British Journal of Pharmacology (1998) 125, 1081–1087
http://www.stockton-press.co.uk/bjp

Examination of somatostatin involvement in the inhibitory action of GIP, GLP-1, amylin and adrenomedullin on gastric acid release using a new SRIF antagonist analogue

[1]Wojciech J. Rossowski, [1]Beng-L. Cheng, [1] Ning-Y. Jiang & [1,2]David H. Coy

[1]Peptide Research Laboratories, Department of Medicine, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, Louisiana 70112-2699, U.S.A.

1 The effect of a new type 2 selective somatostatin (SRIF) receptor antagonist (DC-41-33) on somatostatin-induced inhibition of pentagastrin-stimulated gastric acid secretion in conscious, chronic gastric fistula equipped rats was studied.

2 Infused intravenously, DC-41-33 dose-dependently inhibits SRIF-induced inhibition of pentagastrin-stimulated gastric acid secretion with an IC_{50} of 31.6 ± 1.2 nmol kg^{-1} versus 10 nmol kg^{-1} SRIF and blocks the inhibitory effects of SRIF when simultaneously co-infused. Its effectiveness provides additional evidence that SRIF-inhibition of gastric acid release is a SRIF type 2 receptor-mediated process.

3 DC-41-33 is able to completely reverse the inhibitory effect of glucose-dependent insulinotropic polypeptides, GIP and GIP-$(1-30)NH_2$, and glucagon-like polypeptide, GLP-1(7-36)NH_2, on pentagastrin-stimulated gastric acid secretion thus confirming that they exert these effects through stimulation of endogenous SRIF release.

4 DC-41-33 only partially blocks potent amylin and adrenomedullin-induced inhibition of gastric acid secretion, therefore suggesting that somatostatin may not function as a primary mediator in the action of these peptides.

5 Our results indicate that DC-41-33, is a potent *in vivo* inhibitor of exogenous and endogenous SRIF in rats. It represents a new class of SRIF analogues which should eventually provide excellent tools for further evaluating the many physiological roles of SRIF and its five receptor subtypes.

Keywords: Somatostatin (SRIF) receptor antagonist; SRIF; GIP-(1-42); GIP-(1-30); GLP-1-(7-36)NH_2; rat amylin-(1-37); adrenomedullin-(1-52)

Introduction

In mammals, gastric acid is secreted by the gastric mucosal parietal cells in response to a variety of central and/or mucosal stimulants. Somatostatin (SRIF) is the principal peripheral and central inhibitor of gastric acid secretion and its inhibitory actions are initiated by binding to high affinity membrane-bound receptors (sst) coupled to G protein-dependent signal transduction pathways (Patel *et al*., 1994). Five subtypes of somatostatin receptors have recently been cloned (named sst_1-sst_5) and several high affinity and relatively selective agonists have been described (Raynor *et al*., 1993a; 1993b).

Using these subtype specific SRIF analogues, several important findings have been published. We have found that regulation of gastric acid secretion in rats appears to be mediated through sst_2 (Rossowski *et al*., 1994b) and this observation was later confirmed in several other analogous studies (Aurang *et al*, 1997; Fung & Greenberg, 1997; Lloyd *et al*., 1997). Recently, Martinez *et al*. (1998) using a somatostatin receptor subtype 2 knockout mice model reported that sst_2 is the main subtype whereby endogenous somatostatin suppresses gastric acid secretion through inhibition of gastrin action. It was also found that rat pancreatic glucagon and insulin secretion are selectively mediated by sst_2 and sst_5, respectively (Rossowski & Coy, 1993; 1994a).

Although studies utilizing receptor-selective SRIF agonists have been quite fruitful, they could clearly be much enhanced by the availability of SRIF receptor antagonists. Recently, Bass *et al*. (1996) described the first examples of such a class of compounds by synthesizing analogues of disulphide-bridged SRIF octapeptides with a critical D- rather than L-Cys residue in the 2 position. These analogues quite potently inhibited SRIF-mediated inhibition of cyclic AMP accumulation in a dose-dependent manner and antagonized the somatostatin-stimulated growth of yeast cells expressing the sst_2 subtype, suggesting that they were somatostatin receptor antagonists. Based on this report and previous observations, a series of cyclic and linear octapeptide analogues of SRIF, some of which could effectively block somatostatin effects both *in vitro* and *in vivo* were developed (Murphy *et al*., 1997; Coy *et al*., 1998; Hocart *et al*., 1998). One of the more potent analogues was Nal-c(DCys-Pal-DTrp-Lys-Val-Cys)-Nal-NH_2 (DC-38-48) which completely reversed SRIF (10 nmol kg^{-1} h^{-1}) induced-inhibition of pentagastrin-stimulated gastric acid secretion in conscious rats (Coy *et al*., 1998) at a dose of 5 mmol kg^{-1} h^{-1} i.v. administered. Further structural refining of these 'parental' SRIF analogues led to the formulation of a new analogue DC-41-33 having the structure Fpa-c(DCys-Pal-DTrp-Lys-Tle-Cys)-Nal-NH_2 (Hocart *et al*., manuscript in preparation). This analogue potently reversed SRIF-induced inhibition of GRF-stimulated GH release from primary cultures of rat pituitary cells with an IC_{50} of 1.5 nM. It bound competitively to membranes from CHO cells transfected with human sst_2 subtype with a K_i of 26.4 ± 3.1 nM and was quite selective for this receptor. This analogue was chosen for

[2] Author for correspondence.

further *in vivo* evaluation utilizing a typical sst_2 receptor-mediated system (inhibition of gastric acid secretion) and subsequent gastric acid studies are described in the present paper.

Although SRIF itself is believed to be the principal physiological inhibitor of gastric acid secretion, it is also known that numerous other peptide hormones might also directly or indirectly affect acid secretion. Among these additional gastrointestinal peptides, galanin, glucose-dependent insulinotropic polypeptide (GIP) and calcitonin gene-related peptide (CGRP) have long been recognized as potent gastric acid inhibitors (Brown *et al*., 1975; Tache *et al*., 1984; Rossowski & Coy, 1989). More recently, glucagon-like polypeptide [GLP-1(7-36)NH_2] was shown to be a physiological inhibitor of gastric acid secretion in man (O'Halloran *et al*., 1990) and rat (Eissele *et al*., 1990), and rat amylin and human and rat adrenomedullin were found to be a potent inhibitors of basal and stimulated gastric acid secretion (Rossowski *et al*., 1997) in rats.

