SELECTIVE INHIBITION OF THE BIOLOGICAL ACTIVITY OF GLYCINE-EXTENDED GASTRIN*  

A Novel Effect of Bismuth Ions

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Although bismuth salts have been used for over two centuries for the treatment of various gastrointestinal disorders, the mechanism of their therapeutic action remains controversial. Because gastrins bind two trivalent ferric ions with high affinity, and because ferric ions are essential for the biological activity of glycine-extended gastrin 17, we have investigated the hypothesis that trivalent bismuth ions influence the biological activity of gastrins. Binding of bismuth ions to gastrins was measured by fluorescence quenching and NMR spectroscopy. The effects of bismuth ions on gastrin-stimulated biological activities were measured in inositol phosphate, cell proliferation, and cell migration assays. Fluorescence quenching experiments indicated that both glycine-extended and amidated gastrin 17 bound two bismuth ions. The NMR spectral changes observed on addition of bismuth ions revealed that Glu-7 acted as a ligand at the first bismuth ion binding site. In the presence of bismuth ions the ability of glycine-extended gastrin 17 to stimulate inositol phosphate production, cell proliferation, and cell migration was markedly reduced. In contrast, bismuth ions had little effect on the affinity of the CCK-2 receptor for amidated gastrin 17, or on the stimulation of inositol phosphate production by amidated gastrin 17. We conclude that bismuth ions may act, at least in part, by blocking the effects of glycine-extended gastrin 17 on cell proliferation and cell migration in the gastrointestinal tract. This is the first report of a specific inhibitory effect of bismuth ions on the action of a gastrointestinal hormone.

Since the first recorded medical use of bismuth subsalicylate in 1733 (1), bismuth salts have been used for the treatment of various gastrointestinal disorders, including gastric and duodenal ulcers, dyspepsia, diarrhea, and colitis (2, 3). Not surprisingly, this broad spectrum of effects is associated with a large number of putative mechanisms of action. Bismuth has a direct antibacterial effect on Helicobacter pylori (4), and different combinations of bismuth, gastric acid inhibitors, and antibiotics are variously effective in eradicating the bacterium (5). Although bismuth is no longer a front-line treatment for H. pylori infection, quadruple therapy incorporating bismuth, antibiotics, and a proton pump inhibitor is currently recommended for eradication of H. pylori in patients infected with strains resistant to metronidazole (6). Bismuth salts also preferentially coat ulcer craters preventing back diffusion of H+ ions (2, 7), and additionally accelerate crater repair through stimulation of macrophage influx and prostaglandin synthesis, and through a reduction in pepsin activity (2, 7). In colitis the anti-diarrheal and anti-inflammatory effects of bismuth have proved beneficial (8).

Infection with H. pylori causes an increase in circulating gastrin concentrations via a variety of mechanisms (9, 10). Antral infection directly increases serum gastrin, perhaps via release of inflammatory cytokines, and the increased gastrin in turn increases gastric acidity. In contrast, colonization of the body of the stomach decreases gastric acidity resulting in a reciprocal increase in serum gastrin. The hypergastrinemia and hyperchlorhydria associated with antral H. pylori infection predispose to duodenal ulcer, whereas infection of the gastric body results in atrophic gastritis and an increased risk of gastric cancer. Gastrin has also been reported to stimulate the growth of H. pylori directly (11, 12). The prohormone progastrin is produced by G cells located within the gastric antrum and is processed to shorter peptides, such as glycine-extended gastrin (Ggly)1 and amidated gastrin (Gamide) (Fig. 1) (13). Until recently, amidation of the carboxyl terminus of gastrin was thought to be essential for biological activity (14). However, we and others have reported that Ggly and progastrin are able to induce proliferation and migration of various cell lines in vitro (15–19), and proliferation of the colonic mucosa in vivo (20–22). In addition Ggly acts synergistically with Gamide in the stimulation and maintenance of elevated gastric acid production (23, 24).

