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SHORT COMMUNICATION

Value of the radiolabelled GLP-1 receptor antagonist exendin(9–39) for targeting of GLP-1 receptor-expressing pancreatic tissues in mice and humans

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Abstract
Purpose Radiolabelled glucagon-like peptide 1 (GLP-1) receptor agonists have recently been shown to successfully image benign insulinomas in patients. Moreover, it was recently reported that antagonist tracers were superior to agonist tracers for somatostatin and gastrin-releasing peptide receptor targeting of tumours. The present preclinical study determines therefore the value of an established GLP-1 receptor antagonist for the in vitro visualization of GLP-1 receptor-expressing tissues in mice and humans.
Methods Receptor autoradiography studies with ^{125}I-GLP-1(7–36)amide agonist or ^{125}I-Bolton-Hunter-exendin(9–39) antagonist radioligands were performed in mice pancreas and insulinomas as well as in human insulinomas; competition experiments were performed in the presence of increasing concentration of GLP-1(7–36)amide or exendin(9–39).
Results The antagonist ^{125}I-Bolton-Hunter-exendin(9–39) labels mouse pancreatic β-cells and mouse insulinomas, but it does not label human pancreatic β-cells and insulinomas. High affinity displacement (IC_{50} approximately 2 nM) is observed in mouse β-cells and insulinomas with either the exendin(9–39) antagonist or GLP-1(7–36)amide agonist. For comparison, the agonist ^{125}I-GLP-1(7–36)amide intensively labels mouse pancreatic β-cells, mouse insulinoma and human insulinomas; high affinity displacement is observed for the GLP-1(7–36)amide in all tissues; however, a 5 and 20 times lower affinity is found for exendin(9–39) in the mouse and human tissues, respectively.
Conclusion This study reports a species-dependent behaviour of the GLP-1 receptor antagonist exendin(9–39) that can optimally target GLP-1 receptors in mice but not in human tissue. Due to its overly low binding affinity, this antagonist is an inadequate targeting agent for human GLP-1 receptor-expressing tissues, as opposed to the GLP-1 receptor agonist, GLP-1(7–36)amide.

Keywords Glucagon-like peptide 1 receptor · Exendin · Exendin(9–39) antagonist · Pancreatic tissues · Species selectivity · Peptide receptor tumour targeting

Introduction

Glucagon-like peptide 1 (GLP-1) receptors are expressed physiologically in various tissues. The functionally most prominent among them are the pancreatic islets [1]. GLP-1 receptors have also been found to be overexpressed in specific human tumours, in particular in insulinomas [2]. These receptors constitute the molecular basis for the in vivo targeting of insulinomas using radioactive GLP-1 agonists such as radiolabelled exendin-3 and exendin-4 derivatives [3]. This clinical application has been found particularly useful for the detection and surgical resection of small benign insulinomas that were otherwise hardly detectable by established diagnostic methods [4]. As a future application, it would be extremely elegant if the same method could be used to detect in vivo the GLP-1 receptors of the pancreatic islets in order to assess the β-cell mass in diabetics and other

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patients with diseased, non-neoplastic endocrine pancreas. However, it may be necessary for adequate β-cell imaging to have a more sensitive tracer than those presently available.

It is well known that it is possible to target somatostatin receptor-expressing tissue with specific radiolabelled somatostatin receptor agonists [5]. Interestingly, we have recently observed that sst_2 and sst_3 tumour labelling could be significantly improved when radiolabelled somatostatin receptor antagonists were used instead of agonists [6]. This observation was also extended to gastrin-releasing peptide (GRP) receptor targeting with GRP receptor antagonists [7], suggesting that the phenomenon of targeting peptide receptors with antagonists may have some generality. Recently, Mukai et al. [8] have shown that the GLP-1 receptor antagonist ^{125}I-Bolton-Hunter(BH)-exendin(9–39) was able to visualize pancreatic islets of the mouse in vivo. Although this study is extremely promising, more information is required before entering clinical testing. Indeed, the Mukai et al. study did not compare the β-cell mass visualization using the ^{125}I-BH-exendin(9–39) antagonist with that using a GLP-1 receptor agonist. It is therefore not known whether this antagonist is indeed superior to labelling with established GLP-1-receptor agonists. Moreover, the authors did not investigate if the antagonist tracer that was working in mouse tissue would equally work in human GLP-1 receptor-expressing tissues.