In the present study, for the first time we describe potent, dose-dependent inhibitory effects of a new SRIF antagonist on exogenous and endogenous somatostatin using an *in vivo* gastric acid secretion model. In addition, this analogue is then utilized to probe endogenous SRIF involvement in the inhibitory actions of several important neuro-gastrointestinal peptides.

Methods

Peptides

Fpa-c(DCys-Pal-DTrp-Lys-Tle-Cys)-Nal-NH_2 (DC-41-33), [Fpa, 4-fluorophenylalanine; Pal, 3-pyridylalanine; Tle, tert-leucine; Nal, 3-(2-naphthyl)alanine], somatostatin-14 (SRIF), glucose-dependent insulinotropic polypeptides, GIP and GIP-(1-30), glucagon-like peptide-1(7-36)NH_2 [GLP-1-(7-36)NH_2], rat amylin (r-AMY), and human adrenomedullin-1(1-52) [ADM-(1-52)] were synthesized by standard solid phase methodologies on CS Bio Co. (San Carlos, CA, U.S.A.) model CS 136 or Advanced ChemTech (Louisville, KY, U.S.A.) model 200 automatic peptide synthesizers and purified by preparative r.p.-h.p.l.c. on C18 bonded silica gel columns (Dynamax-300A, 5 or 8 μm, 21.4 × 250 mm). The peptides were hydrolyzed in 4 M methanesulphonic acid containing 0.2% 3-(2-aminoethyl)indole and subjected to amino acid analyses performed using an automatic h.p.l.c. system (Varian, Walnut Creek, CA, U.S.A.). Molecular, weights were determined by matrix assisted laser desorption mass spectrometry using a LaserMat 2000 mass spectrometer (Finnegan MAT, San Jose, CA, U.S.A.) with substance P (1348.7 Daltons) as an internal standard. Pentagstrin (Peptavlon) was purchased from Ayerst Laboratories Inc., Philadelphia, PA, U.S.A.

Animals and experimental procedures

Adult, male CD rats (Harlan Sprague Dawley, Inc. Indianapolis, IN, U.S.A.) weighing 300–400 g were used for all experiments. They were housed under standard conditions and kept in an artificial 12 h light cycle while receiving standard Purina rat chow between experiments. Tap water was given *ad libitum*. The rats were trained in Bollman cages (Plas Labs., Lansig, MI, U.S.A.) for 1 week prior to surgery whereupon they were equipped with chronic gastric fistulae and jugular venous cannulae after an intraperitoneal (i.p.) injection of pentobarbitone (50 mg kg^{-1}; Nembutal Sodium Solution, Abbott Labs., North Chicago, IL, U.S.A.) using completely sterile surgical procedures. After surgery, rats were allowed to recover for 7 days and experiments were then performed twice a week. Each rat was used for four to six

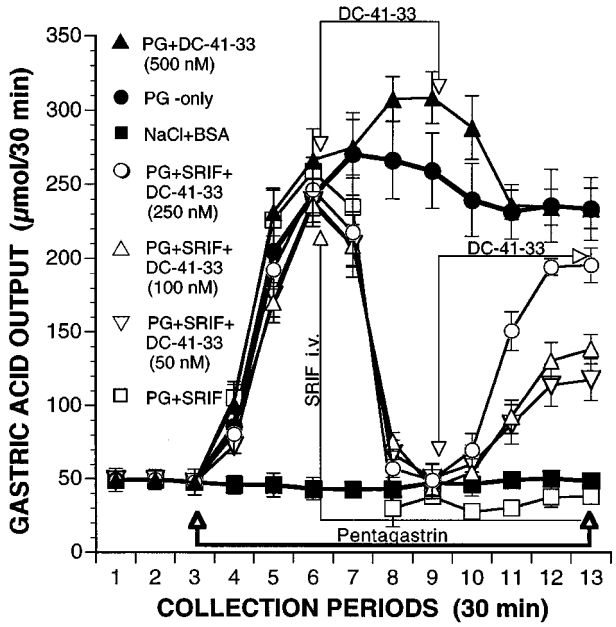

Figure 1 Effects of somatostatin 14 (SRIF) and co-infusion of somatostatin receptor antagonist (DC-41-33) on pentagastrin-stimulated gastric acid secretion. After three basal collections, pentagastrin at the concentration of 18 $\mu g\ kg^{-1}\ h^{-1}$ was i.v. infused and continued until the end of the experiment. Ninety min after pentagastrin infusion was started, i.v. infusion of somatostatin (10 nmol $kg^{-1}\ h^{-1}$) was begun and infusion was continued until the end of the experiment. After SRIF-inhibition reached the lowest level ($P<0.001$), DC-41-33 at doses of 50, 100 and 250 nmol $kg^{-1}\ h^{-1}$ was added to the i.v. infusion solution and continued until the end of the experiment. Dose-dependent inhibitory effects of somatostatin receptor antagonist DC-41-33 on SRIF-induced inhibition of pentagastrin-stimulated gastric acid secretion: 50 nmol $kg^{-1}\ h^{-1}$ ($P<0.01$), 100 nmol $kg^{-1}\ h^{-1}$ ($P<0.01$), and 250 nmol $kg^{-1}\ h^{-1}$ ($P<0.001$). Each point is the mean and vertical lines s.e.mean of six to ten experiments.

Table 1 Comparison of binding affinities of SRIF antagonist DC-41-33 with a typical type 2 and type 5 receptor agonist for the five human receptors present on transfected CHO-K1 cells

	K_i (nM)				
Peptide	$hsst_1$	$hsst_2$	$hsst_3$	$hsst_4$	$hsst_5$
Fpa-c(D-Cys-Pal-D-Trp-Lys-Tle-Cys)-Nal-NH_2 (DC-41-33)	>1000	26.4±3.1	230.5±101.5	>1000	535±116
D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH_2 (BIM-23014)	2414±81	0.75±0.1	97.9±0.5	1826±214	12.7±7.5
c[Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2 (BIM-23268)	12.1±1.3	22.3±3.9	55.5±1.1	35.5±5.7	0.42±0.07
SRIF	1.45±0.35	0.23±0.03	1.17±0.23	1.76±0.28	1.41±0.29
SRIF-28	1.97±0.47	0.39±0.06	1.27±0.29	5.0±2.55	0.40±0.05

experiments and was rotated from one 'peptide' group to another, to avoid multiple exposure to the same peptide. Rats were deprived of food but not water for 18 h prior to an experiment. They were placed in Bollman cages and the stomach was cleansed with warm saline. At this time i.v. infusion of sterile 0.9% sodium chloride was started at a constant infusion rate of 1 ml h^{-1}. After the initial 30 min collection had been discarded, three 30 min basal samples of gastric juice were collected. All gastric juice samples were collected by gravity drainage and all experiments were started at 08 h 30 min and were completed at 16 h 00 min.

