Complexes of Gastrin with In^{3+} , Ru^{3+} or Ga^{3+} Ions are not Recognised by the Cholecystokinin 2 Receptor.

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Abbreviations

ACN, acetonitrile; CCK2R, cholecystokinin 2 receptor; DFO, desferrioxamine; Gamide, amidated gastrin17; Ggly, glycine-extended gastrin17; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PBS, phosphate-buffered saline; PET, positron emission tomography; RPMI, Roswell Park Memorial Institute medium; SEM, standard error of the mean; SPECT, single photon emission computed tomography.

ABSTRACT

The peptide hormone gastrin (Gamide) binds trivalent metal ions, including indium (In), ruthenium (Ru) and gallium (Ga), with high affinity. Complexes of gastrin with chelated isotopes of In and Ga have previously been used for the location of tumours expressing the cholecystokinin 2 receptor (CCK2R). The aim of the present study was to purify the complexes of Gamide with radioactive isotopes of In, Ru or Ga and to investigate their ability to bind to the CCK2R. The radioactive Gamide complexes were purified on Seppak C18 cartridges or by anion exchange HPLC. Binding to the CCK2R was assessed with a stably transfected clone of the gastric carcinoma cell line AGS. The ^{106}Ru-Gamide complex could be eluted from the C18 cartridge; the ^{111}In-Gamide and ^{68}Ga-Gamide complexes bound irreversibly. All three complexes were successfully purified by anion exchange HPLC. The failure to detect binding of the ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes to the CCK2R suggests that formation of these complexes will not be useful for the detection of tumours expressing this receptor, but may instead provide alternative ways to block the actions of Gamide as a growth factor or a stimulant of gastric acid secretion.

1. INTRODUCTION

Amidated gastrin17 (Gamide; ZGPWLEEEEEAYGWMDF-amide) is a gut hormone that stimulates gastric acid secretion [1], and also regulates growth and differentiation of the gastric mucosa [2]. Gamide is thought to play a role in the development of gastrointestinal cancers, affecting proliferation, angiogenesis [3] and apoptosis [4]. The effects of Gamide are mediated through the cholecystokinin 2 receptor (CCK2R) [5, 6]. Glycine-extended gastrin (Ggly; ZGPWLEEEEEAYGWMDFG) is an immature precursor of Gamide with negligible affinity for the CCK2R and no direct effect on acid secretion. However, Ggly can also stimulate cell proliferation, migration, survival and is implicated in the development of colorectal cancer [7, 8].

The CCK2R is a G-protein-coupled 7 transmembrane domain receptor, which is expressed on several tumour types. Reubi and co-workers [9] have demonstrated that almost all medullary thyroid carcinomas and ovarian stromal carcinomas, and more than half of astrocytomas and small cell lung carcinomas, are CCK2R-positive. The use of metal chelate-conjugated gastrin derivatives for the diagnosis of CCK2R-positive tumours has been explored [10, 11], but the harsh conditions required for complex formation may result in some oxidative damage or modification to the peptide [12], which may potentially interfere with the ability of these modified gastrins to bind to CCK2Rs.

Gamide and Ggly both bind two ferric ions [13], the first to Glu7 and the second to Glu8 and Glu9 [14, 15]. The biological activity of Ggly as a stimulant of cell proliferation and migration is dependent on the presence of ferric ions, and thus can be completely blocked either by the substitution Glu7Ala, by competition with bismuth ions, or by treatment with the iron chelator desferrioxamine (DFO) [14], [16, 17]. In contrast, ferric ions are not required for the activation of the CCK2R by Gamide, as the resultant biological activity is unaffected by the substitution Glu7Ala or by treatment with DFO [18].

We recently reported that Gamide and Ggly also bind other trivalent metal ions, including gallium (Ga), indium (In) and ruthenium (Ru). In the case of Gamide, the order of affinities at the first metal ion binding site was In (K_d1 = 6.5 x 10^{-15} M) > Ru (K_d1 = 2.6 x 10^{-13} M) > Fe (K_d1 = 3.0 x 10^{-10} M) > Ga (K_d1 = 3.3 x 10^{-7} M). As complexes of gastrin with chelated isotopes of In and Ga are in clinical use for tumour location by single photon emission computed tomography (SPECT) and positron emission tomography (PET), respectively [10], the possibility arose that complexes of Gamide with appropriate trivalent

metal ions could be used for diagnosis of CCK2R-expressing tumours. The aim of the present study was to utilise radioactive isotopes of indium, ruthenium and gallium to investigate the purification of their Gamide complexes and their subsequent ability to bind to the CCK2R.

2. EXPERIMENTAL PROCEDURES

2.1 Peptides and chemicals

Human amidated gastrin was synthesized by Auspep (Clayton, Australia) to a purity of 88%. The impurities consisted of water and salts. All other consumables were obtained from Sigma Aldrich (Castle Hill, Australia), unless noted otherwise. Radiolabelled indium chloride (^{111}In, 5 mCi in 0.5 ml 50 mM HCl) was purchased from Landauer Australasia Pty Ltd (Sydney, Australia). Radiolabelled ruthenium chloride (^{106}Ru, 1 mCi in 0.5 ml 6M HCl) was purchased from Eckert & Ziegler Isotope Products (Valencia, CA). Radiolabelled gallium (^{68}Ga, 500 µCi in 100 µl 0.05M HCl, 0.25M NaAc) was prepared with a pharmaceutical grade ^{68}Ge/^{68}Ga generator (Eckert & Ziegler Isotope Products) according to the manufacturer's instructions. Solutions of metal ions (Aldrich, St.Louis, MO) were prepared in 10 mM HCl.

2.2 Cell culture

The human gastric cancer cell line, AGS, was obtained from ATCC. AGS cells stably transfected with the cholecystokinin 2 receptor (CCK2R) were kindly provided by Professor Andrea Varro, University of Liverpool, UK [19]. Cells were maintained in RPMI 1640 medium (Life Technologies, Mulgrave, Australia) supplemented with 8% foetal bovine serum and 20 mM HEPES, 0.4% penicillin/streptomycin, and grown at 37^{o}C in 5% CO_2.

