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Radiolabelled GLP-1 analogues for *in vivo* targeting of insulinomas

Maarten Brom[1], **Lieke Joosten**[1], **Wim J.G. Oyen, MD, PhD**[1], **Martin Gotthardt, MD, PhD**[1], and **Otto C. Boerman, PhD**[1]

[1]Department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands

Abstract

For peptide receptor targeting usually internalizing agonists are selected. There is increasing evidence that non-internalizing receptor antagonists can be used for this purpose. We investigated whether the glucagon-like peptide-1 receptor (GLP-1R) antagonist exendin(9-39) can be used for *in vivo* targeting of GLP-1R expressing tumours and compared the *in vitro* and *in vivo* characteristics to the GLP-1R agonists exendin-3 and exendin-4.

The binding and internalization kinetics of labelled [Lys^{40}(DTPA)]exendin-3, [Lys^{40}(DTPA)]exendin-4 and [Lys^{40}(DTPA)]exendin(9-39) were determined *in vitro* using INS-1 cells. The *in vivo* targeting properties of [Lys^{40}(^{111}In-DTPA)]exendin-3, [Lys^{40}(^{111}In-DTPA)]exendin-4 and [Lys^{40}(^{111}In-DTPA)]exendin(9-39) were examined in BALB/c nude mice with subcutaneous INS-1 tumours. ^{nat}In-labelled [Lys^{40}(DTPA)]exendin-3, [Lys^{40}(DTPA)]exendin-4 and [Lys^{40}(DTPA)]exendin(9-39) exhibited similar IC_{50} values (13.5, 14.4 and 13.4 nM, respectively) and bound to 26×10^3, 41×10^3 and 37×10^3 receptors/cell, respectively. [Lys^{40}(^{111}In-DTPA)]exendin-3 and [Lys^{40}(^{111}In-DTPA)]exendin-4 showed rapid *in vitro* binding and internalization kinetics, whereas [Lys^{40}(^{111}In-DTPA)]exendin(9-39) showed lower binding and minimal internalization *in vitro*. In mice, high specific uptake of [Lys^{40}(^{111}In-DTPA)]exendin-3 (25.0 ± 6.0 %ID/g) in the tumour was observed at 0.5 h p.i. with similar uptake up to 4 h p.i.. [Lys^{40}(^{111}In-DTPA)]exendin-4 showed higher tumour uptake at 1 and 4 h p.i. (40.8 ± 7.0 and 41.9 ± 7.2 %ID/g, respectively). Remarkably, [Lys^{40}(^{111}In-DTPA)]exendin(9-39) showed only low specific uptake in the tumour at 0.5 h p.i. (3.2 ± 0.7 %ID/g), rapidly decreasing over time.

In conclusion, the GLP-1R agonists [Lys^{40}(DTPA)]exendin-3 and [Lys^{40}(DTPA)]exendin-4 labelled with ^{111}In could be useful for in *vivo* GLP-1R targeting, whereas [Lys^{40}(DTPA)]exendin(9-39) is not suited for *in vivo* targeting of the GLP-1R.

Author to whom request reprints should be addressed: Maarten Brom, Department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands, Telephone/fax: +31 24 36 15054/+31 24 36 18942, m.brom@nucmed.umcn.nl.

Keywords

GLP-1; exendin; antagonist; agonist; insulinoma

Introduction

Peptide receptor targeting is a successful method for the diagnosis and therapy of neuroendocrine tumours (NET) (1) with somatostatin receptor targeting as the most successful example. In general, agonists are selected for receptor targeting, because agonists usually trigger the internalization of the ligand-receptor complex (2). Internalization of the ligand-receptor complex is the basis for increasing target-to-background ratios, due to retention of the radiolabelled tracer in the target tissue through metabolic trapping (1,3–6). Therefore, internalization is considered to be a crucial step for *in vivo* peptide receptor targeting (5,6). A correlation between internalization rate and *in vivo* accumulation of radiolabelled somatostatin analogues in somatostatin receptor subtype 2 (sstr2) has been found in rats with AR42J tumours (7).

Most peptide receptor antagonist do not internalize, while they do bind to the same receptor with high affinity. Antagonists were considered not be suitable for *in vivo* imaging of tumours due to the lack of internalization. This has been supported by several studies, for example a study showing that a bombesin agonist displayed superior *in vivo* targeting characteristics in comparison to the antagonist (8). However, recent studies showed favourable *in vivo* targeting of radiolabelled bombesin antagonists with higher tumour uptake and longer washout compared to bombesin agonists (9–11). Moreover, a recent study with somatostatin receptor type 2 (sstr 2) and type 3 (sstr 3) ligands showed a remarkably higher uptake in the tumour of the antagonists than the agonists (12). Therefore, there is increasing evidence that peptide receptor antagonists may be useful for the *in vivo* targeting of receptor expressing tissues.

Insulinomas are NET derived from pancreatic beta cells. Although somatostatin receptor scintigraphy is highly efficient in detecting NET and their metastases (13), the sensitivity for the detection of insulinomas is only 40–60% caused by a relatively low incidence of sstr2 and sstr5 receptor subtype expression in insulinomas (13–15). In comparison, the glucacon-like peptide-1 receptor (GLP-1R) is expressed on more than 90% of insulinomas and at a mean density that is two times higher than that of the sst2r (16). Exendin is a GLP-1 (glucagon-like peptide-1) analogue that targets the GLP-1R. For the detection of insulinomas, radiolabelled exendin has been developed recently, with promising preclinical as well as clinical results (17–20). Therefore, exendin analogues should be considered for insulinoma targeting (17–21).

