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Somatostatin sst_2 receptor-mediated inhibition of parietal cell function in rat isolated gastric mucosa

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1 The aim of this study was to determine the location and functional characteristics of the somatostatin (SRIF) receptor type(s) which mediate inhibition of acid secretion in rat isolated gastric mucosa.

2 Gastrin (1 nM-1 μM), dimaprit (10 μM-300 μM) and isobutyl methylxanthine (IBMX, 1 μM-100 μM) all caused concentration-dependent increases in acid output. Responses to gastrin were almost completely inhibited by ranitidine (10 μM) at a concentration which abolished the secretory response to dimaprit. In contrast, responses to IBMX were not changed by ranitidine suggesting that IBMX acts directly on the parietal cell and not indirectly by releasing histamine from enterochromaffin-like (ECL) cells.

3 SRIF-14 (1 nM-1 μM) had no effect on basal acid output, but inhibited acid output produced by gastrin, dimaprit and IBMX in a concentration-dependent manner with respective EC_{50} values of 46, 54 and 167 nM. The peptidase inhibitors, amastatin (10 μM) and phosphoramidon (1 μM), had no effect on SRIF-induced inhibition of dimaprit stimulated gastric acid secretion.

4 The inhibitory effect of a range of SRIF analogues on gastrin-, dimaprit- and IBMX-induced acid secretion was also studied. Irrespective of the secretagogue used to increase acid output, the rank order of potencies was similar (BIM-23027 = seglitide = octreotide > SRIF-14 = SRIF-28 > L-362,855). The linear peptide BIM-23056 was devoid of agonist or antagonist activity in concentrations up to 1 μM.

5 The sst_2 receptor selective peptides, BIM-23027, seglitide and octreotide were the most potent inhibitors of gastrin-, dimaprit- and IBMX-induced acid secretion suggesting that SRIF receptors resembling the recombinant sst_2 receptors are involved. Furthermore, since dimaprit and IBMX stimulate gastric acid secretion independently of histamine release, sst_2 receptor-mediated inhibition must occur at the level of the parietal cell itself.

Keywords: Somatostatin; SRIF; sst_2 receptors; gastric acid secretion; BIM-23027; seglitide; octreotide

Introduction

The tetradecapeptide somatostatin (SRIF-14), is distributed throughout the body but is found in high concentrations in the gastrointestinal tract where its pleiotropic actions include various inhibitory effects on electrolyte and enzyme secretion, neuromodulator effects on motility as well as inhibition of proliferation of gastric and intestinal epithelia (for review, see Lewin, 1992). Immunohistochemical studies have demonstrated SRIF-like immunoreactivity in large amounts in the stomach (Arimura *et al.*, 1975), where it is localised predominantly in the fundic and antral mucosa (Penmann *et al.*, 1983) and where it can inhibit gastric acid and pepsinogen secretion in response to stimulation by pentagastrin (Rossowski *et al.*, 1994; Tanaka & Tani, 1994). The ability of SRIF-14 to inhibit acid secretion has long been known and may involve direct effects mediated by SRIF receptors located on parietal cells, as well as indirect effects on histamine release from enterochromaffin-like (ECL) cells (Park *et al.*, 1987; Schmidtler *et al.*, 1992; Payne & Gerber, 1992; Prinz *et al.*, 1994).

The recent cloning of five distinct SRIF-receptor genes (sst_1-sst_5) encoding a family of G-protein coupled receptors (for review see Hoyer *et al.*, 1994; 1995) has provided the impetus to determine which SRIF-receptor types mediate the diverse biological actions of this peptide (Feniuk *et al.*, 1993; 1994; McKeen *et al.*, 1994). The presence of mRNA for all five sst receptors has been demonstrated in the stomach. Solution hybridisation with nuclease protection analysis (Bruno *et al.*, 1993) has shown that the rat stomach expresses the mRNA for sst_1-sst_4 but not for sst_5 receptor types, whilst reverse transcriptase-polymerase chain reaction (RT-PCR) detected sst_{1-3} and sst_5 but not sst_4 mRNA (Raulf *et al.*, 1994). PCR amplification of a highly enriched ECL cell cDNA library has shown that ECL cells predominantly express sst_2 mRNA (Prinz *et al.*, 1994).

