

H₃-Receptor Regulation of Vascular Gastrin and Somatostatin Releases by the Isolated Rat Stomach

André Bado, Laurent Moizo, Jean-Pierre Laigneau,
Jean Delwaide and Miguel J.M. Lewin^a

Unité de Recherches de Gastroentérologie, INSERM Unité 10,
Hôpital Bichat, Paris, France

(Received March 11, 1994; sent for revision July 15, 1994; accepted September 6, 1994)

We have studied the effects of the H₃-receptor agonist (R) α -methylhistamine [(R) α -MeHA] and the H₃-receptor antagonist thioperamide (Thiop) on basal- and carbachol-stimulated vascular gastrin release (GR) and somatostatin release (SR) by the isolated rat stomach. Carbachol dose-dependently stimulated and inhibited GR and SR, respectively. Maximal stimulation of GR (500 ± 112 percent of basal; $p < .01$), and maximal inhibition of SR (-62 ± 9 percent under basal; $p < .01$) were obtained with $1 \mu\text{M}$ carbachol. Neither (R) α -MeHA nor Thiop, up to $10 \mu\text{M}$, affected GR. However, SR was dose-dependently enhanced by Thiop (25 ± 8 percent for $10 \mu\text{M}$). Carbachol stimulation of GR was strongly inhibited by Thiop (30 ± 7 percent for 100 nM and 73 ± 14 percent for $1 \mu\text{M}$), whereas it was potentiated by (R) α -MeHA. Carbachol inhibition of SR was reversed by Thiop and (R) α -MeHA. However, the reversal effect of (R) α -MeHA was prevented by the CCK_B/gastrin receptor antagonist PD134308. These results support H₃-receptor regulation of basal and cholinergically-stimulated GR and SR.

INTRODUCTION

The groundwork for histamine H₃-receptor was laid by Arrang et al. [1] who reported that histamine inhibits its own release and synthesis in rat brain histaminergic nerves endings. Because this effect was not blocked by H₁- or H₂-receptor antagonists, it was attributed to a novel receptor sub-type [1, 2]. Investigations on the H₃-receptor have now broadened, and it is becoming recognized as a multifunctional receptor with widespread location. Indeed, this receptor sub-type is not restricted to histaminergic neurons since it has been demonstrated on perivascular nerves [3, 4] and on serotonergic, noradrenergic, non-adrenergic, cholinergic and non-cholinergic neurones [5, 6, 7, 8, 9, 10]. Furthermore, it has also been suggested to occur in the skin [11] and the lung [6, 7].

The presence of a H₃-receptor in the gastric mucosa is supported by a body of evidence. We previously reported (R) α -methylhistamine [(R) α -MeHA]^b inhibition of penta-gastrin- and meal-stimulated gastric acid secretion in the gastric fistula cat [12], while others reported (R) α -MeHA inhibition of central cholinergic stimulation of acid secretion in the fistula dog [13]. Furthermore, we provided evidence for (R) α -MeHA inhibition of basal and gastrin-stimulated vascular histamine release in the perfused rat stomach [14]. In addition, we showed that thioperamide (Thiop) enhanced, whereas (R) α -MeHA inhibited, basal histamine release by the isolated rabbit gastric glands [15]. These findings led us to propose the presence of an H₃-receptor on the enterochromaffin-like (ECL) cells.

^aTo whom all correspondence should be addressed.: Miguel J.M. Lewin, Unité de Recherches de Gastroentérologie, INSERM Unité 10, Hôpital Bichat, 75877, Paris, Cedex 18, France; Tel: (33) 1-40-25-83-89; Fax: (33) 1-46-27-85-36.

^bAbbreviations used: (R) α -MeHA, (R) α -methylhistamine; Thiop, thioperamide; ECL, enterochromaffin-like; CCK, cholecystokinin; GR, gastrin release; SR, somatostatin.

This view was recently confirmed by observations suggesting H_3 -receptor regulation of gastrin-induced histamine release from isolated rat ECL cells [16].