For the targeting of the GLP-1R, only agonists (exendin-3 and exendin-4) labelled with ^{111}In via DTPA conjugated to a C-terminal lysine residue have been examined (17,18,20–22). The GLP-1R antagonist exendin(9-39) is an amino-terminally truncated variant prepared by enzymatic degradation of exendin (Table 1). We aimed to investigate whether exendin(9-39) may also be suitable or – in analogy to the sstr2, sstr3 and bombesin antagonists (9–12) – may even be superior for insulinoma imaging as compared to exendin 3

and 4. Therefore, we investigated the *in vitro* and *in vivo* GLP-1R targeting characteristics of the GLP-1R antagonist exendin(9-39) conjugated with DTPA at a C-terminal Lysine: [Lys^{40}(DTPA)]exendin(9-39) and compared these with these of agonists [Lys^{40}(DTPA)]exendin-3 and [Lys^{40}(DTPA)]exendin-4.

Results

Radiolabelling

All peptides could be labelled with ^{111}In with a specific activity of up to 700 GBq/μmol. Radiochemical purity was >99% as determined by HPLC and ITLC. ^{111}In-EDTA eluted from the column after 3 min whereas ^{111}In-labelled [Lys^{40}(DTPA)]exendin-3, [Lys^{40}(DTPA)]exendin-4 and [Lys^{40}(DTPA)]exendin(9-39) had a retention time of ~13 min.

Receptor binding assays

The results of the IC_{50} determination are shown in Figure 1 and Table 2. The IC_{50} of ^{nat}In-labelled [Lys^{40}(DTPA)]exendin-3, [Lys^{40}(DTPA)]exendin-4 and [Lys^{40}(DTPA)]exendin(9-39) were 13.5 nM, 13.4 nM and 14.4 nM, respectively.

The results of the saturation binding assay are summarized in Figure 2 and Table 2. [Lys^{40}(^{111}In-DTPA)]exendin-3 bound 26,000 receptors/cell, [Lys^{40}(^{111}In-DTPA)]exendin-4 bound 41,000 receptors/cell and [Lys^{40}(^{111}In-DTPA)]exendin(9-39) bound 37,000 receptors/cell. The K_d for [Lys^{40}(^{111}In-DTPA)]exendin-3, [Lys^{40}(^{111}In-DTPA)]exendin-4 and [Lys^{40}(^{111}In-DTPA)]exendin(9-39) was 8.7 nM, 17.6 nM and 15.1 nM, respectively.

The large standard deviation for the K_d and B_{max} for [Lys^{40}(^{111}In-DTPA)]exendin(9-39) is due to the lower *in vitro* binding of this tracer, resulting in a low signal in this assay. This finding is in line with the results reported by Waser *et al.*, which also showed lower *in vitro* binding of exendin(9-39) (23).

Internalization assay

The internalization kinetics of [Lys^{40}(^{111}In-DTPA)]exendin-3, [Lys^{40}(^{111}In-DTPA)]exendin-4 and [Lys^{40}(^{111}In-DTPA)]exendin(9-39) are shown in Figure 3. After 0.5 hour 1.1 ± 0.1 fmol [Lys^{40}(^{111}In-DTPA)]exendin-3 was bound to the receptor and 1.1 ± 0.2 fmol was internalized, increasing to 3.3 ± 0.2 fmol cell bound and 1.6 ± 0.1 fmol internalized after 4 hours. The binding kinetics of [Lys^{40}(^{111}In-DTPA)]exendin-4 were similar to that of [Lys^{40}(^{111}In-DTPA)]exendin-3, with maximum binding and internalization after 4 hours: 4.2 ± 0.2 fmol and 2.1 ± 0.2 fmol, respectively. Membrane bound [Lys^{40}(^{111}In-DTPA)]exendin(9-39) after 0.5 h was 0.9 ± 0.3 fmol and decreased to 0.4 ±0.1 fmol after 4 h. Minimal internalization of [Lys^{40}(^{111}In-DTPA)]exendin(9-39) was observed

Biodistribution studies

The results of the biodistribution studies are summarized in Figure 4. [Lys^{40}(^{111}In-DTPA)]exendin-3 showed high uptake in the tumour as soon as 30 min after injection (25.0 ± 6.0 %ID/g) and a similar uptake was observed up to 4 h p.i. (26.5 ± 8.9 %ID/g). There was

no significant difference in tumour uptake between any time points. Co-administration of an excess unlabelled exendin resulted in much lower uptake: 2.4 ± 0.5 %ID/g, indicating that the uptake was GLP-1R-mediated. Specific uptake of [Lys^{40}(^{111}In-DTPA)]exendin-3 was also seen in pancreas, lung and stomach. [Lys^{40}(^{111}In-DTPA)]exendin-4 showed a higher tumour uptake: 30.7 ± 6.1 %ID/g at 0.5 h p.i. increasing to 41.9 ± 7.2 %ID/g at 4 h p.i. and was statistically higher than tumour uptake of [Lys^{40}(^{111}In-DTPA)]exendin-3 at 1 and 4 h p.i. ($p = 0.04$ and 0.02, respectively). There was no significant difference in pancreas, stomach and lung uptake between [Lys^{40}(^{111}In-DTPA)]exendin-3 and [Lys^{40}(^{111}In-DTPA)]exendin-4. In contrast, [Lys^{40}(^{111}In-DTPA)]exendin(9-39) showed much lower uptake in the tumour at 0.5 h p.i.: 3.2 ± 0.7 %ID/g and there was a rapid wash-out of [Lys^{40}(^{111}In-DTPA)]exendin(9-39) from the tumour. The same phenomenon was found in all GLP-1R positive organs (pancreas, lung, stomach and duodenum).

Renal uptake was significantly higher with [Lys^{40}(^{111}In-DTPA)]exendin-3 and [Lys^{40}(^{111}In-DTPA)]exendin-4 compared to renal uptake of [Lys^{40}(^{111}In-DTPA)]exendin(9-39) ($p=0.0003$ and $p=0.0002$ respectively).