2.3 Sep-Pak purification

The ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes were made by incubating 38 nmol of ^{111}In, 250 pmol of ^{106}Ru or 700 pmol of ^{68}Ga with 250 pmol of Gamide in 50 mM Na^+ acetate, pH 4, for 30 min at room temperature. The reaction mixtures were then loaded onto Sep-Pak C18 reverse phase cartridges (Waters Corporation, Milford, MA), which had been activated by precycling with 10 ml of buffer A (100 mM Na^+ acetate (pH 4)), followed by 10 ml of buffer B (100 mM Na^+ acetate (pH 4), 50% acetonitrile (ACN)) and 10 ml of buffer A. After loading, the Sep-Pak cartridge was washed with 10 ml of buffer A to remove unbound ^{111}In, ^{106}Ru or ^{68}Ga, and eluted with buffer B in 5 serial fractions (1 ml

each). The amount of radioactivity in each collected fraction was measured either with a β-counter (Packard, Meriden, CT) or a γ-counter (Perkin Elmer, Waltham, MA).

2.4 HPLC purification

The ^{111}In-Gamide, ^{106}Ru -Gamide and ^{68}Ga-Gamide complexes (made as in 2.3, but not run on a Sep-Pak column) were purified by HPLC on either reverse-phase or anion exchange columns. Reverse-phase HPLC used a C4 column at a flow rate of 1 ml/min with a gradient from 10 mM Na^+ acetate, 100 mM NaCl (pH 4) to 10 mM Na^+ acetate, 100 mM NaCl (pH 4) + 70% ACN over 30 min. The anion exchange HPLC used a Protein-Pak Q 8HR column (Waters, Rydalmere, Australia) at a flow rate of 1 ml/min with a gradient from 10 mM Na^+ acetate (pH 4) to 10 mM Na^+ acetate (pH 4) + 1 M NaCl over 30 min. Fractions were collected every minute and the amount of radioactivity was measured either with a β-counter or a γ-counter. Peptide peaks (line) were observed as an increase in 280 nm absorbance. Radioactivity (%, bars) was determined based on the total amount loaded onto the column as 100%.

2.5 Stability of ^{106}Ru -Gamide complex over time

The Sep-Pak purified ^{106}Ru-Gamide complex was diluted with an equal volume of 50 mM Na^+ HEPES, pH 7.6, and divided into 4 identical aliquots, which were loaded onto new C18 Sep-Pak cartridges after 0 h, 24 h, 48 h and 5 days, respectively. The ^{106}Ru-Gamide complex was eluted as described above, and the radioactivity eluted in fractions 1 and 2 at each time point was expressed as a percentage of the 0 h point and used to calculate the stability of ^{106}Ru-Gamide by curve fitting to the equation for exponential decay.

2.6 Binding assay

AGS wildtype cells (WT) and AGS cells stably expressing the CCK2R (AGS-CCK2R) were seeded at a density of 5 X 10^5 cells/well in 6-well plates. The following day, cells were rinsed with binding buffer (20 mM Na^+ Hepes, 10 mM KCl, 1.25 mM benzamidine, 20 µM $CaCl_2$, 80 µM $MgCl_2$, pH 7.4) and pre-treated with binding buffer or 100 nM Gamide in binding buffer for 15 min at 37°C. ^{111}In-Gamide and ^{106}Ru-Gamide were purified by anion exchange HPLC as described above; purification of ^{68}Ga-Gamide was not attempted because of the short half-life of ^{68}Ga (68 min). ^{111}In-Gamide (48,300 cpm/well), ^{106}Ru-Gamide (4,350 cpm /well), ^{68}Ga-Gamide (81,600 cpm/well), or sulphated cholecystokinin 8 (CCK8) labelled at the N-terminus with ^{125}I-labelled-Bolton and Hunter reagent (20,000 cpm/well, Amersham Biosciences, Castle Hill, Australia) was added to separate wells and incubated for 30 min at

37°C. The cells were washed twice with PBS and lysed with 500 µl of 1M NaOH. The amount of bound radioactivity was determined using either a β-counter or a γ-counter.

2.7 Statistics

Each experiment was repeated at least 3 times. All values are expressed as means ± standard error. Statistical significance was determined by Student's t-test using SigmaStat (Jandel Scientific, San Rafael, CA).

3. RESULTS

Selective binding of indium, ruthenium or gallium ions by Gamide or Ggly was previously demonstrated using absorption spectroscopy and extended X-ray absorption fine structure (EXAFS) spectroscopy [15]. The formation of these non-radioactive complexes suggested that the radioactive forms of these trivalent metals (M^{3+}) bound to Gamide could be utilised to identify tumours expressing the CCK2R. In order to accomplish this task, these complexes were formed as previously described and then purified and tested for stability as detailed below.

3.1 Purification of the ^{106}Ru-Gamide complex using C18 SepPak cartridges.

Radioactive isotopes of Indium (^{111}In), Ruthenium (^{106}Ru) and Gallium (^{68}Ga) were used to quantitate M^{3+}-Gamide complex formation. Purification of the ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes was initially attempted with C18 SepPak reverse phase cartridges, by elution of the M^{3+}-Gamide complex with acetonitrile (ACN). Although no elution of ^{111}In-Gamide (Fig. 1A) or ^{68}Ga-Gamide (Fig. 1C) from the C18 SepPak cartridges was detected, ^{106}Ru-Gamide was clearly bound (Fig. 1B) and was differentially eluted with 50% ACN in fractions 1 and 2. Unbound ^{106}Ru passed through the cartridge in the initial flow through. The ^{111}In-Gamide and ^{68}Ga-Gamide complexes remained bound to the C18 resin as no additional radioactivity was observed in fraction 2 in the presence of Gamide, and as these cartridges remained radioactive after elution.