Receptor expression and transfection

Expression of the cloned human somatostatin receptors, transfection into CHO-K1 cells and radioligand binding assays were kindly performed by Dr John E. Taylor, Biomeasure Inc., Milford, Massachusetts, U.S.A. as described before (Hocart *et al*., 1998).

Effects of DC-41-33 on stimulated gastric acid secretion

After three basal collections, an i.v. infusion of pentagastrin (18 μg kg^{-1} h^{-1}) was started at a constant rate (1 ml h^{-1}) and was continued for the following nine 30 min collection periods. After pentagastrin-stimulated gastric acid secretion reached a plateau (three collection periods), SRIF at a dose of 10 nmol kg^{-1} h^{-1} was added into the infusion solution for the next six to seven collection periods. After SRIF-induced inhibition of pentagastrin-stimulated gastric acid secretion reached the lowest value (three collection periods), DC-41-33 at doses of 50, 100, 250 or 1000 nmol kg^{-1} h^{-1} was added into the infusion solution and continued until the end of the experiment. In another experiment, after pentagastrin-stimulated gastric acid secretion reached a plateau, SRIF (10 nmol kg^{-1} h^{-1}) and DC-41-33 (250 or 1000 nmol kg^{-1} h^{-1}) were added into the infusion solution and i.v. infusion was continued until the end of experiment. Control rats were i.v. infused with (1) 0.9% sodium chloride solution containing 0.1% of bovine serum albumin (fraction V, Sigma Chemical Company, St. Louis MO, U.S.A.); (2) as in (1) plus pentagastrin (18 μg kg^{-1} h^{-1}) after three basal collection periods; (3) and in (2) plus SRIF (10 nmol kg^{-1} h^{-1}) after pentagastrin-induced stimulation of gastric acid secretion reached a plateau; (4) as in (3) but DC-41-33 at dose of 250 nmol kg^{-1} h^{-1} was added instead of SRIF. Samples of gastric juice were collected every 30 min and the volume and pH were measured. Samples were titrated with 0.01 M NaOH to pH 7.0 using an autotitrator (Radiometer, Copenhagen, Denmark). Results are expressed in μmol $[H^+]$ and presented as 30 min outputs.

To study the effect of DC-41-33 on GIP (10 nmol kg^{-1} h^{-1}), GIP(1-30), (50 nmol kg^{-1} h^{-1}), GLP-1-(7-36)NH_2, (1 nmol kg^{-1} h^{-1}), rat amylin (3 nmol kg^{-1} h^{-1}); and human adrenomedullin (10 nmol kg^{-1} h^{-1}) induced inhibition of pentagastrin-stimulated gastric acid secretion, the same protocol as described above for SRIF was employed.

Statistical analysis

Results of the effects of DC-41-33 on inhibition of pentagastrin-stimulated gastric acid secretion by SRIF, GIP, GIP-(1-30), GLP-1-(7-36)NH_2, amylin, and adrenomedullin were

Table 2 Comparison of DC-41-33 effects on SRIF-, GIP-(1-42)-, GIP-(1-30)-, GLP-1-, amylin- and adrenomedullin-induced inhibition of pentagastrin-stimulated gastric acid secretion in conscious rats

Peptide	*Gastric acid output* (μmol/30 min)*	*% Inhibition***	P *value*
Control	52.7 ± 1.1 (n = 75)		
Pentagastrin (PG) (18 μg kg^{-1} h^{-1})	256.7 ± 1.4 (n = 50)		
PG + SRIF (10 nmol kg^{-1} h^{-1})	38.0 ± 1.7 (n = 10)	107.2	< 0.001
PG + SRIF + DC-41-33 (250 nmol kg^{-1} h^{-1})	187.0 ± 13.1 (n = 12)	34.2	< 0.001
PG + SRIF + DC-41-33 (1000 nmol kg^{-1} h^{-1})	273.0 ± 9.1 (10)	0	> 0.05
PG + GIP-(1 – 42) (10 nmol kg^{-1} h^{-1})	33.8 ± 8.3 (n = 6)	109.3	< 0.001
PG + GIP-(1 – 42) + DC-41-33 (500 nmol kg^{-1} h^{-1})	216.5 ± 14.5 (n = 6)	19.7	< 0.001
PG + GIP-(1 – 30) (50 nmol kg^{-1} h^{-1})	51.6 ± 11.0 (n = 8)	100.5	< 0.001
PG + GIP-(1 – 30) + DC-41-33 (1000 nmol kg^{-1} h^{-1})	228.0 ± 12.7 (n = 12)	14.1	< 0.001
PG + GLP-1 (1 nmol kg^{-1} h^{-1})	76.2 ± 7.8 (n = 6)	88.5	< 0.001
PG + GLP-1 + DC-41-33 (1000 nmol kg^{-1} h^{-1})	184.8 ± 10.5 (n = 10)	35.3	< 0.001
PG + GLP-1 + DC-41-33 (1500 nmol kg^{-1} h^{-1})	239 ± 14.5 (n = 6)	8.4	> 0.05
PG + rAMY (3 nmol kg^{-1} h^{-1})	5.0 ± 1.1 (n = 6)	123.4	< 0.001
PG + rAMY + DC-41-33 (1000 nmol kg^{-1} h^{-1})	116.2 ± 6.0 (n = 8)	68.9	< 0.001
PG + hADM (10 nmol kg^{-1} h^{-1})	29.8 ± 7.0 (n = 10)	111.2	< 0.001
PG + hADM + DC-41-33 (1000 nmol kg^{-1} h^{-1})	129.3 ± 10.8 (n = 10)	62.5	< 0.001

*The values represents the means ± s.e.mean of gastric acid output (H^+ μmol/30 min) collected during the ninth collection period.
**Percent of inhibition calculated according to Rhee *et al*. (1991).

analysed using one-way analysis of variance (ANOVA) accompanied by the Bonferroni multiple comparisons test, if applicable (InStat Biostatistics, GraphPad Software). Student's paired *t*-test was used to compare means between two groups. Results are presented as means ± s.e.mean. A probability level of random difference $P<0.05$ was considered significant. IC_{50} values were expressed as the mean of values estimated by non-linear regression analysis of the concentration response curves using the GraphPad computer programme.

