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Regulation of Gastrin, Somatostatin and Bombesin Release from the Isolated Rat Stomach by Exogenous and Endogenous Gamma-Aminobutyric Acid

Key Words
γ-Aminobutyric acid
Gastrin
Somatostatin
Bombesin-related peptides
G cells

Abstract

Background/Aims and Methods: γ-Aminobutyric acid (GABA) is localized in epithelial cells and intrinsic nerve fibers of the gastric mucosa raising the possibility of a regulatory role for this transmitter. Therefore, it was the aim of the present study to examine the effect of exogenous and endogenous GABA on the neuroendocrine functions of the isolated perfused rat stomach. **Results:** Infusion of GABA (10^{-8}, 10^{-6}, 10^{-4} *M*) caused a significant increase in gastrin release by 187 ± 98, 328 ± 43 and 493 ± 84 pg/20 min and a significant decrease in somatostatin secretion by –540 ± 203, –867 ± 96 and –893 ± 195 pg/20 min, respectively. Release of bombesin-like immunoreactivity (BLI) remained unchanged during infusion of GABA at the concentrations employed. The gastrin and somatostatin responses to 10^{-4} *M* GABA were completely inhibited by the $GABA_A$ antagonist bicuculline (10^{-5} *M*) and the cholinergic blocker atropine (10^{-7} *M*), whereas the $GABA_B$ antagonist CGP 35348 (5×10^{-5} *M*) was ineffective. To evaluate the contribution of endogenous GABA in the vagal regulation of gastric neuroendocrine functions, gastrin, somatostatin and BLI responses to electrical stimulation of the vagal nerves were examined in the presence of bicuculline. Vagal stimulation (10 V, 10 Hz, 1 ms) induced a significant inhibition of somatostatin release by –518 ± 78 pg/10 min, which was attenuated to –259 ± 143 pg/10 min ($p < 0.05$) in the presence of bicuculline. Atropine (10^{-7} *M*) turned vagally induced inhibition of somatostatin release into a stimulation by 928 ± 266 pg/10 min which was not altered by additionally infused bicuculline. Vagally stimulated gastrin release was reduced from 397 ± 47 to 217 ± 72 pg/10 min ($p < 0.05$) by bicuculline, while atropine had no effect. Vagally induced BLI release was not altered by bicuculline and atropine. Since the effect of bicuculline on vagally induced gastrin release was independent of cholinergic mechanisms, a potential direct effect of GABA on gastrin release was examined in isolated rabbit antral G cells. In this preparation carbachol (10^{-4} *M*) and neuromedin C (10^{-9} *M*) significantly stimulated gastrin release from 2.6 ± 0.4 to 4.9 ± 0.3 and 8.5 ± 0.9% of the total cellular content, respectively, while GABA (10^{-10}–10^{-3} *M*) changed neither basal nor carbachol- and neuromedin C-stimulated gastrin release. **Conclusion:** The present data confirm that exogenous GABA stimulates gastrin release and inhibits somatostatin release from the isolated rat stomach via $GABA_A$ receptors by activating cholinergic neurotransmission. Furthermore, it was shown for the first time that endogenous GABA contributes to the vagal regulation of gastrin and somatostatin release from the rat stomach. Inhibition of somatostatin secretion by endogenous GABA is mediated by cholinergic mechanisms, whereas stimulation of gastrin release is mediated by pathways unrelated to the cholinergic system and bombesin peptides.

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Introduction

γ-Aminobutyric acid (GABA) has been recognized as a putative neurotransmitter and neuromodulator in the mammalian gastrointestinal tract. Within the enteric nervous system GABA has initially been localized in neurons of the myenteric plexus and in nerve fibers projecting from the myenteric ganglia to the circular muscle layer [1–6]. Therefore experimental studies focussed on the regulatory effects of GABA on intestinal motility. GABA stimulates gut muscular contraction by activating acetylcholine release from neighboring cholinergic neurons [7–9].

In addition, there are several lines of evidence supporting a functional role of GABA in the regulation of gastric endocrine and exocrine functions. Recently GABA was localized to nerve fibers within the gastric mucosal and submucosal layers and to epithelial cells of the gastric mucosa, in particular of the antral mucosa [1, 10–13]. The GABA-immunopositive epithelial cells cannot be seen in the surface epithelium but are confined to the middle and lower parts of the gastric glands and show secretory granules like endocrine cells [11–13]. Furthermore, GABA seems to be synthesized in mucosal and submucosal nerves and epithelial cells of the gastric glands as reflected by the presence of glutamic acid decarboxylase (GAD), the enzyme of GABA synthesis [14, 15]. Furthermore GABA can be released from antral mucosal and submucosal tissue slides by a depolarizing stimulus [10, 16]. Finally, specific $GABA_A$ receptors are localized on epithelial cells of the gastric body and antrum [17].

Pharmacological studies demonstrate that occupation of the $GABA_A$ receptor by exogenous GABA or $GABA_A$ agonists stimulates gastrin release and inhibits somatostatin secretion from isolated antral mucosal fragments [18, 19]. Additionally in the isolated stomach of rats, somatostatin release can be reduced via $GABA_A$ mechanisms, whereas gastrin secretion does not change or increases during infusion of $GABA_A$ agonists [20–22]. Furthermore, activation of $GABA_A$ receptors stimulates acid secretion from the isolated guinea-pig stomach [23]. Modulation of gastric exocrine and endocrine functions by exogenous GABA is mainly mediated by stimulation of cholinergic neurons [10, 18, 20, 22, 23].