3.2 Purification of ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes using HPLC.

As the ^{111}In-Gamide and ^{68}Ga-Gamide complexes were not eluted from the C18 Sep-Pak cartridges, purification of all three complexes was attempted by C4 reverse phase HPLC. The main absorbance peak for Gamide in the absence of added metal ions eluted at 21 min (Fig. 2A). In the presence of metal ions (Fig. 2B-D) unbound metal ions eluted at 4-6 min, the Gamide peak at 21 min was no longer present, and no additional radioactive peaks were eluted. Therefore the ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes presumably remained bound to the C4 resin.

Subsequently, the ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes were purified by anion exchange HPLC. The main absorbance peak for Gamide in the absence of added metal ions eluted at 14.5 min. In the presence of Ru^{3+} ions a new absorbance peak, with associated radioactivity, appeared at 11 min (Fig. 3C). A new early absorbance peak also appeared in the presence of In^{3+} (Fig. 3B) or Ga^{3+}

(Fig. 3D) ions, although in these cases the peak was smaller, and the associated radioactivity tailed extensively. The earlier radioactive peaks eluting in the breakthrough at 1-2 min represent unbound metal ions. The correlation between radioactivity and peptide absorbance indicated that all three M^{3+}-Gamide complexes formed and could be purified. Therefore, appropriate fractions were collected and used to test their stability (in the case of ^{106}Ru-Gamide) and ability to bind selectively to cells expressing the CCK2R.

3.3 The ^{106}Ru-Gamide complex was stable for over 5 days.

Determination of the long-term stability of the M^{3+}-Gamide complexes was only possible for ^{106}Ru ($t_{½}$ = 367 days), as the short half-lives of ^{111}In (2.8 days) and ^{68}Ga (68 min) were limiting. After 30 min incubation the ^{106}Ru-Gamide complex was purified on a C18 SepPak reverse phase cartridge, divided into four aliquots, and each aliquot was subsequently re-purified by the same method after various incubation times at room temperature. The half-life of the ^{106}Ru-Gamide complex obtained by curve fitting assuming exponential decay was 11.1 days at room temperature and pH 7.6 (Fig. 4).

3.4 ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes do not bind to the CCK2R on cultured cells.

Human gastric cancer cells deficient for (AGS-WT), or stably transfected with a plasmid expressing the CCK2R (AGS-CCK2R), were used to determine if any of the purified M^{3+}-Gamide complexes were selectively bound by the CCK2R. Since their introduction by the Varro and Dockray group this pair of cell lines has become a frequently used model for studies of CCK2R binding [19]. Binding to AGS-WT and AGS-CCK2R cells was assessed using ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide diluted into binding buffer in the presence or absence of unlabelled Gamide. Sulphated cholecystokinin 8 labelled at the N-terminus with ^{125}I-Bolton and Hunter reagent (^{125}I-CCK8) was used as a positive control. As expected the amount of ^{125}I-CCK8 radioactivity bound to the CCK2R on AGS-CCK2R cells was decreased in the presence of unlabelled Gamide (Fig. 5D). The absence of any such competition with ^{111}In-Gamide, ^{106}Ru-Gamide or ^{68}Ga-Gamide (Fig. 5A, B, C) in the presence of unlabelled Gamide indicated that none of the radioactive Gamide-metal ion complexes bound to the CCK2R. Presumably the association of Gamide with ^{111}In, ^{106}Ru or ^{68}Ga prevented recognition of the complex by the CCK2R.

4. DISCUSSION

Metal chelate-conjugated gastrin derivatives, such as 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-minigastrin radiolabelled with either ^{111}In or ^{68}Ga, have previously been used for the diagnosis of CCK2R-positive tumours [10]. However, the harsh conditions (pH 4.5, 98^{o}C, 15 min) required to incorporate these metal ions may potentially result in oxidative damage or modification to the peptide [12]. Based on previous work demonstrating high binding affinity of certain trivalent metals to Gamide under relatively mild conditions (pH 4, room temperature, 30 min) [15], we set out to purify M^{3+}-Gamide complexes that might be used to identify cells and tumours that express CCK2Rs.

The ^{106}Ru-Gamide complex was purified on a C18 SepPak cartridge (Fig. 1). In contrast with the ^{111}In-Gamide and ^{68}Ga-Gamide complexes the radioactivity recovered from the C18 SepPaks was always less than what was added to the cartridges, which remained radioactive after elution. These observations suggest that the ^{111}In-Gamide and ^{68}Ga-Gamide complexes may have remained bound to the cartridges, even in the presence of 50% acetonitrile. Similarly none of the M^{3+}-Gamide complexes were eluted from the C4 HPLC column under conditions that eluted Gamide (Fig. 2). This observation is not unexpected as the neutralization of the negative charge of Gamide (6-) by the presence of the positive charge (6+) of two M^{3+} ions would be predicted to increase the hydrophobicity of the complex, causing it to bind more tightly to the column.

In contrast all three M^{3+}-Gamide complexes were successfully purified by anion exchange HPLC (Fig. 3). As expected from the neutralization of the negative charge of Gamide (6-) by the presence of the positive charge (6+) of two M^{3+} ions, the M^{3+}-Gamide complexes eluted from the anion exchange column earlier than Gamide when using a salt gradient.

No binding of the ^{111}In-Gamide, ^{106}Ru-Gamide or ^{68}Ga-Gamide complexes to the CCK2R was detected. This observation was unexpected, as several lines of indirect evidence had previously suggested that binding of Fe^{3+} ions was not essential for recognition of Gamide by the CCK2R. For example the minimum peptide structure required for CCK2R binding was the C-terminal tetrapeptide amide [20], which lacks the pentaglutamate sequence implicated in the binding of Fe^{3+} ions [14]. Furthermore removal of the first Fe^{3+} binding site by the E7A mutation had no significant effect on CCK2R binding or

on biological activity [18], and CCK2R binding and biological activity were unaffected by the Fe^{3+}-selective chelator desferrioxamine [18]. In the case of ^{106}Ru-Gamide, the failure to detect binding does not appear to be the result of dissociation, as the complex was shown to be very stable at neutral pH, with a half-life of 11.1 days (Fig. 4). Similarly, disruption of the ^{111}In-Gamide and ^{106}Ru-Gamide complexes by the 1M NaCl gradient used in anion exchange chromatography appears unlikely, as the extremely high stability constants of the In^{3+} (6.5×10^{-15}) and Ru^{3+} (2.6×10^{-13}) complexes were actually determined in the presence of 100 mM NaCl [15], and the concentration of NaCl required for elution was less than 500 mM (Fig. 3).