Discussion

In this study, the tumour targeting characteristics of the GLP-1 receptor (GLP-1R) antagonist exendin(9-39) and the agonists exendin-3 and exendin-4 were evaluated *in vitro* and *in vivo*. We found that [Lys^{40}(^{nat}In-DTPA)]exendin(9-39) had a similar affinity for the GLP-1R as the agonists [Lys^{40}(^{nat}In-DTPA)]exendin-3 and [Lys^{40}(^{nat}In-DTPA)]exendin-4, as determined in the IC_{50} assay. [Lys^{40}(^{111}In-DTPA)]exendin(9-39) bound to a similar number of receptors per cell *in vitro* as compared to the ^{111}In-labelled agonists. The results of the IC_{50} determination and the Scatchard analysis of exendin-3 were paradoxal: the IC_{50} of exendin-3 (13.5 nM) was significantly higher than the K_d as derived from the Scatchard analysis (8.7 nM). This could be due to the fact that in the Scatchard analysis ^{111}In-labelled exendin-3 was used, while in the IC_{50} nonradioactive exendin-3 was used.

Despite the high affinity, [Lys^{40}(^{111}In-DTPA)]exendin(9-39) showed lower *in vitro* binding compared to the agonists and minimal internalization as compared to the agonists. The biodistribution study showed high uptake of the agonists in the tumour and in GLP-1R positive organs (pancreas, lung and stomach). In contrast, [Lys^{40}(^{111}In-DTPA)]exendin(9-39) showed much lower uptake in GLP-1R expressing tissues at 0.5 h p.i. and the uptake rapidly decreased at later time points. These results suggest that internalization is a prerequisite for specific accumulation of the ligand in GLP-1R expressing tissues *in vitro* and *in vivo*.

Previous studies showed favourable tumour targeting of antagonists compared to agonists, that could be explained by the fact that the antagonists bound to more receptors than the agonists (9,12,24,25). In our study there was no significant difference between the number of receptors bound by [Lys^{40}(^{111}In-DTPA)]exendin(9-39) and agonists [Lys^{40}(^{111}In-DTPA)]exendin-3 and [Lys^{40}(^{111}In-DTPA)]exendin-4. Our study showed that [Lys^{40}(^{111}In-DTPA)]exendin(9-39) is not suitable for the targeting of the GLP-1R *in vivo*. A recent study evaluated the potency of ^{125}I-exendin(9-39) for pancreatic beta cell imaging and showed

high uptake in the pancreas (approximately 20% ID/g) of ddY mice (26). A difference between the two studies is the peptide dose injected. We have previously shown that the optimal peptide dose for the GLP-1R agonist [Lys^{40}(^{111}In-DTPA)]exendin-3 in BALB/c nude mice with a subcutaneous tumour model is < 0.1μg (27). The peptide dose of ^{125}I-exendin(9-39) was 60-fold lower than the peptide dose in our study (0.0017 μg vs. 0.1 μg), which might explain the enhanced pancreatic uptake of ^{125}I-exendin(9-39). A straight comparison between the two tracers is needed to clarify the cause of the paradoxal results in the two studies. Although high pancreatic uptake was observed of ^{125}I-exendin(9-39), the feasibility for beta-cell imaging remains to be determined, because for imaging purposes higher activity doses and thus higher peptide doses will be required.

By *in vitro* autoradiography Waser *et al*. found that ^{125}I-exendin(9-39) had a high affinity for the GLP-1R in murine pancreatic tissue (2.6 ± 0.4 nM), whereas the affinity for the human GLP-1R is much lower (63 ± 47 nM) (28). It should be noted that this study does not provide data to show that GLP-1R antagonists are superior or inferior to GLP-1R agonists.

Our biodistribution study showed high uptake for all peptides in the kidney, which was not GLP-1R-mediated. The renal uptake of the radiolabelled tracer is caused by tubular reabsorbtion after glomerular filtration. The renal uptake of [Lys^{40}(^{111}In-DTPA)]exendin(9-39) was significantly lower than that of [Lys^{40}(^{111}In-DTPA)]exendin-3 and [Lys^{40}(^{111}In-DTPA)]exendin-4, indicating that the 8 N-terminal amino acid sequence caused more efficient tubular reabsorption. Another explanation might be a different uptake mechanism of [Lys^{40}(^{111}In-DTPA)]exendin(9-39) and [Lys^{40}(^{111}In-DTPA)]exendin-3 and [Lys^{40}(^{111}In-DTPA)]exendin-4 in the proximal tubules of the kidney.

Conclusion

The GLP-1R agonists exendin-3 and exendin-4 show high receptor affinity and efficient *in vitro* binding and internalization kinetics. [Lys^{40}(^{111}In-DTPA)]exendin-3 and [Lys^{40}(^{111}In-DTPA)]exendin-4 accumulated efficiently in INS-1 tumours and GLP-1R positive organs and could therefore be useful for detection and therapy of insulinomas. [Lys^{40}(DTPA)]exendin(9-39) exhibited high affinity for the GLP-1R, but did not show internalization and low uptake in INS-1 tumours and GLP-1R positive organs and therefore is not suited for targeting the GLP-1R *in vivo*. This study indicates that internalization is crucial for efficient accumulation and retention in GLP-1R positive tissues.