In the present study we have elucidated the functional characteristics of the SRIF-receptor types mediating inhibition of acid secretion in the rat isolated gastric mucosa with secretagogues such as gastrin, which acts predominantly on the ECL cells to release histamine (Prinz *et al.*, 1994), as well as agents such as isobutyl methylxanthine (IBMX) and dimaprit which act directly on the parietal cell (Welsh *et al.*, 1993). In addition to studying the effects of the two naturally occurring forms of somatostatin, SRIF-14 and SRIF-28, we have compared the effects of a range of smaller SRIF related peptides including BIM-23027, BIM-23056 and L-362,855 which have been shown to display selective affinities for the recombinant sst_2, sst_3 and sst_5 receptors, respectively (Raynor *et al.*, 1993; Patel & Srikant, 1995). Preliminary accounts of some of these findings have been presented to the British Pharmacological Society (Feniuk *et al.*, 1994; Wyatt *et al.*, 1995).

Methods

Preparation of rat isolated gastric mucosa

Rat isolated gastric mucosal preparations were prepared by a method similar to that first described by Main and Pearce (1978). Female Wistar rats (AHA strain, 70-120 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.).

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Following induction of anaesthesia, the stomach was exteriorised, the non-glandular portion removed, and the muscle layer overlying the non-antral region separated from the mucosa by injecting 0.9% NaCl between the two layers. The muscle layer was then cut along the greater curvature and gently teased away from the mucosa. The gastric mucosa was then removed and tied to a perspex perfusion chamber (see Reeves & Stables, 1985) such that the inner mucosal surface could be continuously perfused with an unbuffered Krebs solution, gassed with 100% O_2, at a constant rate of 0.5 ml min^{-1}. The perfusion chamber with the attached mucosa (serosal surface outermost) was immersed in an organ bath containing Krebs solution at 37°C gassed with 95% O_2/5% CO_2. The ionic composition (mM) of the solutions were: serosal, NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 1.3 and glucose 11.1; mucosal, NaCl 144.7, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.3 and glucose 11.1. The serosal solution also contained 2.8 μM indomethacin. The pH of the effluent perfusate was continuously monitored by pH electrodes (Russell, Scotland) attached to a standard pH meter and pen recorder (Radiometer, Copenhagen), and acid output measured as nmol H^+ min^{-1}.

Effects of ranitidine on gastrin-, IBMX- and dimaprit-induced acid secretion

The preparations were washed once and then given a priming dose of pentagastrin (30 nM). Once the response reached a plateau the preparation was washed until a steady baseline was obtained. Cumulative concentration-effect curves were then generated for either gastrin (1 nM–1 μM), IBMX (0.1 μM–100 μM) or dimaprit (1 μM–300 μM). The preparations were washed five times, then twice more at 10 min intervals. After the last wash the preparations were incubated for 45 min with (or without) 10 μM ranitidine. The concentration-effect curves for the same agonist were then repeated.

Effects of SRIF analogues on basal, gastrin-, IBMX- and dimaprit-induced acid secretion

Once a stable baseline was attained, the preparations were stimulated with either gastrin (1 μM), IBMX (30 μM) or dimaprit (200 μM). The tissues were then left to reach a plateau, approximately 45 min for gastrin and IBMX and 30 min for dimaprit. Cumulative concentration-effect curves were then constructed to the SRIF analogues. Doses were administered every 10 min or until a plateau response was reached. Control preparations were not exposed to SRIF analogues allowing the stability of the secretagogue responses to be monitored.

Effects of peptidase inhibitors and antagonists on responses to SRIF

In some experiments the influence of a combination of peptidase inhibitors on the inhibitory effects of SRIF-14 on dimaprit-induced acid secretion was examined. After a stable baseline was achieved the preparation was pre-incubated for 30 min with 10 μM amastatin and 1 μM phosphoramidon, which remained in contact with the preparation before stimulation with dimaprit and throughout the rest of the experiment. Cumulative concentration-effect curves were then constructed to SRIF-14 and compared to preparations not treated with peptidase inhibitors. The same protocol was used to determine the effects of putative antagonists on SRIF-induced inhibition of acid output.

Analyses of results

The inhibitory effects of the SRIF-analogues were expressed as a mean (±s.e. mean) percentage inhibition of the maximum change in H^+ output produced by each secretagogue, defined as the difference between basal acid output measured immediately before secretagogue administration and peak stimulated output. When fully defined concentration-effect curves were obtained, EC_{50} values were determined from individual experiments by non-linear regression, using a four parameter logistic equation (Graph Pad Prism), and are expressed as geometric mean (95% confidence limits), of n observations. When curve maxima could not be achieved, EC_{50} values were not determined. The effect of antagonists or inhibitors was studied by comparing the EC_{50} values for SRIF in the absence/presence of the compound. Tests for statistically significant differences were made by use of a two tailed t test.

