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CCK_1 and CCK_2 receptors regulate gastric pepsinogen secretion

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Abstract

The present study investigated (1) the pharmacological profile of cholecystokinin (CCK) receptor subtypes involved in the regulation of gastric pepsinogen secretion, (2) the influence of gastric acidity on peptic responses induced by CCK-8-sulfate (CCK-8S) or gastrin-I; and (3) the mechanisms accounting for the effects of CCK-like peptides on pepsinogen secretion. In anaesthetized rats, i.v. injection of CCK-8S or gastrin-I increased both pepsinogen and acid secretion. The pepsigogue effect of CCK-8S was higher than that of gastrin-I, whereas acid hypersecretion after CCK-8S was lower than that induced by gastrin-I. Peptic output following CCK-8S was partly blocked by i.v. injection of the CCK_1 receptor antagonist, devazepide (−75.3%), or the CCK_2 receptor antagonist, L-365,260 [3*R*(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3yl)-*N*′-(3-methyl-phenyl)urea; −27.9%], but was fully prevented by combined administration of devazepide and L-365,260. The gastric acid hypersecretory effect of CCK-8S was enhanced by devazepide (+84.5%) and blocked by L-365,260. In contrast, the gastric secretory actions of gastrin-I were insensitive to devazepide, but abolished by L-365,260. Excitatory effects of CCK-8S and gastrin-I were not modified by vagotomy or atropine, whereas cimetidine or α-fluoromethylhistidine (irreversible blocker of histidine decarboxylase) partly prevented acid hypersecretion induced by both peptides without affecting their pepsigogue effects. After pretreatment with omeprazole, both CCK-8S and gastrin-I failed to stimulate acid secretion, while they increased pepsinogen output. In rats with gastric perfusion of acid solutions, CCK-8S or gastrin-I increased peptic output in a pH-independent manner either with or without pretreatment with omeprazole. Ablation of capsaicin-sensitive sensory nerves as well as application of lidocaine to the gastric mucosa failed to modify the excitatory effects of CCK-8S or gastrin-I on pepsinogen and acid secretion. Blockade of the nitric oxide (NO) synthase pathway by N^G-nitro-L-arginine-methyl ester prevented the pepsigogue actions of both CCK-8S and gastrin-I (−61.8% and −71.7%, respectively), without affecting the concomitant increase in acid output. In addition, both these peptides significantly increased the release of NO breakdown products into the gastric lumen. The present results suggest that: (1) both CCK_1 and CCK_2 receptors mediate the peptic secretory responses induced by CCK-like peptides; (2) the excitatory inputs of CCK-8S and gastrin-I to chief cells are not driven through acid-dependent mechanisms or capsaicin-sensitive afferent sensory nerves; and (3) under in vivo conditions, the stimulant actions of CCK-like peptides on pepsinogen secretion are mediated, at least in part, by an increase in NO generation.

Keywords: Pepsinogen secretion; Acid secretion; Cholecystokinin/gastrin receptor; Vagus nerve; Afferent sensory nerve; Nitric oxide (NO)

1. Introduction

Cholecystokinin (CCK), gastrin and CCK-related peptides include a family of hormones which share an identical carboxyl-terminal pentapeptide sequence, and regulate a variety of pathophysiological events, including anxiety, analgesia, satiety, digestive motility, secretions and cell growth (Crawley and Corwin, 1994; Walsh, 1994). At present, all these functions appear to be mediated through specific interactions of CCK-like peptides with at least two receptor subtypes designated as CCK_1 and CCK_2 (Alexander and Peters, 1998). CCK_1 receptors, which are mainly localized in the digestive system and in few areas of the brain, display higher relative affinity for CCK than gastrin and are blocked by the selective antagonist, devazepide. CCK_2 receptors are widely expressed throughout the central nervous system and gastrointestinal tract, exhibit similar affinities for gastrin and CCK, and are selectively blocked by L-365,260 [3*R*(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3yl)-*N*′-(3-methyl-phenyl)urea] (Woodruff and Hughes, 1991;

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Bueno and Junien, 1994). Molecular cloning and structural analysis of genes encoding two distinct CCK receptor subtypes in different mammalian species have lent considerable support to this classification (Blandizzi et al., 1994; Wank, 1995).

In the digestive system, both gastrin and CCK participate in the control of gastric secretory functions (Walsh, 1994). Gastrin stimulates the secretion of hydrochloric acid through activation of CCK_2 receptors located on parietal cells or histamine-releasing enterochromaffin-like cells (Sandvik and Waldum, 1991; Hills et al., 1996). In addition, CCK can either mimic the excitatory action of gastrin on acid secretion (Soll et al., 1984; Patel and Spraggs, 1992), or inhibit the stimulated acid output, this latter effect appearing related to the release of somatostatin from gastric D cells, mediated by CCK_1 receptors (Lloyd et al., 1992; Konturek et al., 1993).

As far as gastric pepsinogen secretion is concerned, both gastrin and CCK exert pepsigogue effects in various experimental models (Kleveland et al., 1986; Tazi-Saad et al., 1992), but the receptor pathways and mechanisms underlying these hypersecretory responses under in vivo conditions are unclear. A major difficulty encountered in experiments dealing with in vivo regulation of pepsinogen secretion is the parallel increase of acid output which usually occurs after administration of gastric secretagogues (Raufman, 1992). Indeed, both in vivo and in vitro studies indicate that the occurrence of pepsinogen secretion upon stimulation of chief cells may require a flow of water and acid from adjacent parietal cells (Hersey, 1987; Sandvik et al., 1987), and that acidity in the stomach lumen is able to stimulate pepsinogen output from the gastric mucosa (Johnson, 1972; Smith and Torres, 1990), suggesting that changes in local hydrogen ion concentration might be responsible for parallel variations of peptic secretory output. In addition, some digestive effects of CCK-like peptides do not depend on a direct interaction with the effector cells, but rather on the activation of CCK receptors located on neural pathways (Raybould and Taché, 1988; Li et al., 1997).

Attempts to discriminate between direct and indirect control exerted by CCK and gastrin on pepsinogen secretion may be of pathophysiological relevance. For instance, pathological conditions associated with hypergastrinemia, such as *Helicobacter pylori*-related gastritis and peptic ulcer (Gillen et al., 1998; Lehmann et al., 1998), could maintain high rates of pepsinogen secretion as well as high levels of serum pepsinogens independently of changes in gastric acid secretion. In addition, it has been suggested that gastrin receptor antagonists may exert beneficial effects in the treatment of peptic ulcer disease (Pendley et al., 1993), but their in vivo influence on pepsinogen secretion remains to be established. Therefore, in the present study an in vivo gastric preparation was used for the evaluation of pepsinogen secretion, under different experimental conditions, in order to: (a) characterize the pharmacological profile of CCK receptor subtypes involved in the regulation of pepsinogen secretion; (b) investigate the influence of gastric acidity on peptic secretory responses induced by administration of CCK-8-sulfate (CCK-8S) or gastrin-I; and (c) gain more insight into the mechanisms underlying pepsigogue actions exerted by CCK-like peptides.

2. Materials and methods

2.1. Animals

The experiments were carried out on male Wistar rats, weighing 200–220 g. The animals were fed standard laboratory chow and tap water ad libitum, and were not used for at least one week after their delivery to the laboratory. The animals were housed, six in a cage, in temperature-controlled rooms on a 12-h light cycle at 22–24°C and 50–60% humidity. Their care and handling as well as the experimental protocol were in accordance with the provisions of the European Economic Community Council Directive 86-609, recognized and adopted by the Italian Government. Twenty-four hours before the experiments, the animals were put in single cages provided with wire net bottoms and deprived of food. Ad libitum access to water was allowed until the beginning of the experiment.

2.2. Perfusion of the gastric lumen in anaesthetized rats

Continuous perfusion of the rat stomach in situ was carried out following the procedure previously described (Blandizzi et al., 1997). The animals were anaesthetized with urethane (1.2 g/kg) administered i.p., the trachea was surgically exposed and cannulated with a polyethylene catheter in order to ensure a patent airway. A polyethylene catheter was introduced into the oesophagus and advanced as far as 5 mm beyond the gastroesophageal junction. Following a midline laparotomy, the proximal duodenum was exposed and its wall was incised. Then, a polyethylene catheter was introduced into the duodenum and pushed forward until its tip was about 5 mm beyond the pylorus. The stomach lumen was perfused continuously at a rate of 1 ml/min with saline solution (154 mM NaCl, $pH = 7.0 \pm 0.2$) at 37°C, unless otherwise stated, and 15-min effluent fractions were collected. The effluent samples were used for the quantitative evaluation of both pepsinogen and acid secretion.