In the isolated rabbit gastric glands, Thiop-induced histamine release was paralleled with a stimulation of ^{14}C -aminopyrine uptake. However, this stimulation was insensitive to the H_2 receptor antagonist ranitidine. Moreover, we found that (R) α -MeHA inhibited, whereas thioperamide enhanced, both histamine- and carbachol-induced ^{14}C -aminopyrine uptake in a ranitidine-insensitive manner [15]. Since histamine and carbachol act directly on the parietal cell, the latter effects would involve post-receptor interactions and further suggest that, in addition to the ECL cell, the parietal cell would also be endowed with a H_3 -receptor. However, a direct evidence for a parietal cell H_3 -receptor is still lacking.

In contrast to histamine, (R) α -MeHA does not enhance intracellular cAMP production (except in millimolar concentrations) on isolated human gastric glands [17], neither does it inhibit histamine stimulated cAMP production (personal observation). Such observations make it unlikely that cAMP is involved in H_3 -receptor signalling. In accord with this, we recently reported that (R) α -MeHA concentration-dependently inhibits basal- and carbachol-stimulated formation of phosphatidylinositides (InsP) InsP_1 , InsP_2 and InsP_3 in the human gastric cell line HGT1 [18]. The H_3 -receptor of the HGT1 cells has been further purified as a 67kDa protein [18], but so far, its associated G protein[s] has not been identified.

Clearly, the above findings support that H_3 -receptor regulation of acid secretion might involve receptors located on different cell types. To further investigate this aspect, the present study addresses the effects of (R) α -MeHA and Thiop on basal and carbachol-stimulated vascular releases of somatostatin and gastrin by the isolated rat stomach. The current findings provide pharmacological evidence supporting the occurrence of an additional H_3 or H_3 -like receptor located on the somatostatin or the gastrin cell or both.

MATERIAL AND METHODS

Animals and surgery

Male Wistar rats (R. Janvier, Le Genest, France) weighing 250-400 g were used in these experiments. The animals were deprived of solid food overnight but allowed water *ad libitum*. Anesthesia was induced by 20 percent urethane injected intraperitoneally, and 1000 IU heparin sodium was subcutaneously injected 15 min before laparotomy. The stomach was isolated and vascularly perfused as previously described [19]. The preparation was maintained at 37°C throughout the experiments by a thermostatically controlled heating block. The entire surgical procedure was completed in 45 min, and the period of ischemia starting with ligation of the aorta above the celiac axis did not exceed one min.

Experimental procedures

The lumen of the stomach was perfused at a rate of 1 ml/min with saline buffered at pH 7.4 with 20 mM NaHCO_3 . The vasculature of the stomach was isolated and perfused at a rate of 2 ml/min with Krebs-buffer solution containing four percent dextran (Sigma Chemicals Co, St Louis, Missouri, USA) and two percent bovine serum albumin (Fraction V, Sigma Chemicals). The composition of the Krebs solution was: NaCl, 80 mM; KCl, 5 mM; MgCl_2 , 1.5 mM; CaCl_2 , 1 mM; NaH_2PO_4 , 0.5 mM; Na_2HPO_4 , 1 mM; NaHCO_3 , 20 mM; Glucose, 4.5 mM; and aprotinin, 100 UI/ml. The perfusion was continuously gassed with 95 percent O_2 -5 percent CO_2 to maintain a pH of 7.4. Samples of vascular effluent was collected every two-min interval and stored at -20°C for subsequent gastrin and somatostatin radioassays. At the end of each experiment, the intactness of the vascular bed was challenged by injecting phenol red through the perfusion catheter, and those experiments in which phenol red was detected in the lumen were discarded. Furthermore, the

functional viability of the isolated stomach was assessed by showing the persistence of carbachol stimulation of gastrin release three hours after the beginning of the experiments, whereas these never overstep 90 min.

The experimental design after surgery and the 30-min equilibration were as follows: *a*) a 10-min-control sampling period; *b*) a 30-min drug infusion period; and *c*) a 10-min period after cessation of drug infusion. In dose-response studies, one concentration of drugs was infused for each stomach during period *b*. In four experiments, no drug was infused, and basal secretion of the peptides was determined for 50-min periods to assess the spontaneous release of gastrin and somatostatin. The effect of H_3 -receptor was assessed by infusing (R) α -MeHA or Thiop either alone or together with carbachol. In another set of experiments, the CCK_B/gastrin-receptor antagonist PD134308 [20, 21] was infused together with (R) α -MeHA and carbachol.