The failure of the ^{111}In-Gamide, ^{106}Ru-Gamide or ^{68}Ga-Gamide complexes to bind to the CCK2R is presumably due to the differences in their structures caused by differences in their ionic radii and coordination state. Indeed a previous EXAFS study has shown that the structure of Ru_2Gamide differs from Fe_2Gamide, with a direct Ru-Ru bond instead of the bridging water molecule between the two ferric ions [15]. One intriguing possibility is that the aspartic acid in the C-terminal moiety of gastrin might interact with indium, ruthenium or gallium ions with consequent inhibition of binding to the CCK2R. In the case of ferric ions and bismuth ions, our NMR data provided convincing evidence that only the glutamic acid residues at positions 7, 8 and 9 of glycine-extended $gastrin_{17}$ were involved in metal binding [14]. Our EXAFS data indicated the presence of carboxylate ligands, but did not discriminate between aspartic and glutamic acid residues in the case of indium, ruthenium and gallium ions [15]. However the binding data presented in the same paper were consistent with competitive inhibition, in which ruthenium (or indium) and ferric ions competed for the same two metal ion binding sites on gastrin. Although attempts to crystallise the gastrin-metal ion complexes have so far been unsuccessful, X-ray crystallography may ultimately be required to identify precisely which gastrin residues are acting as metal ion ligands.

On the other hand, there is now abundant evidence that Fe^{3+} ions are essential for the biological activity of non-amidated gastrins such as progastrin and Ggly [14, 16, 17]. However the identity of the receptors for non-amidated gastrins is still controversial [21-23], and the use of ^{111}In-Ggly, ^{106}Ru-Ggly or ^{68}Ga-Ggly complexes may help to resolve that debate. As non-amidated gastrins stimulate proliferation in the normal colorectal mucosa and accelerate the development of colorectal cancer [7, 8], these receptors may become important targets for improvements in cancer diagnosis and therapy.

This study also suggests a new method of blocking the biological actions of amidated gastrins. Occupation of the metal ion-binding site of amidated gastrins by indium, ruthenium or gallium ions prevents the binding of Gamide to the CCK2R, and so renders the peptide inactive. The high affinity of Gamide for indium and ruthenium ions suggests the possibility that low doses of these ions could be used as specific inhibitors for the treatment of excessive acid production in patients with conditions such as gastrointestinal ulcers, gastro-oesophageal reflux or gastric carcinoid. The same approach might also be used to inhibit G-amide stimulated growth and differentiation of the gastric mucosa, and of gastric cancer [2].

FIGURE LEGENDS

Figure 1: Purification of the ^{106}Ru-Gamide complex on SepPak C18 cartridges.

The reaction mixtures containing ^{111}In-Gamide (A), ^{106}Ru -Gamide (B) or ^{68}Ga-Gamide (C) complexes were passed through C18 Sep-Pak cartridges and the elution of radioactivity was compared to ^{111}In, ^{106}Ru and ^{68}Ga alone, respectively. After passage of the complexes the cartridges were first washed with 10 ml 100 mM Na^+ acetate (pH 4) to remove unbound ^{111}In, ^{106}Ru or ^{68}Ga, and then eluted with 5 x 1 ml 100 mM Na^+ acetate (pH 4) + 50% ACN. The ^{106}Ru-Gamide complex was eluted with 50% ACN in fractions 1 and 2, and the radioactivity in each eluted fraction is expressed as a percentage of the total eluted radioactivity. The ^{111}In-Gamide and ^{68}Ga-Gamide complexes remained bound to the C18 resin as no additional radioactivity was observed in fraction 2 in the presence of Gamide, and the cartridges remained radioactive after elution.

Figure 2: Purification of the ^{111}In-Gamide, ^{106}Ru -Gamide and the ^{68}Ga-Gamide complexes by C4 HPLC.

Because the ^{111}In -Gamide and ^{68}Ga-Gamide complexes remained bound to the C18 resin (Fig. 1), Gamide alone (A) or the ^{111}In-Gamide (B), ^{106}Ru-Gamide (C) or ^{68}Ga-Gamide (D) complexes were run on a C4 reverse phase HPLC column. A gradient from 10 mM Na^+ acetate, 100 mM NaCl, pH 4 to 10 mM Na^+ acetate, 100 mM NaCl, pH 4 + 70% ACN was used over 30 min (dotted line, 70% ACN was increased from 0 to 40% over 5 min (5-10 min), at which point it was increased to 100% (10-30 min)). The main absorbance peak (line) for Gamide in the absence of added metal ions eluted at 21 min (A). In the presence of metal ions (B-D) unbound metal ions eluted at 4-6 min, the Gamide peak at 21 min was no longer present, and no additional radioactive peaks (bars) were eluted. Therefore the ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes presumably remained bound to the C4 resin.

Figure 3: Purification of the ^{111}In-Gamide, ^{106}Ru-Gamide and the ^{68}Ga-Gamide complexes by anion exchange HPLC.