2.3. Evaluation of pepsinogen and acid secretion

Pepsin levels in the gastric effluent were determined as previously reported (Blandizzi et al., 1997). Briefly, 2 ml of 2.5% bovine haemoglobin plus 0.5 ml of 0.3 M HCl and 0.5 ml of gastric effluent were put in separate tubes at

37°C for 10 min and then mixed. The mixtures were then incubated for 10 min at 37°C and the reaction was stopped by the addition of 5 ml 0.3 M trichloroacetic acid. After agitation and filtration, optical density was measured at 280 nm with an Uvikon 930 Spectrophotometer (Kontron Instruments, Milan, Italy). The results were compared to a standard curve, generated in an identical manner using known amounts of porcine pepsin (1 μg = 3 peptic units), and were expressed as μg of pepsin. The acidity in the gastric perfusate was measured with an autotitrator pH meter (PHM 85, Radiometer, Copenhagen, Denmark) by automatic potentiometric titration to pH 7.0 with 0.01 N NaOH, and expressed as μeqH^+.

Following surgical preparation of animals, basal gastric secretion was allowed to stabilize for 30 min. At the end of this period, two consecutive 15-min effluent fractions were collected in order to assess basal secretory values. Both pepsinogen and acid secretions were then monitored at 15-min intervals for an additional 120 min and expressed as μg of pepsin/15 min and μeqH^+/15 min, respectively. The peptic and acid outputs obtained during the whole 120-min period following the collection of basal effluent samples were also calculated and expressed as μg of pepsin/120 min and μeqH^+/120 min, respectively.

2.4. Experimental procedures

The first set of experiments was carried out on rats with intact vagus nerves which were acutely treated with CCK-8S or gastrin-I at the doses of 1.5, 5, 15 and 45 nmol/kg. Both these peptides were administered i.v. as a bolus immediately after the collection of basal effluent samples. In experiments investigating the involvement of muscarinic, histamine H_2 or CCK receptors in the gastric secretory responses elicited by CCK-8S or gastrin-I, the animals were pretreated with atropine (1 μmol/kg i.v.), cimetidine (10 μmol/kg i.v.), devazepide (1.25–2.5 μmol/kg i.v.) or L-365,260 (2.5–5 μmol/kg i.v.) 10 min before ending the collection of the second basal effluent sample. Additional experiments were performed in animals pretreated with the irreversible inhibitor of histidine decarboxylase, α-fluoromethylhistidine (450 μmol/kg i.p. twice daily for two consecutive days), in order to suppress endogenous histamine production from digestive enterochromaffin-like cells (Andersson et al., 1992; Fujimoto et al., 1995). The effects of CCK-8S were also examined in the presence of somatostatin-14 (15 nmol/kg/h i.v.) infused for 2 h, starting at the beginning of the second basal effluent sample.

The second series of experiments was performed on rats whose vagus nerves were carefully separated from the carotid arteries and cut at the cervical level 30 min before the collection of basal effluent samples was started. In order to ensure that vagal trunks could not still favour the transmission of afferent or efferent nervous activity after cutting, care was taken to produce mechanical damage to both the proximal and distal end of each vagal trunk by crushing them with forceps. The effects of CCK-8S or gastrin-I on gastric secretions were assessed in animals undergoing the vagotomy procedure.

The third set of experiments was designed in order to assess the effects of CCK-8S or gastrin-I on pepsinogen secretion in the presence of complete blockade of the acid secretory function of gastric parietal cells. For this purpose, 90 min before the collection of basal effluent samples started, the animals were pretreated with omeprazole (90 μmol/kg i.v.), a benzimidazole derivative which inhibits gastric acid secretion through a selective blockade of H^+:K^+-adenosin-triphosphatase (Fellenius et al., 1981) without interfering with receptor or signal transduction pathways of chief cells (Clissold and Campoli-Richards, 1986). The dose of omeprazole was selected because of its ability to acutely suppress both basal and stimulated gastric acid secretion in anaesthetized rats (Blandizzi et al., 1997).

In the fourth series, the effect on pepsinogen secretion of topical application of acid solutions at different pH on the surface of gastric mucosa was studied under basal conditions as well as after administration of CCK-8S or gastrin-I. Experiments were carried out either in the absence or in the presence of pretreatment with omeprazole in order to suppress endogenous acid production. In both cases the gastric lumen was perfused with saline up to the collection of basal effluent samples and then continued until the end of the experimental period with one of the following acid solutions: 0.1 mM HCl plus 153.9 mM NaCl (pH = 4.0); 1 mM HCl plus 153 mM NaCl (pH = 3.0); 10 mM HCl plus 144 NaCl (pH = 2.0); 100 mM HCl plus 54 mM NaCl (pH = 1.0).

The fifth group of experiments was designed to assess whether CCK-8S or gastrin-I was able to affect gastric pepsinogen and acid secretion in the presence of systemic ablation of capsaicin-sensitive sensory nerve fibres. For this purpose, some animals were given a dose of 125 mg/kg capsaicin s.c., as previously reported by Pabst et al. (1993). Ten days after this treatment the animals were used for the assessment of gastric pepsinogen and acid secretions. One day before the experiment, the efficacy of capsaicin treatment was checked by instilling a drop of a capsaicin solution (0.1 mg/ml in saline solution) into one eye of each rat. Capsaicin-treated rats were expected not to react by wiping their eyes but, whenever an animal responded with wiping, the afflicted eye was immediately and extensively rinsed with water.

The sixth series of experiments was performed to investigate the effects of CCK-8S or gastrin-I on both pepsinogen and acid secretions following topical application of the local anaesthetic, lidocaine, to the gastric mucosal surface. In this case, pre-exposure of the gastric mucosa to lidocaine was carried out according to the procedure reported by Mercer et al. (1994) with minor modifications. After

the collection of two basal effluent samples, the perfusion was interrupted, the gastric lumen was emptied and then filled with 3 ml of 4% lidocaine. Fifteen minutes later the stomach was emptied, gently rinsed with 6 ml saline and filled again with 3 ml saline. Perfusion of the gastric lumen with saline was then resumed and continued up to the end of the experimental period. An interval of 5 min was allowed to elapse between the restart of gastric perfusion and the administration of CCK-8S or gastrin-I.

In the seventh set of experiments the putative involvement of the nitric oxide (NO) pathway in the gastric secretory effects of CCK-8S or gastrin-I was investigated. Two experimental approaches were used to address this point. In the first procedure, animals receiving CCK-8S or gastrin-I were pretreated with the NO synthase inhibitor, N^G-nitro-L-arginine-methyl ester (L-NAME, 75 μmol/kg i.v.), 10 min before ending the collection of second basal effluent sample. L-NAME was given either alone or in the presence of L-arginine (specific substrate of NO synthase) or D-arginine (the inactive enanthiomer of L-arginine, not able to act as a substrate for NO synthase) both administered at the dose of 2 mmol/kg i.v. 15 min before the injection of L-NAME. In this case, 15-min fractions of gastric perfusate were collected and used for quantitative evaluation of both pepsinogen and acid secretions. In the second procedure the breakdown products of NO (nitrites plus nitrates, NO_x), released into the gastric lumen, were quantitatively estimated after treatment of animals with CCK-8S or gastrin-I, either alone or in the presence of L-NAME (75 μmol/kg i.v.). For this purpose, experiments were performed as previously reported by Saperas et al. (1995), with minor modifications. Briefly, after surgical preparation and 30-min stabilization, the perfusion was interrupted and the gastric lumen was gently emptied. The stomach was then filled with 3 ml of saline solution, and 30 min later the gastric luminal contents were recovered. This procedure was repeated at 30-min intervals six times. CCK-8S or gastrin-I was administered at the end of the second collection period, whereas L-NAME was injected 10 min before the administration of peptides. NO_x concentration in aliquots of the recovered gastric contents was measured by the Griess method (Green et al., 1982) after reduction of nitrates to nitrites with nitrate reductase in the presence of 5 mM NADPH for 1 h at 37°C. Nitrites were incubated with Griess reagent [0.1% *N*-(1-naphthyl)ethylenediamine dyhydrochloride and 1% sulfanilamide in 2.5% H_3PO_4) for 10 min at 25°C, and absorbance was measured by spectrophotometer at 540 nm. Standards were prepared with sodium nitrate and taken through the full assay procedure. The results were expressed as nmol of NO_x.