All these findings support the concept of endogenous GABA being a neurocrine or paracrine modulator in the stomach, however, its functional importance has not yet been evaluated. Therefore, it was the aim of the present study to examine the effect of endogenously activated GABA on vagally induced release of gastrin, somatostatin and bombesin-related peptides from the stomach. The experiments were performed in vitro in the isolated vascularly perfused rat stomach, an extrinsically denervated preparation, in which the integrity of intramural neurons and paracrine pathways is preserved. Furthermore, enriched antral G cells in primary culture were used to study more direct GABA effects on gastrin release in a system in which neural mechanisms and cell-to-cell interactions are excluded or largely reduced.

Materials and Methods

Isolated Rat Stomach

Preparation of the Stomach

After overnight fasting, stomachs were isolated from rats (male Wistar rats; body weight 250–300 g; Charles River Wiga GmbH, Sulzfeld, Germany) anesthetized with pentobarbital sodium (60 mg/kg i.p.) employing a modification [24] of the detailed description by McIntosh et al. [25]. Isolated stomachs were vascularly perfused through the celiac artery in a single-pass perfusion system at a rate of 1.5 ml/min with a modified Krebs-Ringer buffer solution. The perfusion medium contained 4% dextran T-70, 0.2% bovine serum albumin and 5.5 m*M* glucose and was gassed with 95% O_2 and 5% CO_2. The gastric venous effluent was collected via a catheter in the portal vein at 2-min intervals and frozen immediately for subsequent radioimmunological determination of gastrin, somatostatin and bombesin-related peptides. Additional catheters were placed in the stomach via the esophagus with the tip at the cardia and at the ligated pylorus to drain gastric contents. After insertion of the gastric catheters the lumen of the stomach was gently rinsed with isotonic saline until clear. Thereafter the gastric lumen was continuously perfused with saline p (1.5 ml/min) to maintain constant intragastric pH.

Experimental Design

After an equilibration period of 25 min and a basal period of 10 min GABA was added to the vascular perfusate for 20 min at concentrations of 10^{-10}, 10^{-8}, 10^{-6} and 10^{-4} *M* followed by another 15-min perfusion period without GABA. In further experiments the effect of 10^{-4} *M* GABA was examined in the presence of the $GABA_A$-receptor antagonist bicuculline (10^{-5} *M*), the $GABA_B$-receptor antagonist CGP 35348 (5×10^{-5} *M*) [26] and the cholinergic blocker atropine (10^{-7} *M*). Infusion of the respective antagonists started with the equilibration period and continued during the entire experiment. In control experiments the effect of bicuculline, CGP 35348 and atropine was examined on basal neuroendocrine functions. Therefore saline or the respective antagonists were added to the vascular perfusate after the basal period for a 20-min perfusion interval. In another experimental group the effect of bicuculline was examined on carbachol (10^{-4} *M*)-induced changes in gastrin, somatostatin and bombesin-like immunoreactivity (BLI) release.

In a second series of experiments electrical stimulation of the vagal nerves was performed. For that purpose the vagus nerves were dissected free of tissue, and 2 bipolar platinum electrodes were placed on the distal end of each vagal trunk. After the basal period both vagal nerves were stimulated with monophasic square wave pulses (10 V, 10 Hz, 1 ms) for a stimulation period of 10 min using an

electronic stimulator (Grass S11; Grass Medical Instruments, Quincy, Mass., USA). Vagal stimulation was also performed during a background infusion of bicuculline (10^{-5} *M*) and atropine (10^{-7} *M*). The antagonists were added to the perfusate alone or in combination.

Only one experiment was performed on each stomach.

Rabbit Antral G Cells in Primary Culture

Cell Isolation, Enrichment and Culture

For culture of antral G cells, rabbits were used instead of rats because the cell yield from the small rat antra was too low. Rabbit antral epithelial cells were isolated for culture as previously described [27]. The stomach of male New Zealand White rabbits (2.5–3.0 kg; Savo, Kisslegg, Germany) was excised and the antrum was carefully dissected from the corpus. Antral mucosa was separated from the submucosa, minced into small pieces and dispersed by sequential enzymatic digestion in oxygenated basal medium Eagle (BME) containing 10 m*M* HEPES, 0.1% bovine serum albumin and 300 U/ml of type-I and 300 U/ml of type-XI collagenase in a shaking bath at 37°C. After 30 min the medium containing mainly mucous cells was discarded. The mucosal fragments were further digested by four sequential 60-min periods in BME containing 600 U/ml type-I and 600 U/ml type-XI collagenase in a shaking bath at 37°C. Each 60-min period was followed by a 10-min period with addition of EDTA at a final concentration of 5 m*M*. The isolated cells of each collagenase-EDTA digest were filtered through a nylon mesh (240 μm), washed three times in HBSS containing 10 m*M* HEPES, 0.1% bovine serum albumin, 100 mg/l dithiothreitol and 10 mg/l DNase I and pooled in the same buffer. Thereafter, this crude cell suspension was adjusted to 1.0×10^8 cells/25 ml, filtered through a fine nylon mesh (64 μm) and separated by velocity sedimentation using a Beckman elutriator (rotor JE-6B, run in a J2-21M/E centrifuge; Beckman Instruments, Glenrothes, UK) under sterile conditions. HBSS containing 0.1% bovine serum albumin and 10 m*M* HEPES was used as eluant.