We recently reported that Ggly specifically bound two trivalent ferric ions (25), and that binding of ferric ions was essential for biological activity (26). Because bismuth ions are also trivalent, and have been shown to substitute for ferric ions in binding to lactoferrin (27) and transferrin (28), we hypothesized that gastrins might also bind bismuth ions and that binding might influence biological activity. To investigate this

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¶The abbreviations used are: Ggly, glycine-extended gastrin 17; BH, Bolton and Hunter; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; Gamide, amidated gastrin 17; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
question, we have studied the fluorescence and NMR spectra of gastrins in the presence of bismuth salts. We have also investigated the effect of bismuth ions on gastrin-induced inositol phosphate production, cell proliferation, and cell migration, and on the binding of gastrins to their receptors. Our results indicate that bismuth ions selectively inhibit the biological activity of Ggly, but have little effect on the actions of Gamide. This novel effect of bismuth has implications for treatment of peptic ulcer disease and colorectal cancer.

EXPERIMENTAL PROCEDURES

Chemicals and Cell Lines—Ggly and Gamide were purchased from Auspep (Melbourne, Australia) and Bachem (Bubendorf, Switzerland), respectively. The T lymphoblastoid cell line Jurkat (29) (kindly provided by Dr. C. Oiry, Université de Montpellier I, France) was grown at 37 °C in 25-cm² culture flasks in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 µg/ml streptomycin. The human colorectal carcinoma cell line HT29 was obtained from the American Type Culture Collection, and was grown at 37 °C in 175-cm² culture flasks in RPMI 1640 medium containing 5% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. COS-7 cells were grown at 37 °C in 175-cm² culture flasks in Dulbecco’s modified Eiger’s medium (DMEM) containing 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. COS-7 cells were transiently transfected with a plasmid encoding the CCK-2 receptor as described previously (30).

The IMGE-5 cell line was established from the gastric mucosa of mice transgenic for a temperature-sensitive mutant of the SV40 large T antigen as described previously (31). IMGE-5 cells were generally grown at 33 °C in DMEM containing 1% fetal bovine serum (FBS), 1% penicillin, 100 units/ml penicillin, and 100 µg/ml streptomycin. The human colorectal carcinoma cell line HT29 obtained from the American Type Culture Collection, and was grown at 37 °C in 175-cm² culture flasks in RPMI 1640 medium containing 5% FBS, 10 units/ml penicillin, and 100 µg/ml streptomycin. COS-7 cells were grown at 37 °C in 175-cm² culture flasks in Dulbecco’s modified Eiger’s medium (DMEM) containing 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. COS-7 cells were transiently transfected with a plasmid encoding the CCK-2 receptor as described previously (30).

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so that a plot of $y/\gamma$ versus $\gamma$ at different $G$ values would be expected to yield intercepting straight lines. If cell counts in the test well are expressed as a percentage of the control value in the absence of Ggly or bismuth ions the following equation applies.

$$\frac{1/\gamma}{y} = \frac{1/\alpha}{\gamma} (test - control) \quad (Eq. 6)$$

Migration Experiments—To assess the effects of bismuth ions on Ggly-induced cell migration, wound healing experiments were performed as detailed elsewhere (18). In brief, IMGE-5 cells were grown in 12-well plates in DMEM at 33 °C until they reached 80% confluence, then shifted to 39 °C and serum starved for 24 h. The confluent monolayer was wounded using a 20-μl pipette tip, and cells were then washed 3 times with phosphate-buffered saline (2.7 mM KCl, 1.5 mM KH$_2$PO$_4$, 142 mM NaCl, and 10 mM Na$_2$HPO$_4$, pH 6.95) and treated with or without different compounds in DMEM with antibiotics. Morphology and migration of cells were observed and photographed immediately, and after treatment for 17 and 24 h. Wound width was measured at six different positions on the 24-h photographs, and averages were calculated.

