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Role of endogenous gastrin in gastroprotection

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Abstract

Gastrin has a potent influence on gastric acid secretion and mucosal growth but its role in mucosal integrity has been little studied. This study investigated in rats whether gastrin protects the gastric mucosa against the damage by 100% ethanol and what are the possible mechanisms of this protection. Exogenous gastrin-17 (0.6–5.0 pmol/kg) injected subcutaneously (s.c.) reduced dose dependently ethanol-induced mucosal damage and the dose decreasing the ethanol lesions by 50% was about 1.8 pmol/kg. The protection afforded by gastrin-17 was accompanied by a dose-dependent increase in gastric blood flow and these effects were almost completely abolished by the pretreatment with specific CCK_B (L-365,260) but not CCK_A receptor antagonist (loxiglumide). Endogenous gastrin released by intragastric (i.g.) peptone meal or s.c. injection of gastrin-releasing peptide prevented the formation of acute ethanol-induced lesions and these effects were also abolished by the pretreatment with L-365,260 but not by loxiglumide. The inhibition of nitric oxide (NO) synthase, by N^G-nitro-L-arginine methyl ester almost completely eliminated both the protective and hyperemic effects of gastrin-17 and the addition of L-arginine (but not D-arginine) to N^G-nitro-L-arginine-methyl ester restored, in part, these effects of gastrin-17. Deactivation of sensory nerves with capsaicin did not influence the protective or hyperemic effects of gastrin-17. We conclude that both exogenous and endogenous gastrin exert its protective activity against ethanol damage of gastric mucosa and this effect is mediated through the interaction with specific CCK_B receptors and arginine-NO pathway, but does not involve sensory nerves.

Keywords: Gastrin-17; CCK (cholecystokinin); Gastroprotection; Stomach; Capsaicin; Nitric oxide (NO)

1. Introduction

Gastrin is known to stimulate gastric acid secretion and mucosal growth (Walsh, 1994) but its role in the maintenance of mucosal integrity has been little studied. Pentagastrin (Konturek et al., 1983; Stroff et al., 1994) and its natural analog cholecystokinin (CCK) (Evangelista et al., 1987; Evangelista and Maggi, 1991) were reported to protect the mucosa against the damage by acid-independent irritant such as absolute ethanol while enhancing the lesions produced by acid-dependent ulcerogens such as aspirin or cysteamine (Konturek et al., 1983; Evangelista and Maggi, 1991; Pendley et al., 1993). The protective effect of pentagastrin was almost completely abolished by specific antagonists of CCK_B receptors such as L-365,260 but not by the antagonist of CCK_A receptors, while the protection afforded by CCK was eliminated by CCK_A but not CCK_B receptor antagonist. Furthermore, the gastroprotective effects of CCK were attenuated by the vagotomy and the inactivation of sensory nerves with capsaicin suggesting the involvement of vagal capsaicin-sensitive fibers in this protection (Evangelista and Maggi, 1991). No study was undertaken so far to determine the physiological role of endogenous gastrin in the protection of gastric mucosa against the damage by necrotizing substance such as absolute ethanol and acid-dependent irritant such as aspirin.

This study was designed (1) to assess the gastroprotective and circulatory activities of exogenous gastrin against the mucosal damage induced by absolute ethanol or aspirin, (2) to evaluate the possible gastroprotective actions of endogenous gastrin released by peptone meal or gastrin-releasing peptide and (3) to determine the possible mechanisms of protective effects of gastrin, particularly the role of endogenous prostaglandins, nitric oxide and sensory nerves.

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2. Materials and methods

Male Wistar rats, weighing 200–250 g and fasted for 24 h, were used in gastric secretory tests and in studies on gastroprotection.

2.1. Gastric secretory studies

The effects of gastrin-17 (UCB Bioproducts, Braine-l'Allend, Belgium) without or with pretreatment with CCK_A receptor antagonist, loxiglumide (gift of Dr. L. Rovati, Rotta Research Laboratories, Monza, Italy) or CCK_B receptor antagonist, L-365,260 (gift of Dr P.S. Anderson, Merck Sharp and Dohme, West Point, PA, USA) on gastric acid secretion were examined in ten conscious rats equipped about 1 month earlier with gastric fistulas as described previously (Konturek et al., 1990). The animals 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. The gastric fistula was opened, 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 gastrin-17 was administered subcutaneously (s.c.) in various doses ranging from 0.6 pmol/kg to 10 nmol/kg of gastrin-17, each dose being dissolved in 1 ml of saline and given on a separate test day. In control tests, vehicle (saline) was given s.c. in a volume of 1 ml in the same manner as in tests with each secretagogue. In tests with CCK_A or CCK_B receptor antagonists, a standard dose of gastrin-17 (2.5 pmol/kg) was injected s.c. 30 min after intraperitoneal (i.p.) injection of L-365,260 or loxiglumide given in a standard dose (25 μmol/kg), the collection of gastric juice being continued for the final 60 min. The volume and acid concentration of each collected sample of gastric juice were measured and acid outputs (expressed in term of μmol of acid per 30 min) were determined as described before (Konturek et al., 1990, Konturek et al., 1991b).