Results

Comparison of binding affinities of the DC-41-33 antagonist analogue to cell membranes of the five CHO-K1 cell types expressing human somatostatin receptor subtypes sst_1-sst_5 indicate preferential binding to the $hsst_2$, while antagonist affinity to the $hsst_3$ and $hsst_5$ was nine and more than 21 times lower, respectively, and is compared with the very high affinity to a typical type 2 receptor agonist (BIM-23014, Table 1).

Pentagastrin infused intravenously at a dose of 18 μg kg^{-1} h^{-1} stimulated gastric acid secretion from a basal value of 52.7 ± 1.1 μmol^{-1} 30 min ($n=75$) to 270.5 ± 27.7 ($n=10$) μmol^{-1} 30 min ($P<0.0001$) and continued to stay at the elevated level with only a slight decrease throughout the 4 h experiment (Figure 1). Somatostatin-14 infused i.v. at doses of 1, 3 and 10 nmol kg^{-1} h^{-1}, dose- and time-dependently inhibited pentagastrin-stimulated gastric acid secretion ($P<0.001$) in conscious, chronic gastric fistulae equipped rats with an IC_{50} value of 0.83 ± 0.22 nmol kg^{-1} h^{-1}.

Intravenous infusion of DC-41-33 dose-dependently reversed inhibitory effects of SRIF on pentagastrin-stimulated gastric acid secretion (Figure 1) with an IC_{50} value of 31.6 ± 1.2 nmol kg^{-1} h^{-1} vs SRIF 10 nmol kg^{-1} h^{-1} ($P<0.001$) and, at the highest tested dose of 1000 nmol kg^{-1} h^{-1}, reached the control pentgastrin-stimulated gastric acid secretion level ($P>0.05$) thus displaying complete blockade of the effect of SRIF (Table 2). In addition, when DC-41-33 was infused alone into pentagastrin-stimulated rats at doses of 500 nmol kg^{-1} h^{-1} there was consistent additional but non-significant elevation of the pentagastrin-stimulated gastric acid secretion above pentagastrin-alone levels (Figure 1).

Intravenous infusion of porcine GIP-(1-42) dose- and time-dependently inhibited pentagastrin-stimulated gastric acid secretion ($P<0.001$) with an IC_{50} of 0.96 ± 0.26 nmol kg^{-1} h^{-1}. Although significantly less potent in inhibiting gastric acid secretion than its parent peptide, GIP-(1-30) also significantly inhibited pentagastrin-stimulated gastric acid secretion ($P<0.001$) ($IC_{50}=10.0\pm3.1$ nmol kg^{-1} h^{-1}). The inhibitory effects of both peptides could be reversed or prevented by late or early i.v. infusion of DC-41-33 at doses of 500 and 1000 nmol kg^{-1} h^{-1}, respectively ($P<0.001$) (Figure 2 and 3 and Table 2). GLP-1(7-36)NH_2 was i.v. infused at doses of 0.1, 0.5, 1 and 3 nmol kg^{-1} h^{-1} and significantly inhibited pentagastrin-stimulated gastric acid secretion in a dose- and time-dependent manner (figure not shown) with an IC_{50} of 0.33 ± 0.1 nmol kg^{-1} h^{-1}. Infusion of DC-41-33 (1000 nmol kg^{-1} h^{-1}) together with GLP-1-(7-36)NH_2 (1 nmol kg^{-1} h^{-1}) after pentagastrin-stimulated gas-

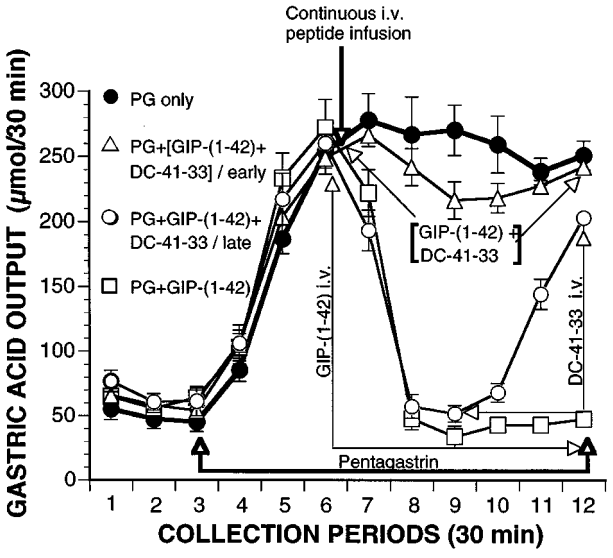

Figure 2 Effect of somatostatin receptor antagonist DC-41-33 on glucose-dependent insulinotropic polypeptide [GIP-(1-42)]-induced inhibition of pentagastrin-stimulated gastric acid secretion. After three basal collections of gastric juice, pentagastrin (18 μg kg^{-1} h^{-1}) was i.v. infused for the following 4.5 h. After maximal stimulation was reached (three collection periods), i.v. infusion of porcine GIP-(1-42) at dose of 10 nmol kg^{-1} h^{-1} was added into the solution and infusion was continued until the end of the experiment. The effect of SRIF receptor antagonist DC-41-33 on GIP-(1-42)-induced inhibition of pentagastrin-stimulated gastric acid secretion was examined by two modes of infusion: simultaneously with GIP-(1-42), to block inhibitory effect of GIP-(1-42), or after maximal GIP-(1-42)-induced inhibition of pentagastrin-stimulated gastric acid secretion was reached (three collection periods) to demonstrate that GIP-induced inhibitory effect is reversible. DC-41-33 (500 nmol kg^{-1} h^{-1}) almost completely blocked early or late inhibitory effect of GIP-(1-42) on pentagastrin-stimulated gastric acid secretion ($P<0.001$). Each point is the mean and vertical lines s.e.mean of six experiments.