Experimental

Radiolabelling of exendin

[Lys^{40}(DTPA)]exendin-3, [Lys^{40}(DTPA)]exendin-4 and [Lys^{40}(DTPA)]exendin(9-39) were purchased from Peptide Specialty Laboratories (Heidelberg, Germany). Labelling of DTPA-conjugated [Lys^{40}]exendin-3, [Lys^{40}]exendin-4 or [Lys^{40}]exendin(9-39) was performed under identical conditions. 150 MBq $^{111}InCl_3$ was added to 1 μg of exendin in 5 volumes of 0.1 M MES buffer, pH 5.5. After 30 min of incubation at room temperature, EDTA was added to a final concentration of 5 mM and incubated at room temperature for 5 min. Tween80 was added to a final concentration of 0.1% and quality control was performed

using reversed-phase high performance liquid chromatography (RP-HPLC) on a C_{18} reversed-phase column (Zorbax Rx-C18; 4.6 mm × 25 cm; Agilent echnologies). The column was eluted with 10 mM NH_4Ac, pH 5.5 with a linear gradient to 100% acetonitrile over 10 min with a flow rate of 1 ml/min.

ITLC was performed on silica gel ITLC strips (Pall Corporation Life Sciences, New York, NY, USA). Two mobile phases were used: 0.1 M EDTA in 0.1 M NH_4Ac, pH 5.5 (R_f ^{111}In-labelled exendin = 0, R_f ^{111}In-EDTA = 1) and 0.25 M NH_4Ac, pH 5.5:methanol (1:1) (R_f colloidal residues = 0, R_f ^{111}In-labelled [Lys^{40}(DTPA)]exendin = 0.2–0.8: and R_f ^{111}In-EDTA = 1).

Cell culture

The rat insulinoma cell line INS-1 (29) was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 10 mM HEPES, 50 μM β-mercaptoethanol, 1 mM sodium pyruvate, 100 units/ml penicillin and 100 μg/ml streptomycin, in a humidified 5% CO_2 atmosphere at 37 °C. The cells were harvested by trypsinization with trypsin/EDTA.

Receptor binding assays

The 50% inhibitory concentration (IC_{50}) was determined for [Lys^{40}(^{nat}In-DTPA)]exendin-3, [Lys^{40}(^{nat}In-DTPA)]exendin-4 and [Lys^{40}(^{nat}In-DTPA)]exendin(9-39) using suspensions of INS-1 cells. [Lys^{40}(DTPA)]exendin-3 and [Lys^{40}(DTPA)]exendin-4 were labelled with ^{nat}In by adding 21 μl $^{nat}InCl_3$ (0.5 mM in 0.02 M HCl) to 105 μl 0.1 M MES buffer, pH 5.5 containing 10 μg peptide, representing equimolar conditions. [Lys^{40}(DTPA)]exendin(9-39) was labelled by adding 25 μl $^{nat}InCl_3$ (0.5 mM in 0.02 M HCl) to 125 μl 0.1 M MES buffer, pH 5.5 containing 10 μg peptide. The reaction mixtures were incubated at room temperature for 20 min and 64 μl 1% Tween80 in PBS was added. Serial dilutions of [Lys^{40}(^{nat}In-DTPA)]exendin-3, [Lys^{40}(^{nat}In-DTPA)]exendin-4 and [Lys^{40}(^{nat}In-DTPA)]exendin(9-39) were prepared in binding buffer (RPMI 1640 medium containing 1% BSA). INS-1 cells were resuspended in binding buffer to a concentration of 3 million cells/0.5 ml. After 10 min incubation on ice, [Lys^{40}(^{nat}In-DTPA)]exendin-3, [Lys^{40}(^{nat}In-DTPA)]exendin-4 and [Lys^{40}(^{nat}In-DTPA)]exendin(9-39) were added at final concentrations ranging from 0.10–350 nM along with radiolabelled [Lys^{40}(^{111}In-DTPA)]exendin-3 (1,000 Bq). To prevent internalization, cells were incubated on ice for 4 h. The cell suspension was centrifuged at 3,000 g for 5 min and the supernatant was discarded. The cell pellet was washed with 500 μl ice-cold PBS, centrifuged at 3,000 g for 5 min and the supernatant was discarded. The radioactivity in the cell pellet was determined in a gamma counter (Wizard, Wallac-Oy, Uppsala, Sweden) and the bound fraction was determined. Saturation binding assays were performed for [Lys^{40}(^{111}In-DTPA)]exendin-3, [Lys^{40}(^{111}In-DTPA)]exendin-4 and [Lys^{40}(^{111}In-DTPA)]exendin(9-39) in suspensions of INS-1 cells. Serial dilutions of the radiolabelled peptides, ranging from 0.1–125 nM, were added to 7×10^6 INS-1 cells in 600 μl binding buffer. The cells were incubated on ice, to prevent internalization, for 4 h. After incubation the cells were centrifuged at 3,000 g for 5 min, the supernatant was discarded and the cells were washed with 500 μl ice-cold PBS. The radioactivity in the cell pellet was determined in a counter. The bound fraction (in receptors/cell) was plotted against the tracer

concentration added. In the scatchard plot the bound/free was plotted against the bound fraction and the dissociation constant (K_d) and the maximum number of binding sites was calculated by nonlinear regression using GraphPad Prism (version 4).

Internalization assay

The kinetics of internalization of [Lys^{40}(^{111}In-DTPA)]exendin-3, [Lys^{40}(^{111}In-DTPA)]exendin-4 and [Lys^{40}(^{111}In-DTPA)]exendin(9-39) were determined using suspensions of INS-1 cells as described above. Approximately 1,000 Bq of ^{111}In-labelled peptide (55 fmol) was added and incubated for 0.5, 1, 2 or 4 h at 37 °C. After incubation, the cells were centrifuged and the supernatant was discarded. The cells were washed twice with 0.5 ml ice-cold PBS and subsequently 0.5 ml acid buffer (0.1 M acetic acid, 154 mM NaCl, pH 2.5) was added. After 10 min incubation at 4 °C, cells were spun down and the pellet was washed twice with ice-cold PBS. The radioactivity associated with the cell pellet (internalized fraction) and the supernatants (receptor-bound fraction) was determined.

