ation of a tumor-derived human pancreastatin-related protein. *Biochem Biophys Res Commun* 1989, **164**, 141–148.
29. Flanagan T, Taylor L, Poulter L, Viveros OH, Diliberto EJ. A novel 1745-dalton pyroglutamyl peptide derived from chromogranin B is in the bovine adrenomedullary chromaffin vesicle. *Cell Mol Neurobiol* 1990, **10**, 507–523.
30. Galindo E, Rill A, Bader M-F, Aunis D. Chromostatin,a 20-amino acid peptide derived from chromogranin A, inhibits chromaffin cell secretion. *Proc Natl Acad Sci USA* 1991, **88**, 1426–1430.

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Inhibition of Gastrin-stimulated Growth of Gastrointestinal tumour cells by Octreotide and the Gastrin/Cholecystokinin Receptor Antagonists, Proglumide and Lorglumide

Susan A. Watson, David L. Morris, Lindy G. Durrant, John F. Robertson and Jack D. Hardcastle

The rat pancreatic cell line, AR42J possessed high-affinity gastrin and somatostatin receptors and its growth was stimulated by physiological gastrin-17 concentrations between 5×10^{-11} mol/l and 10^{-9} mol/l as measured by [^{75}Se]selenomethionine uptake. The somatostatin analogue, octreotide (2×10^{-7} to 2×10^{-11} mol/l), reduced this stimulated growth. Gastrin-stimulated AR42J growth was also inhibited by proglumide (3×10^{-4} mol/l) and lorglumide (3×10^{-5} mol/l) at maximal G17 concentrations of 5×10^{-11} and 10^{-10} mol/l, respectively, and the analogues competed with [^{125}I] gastrin-17 (5×10^{-10} mol/l) for binding to gastrin receptors on AR42J (50% inhibitory concentrations, $\leq 10^{-3}$ mol/l and 4×10^{-6} mol/l, respectively. Octreotide reduced the basal growth of the human gastric cell line, MKN45G, (which is associated with intracellular gastrin immunoreactivity) in serum-free medium to 73% of control at a concentration of 2×10^{-8} mol/l, which was reversed by gastrin-17 (10^{-10} mol/l). Lorglumide (3×10^{-5} mol/l) also reduced the basal growth to 30% of control, which was reversed to 78% by 10^{-5} mol/l gastrin. Proglumide had no effect on the basal growth of MKN45G.
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INTRODUCTION

THE POLYPEPTIDE hormone, gastrin, has both endocrine [1, 2] and paracrine/autocrine [3–6] growth modulatory effects on human gastrointestinal (GI) adenocarcinomas. Thus potential therapies of such hormone-responsive tumours need to inhibit both mechanisms of gastrin-stimulated growth. Gastrin/cholecystokinin (CCK) receptor antagonists have been described, which include glutamic acid derivatives such as proglumide [7] and benzodiazepam-like compounds such as L-365 260 [8]. For such antagonists to be effective they must bind with high affinity to gastrin receptors (GR) or be non-toxic so they can be administered at high enough concentrations to compete with gastrin for receptor occupation. Receptor antagonists may have to compete with both circulating gastrin, (which may be elevated in GI cancer patients [9]) and unknown concentrations of tumour-associated gastrin.

The hormone, somatostatin, is known to suppress several endocrine functions. These include inhibition of release of peptide hormones [10, 11] and direct effects on the growth of GI mucosa which is partly due to the effect of somatostatin on gastrin release [12, 13]. Long-acting derivatives of somatostatin have been derived, such as octreotide [14] and RC-160 [15].

The purpose of this study is to compare the abilities of the CCK/GR antagonists; proglumide and lorglumide (CR1409) and the somatostatin analogue; octreotide to inhibit GI tumour growth stimulated by gastrin firstly in an endocrine and secondly in a paracrine/autocrine manner.

MATERIALS AND METHODS

Cell lines

AR42J is a rat pancreatic acinar cell line [16]. MKN45G was derived from a human gastric adenocarcinoma, MKN45 [17] and was found to be associated with production of a gastrin-like peptide [4, 5]. The cell lines were maintained in RPMI culture

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medium (Gibco) containing 10% fetal calf serum (FCS, Gibco) at 37°C in a humidified incubator gassed with CO_2.

CCK/GR antagonists and octreotide

Proglumide and lorglumide were provided by Rotta Research Laboratorium, Milan. The compounds were dissolved in distilled water and 100% ethanol, respectively and infused into culture medium at the required concentrations. Ethanol controls were included for lorglumide and results were expressed as a percentage of the growth in ethanol.

Octreotide was provided by Sandoz Pharma, Basel, and was diluted in distilled water and infused into growth medium at the required concentrations.

Measurement of somatostatin binding sites

[^{125}I]iodo-tyrosyl somatostatin-14 (Amersham) of specific activity, 74 TBq/mmol was used to measure somatostatin-14 binding sites on fresh whole cell preparations.