The aim of the present study was therefore to evaluate the binding characteristics of the ^{125}I-BH-exendin(9–39) antagonist in vitro in comparison to the currently used, well established agonist ^{125}I-GLP-1(7–36)amide in GLP-1 receptor-expressing tissues in mice and humans, using in vitro receptor autoradiography. Firstly, this should allow one to directly compare the antagonist binding properties with those of the agonist and assess its possible advantages; secondly, it should allow one to predict whether the antagonist may be a suitable tracer for human tissues, a prerequisite before human studies can be started with this compound.

Materials and methods

Tissues

The following tissues were used for in vitro experiments: normal mouse pancreas and insulinomas grown in the Rip1Tag2 mouse model [9] and human insulinomas surgically resected from patients [4]. These tissues had all been used and characterized for GLP-1 receptor expression in previous studies [4, 9]. Informed consent was available for the human tissue study.

Table 1 Binding assay with the ^{125}I-BH-exendin(9–39) antagonist

Tissue	Binding affinity (IC_{50} in nM; mean ± SEM; $n \geq 3$)	
	GLP-1(7–36) amide agonist	Exendin(9–39) antagonist
Mouse pancreatic islets	2.4±0.6	2.6±0.4
Mouse insulinoma	1.9±1.9	2.3±0.4
Human insulinoma	Not detectable	Not detectable

GLP-1 receptor autoradiography

GLP-1 receptor autoradiography was performed as reported previously [2] using the agonist ^{125}I-GLP-1(7–36)amide [74 TBq/mmol (2,000 Ci/mmol); Anawa,

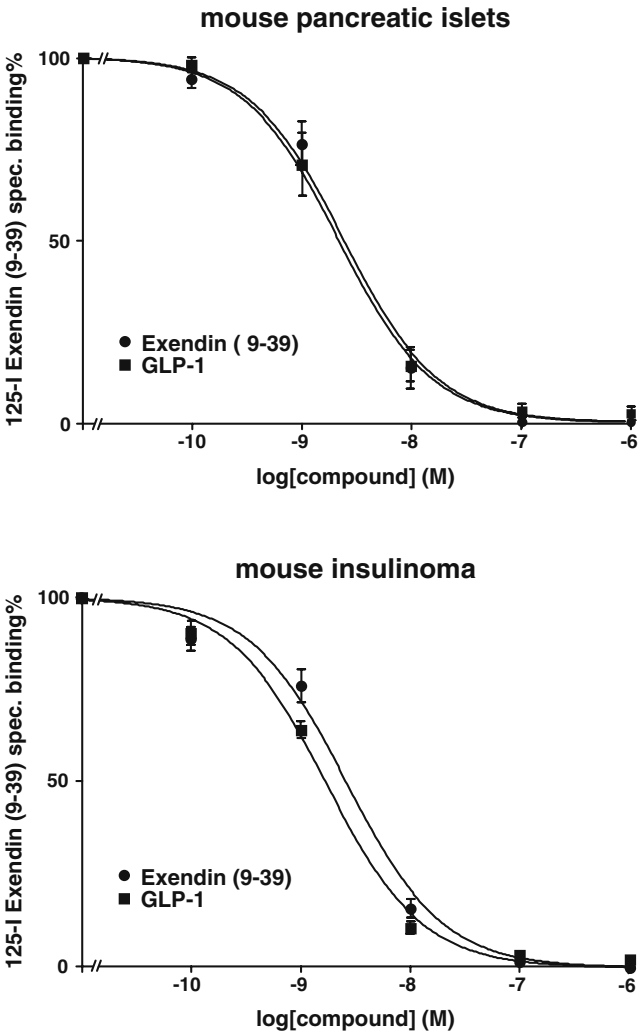

Fig. 1 Competition experiments in mouse pancreatic islets and mouse insulinoma. In both examples, high affinity displacement of the ^{125}I-BH-exendin(9–39) antagonist tracer by the GLP-1 receptor-selective agonist GLP-1(7–36)amide (■) and exendin(9–39) antagonist (●) is illustrated. Mean ± SEM of > 3 independent experiments