Radioimmunoassays of gastrin and somatostatin

Gastrin immunoassay was run in duplicate using ^{125}I -Gastrin-17 as a tracer (Amersham, England). The rabbit gastrin antiserum used recognized the C-terminus of all matured forms of gastrin. Cross-reactivities with CCK₄ was less than 0.001 percent and less than two percent with CCK₈ and CCK₃₃. Separation of free and bound tracer was achieved using a mixture of charcoal and Dextran. Detection limit was 0.1 pg/ml of standard Human gastrin-17.

Somatostatin was assayed in duplicate as described in detail elsewhere [22]. Briefly, the antibody raised in sheep (gift from P. Brazeau, Montreal) recognized both somatostatin-14 and somatostatin-28 and was used at a final dilution of 1/60,000; ^{125}I -Tyr1-somatostatin was used as a tracer with an assay sensitivity of 10 pg/tube. Tyr1-somatostatin-14 (Sigma chemicals, St-Louis Missouri, USA) was iodinated according to the chloramine T method [23] and purified by chromatography on a carboxymethyl cellulose (CM52) column. Somatostatin concentrations for each sample were determined using somatostatin-14 as standard. Synthetic somatostatin-14 or gastrin-17 was added to the perfusate and the serial dilution of the sample produced a curve paralleling that of the standard curves for somatostatin and gastrin.

DATA EXPRESSION AND STATISTICAL ANALYSIS

Basal gastrin release (GR) and somatostatin release (SR) varied from stomach to stomach but maintained during the 50-min observation periods. The mean gastrin concentrations in portal effluent during the drugs infusion period were calculated and

Table 1. Summary of the effects of the H_3 -receptor agonist and antagonist, respectively, (R) α -MeHA and Thioperamide, on gastrin and somatostatin releases from the isolated perfused rat stomach. Results are the means of \pm SEM of the average values from 10 to 20 min (i.e., periods of the infusion of H_3 ligands) and are expressed as percentage change above or below basal.

	Somatostatin	Gastrin
Basal		
Thioperamide 1 μM	+20 \pm 4%	-12 \pm 8%
(R) α -MeHA	-10 \pm 6%	+2 \pm 8%
Stimulated		
Carbachol 1 μM	-63 \pm 2%	+380 \pm 34%
+ (R) α -MeHA 1 μM	-34 \pm 11%	+996 \pm 93%
+ Thioperamide 100 nm	-22 \pm 8%	+214 \pm 30%
+ Thioperamide 1 μM	+15 \pm 3%	+71 \pm 15%

FIGURE 1

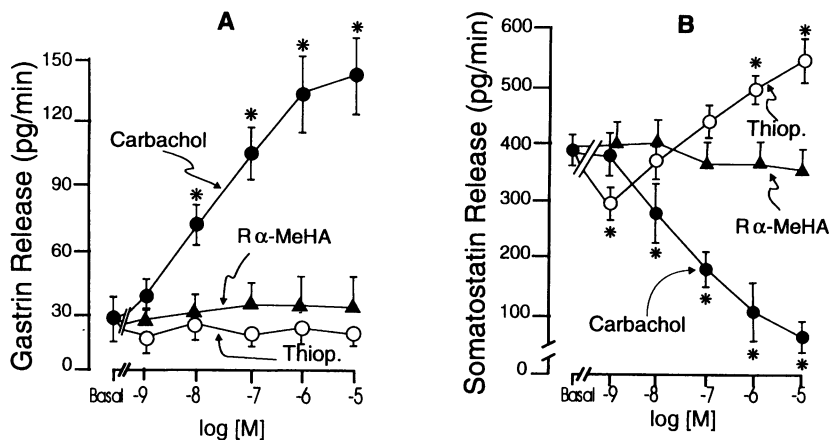


Figure 1. Dose-response curves for carbachol, H_3 -receptor agonist (R) α -MeHA or antagonist thioperamide on gastrin (A) and somatostatin (B) release from the isolated perfused rat stomach. Results are expressed in picograms/min. Values are means \pm 1 SEM of six to eight experiments. ($p < .05$ vs control)

expressed as a percentage of basal secretion (10-min period). Somatostatin secretion (generally a decrease) was evaluated in the same manner, and the data were expressed as a percentage of decrease under basal secretion. The data were analyzed by Student's t-test for paired values, and $p < .05$ was considered significant.