Cell lines were grown as adherent monolayers until semi-confluent and harvested by either gentle scraping (MKN45G) or 0.025% EDTA (Sigma, AR42J). A single cell suspension was achieved by vigorous pipetting. The cells were washed with 10 mmol/l Hepes buffer, pH 7.6 containing 0.5% bovine serum albumen (BSA, Sigma) and 10 μg/ml aprotinin (Sigma) and aliquoted into plastic eppendorf tubes (Northern Media, Nottingham) at a concentration of 2×10^5 cells/tube. Triplicate tubes were used per determination.

[^{125}I] somatostatin-14 was added to the cells at a final concentration of 5×10^{-10} mol/l. Specific binding was determined by co-incubating the cells with unlabelled somatostatin-14 (Novobiochem, Nottingham), at concentrations from 10^{-10} to 10^{-7} mol/l, and measuring displacement of labelled somatostatin.

Cells were incubated for 1 h at room temperature before being washed with ice cold Hepes buffer to terminate the reaction. Associated radioactivity was measured on a γ-counter.

Scatchard analyses were performed [18] by the "Radlig" ligand pc curve-fitting programme [19] allowing the affinity of binding (K_d) and receptor density (B_{max}) to be determined.

Measurement of gastrin-17 (G-17) binding sites

[^{125}I]iodo tyrosyl human gastrin I (NEN-Dupont, Stevenage, Herts) of specific activity 81 TBq/mmol was used to measure G17 binding sites on whole cell preparations.

The cells were prepared as for the somatostatin binding study previously described, washed and suspended in Eagles culture medium (Flow) containing 0.5% BSA.

[^{125}I] G17 was added to the cells at a final concentration of 5×10^{-10} mol/l and specific binding was measured by competing with unlabelled G17 (Sigma) at concentrations from 10^{-10} to 10^{-6} mol/l. Cells were incubated for 1 h at room temperature before being washed in ice cold Eagles medium. Associated radioactivity was measured and Scatchard analyses performed as described for the somatostatin binding studies.

Competition assays were performed with [^{125}I] G17 (5×10^{-10} mol/l) and increasing concentrations of proglumide (4×10^{-6} to 5×10^{-3} mol/l) and lorglumide (2×10^{-7} to 2×10^{-4} mol/l). Displacement of [^{125}I] G17 was measured at each unlabelled ligand concentration.

Effect of the CCK/GR antagonists and octreotide on the basal and gastrin-stimulated growth of AR42J

Initially, experiments were performed to compare two methods of assessing *in vitro* cell growth; direct cell counts and [^{75}Se]selenomethionine uptake [20]. For direct cell counts, cells were seeded into 96 well flat-bottomed tissue culture plates (Flow) in Hams F12 culture medium (Flow) in a 1:1 mixture with Eagles medium containing 0.5% BSA. The cells were then incubated to allow cell division, harvested with 0.025% EDTA and the number of cells per well counted by haemocytometer (three replicates per drug dilution).

For [^{75}Se]selenomethionine incorporation, cells were seeded into 96-well tissue culture plates in Hams F12 Eagles culture medium described above (five replicates/drug dilution). After incubation, the cells were pulsed for 18 h with [^{75}Se]selenomethionine (3.7 kBq/well, Amersham). The cells were then washed and associated radioactivity counted.

For a comparison of cell counts with [^{75}Se]selenomethionine uptake, cells were plated at initial concentrations of 1×10^3 to 5×10^4/well. After 48 h incubation, cells from identical plates were either counted or labelled with [^{75}Se]selenomethionine and a correlation between the two methods was made.

For assessment of cell growth in the presence of G17 with or without CCK/GR antagonists or octreotide, semi-confluent monolayers of AR42J were harvested with 0.025% EDTA, washed and suspended in the serum free culture medium as previously described. The cells were seeded into 96-well, tissue culture plates at a concentration of 10^4 cells/well (five replicates per drug dilution). After allowing for cell adherence, human G17 at concentrations of 5×10^{-11} to 5×10^{-10} mol/l (which approximate to physiological gastrin levels in man, 21) was added to the cells in the absence/presence of proglumide (3×10^{-6} to 3×10^{-4} mol/l), lorglumide (3×10^{-6} to 3×10^{-5} mol/l) and octreotide (2×10^{-11} to 2×10^{-7} mol/l). The cells were incubated for up to 5 days before being pulsed with [^{75}Se]selenomethionine.

Effect of CCK/GR antagonists and octreotide on the basal growth of MKN45G

Semi-confluent monolayers of MKN45G were harvested by 0.025% trypsin (Sigma)/0.5% EDTA and seeded into 96-well plates at a cell concentration of 10^4/well in the serum free cell culture medium described.

Proglumide (5×10^{-5} to 5×10^{-3} mol/l), lorglumide (2×10^{-6} to 3×10^{-5} mol/l) and octreotide (2×10^{-11} to 2×10^{-7} mol/l) were incubated with the cells for up to 5 days (octreotide was replenished daily by aspiration of the spent growth medium and replacement with fresh medium containing the compound at the required concentrations). Reversal of growth inhibition with G17 was achieved by co-incubation of the CCK/GR antagonists and octreotide with excess G17.