1.0×10^8 cells/run were loaded into the separation chamber of the elutriator at a rotor speed of 2,600 rpm and a flow rate of 21 ml/min. After washing out cell fragments, bacteria and smaller cells during 5 min at a rotor speed of 2,400 rpm and a flow rate of 30 ml/min, 300 ml eluant were collected at 1,800 rpm and 50 ml/min in sterile 150-ml tubes in a laminar flow hood. These cells were spun, washed with sterile DMEM/F-12 containing gentamicin (50 μg/ml), penicillin (100 U/ml) and streptomycin (100 μg/ml), centrifuged again and resuspended in prewarmed (37°C) culture medium DMEM/F-12 supplemented with nuserum (10%), glutamine (2 m*M*), hydrocortisone (1 μg/ml), insulin (10 μg/ml), transferrin (10 μg/ml), selenite (10 ng/ml), gentamycin (50 μg/ml), penicillin (100 U/ml) and streptomycin (100 μg/ml). At a density of 1.0×10^6/well the cells were seeded in 1 ml medium onto 24-multiwell tissue culture plates (well diameter 16 mm; Becton-Dickinson, Heidelberg, Germany) coated with sterile rat tail collagen. The cells were maintained in culture at 37°C in a humidified atmosphere of 5% CO_2 and 95% O_2 for 24–36 h.

Release Study

After 24–36 h the seeded cells formed a confluent monolayer. At that time the culture medium was aspirated and adherent cells were washed twice with 1 ml prewarmed release medium (DMEM/F-12 supplemented with 0.1% bovine serum albumin) to remove secreted mucus and cell debris. Thereafter adherent cells were incubated in 1 ml release medium and the test agents were added at a volume of 25 μl to duplicate wells. After incubation for 2 h at 37°C in a humidified atmosphere of 5% CO_2 and 95% O_2 release medium was completely aspirated and centrifuged. The supernatant was collected and frozen immediately for subsequent radioimmunoassay. In 2 control wells of each plate the adherent monolayer was incubated in 1 ml release medium containing crude collagenase D (1 mg/ml) for a further 20 min incubation. Thereafter the cells were completely removed by vigorous repipetting, heated to 100°C for 10 min and centrifuged. The supernatant was collected and stored at –20°C for measurement of total intracellular gastrin.

Immunocytochemistry

After the release study cultured cells were fixed in Bouin's solution for 10 min, washed three times with phosphate-buffered saline (PBS) and incubated in PBS containing 0.3% H_2O_2 for 30 min to block endogenous peroxidase and to prevent artifactual staining. After washing in PBS the G cells were stained by the avidin-biotinylated enzyme complex method (Vector Laboratories, Burlingame, Calif., USA) originally developed by Hsu et al. [28] using a mouse monoclonal antibody against the N-terminal region of human gastrin-17 (Biomol, Hamburg, Germany) at 1:200 dilution, a secondary polyclonal biotinylated horse anti-mouse antibody at 1:200 dilution and an avidin-biotinylated enzyme complex at 1:100 dilution. G cells were counterstained by diaminobenzidine (0.5 mg/ml 0.1 *M* Tris buffer, pH 7.6, containing 0.02% H_2O_2). Finally, the culture wells were rinsed in PBS and the total number of immunoreactive G cells was counted in at least 1,000 adherent cells/well and the percentage of stained cells was calculated. Furthermore, 2 wells of each culture plate were stained additionally with periodic acid-Schiff reagent to identify the mucous cells.

In addition, immunocytochemistry of freshly isolated cells was performed before and after elutriation. Slides with 100,000 cells were prepared using a cytocentrifuge (Shandon Cytospin; Shandon Instruments, Sewickley, Pa., USA) and fixed in Bouin's solution for 10 min. Thereafter the G cells were stained on the slides by the avidin-biotinylated complex method as mentioned above.

Radioimmunoassay

Gastrin levels in the release medium of the cultured antral cells and in the vascular perfusate of the isolated stomach were measured by radioimmunoassay as described [29] employing a commercial kit (Becton-Dickinson, Heidelberg, Germany).

Somatostatin was determined as described [30] employing antibody 80C generously provided by Dr. R.H. Unger (Dallas, Tex., USA).

BLI was measured by radioimmunoassay as described [24] using an antibody against Lys^4-bombesin [31] generously provided by Dr. M. Brown (Salk Institute, La Jolla, Calif., USA). Tyr^4-bombesin for the preparation of labelled bombesin and synthetic bombesin-14 as standard were generously supplied by Dr. J. Rivier (Salk Institute, La Jolla, Calif., USA).

Drugs and Chemicals

GABA, bicuculline, atropine, carbachol, neuromedin C, BME, collagenase I (type I), collagenase XI (type XI), dithiothreitol, glutamine, hydrocortisone, insulin, transferrin, sodium selenite, gentamycin, penicillin, streptomycin and rat tail collagen were obtained from