RESULTS

Dose-response effects of carbachol, [R] α -MeHA and Thioperamide on gastrin and somatostatin releases

Carbachol dose-dependently stimulated GR from the perfused rat stomach. Half-maximal and maximal responses were achieved for 20 nM and 10 μ M, respectively (Figure 1A). (R) α -MeHA at concentrations ranging from 1 nM to 10 μ M were without any effect by its own on basal GR, whereas Thiop slightly, but non-significantly, reduced this release (Table I).

Carbachol dose-dependently inhibited SR. Half-maximal and maximal effects were achieved for 30 nM and 10 μ M, respectively. (R) α -MeHA had no effect on basal SR, up to 10 μ M. However, Thiop dose-dependently increased basal SR with a maximal effect reaching 25 ± 8 percent for 10 μ M. (Figure 1B).

Effects of [R] α -MeHA and Thiop on carbachol-induced gastrin and somatostatin releases

Carbachol produced a rapid and time-dependent basal GR increase, levelling off during the infusion at 500 ± 112 percent of basal ($p < .01$ vs. control). GR returned to control level on cessation of carbachol infusion. Thiop dose-dependently inhibited carbachol stimulation of gastrin release by 30 ± 7 percent and 73 ± 14 percent ($p < .01$ vs. carbachol alone) for 100 nM and 1 μ M, respectively. On cessation of Thiop infusion, GR returned to control (Figure 2).

Carbachol time-dependently decreased basal SR. This decrease was significant as soon as four min after the start of the infusion, and maximal inhibition reached 62 ± 9 percent under basal ($p < .01$). However, carbachol inhibition was totally blocked by 100 nM

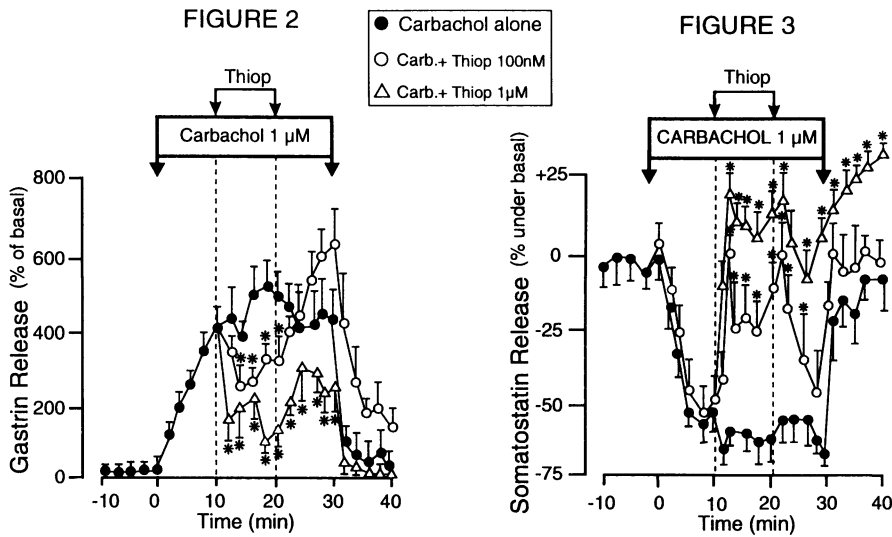


Figure 2. Effect of H_3 -receptor antagonist thioperamide on carbachol-stimulated gastrin release from the isolated perfused rat stomach. Results are expressed as a percentage of basal. Values are means \pm 1 SEM of six to eight experiments. ($p < .05$ vs. carbachol alone).

Figure 3. Effect of H_3 -receptor antagonist thioperamide on the carbachol inhibition of somatostatin release by the isolated perfused rat stomach. Results are expressed as a percentage under basal. Values are means \pm 1 SEM of six to eight experiments ($p < .05$ vs. carbachol alone).