All assessments of growth were made by [^{75}Se]selenomethionine incorporation as previously described. Assessments of viability were made by trypan blue exclusion.

Statistical analysis

The Student's t-test was performed on all replicate results using the SPSS/PC + statistical package for the IBM PC. The data were shown to be distributed normally. Significance level was set at $P < 0.05$.

RESULTS

GR status of AR42J and competitive displacement of G17 by proglumide and lorglumide

Scatchard analysis revealed that AR42J possessed GR of K_d, 4.6×10^{-10} mol/l and B_{max}, 5×10^4/cell, respectively.

In a competitive ligand binding assay in which [^{125}I] G17

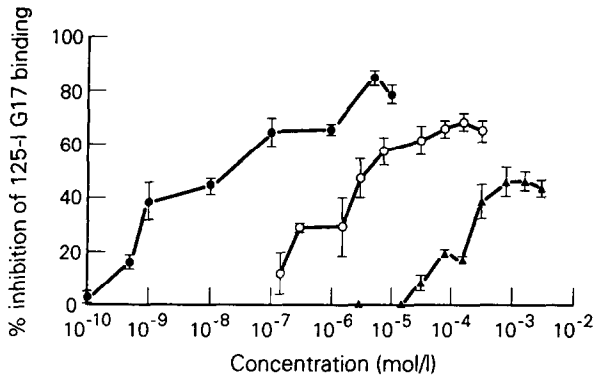

Fig. 1. A competition assay for GR binding to AR42J cells between a fixed concentration of [^{125}I] G17 (5×10^{-10}mol/l) and increasing concentrations of unlabelled G17 ●–●, lorglumide ○–○, and proglumide ▲–▲. Lorglumide was 200 × less effective and proglumide ≤ 50,000 less effective that G17 itself for binding to GR on AR42J cells when comparing the concentrations inducing 50% inhibition of [^{125}I] G17 binding. The data is the mean of five separate experiments with interexperimental variation expressed by standard errors.

(5×10^{-10} mol/l) was competed with increasing concentrations of either unlabelled G17, lorglumide or proglumide, it was found that the concentrations inducing 50% inhibition of [^{125}I] G17 binding (IC_{50}) were 2×10^{-8} mol/l, 4×10^{-6} mol/l and $\leq 10^{-3}$ mol/l, respectively for the three ligands. Figure 1 shows the mean of five separate experiments with inter-experimental variation expressed by the standard errors.

Effect of CCK/GR antagonists on the gastrin-stimulated growth of AR42J

[^{75}Se]selenomethionine uptake was compared with direct cell counts in both AR42J and MKN45G cells. Cells were initially seeded at concentrations between 1×10^3 and 5×10^4/well and label uptake and cell counts were measured after a 48 h culture period. Regression analysis of the data gave an r value of 0.992 for MKN45G ($P < 0.001$) and an r value of 0.995 for AR42J ($P < 0.001$, Fig. 2).

Gastrin was found to increase the growth of AR42J over a 5 day period as assessed by direct cell counts and by [^{75}Se]selenomethionine incorporation (Table 1). The G17 concentration inducing the maximal mitogenic response was 10^{-10} mol/l and the level of increased mitogenesis varied between 43% and 173% above the untreated control in the experiments shown however the maximal increase achieved is around 250% above control (as cited in Fig. 3). As the level of mitogenesis obtained with both techniques was comparable (with respect to the dose response and level of variability of magnitude of the response) it was decided to use [^{75}Se]selenomethionine uptake as an assessment of cell number increase for all further experiments as it was less time-consuming and subjective when compared to direct cell counts.

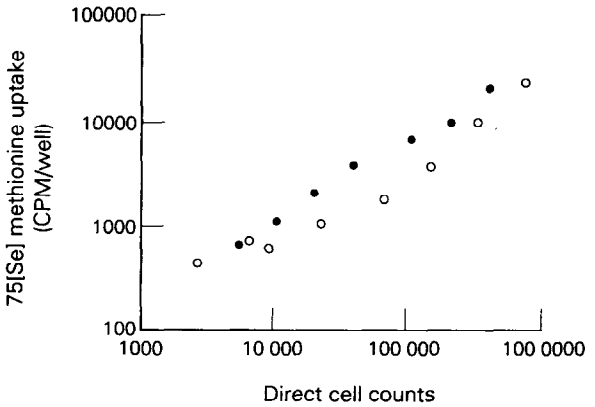

Fig. 2. A comparison of [^{75}Se]selenomethionine uptake (cpm/well) with direct cell counts for ●–● MKN45G and ○–○ AR42J after 48 h culture in serum free culture medium. Regression analysis gave an r value of 0.992 ($P < 0.001$) for MKN45G and an r value of 0.995 ($P < 0.001$) for AR42J. The means are expressed ($n = 5$ replicates).