Biodistribution studies

Animal experiments were performed after approval of the local ethical committee (RUDEC) for animal experiments (RUDEC). Six to eight weeks old female BALB/c nude mice were injected subcutaneously with 0.2 ml of a 1×10^7 cells/ml suspension of INS-1 cells. When the tumours were 2–5 mm in diameter, mice were randomly divided in groups of 5 mice. Mice were intravenously injected with 370 kBq (peptide dose: 20 pmol) of ^{111}In-labelled [Lys^{40}(DTPA)]exendin-3, [Lys^{40}(DTPA)]exendin-4 or [Lys^{40}(DTPA)]exendin(9-39) via a tail vein. At various time points (0.5, 1, 2 and 4 hours) after injection, mice were euthanized by CO_2 suffocation and blood, tumour and relevant tissues (muscle, heart, lung, spleen, pancreas, kidney, liver, stomach and duodenum) were dissected, weighed and counted in a gamma counter. The percentage injected dose per gram tissue (%ID/g) was determined for each tissue. To determine whether the exendin uptake is GLP-1 receptor-mediated, a 100-fold excess (2 nmol) of unlabelled [Lys^{40}]exendin-3 was co-injected in a separate group of mice (n=5).

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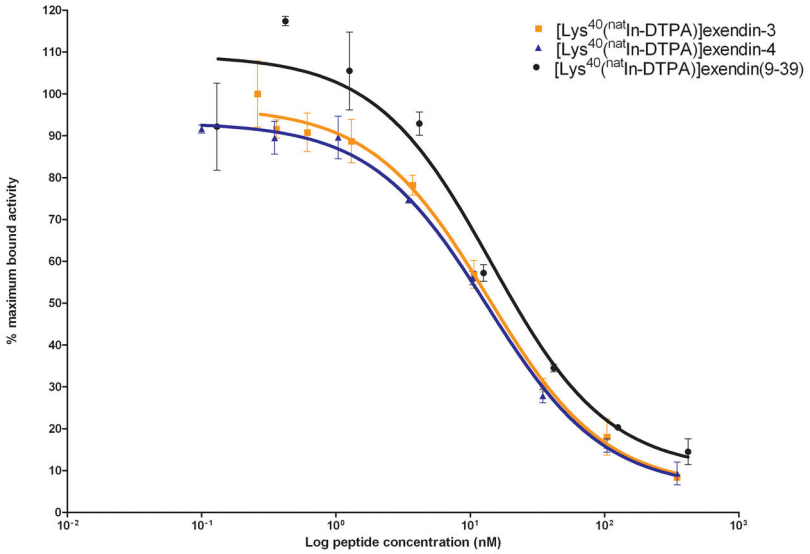


Figure 1.

Competition binding assay (IC_{50}) of [Lys^{40}(^{nat}In-DTPA)]exendin-3, [Lys^{40}(^{nat}In-DTPA)]exendin-4 and [Lys^{40}(^{nat}In-DTPA)]exendin(9-39). [Lys^{40}(^{111}In-DTPA)]exendin-3 was used as tracer.

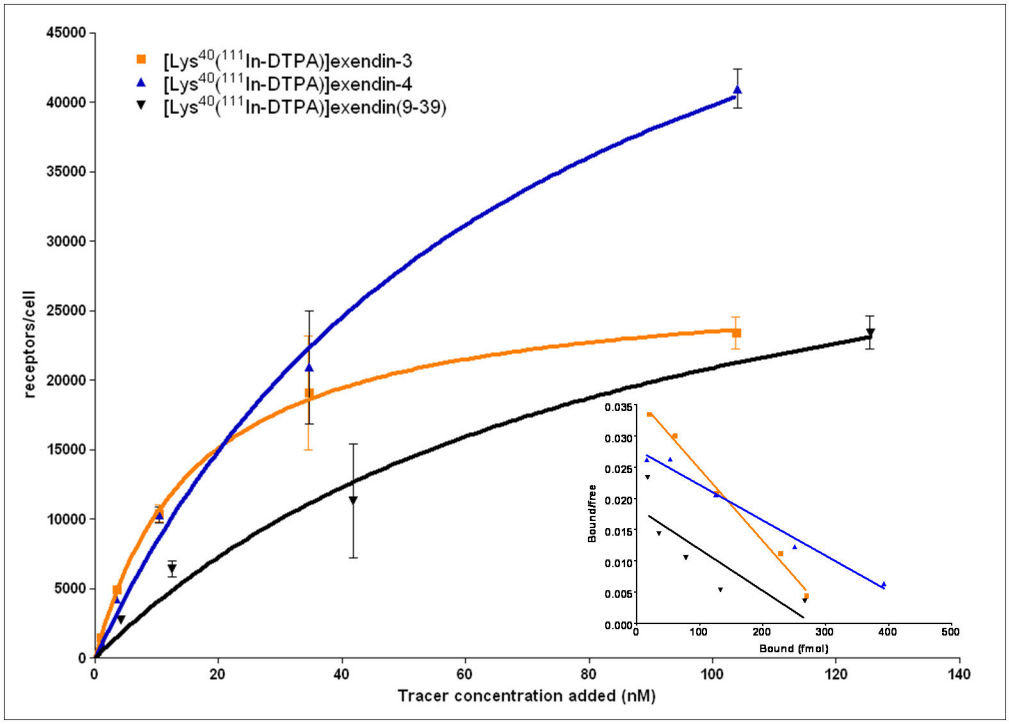


Figure 2.

Saturation binding assay and Scatchard plot (insert) of [Lys^{40}(^{111}In-DTPA)]exendin-3, [Lys^{40}(^{111}In-DTPA)]exendin-4 and [Lys^{40}(^{111}In-DTPA)]exendin(9-39).