Gamide alone (A) or the ^{111}In-Gamide (B), ^{106}Ru-Gamide (C) and ^{68}Ga-Gamide (D) complexes were run on an anion exchange HPLC column using a gradient from 10mM Na^+ acetate (pH 4) to 10mM Na^+ acetate (pH 4) + 1M NaCl over 30 min (dotted line, 1 M NaCl was increased from 0 to 40% over 5 min (5-10 min), at which point it was increased to 100% (10-30 min)). The main absorbance peak (line) for Gamide in the absence of added metal ions eluted at 14 min (A). In the presence of Ru^{3+} ions a new peak, with associated radioactivity (bars), appeared at 11 min (C). The new early peak also appeared in the presence of Ga^{3+} (D) or In^{3+} (B) ions, although in these cases the peak was smaller, and the

associated radioactivity tailed extensively. The earlier radioactive peaks eluting in the breakthrough at 1-2 min represent unbound metal ions.

Figure 4: Stability of ^{106}Ru-Gamide complex

The Sep-Pak-purified ^{106}Ru-Gamide complex was diluted with an equal volume of 50 mM Na^+ HEPES, pH 7.6, and divided into 4 aliquots, which were incubated for 0 h, 24 h, 48 h or 5 days at room temperature, and loaded onto new C18 Sep-Pak cartridges. The remaining ^{106}Ru-Gamide complex was eluted as described above, and the radioactivity eluted in fractions 1 and 2 at each time point was expressed as a percentage of the 0 h point. The half-life of the ^{106}Ru-Gamide complex obtained by curve fitting assuming exponential decay was 11.1 days at room temperature and pH 7.6.

Figure 5: The ^{111}In-Gamide, ^{106}Ru-Gamide and ^{68}Ga-Gamide complexes do not bind to the CCK2R.

^{111}In-Gamide and ^{106}Ru-Gamide were purified by anion exchange HPLC as described in Experimental Procedures; purification of ^{68}Ga-Gamide was not attempted because of the short half-life of ^{68}Ga (68 min). Binding of the ^{111}In-Gamide (A, 48,300 cpm/well), ^{106}Ru-Gamide (B, 4,350 cpm/well), or ^{68}Ga-Gamide (C, 81,600 cpm/well) complexes to wild type (WT) or the CCK2R expressing AGS cells in the presence, or absence, of 100 nM unlabelled Gamide was determined as described under Experimental Procedures. Binding of ^{125}I-CCK8 (D, 20,000 cpm/well) in the presence, or absence, of 100 nM unlabelled Gamide was assessed as a positive control. Although the presence of 100 nM unlabelled Gamide reduced the binding of ^{125}I-CCK8 to the CCK2R by more than 90% (**, $p < 0.01$), no evidence was obtained for binding of M^{3+}- Gamide complexes to the CCK2R.

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Figure 1

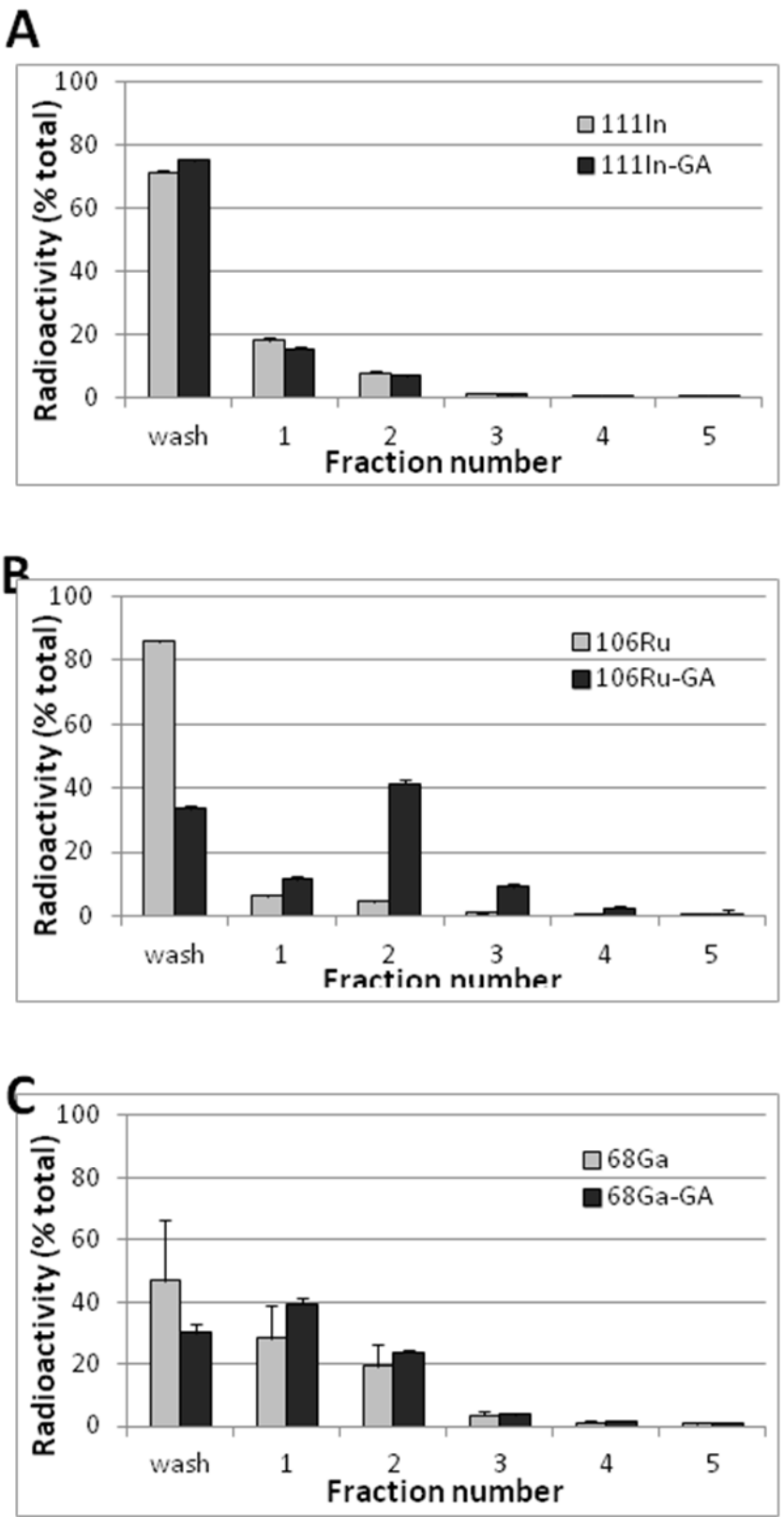

A
100
80
60
40
20
0
Radioactivity (% total)
111In
111In-GA
wash
1
2
3
4
5
Fraction number
B
100
80
60
40
20
0
Radioactivity (% total)
106Ru
106Ru-GA
wash
1
2
3
4
5
C
100
80
60
40
20
0
Radioactivity (% total)
68Ga
68Ga-GA
wash
1
2
3
4
5
Fraction number