Table 1. A comparison of the effect of G17 on the growth of AR42J cells in serum free in vitro *culture as assessed by (a) direct cell count and (b) [^{75} Se]selenomethionine uptake*

G17 mol/l	% Untreated control (S.E.) Experiment 1	Experiment 2	Experiment 3
(a) Direct cell counts*			
0	100(7)	100(12)	100(7)
5×10^{-11}	152(15)	197(26)	165(6)
10^{-10}	169(18)	243(9)	212(12)
5×10^{-10}	137(6)	231(27)	193(13)
(b) [^{75}Se]selenomethionine incorporation†			
0	100(3)	100(1)	100(4)
5×10^{-11}	143(6)	212(15)	210(21)
10^{-10}	152(8)	222(11)	273(19)
5×10^{-10}	138(4)	187(6)	243(16)

*Means of triplicates.
†Means of five replicates.
For direct cell counts and ^{75}[Se]selenomethinione uptake, AR42J cells were plated into 24 and 96 well flat bottomed tissue culture plates at cell concentrations of 5×10^4 cells/well, respectively, in serum free culture medium. Human G17 was added to cells at concentrations between 5×10^{11} and 5×10^{-10}mol/l and incubated for up to 5 days. For direct cell counts, cells were harvested with 0.025 % EDTA and counted with a haemocytometer and for label uptake, cells were pulsed with ^{75}[Se] selenomethionine (3.7 kBq/well) for 18 h, washed and associated radioactivity counted.

The effect of proglumide (3×10^{-6} to 3×10^{-4} mol/l) on the basal and gastrin-stimulated growth of AR42J was investigated. Higher concentrations of proglumide had non-specific cytotoxic effects on the growth of AR42J (as assessed by trypan blue exclusion). Proglumide had no significant effect on the basal growth of AR42J (Fig. 3a). Proglumide significantly ($P < 0.001$) inhibited the increased label uptake of AR42J induced by 5×10^{-11} mol/l G17 at concentrations of 3×10^{-5} and 3×10^{-4} mol/l (161% reduced to 103% untreated control value). However, the proglumide concentrations examined had no significant effect on the increased uptake induced by 10^{-10} mol/l G17 (Fig. 3a).

Lorglumide at concentrations of 3×10^{-6} to 3×10^{-5} mol/l, (3×10^{-5} mol/l was the maximal concentration that avoided large non-specific effects of the ethanol diluent) had no effect on the basal growth of AR42J but reduced the increased label uptake observed with 5×10^{-11} mol/l G17 (315% down to 207%, $P < 0.001$) and 10^{-10} mol/l G17 (363% down to 296%, $P < 0.001$) at the highest dose of lorglumide used (3×10^{-5} mol/l). However, at a G17 concentration of 5×10^{-10} mol/l, the stimulated label uptake was not signifi-

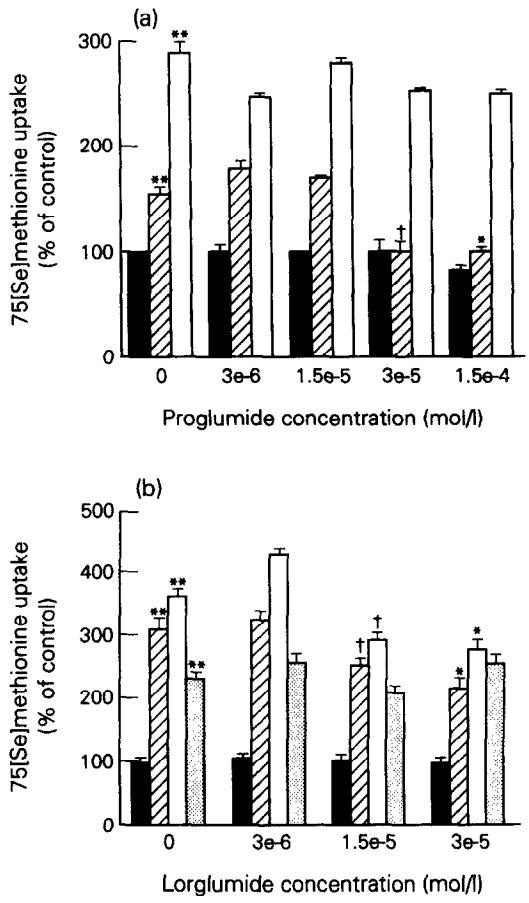

Fig. 3. Effect of (a) proglumide and (b) lorglumide on the basal growth of AR42J ■, and on the response of AR42J to 5×10^{-11} mol/l G17 ▨, 10^{-10} mol/l G17 □ and 5×10^{-10} mol/l G17 ▩, as measured by [^{75}Se]selenomethionine incorporation. The mean and S.E. are expressed ($n = 5$ replicates). *$P < 0.001$, when compared to G17 treatment, **$P < 0.001$ when compared with untreated controls and † signifies borderline non-significant differences as assessed by the Student's t-test. Proglumide significantly inhibited the increased label uptake of AR42J induced by 5×10^{-11} mol/l G17 at concentrations of 3×10^{-5} and 3×10^{-4} mol/l but did not inhibit increased label uptake induced by higher G17 concentrations. Lorglumide inhibited the increased label uptake of AR42J induced by 5×10^{-11} and 10^{-10} mol/l G17 at a concentration of 3×10^{-5} mol/l.

cantly inhibited (Fig. 3b). These are single typical experiments from a series of three that were performed.