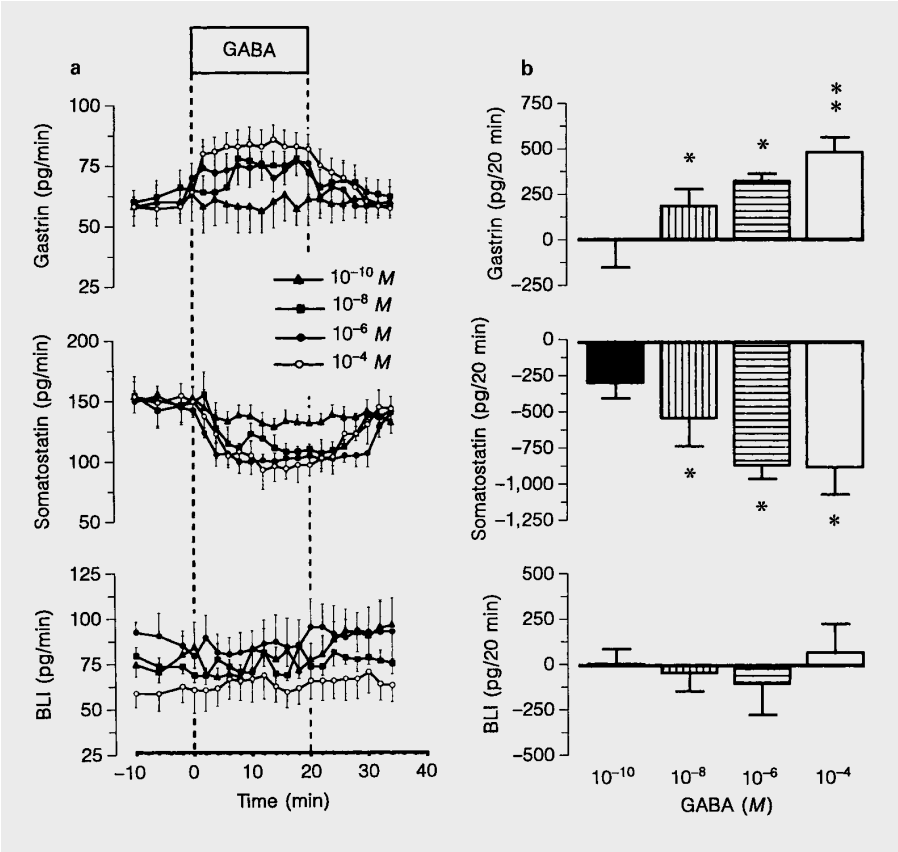

Fig. 1. Release of gastrin, somatostatin and bombesin-like immunoreactivity (BLI) from isolated perfused rat stomachs during infusion of GABA at 10^{-10} *M* (n = 8), 10^{-8} *M* (n = 8), 10^{-6} *M* (n = 8) and 10^{-4} *M* (n = 14). **a** Peptide release is expressed as picograms per minute in the course of time. **b** Integrated peptide secretion during infusion of GABA at the indicated concentrations is calculated as the sums of differences of each time point during GABA infusion to the mean value of the preceding baseline period (mean ± SEM). * Significant difference of $p < 0.05$ or less; ** significant difference of $p < 0.01$ or less to the respective basal level.

Sigma (Munich, Germany). HEPES, bovine serum albumin and EDTA were purchased from Serva (Heidelberg, Germany). HBSS and DMEM/F-12 (1:1) were products of Gibco (Eggenstein, Germany). DNase I and collagenase D were obtained from Boehringer (Mannheim, Germany). Nuserum IV culture supplement (contains 25% fetal bovine serum) was purchased from Collaborative Biomedical Products (Bedford, Mass., USA). Diaminobenzidine was produced by Merck (Darmstadt, Germany). Pentobarbital sodium was obtained from Rhone-Merieux (Cologne, Germany). Dextran T-70 was a product of Pharmacia (Uppsala, Sweden). CGP 35348 was kindly provided by Ciba-Geigy (Basel, Switzerland).

Data Analysis

All data are given as the mean ± SEM of the indicated number of experiments. Gastrin release from cultured G cells was expressed in percent of total cellular content because of considerable interdonor variations in the gastrin content of the cell cultures. Gastrin, somatostatin and BLI release from the isolated stomach was expressed as picograms per minute. Integrated peptide secretion from the isolated stomach was calculated as the sum of the differences between each time point during stimulation and the mean value of the preceding baseline period.

For statistical evaluation of the point-to-point variations the Friedman two-way analysis of variance was used, followed by the Wilcoxon matched-pairs signed-rank test if the former allowed rejection of the null hypothesis. The difference of the values between the treatment groups was statistically evaluated by analysis of variance for multiple determinations. Differences resulting in p values of 0.05 or less were considered significant.

Results

Effect of GABA on the Release of Gastrin, Somatostatin and BLI from the Isolated Rat Stomach

Infusion of GABA elicited a significant increase in gastrin release from a mean baseline of 63 ± 5 to a maximum of 78 ± 6 pg/min ($p < 0.05$) at 10^{-8} *M* (n = 8), from 61 ± 7 to 77 ± 6 pg/min ($p < 0.05$) at 10^{-6} *M* (n = 8) and from 59 ± 4 to 86 ± 6 pg/min ($p < 0.01$) at 10^{-4} *M* (n = 14; fig. 1a). Gastrin secretion remained elevated throughout GABA application and returned promptly to basal values after cessation of GABA infusion. Incremental gastrin release during GABA infusion was 187 ± 98 pg/20 min ($p < 0.05$ vs. basal) at 10^{-8} *M*, 328 ± 43 pg/20 min ($p < 0.05$ vs. basal) at 10^{-6} *M* and 493 ± 84 pg/20 min ($p < 0.01$ vs. basal) at 10^{-4} *M* (fig. 1b). Infusion of 10^{-10} *M* GABA (n = 8) did not change basal gastrin release.