2.5. Drugs

The following drugs and reagents were used: [Tyr-$(SO_3H)^{27}$]carboxyl terminal octapeptide-(26–33) of cholecystokinin (Asp-Tyr[SO_3H]-Met-Gly-Trp-Met-Asp-Phe-NH_2; CCK-8S), rat gastrin-I (pGlu-Arg-Pro-Pro-Met-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2), somatostatin-14 (Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys), urethane ethyl carbamate, crystalline porcine pepsin, lyophilized bovine haemoglobin, N^G-nitro-L-arginine-methyl ester, L-arginine, D-arginine, capsaicin, aminophylline, terbutaline, lidocaine, NADPH, nitrate reductase (from *Aspergillus*), *N*-(1-naphthyl)ethylenediamine dihydrochloride, and sulfanilamide (Sigma, St. Louis, MO, USA); atropine sulfate (BDH Chemicals, Poole, England); cimetidine (Italfarmaco, Milan, Italy); α-fluoromethylhistidine (RBI, Natick, MA, USA); devazepide and L-365,260 [3*R*(+)-*N*-(2,3-dihydro-1-methyl2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3yl)-*N*′-(3-methyl-phenyl)urea] were kindly provided by Merck Research Laboratories, Rahway, NJ, USA; omeprazole was kindly provided by Malesci, Florence, Italy. Other reagents were of analytical grade. CCK-8S, gastrin-I, somatostatin-14, atropine, cimetidine, α-fluoromethylhistidine, N^G-nitro-L-arginine-methyl ester, L-arginine, D-arginine, and lidocaine were dissolved in saline immediately before use. Both devazepide and L-365,260 were dissolved in 1:1 dimethylsulphoxide (DMSO):Tween 80; the solution was sonicated and then diluted with saline to a final concentration of 8% DMSO and Tween 80. Omeprazole was initially dissolved in polyethyleneglycol (molecular weight = 400) and then diluted with 7 mM $NaHCO_3$ to a final concentration of 50% polyethyleneglycol (v/v). All drugs administered i.v. were injected in a volume of 0.25 ml/rat. Capsaicin was dissolved (12.5 mg/ml) in a vehicle composed of 10%

Table 1
Anaesthetized rats with perfused gastric lumen

Treatment	Pepsinogen output	Acid output
Saline	651.3 ± 63.7	27.5 ± 3.2
Vagotomy	628.6 ± 71.8	24.7 ± 4.1
Atropine	645.4 ± 82.3	21.8 ± 3.7
Cimetidine	674.5 ± 84.6	11.7 ± 1.8*
α-FMH	667.2 ± 89.5	14.3 ± 2.3*
Omeprazole	633.3 ± 75.9	2.9 ± 0.7*
Devazepide	624.1 ± 92.2	28.7 ± 4.5
L-365,260	661.5 ± 78.3	23.2 ± 3.6
L-NAME	612.7 ± 54.8	32.6 ± 5.1
L-arginine	683.2 ± 84.6	22.8 ± 5.4
D-arginine	649.7 ± 71.3	29.1 ± 4.2
Somatostatin-14	665.3 ± 74.7	19.5 ± 3.8

Effects of bilateral cervical vagotomy, atropine (1 μmol/kg i.v.), cimetidine (10 μmol/kg i.v.), α-fluoromethylhistidine (α-FMH, 450 μmol/kg i.p. twice daily for 2 days), omeprazole (90 μmol/kg i.v.), devazepide (2.5 μmol/kg i.v.), L-365,260 (5 μmol/kg i.v.), L-NAME (75 μmol/kg i.v.), L-arginine (2 mmol/kg i.v.), D-arginine (2 mmol/kg i.v.) or somatostatin-14 (15 nmol/kg/h i.v.) on basal pepsinogen (μg of pepsin/120 min) and acid (μeqH^+/120 min) secretory output.
Results are given as means ± S.E.M. ($n = 6$ for each value); *$P < 0.05$: significant difference from control values obtained with saline-treated animals.

ethanol, 10% Tween 80 and 80% saline solution (v/v/v). The total dose of capsaicin (125 mg/kg s.c.) was administered under ether anaesthesia in four injections over two consecutive days (first day: 25 mg/kg in the morning and 25 mg/kg in the late afternoon; second day: 25 mg/kg in the morning and 50 mg/kg in the late afternoon). In order to counteract the respiratory impairment associated with the administration of capsaicin, the rats received atropine (0.2 mg/kg i.p.), terbutaline (0.2 mg/kg i.p.) and aminophylline (20 mg/kg i.p.) 10 min before the first and third capsaicin injection.

2.6. Statistical analysis

The results are given as means ± S.E.M. The significance of differences was evaluated by Student's *t*-test or one-way analysis of variance (ANOVA) followed by post hoc analysis by Student–Newman–Keuls test, and *P* values lower than 0.05 were considered significant; '*n*' indicates the number of experiments.

3. Results

3.1. Evaluation of basal pepsinogen and acid secretions

In control animals with intact vagus nerves, basal gastric pepsinogen and acid secretions, assessed after 30-min stabilization, accounted for 82.4 ± 10.1 μg of pepsin/15 min and 3.7 ± 0.9 μeqH^+/15 min, respectively ($n = 6$), and these values did not vary up to the end of the experiments (120 min). In preliminary experiments, both basal pepsinogen and acid outputs were monitored in rats after either bilateral vagotomy ($n = 6$) or pretreatment with atropine, cimetidine, α-fluoromethylhistidine, omeprazole, devazepide, L-365,260, somatostatin-14, L-NAME, L-arginine or D-arginine ($n = 6$ for each drug). A significant inhibition of basal acid secretion, but not peptic output, was detected in rats treated with cimetidine, α-fluoromethylhistidine or omeprazole, whereas vagotomy, atropine, devazepide, L-365,260, somatostatin-14, L-NAME, L-arginine or D-arginine did not significantly affect basal peptic or acid secretory activity (Table 1).

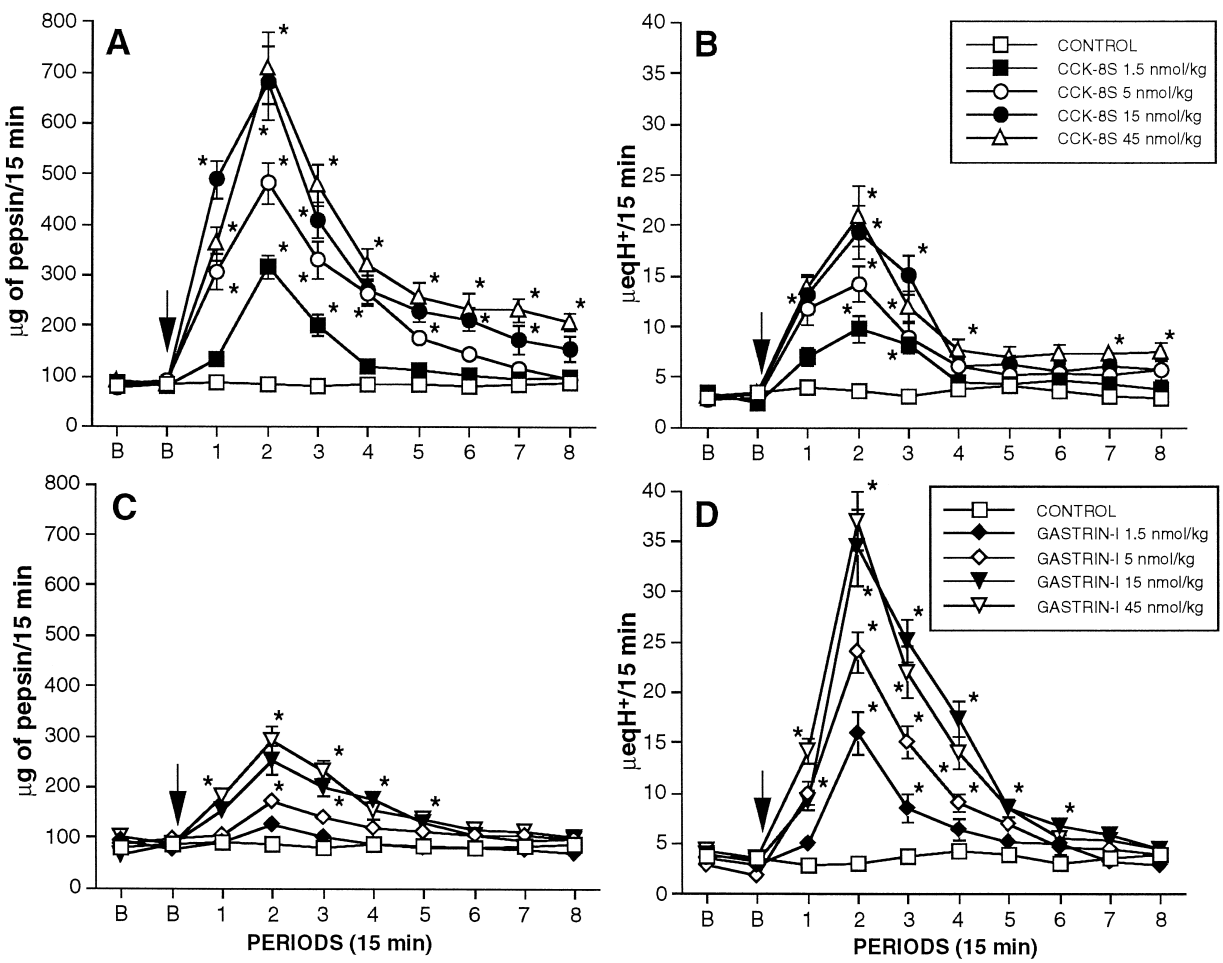

Fig. 1. Anaesthetized rats with perfused gastric lumen. Effects of CCK-8S (1.5, 5, 15 and 45 nmol/kg i.v.) or gastrin-I (1.5, 5, 15 and 45 nmol/kg i.v.) on basal pepsinogen [A, C] and acid secretion [B, D]. Each point represents the mean value obtained from 8–10 animals ± S.E.M. (vertical lines). The single arrow indicates the time of CCK-8S or gastrin-I administration. B = basal value. $^*P < 0.05$: significant difference from control values.