Drugs and solutions

The following drugs were used: dimaprit diHCl (RBI), sodium pentobarbitone (RhoneMerieux), isobutyl methylxanthine, indomethacin, gastrin 1, pentagastrin, amastatin and phosphoramidon (Sigma), SRIF-14 and SRIF-28 (Peninsula Laboratories Europe Ltd.), octreotide (Sandoz), ranitidine HCl, seglitide (MK678), BIM-23027 (c[N-Me-Ala-Tyr-D-Trp-Lys-Abu-Phe]), BIM-23056 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2) and L-362,855 (c[Aha-Phe-Trp-D-Trp-Lys-Thr-Phe]) were synthesized by Dr J. Murray's team (GlaxoWellcome Chemistry Unit, Department of Chemistry, University of Cambridge). All buffer salts were Fisons AR grade. Indomethacin was dissolved in 10% sodium bicarbonate and isobutyl methylxanthine in 30% ethanol, all other drugs were dissolved in distilled water. All peptides were initially dissolved in distilled water, divided into aliquots and stored at −20°C. Fresh aliquots were used on each experimental day.

Results

Responses to gastrin, dimaprit and IBMX

The cumulative administration of gastrin to the rat isolated gastric mucosa produced concentration-dependent increases in acid output (Figure 1). Successive cumulative concentration-effect curves to gastrin were very reproducible. The first gastrin concentration-effect curve had an EC_{50} of 30 nM (22–40) with a maximum secretory response of 105±16 nmol min^{-1} (n=10), whilst the second concentration-effect curve had an EC_{50} of 35 nM (26–48) and a maximum response which was 87±13% of the first maximum. Dimaprit (1–300 μM) and IBMX (0.1–100 μM) also caused concentration-dependent increases in acid output but maximum secretory responses could not be achieved with the highest concentrations used (Figure 1). Dimaprit (200 μM) produced an increase in H^+ output of 55±12 (n=4) and IBMX (100 μM) an increase of 76±12 nmol min^{-1} (n=4). The second concentration-effect curves to both dimaprit and IBMX were both very reproducible, with concentration ratios (measured at 50% of the response obtained at the highest concentration tested on the linear portion of the curves) of 2.3 (0.2–4.4) and 1.6 (0.9–2.7), respectively.

Effects of ranitidine

The selective H_2 receptor antagonist ranitidine (10 μM) caused an insurmountable antagonism of the response to gastrin (Figure 1). The maximum response to gastrin in the presence of 10 μM ranitidine was reduced to 11±6% of the response seen in its absence. Secretory responses to dimaprit were abolished in the presence of 10 μM ranitidine (Figure 1). Ranitidine (10 μM) had no effect on the secretory response to IBMX (concentration ratios were 2.2 (1.5–3.3) in the presence and 1.6 (0.9–2.7) in the absence of ranitidine).

Effects of SRIF on basal and stimulated acid secretion

Basal gastric acid secretion ranged from 16.2 nmol min^{-1} to 88.9 nmol min^{-1} over all the experimental procedures. Cu-