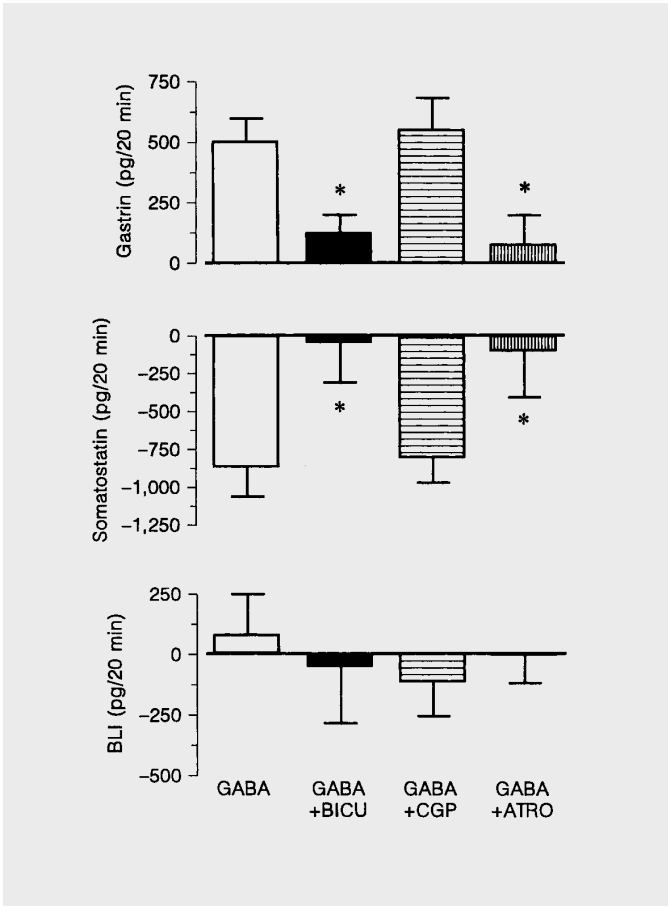

Fig. 2. Effect of bicuculline (BICU; 10^{-5} *M*; n = 8), CGP 35348 (CGP; 5×10^{-5} *M*; n = 8) and atropine (ATRO; 10^{-7} *M*; n = 8) on integrated release of gastrin, somatostatin and bombesin-like immunoreactivity (BLI) from isolated perfused rat stomachs in response to 10^{-4} *M* GABA (n = 14) (mean ± SEM).* Significant difference of $p < 0.05$ or less to 10^{-4} *M* GABA.

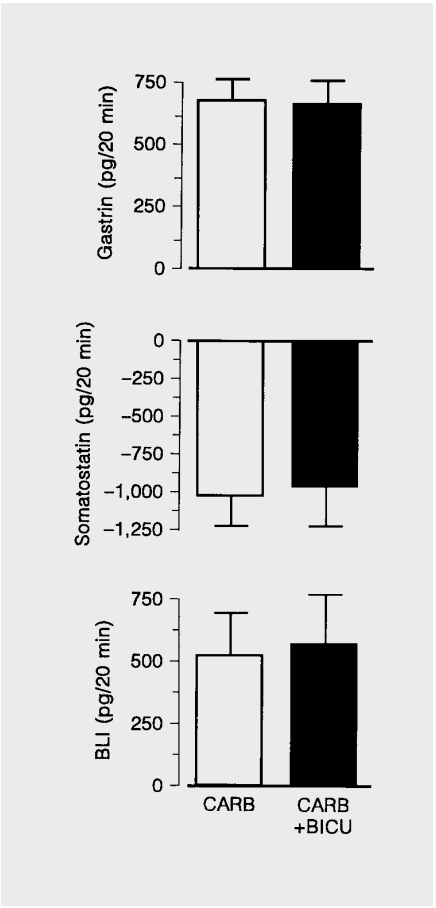

Fig. 3. Effect of bicuculline (BICU; 10^{-5} *M*; n = 6) on integrated release of gastrin, somatostatin and bombesin-like immunoreactivity (BLI) from isolated perfused rat stomachs in response to 10^{-4} *M* carbachol (CARB; n = 6). No significant differences between the two experimental groups were observed (mean ± SEM).

In the same experiments somatostatin secretion decreased during GABA infusion from a mean basal level of 150 ± 9 to a minimum of 106 ± 11 pg/min ($p < 0.05$) at 10^{-8} *M*, from 148 ± 8 to 99 ± 7 pg/min ($p < 0.05$) at 10^{-6} *M* and from 151 ± 15 to 93 ± 9 pg/min ($p < 0.05$) at 10^{-4} *M*. GABA-induced decrement of somatostatin release was −540 ± 203 pg/20 min ($p < 0.05$ vs. basal) at 10^{-8} *M*, −867 ± 96 pg/20 min ($p < 0.01$ vs. basal) at 10^{-6} *M* and −893 ± 195 pg/20 min ($p < 0.05$ vs. basal) at 10^{-4} *M*. GABA 10^{-10} *M* was without effect on somatostatin release.

BLI release from the isolated rat stomach remained unchanged during infusion of GABA at the concentrations employed.

Effect of $GABA_A$-, $GABA_B$- and Cholinergic Receptor Antagonists on the Release of Gastrin, Somatostatin and BLI from the Isolated Rat Stomach during Infusion of GABA

Gastrin response to 10^{-4} *M* GABA (493 ± 84 pg/20 min; n = 14) was completely abolished by the $GABA_A$ receptor antagonist bicuculline (10^{-5} *M*; 116 ± 79 pg/20 min; n = 8; n.s. vs. basal) and the cholinergic blocker atropine (10^{-7} *M*; 74 ± 107 pg/20 min; n = 8; n.s. vs. basal), but remained unchanged during blockade of $GABA_B$ receptors by CGP 35348 (5×10^{-5} *M*; 541 ± 120 pg/20 min; n = 8; n.s. vs. GABA alone) employed at a concentration sufficient to block $GABA_B$ receptors as shown in previous in vitro studies [26] (fig. 2).