3.2. Effects of CCK-8S and gastrin-I

In animals with intact vagus nerves, CCK-8S (1.5, 5, 15 and 45 nmol/kg i.v.) caused a dose-dependent and parallel increase in both pepsinogen and acid secretions, with maximal effect at the dose of 15 nmol/kg (Fig. 1A,B). The excitatory responses to CCK-8S of both peptic and acid outputs, were mimicked with gastrin-I (1.5, 5, 15 and 45 nmol/kg i.v.) which induced a maximal increase in gastric secretions also at the dose of 15 nmol/kg (Fig. 1C,D). However the pepsigogue effect of CCK-8S (peak effect at 15 nmol/kg: 679.6 ± 74.3 μg of pepsin/15 min; $n = 10$) was significantly higher than that obtained in the presence of gastrin-I (peak value at 15 nmol/kg: 252.7 ± 27.1 μg of pepsin/15 min; $n = 10$; $P < 0.001$ versus CCK-8S-induced pepsinogen secretion), whereas the acid hypersecretion produced by CCK-8S (peak response at 15 nmol/kg: 19.4 ± 2.1 μeqH^+/15 min) was significantly lower than that evoked by gastrin-I (peak increase at 15 nmol/kg: 34.4 ± 3.8 μeqH^+/15 min; $P < 0.01$ versus acid secretion stimulated by CCK-8S).

3.3. Effects of CCK-8S or gastrin-I on animals pretreated with CCK receptor antagonists

In animals with intact vagus nerves, the increase in pepsinogen output following CCK-8S 15 nmol/kg was partly antagonized by i.v. injection of devazepide (1.25 and 2.5 μmol/kg; −75.3% at the highest dose tested) or L-365,260 (2.5 and 5 μmol/kg; −27.9% at the highest dose tested). However, the combined administration of devazepide 2.5 μmol/kg and L-365,260 5 μmol/kg completely prevented the pepsigogue effect evoked by CCK-8S (−98%; Fig. 2A). The acid hypersecretory effect of CCK-8S 15 nmol/kg was significantly enhanced by devazepide (+84.5% at the dose of 2.5 μmol/kg), whereas it was fully antagonized by L-365,260 (2.5 and 5 μmol/kg). The combined treatment of animals with devazepide plus L-365,260 also resulted in complete inhibition of CCK-8S-induced acid hypersecretion (Fig. 2B).

Under the same conditions, the stimulant actions of gastrin-I 15 nmol/kg on both peptic and acid secretions were not affected by i.v. injection of devazepide (1.25 and

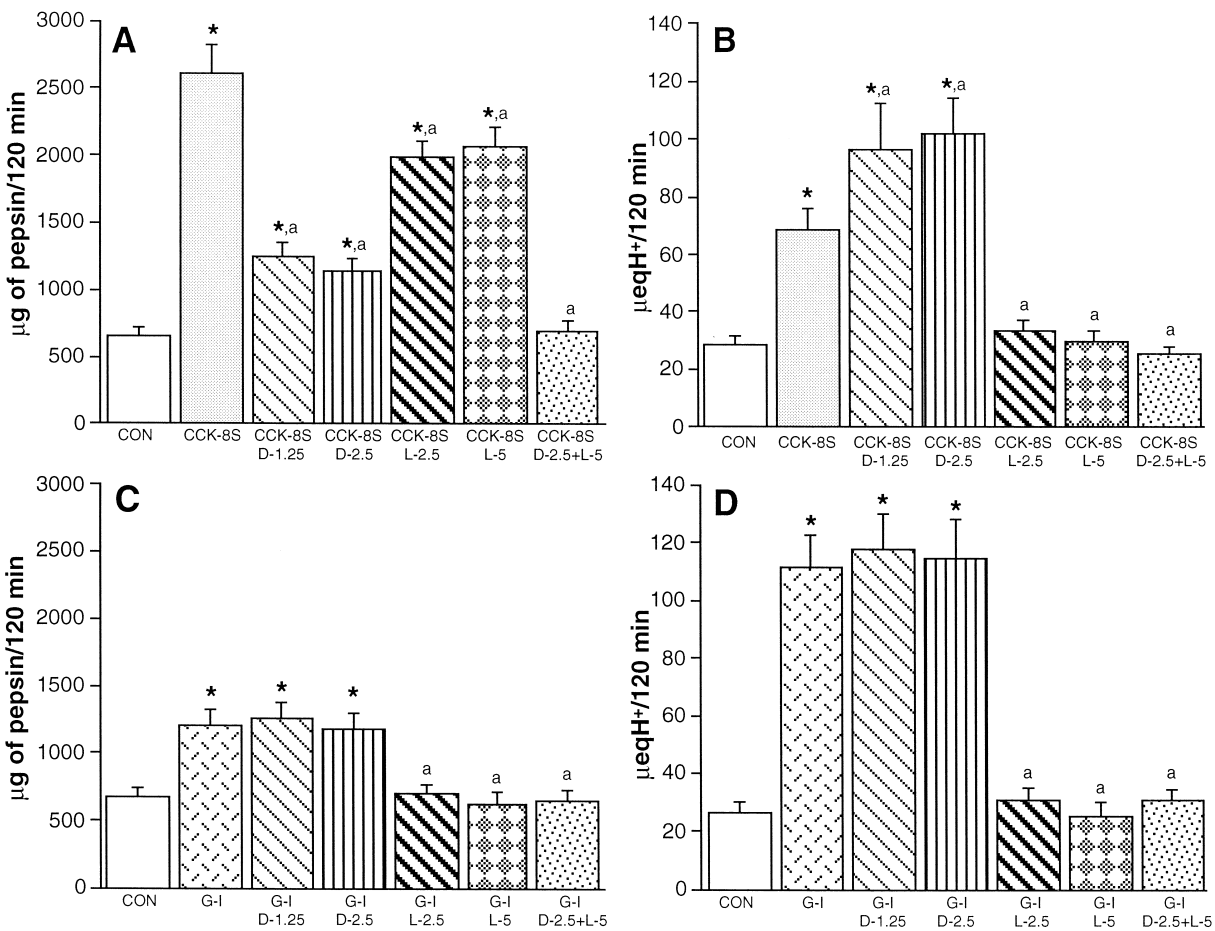

Fig. 2. Anaesthetized rats with perfused gastric lumen. Effects of CCK-8S 15 nmol/kg i.v. or gastrin-I 15 nmol/kg i.v. (G-I), administered either alone or in the presence of devazepide 1.25 μmol/kg i.v. (D-1.25), devazepide 2.5 μmol/kg i.v. (D-2.5), L-365,260 2.5 μmol/kg i.v. (L-2.5), L-365,260 5 μmol/kg i.v. (L-5) and devazepide 2.5 μmol/kg plus L-365,260 5 μmol/kg (D-2.5 + L-5) on basal pepsinogen [A, C] and acid secretion [B, D]. Each column represents the mean value obtained from 8–10 animals ± S.E.M. (vertical lines). *$P < 0.05$: significant difference from control values (CON); [a]$P < 0.05$: significant difference from either CCK-8S alone [A, B] or gastrin-I alone [C, D].