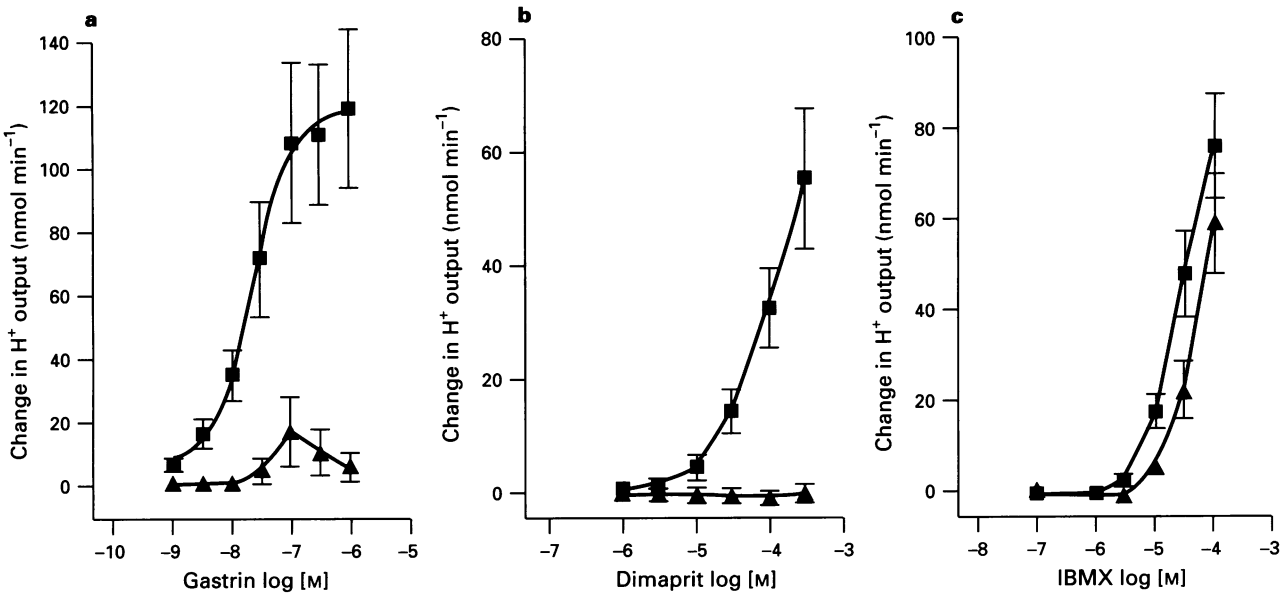

Figure 1 Cumulative concentration-effect curves to (a) gastrin, (b) dimaprit and (c) isobutyl methylxanthine (IBMX) for increasing acid secretion in rat isolated gastric mucosa in the absence (■) or presence of (▲) 10 μM ranitidine. Values are mean ± s.e.mean (vertical lines) from 4 experiments.

mulative administration of SRIF-14 (0.1 nM to 1 μM) to unstimulated mucosae had little effect on H^+ secretion. The maximum change in H^+ being only 1.7 ± 2.7 mmol min^{-1}. Stimulation of gastric acid secretion with a single concentration of gastrin (1 μM), dimaprit (200 μM) or IBMX (30 μM) produced a sustained increase in acid output which was maintained (less than 10% decline in response) for 60–70 min. SRIF-14 (1 nM–1 μM) produced a concentration-dependent inhibition of gastrin-, dimaprit- and IBMX-induced acid secretion with EC_{50} values of 46 nM (34–61), 54 nM (37–79) and 167 nM (98–283) and maximum inhibitions of 118 ± 16, 81 ± 6 and 50 ± 9%, respectively (Figure 2). The magnitude of the increases in $[H^+]$ secretion to gastrin, dimaprit and IBMX were 61 ± 14, 43 ± 6 and 56 ± 7 nmol min^{-1}, respectively (mean ± s.e. mean from 5–8 experiments). The inhibitory effects of SRIF-14 on acid secretion were well maintained throughout its presence.

Effects of peptidase inhibitors

The peptidase inhibitors, amastatin (10 μM) and phosphoramidon (1 μM), had no effect on SRIF-induced inhibition of dimaprit-induced acid secretion (Figure 3). In the presence of these inhibitors SRIF-14 had an EC_{50} of 30 nM (12–77) and caused a maximum inhibition of 77 ± 11% ($n = 6$). These values were not significantly different from values obtained in the absence of peptidase inhibitors ($P < 0.05$) (see above).

Effects of SRIF analogues

In order to characterize the receptors mediating inhibition of acid secretion, the effects of some SRIF receptor selective peptides were studied on gastrin-, dimaprit- and IBMX-induced acid secretion.

Irrespective of the secretagogue used to stimulate acid secretion, the rank orders of the SRIF agonists at inhibiting secretion were similar. Thus the sst_2 receptor selective peptides, BIM-23027 (Figure 4), seglitide and octreotide, were the most potent inhibitors of acid secretion being approximately 5–10 times more potent than SRIF-14. The sst_5 receptor selective peptide, L-362,855, was at least 10 times weaker than SRIF-14 and BIM-23056 which has a similar affinity for the human recombinant sst_3, sst_4 and sst_5 was devoid of agonist activity in concentrations up to 1 μM (Figure 4). It is, however, noteworthy that the maximum inhibitory effect of SRIF-28 and BIM-23027 was less than that of SRIF-14 when IBMX was used as the secretory agent. The quantified data from all of the agonists examined are summarised in Table 1.