2.2. Gastroprotection studies

Acute gastric lesions were induced by an intragastric (i.g.) application of 100% ethanol or acidified aspirin (200 mg/kg) as described previously (Konturek et al., 1994). Briefly, 1.5 ml of 100% ethanol or 1.5 ml of 200 mM HCl solution containing 200 mg/kg of aspirin was administered i.g. to rats by means of a metal oro-gastric tube. After 60 min, the animals were lightly anesthetized with ether, the abdomen was opened and the gastric blood flow was measured using laser Doppler technique (Laserflow, BP403B, Vasamedics, St. Paul, MN, USA) as described previously (Konturek et al., 1994). The values of gastric blood flow were expressed as percent change from the values recorded in vehicle-treated control animals with intact mucosa. Then, the stomach was removed, rinsed with water and pinned open for macroscopic examination. The area of necrotic lesions in oxyntic mucosa was measured using computerized planimetry (Morphomat, Carl Zeiss, FRG) under blinded conditions.

2.3. Experimental groups of rats

Three major series (A, B and C) of experiments were carried out.

Series A was designed to determine the effects of s.c. injection of exogenous gastrin-17 (0.6–5.0 pmol/kg) without or with the pretreatment with L-365,260 or loxiglumide on ethanol- or aspirin-induced gastric lesions.

Series B was used to examine the influence of endogenous gastrin released by intragastric (i.g.) administration of peptone meal or s.c. injection of gastrin releasing peptide without or with the pretreatment with specific antagonist of CCK_B (L-365,260), CCK_A (loxiglumide) or bombesin (RC-3095) receptors (Dembinski et al., 1991) on the area of gastric mucosal lesions induced by 100% ethanol.

Series C was used to investigate the involvement of endogenous prostaglandins, nitric oxide (NO) and capsaicin-sensitive nerves in the protection afforded by gastrin-17 against ethanol damage.

The following groups of rats in series A were used: (I) vehicle (1 ml of saline s.c.) followed 30 min later by 100% ethanol; (II) gastrin-17 (0.6–5.0 pmol/kg) followed 30 min later by 100% ethanol; (III) gastrin-17 (2.5 pmol/kg s.c.) followed 30 min later by 100% ethanol; (IV) loxiglumide or L-365,260 (25 μmol/kg i.p.) followed 30 min later by gastrin-17 (2.5 pmol/kg s.c.) and then finally 30 min later by 100% ethanol.

Rats of group B were used to study the effects of endogenous gastrin, i.g. administration of peptone (1–8%) meal or s.c. injection of gastrin-releasing peptide without or with specific CCK_B receptor antagonist, L-365,260 or with specific gastrin-releasing peptide antagonist, RC 3095 (Jaworek et al., 1992), followed 30 min later by 100% ethanol. In tests with peptone meal, 1 ml of 1, 5 or 8% peptone (Bacto-Peptone, Difco Laboratories, Detroit, MN, USA) was introduced into the stomach using oro-gastric tube. NaCl was added when appropriate to achieve isoosmolarity of peptone solution as measured by freezing depression point with Fiske osmometer (Fiske Associates, Denver, MA, USA). The pH of the test meal was adjusted to pH 7.0 using 1 mM NaOH. In separate tests, the rats were lightly anesthetized with ether, the abdomen was opened and 1 ml of 8% peptone meal or vehicle (saline) (pH 7.0 and osmolarity adjusted to about 300 mOsm/l) was instilled through the needle into the duodenum, while the pyloric sphincter was temporarily

occluded (by clamping) to avoid any escape of test meal into the stomach. Then, the abdomen was closed by suture and 30 min later 1.5 ml of 100% ethanol was given i.g.

Each test solution given i.g. or i.d. contained phenol red used as a marker to determine (by spectrophotometry at 520 nm after being alkalinized to pH 11.5) the presence of the test meal in the stomach or the reflux of the duodenal meal into the stomach.

In series C, the role of endogenous prostaglandins in the protection induced by gastrin-17 was examined (1) using indomethacin to inhibit endogenous prostaglandins in an attempt to reverse the gastroprotective activity of gastrin-17 and (2) by direct measuring mucosal generation of prostaglandin E_2 by radioimmunoassay (Konturek et al., 1994) in tests with indomethacin. The following groups of rats were used; (I) vehicle (saline 1 ml i.p.) followed 90 min later by 100% ethanol, (II) vehicle (saline) followed 60 min later by gastrin-17 (2.5 pmol/kg s.c.) and then 30 min later by 100% ethanol, (III) indomethacin (5 mg/kg i.p.) followed 60 min later by vehicle (saline s.c.) and finally 30 min later by 100% ethanol; (IV) indomethacin (5 mg/kg i.p.) followed 60 min later by gastrin-17 (2.5 pmol/kg s.c.) and finally 30 min later by 100% ethanol.

To assess the role of NO in gastroprotection induced by gastrin-17 against ethanol damage, rats were pretreated with N^G-nitro-L-arginine-methyl ester (L-NAME), a specific inhibitor of NO synthase (Clinalfa AG, Laufelfingen, FRG) injected intravenously (i.v.) without or with addition of L-arginine or D-arginine (Sigma Chemical Co, St Louis, MO, USA), the substrate of NO synthase as described before (Konturek et al., 1994). The following groups of rats were used; (I) vehicle (saline 1 ml i.v.) followed 60 min later by 100% ethanol, (II) vehicle (saline (i.v.) followed 30 min later by gastrin-17 (2.5 pmol/kg s.c.) and then 30 min later by 100% ethanol, (III) N^G-nitro-L-arginine-methyl ester (5 mg/kg i.v.) followed 15 min later by vehicle (saline s.c.) and then 30 min later by 100% ethanol, (IV) N^G-nitro-L-arginine-methyl ester (5 mg/kg i.v.) followed 15 min later by gastrin-17 (2.5 pmol/kg s.c.) and then 30 min later by 100% ethanol, (V) L-arginine or D-arginine (300 mg/kg i.v.) followed 15 min later by N^G-nitro-L-arginine-methyl ester (5 mg/kg i.v.) and then 15 min later by gastrin-17 (2.5 pmol/kg s.c.) and finally 30 min later by 100% ethanol.