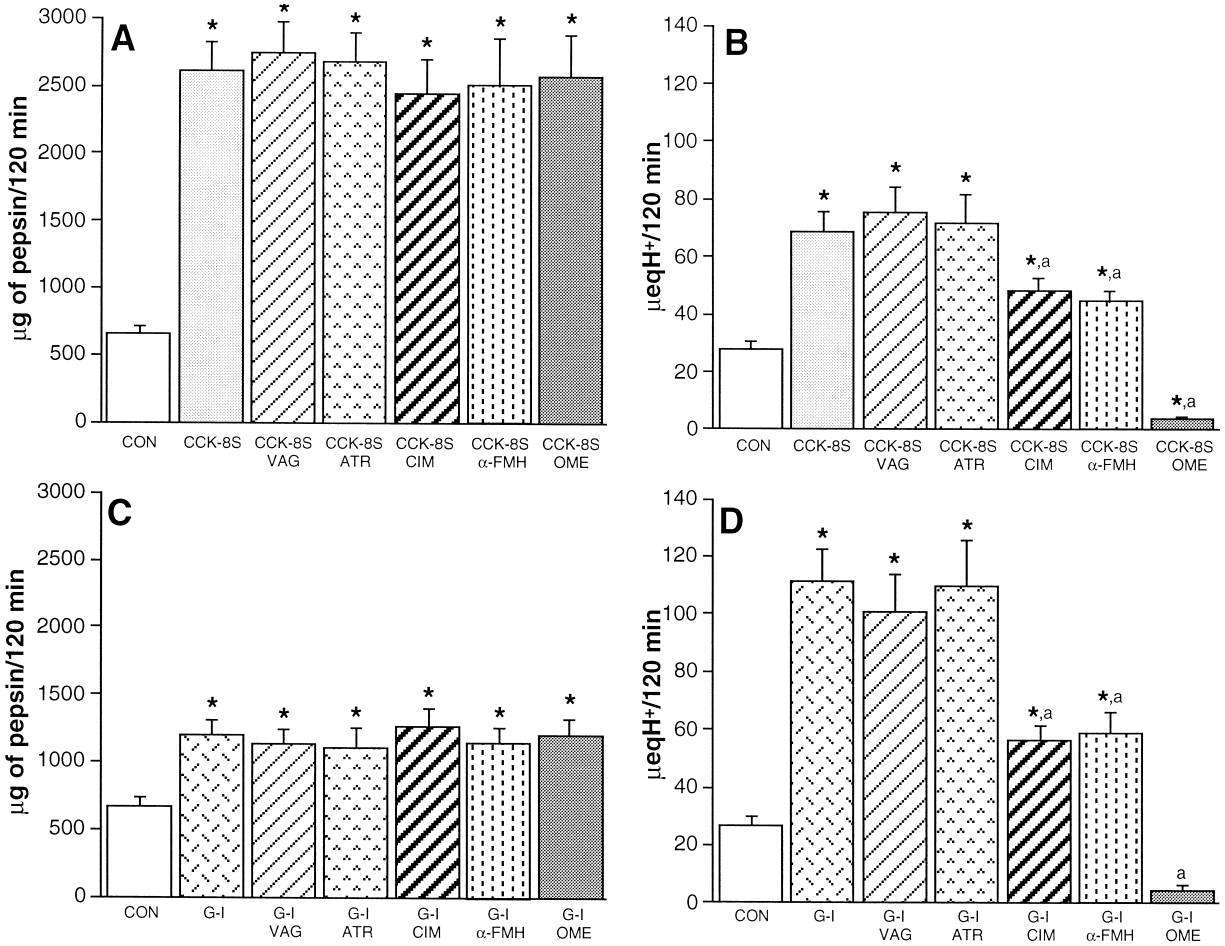

Fig. 3. Anaesthetized rats with perfused gastric lumen. Effects of CCK-8S 15 nmol/kg i.v. or gastrin-I 15 nmol/kg i.v. (G-I), administered either alone or after bilateral cervical vagotomy (VAG), atropine 1 μmol/kg i.v. (ATR), cimetidine 10 μmol/kg i.v. (CIM), α-fluoromethylhistidine 450 μmol/kg i.p. twice daily for 2 days (α-FMH), or omeprazole 90 μmol/kg i.v. (OME), on basal pepsinogen [A, C] and acid secretion [B, D]. Each column represents the mean value obtained from 8–10 animals ± S.E.M. (vertical lines). $^{*}P < 0.05$: significant difference from control values (CON); $^{a}P < 0.05$: significant difference from either CCK-8S alone [A, B] or gastrin-I alone [C, D].

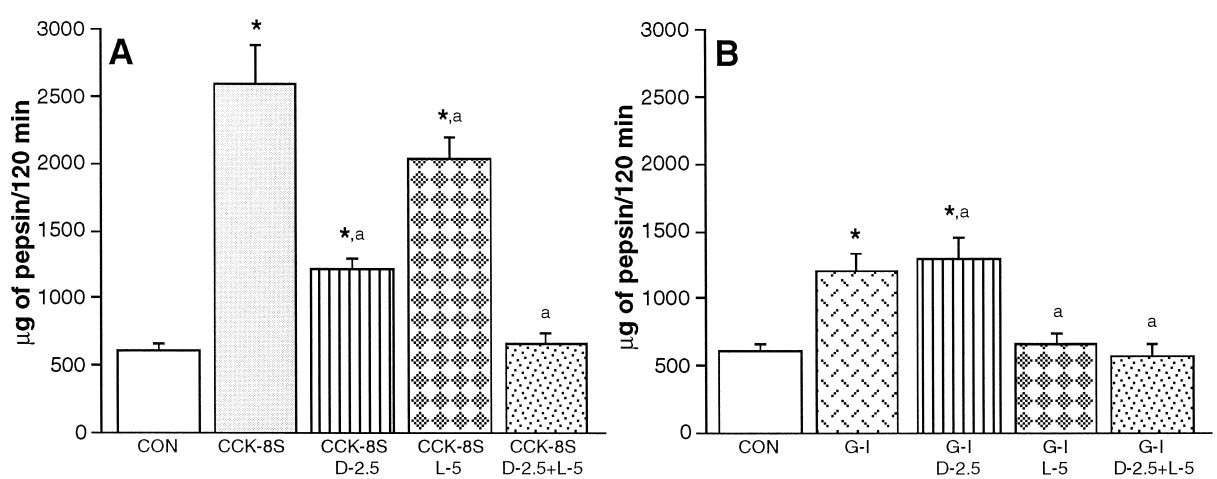

Fig. 4. Anaesthetized rats with perfused gastric lumen and pretreatment with omeprazole (90 μmol/kg i.v.). Effects of CCK-8S 15 nmol/kg i.v. [A] or gastrin-I 15 nmol/kg i.v. (G-I) [B], administered either alone or in the presence of devazepide 2.5 μmol/kg i.v. (D-2.5), L-365,260 5 μmol/kg i.v. (L-5) or devazepide 2.5 μmol/kg plus L-365,260 5 μmol/kg (D-2.5 + L-5), on basal pepsinogen secretion. Each column represents the mean value obtained from 8–10 animals ± S.E.M. (vertical lines). $^{*}P < 0.05$: significant difference from control values (CON); $^{a}P < 0.05$: significant difference from either CCK-8S alone [A] or gastrin-I alone [B].

2.5 μmol/kg), whereas they were prevented by L-365,260 (2.5 and 5 μmol/kg). In animals pretreated with devazepide plus L-365,260, gastrin-I failed to stimulate either peptic or acid outputs (Fig. 2C,D).

3.4. Effects of CCK-8S or gastrin-I on animals subjected to vagotomy, blockade of histidine decarboxylase, pretreatment with antisecretory drugs, or somatostatin infusion

The effects elicited by 15 nmol/kg of CCK-8S or gastrin-I were not significantly modified by bilateral cervical vagotomy or pretreatment with atropine (1 μmol/kg i.v.). Cimetidine (10 μmol/kg i.v.) partly prevented the acid stimulant effects of CCK-8S or gastrin-I (−48.8% and −64.6%, respectively), without affecting their pepsigogue actions. In animals pretreated with α-fluoromethylhistidine (450 μmol/kg i.p. twice daily for 2 days), the effects of both peptides were similar to those obtained in the presence of cimetidine (Fig. 3).

When tested on animals with the gastric proton pump blocked by omeprazole (90 μmol/kg i.v.), CCK-8S or gastrin-I failed to stimulate acid secretion, whereas they still increased pepsinogen output (Fig. 3). Additional experiments on omeprazole-pretreated animals further confirmed that full blockade of CCK-8S-induced pepsinogen secretion required combined administration of devazepide (2.5 μmol/kg i.v.) plus L-365,260 (5 μmol/kg i.v.), while the pepsigogue action of gastrin-I was insensitive to devazepide but could be prevented by L-365,260 (Fig. 4).