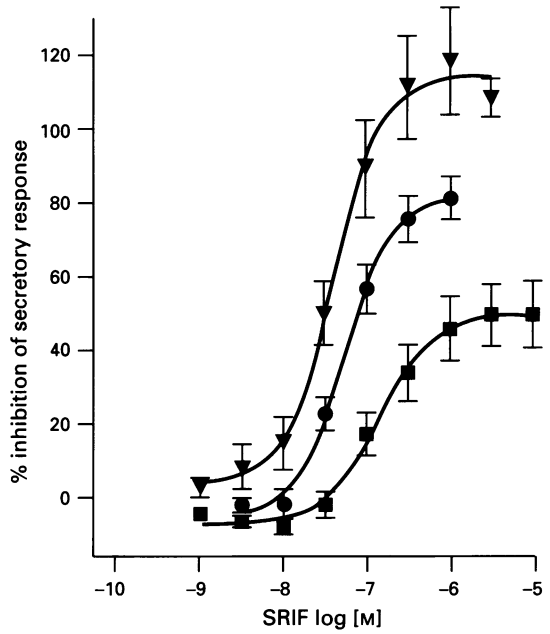

Figure 2 Comparison of the effects of SRIF-14 on gastrin- (▼), dimaprit- (●) and isobutyl methylxanthine (IBMX)- (■) induced increases in acid secretion. The EC_{50} (nM) values were 46 (34–61), 54 (37–79) and 167 (98–283), respectively. Values are mean ± s.e.mean (vertical lines) from 6, 5 and 8 experiments, respectively.

BIM-23056 as an antagonist

Since it lacked agonist activity, BIM-23056 was tested as an antagonist against SRIF-14-induced inhibitions of dimaprit-stimulated gastric acid secretion. Preincubation with BIM-23056 (1 μM) had no effect on SRIF-induced inhibition of dimaprit-induced gastric acid secretion (Figure 3).

Discussion

The ability of SRIF-14 to inhibit gastric acid secretion is well documented and involves both a direct effect on gastric parietal cells and an indirect effect involving inhibition of histamine release from gastric ECL cells (Park *et al.*, 1987; Schmidtler *et al.*, 1992; Prinz *et al.*, 1994). Although the mRNA for all five SRIF receptor subtypes have been identified in the stomach (Bruno *et al.*, 1993; Raulf *et al.*, 1994), the functional significance of each receptor subtype is still unknown. *In vivo* studies (Rossowski *et al.*, 1994; Lloyd *et al.*, 1995) with a range of SRIF receptor selective peptides have suggested that receptors similar to the recombinant sst_2 receptor mediate an inhibition of pentagastrin stimulated acid secretion in the rat. However such studies could not differentiate between effects of SRIF which could be mediated at the level of either the parietal cell, the ECL cell or both and may be additionally compromised by metabolism of these peptides in either the plasma or peripheral organs such as the lung and liver (Ruggere *et al.*, 1985; Taborsky & Ensink, 1983). Subsequent studies on rat isolated gastric ECL cells have provided good evidence for the role of the sst_2 receptor in mediating SRIF-induced inhibition of gastrin stimulated histamine release, by blocking gastrin-stimulated increases in intracellular calcium (Prinz *et al.*, 1994). In the mouse isolated perfused stomach, the metabolically more stable SRIF analogue, octreotide, which displays high affinity for the recombinant sst_2 receptor (Raynor *et al.*, 1993; Hoyer *et al.*, 1995) was ineffective at decreasing histamine-induced acid secretion (Buhl *et al.*, 1994), suggesting that SRIF receptors other than sst_2 may be important in regulating acid secretion from parietal cells.