Wangen, Switzerland] or the antagonist ^{125}I-Bolton-Hunter-exendin(9–39) [74 TBq/mmol (2,000 Ci/mmol); Anawa, Wangen, Switzerland] as radioligands, used under identical experimental conditions. The GraphPad Prism program was used for curve fitting.

Results

Table 1 shows the excellent binding affinities of the GLP-1 receptor agonist GLP-1(7–36)amide and of the exendin (9–39) antagonist in mouse pancreatic islets and mouse insulinomas in competition experiments using ^{125}I-BH-exendin(9–39) antagonist as tracer. Figure 1 shows competition curves illustrating the almost identical high affinity displacement of the potent GLP-1 receptor agonist GLP-1(7–36)amide and the antagonist exendin(9–39) in these tissues. Table 1, however, also reveals that under identical conditions no measurable binding of ^{125}I-BH-exendin(9–39) was detected in the GLP-1 receptor-expressing human insulinomas.

Table 2 shows the binding affinities of the GLP-1 receptor agonist GLP-1(7–36)amide and the exendin(9–39) antagonist in mouse pancreatic islets and mouse insulinomas in competition experiments using the ^{125}I-GLP-1(7–36)amide agonist tracer. The data resemble the data obtained with the ^{125}I-BH-exendin(9–39) antagonist (Table 1), except that the IC_{50} values for the binding affinity are found to be approximately five times higher for the antagonist exendin(9–39) than for the agonist GLP-1(7–36)amide. This is also illustrated in competition experiments for mouse pancreatic islets in Fig. 2.

More importantly, Table 2 also shows, surprisingly, that in human insulinomas the IC_{50} values for the exendin(9–39) antagonist is more than 20 times higher than for the agonist GLP-1(7–36)amide. This is also illustrated in competition experiments for human insulinomas in Fig. 2.

Figure 3 shows examples of GLP-1 receptor autoradiography which illustrate the above-mentioned observations on tissue sections. Using the agonist ^{125}I-GLP-1(7–36)amide as tracer, Fig. 3 shows that all tested tissues, namely mouse pancreatic islets, mouse Rip1Tag2 insulinomas and two different human insulinomas express a very high density of GLP-1 receptors; the labelling is completely abolished in the presence of 100 nM GLP-1 (nonspecific binding), indicating the presence of specific GLP-1 receptors. Conversely, the ^{125}I-BH-exendin(9–39) antagonist tracer does label the GLP-1 receptors of the mouse pancreatic islets and mouse insulinomas, albeit with lower intensity than the agonist tracer. In line with the above-mentioned competition experiments, it does not or only very weakly label the GLP-1 receptors in the human insulinomas, despite the high density of GLP-1 receptors measured with the agonist tracer in these tissues.

Table 2 Binding assay with the ^{125}I-GLP-1(7–36)amide agonist

Tissue	Binding affinity (IC_{50} in nM; mean ± SEM; $n \geq 3$)	
	GLP-1(7–36) amide agonist	Exendin(9–39) antagonist
Mouse pancreatic islets	3.0±0.4	13±1.3
Mouse insulinoma	4.5±1.0	26±4.8
Human insulinoma	3.1±0.4	63±47