Somatostatin receptor (SR) status of AR42J

Scatchard analysis revealed that AR42J possessed SR (that bound [^{125}I] somatostatin-14) of Kd, 6.4×10^{-10} mol/l and B_{max}, 6.7×10^4/cell.

Effect of octreotide on the gastrin-stimulated growth of AR42J

Octreotide at concentrations of 2×10^{-7} mol/l to 2×10^{-11} mol/l, had no effect on the basal growth of AR42J in serum-free culture medium as measured by label uptake but reduced the increased label uptake stimulated by 10^{-10}, 5×10^{-10} mol/l and 10^{-9} mol/l G17 (Fig. 4). The maximum reduction in G17-stimulated uptake was achieved at octreotide concentrations of 2×10^{-8} mol/l (with 10^{-10} mol/l G17 from 174% to 56% of the control, $P < 0.001$ and with 5×10^{-10} mol/l G17 from 138% to 51% of control, $P < 0.001$ and with 10^{-9} mol/l G17 from 124% to 81% of the control, $P < 0.001$). However, significant reduction in the G17 stimulated uptake was also achieved with all other octreotide concentrations examined.

The effect of increasing concentrations of octreotide (2×10^{-11} to 2×11^{-7} mol/l) on the binding of 5×10^{-10} mol/l [^{125}I] G17 to GR on AR42J was examined and octreotide was found to have no effect on G17 binding to GR even at the highest octreotide concentrations examined (data not shown).

GR and SR status of MKN45G

MKN45G possessed both SR and GR as assessed by Scatchard analyses. The Kd and B_{max} for G17 binding were 1.2×10^{-9} mol/l and 1.1×10^4/cell, respectively and for somatostatin-14 binding 1.4×10^{-9} mol/l and 3.3×10^5/cell, respectively.

The effect of CCK/GR antagonists and octreotide on the basal growth of MKN45G cells

Proglumide had no significant effect on the basal growth of MKN45G in serum-free medium (data not shown). Lorglumide at a concentration of 2×10^{-5} mol/l induced a significant inhibition of growth to 88% of the control ($P < 0.02$) and a concentration of 3×10^{-5} mol/l induced inhibition to 30% of control ($P < 0.001$) as assessed by label uptake (Fig. 5). This was not a direct cytotoxic effect affecting the viability of the cells (as assessed by trypan blue exclusion) as the cells remaining were viable but did not divide, i.e. were quiescent. The inhibition observed with 3×10^{-5} mol/l lorglumide was reversed to 60% of the untreated control by co-incubation with 10^{-5} mol/l G17 ($P < 0.001$) (Fig. 4).

The effect of octreotide on the growth of MKN45G was also analysed. At an octreotide concentration of 2×10^{-7} mol/l, no significant effect on the basal growth of MKN45G in serum free medium was achieved (Table 2). At an octreotide concentration of 2×10^{-8} mol/l, the inhibition of MKN45G was reduced to 73% of the untreated control value ($P < 0.001$) at day 4 (as measured by label uptake). The inhibition was also maintained at day 6 (77% of control, $P < 0.001$). At 2×10^{-9} mol/l, the basal growth of MKN45G was significantly inhibited to 83% of

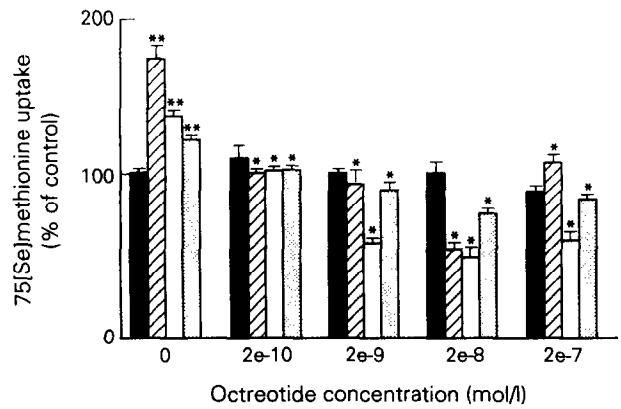

Fig. 4. Effect of octreotide (2×10^{-7} to 2×10^{-11} mol/l) on the basal growth of AR42J ■, and on the response of AR42J to 10^{-10} mol/l G17 ▨, and 5×10^{-10} mol/l G17 □, and 10^{-9} mol/l G17 ▩, as measured by [^{75}Se]selenomethionine incorporation. The mean and S.E. are expressed ($n = 5$ replicates). *$P < 0.001$ when compared with G17 treatment, **$P < 0.001$ when compared with untreated controls and † signifies borderline non-significant differences as assessed by the Student's t-test. Octreotide reduced the increased label uptake induced by 10^{-10}, 5×10^{-10} and 10^{-9} mol/l G17 with the maximal level of inhibition being achieved at an octreotide concentration of 2×10^{-8}mol/l.