In the same experiments GABA-induced inhibition of somatostatin secretion (–893 ± 195 pg/20 min) was also completely blocked by bicuculline (–34 ± 273 pg/20 min; n.s. vs. basal) and atropine (–100 ± 307 pg/20 min; n.s. vs. basal) and was not attenuated by CGP 35348 (–830 ± 158 pg/20 min; n.s. vs. GABA alone).

BLI release was not altered by GABA also in the presence of bicuculline, CGP 35348 or atropine.

In control experiments (n = 6) gastrin, somatostatin and BLI secretion remained at baseline levels throughout the entire experimental period. Additionally no significant differences with the respective basal peptide release were observed during infusion of bicuculline (10^{-5} *M*; n = 6), CGP 35348 (5×10^{-5} *M*; n = 6) and atropine (10^{-7} *M*; n = 6; table 1).

Table 1. Effect of bicuculline (10^{-5} *M*), CGP 35348 (5×10^{-5}) and atropine (10^{-7} *M*) on basal release of gastrin, somatostatin and bombesin-like immunoreactivity (BLI) from isolated perfused rat stomachs

Antagonist	Gastrin release	Somatostatin release	BLI release
Saline	60 ± 40	110 ± 130	122 ± 107
Bicuculline	50 ± 85	150 ± 125	99 ± 133
CGP 35348	85 ± 99	200 ± 175	144 ± 135
Atropine	66 ± 89	125 ± 144	166 ± 180

Peptide release (mean ± SEM) was calculated as the sums of differences of each time point during the 20-min infusion of saline (n = 6) or the respective antagonist (n = 6) to the mean value of the preceding baseline period and is expressed as picograms per 20 min.

No significant differences compared to the respective basal values and between the different groups were observed.

Effect of Bicuculline on the Release of Gastrin, Somatostatin and BLI from the Isolated Rat Stomach during Infusion of Carbachol

To exclude the anticholinergic effects of the $GABA_A$ receptor antagonist bicuculline, the effect of bicuculline on carbachol-induced changes of gastrin, somatostatin and BLI release from the isolated rat stomach was examined (fig. 3). Carbachol infused at a maximal effective concentration of 10^{-4} *M* stimulated gastrin release (642 ± 64 pg/20 min; $p < 0.01$ vs. basal) and BLI release (525 ± 165 pg/20 min; $p < 0.05$ vs. basal) and inhibited somatostatin secretion (–1,044 ± 198 pg/20 min; $p < 0.05$ vs. basal; n = 6). As shown in figure 3, bicuculline (10^{-5} *M*; n = 6) had no significant effect on carbachol-induced changes in gastrin, somatostatin and BLI secretion.

Effect of Bicuculline and Atropine on the Release of Gastrin, Somatostatin and BLI from the Isolated Rat Stomach during Vagal Stimulation

In control experiments (n = 8) electrical stimulation of the vagal nerves elicited a maximal rise in gastrin levels by 58 ± 7 pg/min ($p < 0.01$ vs. basal) and an integrated increase of 397 ± 47 pg/10 min ($p < 0.01$ vs. basal; fig. 4). Vagally stimulated gastrin secretion was reduced to 217 ± 72 pg/10 min ($p < 0.05$ vs. controls) during blockade of $GABA_A$ receptors by bicuculline (n = 8) and to 213 ± 29 pg/10 min ($p < 0.05$ vs. controls) during combined infusion of bicuculline and atropine (n = 8), respectively. In contrast, cholinergic blockade by atropine alone (n = 8) was without effect on vagally stimulated gastrin release (455 ± 93 pg/10 min; n.s. vs. controls).

Somatostatin secretion decreased during vagal stimulation by –518 ± 78 pg/10 min ($p < 0.01$ vs. basal). The decrement of somatostatin was significantly attenuated to –259 ± 143 pg/10 min ($p < 0.05$ vs. control) by bicuculline and reversed into a stimulation of 928 ± 266 pg/10 min by infusion of atropine alone and a stimulation of 739 ± 349 pg/10 min by combined infusion of atropine and bicuculline.

BLI release rose significantly by 666 ± 148 pg/10 min ($p < 0.01$) during vagal stimulation. The BLI response to vagal stimulation was not altered by separate or combined infusion of bicuculline and atropine, respectively.

Enrichment of G Cells by Separation and Culture

After 24–36 h of culture 13.4 ± 2.7% of total adherent cells stained positively for gastrin as shown by immunocytochemistry, whereas G cells accounted only for 0.7 ± 0.3% of total cells in the acutely isolated cell suspension and for 1.7 ± 0.3% in the elutriated cell fraction. Thus, compared to the acutely isolated suspension G cells were enriched 19-fold by elutriation and primary culture. The majority of the remaining cells was identified as mucus cells (84.5 ± 12.5%).

