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[111]In-exendin Uptake in the Pancreas Correlates With the β-Cell Mass and Not With the α-Cell Mass

<div style="float:left; writing-mode:vertical">ISLET STUDIES</div>

Targeting of the GLP-1 receptor with ^{111}In-labeled exendin is an attractive approach to determine the β-cell mass (BCM). Preclinical studies as well as a proof-of-concept study in type 1 diabetic patients and healthy subjects showed a direct correlation between BCM and radiotracer uptake. Despite these promising initial results, the influence of α-cells on the uptake of the radiotracer remains a matter of debate. In this study, we determined the correlation between pancreatic tracer uptake and β- and α-cell mass in a rat model for β-cell loss. The uptake of ^{111}In-exendin (% ID/g) showed a strong positive linear correlation with the BCM (Pearson $r = 0.82$). The fraction of glucagon-positive cells in the total endocrine mass was increased after alloxan treatment (26% ± 4%, 43% ± 8%, and 69% ± 21% for 0, 45, and 60 mg/kg alloxan, respectively). The uptake of ^{111}In-exendin showed a negative linear correlation with the α-cell fraction (Pearson $r = -0.76$). These data clearly indicate toward specificity of ^{111}In-exendin for β-cells and that the influence of the α-cells on ^{111}In-exendin uptake is negligible.

The role of the β-cell mass (BCM) in the development and progression of type 1 and type 2 diabetes remains poorly understood. Our knowledge is mainly based on autopsy studies and studies in pancreatic specimens obtained in patients undergoing pancreatectomy (1), representing information obtained at only one point in time during the course of the disease without further follow-up. Therefore, a method enabling longitudinal noninvasive determination of the BCM would represent a major breakthrough for diabetes research as it would

allow better elucidation of the pathophysiology underlying the development of both types of diabetes (2). Such a noninvasive imaging technology for determination of the BCM in vivo has been developed based on a radiolabeled GLP-1 analog (^{111}In-exendin). We have previously demonstrated that ^{111}In-exendin uptake in the pancreas correlates linearly with the BCM in a rat model for β-cell loss, and the first clinical proof-of-principal revealed a clearly reduced uptake of the radiotracer in the pancreas of long-standing type 1 diabetic patients as compared with healthy volunteers (3). Despite these promising initial preclinical and clinical results, the specificity of radiolabeled exendin toward β-cells has been a matter of debate. Although relevant GLP-1 receptor (GLP-1R) expression in the exocrine pancreas was claimed (4–6), recent studies showed only low expression in acinar cells and no expression in ductal cells, whereas high GLP-1R expression could be demonstrated in the pancreatic islets, as determined by in vitro autoradiography with ^{125}I-GLP-1 (7) and by immunohistochemistry with an extensively validated anti–GLP-1R antibody (8). We have recently shown by ex vivo autoradiography that ^{111}In-exendin specifically accumulates in the islets of Langerhans of rats and that GLP-1R mRNA expression is much higher in the islets compared with the exocrine pancreas. Moreover, the endocrine-to-exocrine ratio is even more favorable in humans than in rats in which the validity of the method for noninvasive determination of BCM has been demonstrated (3). Although these data clearly indicate toward specificity of ^{111}In-exendin accumulation in islets, they do not rule out the potential influence of

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other endocrine cells on the accumulation of the tracer. One concern is the presence of GLP-1R on α-cells, reported in some studies (9–11), although other studies contradict these findings (12–14). The expression of GLP-1R on α-cells could potentially lead to accumulation of ^{111}In-exendin in the α-cells and thus to an overestimation of the BCM as determined by this technique. To date, the influence of the presence of GLP-1R on α-cells on the uptake of ^{111}In-exendin in the endocrine pancreas has not been studied. Therefore, we have examined the contribution of the α-cell mass on the accumulation of radio-labeled exendin in a rat model of alloxan-induced β-cell loss. The α-cell mass and BCM, determined by morphometric analysis, was compared with the uptake of ^{111}In-exendin in healthy and alloxan-induced diabetic rats.