The role of afferent nerves in gastroprotection by gastrin-17 was tested in rats with capsaicin-induced deactivation of these nerves. For this purpose the animals were pretreated with capsaicin (Sigma Co., St. Louis, MO) injected s.c. for 3 consecutive days at a dose of 20, 30 and 50 mg/kg about 2 weeks before the experiment (Esplugues and Whittle, 1990; Yonei et al., 1990). All injections of capsaicin were performed under ether anesthesia to counteract the respiratory impairment associated with injection of this agent. To check the effectiveness of the capsaicin denervation, a drop of 0.1 mg/ml solution of capsaicin was instilled into the eye of each rat and the protective wiping movements were counted as previously described (Yonei et al., 1990). Control rats received a vehicle injection. All animals pretreated with capsaicin showed negative wiping movement test, confirming functional denervation of the capsaicin-sensitive nerves. The following study groups were used; (I) vehicle (saline 1 ml s.c.) followed 30 min later by 100% ethanol in rats with intact afferent nerves, (II) gastrin-17 (2.5 pmol/kg s.c.) followed 30 min later by 100% ethanol in rats with intact afferent nerves; (III) vehicle (saline) followed 30 min later by 100% ethanol in rats with capsaicin inactivated afferent nerves and (IV) gastrin-17 (2.5 pmol/kg s.c.) followed 30 min later by 100% ethanol in rats with capsaicin-inactivated afferent nerves.

2.4. Measurement of gastric blood flow

In some rats of series A, B and C, the gastric blood flow was determined using laser Doppler flowmetry (Laserflo, BPM403B, Vasamedics, St. Paul, MN, USA) as described previously (Konturek et al., 1994). The rats were anesthetized with ether, the abdomen was opened, and the gastric contents were gently evacuated to the exterior through the cut in the forestomach. The optical flow probe was placed on the mucosa in the oxyntic gland area to monitor gastric blood flow. The values of gastric blood flow are expressed as percent change from the values recorded in the oxyntic gland area of the stomach in vehicle treated rats.

2.5. Determination of mucosal generation of prostaglandin E_2

In rats of series C, the mucosal samples of the oxyntic gland area were taken by biopsy (about 50 mg) immediately after the animals were killed to determine the mucosal generation of prostaglandin E_2 by specific radioimmunoassay as described previously (Konturek et al., 1990, 1994). The mucosal sample was placed in preweighed Eppendorf vials and 1 ml of Tris buffer (50 mM, pH 3.5) was added to each vial. The samples were finally minced (about 15 s) with scissors, washed, and centrifuged for 10 s, the pellet being resuspended again in 1 ml of Tris. Then each sample was incubated on a Vortex mixer for 1 min and centrifuged for 15 s. The pellet was weighed and the supernatant was transferred to a second Eppendorf vial containing indomethacin (10 mM) and kept at $-20°C$ until radioimmunoassay. Prostaglandin E_2 was measured in duplicate using radioimmunoassay kit (New England Nuclear, Munich, Germany). The capability of the mucosa

to generate prostaglandin E_2 was expressed in nanograms of wet tissue weight.

At the termination of experiments in rats of series A and B with s.c. administration of gastrin-17 or gastrin-releasing peptide and i.g. instillation of peptone meal (followed 30 min later by i.g. application of 100% ethanol), the blood samples (about 5 ml) were taken from the jugular vein (into tubes containing 2500 U Trasylol, Bayer, FRG and 0.5 mg of EDTA). For comparison, vehicle-treated rats (given only s.c. saline) were also anesthetized with ether and the blood samples were collected for the determination of control values of plasma levels of gastrin. The blood samples were stored at −50° C to measure the concentration of gastrin as described previously (Konturek et al., 1992). The assay system was sufficiently sensitive to detect about 1 pM of gastrin.

Results are presented as means ± S.E.M. Statistical evaluation was made, by analysis of variance followed by Student's *t*-test. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Effects of exogenous gastrin on gastric acid secretion

The effects of graded doses of gastrin-17 on gastric acid secretion from the gastric fistula in conscious rats are shown on Table 1. In control rats injected s.c. with vehicle (saline), basal acid output averaged about 120 ± 15 μmol/30 min. Gastrin-17 injected s.c. in gradually increasing doses ranging from 0.6 to 10.0 pmol/kg failed to affect significantly gastric acid secretion at this dose range. The maximal gastric acid response to gastrin-17 was attained at a dose of 10 nmol/kg given s.c. in these animals (Table 1). Doubling of this dose did not produce any further increase in acid output and these results are not included). Pretreatment with L-365,260 reduced significantly basal gastric acid secretion and that in response to gastrin-17 (2.5 pmol/kg s.c. or 10 nmol/kg s.c.). Pretreatment with loxiglumide failed to affect basal or gastrin-17-stimulated acid secretion (Table 1).