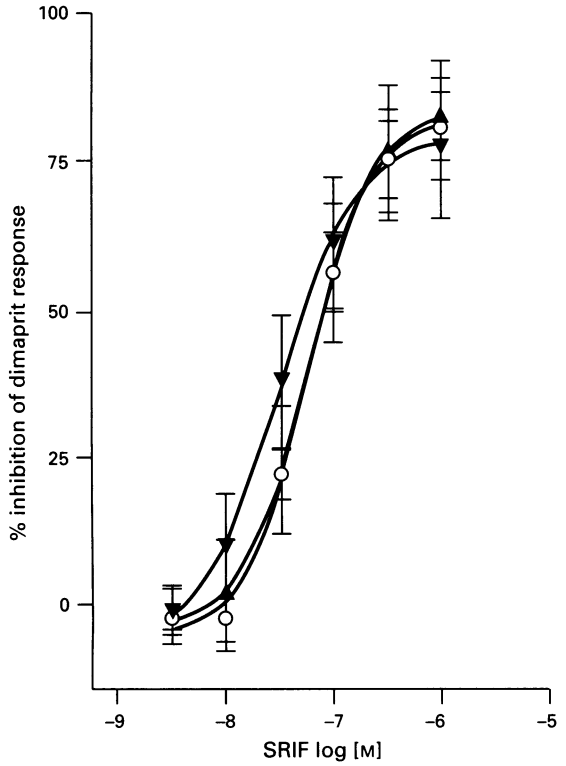

Figure 3 SRIF-14-induced inhibition of dimaprit stimulated acid secretion in the absence (○) and presence of a combination of amastatin (1 μM) and phosphoramidon (10 μM) (▼) or BIM23056 (1 μM) (▲). Values are mean ± s.e.mean (vertical lines) from 4 experiments.

In the present study we have compared the effect of SRIF-14 and a range of SRIF analogues displaying varying degrees of selectivity for different recombinant SRIF receptor subtypes on gastrin-, dimaprit- and IBMX-induced increases in acid secretion in the rat isolated gastric mucosa, in order to determine the identity of the SRIF receptor type(s) mediating an inhibition of acid secretion when stimulated at the level of either the ECL cell (gastrin) or the parietal cell (dimaprit and IBMX).

Gastrin, dimaprit and IBMX all caused concentration-dependent and stable increases in acid secretion in the rat isolated gastric mucosa. Responses to gastrin were markedly attenuated by the histamine H_2 receptor blocking drug, ranitidine, at concentrations (10 μM) which abolished the effect of the H_2 receptor agonist, dimaprit, suggesting that the effect of gastrin was largely, if not exclusively mediated indirectly *via* the release of histamine from ECL cells. Such a conclusion is supported by the observation that gastrin has no secretory effect on rat isolated parietal cells (Schmidtler *et al.*, 1992). SRIF-14 and a range of SRIF analogues which had little or no effect on basal acid output (data not shown) caused a con-

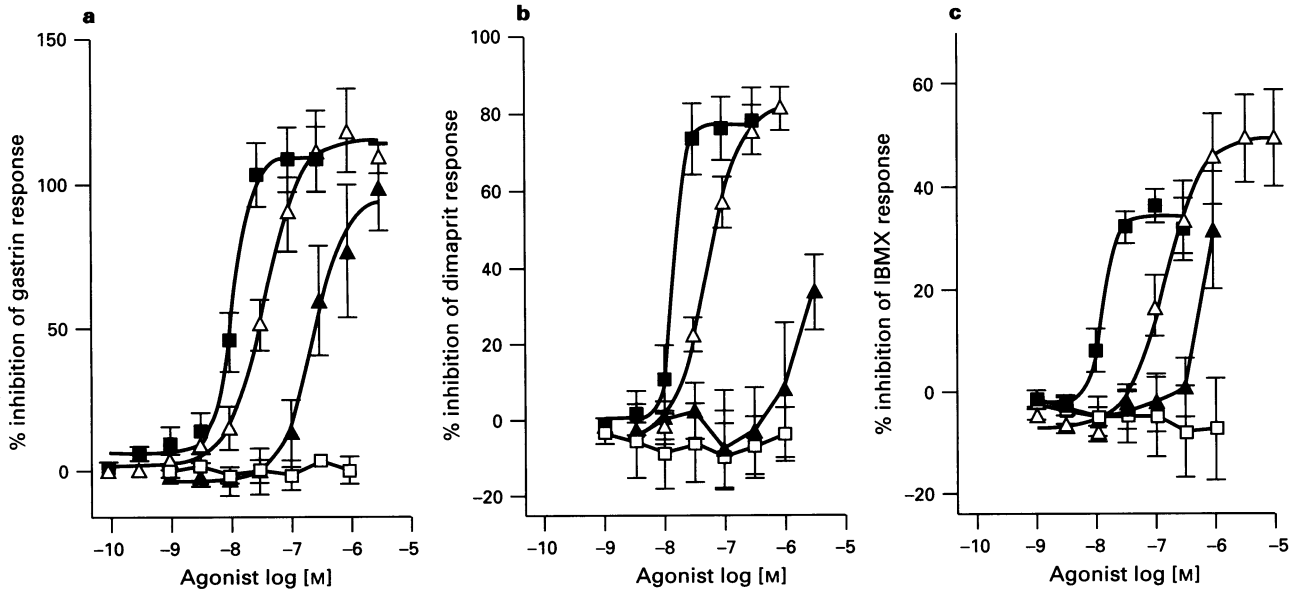

Figure 4 Inhibitory effects of SRIF-14 (△), BIM-23027 (■), L-362, 885 (▲) and BIM-23056 (□) on (a) gastrin-, (b) dimaprit- and (c) isobutyl methylxanthine (IBMX)-induced acid secretion. Values are mean ± s.e.mean (vertical lines) from 4 or more experiments.