Effect of GABA on Gastrin Release from Isolated G Cells in Primary Culture

The total cellular content (TCC) of gastrin ranged from 3,955 ± 345 to 4,497 ± 385 pg/well in the three series of experiments presented in this study. Addition of GABA (fig. 5) was without significant effect on basal gastrin release from cultured antral G cells at all concentrations tested (10^{-10}–10^{-3} *M*), whereas carbachol (10^{-4} *M*) and

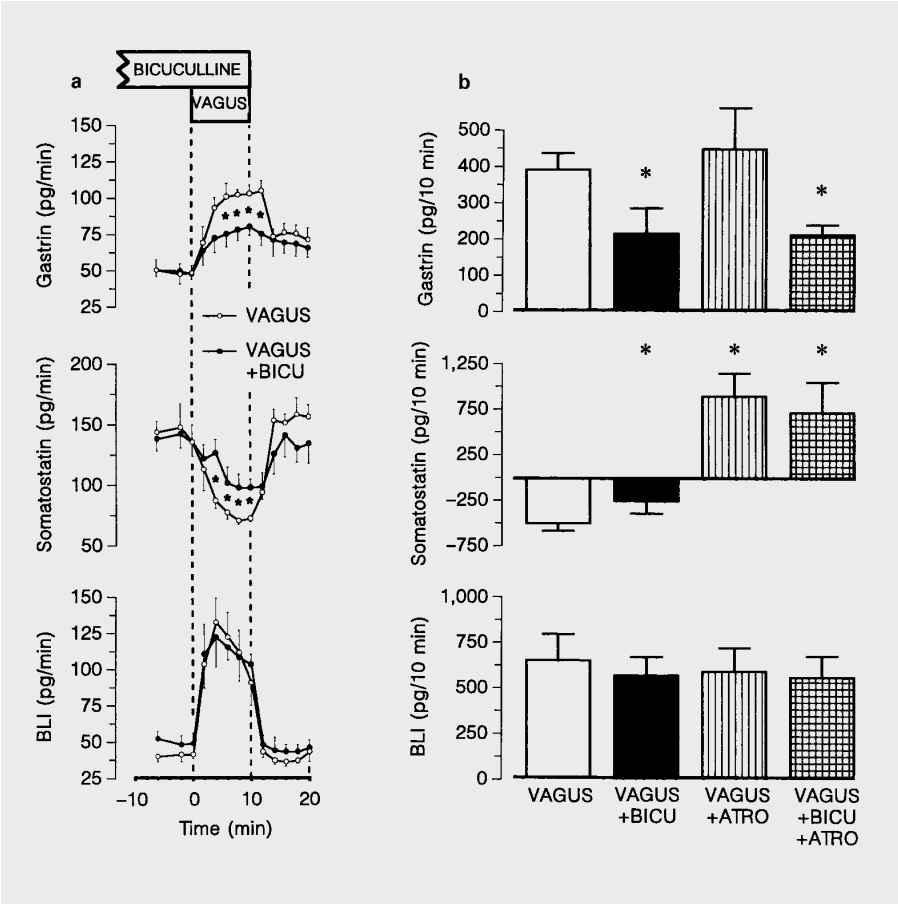

Fig. 4. Effect of electrical stimulation of the vagal nerves (10 V, 10 Hz, 1 ms) on release of gastrin, somatostatin and bombesin-like immunoreactivity (BLI) from isolated perfused rat stomachs in control experiments (VAGUS; n = 8) and during perfusion with bicuculline (BICU; 10^{-5} *M*; n = 8), atropine (ATRO; 10^{-7} *M*; n = 8) and a combination of 10^{-5} *M* bicuculline and 10^{-7} *M* atropine (n = 8). **a** The effect of bicuculline on vagally induced peptide release in the course of time expressed as picograms per minute. **b** Integrated peptide secretion during vagal stimulation in control experiments and in the presence of bicuculline, atropine and the combination of bicuculline and atropine (mean ± SEM). * Significant difference of $p < 0.05$ or less to the respective controls.

neuromedin C (10^{-9} *M*) significantly stimulated gastrin release from 2.6 ± 0.4 to 4.9 ± 0.3% ($p < 0.05$) and 8.5 ± 0.9% TCC ($p < 0.01$), respectively. Gastrin response to 10^{-4} *M* carbachol and 10^{-9} *M* neuromedin C was not significantly changed by GABA (10^{-10}–10^{-3} *M*; fig. 6).

Discussion

The present study is in accordance with previous reports demonstrating that exogenous GABA stimulates gastrin release and inhibits somatostatin secretion from isolated antral fragments and isolated perfused stomachs of rats [18–20]. In contrast, Koop and Arnold [22] and Guo et al. [21] reported on the inhibition of somatostatin release from the isolated perfused rat stomach without concomitant stimulation of gastrin release in response to GABA. The reason for these discrepancies is unknown and might be due to differences in the experimental procedure.

The gastrin and somatostatin response to exogenous GABA can be abolished by the selective $GABA_A$ receptor antagonist bicuculline as well as by the cholinergic blocker atropine as shown previously [18, 20, 22]. Since bicuculline has no anticholinergic effect, these results suggest that exogenous GABA modulates gastric endocrine function through $GABA_A$-receptor-mediated activation of the cholinergic neurotransmitter acetylcholine, which directly stimulates gastrin release and inhibits somatostatin secretion from isolated G and D cells [27, 32, 33]. This mechanism of action is also supported by experiments demonstrating stimulation of acetylcholine release from antral mucosal and submucosal fragments and from neurons of the myenteric plexus in response to exogenous GABA through a $GABA_A$-receptor-mediated mechanism [9, 10].