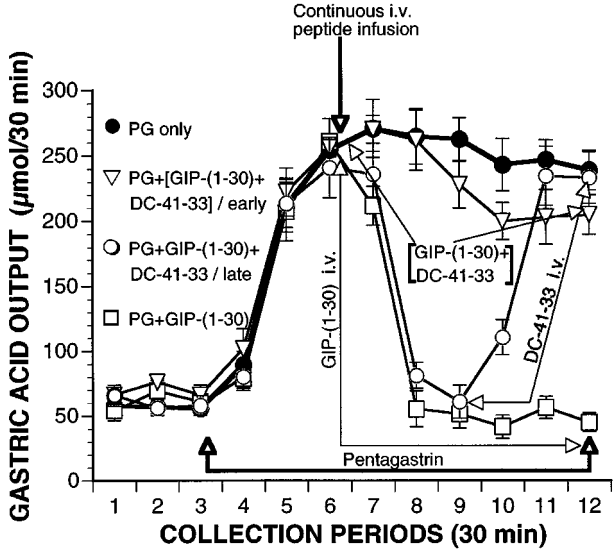

Figure 3 Effect of somatostatin receptor antagonist DC-41-33 on GIP-(1-30)-induced inhibition of pentagastrin-stimulated gastric acid secretion. After three basal collections of gastric juice, pentagastrin (18 μg kg^{-1} h^{-1}) was i.v. infused for the following 4.5 h. After maximal stimulation of gastric acid was reached (three collection periods), i.v. infusion of GIP-(1-30) (50 nmol kg^{-1} h^{-1}) was added and continued until the end of the experiment. After maximal inhibition was reached, DC-41-33 (1000 nmol kg^{-1} h^{-1}) was added and continued until the end of the experiment (late effect). In separate experiments, 90 min after pentagastrin infusion was started, GIP-(1-30) (50 nmol kg^{-1} h^{-1}) was co-infused together with DC-41-33 (1000 nmol kg^{-1} h^{-1}) until the end of the experiment (early effect). Co-infusion of DC-41-33 (1000 nmol kg^{-1} h^{-1}) together with GIP-(1-30), or after GIP-(1-30)-induced inhibition of pentagastrin-stimulated gastric acid have reached the maximum, almost completely prevented inhibitory action of GIP-(1-30), ($P<0.001$). Each point is the mean and vertical lines s.e.mean of 12 experiments.

tric acid secretion had reached a plateau, significantly inhibited effect of the latter ($P<0.001$) and at dose of 1500 nmol kg^{-1} h^{-1} reaches the level of the pentagastrin-stimulated gastric acid secretion (control) ($P<0.05$) and completely blocked the inhibitory effect of glucagon-like peptide-1 (Figure 4 and Table 2).

Intravenous infusion of rat amylin at doses of 0.1–3.0 nmol kg^{-1} h^{-1} in the same pentagastrin-induced stimulated model resulted in rapid and extraordinarily potent inhibition of gastric acid secretion ($IC_{50}=0.088\pm0.023$ nmol kg^{-1} h^{-1}). After pentagastrin-stimulated gastric acid secretion reached the lowest amylin-inhibited level, i.v. infusion of DC-41-33 (1000 nmol kg^{-1} h^{-1}) resulted in time-dependent, statistically significant ($P<0.0004$), but only partial recovery of amylin-inhibited pentagastrin-stimulated gastric acid secretion (Figure 5). Similarly, DC-41-33 (1000 nmol kg^{-1} h^{-1}) i.v. co-infused with rat amylin (3 nmol kg^{-1} h^{-1}) after pentagastrin-stimulation of gastric acid secretion reached a plateau, only attenuates but not reverses the inhibitory effect of amylin (Figure 5 and Table 2).

Human adrenomedullin also potently inhibited pentagastrin-stimulated gastric acid secretion ($IC_{50}=1.67\pm0.11$ nmol kg^{-1} h^{-1}) and DC-41-33 (1000 nmol kg^{-1} h^{-1}) when co-infused with 10 nmol kg^{-1} h^{-1} adrenomedullin was able to only attenuate the inhibitory effect of adrenomedullin and at the used doses never could reach control pentagastrin-stimulated gastric acid level (Figure 6 and Table 2).

Discussion

DC-41-33 is a new SRIF-receptor antagonist with the sequence, Fpa-c(DCys-Pal-DTrp-Lys-Tle-Cys)-Nal-NH_2. As already mentioned, it is one of the most potent antagonist analogues that we have examined (Hocart *et al*. manuscript in preparation), benefitting from the optimum side-chain structures of the aromatic amino acids in positions 1 and 8 and the presence of 3-pyridylalanine (Pal) and tert-leucine (Tle) within the peptide ring. The hydrophilic, basic amino acid, Pal, also has the advantage of improving the solubility of the peptide - an important consideration for the present *in vivo* studies where high peptide concentrations were often required. No acute toxicity was observed with the analogue even at very high infusion doses. The *in vivo* dose response studies with this peptide closely parallel results observed using *in vitro* inhibition of SRIF effects on isolated rat pituitary cells. Thus, its *in vivo* potency is more then ten times higher than a previous analogue investigated in this rat gastric acid assay system (Coy *et al*, 1998) making it an excellent choice for further examining some of the physiological roles of SRIF previously approached through the use of immunoneutralization procedures. Its ability to so efficiently block SRIF effects on gastric acid secretion support it being a rat sst_2 receptor antagonist in accordance with its selectivity for the human sst_2 receptor on transfected cells (Table 1).

The value of this type of compound is illustrated in the subsequent work aimed at demonstrating its utility in confirming or eliminating endogenous SRIF as a mediator of the inhibitor effects which several other neuro-gastrointestinal peptides are known to have on gastric acid release. One of these was GIP (Brown *et al*., 1975) which has long been known to have quite potent inhibitory effects on gastric acid secretion