Infusion with somatostatin-14 (15 nmol/kg/h i.v. for 120 min) did not significantly modify the pepsigogue effect of CCK-8S 15 nmol/kg i.v. (CCK-8S alone: 2617.3 ± 209.7 μg of pepsin/120 min; CCK-8S plus somato-

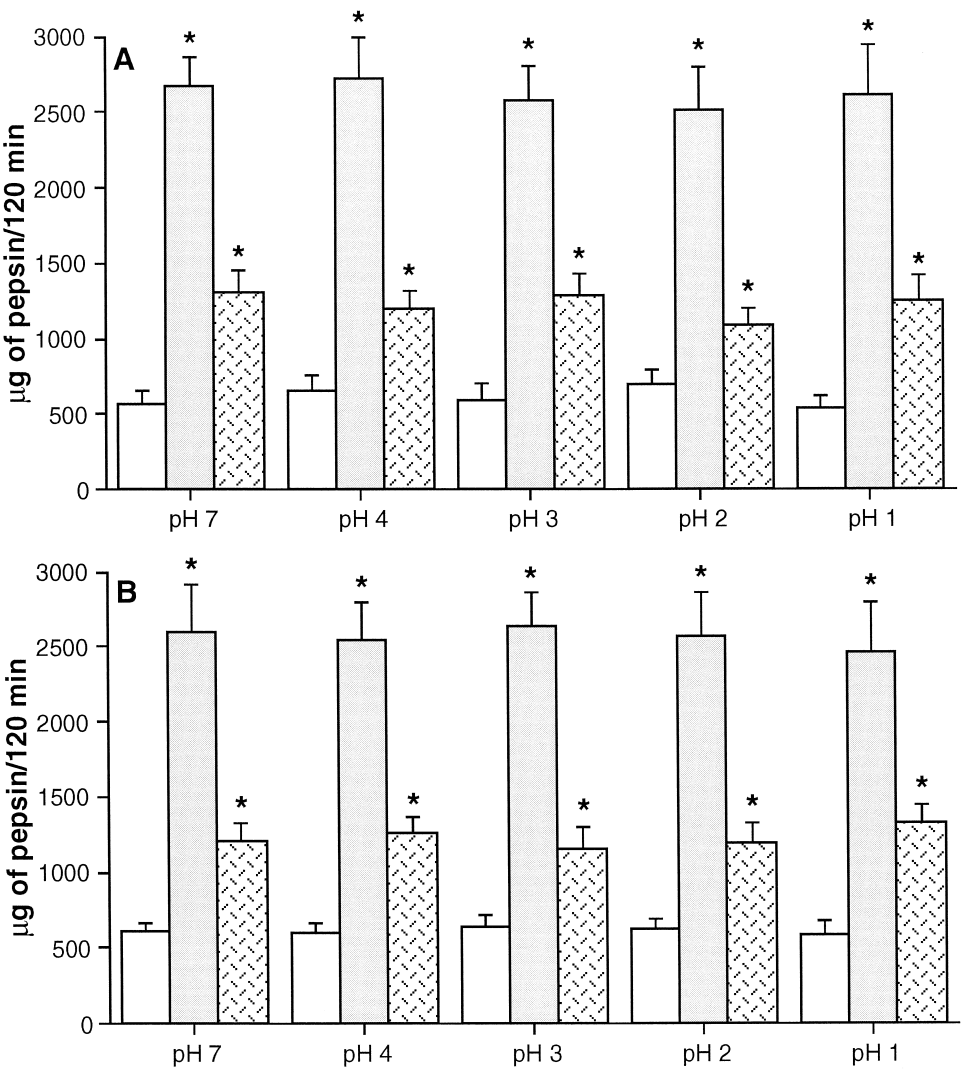

Fig. 5. Anaesthetized rats with perfused gastric lumen. Effects of CCK-8S 15 nmol/kg i.v. ( ) or gastrin-I 15 nmol/kg i.v . ( ) on pepsinogen secretion in the presence of gastric intraluminal perfusion with solutions at pH 7, 4, 3, 2 or 1. Experiments were performed either in the absence [A] or in the presence [B] of pretreatment with omeprazole (90 μmol/kg i.v.). Each column represents the mean value obtained from 6–8 animals ± S.E.M. (vertical lines). $^{*}P < 0.05$: significant difference from control values ( ).

statin-14: 2503.8 ± 311.3 μg of pepsin/120 min; $n = 6$ for each group).

3.5. Effects of CCK-8S or gastrin-I on animals during perfusion of gastric lumen with acid solutions

The effect on pepsinogen secretion of the continuous perfusion of the gastric lumen with solutions at different pH values was studied either in the absence or in the presence of pretreatment with omeprazole. In neither case did intragastric application of solutions at pH 4, 3, 2 or 1 modify the basal peptic secretory activity detected at pH 7 (Fig. 5). In addition, when gastric perfusion with one of these solutions was combined with i.v. administration of CCK-8S or gastrin-I at 15 nmol/kg, the pepsinogen output increased in a pH-independent manner, and in no case were secretory values significantly different from those obtained at pH 7 (Fig. 5).

3.6. Effects of CCK-8S or gastrin-I on animals pretreated with capsaicin, lidocaine or L-NAME

Functional ablation of capsaicin-sensitive sensory neurons by systemic pretreatment with capsaicin failed to modify the excitatory effects of CCK-8S or gastrin-I (both at the dose of 15 nmol/kg) on pepsinogen and acid secretions (Fig. 6). Similar results were obtained when CCK-8S or gastrin-I was tested on animals subjected to topical intragastric application of lidocaine in order to induce surface anaesthesia of the gastric mucosa (Fig. 6A,B).

Blockade of the NO synthase pathway by systemic administration of L-NAME (75 μmol/kg i.v.) prevented the stimulant actions of CCK-8S or gastrin-I on pepsinogen secretion (−61.8% and −71.7%, respectively), without affecting the concomitant increase of acid output (Fig. 6C,D). Pretreatment with L-arginine (2 mmol/kg i.v.), but