Table 1
Gastric acid secretion in conscious rats with gastric fistula injected s.c. with various doses of gastrin-17 without or with pretreatment with loxiglumide or L-365,260

Test substance	Gastric acid output (μmol/30 min)
Vehicle (control)	120 ± 15
Gastrin-17 (pmol/kg s.c.)	
0.6	128 ± 20
1.2	130 ± 15
2.5	136 ± 18
5.0	122 ± 26
10.0	138 ± 18
Gastrin-17 (nmol/kg s.c.)	
10.0	284 ± 22 [a]
Loxiglumide (25 μmol/kg i.p.)	115 ± 8
L-365,260 (25 μmol/kg i.p.)	43 ± 2 [a,b]
L-365,260 + gastrin-17 (2.5 pmol/kg s.c.)	52 ± 3 [a,b]
L-365,260 + gastrin-17 (10.0 nmol/kg s.c.)	109 ± 18 [b]
Loxiglumide + gastrin-17 (2.5 pmol/kg s.c.)	125 ± 21

Means ± SEM of ten tests on ten rats. [a] $P < 0.05$ when compared to the vehicle control values. [b] $P < 0.05$ when compared to the values obtained in tests with gastrin-17 but without pretreatment with L-365,260 or loxiglumide.

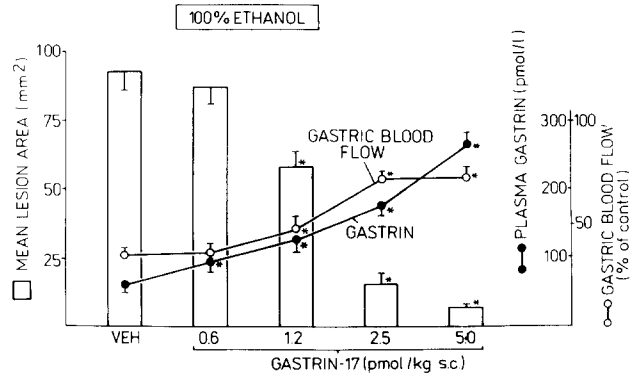

Fig. 1. Area of ethanol-induced gastric lesions, gastric blood flow and serum gastrin level in rats pretreated with vehicle (control) or with graded doses of gastrin-17. Means ± S.E.M. of eight to ten rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated rats.

3.2. Gastroprotective effects of exogenous gastrin

The effects of graded doses of gastrin-17 on the formation of acute gastric lesions induced by 100% ethanol are shown on Fig. 1. Gastrin-17 given s.c. reduced dose dependently the area of gastric lesions caused by 100% ethanol. The threshold (significant) reduction by gastrin-17 occurred at a dose of 1.2 pmol/kg s.c. and the dose reducing the area of gastric lesions by about 50% (ID_{50}) averaged about 1.8 pmol/kg of gastrin-17. Plasma levels of immunoreactive gastrin in tests with gastrin-17 showed a dose-dependent increments with increasing doses of administered peptide, the significant rise (over basal) being reached at a dose of 0.6 pmol/kg of gastrin-17 (Fig. 1). In further studies, gastrin-17 was used in a standard dose of 2.5 pmol/kg s.c. that caused over 80% reduction in the area of ethanol-induced lesions and the increase in plasma gastrin to the level observed after the administration of 8% peptone meal.

Gastric blood flow was reduced to about 40% in the stomach of rats given i.g. 100% ethanol. With gradually increasing doses of gastrin-17 administered before 100% ethanol, the gastric blood flow showed a dose-dependent increase, the rise being significant starting with 1.2 pmol/kg of gastrin-17 (Fig. 1).

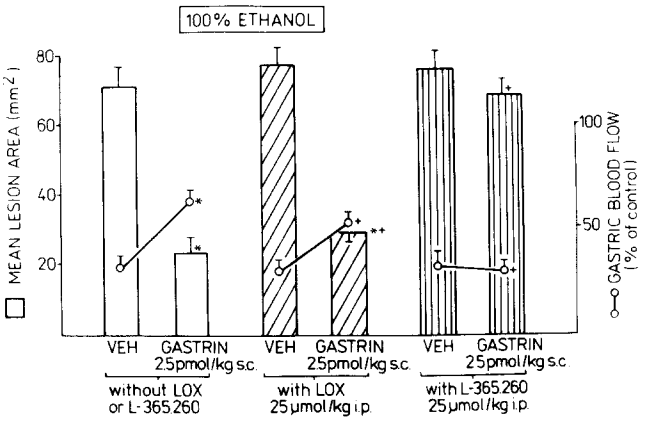

Fig. 2. Area of ethanol-induced gastric lesions, gastric blood flow and serum gastrin level in rats pretreated with vehicle (control) or standard dose of gastrin-17 (2.5 pmol/kg s.c.) in tests without and with administration of loxiglumide (25 μmol/kg) or L-365,260 (25 μmol/kg i.p.). Means ± S.E.M. of eight to ten rats. Asterisk indicates significant change as compared to vehicle control and cross indicates significant change as compared to the values obtained in rats with the administration of loxiglumide or L-365,260.