RESEARCH DESIGN AND METHODS

Radiolabeling
Radiolabeling and quality control of [Lys^{40}(DTPA)]exendin-3 (Peptide Specialty Laboratories, Heidelberg, Germany) with ^{111}InCl$_3$ (Mallinckrodt Medical, Petten, the Netherlands) was performed as previously described (15).

Animals, Alloxan Treatment, and Biodistribution Studies
Female Brown Norway rats of 6–8 weeks were purchased from Harlan (Horst, the Netherlands). Animal experiments were performed after approval of the local ethical committee for animal experiments (RUDEC). Alloxan was injected intravenously (45 or 60 mg/kg, $n = 4$ per group) as previously described (3). A separate group was injected with vehicle only as a control ($n = 4$).

One week after alloxan injection, rats were injected intravenously with 15 MBq ^{111}In-exendin (peptide dose 0.1 μg/rat). One hour after ^{111}In-exendin administration, the rats were killed and the pancreas was dissected, weighed, and fixed in formalin. The radioactivity concentration in the pancreas was measured using an automated well-type gamma counter (Wallac 1480-Wizard; Perkin-Elmer, Boston, MA), and the uptake of ^{111}In-exendin was calculated and expressed as the percentage of the administered dose per gram of tissue (% ID/g).

Histology and Determination of the BCM and α-Cell Mass
After 48 h fixation in formalin, pancreata were embedded in paraffin, and 4-μm sections were cut at three levels 100 μm apart. One section of each level was stained for insulin, and the BCM was determined by morphometric analysis as previous described (3). A consecutive section was stained for glucagon: the sections were rehydrated using xylene for 10 min and washed with xylene, washed twice with 100% ethanol, 75% ethanol, 50% ethanol, and water. Antigen retrieval was performed by a microwave treatment in 10 mmol/L sodium citrate buffer, pH 6.0, for 10 min. Endogenous peroxidase activity was blocked by 10 min incubation with 3% H$_2$O$_2$ in PBS. The sections were washed twice with demineralized water and three

times with PBS and incubated with 5% normal goat serum (Bodinco, Alkmaar, the Netherlands) for 30 min. After removal of the goat serum, the sections were washed three times with PBS and incubated with 50 μL antiglucagon antibody (1:500 diluted in PBS containing 1% BSA weight for volume) (catalog no. 2760; Cell Signaling, Leiden, the Netherlands) for 60 min. After washing three times with PBS, 50 μL goat anti-rabbit IgG-biotin conjugate (Vector, Burlingame, CA) diluted in PBS containing 1% BSA (1:200) was added and incubated for 30 min. The sections were washed three times with PBS, and the sections were incubated with 50 μL ABC complex (VECASTAIN, Elite ABC kit; Vector Laboratories, Burlingame, CA) for 30 min. The bound antiglucagon antibody was visualized using diaminobenzidine (Bright DAB; Sigma-Aldrich, St. Louis, MO) as a chromogen after washing three times with PBS. All slides were counterstained with hematoxylin and mounted with mounting fluid (Permount; Fisher Scientific, Waltham, MA). All steps were performed at room temperature in the dark.

The absolute α-cell mass was determined analogous with the BCM determination described above. The relative α-cell mass was calculated by dividing the α-cell mass by the total endocrine mass (BCM and α-cell mass).

Statistical Analysis
All mean values are expressed as mean $\pm$ SD. Statistical analysis was performed using unpaired, two-tailed Student t test using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). The level of significance was set at $P < 0.05$.

Correlation between the BCM and pancreatic uptake was determined by the Pearson correlation coefficient (r) using two-tailed ANOVA with GraphPad Prism. The level of significance was set at $P < 0.05$.