Table 1 Potencies of somatostatin (SRIF) analogues at inhibiting gastrin-, dimaprit- and isobutyl methylxanthine (IBMX)-induced acid secretion in rat isolated gastric mucosa

	Gastrin		*Dimaprit*		*IBMX*	
Agonist	EC_{50} (nM)	*Max. % inhibition*	EC_{50} (nM)	*Max. % inhibition*	EC_{50} (nM)	*Max. % inhibition*
SRIF-14	46 (34–61)	118±16	54 (37–79)	81±6	167 (98–283)	50±9
SRIF-28	27 (14–52)	86±8*	295 (85–1023)	85±9	252 (87–729)	26±9
Seglitide	6 (4–8)	127±15	17 (11–26)	77±7	13 (4–41)	64±2
BIM-23027	11 (7–17)	108±11	16 (11–25)	78±9	11 (9–13)	36±3
Octreotide	15 (11–22)	103±9	13 (8–23)	84±6	22 (9–51)	62±11
L-362,855	428 (100–1840)	97±14	>1000	⩾36	>300	>32
BIM-23056	>1000	3±1	>1000	No inhibition	>1000	No inhibition
Angiopeptin	18 (9–36)	93±10	ND		35 (17–76)	59±11

EC_{50} values are geometric means with 95% CL, and maximum % inhibitions are means±s.e.mean of 5–8 observations. *Significantly different from SRIF-14 response ($P<0.05$) by a two tailed t test. ND, not determined.

centration-dependent inhibition of gastrin-induced acid output. The sst_2 receptor selective peptides BIM-23027, seglitide and octreotide (Raynor *et al.*, 1993) were all more potent than either of the naturally occurring forms of somatostatin, SRIF-14 and SRIF-28; at the highest concentrations tested gastrin stimulated acid secretion was abolished. The potency of SRIF-14 was not modified when experiments were repeated in the presence of the aminopeptidase inhibitors, phosphoramidon and amastatin, in concentrations which can markedly enhance potency in isolated tissue preparations (Feniuk *et al.*, 1993), suggesting that the potency of SRIF-14 was not underestimated as a consequence of peptide breakdown. Peptides such as L-362,855 which has high affinity for the recombinant sst_5 receptor and BIM-23056 which has low affinity for sst_2 receptors were either weaker inhibitors or had little effect on gastrin-mediated increased in acid output. In addition BIM-23056, which we have recently shown to be a potent antagonist on the human recombinant sst_5 receptor (Wilkinson *et al*,, 1996), was devoid of antagonistic effects on SRIF-14-induced inhibition of dimaprit-stimulated acid secretion. The results from these studies suggest that SRIF-induced inhibition of gastrin stimulated acid secretion is mediated by receptors which are similar to the recombinant sst_2 receptor and confirm the conclusions reached from earlier studies performed *in vivo* (see above). Although we did not measure the release of histamine, the results from our study would be consistent with the hypothesis that activation of sst_2 receptors on ECL-cells by SRIF-14 inhibits gastrin-induced histamine release (Prinz *et al.*, 1994). However, the effect of any histamine which reached the parietal cell would also be inhibited by SRIF through an sst_2 receptor mechanism (see below).