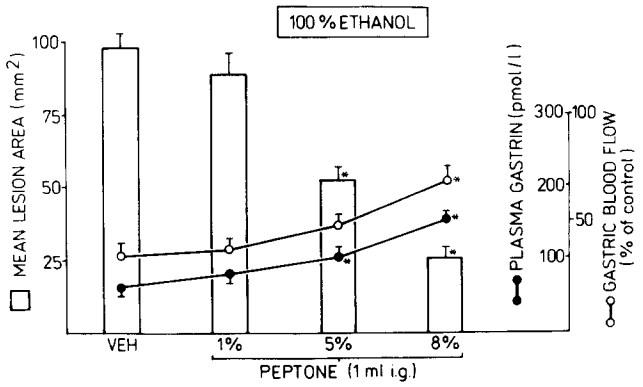

Fig. 3. Area of ethanol-induced gastric lesions, gastric blood flow and serum gastrin level in rats pretreated with i.g. vehicle (saline) or gradually increasing concentrations of peptone. Means ± S.E.M. of eight to ten rats. Asterisk indicates significant change as compared to the value obtained in vehicle-treated rats.

Pretreatment with L-365,260 or loxiglumide (25 μmol/kg i.p.) did not influence the area of gastric lesions or the fall in gastric blood flow caused by 100% ethanol alone in rats pretreated with vehicle (Fig. 2). Pretreatment with L-365,260 almost completely eliminated both the protective and hyperemic effects of gastrin-17, while loxiglumide only partly reduced the protection and gastric hyperemia caused by gastrin-17.

3.3. Gastroprotective effects of i.g. peptone or s.c. gastrin-releasing peptide

Peptone solution administered i.g. in various concentrations (1–8%) resulted in a concentration-dependent reduction in the area of gastric lesions induced by ethanol starting with the 5% peptone and reaching with 8% peptone about 25% of the vehicle control value (Fig. 3). Plasma gastrin levels showed significant increments at 5% and 8% peptone solutions and gastric blood flow was significantly elevated (above the value in rats treated with vehicle and followed by 100% ethanol) at 8% peptone. Pretreatment with L-365,260 almost completely abolished the protective and hyperemic effects of 8% peptone, while the administration of loxiglumide only partly reversed these effects (Fig. 4). Intraduodenal admnistration of 8% peptone meal failed to affect gastric lesions and the fall of gastric blood flow caused by ethanol or to alter serum gastrin level and these results were not included. Phenol red measured in the content evacuated from the stomach failed to detect any significant amounts of test solution in the stomach in these animals.

Gastrin-releasing peptide administered at a dose of 10 nmol/kg, which in our previous experiments on rats caused maximal pancreatic protein secretion (Jaworek et al., 1992) reduced ethanol-induced gastric damage by about 65% and this was accompanied by a significant elevation of plasma gastrin and the rise in gastric blood flow (Fig. 5). The protective activity and the rise in gastric blood flow and serum gastrin level induced by gastrin-releasing peptide were significantly reduced by the pretreatment with RC 3095, a selective blocker of bombesin receptors (Konturek et al., 1991a). L-365,260 abolished almost completely the protective and hyperemic effects of gastrin-releasing peptide but failed to influence the rise in serum gastrin response to gastrin- releasing peptide.

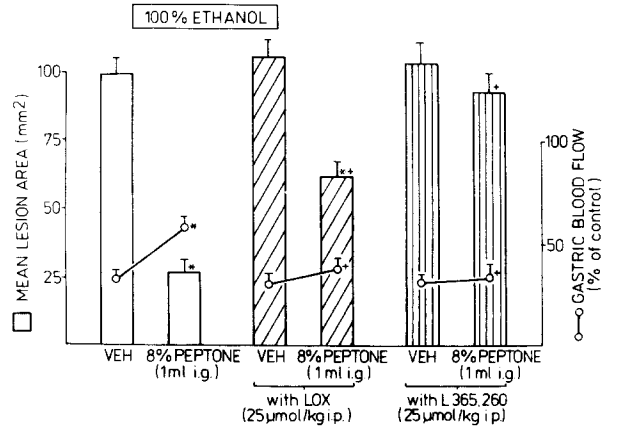

Fig. 4. Area of ethanol-induced gastric lesions, gastric blood flow and serum gastrin levels in rats pretreated with vehicle or 1 ml of 8% peptone in tests without or with administration of loxiglumide (25 μmol/kg) or L-365,260 (25 μmol/kg). Means ± S.E.M. of nine to ten rats. Asterisk indicates significant change as compared to the vehicle control and cross indicates significant change as compared to the values obtained in rats without administration of loxiglumide or L-365,260.

Table 2
Area of ethanol-induced gastric lesions, mucosal contents of prostaglandin E_2 and gastric blood flow (GBF) in rats injected with vehicle (saline), gastrin-17 (2.5 pmol/kg s.c.) in tests without or with administration of indomethacin (5 mg/kg i.p.) or aspirin-induced gastric lesions and mucosal prostaglandin E_2 or gastric blood flow without or with pretreatment with L-365,260 or loxiglumide (25 μmol/kg i.p.)

	Lesion area (mm^2)	Prostaglandin E_2 (ng/g)	GBF (% control)
Ethanol-induced lesions			
Vehicle + 100% ethanol	78 ± 12	280 ± 38	21 ± 3
Gastrin-17 + 100% ethanol	10 ± 4 [a]	316 ± 48	61 ± 7 [a]
Indo + 100% ethanol	84 ± 15	56 ± 8 [a]	23 ± 4
Indo + G-15 + 100% ethanol	12 ± 4 [a]	40 ± 6 [a]	35 ± 4 [a]
Aspirin-induced lesions			
Vehicle + aspirin	41 ± 7	36 ± 6 [a]	43 ± 8 [a]
Gastrin-17 + aspirin	45 ± 8	32 ± 5 [a]	46 ± 7 [a]
L-365,260 + aspirin	23 ± 5 [a]	28 ± 5 [a]	72 ± 15 [a,b]
Loxiglumide + aspirin	52 ± 9	34 ± 5 [a]	48 ± 8 [a,b]
L-365,260 + gastrin-17 + aspirin	20 ± 4	39 ± 8 [a]	74 ± 11 [b]
Loxiglumide + gastrin-17 + aspirin	40 ± 6	41 ± 6	46 ± 7

Means ± S.E.M. of eight tests on eight rats. [a] $P < 0.05$ when compared to the values obtained with vehicle + 100% ethanol or vehicle + aspirin. [b] $P < 0.05$ when compared to the value obtained with vehicle (saline) + aspirin.