CCK-2 Receptor Binding Assays—Binding of Gamide to the human CCK-2 receptor on transiently transfected COS-7 cells was measured in triplicate by competition for $^{125}$I-labeled Bolton and Hunter-CCK$_B$ (125I-Bolton-CCK$_B$) binding as described previously (30). Transfected COS-7 cells were grown to 60–70% confluence as described above, washed once with phosphate-buffered saline, and then incubated for 45 min at 37 °C in 500 μl of DMEM containing 0.1% bovine serum albumin and [125I]Bolton-CCK$_B$ (100,000 cpm, 14.5 fmol, Amersham Biosciences), with or without unlabeled compounds. Nonspecific binding was evaluated with 1 μM unlabeled CCK$_B$. Incubation was stopped by adding 2 ml of ice-cold phosphate-buffered saline containing 2% bovine serum albumin. After centrifugation, supernatants were removed and the radioactivity bound to the cell membranes was measured in a γ-counter (LKB-Wallac, Turku, Finland). Estimates of IC$_{50}$ values, and of the levels of [125I]Bolton-CCK$_B$, bound in the absence of competitor, were obtained by nonlinear regression with the program Sigmaplot (Jandel Scientific, San Rafael, CA) to the equation,

$$y = a(1 + x/b) \quad (Eq. 7)$$

where $y$ is the binding expressed as a percentage of the value $a$ observed in the absence of peptide competitor, $x$ is the concentration of peptide competitor, and $b$ is the IC$_{50}$ value.

Ggly Receptor Binding Assay—Ggly was iodinated using the IODOGEN method and purified by reversed phase high performance liquid chromatography (38). Binding assays with mono-[125I]Ggly were carried out by methods described previously (39). Briefly, 5 × 10$^5$ IMGE-5 cells/well were seeded in 6-well plates and grown at 33 °C for 24 h. The next day they were serum starved and shifted to 39 °C for 24 h. The next day they were incubated for 60 min at 39 °C in 200 μm Tris-HCl (pH 7.2) containing 100 mM KCl, 2 mM MgCl$_2$, 1 mM dithiothreitol, 1 mM benzamidine, 0.1% bovine serum albumin, and 5 × 10$^5$ cpm of [125I]Ggly with or without cold compounds. Nonspecific binding was evaluated with 1 μM cold Ggly. Cells were then washed three times in ice-cold phosphate-buffered saline and lysed with 1 N NaOH, and the amount of radioactivity bound was measured in a γ-counter.

Statistics—Results are expressed as the mean ± S.E. Data were analyzed by one-way analysis of variance, or by one-way repeated measures analysis of variance, as appropriate. If there was a statistically significant difference in the data set, individual values were compared with the appropriate value without bismuth ions by Bonferroni’s $t$ test. Differences with $p$ values of <0.05 were considered significant.

RESULTS

We have previously utilized quenching of tryptophan fluorescence to show that Ggly bound two trivalent ferric ions in aqueous solution (25). Examination of the structure of Ggly in aqueous solution by $^1$H NMR spectroscopy indicated that the first ferric ion bound to Glu-7, and the second ferric ion to Glu-8 and Glu-9 (26). The same techniques were used to investigate the hypothesis that gastrins bind bismuth ions.

**Gastrins Bind Two Bismuth Ions**—Ggly binds two ferric ions, and the binding data are well fitted by a model with two equivalent but independent binding sites, with $K_d$ = 0.62 μM (25). To test the hypothesis that gastrins bound bismuth ions, we compared the ability of trivalent bismuth, chromium, and ferric ions to quench the fluorescence of Gamide and Ggly. Bismuth ions quenched Gamide fluorescence, but the quenching was less marked than with ferric ions; in contrast chromium ions had little effect (Fig. 2A). Similar results were obtained with Ggly (data not shown). When the bismuth data were fitted by non-linear regression to models with either one binding site or two binding sites with identical affinity, the better fit was obtained with a 2-site model (Fig. 2B); when a model with two non-identical binding sites was used the $K_d$ values converged. We conclude that both Gamide and Ggly bind two bismuth ions, and that the two binding sites have very similar affinities. The deviation observed between the experimental data and the lines of best fit may indicate the presence of some co-operativity between binding of the first and second bismuth ions, or of polymeric species because bismuth compounds have a propensity to polymerize. The $K_d$ values obtained for the binding of bismuth ions to Gamide and Ggly were similar to each other, and were 41- and 10-fold higher than the $K_d$ values for the binding of ferric ions to Gamide and Ggly, respectively (Table I).