RESULTS

Histology
In the pancreas of healthy rats, scarce glucagon staining was observed at the periphery (Fig. 1A) and abundant insulin staining in the core of the islets (Fig. 1B). In alloxan-treated rats, the number of insulin-positive cells per islet was markedly reduced (Fig. 1D) and the islets mainly consist of glucagon-positive cells (Fig. 1C).

α-Cell Mass and BCM and ^{111}In-exendin Uptake
The total mass of the endocrine pancreas was reduced in the alloxan-treated rats (8.5 $\pm$ 1.9, 5.5 $\pm$ 1.7, and 2.8 $\pm$ 0.8 mg for 0, 45, and 60 mg/kg alloxan, respectively). The loss of endocrine mass is due to β-cell loss in alloxan-treated rats (BCM 6.3 $\pm$ 1.7 mg in healthy rats and 3.2 $\pm$ 1.5 and 1.0 $\pm$ 0.9 in rats treated with 45 and 60 mg/kg, respectively) (Fig. 2A), since the absolute α-cell mass was not significantly different in rats treated with 45 or 60 mg/kg alloxan (2.2 $\pm$ 0.4, 2.3 $\pm$ 0.3, and 1.8 $\pm$ 0.3 mg for healthy and 45 and 60 mg/kg alloxan, respectively; $P = 0.81$ and $P = 0.21$, respectively) (Fig. 2B). Although the absolute α-cell mass did not change, the
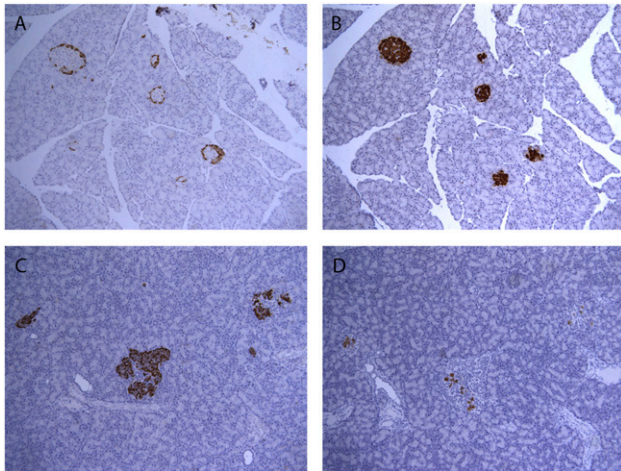
Figure 1—Immunohistochemical staining of pancreatic sections for glucagon (*A* and *C*) and insulin (*B* and *D*) of healthy rats (*A* and *B*) and rats treated with 60 mg/kg alloxan (*C* and *D*). In healthy rats, scarce glucagon staining in the periphery of the islet (*A*) and abundant insulin staining in the core of the islet (*B*) is observed. In severely diabetic rats, the islets consist predominantly of glucagon-producing cells (*C*), and only a few insulin-positive cells could be observed (*D*).

relative α-cell fraction (represented as the percentage of α-cells of the total endocrine mass) was increased as a result of β-cell loss (and thereby loss of total endocrine mass) when rats were treated with 45 or 60 mg/kg alloxan (43% ± 8% and 69% ± 21%, respectively, vs. 26% ± 4% in healthy rats) (Fig. 2*C*). The pancreatic uptake of ^{111}In-exendin showed a linear correlation with the BCM (Pearson r = 0.82, $P < 0.005$) (Fig. 3*A*). There was no significant correlation between the absolute α-cell mass and ^{111}In-exendin uptake in the pancreas (Pearson r = 0.18, $P = 0.59$) (Fig. 3*B*). The relative α-cell fraction (fraction of α-cell of the total endocrine mass) showed a negative linear correlation with pancreatic ^{111}In-exendin uptake (Pearson r = −0.76, $P < 0.01$) (Fig. 3*C*).