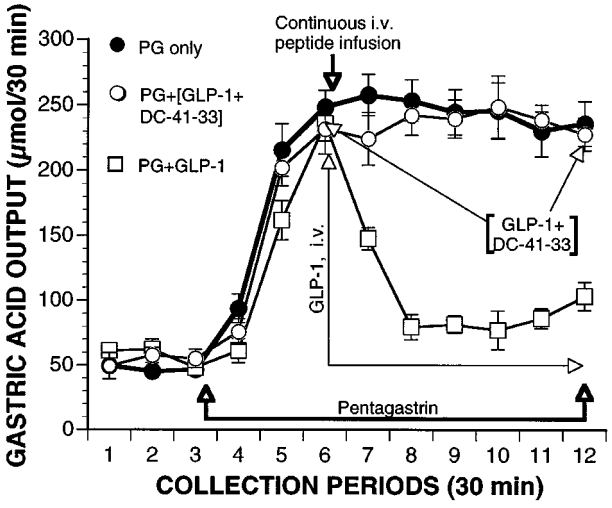

Figure 4 Effect of SRIF receptor antagonist DC-41-33 on glucagon-like peptide-1 [GLP-1-(7-36)NH_2]-induced inhibition of pentagastrin-stimulated gastric acid secretion. After three basal collections of gastric acid juice, pentagastrin (18 μg kg^{-1} h^{-1}) was i.v. infused for the following 4.5 h. After maximal stimulation of gastric acid was reached, i.v. infusion of GLP-1-(7-36)NH_2 at dose of 1.0 nmol kg^{-1} h^{-1}, was begun and continued until the end of experiments. GLP-1-(7-36)NH_2 at dose of 1 nmol kg^{-1} h^{-1} potently inhibited the pentagastrin-induced gastric acid secretion (88%), ($P<0.001$). DC-41-33 at dose of 1500 nmol kg^{-1} h^{-1} was co-infused with GLP-1-(7-36)NH_2 (1.0 nmol kg^{-1} h^{-1}) until the end of the experiment. DC-41-33 at dose of 1500 nmol kg^{-1} h^{-1}, completely blocks inhibitory action of glucagon-like peptide-1. Each point is the mean and vertical lines s.e.mean of six experiments.

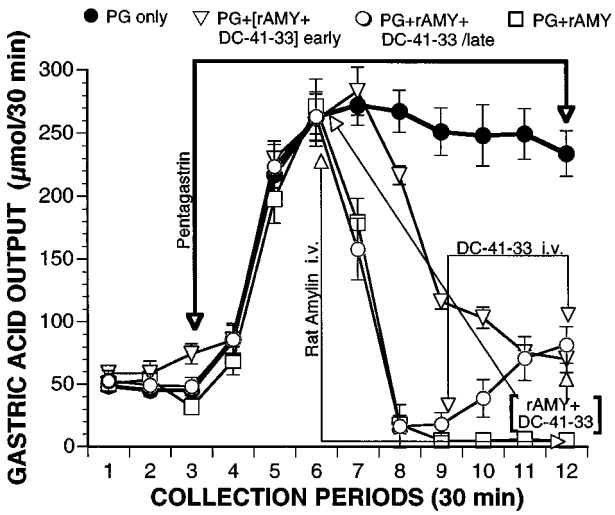

Figure 5 Effect of somatostatin receptor antagonist DC-41-33 on rat amylin-induced inhibition of pentagastrin-stimulated gastric acid secretion. After three basal gastric juice collection periods, pentagastrin (18 μg kg^{-1} h^{-1}) was i.v. infused, and continued until the end of the experiment. After pentagastrin stimulation of gastric acid secretion have reached a plateau, rat amylin [rAMY-(1-37)], at dose of 3 nmol kg^{-1} h^{-1} was added into the infusion solution and the infusion was continued until the end of the experiment. The effect of SRIF-receptor antagonist on rat amylin-induced inhibition of pentagastrin-stimulated gastric acid secretion was tested after rat-amylin-induced gastric acid inhibition had reached its lowest value, DC-41-33 (1000 nmol kg^{-1} h^{-1}) was then added to the infusion solution and infusion was continued until the end of the experiment (late effect). In separate experiments, when pentagastrin stimulation had reached a plateau r-AMY-(1-37) (3 nmol kg^{-1} h^{-1}) was co-infused with DC-41-33 (1000 nmol kg^{-1} h^{-1}) until the end of experiment (early effect). DC-41-33 (1000 nmol kg^{-1} h^{-1}) in both experimental conditions partially reverses the inhibitory effect of rat amylin on pentagastrin-stimulated gastric acid secretion ($P<0.01$). Each point is the mean and vertical lines s.e.mean of six to eight separate experiments.

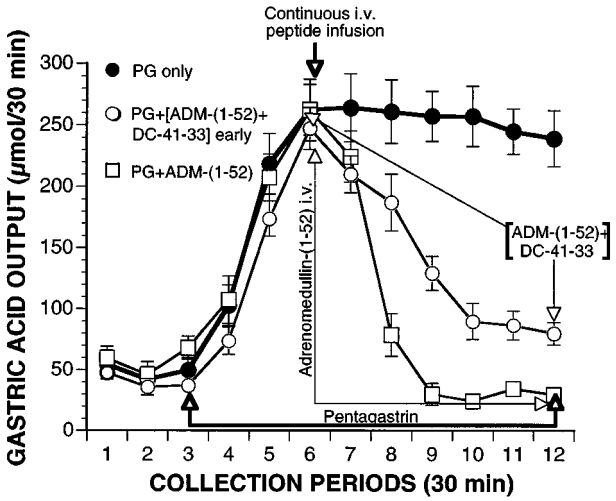

Figure 6 Effect of somatostatin receptor antagonist DC-41-33 on adrenomedullin-induced inhibition of pentagastrin-stimulated gastric acid secretion. After three basal gastric juice collection periods, pentagastrin i.v. infusion (18 μg kg^{-1} h^{-1}) was started and continued for the rest of the experiment. Ninety min after pentagastrin stimulated was started, adrenomedullin-(1-52) at dose of 10 nmol kg^{-1} h^{-1} was added alone or together with DC-41-33 (1000 nmol kg^{-1} h^{-1}) to the infusion solution and infusion was continued until the end of experiment. Addition of SRIF receptor antagonist DC-41-33 at dose of 1000 nmol kg^{-1} h^{-1} at the early stage of adrenomedullin infusion results in partial, but significant attenuation of ADM-induced inhibition of pentagastrin-stimulated gastric acid secretion ($P=0.002$). Each point is the mean and vertical lines s.e.mean of ten experiments.

from several species whilst actually potentiating insulin secretion. There appears to be no direct proof of SRIF mediation of the inhibitory gastric acid effect, although this has been inferred from studies (Holst *et al*., 1983) revealing GIP-stimulated SRIF release, presumably from antral D-cells, and reduced gastric acid and gastrin release from perfused pig antrum which occurred when physiological concentrations of GIP were administered. In the present study the ability of the SRIF antagonist to readily abolish the inhibitory gastric acid response (Figure 2) fully supports this conclusion and suggests no direct effect of GIP on the parietal cells. The GIP analogue, GIP(1-30)NH_2, was reported (Rossowski & Coy, 1994a) to have relatively more potent effects on insulin rather than gastric acid secretion. As expected, DC-41-33 also blocked the inhibitory effects of this fragment on stimulated gastric acid release.