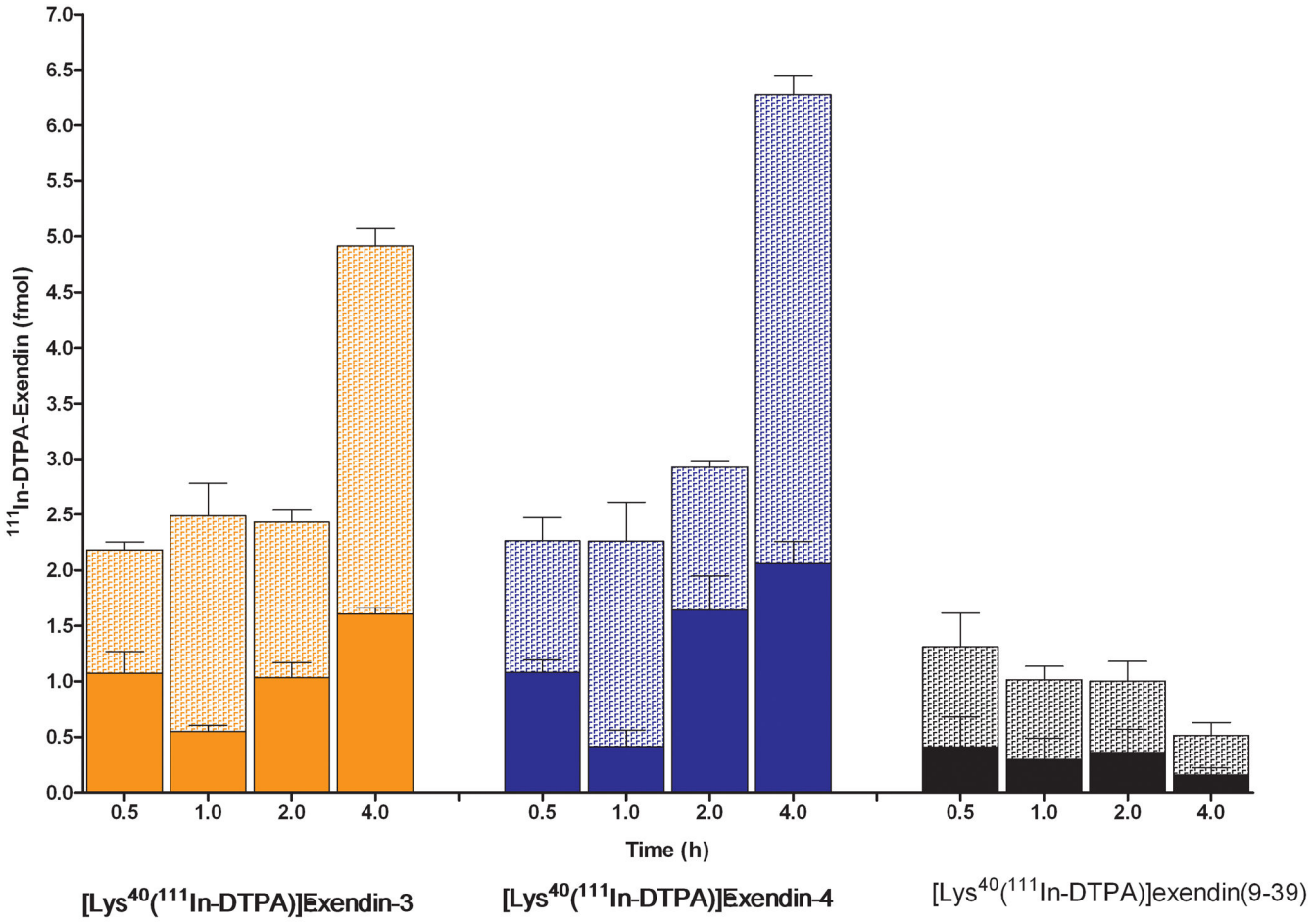
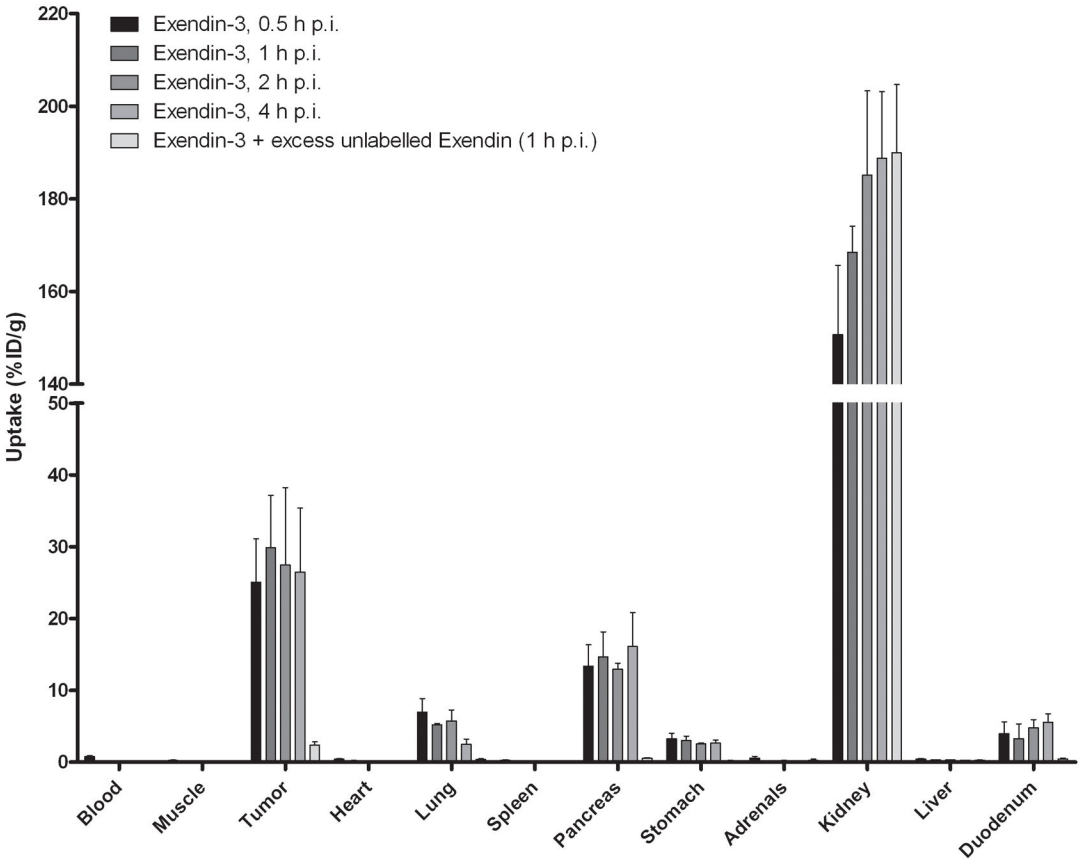