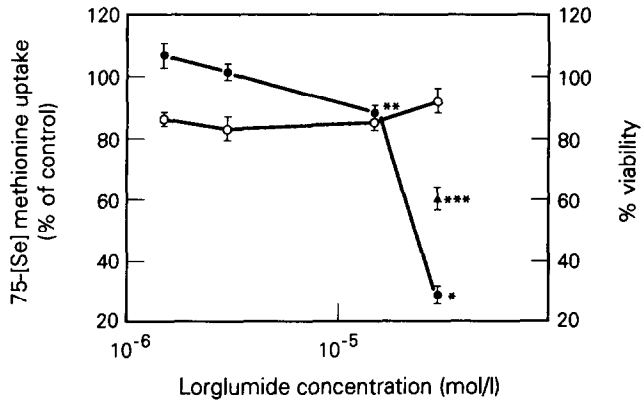

Fig. 5. Effect of lorglumide on the [^{75}Se]selenomethionine uptake ●–● and the viability ○–○ of MKN45G grown in serum free culture medium and the effect of reversal of the inhibitory effect of lorglumide at 3 × 10^{-5} mol/l with 10^{-5} mol/l G17, ▲ (as measured by label uptake). The mean and S.E. are expressed (n = 5 replicates). *$P < 0.001$, **$P < 0.02$ when compared to the untreated control and ***$P < 0.001$ when compared with the inhibition induced with 3 × 10^{-5}mol/l lorglumide, as analysed by the Student's t-test. Lorglumide at a concentration of 3 × 10^{-5} mol/l inhibited the basal growth of MKN45G, although the viability of the remaining cells was unaffected. Partial reversal of the inhibition was achieved by co-incubation with 10^{-5} mol/l G17.

control at day 2 ($P < 0.001$) yet this was not maintained at day 4 and 6 despite daily re-feeding.

When G17 was present in the serum-free medium at 10^{-10} mol/l, no inhibition of MKN45G with octreotide was observed (data not shown).

DISCUSSION

Somatostatin plays an important modulatory role in the secretion and growth-promoting functions of many hormones and this role may be of particular importance as several GI hormones have been shown to stimulate the growth of tumours arising in the GI tract [1].

Somatostatin may influence tumour growth directly at the intracellular level via interaction with membrane receptors [22] or indirectly via suppression of hormones acting as tumour growth factors [23].

Table 2. The effect of octreotide on the basal growth of MKN45G in serum free growth medium with daily refeeding

	[^{75}Se]selenomethionine incorporation [% control (S.E.)]		
Octreotide (mol/l)	Day 2	Day 4	Day 6
2 × 10^{-7}	94(3)	84(7)	94(8)
2 × 10^{-8}	87(3)*	73(4)†	77(2)†
2 × 10^{-9}	83(2)†	80(9)	89(3)

*$P < 0.01$; †$P < 0.001$; when comparing significance to untreated controls.

MKN45G cells were seeded into 96-well, flat bottomed tissue culture-treated plates at cell concentrations of 10^4/well in serum-free culture medium. Octreotide (2 × 10^{-11} to 2 × 10^{-7}mol/l) was incubated with the cells for up to 6 days (octreotide was replenished daily by aspiration of the spent growth medium and replacement with fresh medium containing octreotide at the required concentration). Cells were then pulsed with [^{75}Se]selenomethionine (3.7 kBq/well) for 18 h, washed and associated radioactivity counted.

Somatostatin receptors have been shown to be present on GI tumours; Viguerie *et al.* [24] showed SR on AR42J cells, Reyl-Desmals *et al.* [25] showed SR on a human gastric cell line (HGT-1) and Miller *et al.* [26] have recently shown SR on human colo-rectal primary tumours. In the present study, both AR42J and the human gastric cell line, MKN45G were shown to possess high affinity SR.

[^{75}Se]selenomethionine uptake of proliferating cells was shown to correlate with direct cell counts in the present study which agrees with the findings of Brook *et al.* [20] and Durrant *et al.* [27]. This was then used to assess the effect of the CCK/GR antagonists and octreotide on the basal and/or the gastrin-stimulated growth of AR42J and MKN45G.

Octreotide was shown to reduce both AR42J cell growth stimulated by exogenously applied physiological gastrin concentrations [21] and MKN45G cell growth in serum-free medium where it has been shown that gastrin produced in an autocrine/paracrine manner is partially responsible for maintaining cell growth [4, 5].