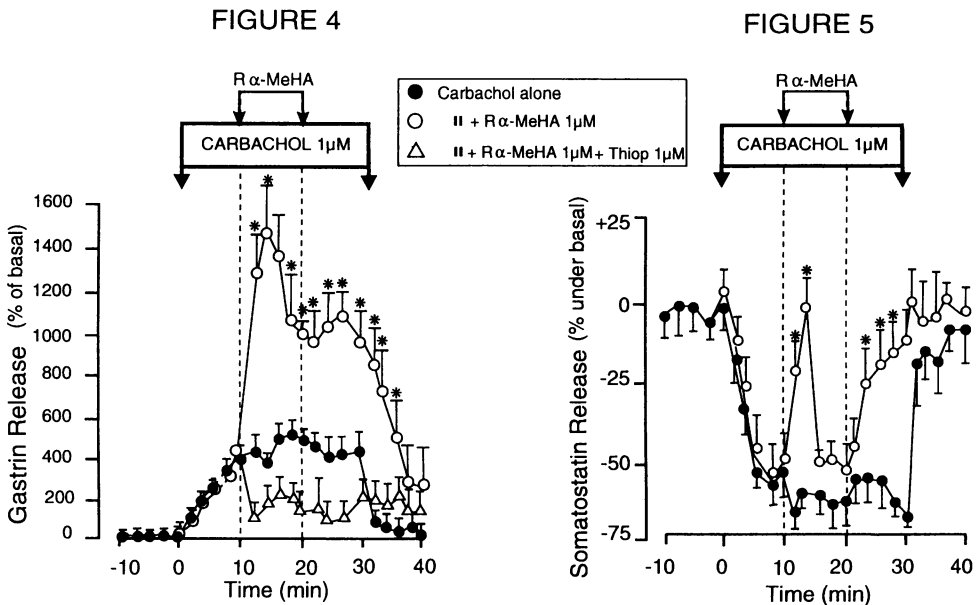


Figure 4. Effect of H_3 -receptor agonist (R) α -MeHA alone or in combination with thioperamide on carbachol-stimulated gastrin release by the isolated perfused rat stomach. Results are expressed as a percentage over basal. Values are means \pm 1 SEM of six to eight experiments. ($p < .05$ vs. carbachol alone).

Figure 5. Effect of H_3 -receptor agonist (R) α -MeHA on the carbachol inhibition of somatostatin release by the isolated perfused rat stomach. Results are expressed as a percentage under basal. Values are means \pm 1 SEM of six to eight experiments. ($p < .05$ vs. carbachol alone)

Thiop. This blockade effect occurred within the first four min and lasted for the whole duration of Thiop infusion. Interestingly, 1 μ M Thiop not only reverted carbachol inhibition but also produced a significant stimulation of SR (Figure 3).

Addition of 1 μ M (R) α -MeHA to carbachol resulted in a further increase in GR. The effect was rapid (within the first two min of the infusion) and maintained for a while after the end of infusion. It corresponded to a potentiation since the effect of (R) α -MeHA together with carbachol was higher than the summation of the effect of each drug alone. Furthermore, it was H₃-receptor specific since it was completely abolished by the addition of 1 μ M thioperamide (Figure 4).

On the other hand, (R) α -MeHA reversed carbachol-induced inhibition of SR (Figure 5). To evaluate whether this reversal effect could be secondary to increased GR, we repeated the experiment in the presence of the CCK_B/gastrin receptor antagonist PD134308. As shown in Figure 6, PD134308 did not affect the effect of (R) α -MeHA on carbachol-induced GR. However, it suppressed the effect of (R) α -MeHA on SR and completely restored carbachol inhibition of SR, producing even a further decrease of this release as compared to carbachol alone.

DISCUSSION

The putative role of histamine as a regulator of gastric releases of somatostatin and gastrin is still an open question. Using the perfused rat stomach model, two different groups have reported evidence for histamine stimulation of SR [24, 25, 26]. This stimulation was increased by micromolar concentrations of cimetidine which, on the other hand, blocked cholinergic inhibition of SR [26, 27]. However, a lack of effect of H₂-receptor blockade on basal SR has also been reported [24].