Figure 2

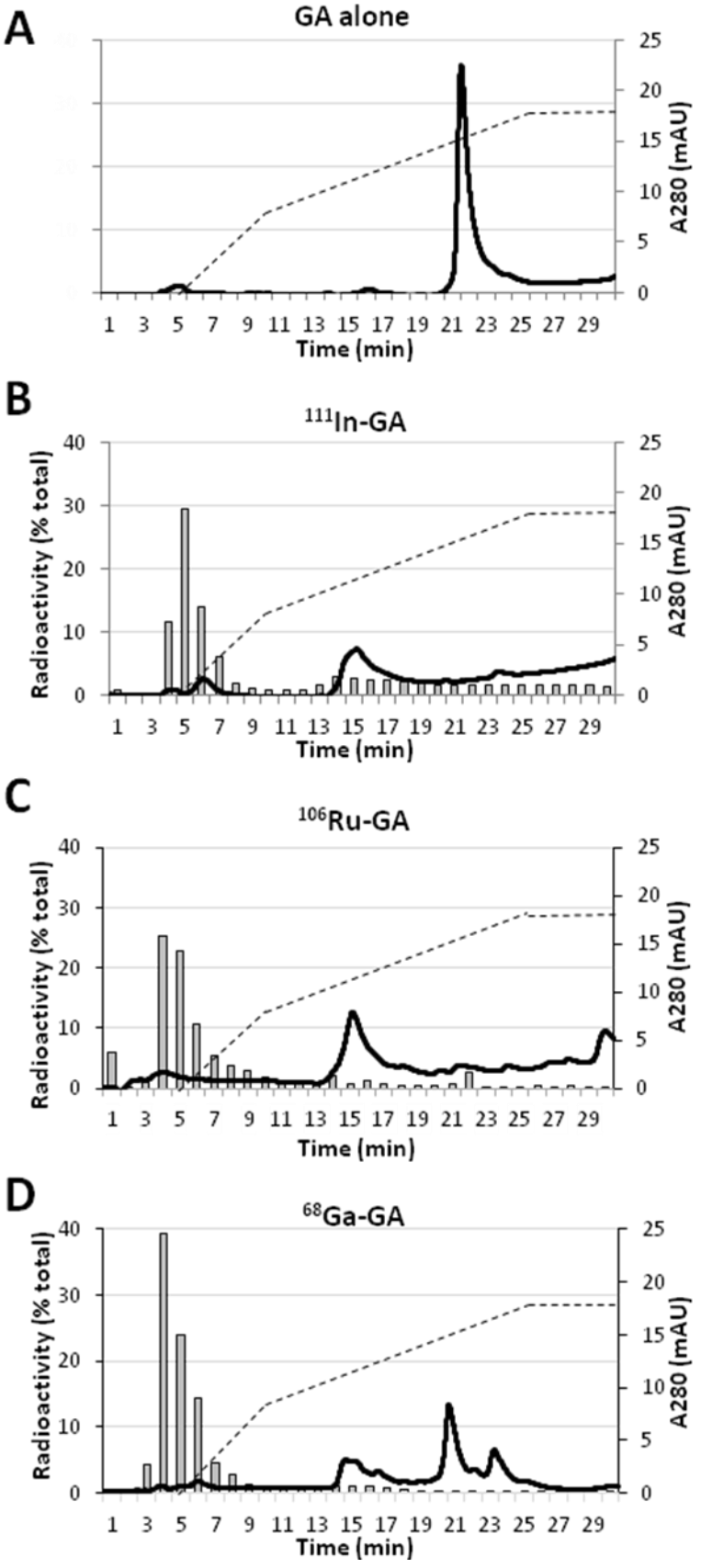

A
GA alone
B
^{111}In-GA
C
^{106}Ru-GA
D
^{68}Ga-GA
Radioactivity (% total)
A280 (mAU)
Time (min)
0
10
20
30
40
0
5
10
15
20
25
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29