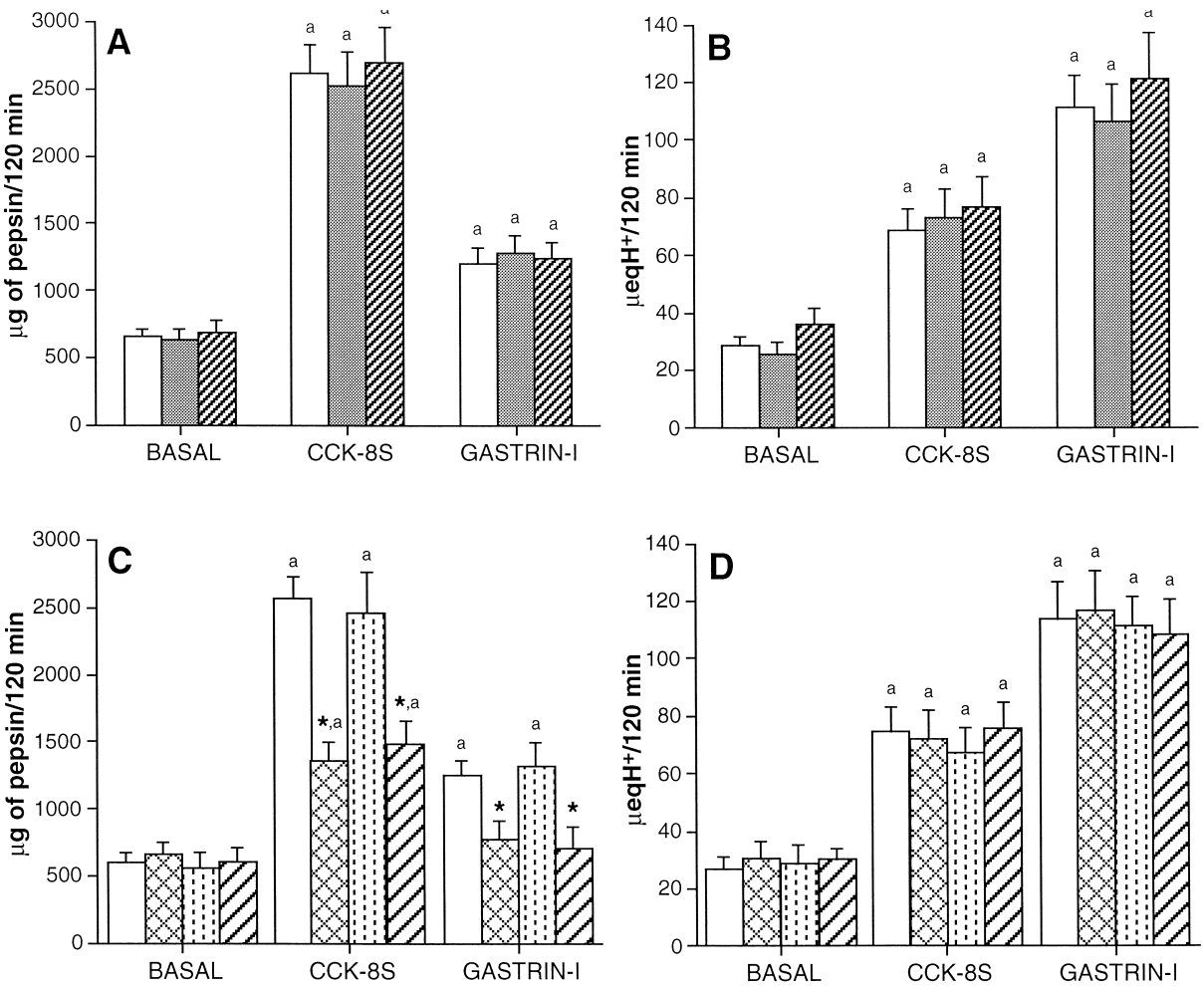

Fig. 6. Anaesthetized rats with perfused gastric lumen. Effects of systemic capsaicinization ( ), intragastric applicatio n of lidocaine ( ), pretreatment with L-NAME 75 μmol/kg i.v.( ), L-NAME 75 μmol/kg i.v. plus L-arginine 2 mmol/kg i.v. ( ), and L-NAME 75 μmol/kg i.v. plus D-arginine 2 mmol/kg i.v. ( ) on pepsinogen [A, C] and acid secretion [B, D] under both basal conditions and treatment with CCK-8S 15 nmol/kg i.v. or gastrin-I 15 nmol/kg i.v. Each column represents the mean value obtained from 6–8 animals ± S.E.M. (vertical lines). $^{*}P < 0.05$: significant difference from control values ( ); $^{a}P < 0.05$: significant difference from basal values.

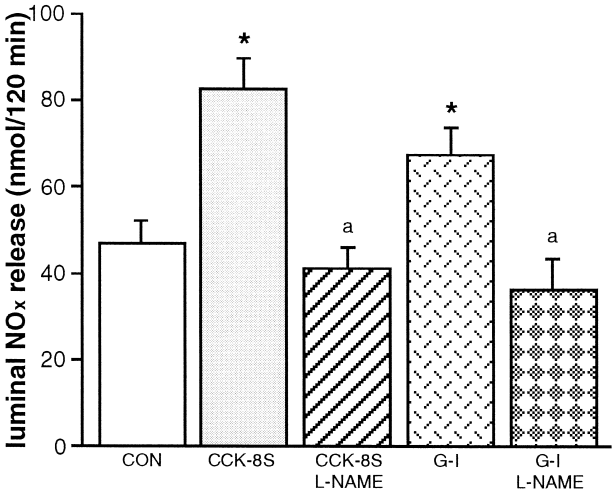

Fig. 7. Anaesthetized rats. Effects of CCK-8S 15 nmol/kg i.v. or gastrin-I 15 nmol/kg i.v. (G-I), administered either alone or in the presence of L-NAME 75 μmol/kg i.v., on gastric luminal release of NO_x. Each column represents the mean value obtained from 5–6 animals ± S.E.M. (vertical lines). $^{*}P < 0.05$: significant difference from control values (CON); $^{a}P < 0.05$: significant difference from either CCK-8S alone or gastrin-I alone.

not D-arginine (2 mmol/kg i.v.), completely restored the pepsigogue effects of both CCK-8S and gastrin-I (Fig. 6C,D).

3.7. Effects of CCK-8S or gastrin-I on gastric luminal NO_x release

In control animals, the basal gastric NO_x output accounted for 12.3 ± 1.7 nmol/30 min ($n = 6$), and this value did not vary significantly throughout the experiments. Under these conditions, CCK-8S or gastrin-I, both at the dose of 15 nmol/kg i.v., significantly increased the release of NO breakdown products into the gastric lumen. The stimulant actions of CCK-8S and gastrin-I on gastric NO_x output were no longer observed when these peptides were administered to animals pretreated with L-NAME (75 μmol/kg i.v.) (Fig. 7).

4. Discussion

Peptides of the CCK/gastrin family regulate digestive secretory and motor functions either by direct actions on effector cells or indirectly through an involvement of neural pathways and multicellular effector mechanisms (Walsh, 1994; Heinemann et al., 1996). In addition, acid hypersecretory responses induced by gastric stimulants seem to play a pivotal role in driving concomitant increases of pepsinogen secretion (Kleveland et al., 1986; Smith and Torres, 1990). In the present study, use of omeprazole (Fellenius et al., 1981) made it possible to dissociate the gastric acid from peptic secretory functions in a complex integrated model. Under these conditions, both CCK_1 and CCK_2 receptor subtypes seem to contribute to the increase in pepsinogen secretion evoked by CCK-8S or gastrin-I, and such stimulant actions are independent of gastric acidity.

The effects of both CCK and gastrin on acid secretion have been extensively investigated and much is known about the mechanisms through which CCK-like peptides regulate this function (Lloyd and Debas, 1994; Soll and Berglindh, 1994). Nevertheless, although the present study dealt mainly with the control of pepsinogen secretion by CCK-8S and gastrin-I, care was taken to monitor gastric acid output also, for two reasons: (1) to verify the suitability of the present experimental model; and (2) to interpret the patterns of pepsinogen secretion in the presence of concomitant variations of acid secretory activity. Under basal conditions, the acid output was partly reduced by cimetidine or α-fluoromethylhistidine, whereas it remained unchanged in the presence of bilateral cervical vagotomy or atropine. These findings are consistent with results of previous studies indicating that urethane anaesthesia depresses vagal cholinergic outflow to the stomach, whereas a basal release of histamine from gastric mucosa still occurs even in whole isolated stomachs (Maggi and Meli, 1986; Sandvik and Waldum, 1991). In addition, the lack of effects of CCK receptor antagonists, devazepide (CCK_1) or L-365,260 (CCK_2) is in line with previous data showing that, in urethane-anaesthetized rats, basal acid secretion is not subjected to tonic control mediated by CCK_1 or CCK_2 receptors, and that, in isolated stomach preparations, these receptor subtypes are not tonically implicated in the regulation of basal histamine release (Hayward et al., 1991; Sandvik and Waldum, 1991; Heinemann et al., 1995). The present results concerning the excitatory effects of both gastrin-I and CCK-8S on basal acid secretion were also not unexpected. Indeed, the fact that the marked stimulant effect of gastrin-I was totally blocked by L-365,260 agrees with general theory that gastrin stimulates acid secretion both indirectly, via histamine release, and directly, by interacting with CCK_2 receptors located on enterochromaffin-like and parietal cells, respectively (Soll and Berglindh, 1994; Hills et al., 1996). On the other hand, CCK-8S acted with lower efficacy than, but with similar potency to, gastrin-I to stimulate gastric acid output, and it was able to induce a full excitatory action only when administered in the presence of the CCK_1 receptor blocker, devazepide. These observations are consistent with previous observations indicating that, although CCK-8S shares with gastrin-I a similar affinity for CCK_2 receptors (Kopin et al., 1992; Patel and Spraggs, 1992), which are responsible for the increase of acid output, it also acts on CCK_1 receptors located on gastric D cells, thus inhibiting the acid secretory activity of parietal cells via somatostatin release (Soll et al., 1985; Lloyd et al., 1992). In support of this view, CCK-8S was proven to be a poor stimulant of acid

secretion in various mammalian species, including the human, but its secretory ability increased significantly after pretreatment with selective CCK_1 receptor antagonists (Burckhardt et al., 1994; Konturek et al., 1995).

In the present study, the basal secretion of pepsinogen was not affected by bilateral vagotomy, atropine, cimetidine, α-fluoromethylhistidine, devazepide or L-365,260, confirming the view that, under basal conditions, there is a continuous, low-rate peptic secretory activity which is constitutive in nature and is not subject to tonic regulation by either excitatory or inhibitory pathways (Hersey, 1994). In addition, despite total inhibition of acid output, the basal pepsinogen secretion continued unchanged after omeprazole. This latter observation agrees with data obtained from fundic mucosal sheets (Basson et al., 1988), and suggests that, in the present model, the basal peptic secretory rate is independent of acid secretion.

When the effects of CCK-8S and gastrin-I on pepsinogen secretion were tested, patterns of stimulant actions opposite to those observed for the acid hypersecretory effects were obtained. CCK-8S induced a marked increase in peptic output, whereas gastrin-I, although displaying a potency similar to that of CCK-8S, caused a moderate increase of basal peptic output. The results obtained with devazepide and L-365,260 suggest that an interaction of CCK-8S and gastrin-I with distinct receptor populations might account for the 'partial agonist' profile exhibited by gastrin in this study. Indeed, the pepsigogue action of gastrin-I was sensitive to L-365,260, but not to devazepide, whereas the stimulant activity of CCK-8S was partly antagonized by devazepide or L-365,260, and complete suppression of its effect could be achieved only after treatment with both devazepide and L-365,260. These results indicate that peptic stimulation by CCK-8S depends mainly on activation of CCK_1 receptors, and that CCK_2 receptors also play a role in this effect. Since gastrin-I displays a low affinity for CCK_1 receptors (Woodruff and Hughes, 1991; Bueno and Junien, 1994), the moderate pepsigogue action of this peptide can be ascribed to its interaction with only CCK_2 receptor subtypes.