3.4. Effects of the suppression of prostaglandin E_2 generation on gastroprotection afforded by gastrin

Administration of indomethacin (5 mg/kg i.p.) 90 min before i.g. application of 100% ethanol caused a small and non-significant increase in the mean area of gastric lesions. The generation of prostaglandin E_2 in the gastric mucosa of intact rats averaged 260 ng/g of wet tissue weight and this was not significantly affected by the exposure of the mucosa to 100% ethanol (Table 2). The pretreatment with indomethacin resulted in a significant reduction (by about 80%) in mucosal generation of prostaglandin E_2. Pretreatment with gastrin-17 (2.5 pmol/kg) caused similar reduction in the mean lesion area in rats without, as in those with, the administration of indomethacin. Mucosal content of prostaglandin E_2 was significantly reduced only in rats pretreated with indomethacin (Table 2).

Unlike ethanol injury, aspirin-induced mucosal damage is acid-dependent and i.g. administration of acidified aspirin produced gastric lesions of the mean area (41 ± 17 mm^2) about 50% smaller than that caused by 100% ethanol (Table 2). Pretreatment with gastrin-17 did not affect significantly the area of aspirin-induced gastric lesions, the mucosal prostaglandin E_2 or gastric blood flow as compared to the values obtained in vehicle-pretreated controls receiving aspirin alone. The addition of L-365,260 significantly reduced aspirin-induced gastric injury in tests without or with injection of gastrin-17. The mucosal generation of prostaglandin E_2 was reduced by about 90% in all aspirin-treated rats as compared to that recorded in intact vehicle-treated animals. The pretreatment with gastrin-17 and/or loxi-

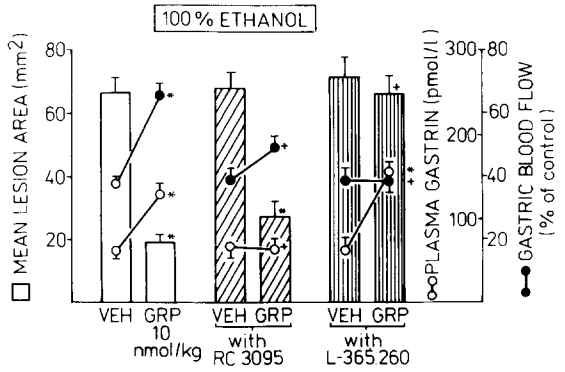

Fig. 5. Area of ethanol-induced gastric lesions, gastric blood flow and serum gastrin level in rats injected s.c. with vehicle (saline) or gastrin-releasing peptide (10 nmol/kg s.c.) in tests without or with the pretreatment with RC-3095 (50 nmol/kg i.p.). Means ± S.E.M. of eight to ten rats. Asterisk indicates significant difference as compared to the value obtained in vehicle-treated rats. Cross indicates significant change as compared to the value obtained with gastrin-releasing peptide but without RC-3095.

Table 3
Area of ethanol-induced gastric lesions and gastric blood flow (GBF) in rats injected with vehicle (saline) or gastrin-17 (2.5 pmol/kg s.c.) in tests without or with pretreatment with N^G-nitro-L-arginine-methyl ester (L-NAME) (5 mg/kg i.v.) alone or in combination with L-arginine or D-arginine (300 mg/kg i.v.)

	Lesion area (mm^2)	GBF (% control)
Vehicle + 100% ethanol	82 ± 12	31 ± 3
Gastrin-17 + 100% ethanol	14 ± 4 [a]	55 ± 8 [a]
L-NAME + 100% ethanol	108 ± 14 [a]	26 ± 8
L-NAME + gastrin-17 + 100% ethanol	68 ± 8 [b]	25 + 4 [b]
L-NAME + L-Arg + 100% ethanol	104 ± 14	27 ± 4
L-NAME + D-Arg + 100% ethanol	98 ± 10	29 ± 6
L-NAME + L-Arg + gastrin-17 + 100% eth.	12 ± 4 [a]	72 ± 4 [a]
L-NAME + D-Arg + gastrin-17 + 100% eth.	72 ± 9 [b]	32 ± 4 [b]

Means ± S.E.M. of eight tests on eight rats. [a] $P < 0.05$ when compared to the values obtained with vehicle + 100% ethanol. [b] $P < 0.05$ when compared to the values obtained with gastrin-17 + 100% ethanol.

Table 4
Area of ethanol-induced gastric lesions and gastric blood flow (GBF) in rats injected with vehicle (saline), gastrin-17 (2.5 pmol/kg s.c.) without or with pretreatment with L-365,260 (25 μmol/kg i.p.) in rats with intact and capsaicin-deactivated afferent nerves.