Figure 3

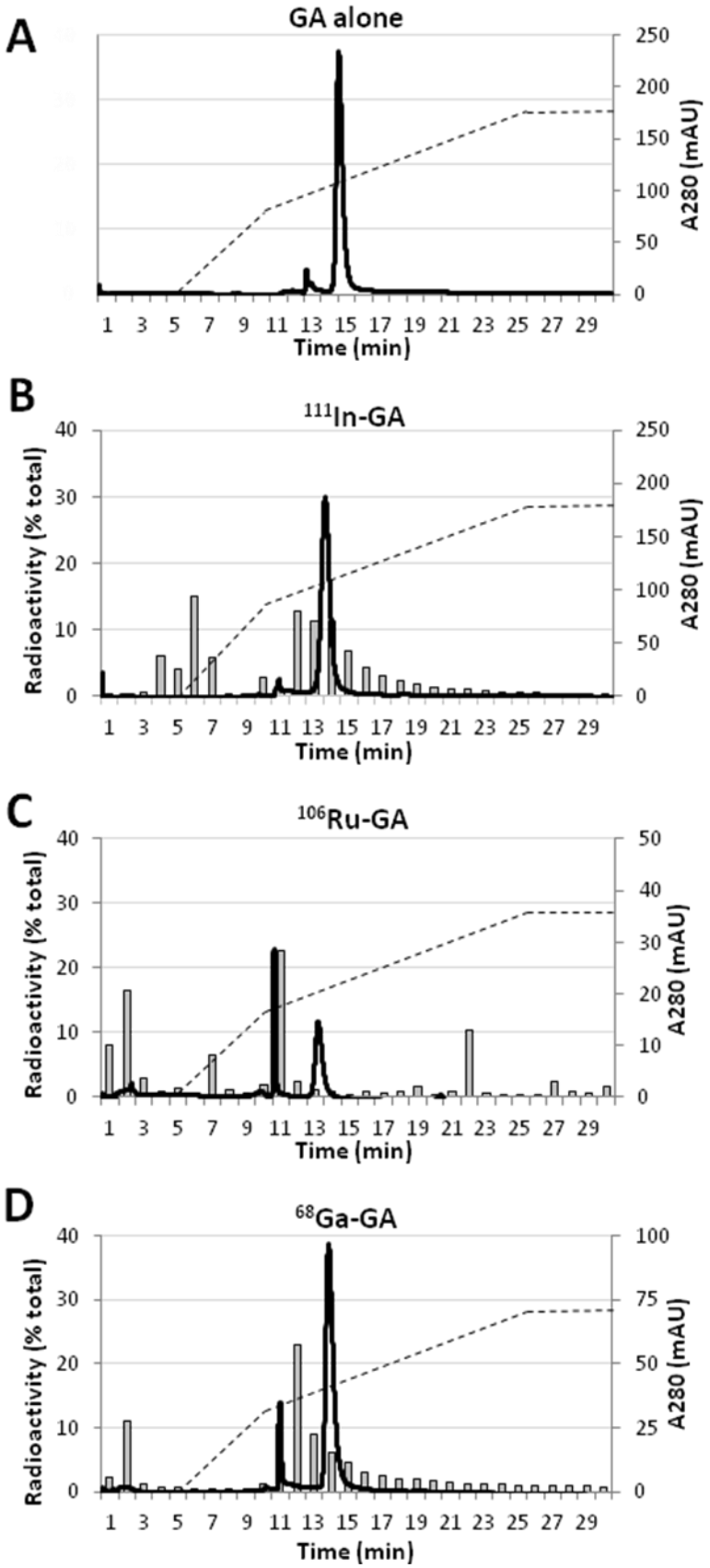

A
GA alone
A280 (mAU)
Time (min)
B
^{111}In-GA
Radioactivity (% total)
A280 (mAU)
Time (min)
C
^{106}Ru-GA
Radioactivity (% total)
A280 (mAU)
Time (min)
D
^{68}Ga-GA
Radioactivity (% total)
A280 (mAU)
Time (min)