**Glutamates 7–9 Act as Bismuth Ligands**—To define the ligands involved in bismuth ion binding, we next investigated the effect of bismuth ions on the Ggly NMR spectrum (Fig. 3). Addition of 4 mol of bismuth ions/mol of Ggly broadened beyond detection the resonances of Glu-7, -8, and -9, without significantly affecting other resonances. These results suggested that the carboxyl groups of Glu-7, -8, and -9 were acting as ligands for the bismuth ion, and are in agreement with our previous
Bismuth Ions and Gastrin Activity

Table I
Affinity of gastrins for bismuth and ferric ions

The tryptophan fluorescence of Gamide and Ggly was measured in the presence of increasing concentrations of bismuth or ferric ions at pH 4.0. Values of the apparent dissociation constants (K_d) were obtained from data similar to that presented in Fig. 2B by nonlinear regression to the equation for a model with two identical independent binding sites with the program Sigmaplot. Values from the indicated number of independent experiments were combined to obtain the mean values (±S.E.) presented above. The K_d value for the binding of ferric ions to Ggly is taken from Ref. 25.

| Peptide | Bismuth K_d (μM) | Replicates | Ferric K_d (μM) | Replicates |
|---------|-----------------|------------|----------------|------------|
| Gamide  | 8.2 ± 0.8       | 5          | 0.2 ± 0.1      | 3          |
| Ggly    | 5.8 ± 1.4       | 5          | 0.6 ± 0.2      | 12         |

Conclusion that Glu-7 acts as a ligand for the first ferric ion binding site, and that Glu-8 and -9 act as ligands for the second ferric ion binding site (26).

Bismuth Ions Selectively Inhibit Ggly-induced Inositol Phosphate Production—To assess whether the binding of bismuth ions influenced the biological activity of either Ggly or Gamide, we next investigated inositol phosphate production. Ggly-stimulated inositol phosphate production was measured in HT29 human colorectal carcinoma cells. AlF_4^-, which constitutively activates G proteins upstream of phospholipase C, induced a 2.6-fold increase in inositol phosphate production in this system (Fig. 4A). Ggly significantly stimulated inositol phosphate production (225% compared with the control). In this model, Ggly is active in the absence of added ferric ions as the DMEM contains 248 nM Fe^{3+}. Addition of increasing concentrations of bismuth ions (2, 8, and 32 mol of Bi^{3+}/mol of Ggly) significantly reduced this stimulation. Similar inhibitory effects of bismuth ions on Ggly-stimulated inositol phosphate production were also observed in IMGE-5 gastric epithelial cells, but the fact that Ggly stimulation was only 110% compared with the control made quantitation more difficult (data not shown). Gamide-stimulated inositol phosphate production was measured in COS-7 cells transfected with the CCK-2 receptor. AlF_4^- induced a 5-fold increase in inositol phosphate production in this system (Fig. 4B). Gamide also significantly stimulated inositol phosphate turnover, by a factor of 4-fold. In contrast to Ggly, addition of increasing concentrations of bismuth ions (2, 8, and 32 mol of Bi^{3+}/mol of Gamide) had no significant effect on inositol phosphate production. Bismuth ions alone had no effect on inositol phosphate production in HT29 cells or in CCK-2 receptor-transfected COS-7 cells even at the highest concentration tested.

Bismuth Ions Selectively Inhibit Ggly-induced Cell Proliferation—We then investigated whether bismuth ions had any effect on cell proliferation. As reported previously by Dickinson and co-workers (17) Ggly-stimulated HT29 cell proliferation, by 124% compared with the control using a MTT assay (Fig. 5A). As with inositol phosphate production, addition of increasing concentrations of bismuth ions significantly decreased the proliferation induced by Ggly (Fig. 5A). Bismuth ions alone had no effect in this assay even at the highest concentrations tested. Similar inhibitory effects of bismuth ions were also observed when HT29 cell proliferation was measured by cell counting (Fig. 5B), and on Ggly-stimulated proliferation of the gastric carcinoma cell line AGS and the gastric epithelial cell line IMGE5 as assessed by MTT assay (data not shown).