	Lesion area (mm^2)		GBF (% control)	
	Intact	Deactivated	Intact	Deactivated
Vehicle + 100% ethanol	78 ± 11	92 ± 16	34 ± 6	25 ± 3 [a]
Gastrin-17 + 100% ethanol	15 ± 3 [a]	18 ± 4 [a]	62 ± 10 [a]	48 ± 8 [a]
L-365 + gastrin-17 + 100% eth.	72 ± 14 [b]	58 ± 8 [b]	35 + 17 [b]	30 ± 4 [b]

Means ± S.E.M. of ten tests on ten rats. [a] $P < 0.05$ when compared to the values obtained with vehicle + 100% ethanol. [b] $P < 0.05$ when compared to the values obtained with gastrin-17 + 100% ethanol.

glumide and L-365,260 did not alter significantly this aspirin-induced suppression of generation of prostaglandin E_2 (Table 2).

3.5. The role of endogenous NO in gastrin-induced gastroprotection

Intravenous administration of N^G-nitro-L-arginine-methyl ester (5 mg/kg i.v.) before 100% ethanol caused a small but significant increase in the area of ethanol-induced gastric lesions (Table 3). When N^G-nitro-L-arginine-methyl ester (5 mg/kg i.v.) was injected prior to gastrin-17 in a standard dose, the area of ethanol-induced gastric injury was significantly increased and the gastric blood flow was significantly attenuated as compared to those obtained in rats treated with gastrin-17. Addition to N^G-nitro-L-arginine-methyl ester of L-arginine, but not D-arginine, restored the gastroprotective and hyperemic effects of gastrin-17 in rats with ethanol-induced gastric lesions.

3.6. Effect of deactivation of afferent nerves with capsaicin on gastroprotection afforded by gastrin

Deactivation of primary afferent nerves achieved with parenteral capsaicin about 2 weeks before the experiment did not affect the area of ethanol-induced gastric injury but significantly reduced the gastric blood flow as compared to that observed in ethanol-treated rats with intact sensory nerves (Table 4). Pretreatment of rats with deactivated afferent nerves with gastrin-17 in a standard dose resulted in a similar reduction in the lesion area and gastric blood flow to those found in animals with intact nerves. The addition of L-365,260 to gastrin-17 in capsaicin-denervated rats caused similar reduction in gastroprotection afforded by gastrin-17 to that observed in rats with intact sensory nerves.

4. Discussion

Peptides of gastrin/CCK family are released from the stomach and proximal portion of the gut upon normal ingestion of certain nutrients such as proteins and fat and affect various physiological functions such as gastric secretion and mucosal growth (Walsh, 1994; Liddle, 1994). Recently, it has been demonstrated that CCK may increase gastric mucosal integrity (Evangelista et al., 1987; Evangelista and Maggi, 1991; Pendley et al., 1993) acting through peripheral CCK_A receptors probably present in afferent vagal capsaicin-sensitive nerves because both vagotomy and capsaicin-induced deactivation of sensory fibers reduced the protective effects afforded by CCK. Other studies showed that the capsaicin-sensitive nerves are implicated in a local mucosal defense mechanism against various acute gastroduodenal lesions (Holtzer and Lippe, 1988; Holtzer et al., 1990).

Pentagastrin was reported a long time ago to prevent ethanol-induced gastric lesions (Konturek et al., 1983) and this finding has been confirmed recently (Stroff et al., 1994). However, no attempts were made to compare the protective efficacy of exogenous and endogenous gastrin and to determine the possible mechanism of this protection, especially the role of endogenous prostaglandins, nitric oxide (NO) and sensory nerves.

In this study we assessed the protective ability of gastrin-17, the major form of gastrin released postprandially in rats (Walsh, 1994) and found that the protective efficacy of gastrin-17 is several orders of magnitude higher on a molar basis than that of pentagastrin (Konturek et al., 1983; Stroff et al., 1994). This protection by gastrin-17 occurred at a dose range of the peptide which was below the threshold for the stimulation of gastric acid secretion in conscious rats, emphasizing the true cytoprotective activity of gastrin-17 because this protection had nothing to do with the alteration in gastric acid secretion (Robert et al., 1979).

The protective activity of gastrin-17 and that released endogenously by peptone meal or gastrin-releasing peptide can be abolished by L-365,260, which is considered a highly specific CCK_B receptor antagonist. In contrast, loxiglumide, that is a specific CCK_A receptor antagonist and that was shown to eliminate almost completely the protection afforded by CCK (Evangelista and Maggi, 1991), was less effective in the reduc-

tion of the protection attained with gastrin-17. Our results indicate that the receptors involved in the regulation by gastrin of the susceptibility of the mucosa to topical injury are predominantly of the CCK_B type.

As the protective activity of gastrin-17 occurred at the picomolar doses of this peptide, that raised plasma gastrin concentration to the levels similar to those observed after peptone meal in rats, we may conclude that gastrin probably plays a physiological role in maintaining the gastric mucosal integrity and in the protection of the mucosa against topical irritants such as ethanol.

It is noteworthy, that both CCK_A and CCK_B receptors are also involved in the control of gastric acid secretion in rats (Lloyd et al., 1992; Corwin and Smith, 1993) though not to the same extent. In our study, gastrin-17 administered alone in gastroprotective doses (0.6–10.0 pmol/kg s.c.) failed to affect gastric acid secretion. Pretreatment with loxiglumide did not influence gastric acid secretion when gastrin-17 was used in lower gastroprotective doses, but pretreatment with L-365,260 significantly reduced basal gastric acid secretion and that induced by gastrin-17 at its higher dose. This suggests that the action of gastrin-17 on gastric acid secretion is mediated entirely by CCK_B receptors (blocked by L-365,260).