FIGURE 6

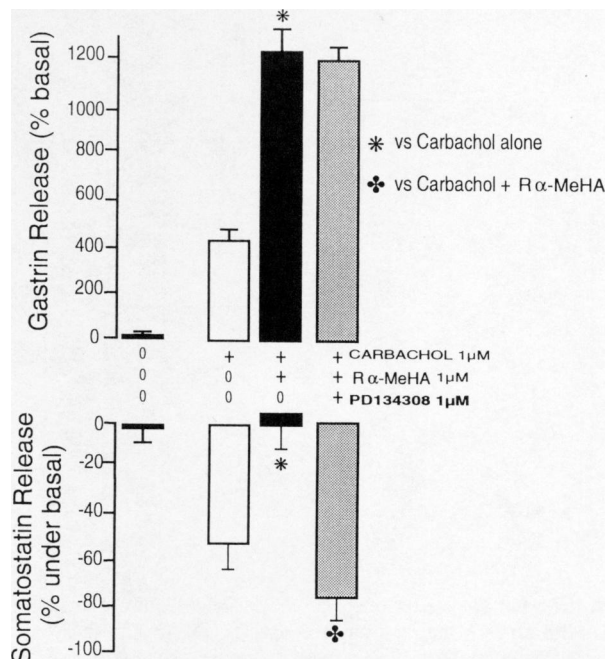


Figure 6. Effect of H₃-receptor agonist (R) α -MeHA alone or in combination with the CCK_B/Gastrin receptor antagonist PD134308 on the carbachol stimulation of gastrin and inhibition of somatostatin releases by the isolated rat perfused stomach. Values are means \pm 1 SEM of eight experiments, and results are expressed as a percentage of basal (gastrin) or under basal (somatostatin).

The present findings showing Thiop enhancement of basal SR are consistent with previous reports from our and other laboratories on isolated rabbit gastric glands [15] and isolated mouse stomachs [25]. As far as the fundic part of the stomach is concerned, they could be explained by Thiop stimulation of histamine release due to inhibition H_3 -receptors on the ECL cells [15, 16] and histamine inhibition of SR through a putative D-cell receptor.

Under our experimental conditions, with the lumen of the isolated stomach being maintained at pH 7, we clearly found that carbachol inhibited SR and stimulated GR in the absence of any feed back due to acid secretion. This is in agreement with previous studies [19, 28, 29, 30]. In our study, (R) α -MeHA fails to affect basal gastrin release. We have no explanation for this, but it is likely that it reflects the level of endogenous-released histamine or that the whole stomach preparation is not adequate to demonstrate such an effect. However, we found that (R) α -MeHA produced a dramatic potentiation of carbachol stimulation of GR, which is consistent with preliminary observations [28, 31]; whereas Thiop both prevented carbachol inhibition of SR and carbachol stimulation of GR (Table 1). These observations cannot be unequivocally explained with the experimental model used. First, it is not known, whether the effects of carbachol on GR and SR are due to direct interactions with the somatostatin and the gastrin cells or are indirectly exerted. For instance, these effects could be secondary to histamine release from the ECL cells or mediated by intrinsic neurons. Second, since the gastrin cell is equipped with an inhibitory somatostatin receptor and the somatostatin cell with a stimulatory gastrin receptor [32, 33, 34], SR and GR are interacting each other. Taking this into consideration, a simple hypothesis is that the effects of the H_3 ligands on SR are just the consequence of those produced on GR. Consistent with this view is the observation that the paradoxical effect produced by (R) α -MeHA on carbachol inhibition of SR, i.e., an apparent reversal of this inhibition in spite of the large increase observed for GR (Figure 6), is prevented by the CCK_B /gastrin receptor antagonist PD134308.

In conclusion, the present study provides a new insight into H_3 -receptor regulation of gastric functions. In addition to the H_3 -receptor, which has been pharmacologically described on the ECL cell [16, 35] and to that suggestively present on the parietal cell [15], it appears that H_3 or H_3 -resembling receptors may be also involved in cholinergic regulation of gastrin and somatostatin secretions. However, whether these putative receptors are located on the gastrin and/or somatostatin cells or are beared by other cell types, e.g., intrinsic neurons, remains to be elucidated.

REFERENCES.