GLP-1-(7-36)NH_2 is another perhaps more important gastrointestinal/pancreatic peptide which also inhibits gastric acid secretion (Schjoldager *et al*., 1989; O'Halloran *et al*., 1990) whilst acting as a potent incretin (Holst *et al*., 1987). As with GIP, it has been shown that GLP-1 peptides stimulate gastric SRIF release in rats (Eissele *et al*., 1990) and it is thus inferred that SRIF mediates the inhibitory effects of GLP-1 on gastric acid release. Our results showing complete blockade of GLP-1 effects on stimulated gastric acid release (Figure 4) by the specific SRIF antagonist confirms this conclusion and, once again, suggests no direct effect of GLP-1(7-36)NH_2 on the acid secreting parietal cells. This is particularly interesting since GLP-1 peptides have recently been shown (Fung *et al*., 1998) to be actual physiological mediators of fat-induced inhibition of gastric acid release in dogs suggesting a cascade of events ultimately controlling gastric SRIF release.

More recent players in the complicated field of gastric acid regulation are the members of the CGRP family of peptides which include amylin (Cooper *et al*., 1989) and the recently discovered adrenomedullin (Kitamura *et al*., 1993). Amylin and adrenomedullin have been shown to be present throughout the gastrointestinal tract of rats and human and to be present in major populations of SRIF and/or serotonin-staining cells in the gastric mucosa (Kaneko *et al*., 1996; Miyazato *et al*., 1991; Mulder *et al*., 1994; 1996). We have recently reported that CGRP itself, rat amylin and human and rat adrenomedullin are potent inhibitors of basal and stimulated gastric acid secretion (Rossowski *et al*., 1997) in the same conscious rat model used in the present study. It has also been reported that amylin administered peripherally or intracerebroventricularly suppresses gastric acid secretion in a dose-dependent manner in anaesthetized, pylorus-ligated rats and that centrally administered amylin significantly reduces gastric acid secretion in cystamine-induced somatostatin-depleted rats, suggesting that amylin acts independently from somatostatin (Guidobono *et al*., 1994). On the other hand, results presented by Makhlouf *et al*. (1996) on antral mucosal segments from human, dog, and rat stomach suggests that release of amylin from somatostatin cells enhances somatostatin secretion that leads to inhibition of gastrin release.

None of these studies was able to pinpoint an exact mechanism of action for these potent effects, although they could involve the same receptor since all members of this family of peptides appear to have profoundly overlapping receptor binding profiles to presumably multiple as yet uncharacterized receptors. The SRIF antagonist, whilst having a partial inhibitory effect on the inhibitory action of amylin and adrenomedullin on stimulated gastric acid release, was unable (Figures 5 and Figure 6) to totally overcome this at high infusion doses (1000 nmol kg^{-1} h^{-1}). Our study suggests, therefore, that any effects of amylin or adrenomedullin on gastric acid production are not primarily SRIF-mediated and could be due to direct effects on gastrin and/or histamine release. We conclude that the action of these peptides is quite different from either GIP or GLP-1 and this has interesting implications for the mechanisms maintaining gastric acid/gastrin homeostasis.

We would like to thank John E. Taylor, Ph D, Biomeasure Inc., Milford, Massachusetts, U.S.A. for performing binding affinity analysis of DC-41-33 for the five human somatostatin receptor subtypes.

References

AURANG, K., WANG, J. & LLOYD, K.C.K. (1997). Somatostatin inhibition of acid and histamine release by activation of somatostatin receptor subtype 2 receptors in rats. *J. Pharmacol. Exp. Ther.*, **281,** 245–252.

BASS, R.T., BUCKWALTER, B.L., PAUSCH, M.H., PRICE, L.A., STRNAD, J. & HADCOCK, J.R. (1996). Identification and characterization of novel somatostatin antagonists. *Mol. Pharmacol.*, **50,** 709–715.

BROWN, J.C., DRYBURGH, J.R., ROSS, S.A. & DUPRE, J. (1975). Identification and actions of gastric inhibitory polypeptide. *Recent. Prog. Horm. Res.*, **31,** 487–532.

COOPER, G.J.S., DAY, A.J., WILLIS, A.C., ROBERTS, A.N. & REID, K.B.M. (1989). Amylin and the amylin gene: structure, function and relationship to islet-amyloid polypeptide and to diabetes mellitus. *Biochim. Biophys. Acta,* **1014,** 247–258.

COY, D.H., MURPHY, W.A., ROSSOWSKI, W.J., HOCART, S.J., JAIN, R., FUSELIER, J. & TAYLOR, J.E. (1998). Somatostatin receptor antagonists based on a mixed neuromedin B antagonist/somatostatin agonist. 15th American Peptide Symposium. In press.

EISSELE, R., KOOP, H. & ARNOLD, R. (1990). Effect of glucagon-like peptide-1 on gastric somatostatin and gastrin secretion in the rat. *Scand. J. Gastroenterol.,* **25,** 449–454.

FUNG, L.C., CHISHOLM, C. & GREENBERG, G.R. (1998). Glucagon-like peptide1-(7-36) amide and peptide YY mediate introduodenal fat-induced inhibition of acid secretion in dogs. *Endocrinology,* **139,** 189–194.

FUNG, L.C. & GREENBERG, G.R. (1997). Characterization of somatostatin receptor subtypes mediating inhibition of nutrient-stimulated gastric acid and gastrin in dogs. *Regulatory Peptides,* **68,** 197–203.

GUIDOBONO, F., COLUZZI, M., PAGANI, F., PACILE, A. & NETTI, C. (1994). Amylin given by central and peripheral routes inhibits acid gastric secretion. *Peptides,* **15,** 699–702.

HOLST, J.J., ORSKOV, C., NIELSEN, O.V. & SCHWARTZ, T.W. (1987). Truncated glucagon-like peptide-1, an insulin-releasing hormone from the distal gut. *FEBS Letts.,* **211,** 169–173.

HOLST, J.J., JENSEN, S.L., KNUHTSEN, S., NIELSEN, O.V. & REHFELD, J.F. (1983). Effect of vagus, gastric inhibitory polypeptide, and HCl on gastrin and somatostatin release from perfused pig antrum. *Am. J. Physiol. (Gastrointest. Liver Physiol.)* **7,** G515–G522.