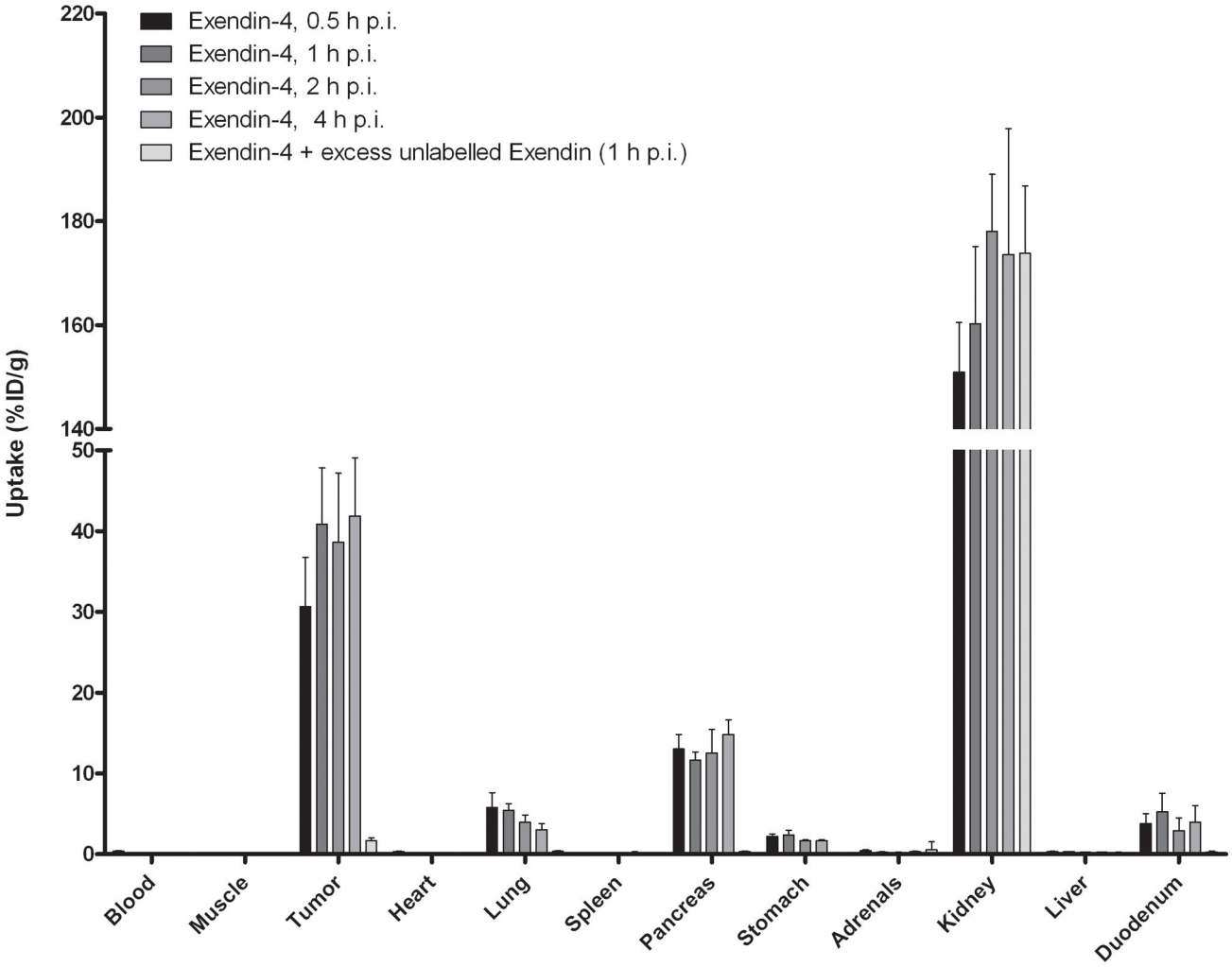