1. Arrang, J.M., Garbarg, M., and Schwartz, J.C. Auto-inhibition of brain histamine release by a novel class (H_3) of histamine receptor. *Nature* 302:832-837, 1983.
2. Arrang, J.M., Garbarg, M., Lancelot, J.C., Lecomte, J.M., Pollard, H., Robba, M., Schunack, W., and Schwartz, J.C. Highly potent and selective ligands for histamine H_3 -receptors. *Nature* 327:117-123, 1987.
3. Ishikawa, S. and Sperelakis, N. A novel class [H_3] of histamine receptors on perivascular nerve terminals. *Nature* 327:158-160, 1987.
4. Ea-Kim, L. and Oudart, N. A highly potent and selective H_3 agonist that relaxes rabbit middle cerebral artery, *in vitro*. *Eur. J. Pharmacol.* 150:393-396, 1988.
5. Schlicker, E., Fink, K., Hinterthaler, M., and Göthert, M. Inhibition of noradrenaline release in the rat brain cortex via presynaptic H_3 receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 340:633-637, 1989.
6. Ichinose M, Belvisi, M.G. and Barnes, P.J. Histamine H_3 -receptors inhibit neurogenic microvascular leakage in airways. *J. Appl. Physiol.* 68:21-25, 1990.
7. Ichinose, M., Stretton, C.D., Schwartz, J.C., and Barnes, P.J. Histamine H_3 -receptors inhibit cholinergic neurotransmission in guinea pig airways. *Brit. J. Pharmacol.* 97:13-15, 1989.

8. Lees, G.M. and Steel, M.C. Histamine H₃-receptor mediated inhibition of cholinergic and non-adrenergic non-cholinergic neuroeffector transmission in guinea-pig isolated ileum. *Eur. J. Pharmacol.* 183:1145-1149, 1990.
9. Menkveld, G.J. and Timmerman, H. Inhibition of electrically evoked contractions of guinea pig ileum preparations mediated by the histamine H₃-receptor. *Eur. J. Pharmacol.* 186: 343-347, 1990.
10. Poli, E., Coruzzi, G., and Bertaccini, G. Histamine H₃ receptors regulate acetylcholine release from the guinea-pig ileum myenteric plexus. *Life Sci.* 48:63-68, 1991.
11. Archer, C.B. and Greaves, M.W. H₃ receptors and regulation of histamine turnover in skin: a new approach? *Skin Pharmacol.* 6:81-84, 1993.
12. Bado, A., Hervatin, F., and Lewin, M.J.M. Evidence for histamine H₃ receptors in the regulation of gastric acid secretion in the cat. *Am. J. Physiol.* 260 [Gastrointest. Liver Physiol.23]: G631-635, 1991.
13. Soldani, G., Mengozzi, G., Intorre, L., De Giorgi, Coruzzi, G., and Bertaccini, G. Histamine H₃ receptor-mediated inhibition of gastric acid secretion in conscious dogs. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 347:61-65, 1993.
14. Sandvik, A.K., Lewin M.J.M., and Waldum, H.L. Histamine release in the isolated vascularly perfused stomach of the rat: regulation by autoreceptors. *Brit. J. Pharmacol.* 96:557-562, 1989.
15. Bado, A., Moizo, L., Laigneau, J.P., and Lewin, M.J.M. Pharmacological characterization of histamine H₃-receptors on isolated rabbit gastric glands. *Am. J. Physiol.* 262 (Gastrointest. Liver Physiol. 25):G56-G61, 1992.
16. Prinz, C., Kajimura, M., Scott, D.R., Mercier, F., Helander, H.F., and Sachs, G. Histamine secretion from rat enterochromaffinlike cells. *Gastroenterology* 105:449-461, 1993.
17. Gespach, C., Fagot, D., and Emami, S. Pharmacological control of the human gastric histamine H₂ receptor by famotidine: comparison with H₁, H₂, and H₃ receptor agonists and antagonists. *Eur. J. Clin. Invest.* 19:1-10, 1988.
18. Cherifi, Y., Pigeon, C., Le Romancer, M., Bado, A., Reyl-Desmars, F., and Lewin, M.J.M. Purification of a Histamine H₃-receptor negatively coupled to phosphoinositide turnover in the human gastric cell line HGT1. *J. Biol. Chem.* 267:25315-25320, 1992.
19. Saffouri, B., Weir, G.C., Bitar, K.N., and Makhlof, G.M. Gastrin and somatostatin secretion by perfused rat stomach: functional linkage of antral peptides. *Am. J. Physiol.* 238 (Gastrointest. Liver Physiol. 1):G495-G501, 1980.
20. Hughes, J., Boden, P., Costall, B., Domeney, A., Kelly, E., Horwell, D.C., Hunter, J.C., Pinnock, R.D., and Woodruff, G.N. Development of a class of selective cholecystokinin type B antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. USA* 87:6728-6732, 1990.
21. Eissele, R., Koop, H., Bothe-Sandfort, E., and Arnold, R. Effect of gastrin receptor antagonists on gastric acid secretion and gastrin and somatostatin release in the rat stomach. *Digestion* 53:179-188, 1992
22. Rorstadt, O.P., Epelbaum, J., Brazeau, P., and Martin, J.B. Chromatographic and biological properties of immunoreactive somatostatin (IRS) in hypothalamic and extrahypothalamic brain regions of the rat. *Endocrinology* 105:1083-1092, 1979.
23. Hunter, W.M. and Greenwood, F.C. Preparation of iodine I131 labeled growth hormone of high specific activity. *Nature Lond.* 194:495-496, 1962.
24. McIntosh, C.H.S., Bakich, V., Kwok, Y.N., Wong, J., and Brown, J.C. The effects of substance P, histamine antagonists on somatostatin and gastrin release from the isolated perfused rat stomach. *Regul. Pept.* 19:253-263, 1987.
25. Vuyyuru, L. and Schubert, M.L. Participation of histamine H₃ receptors of the fundus in the regulation of gastric acid secretion (Abstract). *Gastroenterology* 104:A220, 1993 .
26. Madaus, S., Bender, H., Schusdziarra, V., Kehe, K., Munzert, G., Weber, G., and Classen, M. Vagally induced release of gastrin, somatostatin and bombesin-like immunoreactivity from perfused rat stomach. Effect of stimulation frequency and cholinergic mechanisms. *Regul. Pept.* 30:179-192, 1990.
27. Schusdziarra, V., Bender, H., Pfeffer, A., and Pfeiffer, E.F. Modulation of acetylcholine-induced secretion of gastric bombesin like immunoreactivity by cholinergic and histamine H₂-receptors, somatostatin, and intragastric pH. *Regul. Pept.* 8:189-198, 1984.
28. Schubert, M.L., Harrington, L., and Makhlof, G.M. Histamine H₃-receptors are coupled to inhibition of somatostatin secretion in the fundus and antrum of the stomach (Abstract). *Gastroenterology* 104:A854, 1993.
29. Martindale, R., Gordon, L., Kauffman, L., Levin, S., Walsh, J.H., and Yamada, T. Differential regulation of gastrin and somatostatin secretion from isolated perfused rat stomachs. *Gastroenterology* 83:240-244, 1982.