A putative contribution of GABA to the regulation of gastric endocrine function is also supported by histological studies which have localized GABA and the GABA-synthesizing enzyme GAD to intrinsic nerve fibers within

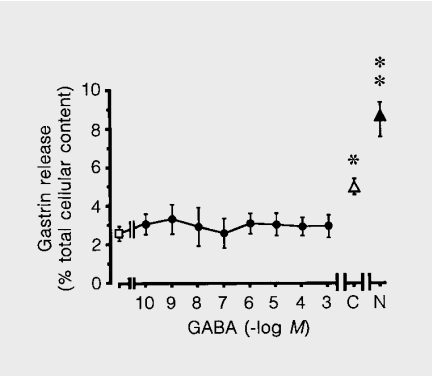

Fig. 5. Effect of GABA on basal gastrin release from rabbit antral G cells in primary culture. The cells were incubated for 120 min under basal conditions (□), or in the presence of GABA (●) at the indicated concentrations or at 10^{-4} *M* carbachol (△) and 10^{-9} *M* neuromedin C (▲) (n = 5; mean ± SEM). *Significant difference of $p < 0.05$ or less; ** significant difference of $p < 0.01$ or less to the respective basal level.

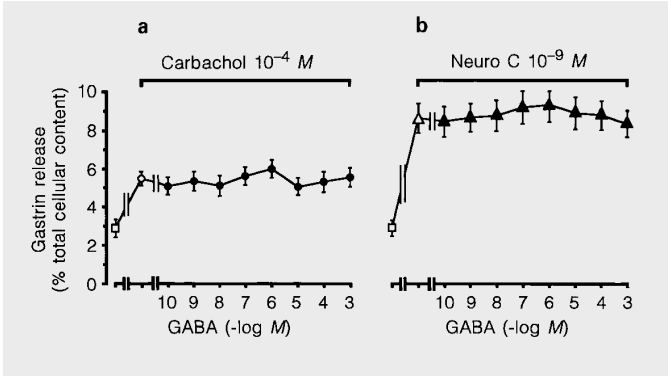

Fig. 6. Effect of GABA on carbachol- and neuromedin C-induced gastrin release from rabbit antral G cells in primary culture. **a** The cells were incubated for 120 min under basal conditions (□), or in the presence of 10^{-4} *M* carbachol alone (○), or in the presence of 10^{-4} *M* carbachol plus GABA at the indicated concentrations (●). **b** The cells were incubated for 120 min under basal conditions (□), or in the presence of 10^{-9} *M* neuromedin C alone (△), or in the presence of 10^{-9} *M* neuromedin C plus GABA at the indicated concentrations (▲) (n = 5; mean ± SEM).

the gastric mucosa and submucosa and to epithelial cells of the gastric glands in the antrum [1, 10–14]. In the rat gastric mucosa GABA- and GAD-immunopositive epithelial cells seem to be identical with endocrine cells [11–14]. It is speculated that GABA is colocalized, at least in part, with other regulatory transmitters in the endocrine cells. In contrast to G cells, several D cells of the gastric mucosa contain GABA as shown by immunohistochemical staining [12]. Furthermore, specific $GABA_A$ receptors have been demonstrated in the gastric mucosa [17]. These findings raise the possibility that GABA acts in the gastrointestinal tract as an endocrine or paracrine transmitter in addition to its role as a neurotransmitter and neuromodulator in the gastrointestinal nervous system. However, to our knowledge the functional role of endogenous GABA on gastric neuroendocrine function is completely unknown.

Bicuculline-induced blockade of $GABA_A$ receptors attenuated the increment of gastrin release and the decrement of somatostatin secretion in response to electrical stimulation of the vagal nerves. These data demonstrate for the first time that endogenous GABA is activated by vagal stimulation and contributes to the vagal regulation of gastrin and somatostatin release via activation of $GABA_A$ receptors.

Vagally induced inhibition of somatostatin release is mediated by cholinergic mechanisms [34, 35]. During blockade of the cholinergic system bicuculline did not change the somatostatin response to vagal stimulation suggesting that the effect of endogenous GABA on D-cell function is mediated by cholinergic mechanisms. In contrast, endogenous GABA contributes to vagal stimulation of gastrin release independent of cholinergic pathways, since bicuculline-induced inhibition of gastrin response to vagal stimulation remained unchanged by additionally infused atropine. Therefore, endogenous GABA seems to activate other pathways involved in gastrin release than exogenously infused GABA. This difference emphasizes that results obtained with an exogenously administered neurotransmitter do not necessarily reflect the role of the endogenously released substance.

Since a direct effect of GABA on G cells was excluded by our experiments in cultured G cells, an interaction of GABA with other noncholinergic stimulatory mechanisms has to be postulated. Therefore we have examined interactions of GABA with bombesin-related peptides, such as gastrin-releasing peptide and neuromedin C. These peptides are localized in neurons of the intrinsic nervous system of the stomach [36, 37] and contribute to vagally induced gastrin release [38–40]. However, in the present study basal and vagally induced release of bombesin-related peptides as well as stimulation of gastrin release from isolated G cells by the bombesin-peptide neuromedin C remained unchanged during infusion of

GABA or GABA antagonists, respectively. Therefore, endogenous GABA stimulates gastrin release through noncholinergic and bombesin-unrelated mechanisms, which have to be determined in future studies.

In summary, the present data demonstrate for the first time that endogenous GABA is involved in vagal regulation of gastrin and somatostatin release from the isolated perfused rat stomach. Activation of $GABA_A$ receptors by endogenous GABA inhibits somatostatin release via atropine-sensitive mechanisms and stimulates gastrin release via noncholinergic and bombesin-unrelated pathways.

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