DISCUSSION

In this study, we examined the contribution of the α-cells to the uptake of ^{111}In-exendin, a radiotracer potentially enabling noninvasive determination of BCM, in a rat model of diabetes. We demonstrated that there is no correlation between the absolute α-cell mass and pancreatic tracer uptake and a negative linear correlation between the relative α-cell mass and ^{111}In-exendin uptake, indicating a negligible influence of the α-cells on tracer accumulation in the pancreas.

The expression of GLP-1R on α-cells remains a matter of debate. Specific in vitro binding of ^{125}I-labeled GLP-1 to insulin and glucagon immunoreactive cells was found by combined autoradiography and immunohistochemical analysis of pancreatic tissue specimens of rats (10). A later study confirmed these data by showing that a small portion of the α-cells express GLP-1R as determined by RT-PCR
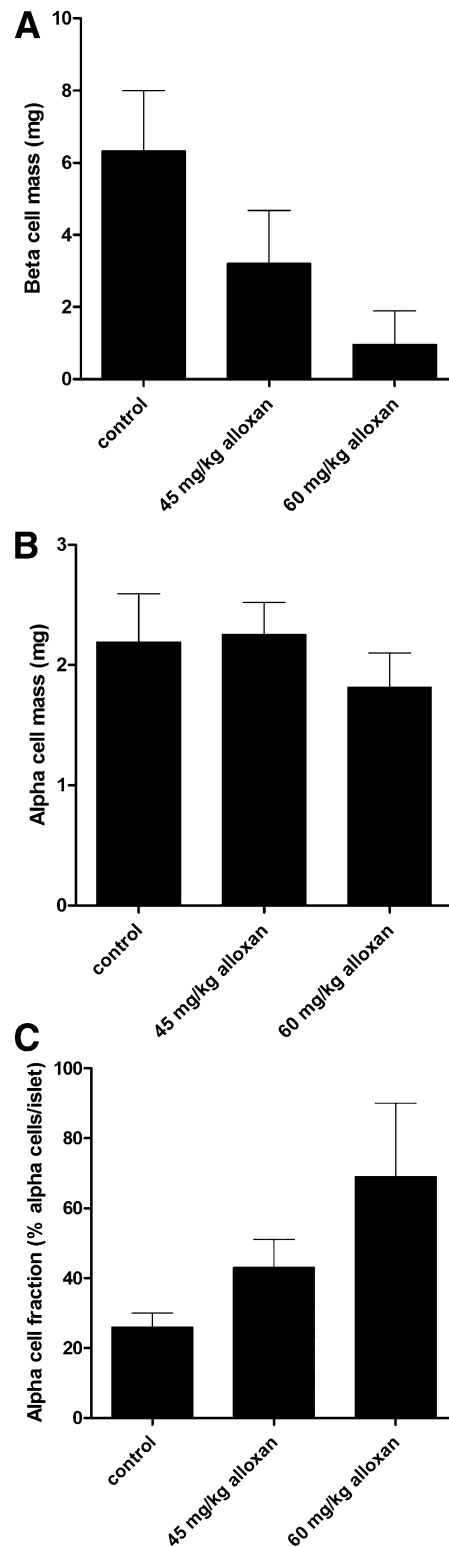