It is known that the somatostatin analogue, RC-160 reduces EGF-stimulated pancreatic tumour cell growth by inducing a tyrosine phosphatase activity which dephosphorylates both the receptor (which autophosphorylates upon activation) and the substrate, thereby interrupting the intracellular signalling events which ultimately end in cell mitogenesis [22]. However, in the same study it was shown that octreotide did not induce such effects. Conversely Viguerie *et al.* [28] showed that octreotide did reduce EGF-stimulated mitogenesis of AR42J cells. They found that the mechanism involved in the inhibition did not involve a GTP-binding protein and they postulated that there may have been an effect on the EGF-stimulated tyrosine kinase. Thus there is some confusion in the literature as to the exact mechanism by which octreotide induces its growth inhibitory effects.

It is known that G17 may be phosphorylated by an EGF-stimulated tyrosine kinase. This phosphorylation event is believed to be important in the effect of G17 on GI cell growth [29]. If octreotide has the ability to alter this phosphorylation then this may go towards explaining its effect on the gastrin-stimulated growth of AR42J.

With respect to MKN45G, as gastrin produced by the cells appears to be important in maintaining cell growth [4, 5], octreotide may be inducing an anti-secretory effect on cell associated gastrin which is then reversed when exogenous gastrin is added. Intracellular gastrin immunoreactivity however appears to be unaffected by octreotide treatment *in vitro* [30] indicating that the gastrin is being synthesised but may not be able to get to its site of action. This may involve secretion, if the gastrin binds to external GR which have been shown to be present on MKN45G in the present study.

The CCK/GR antagonists, proglumide and benzotript have previously been shown to inhibit the growth of human colon cancer cell lines in both serum-supplemented and serum-free growth medium [31]. This inhibition was shown to be reversible in the case of proglumide by the addition of exogenous gastrin. In the present study, lorglumide inhibited the growth of MKN45G in serum-free medium but not AR42J, indicating growth of the former but not the latter cell line was dependent on gastrin when grown in serum free conditions. This inhibitory effect was also partially reversible and therefore shows some specificity to gastrin.

Both lorglumide and proglumide were shown to reduce the G17-stimulated growth of AR42J cells. Proglumide (3×10^{-4} and 3×10^{-5} mol/l) was only competitive at G17 concentrations of 5×10^{-11} mol/l and lorglumide (3×10^{-5} mol/l) at G17 concentrations of 5×10^{-11} and 10^{-10} mol/l. At higher G17 concentrations the antagonists failed to compete with G17 for GR binding. Seva *et al.* [32] have recently shown that lorglumide blocked the gastrin-stimulated growth of AR42J cells.

In therapy terms, due to its potential effect at an intracellular level, octreotide may have more potential inhibitory effects on gastrin stimulated growth than GR antagonists which to compete with gastrin for binding to GR will have to achieve high enough serum concentrations and thus be non-toxic.

Lorglumide inhibited the basal growth of MKN45G in serum-free medium, which was reversible by exogenous G17. This indicates that lorglumide was able to compete for GR binding with gastrin produced by the tumour cells themselves and that locally produced gastrin is present at such concentrations as to allow such competition.

In summary, both octreotide and the lorglumide can inhibit GI tumour growth stimulated by gastrin in an endocrine and paracrine/autocrine manner. Thus therapy of gastrin-responsive GI tumours with such agents should be clinically beneficial.