30. Schubert, M.L., Saffouri, B., and Makhlof, G.M. Identical patterns of somatostatin secretion from isolated antrum and fundus of rat stomach. *Am. J. Physiol.* 254 (Gastrointest. Liver Physiol. 17):G20-G24, 1988.
31. Bado, A., Moizo, L., Laigneau, J.P., and Lewin, M.J.M. Regulation of gastrin and somatostatin by the gastric histamine H₃-receptor from the isolated perfused rat stomach. *Gastroenterology* 102:916A, 1992.
32. Chiba, T. and Yamada, T. Mechanisms for the stimulatory and inhibitory effects of carbamylcholine on canine gastric D-cells. *Biochem. Biophys. Res. Comm.* 147:140-144, 1987.
33. Delvalle, J., Chiba, T., Park, J., and Yamada, T. Distinct receptors for cholecystokinin and gastrin on canine fundic D-cells. *Am. J. Physiol.* 264:G811-G815, 1993.
34. Soll, A.H., Amirian, D.A., Park, J., Elashoff, J.D., and Yamada, T. Cholecystokinin potently releases somatostatin from fundic mucosal cells in short-term culture. *Am. J. Physiol.* 248:G569-G573, 1985.
35. Hollande, F., Bali, J.P., and Magous, R. Autoregulation of histamine synthesis through H₃-receptors in isolated fundic mucosal cells. *Am. J. Physiol.* 265 (Gastrointest. Liver Physiol. 28):G1039-G1044, 1993.