To determine the type of inhibition the reciprocal of the proliferation data was plotted against the concentration of bismuth ions (Fig. 5C). With this transformation the family of straight lines obtained at different concentrations of Ggly either intercept above the ordinate in the case of competitive inhibition, on the ordinate in the case of noncompetitive inhibition, or are parallel in the case of uncompetitive inhibition (37). The observation of an intersection point above the ordinate was consistent with competitive inhibition (Fig. 5C). The value of the inhibition constant K_i obtained by curve fitting of the data obtained for 1 and 10 nM Ggly to the untransformed equation presented under “Experimental Procedures” was 0.29 ± 0.08 nM (mean ± S.E., n = 6).

Gamide significantly stimulated cell proliferation in the T-lymphoblastoid cell line Jurkat, by 176% compared with the control (Fig. 5D). In contrast to Ggly, addition of increasing concentrations of bismuth ions (2, 8, and 32 mol of Bi^{3+}/mol of Gamide) had no significant effect on Gamide-induced cell proliferation. At the highest concentration tested, bismuth ions alone significantly stimulated the proliferation of Jurkat cells.

Bismuth Ions Inhibit Ggly-induced Cell Migration—To determine whether or not the inhibition of Ggly biological activity by bismuth ions was a general phenomenon, we next investigated the effect of bismuth ions on Ggly-induced cell migration. At concentrations of 2, 8, or 32 mol/mol of Ggly, bismuth ions completely blocked Ggly-induced migration of IMGE-5 gastric epithelial cells as measured in wound healing assays (Fig. 6). The maximum concentration of bismuth ions alone had no effect in this assay. Gamide was not tested in this assay because we have shown previously that this hormone had no effect on IMGE-5 cell migration (18).

Bismuth Ions Inhibit Binding of Ggly to Its Receptor—Because bismuth ions had significant inhibitory effects on inositol phosphate production, cell proliferation, and migration, we investigated the effect of these ions on Ggly binding to IMGE-5 gastric epithelial cells. We measured the ability of 10 μM Ggly to compete with [125I]Ggly with or without various concentrations of bismuth ions (2, 8, and 32 mol of Bi^{3+}/mol of Ggly). As expected from the effects on Ggly-stimulated biological activity, bismuth ions inhibited the binding of [125I]Ggly to the Ggly receptor. Indeed, even 2 mol of Bi^{3+}/mol of Ggly inhibited [125I]Ggly binding by 50%, and complete inhibition was observed in the presence of 8 or 32 mol of Bi^{3+}/mol of Ggly (Fig. 7A).

Bismuth Ions Have Little Effect on Binding of Gamide to the CCK-2 Receptor—The absence of a major inhibitory effect of bismuth ions on the biological activities of Gamide suggested that bismuth ions would not inhibit significantly the binding of Gamide to the CCK-2 receptor. To test this hypothesis we measured the effect of bismuth ions on the ability of Gamide to compete with [125I]BH-CCK_2 for binding to CCK-2 receptors on transiently transfected COS-7 cells. No significant change in the binding of [125I]BH-CCK_2 was observed in the presence of increasing concentrations of bismuth ions (Fig. 7B).

 Discussion

Although bismuth has been used as a gastrointestinal therapeutic for over two centuries, there is still no established consensus on its mechanism of action. We have previously observed that gastrins bind two trivalent ferric ions with high affinity (25), and that ferric ion binding is essential for the biological activity of Ggly (26). We therefore hypothesized that trivalent bismuth ions might compete with ferric ions for the Ggly binding sites, and hence that bismuth ions might block the biological activity of Ggly.

The chemistry of aqueous solutions of bismuth is complex (40). Inorganic bismuth salts hydrolyze under weakly acidic to basic conditions with formation of the bismuthyl ion (BiO^3^-), which has low aqueous solubility (40). Extensive study of the complexes between bismuth and simple carboxylic acids such as nitroacetate has revealed an even greater complexity. The high coordination numbers accessible to bismuth permit...
extensive intermolecular interactions, and hence polymer formation (41). For these reasons we have restricted our biophysical analysis of the bismuth-gastrin complex to pH 4.0, to allow direct comparison with our previous study of the iron-gastrin complex. The pH value of 4.0 was originally chosen as a compromise to minimize the precipitation of gastrins that occurs at lower pH values, and the precipitation of ferric hydroxides that occurs at higher pH values (25). However, as discussed below, curve fitting of biological data (Fig. 5) yields an apparent dissociation constant in the nanomolar range for the bismuth-gastrin complex at neutral pH.