1. Townsend CM, Singh P, Thompson JC. Possible role of gut hormones in cancer. In: Thompson JC *et al.*, eds, *Gastrointestinal Endocrinology*. New York, McGraw-Hill, 1987, 178–183.
2. Watson SA, Durrant LG, Crosbie JD, Morris DL. The *in vitro* growth response of primary human colorectal and gastric cancer cells to gastrin. *Int J Cancer* 1989, **43**, 692–696.
3. Hoosein NM, Kiener PA, Curry RC, Brittain MG. Evidence for autocrine growth stimulation of cultured colon tumour cells by a gastrin/cholecystokinin-like peptide. *Exp Cell Res* 1990, **186**, 15–21.
4. Watson SA, Hall AR, Durrant LG, Morris DL. Characterisation of a human gastric adenocarcinoma, MKN45G, which is associated with intracellular gastrin, and its response to anti-gastrin antibodies. *Gut* 1990, **31**, 595.
5. Watson SA, Durrant LG, Wencyk P, Watson AL, Morris DL. Intracellular gastrin in human Gastrointestinal (GI) tumour cells: a possible autocrine/paracrine role. *JNCI* 1991, **83**, 866.
6. Finley G, Koski R, Melhem M, Pipas J, Meisler A. Expression of gastrin in colonic adenocarcinoma and normal mucosa. *Proc Amer Assoc Canc Res* 1990, **31**, 39.
7. Rovati AL. The relationship between chemical structure of a new dicarboxylic amino acid derivative and anti-gastrin activity in the rat. *Br J Pharmacol* 1986, **34**, 677–682.
8. Bock MG, DiPardo RM,Evans BE, *et al.* Benzodiazepine gastrin and brain cholecystokinin receptor ligands: L-365,260. *J Med Chem* 1989, **32**, 13–16.
9. Smith JP, Wood JG, Soloman TE. Elevated gastrin levels in patients with colorectal cancer and adenomatous polyps. *Gastroenterology* 1987, **92**, 1646.
10. Arnold R, Lankisch PG. Somatostatin and the gastrointestinal tract. *Clin Gastroenterol* 1980, **9**, 733–753.
11. Kawai K, Ipp E, Orci L, Perrelet A, Unger RH. Circulating somatostatin acts on the islets of Langerhans by way of a somatostatin poor compartment. *Science* 1982, **218**, 477–478.
12. Lehy T, Dubrasquet P, Brazeau P, Bonfils S. Inhibitory effect of prolonged administration of long-acting somatostatin on gastrin-stimulated fundic epithelial cell growth in the rat. *Digestion* 1982, **24**, 246–255.
13. Dembinski A, Warzecha Z, Konturek SJ, Schally AV. Effect of somatostatin on the growth of gastrointestinal mucosa and pancreas in rats. Role of endogenous gastrin. *Gut* 1987, **28**, 227–232.
14. Bauer W, Briner U, Doepfner W, *et al.* SMS-201-995: A very potent and selective octapeptide analogue of somastatin with prolonged action. *Life Sci* 1982, **31**, 1133–1140.
15. Cai RZ, Szoke B, Lu R, Fu D, Redding TW, Schally AV. Synthesis and biological activity of highly potent octapeptide analogues of somatostatin. *Proc Natl Acad Sci USA* 1986, **83**, 1896–1900.
16. Jessop NW, Hay RJ. Characteristics of two rat pancreatic exocrine cell lines derived from transplantable tumours. *In Vitro* 1980, **16**, 212.
17. Hojo J. Establishment of cultured cell lines of human stomach cancer origin and their morphological characteristics. *Niigata Igakukai Zasshi* 1977, **91**, 737.
18. Scatchard G. The attractions of protein for small molecules and ions. *Ann NY Acad Sci* 1949, **51**, 660–671.
19. Munson PJ. Robard D. Ligand, a versatile computerised approach for characterisation of ligand binding systems. *Annal Biochem* 1980, **107**, 220–239.
20. Brooks CG. Rees RC, Robins RA. Studies on the microcytotoxicity test II. *J Immunol Mets* 1978, **21**, 111–124.
21. Mahachai V, Walker K, Serelius H, Thomson ABR. Antisecretory and serum gastrin lowering effect of enprostil in patients with duodenal ulcer disease. *Gastroenterology* 1985, **89**, 555–561.
22. Liebow C, Reilly C, Serrano M, Schally AV. Somatostatin analogues inhibit growth of pancreatic cancer by stimulating tyrosine phosphatase. *Proc Natl Acad Sci USA* 1989, **86**, 2003–2007.
23. Schally AV. Oncological application of somatostatin analogues. *Cancer Res* 1988, **48**, 6977.
24. Viguerie N, Tahiri-Jouti N, Esteve J, *et al.* Functional somatostatin receptors on a rat pancreatic acinar cell line. *Am J Physiol (Gastrointest Liver Physiol 18)* 1988, **255**, G113–G120.
25. Reyl-Desmars F. Le Roux S, Linard C, Benkouka F, Lewin MJM. Purification jusquti apparente homogeneite du recepteur de la somatostatin de la lignée cellulaire humaine HGT-1 a'origine gastrique. *ER Acad Sci Paris* 1989, **308**, 251–254.
26. Miller GV, Woodhouse L, Farmery S, Primrose JM. Somatostatin receptors (SR) in human colorectal cancer (CRC). *Br J Surg* 1990, 77, A700.
27. Durrant LG, Watson SA, Hall A, Morris DL. Co-stimulation of gastrointestinal tumour cell growth by gastrin, transforming growth factor α and insulin like growth factor I. *Br J Cancer* 1991, **63**, 67–70.
28. Viguerie N, Tahiri-Jouti N, Ayral AM, *et al.* Direct inhibitory effects of a somatostatin analogue, octreotide, on AR42J cell proliferation via pertussis toxin-sensitive guanosine triphosphate-binding protein-independant mechanism. *Endocrinology* 1989, **124**, 1017–1025.
29. Baldwin GS, Knesel J, Monckton JM. Phosphorylation of gastrin-17 by epidermal growth factor-stimulated tyrosine kinase. *Nature* 1983, **301**, 435–437.
30. Watson SA, Durrant LG, Morris DL. The effect of the E_2 prostoglandin enprostil, and the somatostatin analogue, octreotide, on the growth of a human gastric cell line, MKN 45G. *Int J Cancer* 1990, **45**, 90–95.
31. Hoosein NM, Kiener PA, Curry RC, Rovati LC, McGilbra DK, Brattain MG. Antiproliferative effects of gastrin receptor antagonists and antibodies to gastrin on human colon carcinoma cell lines. *Cancer Res*, 1988, **48**, 7179–7183.
32. Seva C, Scemama JL, Bastié MJ, Pradayrol L, Vaysse N. Lorglumide and loxiglumide inhibit gastrin-simulated DNA synthesis in a rat tumoral acinar pancreatic cell line (AR42J) *Cancer Res* 1990, **50**, 5829–5833.

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