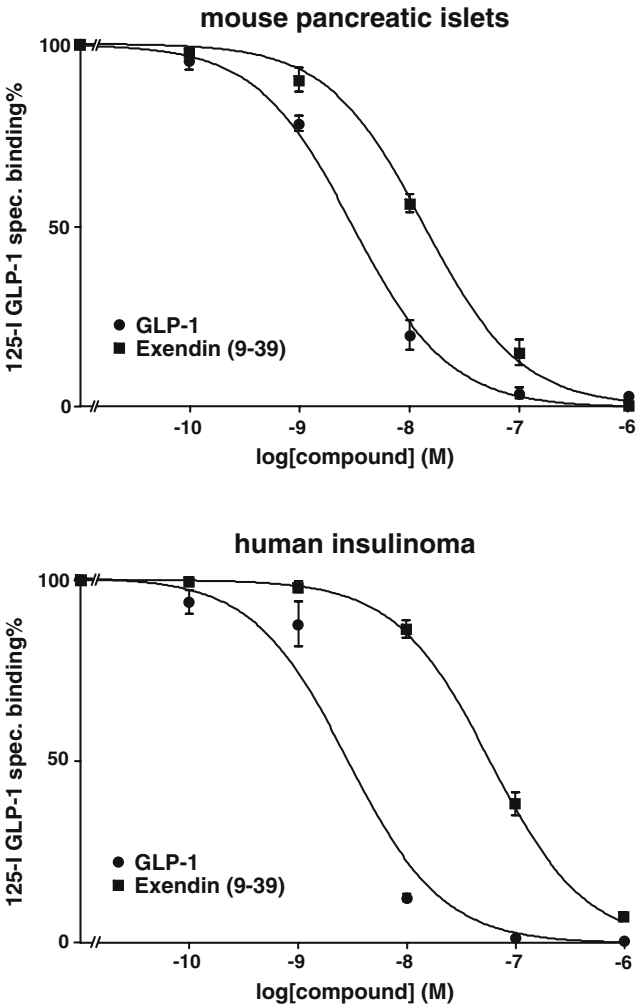

Fig. 2 Competition experiments in mouse pancreatic islets and human insulinoma tissues. High affinity displacement of the ^{125}I-GLP-1(7–36)amide tracer (^{125}I-GLP-1) is observed by the GLP-1 receptor-selective agonist GLP-1(7–36)amide (●) in both tissues, while the antagonist exendin(9–39) (■) reveals a lower affinity, especially in the human insulinoma tissues. Mean ± SEM of > 3 independent experiments