HOCART, S.J., JAIN, R., MURPHY, W.A., TAYLOR, J.E., MORGAN, B. AND COY, D.H. (1998). Potent antagonists of somatostatin, synthesis and biology, *J. Med. Chem.,* **41,** 1146–1154.

KANEKO, H., RHUE, N., NAGAI, N., MORI, S., YAMASHITA, K., YAMAGUSHI, C., TACHE, Y. & MITSUMA, T. (1996). Central distribution and action of adrenomedullin (AM) to induce gastric protection against ethanol in rats, Abstract #2097. *Digestive Disease Week,* San Francisco, May 19–22. A-525.

KITAMURA, K., KANGAWA, K., KAWAMOTA, M., ICHIKI, Y., NAKAMURA, S., MATSUO, H. & ETO, T. (1993). Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res., Commun.,* **192,** 553–560.

LLOYD, K.C.K., AMIRMOAZZAMI, S., FRIEDIK, F., CHEW, P. & WALSH, J.H. (1997). Somatostatin inhibits gastrin release and acid secretion by activating sst2 in dogs. *Am. J. Physiol.,* **272,** G1481–G1488.

MAKHLOUF, P.C., ZAKI, M., HARRINGTON, L., MCCUEN, R. & SCHUBERT, M.L. (1996). Endogenous amylin stimulates somatostatin (SST) and inhibits gastrin secretion from the antrum of human, dog and rat stomach. *Gastroenterology,* **110,** A1096.

MARTINEZ, V., CURI, A.P., TORKIAN, B., SCHAEFFER, J.M., WILKINSON, H.A., WALSH, J.H. & TACHE, Y. (1998). High basal gastric acid secretion in somatostatin receptor subtype 2 knockout mice. *Gastroenterology,* **114,** 1125–1132.

MIYAZATO, M., NAKAZATO, M., SHIOMI, K. (1991). Identification and characterization of islet amyloid polypeptide in mammalian gastrointestinal tract. *Biochem. Biophys. Res. Commun.,* **181,** 293–300.

MULDER, H., AHREN, B., KARLSSON, S., SUNDLER, F. (1996). Adrenomedullin: Localization in the gastrointestinal tract and effects on insulin secretion. *Regul. Pept.,* **62,** 107–112.

MULDER, H., LINDH, A.C., EKBLAD, E., WESTERMARK, P. & SUNDLER, F. (1994). Islet amyloid polypeptide is expressed in endocrine cells of the gastric mucosa in the rat and mouse. *Gastroenterology,* **107,** 712–719.

MURPHY, W.A., ROSSOWSKI, W.J. & COY, D.H. (1997). Octapeptide somatostatin (SRIF) analogs with in vitro and in vivo antagonist activity in the rat. 79th Annual Meeting The Endocrine Society, June 11–14, 1997, Minneapolis, Minnesota. Program @ Abstracts, 18p.147, 1–49.

O'HALLORAN, D.J., NIKOU, G.C., KREYMANN, B., GHATEI, M.A. & BLOOM, S.R. (1990). Glucagon-like peptide-1 (7-36)-NH2: a physiological inhibitor of gastric acid secretion in man. *J. Endocrinology,* **126,** 169–173.

PATEL, Y.C., GREENWOOD, M.T., WARSZYNSKA, A., PANETTA, R. & SRIKANT, C.B. (1994). All five cloned human somatostatin receptors (hSSTR1-5) are functionally coupled to adenylyl cyclase. *Biochem. Biophys. Res. Commun.,* **198,** 605–612.

RAYNOR, K., MURPHY, W.A., COY, D.H., TAYLOR, J.E., MOREAU, J.-P., YASUDA, K., BELL, G.L. & REISINE, T. (1993a). Cloned somatostatin receptors: Identification of subtype-selective peptides and demonstration of high affinity binding of linear peptides. *Mol. Pharmacol.,* **43,** 838–844.

RAYNOR, K., O'CARROL, A.M., KONG, H., YASUDA, K., MAHAN, L.C., BELL, G.I. & REISINE, T. (1993b). Characterization of cloned somatostatin receptors SSTR4 and SSTR5. *Mol. Pharmacol.,* **44,** 380–384.

RHEE, J.C., CHANG, T.M., LEE, K.V., JO, Y.H. & CHEY, W.Y. (1991). Mechanism of oleic acid-induced inhibition of gastric acid secretion in rats. *Am. J. Physiol.,* **260,** G564–G568.

ROSSOWSKI, W.J., JIANG, N.-Y. & COY, D.H. (1997). Adrenomedullin, amylin, calcitonin gene-related peptide and their fragments are potent inhibitors of gastric acid secretion in rats. *Eur. J. Pharmacol.,* **336,** 51–63.

ROSSOWSKI, W.J. & COY, D.H. (1994a). Specific inhibition of rat pancreatic insulin or glucagon release by receptor-selective somatostatin analogs. *Biochem. Biophys. Res. Commun.,* **205,** 341–346.

ROSSOWSKI, W.J., GU, Z.-F., AKARCA, U.S., JENSEN, R.T. & COY, D.H. (1994b). Characterization of somatostatin receptor subtypes controlling rat gastric acid and pancreatic amylase release. *Peptides,* **15,** 1421–1424.

ROSSOWSKI, W.J. & COY, D.H. (1993). Potent inhibitory effect of a type four receptor-selective somatostatin analog on rat insulin release. *Biochem. Biophys. Res. Commun.,* **197,** 366–371.

ROSSOWSKI, W.J. & COY, D.H. (1989). Inhibitory action of galanin on gastric acid secretion in pentobarbital-anesthetized rats. *Life Sci.,* **44,** 1807–1813.

SCHJOLDAGER, B.G.T., MORTENSEN, P.E., CHRISTIANSEN, J., ORSKOV, C. & HOLST, J.J. (1989). GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. *Dig. Dis. Sci.,* **35,** 703–708.

TACHE, Y., PAPPAS, T., LAUFFENBURGER, M., GOTO, Y., WALSH, J.H. & DEBAS, H. (1984). Calcitonin gene-related peptide: Potent peripheral inhibitor of gastric acid secretion in rats and dogs. *Gastroenterology,* **87,** 344–349.

(Received April 30, 1998
Revised August 10, 1998
Accepted August 11, 1998)