Figure 3.

Internalization kinetics of [Lys^{40}(^{111}In-DTPA)]-exendin-3, [Lys^{40}(^{111}In-DTPA)]-exendin-4 and [Lys^{40}(^{111}In-DTPA)]exendin(9-39). Specifically internalized (solid bars) and specifically cell surface bound (hatched bars) tracer are expressed in fmol.





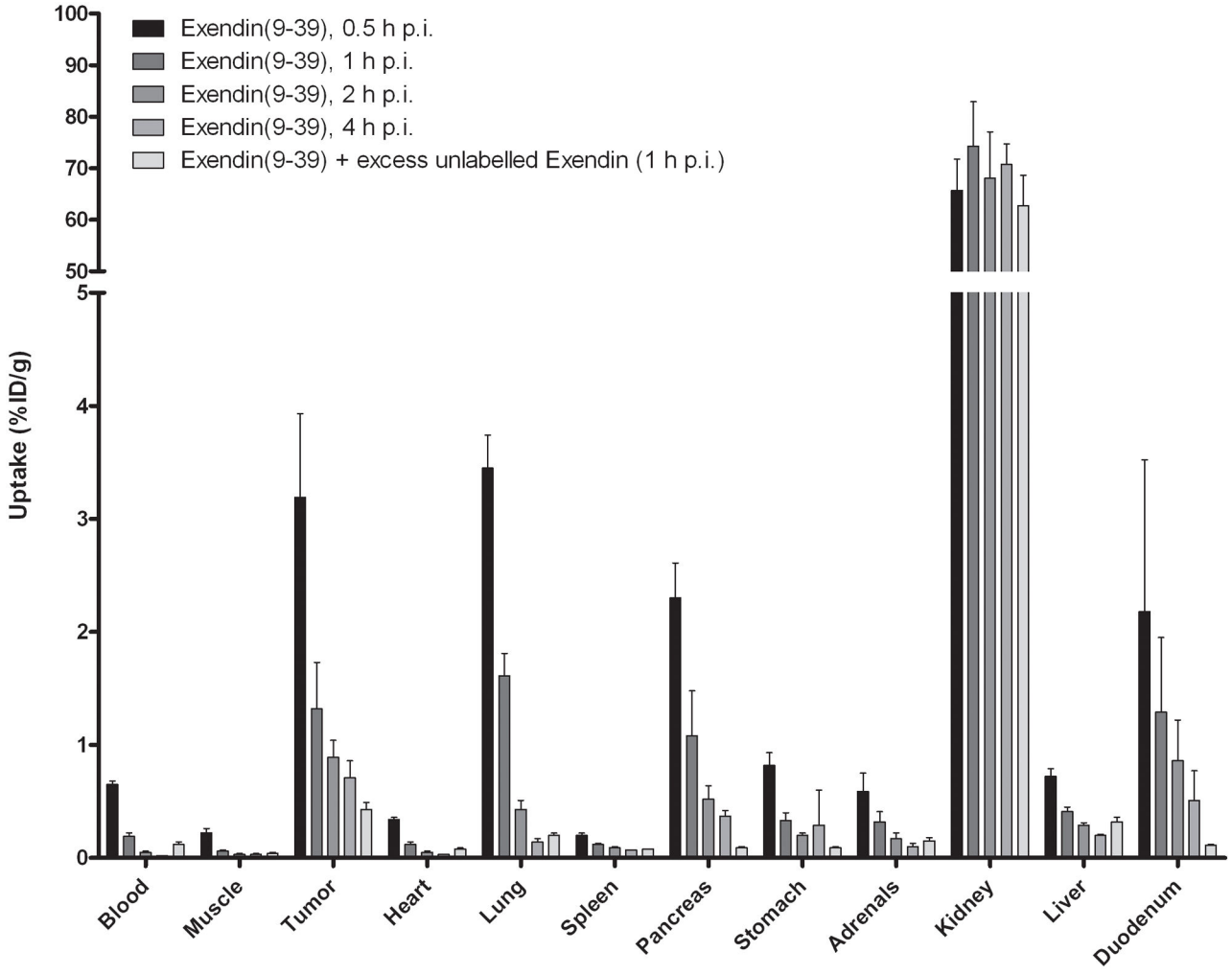


Figure 4.

Biodistribution study of [Lys^{40}(^{111}In-DTPA)]exendin-3 (A), [Lys^{40}(^{111}In-DTPA)]exendin-4 (B) and [Lys^{40}(^{111}In-DTPA)]exendin(9-39) (C) in BALB/c nude mice bearing a subcutaneous INS-1 tumour. Values are expressed as percentage injected dose per gram tissue (%ID/g). Blocking was performed by co-injection of a 100-fold molar excess of unlabelled [Lys^{40}]exendin-3. Mice were dissected 4 hours after injection.

Table 1

Amino acid sequence of GLP-1, exendin-3, exendin-4 and exendin(9-39). The homologous amino acids compared to GLP-1 are underlined.

Peptide	Amino acid sequence
GLP-1	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG
exendin-3	HSDGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2
exendin-4	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2
exendin(9-39)	DLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2

Table 2

IC_{50} values (with the 95% confidence interval in parenthesis) of [Lys^{40}(^{nat}In-DTPA)]exendin-3, [Lys^{40}(^{nat}In-DTPA)]exendin-4 and [Lys^{40}(^{nat}In-DTPA)]exendin(9-39) determined in a competition binding assay in INS-1 cells. The dissociation constant (K_d) and the B_{max} (with the 95% confidence interval in parenthesis) of [Lys^{40}(^{111}In-DTPA)]exendin-3, [Lys^{40}(^{111}In-DTPA)]exendin-4 and [Lys^{40}(^{111}In-DTPA)]exendin(9-39) determined by a binding saturation experiment in INS-1 cells.

Compound	**IC_{50} (nM)**	**K_d (nM)**	**B_{max} (receptors/cell)**
[Lys^{40}(DTPA)]exendin-3	13.5 (9.9 – 18.5)	8.7 (7.6 – 10.1)	26,000 (24×10^3 – $29 \times 10^{3)}$
[Lys^{40}(DTPA)]exendin-4	13.4 (10.5 – 17.0)	17.6 (14.2 – 23.3)	41,000 (35×10^3 – $50 \times 10^{3)}$
[Lys^{40}(DTPA)]exendin(9-39)	14.4 (4.8 – 43.2)	15.1 (6.9 – 80.7)	37,000 (13×10^3 – $60 \times 10^{3)}$