The major finding of this report is an observation that gastrin released endogenously by i.g. peptone meal or s.c. injection of gastrin-releasing peptide was as effective in inducing gastroprotection and gastric hyperemia as exogenous gastrin-17. It is of interest that the increments in plasma gastrin achieved by gastric peptone meal, raised plasma hormone levels to the values similar to those achieved with exogenous peptide at its protective doses. The mediation of gastrin in gastroprotection afforded by intragastric peptone is supported by our observation showing that intraduodenal application of this peptone that failed to affect plasma gastrin level was also without any protective influence on gastric damage produced by ethanol. Our results seem to reinforce our conclusion that the protective activity of gastrin should be considered as a physiological phenomenon and that this hormone plays an important role in the maintenance of mucosal integrity. Furthermore, the protective effects of both exogenous and endogenous gastrin appear to be mediated by the same CCK_B receptors because the pretreatment with L-365,260 completely abolished the protection afforded by s.c. injection of gastrin-17 or gastrin-releasing peptide and by gastric peptone but was only slightly reduced by loxiglumide, blocking CCK_A receptors. In case of gastrin-releasing peptide, which was also protective while enhancing plasma gastrin level, we used a highly specific bombesin receptor antagonist, originally described by Coy et al. (1989). Such an antagonist of bombesin receptors given before 100% ethanol failed to affect the extent of mucosal damage caused by ethanol, thus, eliminating the possible involvement of endogenous bombesin-like peptides in the formation of this type of mucosal damage but reduced in part the protective and gastrin-releasing property of gastrin-releasing peptide against ethanol injury. However, pretreatment with L-365,260 completely eliminated the protective activity of gastrin-releasing peptide without affecting serum gastrin response to gastrin-releasing peptide, indicating that gastrin-releasing peptide protects the gastric mucosa mainly through the release of endogenous gastrin.

Gastroprotection has been originally attributed to prostaglandins and endogenous prostaglandins have been proposed to play a major role in gastroprotective mechanisms (Robert et al., 1979; Robert, 1979). Our finding that the pretreatment with indomethacin, that suppressed mucosal generation of prostaglandin E_2 by about 80% did not affect the protection afforded by gastrin-17 excludes any major role of endogenous prostaglandins in this protection. The observation that acute gastric lesions, induced by aspirin in a dose that almost completely abolished the mucosal generation of prostaglandin E_2, were not prevented by the parenteral administration of gastrin-17 could be explained by the enhancement of damaging action of aspirin possibly due to the stimulation of endogenous gastric acid secretion not detected in tests on rats with gastric fistula. This is supported by the fact that the inhibition of this secretion by the blockade of CCK_A receptors with L-365,260 significantly reduced aspirin-induced gastric damage. The pretreatment with gastrin-17 had no influence on the reduction in aspirin-induced damage by L-365,260 probably because too low dose of this peptide was used and a higher dose of gastrin-17 (not used in the study with aspirin) could reverse the protective effects of L-365,260 against aspirin-induced gastric damage.

The protection induced by exogenous gastrin-17 and by endogenous hormone released by gastric peptone was accompanied by a significant elevation of gastric mucosal blood flow. Since the maintenance of mucosal circulation was shown to play a crucial role in gastroprotection by prostaglandins (Leung et al., 1985) and endogenous NO released constitutively (Palmer et al., 1988; Brown et al., 1992) in the mucosa was postulated to mediate gastroprotection and gastric hyperemia (Peskar et al., 1991; Brzozowski et al., 1992; Whittle et al., 1992), we studied, therefore, whether the blockade of NO synthase by N^G-nitro-L-arginine-methyl ester affects the protection and mucosal blood flow induced by gastrin. Indeed, the pretreatment with N^G-nitro-L-arginine-methyl ester reduced significantly the gastric blood flow induced by gastrin-17 and prevented its protective activity. These effects of N^G-nitro-L-arginine-methyl ester were reversed by concurrent ad-

ministration of L-arginine (but not D-arginine), a substrate of NO synthase. These results indicate that NO plays a crucial role both in mucosal hyperemia and gastroprotection afforded by gastrin.

In an attempt to elucidate further the mechanism of gastrin-17-induced protection, we studied the possible involvement of sensory nerve fibers that have been proposed previously to mediate this protection by CCK (Evangelista and Maggi, 1991). However, the pretreatment with parenteral capsaicin in a dose that inactivated the sensory fiber (Holtzer et al., 1990) did not counteract the protective activity of gastrin-17. Furthermore, the pretreatment with L-365,260 abolished the protection afforded by gastrin-17 in capsaicin-denervated rats to the same extent as in intact animals. We postulate, therefore, that the protective effect of gastrin is not mediated by capsaicin-sensitive afferent nerves.

In conclusion, (1) systemic gastrin protects the rat gastric mucosa against ethanol-induced injury and this peptide interacts on the mucosal integrity; (2) this protective effect is seen also after pretreatment with indomethacin excluding the major role of endogenous prostaglandins in this protection, (3) gastrin-induced protection does not seem to involve afferent capsaicin-sensitive nerves but appears to be mediated by separate CCK_B receptors and NO generated within the gastric mucosa and (4) gastrin does not prevent aspirin-induced gastric damage probably due to the stimulation of gastric acid secretion mediated mainly by CCK_B receptors.

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