The fluorescence and NMR spectroscopic data presented in this article are consistent with the hypothesis that bismuth ions might compete with ferric ions for the Ggly binding sites. Fluorescence quenching experiments indicate that Ggly binds two bismuth ions at pH 4.0, with an affinity (5.8 μM) 10-fold lower than for ferric ions. Although the affinity of Ggly for bismuth or ferric ions at pH 7.6 cannot be calculated exactly from the value determined at pH 4.0 for reasons discussed previously (25), an estimate of the affinity can be made based on a comparison of the apparent first dissociation constants for the complex between ferric ions and nitrilotriacetic acid, which are 830 and 0.19 nM at pH 4.0 and 7.6, respectively. Based on the same ratio the affinity of Ggly for bismuth ions would be 1.5 nM at pH 7.6.

An independent estimate of the apparent affinity of Ggly for bismuth ions at neutral pH was obtained by linear transformation of proliferation data obtained in the presence of increasing concentrations of bismuth ions (Fig. 5C). The value of 0.29 ± 0.08 nM (mean ± S.E., n = 6) obtained by curve fitting of the data for 1 and 10 nM Ggly as described under “Experimental Procedures” was in reasonable agreement with the extrapolated value of 1.5 nM obtained from the fluorescence data as described in the previous paragraph. Under the conditions of the bioassays reported in Figs. 4–7 (i.e. total [Ggly] = 10 nM and total [bismuth] = 80 nM), the occupancy of the Ggly bismuth binding sites can be calculated from the fitted value of the apparent affinity of 0.29 nM to be greater than 99%. It must be emphasized that the fitted value should be regarded as an estimate only because in the absence of bismuth ions the stimulation by 1 nM Ggly was greater than that by 10 nM Ggly (Fig. 5C). This phenomenon of high dose inhibition by Ggly, which...
has been reported previously in the colon cell line YAMC (16), prevents simultaneous fitting of the data obtained at different concentrations of Ggly, as would be possible in a classical competitive inhibition model.

The changes observed in the NMR spectrum of Ggly on addition of bismuth ions reveal that glutamates 7, 8, and 9 act as bismuth ion ligands. These observations are similar to the previously reported effects of ferric ions on the NMR spectrum of Ggly (26). Because addition of 1 molar eq of ferric ions broadened the resonances of glutamate 7, without significantly affecting other resonances, we concluded that glutamate 7 acts as a ligand for the first ferric ion. Similarly, because addition of a second molar equivalent of ferric ions broadened the resonances of glutamates 8 and 9, without significantly affecting other resonances, we concluded that glutamates 8 and 9 act as ligands for the second ferric ion. Thus the Ggly side chains acting as bismuth ion ligands are similar to the side chains acting as ferric ion ligands, despite the greater ionic radius of the Bi^{3+} ion (0.096 nm) compared with the Fe^{3+} ion (0.064 nm).

Our results are consistent with the suggestion that bismuth and ferric ions compete for the same metal ion binding site on Ggly.

The biological data presented in this article are consistent with the second hypothesis that bismuth ions block the biological activity of Ggly. Thus the addition of bismuth ions reveal that glutamates 7, 8, and 9 act as bismuth ion ligands. These observations are similar to the previously reported effects of ferric ions on the NMR spectrum of Ggly (26). Because addition of 1 molar eq of ferric ions broadened the resonances of glutamate 7, without significantly affecting other resonances, we concluded that glutamate 7 acts as a ligand for the first ferric ion. Similarly, because addition of a second molar equivalent of ferric ions broadened the resonances of glutamates 8 and 9, without significantly affecting other resonances, we concluded that glutamates 8 and 9 act as ligands for the second ferric ion. Thus the Ggly side chains acting as bismuth ion ligands are similar to the side chains acting as ferric ion ligands, despite the greater ionic radius of the Bi^{3+} ion (0.096 nm) compared with the Fe^{3+} ion (0.064 nm). Our results are consistent with the suggestion that bismuth and ferric ions compete for the same metal ion binding site on Ggly.

