final mediator of the afferent nerve-induced gastroprotection. In addition, activation of bombesin/GRP and afferent neurons releases somatostatin and/or by an unknown mechanism contributes to or facilitates the protective effect of gastrin. Activation of cholinergic neural pathways contributes to peptone-evoked protection indirectly by promotion of gastrin release as well as possibly by a direct mechanism resulting from stimulation of prostaglandin generation in the stomach. The precise mechanisms by which NO and prostaglandins mediate protective effects have not been elucidated so far but obviously do not rely on elevated gastric mucosal blood flow. Our findings also show that, although selective inhibitors of COX-2 do not produce gastric mucosal lesions, they significantly attenuate the protective activity of intragastric peptone, suggesting that prostaglandins mediating the protection are generated via the COX-2 isoform. These findings provide evidence that prostaglandins biosynthesized by a constitutive COX-2 enzyme are crucially involved in physiological functions that are essential for homeostasis reactions in the gastrointestinal tract.

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