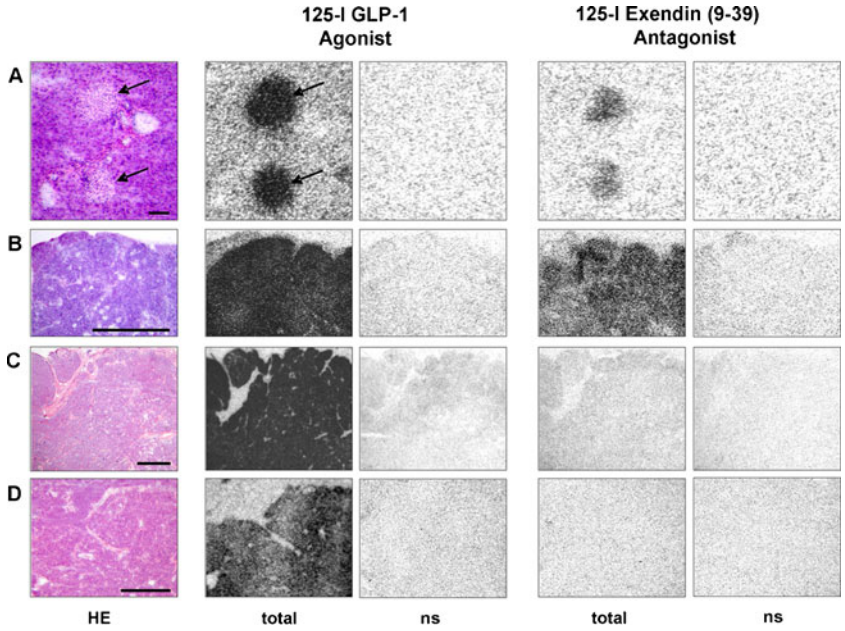

Fig. 3 Comparative in vitro GLP-1 receptor autoradiography with the ^{125}I-GLP-1(7–36)amide agonist tracer (^{125}I-GLP-1) and with the ^{125}I-BH-exendin(9–39) antagonist tracer in mouse tissue (*A* mouse pancreatic islets, *B* mouse insulinoma) and human tissues (*C* and *D* two human insulinomas). *Left column*: haematoxylin and eosin (*HE*)-stained sections with pancreatic islets (*arrows*) in *A* (*bar*=0.1 mm) and insulinoma tumours in *B–D* (*bars*=1 mm). The "*total* binding" column with the ^{125}I-GLP-1 agonist shows high density of GLP-1 receptors in mouse pancreatic islets (*arrows*) and in each of the insulinoma. The "*ns*" column represents nonspecific binding in the presence of 100 nM GLP-1. Conversely, the "*total* binding" column with the ^{125}I-BH-exendin(9–39) antagonist shows moderate density of GLP-1 receptors in mouse pancreatic islets and in mouse insulinoma but no binding at all in the two human insulinomas. The "*ns*" column represents nonspecific binding in the presence of 100 nM GLP-1

Discussion

The present data indicate that the ^{125}I-BH-exendin(9–39) antagonist is a good tracer of GLP-1 receptors expressed in normal and neoplastic pancreatic β-cells in mice tissues. These data confirm under different conditions and extend to other systems the findings of Mukai et al. [8]. In addition, the antagonist exendin(9–39) shows a high affinity competition comparable to that of the reference GLP-1 receptor agonist GLP-1(7–36)amide in mice tissues, with an excellent IC_{50} value of approximately 2 nM, when ^{125}I-BH-exendin (9–39) is used as tracer. Conversely, however, our data also show, most importantly, that the ^{125}I-BH-exendin(9–39) antagonist is not a good tracer for the labelling of human GLP-1 receptor-expressing pancreatic tissues: this radiolabelled antagonist does not label the GLP-1 receptor-expressing human insulinomas, while under identical conditions it labels both mice pancreatic β-cells and insulinomas very well.

The comparison of binding affinities of the exendin (9–39) antagonist and the GLP-1 receptor agonist using ^{125}I-GLP-1(7–36)amide tracer explains the above-mentioned results obtained with the ^{125}I-BH-exendin(9–39) tracer. While the binding affinity values for exendin(9–39) tend to be lower than for GLP-1(7–36)amide in mice, this difference is markedly higher and reaches approximately a factor of 20 when tested in humans. The high IC_{50} value of 63 nM for exendin(9–39) in human insulinomas is therefore the likely explanation for the failure of in vitro visualization of GLP-1 receptors in this tissue with the antagonist tracer.

Our data indicate that the low binding affinity of the exendin(9–39) antagonist for human insulinomas is a sufficient reason to explain the absence of GLP-1 receptor visualization in these tissues. It gives, however, no clues as to whether, in general terms, a GLP-1 receptor antagonist is better or less adequate than a GLP-1 receptor agonist for GLP-1 receptor targeting in humans. For such a comparison, agonist and antagonist candidates with similar in vitro and in vivo binding characteristics should be compared, as was the case in the somatostatin and GRP receptor targeting studies [6, 7].

The present data are a further example for species differences related to receptor binding characteristics of peptide analogs; this was shown previously for other peptides and more recently for GRP receptor analogs [10]. It indicates the need for including human tissues and human receptors in the preclinical testing of novel tracer candidates, either using cell lines expressing the human receptor or using resected human tumours, as shown in the present study. Hopefully, future studies will identify GLP-1 receptor antagonists with radiotargeting characteristics adequate for human tissue visualization.

Conflicts of interest None.

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