The nature of cellular receptors through which peptides of the CCK/gastrin family regulate pepsinogen secretion is still being discussed (Hersey, 1994). Based on agonist selectivity for stimulation of peptic output, Hersey et al. (1983) proposed that the chief cell is provided with CCK_1 receptors similar to those found in the pancreas, and that gastrin acts as a partial agonist on these receptor sites (Tang et al., 1993). In contrast, other investigators suggested that gastric chief cells possess distinct receptors for CCK and gastrin: C receptors, able to bind CCK-8S, and G receptors, interacting with gastrin-I (Cherner et al., 1988). Subsequently, both binding and functional studies provided evidence that chief cells are equipped with typical CCK_1 and CCK_2 receptors, accounting for about 80–90% and 10–20%, respectively, of the maximal response to CCK-8S (Lin et al., 1992; Qian et al., 1993; Pradhan et al., 1995). In support of this view, both molecular biology and immunohistochemistry studies indicated that CCK_1 and CCK_2 receptor subtypes are expressed on gastric chief cells (Reuben et al., 1994; Tarasova et al., 1996). Our results are consistent with these observations and suggest that, under in vivo conditions, parenterally administered CCK-like peptides stimulate pepsinogen secretion by activating CCK_1 and/or CCK_2 receptors.

An important point addressed in this study was to establish whether some endogenous systems commonly implicated in the regulation of gastric secretion, such as acetylcholine, histamine or somatostatin, could interfere with the pepsigogue effects induced by CCK-8S or gastrin-I. Under the present conditions, the peptic secretory effects of these peptides were not affected by bilateral cervical vagotomy or pretreatment with atropine, indicating a lack of involvement of endogenous cholinergic pathways. A similar conclusion seems to apply to endogenous histamine since: (a) pretreatment with α-fluoromethylhistidine, at doses that greatly decreased histidine decarboxylase activity and histamine concentration in digestive tissues (Andersson et al., 1992; Fujimoto et al., 1995), or with cimetidine did not interfere with CCK-8S- or gastrin-I-induced peptic hypersecretion; and (b) exogenous histamine (up to 15 μmol/kg i.v.) stimulated basal pepsinogen secretion in a cimetidine-sensitive manner (Blandizzi, unpublished results).

As far as somatostatin is concerned, the infusion of somatostatin-14 did not modify either basal or CCK-8S-induced pepsinogen secretion. This finding is consistent with results of previous investigations showing that: (a) somatostatin-14 did not influence (or it even stimulated, at the highest doses tested) pepsinogen secretion under basal conditions or in the presence of pentagastrin in rats (Seefried et al., 1988); and (b) the activation of somatostatin receptors located on isolated chief cells did not interfere with basal or CCK-8S-stimulated pepsinogen secretion (Felley et al., 1994). Thus, according to the above data, it appears that somatostatin is not significantly implicated in the pepsigogue effects of CCK-like peptides.

Changes in hydrogen ion concentrations, occurring either near chief cells or in the gastric lumen, appear to increase peptic secretory activity (Johnson, 1972; Smith and Torres, 1990). Therefore, the present study investigated whether the acid stimulant properties of CCK-8S and gastrin-I might be responsible for their pepsigogue actions. However, some evidence indicates that this is not the case in the present experimental model: (1) the acid secretory patterns of CCK-8S and gastrin-I differed consistently from the pepsigogic ones; indeed, as already pointed out above, CCK-8S evoked a marked increase of peptic output which was associated to a moderate acid response, whereas a poor increase in pepsigogue activity corresponded with a marked acid hypersecretion induced by gastrin-I; (2) no differences were detected in the peptic responses of chief cells when the effects of CCK-8S or gastrin-I were tested

either in the absence or in the presence of pretreatment with omeprazole; (3) in the absence or in the presence of omeprazole-induced inhibition of endogenous acid production, exposure of gastric mucosal surface to solutions with pH ranging from 4 to 1 was associated with no pH-dependent changes in peptic secretory activity either under basal conditions or upon administration of CCK-8S or gastrin-I. Overall, hydrogen ions do not seem necessary to promote secretion of pepsinogen either basally or when CCK receptors are activated by peptides of the CCK/gastrin family. It appears instead that parietal cells and chief cells can be stimulated simultaneously by these agents only by virtue of their sharing similar receptors. In line with these conclusions, previous studies indicated a lack of direct excitatory influence by gastric acidity on the secretory functions of chief cells (Kleveland et al., 1986; Blandizzi et al., 1997).

There is strong evidence to support the view that some digestive actions of CCK, including regulation of gastric emptying (Raybould and Taché, 1988), gastric mucosal protection (Evangelista and Maggi, 1991), post-prandial hyperemia (Heinemann et al., 1996), and pancreatic secretion (Li et al., 1997) are mediated by vagal afferent capsaicin-sensitive fibers. Accordingly, it has been demonstrated that both CCK_1 and CCK_2 receptors, expressed on cell soma of afferent neurons in the nodose ganglion, are transported toward peripheral nerve endings and are lost after treatment with capsaicin (Moriarty et al., 1997). Therefore, these receptors may serve as target sites for mediating several effects induced by CCK-like peptides. It is also noteworthy that capsaicin-sensitive afferent fibers are significantly implicated in the regulation of gastric acid secretion (Raybould et al., 1991). In particular, acute intragastric application of capsaicin decreases acid secretion in rats (Lippe et al., 1989), and the ablation of capsaicin-sensitive fibers inhibits the acid hypersecretion evoked by gastric distension (Raybould and Taché, 1989). Nevertheless, the present data, which show that CCK-8S or gastrin-I were still able to induce concomitant increases of peptic and acid outputs after bilateral cervical vagotomy or pretreatment with capsaicin, indicate that systemic capsaicinization did not influence the functional status of chief and parietal cells, and suggest that capsaicin-sensitive sensory nerves are not implicated in the mechanisms underlying the gastric secretory effects of CCK-8S and gastrin-I. In line with this view, previous studies provided consistent evidence that neither vagal nor systemic capsaicinization affected the acid hypersecretory response induced by pentagastrin in urethane-anaesthetized rats (Raybould and Taché, 1989; Livingston and Holzer, 1993). It must also be noted that pretreatment with lidocaine did not impair the ability of parietal or chief cells to respond to CCK-8S or gastrin-I, suggesting that local afferent fibers do not drive the gastric secretory input activated by these peptides.

Constitutive NO synthase is expressed in rat gastric surface epithelial cells (Kugler and Drenckhahn, 1994), and maximal NO synthase activity occurs in gastric cell fractions enriched in mucous and chief cells (Brown et al., 1992). Furthermore, NO generation appears to play a crucial role in both mucosal hyperemia and gastroprotection afforded by CCK-like peptides (Stroff et al., 1994; Heinemann et al., 1996). Accordingly, we studied whether NO might be involved in the gastric hypersecretory effects of CCK-8S and gastrin-I. It was thus observed that the blockade of NO synthase by L-NAME significantly counteracted the peptic, but not the acid, stimulant actions of both peptides, the inhibitory effects of L-NAME being specifically overcome by administration of L-arginine. In addition, both the CCK-8S- and gastrin-I-stimulated gastric luminal output of NO_x, taken as an index of gastric mucosal NO synthesis, were prevented by L-NAME. These findings suggest that CCK-like peptides stimulate pepsinogen secretion in rats partly through an increase of NO synthase activity. In support of this view, it was previously shown that application of CCK-8S or gastrin to isolated chief cells stimulates a Ca^{2+}-calmodulin-dependent constitutive NO synthase, leading to NO generation and activation of soluble guanylate cyclase, and that pepsinogen secretion, induced by Ca^{2+}-releasing agonists, is prevented by NO synthase blockade (Fiorucci et al., 1995).

In conclusion, the present results suggest that, under in vivo conditions, both CCK_1 and CCK_2 receptor subtypes contribute to the peptic secretory responses elicited by peptides of the CCK/gastrin family. According to our findings, it appears that the excitatory inputs of CCK-8S and gastrin-I to chief cells are not driven through acid-dependent mechanisms or capsaicin-sensitive vagal afferent fibers. It is proposed instead that the stimulant actions of CCK-like peptides on pepsinogen secretion are mediated, at least in part, by an increase of gastric NO generation.

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