The biological data presented in this article are consistent with the second hypothesis that bismuth ions block the biological activity of Ggly. Thus the addition of bismuth ions significantly inhibited both Ggly-stimulated inositol phosphate production (Fig. 4A) and proliferation (Fig. 5, A and B) in the human colorectal carcinoma cell line HT29, and migration of the gastric epithelial cell line IMGE5 (Fig. 6). Linear transformation of the proliferation data indicated that the inhibition was competitive in nature (Fig. 5C). Similar inhibitory effects of bismuth ions on Ggly-stimulated inositol phosphate produc-
Bismuth Ions and Gastrin Activity

The effect of bismuth ions on the binding of gastrins to their receptors. The effect of bismuth ions (Bi) at a concentration of 2, 8, or 32 nM on specific binding of [125I]Gly to the Gly receptor on IMGE-5 cells (A) or of [125I]BIH-CCK, to CCK-2 receptors on transiently transfected COS-7 cells (B) was measured as described under “Experimental Procedures.” Data are mean ± S.E. from three independent experiments, each in triplicate. Counts bound were 1.5 ± 0.2% (A) and 9.5 ± 0.2% (B) of counts bound. Nonspecific binding was not affected by the presence of bismuth ions. Statistical significance relative to the control (** p < 0.01) was assessed by one-way repeated measures of analysis of variance, followed by Bonferroni’s t test. Bismuth ions significantly inhibited binding of Gly to the Gly receptor, but had no effect on binding of Gamide to the CCK-2 receptor.

In contrast, bismuth ions do not affect the biological activity of Gamide. Thus the addition of bismuth ions did not significantly inhibit either Gamide-stimulated inositol phosphate production in COS-7 cells transiently transfected with the CCK-2 receptor (Fig. 4B) or proliferation (Fig. 5D) in Jurkat cells that express the CCK-2 receptor. These observations are consistent with our previous demonstration that binding of ferric ions to Glu-7 of Gamide is essential for biological activity (26), and with the early demonstration by Tracy and Gregory (42) that the C-terminal tetrapeptide amide is the minimum biologically active fragment of amidated gastrin.

The differential effects of bismuth ions on the biological activities of Gly and Gamide provide further evidence that the Gly receptor and the CCK-2 receptor are distinct. This distinction has previously been demonstrated by the inability of selective CCK-2 receptor antagonists to block Gly binding to cell lines (15, 16, 18) and to isolated colonic crypts (38), or to inhibit Gly-induced cell proliferation (15, 16, 18). The absolute requirement for ferric ions for Gly biological activity (26), when compared with the absence of any such requirement for ferric ions for the biological activity of Gamide, provides additional support for the conclusion that none of the effects of Gly are mediated via binding to the CCK-2 receptor.

The relationship between the selective inhibition of Gly bioactivity by bismuth and the therapeutic effect of bismuth is unclear, in part because the biological roles of Gly are still the subject of debate, and the nature of the Gly receptor has not been established. In the gastric lumen the antibacterial effect of bismuth on H. pylori might be partly mediated by an inhibition of the stimulatory effects of luminal gastrins on bacterial growth (11). Bismuth concentrations of ~30 nM have been reported in the serum of patients taking therapeutic doses of colloidal bismuth subcitrate for 15 days (43). On the basis of this concentration and the affinity of Gly for bismuth of 0.29 nM at neutral pH determined above, the occupancy of the two Gly binding sites can be calculated to be greater than 90%. This figure will of course be modified by the iron status of the patient because bismuth is also known to bind to apotransferrin (28), but in principle a significant proportion of the trivalent metal binding sites of Gly in serum may be occupied by bismuth. In terms of ulcer disease, inhibition of Gly activity by bismuth might then modulate the long-term acid stimulatory effects of an increased serum Gamide, because Gly seems to be required for a sustained Gamide-mediated hyperchlorhydria (24). In terms of proliferative disorders, Gly stimulates growth and migration in gastric and colonic cell lines (15–19), and accelerates colon carcinogenesis in rats treated with azoxymethane (44, 45). Bismuth has not to our knowledge been tested for the treatment of experimental or clinical colon cancer, but the data presented herein suggest that bismuth ions may reverse the stimulatory effects of Gly in animal models of colon carcinogenesis.

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