Figure 4

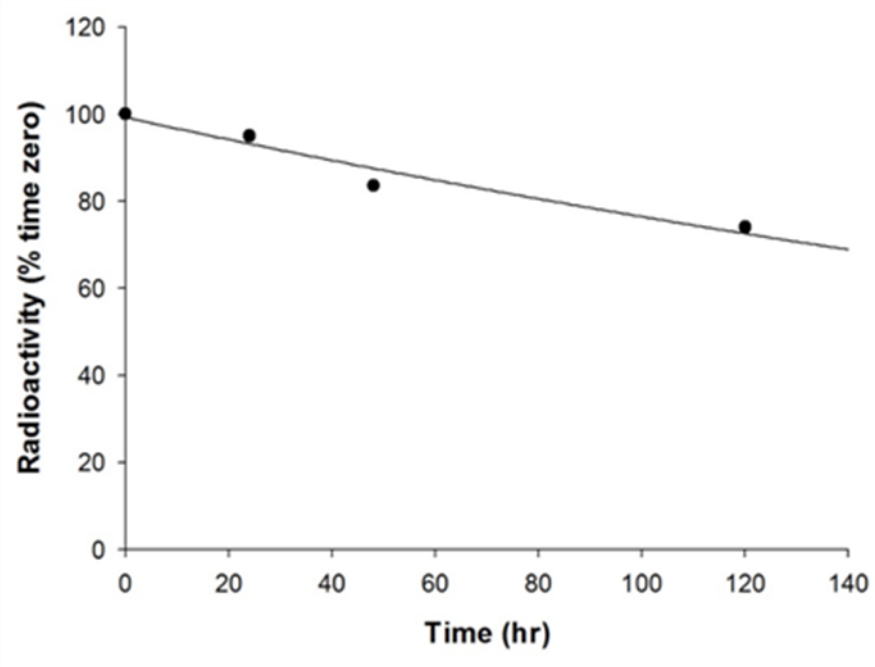
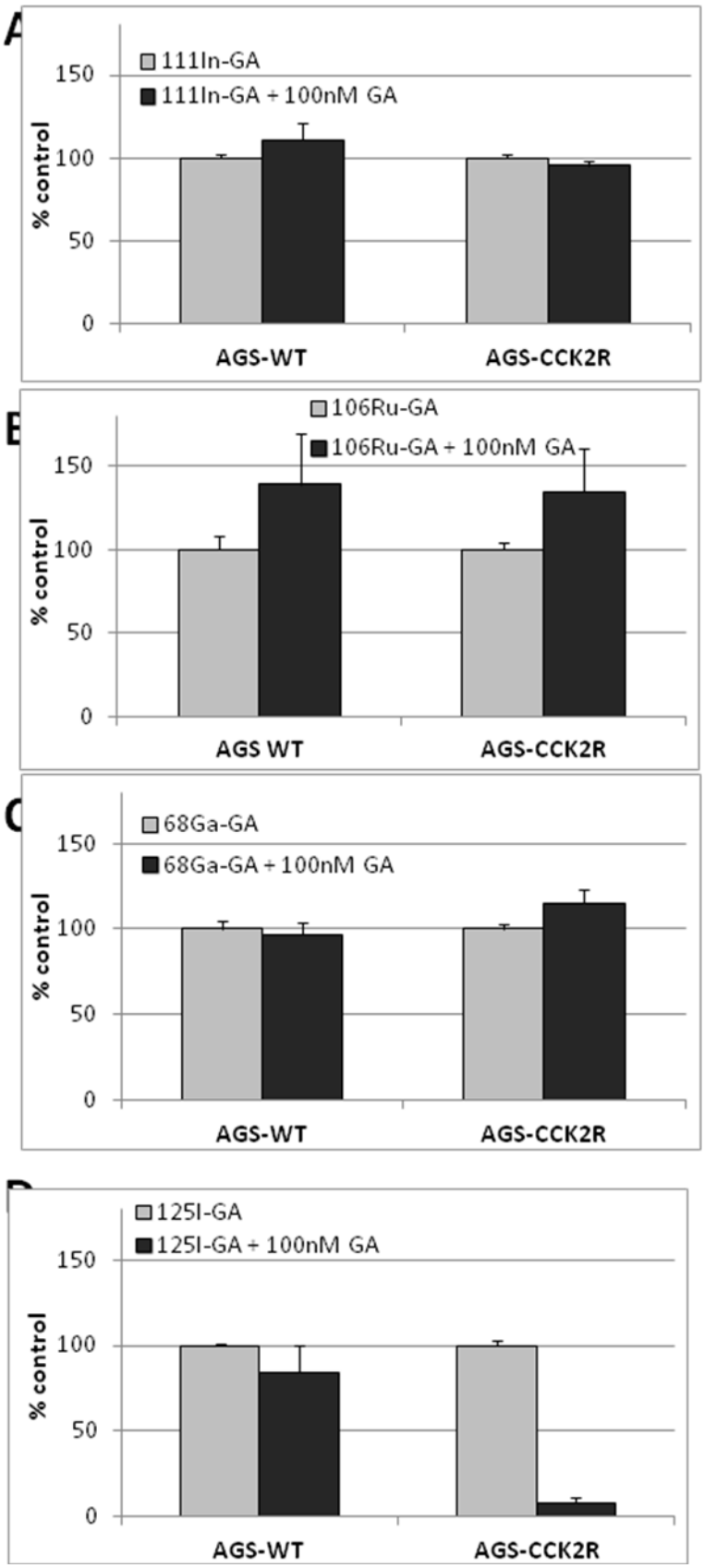
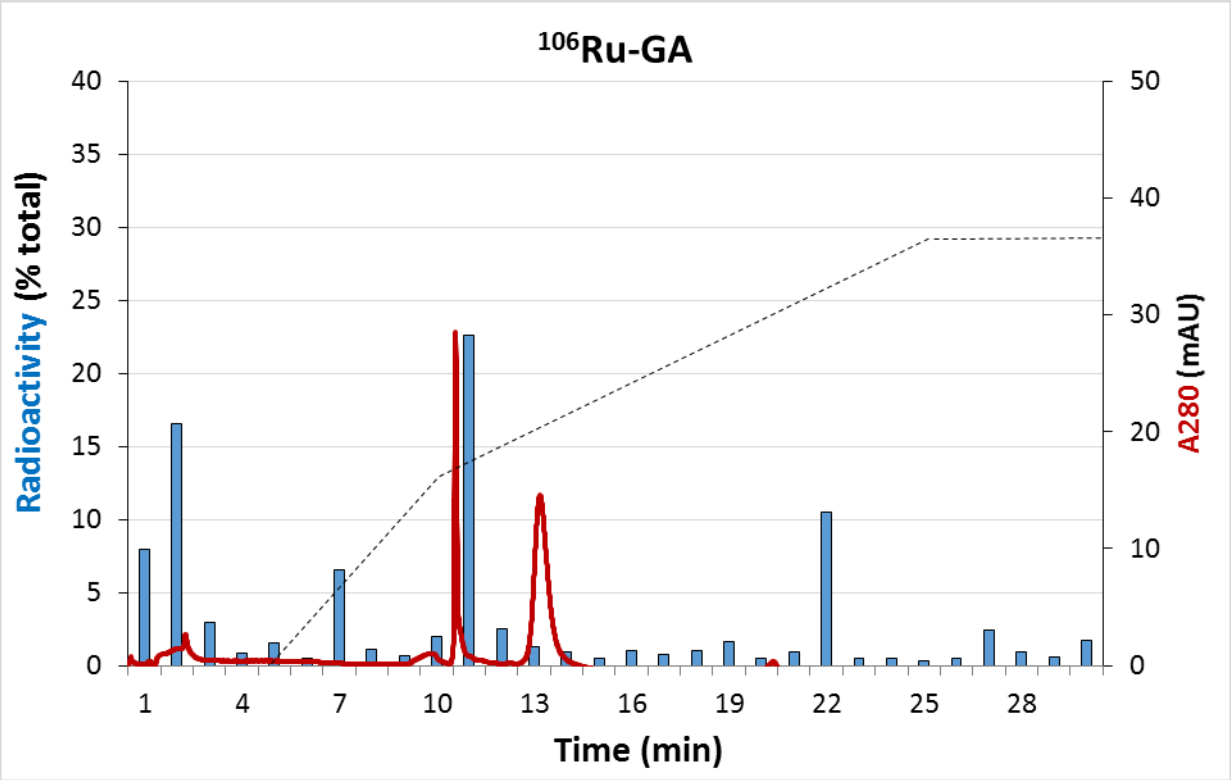

Radioactivity (% time zero)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Time (hr)

Figure 5



The complexes between the hormone gastrin and radioactive ^{111}In, ^{106}Ru or ^{68}Ga ions were purified by anion-exchange HPLC using a NaCl gradient. The failure to detect binding of the complexes to the

cholecystokinin2 receptor suggests that metal ion treatment may provide novel approaches to block the biological actions of gastrin.



Author/s:
Laval, M; Marshall, KM; Sachinidis, J; Scott, A; Eutick, M; Baldwin, GS

Title:
Complexes of gastrin with In3+, Ru3+ or Ga3+ ions are not recognised by the cholecystokinin 2 receptor

Date:
2017-10-01

Citation:
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