Although the mRNA for sst_2 receptors has been identified on ECL cells, the ability of SRIF to inhibit acid secretion induced by the H_2 receptor agonist, dimaprit, in the present study, suggests that SRIF receptors are also located on rat parietal cells. Indeed SRIF-14 has been shown to inhibit histamine and forskolin stimulated acid secretion in an enriched isolated parietal cell preparation of the rat (Schmidtler *et al.*, 1992). However, the identity of the SRIF receptor involved is unknown. Recently, mRNA for sst_2, sst_3, sst_4 and sst_5 receptors have been identified in rat isolated parietal cells (Le Romancer *et al.*, 1996). In an attempt to characterize functionally the receptor mediating inhibition of acid secretion in parietal cells, we also determined the effect of SRIF-14 and the SRIF receptor selective peptides on dimaprit- and IBMX-induced increases in acid output. The IBMX-induced increase in acid output was resistant to blockade by ranitidine suggesting that its effect was independent of histamine release and mediated by the inhibition of phosphodiesterase- catalysed breakdown of cyclic AMP produced by the basal activity of adenylyl cyclase in the parietal cell (see Welsh *et al.*, 1993). In contrast to the studies on the mouse isolated perfused stomach preparation (Buhl *et al.*, 1994), octreotide and other peptides displaying high affinity for the recombinant sst_2 receptor (BIM-23027 and seglitide) were all more potent than SRIF-14 at inhibiting dimaprit- and IBMX-induced increases in acid output in the rat isolated mucosa. This provides good evidence that receptors similar to the recombinant sst_2 receptor are also localized to the parietal cell where they mediate inhibition of acid secretion. The potencies of the agonists studied were similar against both dimaprit- and IBMX-induced increases in acid secretion, although the maximum degree of inhibition was less when IBMX was used as the secretagogue. The reason for this difference in the maxima is likely to have resulted from the larger magnitude of the secretory response to IBMX. Our initial intention was to use a similar level of secretory response for gastrin, dimaprit and IBMX and although this was achieved for gastrin and IBMX, the maximum secretory response to dimaprit at the highest concentration used was approximately 50% of the response to IBMX and gastrin. It was impractical to use lower concentrations of IBMX and gastrin, since secretory responses were not well maintained (unpublished observations). Although the magnitudes of the secretory response to gastrin and IBMX were similar, the magnitude of the inhibition produced by all of the analogues examined was lower when IBMX was used as the secretory stimulus. Although this could in part be explained by a dual inhibitory effect against gastrin-induced acid secretion in two locations, it is also possible that the SRIF receptor density is lower on the parietal cell compared with the ECL cell.

Receptors similar to the recombinant sst_2 receptor also mediate inhibition of electrogenic ion transport in rat isolated colonic mucosa (McKeen *et al.*, 1995) but it is noteworthy that the potencies of SRIF-14 and selective sst_2 ligands such as BIM-23027, seglitide and octreotide were approximately ten times greater than those observed in the present study on rat isolated gastric mucosa. In view of the fact that peptidase inhibitors did not modify the potency of SRIF-14 in the gastric mucosa, it is possible that either the receptor density in the gastric mucosa is low or that the receptors are less well coupled to the transduction-effector process. The former explanation would seem possible since we could not detect specific [^{125}I]-Tyr^{11}-SRIF binding sites in membrane preparations of the gastric mucosa (unpublished observation) whilst they could readily be detected in the colonic mucosal (McKeen *et al.*, 1995).

Although many of the peripheral actions of SRIF appear to be mediated by receptors which resemble the recombinant sst_2 receptor (McKeen *et al.*, 1994; 1995; Feniuk *et al.*, 1995), these various responses can be differentiated by their susceptibility to agonist-induced desensitization. In this respect the SRIF receptor mediating inhibition of acid secretion appears similar to the receptor inhibiting secretion in the rat colonic mucosa, where the inhibitory effect of SRIF-14 is well maintained in the continuous presence of agonist. It has been demonstrated that the mouse sst_2 receptor gene can be alternatively spliced to give

two gene products termed sst_{2a} and sst_{2b} (Vanetti *et al.*, 1992). Both of these spliced variants bind the stable cyclic peptides such as BIM-23027, seglitide and octreotide with high affinity but the sst_{2b} isoform is more resistant to agonist induced desensitization. Although sst_2 mRNA has been identified in the stomach (Bruno *et al.*, 1993; Raulf *et al.*, 1994), attempts to identify which of the splice variants exists in the stomach have not been performed. It is clearly tempting to speculate that it is the sst_{2b} receptor which mediates inhibition of acid secretion in the rat isolated gastric mucosa. The significance of the mRNA for the other SRIF receptor types found in the gastric mucosa (Le Romancer *et al*,, 1996) also remains to be determined.

In conclusion, these studies provide good evidence that SRIF receptors resembling the recombinant sst_2 receptor mediate inhibition of acid secretion in rat isolated gastric mucosa. More importantly, these receptors are localized not only on ECL cells but also on the acid secreting parietal cells.

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