Figure 2—Absolute β-cell (*A*) and α-cell (*B*) mass in control and alloxan-treated Brown Norway rats. The relative α-cell fraction (*C*) was determined by dividing the absolute α-cell mass by the sum of the absolute α-cell mass and BCM.
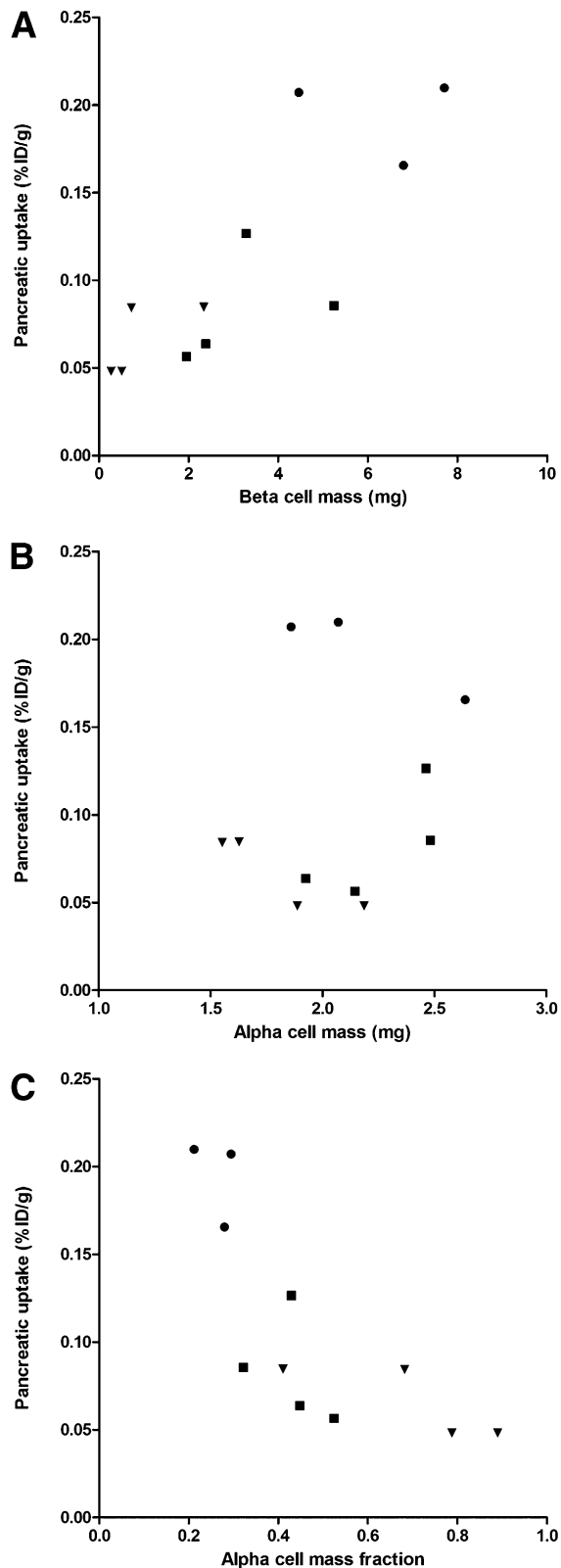
and immunohistochemical analysis (9). However, several other studies showed that GLP-1R is specifically expressed in β-cells in mice, rats, and humans and not in α-cells (12–14). Another study showed that GLP-1 has a direct inhibitory effect on glucagon excretion by α-cells, but that GLP-1R is only expressed on ~1% of the α-cells (11). In a more recent study in which a highly specific anti–GLP-1R antibody was validated, the specificity of most (commercially) available GLP-1R antibodies was questioned (8). Therefore, based on the results presented in the literature, it remains a matter of debate whether GLP-1R is expressed on α-cells. It should be noted that in the case of peptide-based tracers, such as exendin, the tracer accumulation is dependent not only on the receptor expression density but also the internalization rate (16), meaning that if the expression of GLP-1R on α-cells or the internalization rate is low, the receptor expression itself might have a negligible influence on the accumulation of ^{111}In-exendin in the islets.

The aim of our study was not to demonstrate the presence or absence of GLP-1R on α-cells but to evaluate the potential influence of the presence of α-cells in the islets of Langerhans on the accumulation of ^{111}In-exendin in the pancreas. Our findings indicate that even if GLP-1Rs are present on α-cells, they only exert a minor influence on ^{111}In-exendin uptake. This further supports the idea that measurement of the pancreatic uptake of ^{111}In-exendin can indeed serve as an imaging biomarker for BCM.

Not only the BCM but also the number of other endocrine cells might change during the progression of diabetes. Noninvasive methods to longitudinally determine the endocrine cell mass and confirmation might provide important information on the role of these cells in the pathogenesis of diabetes. Recently, Eriksson et al. (17) showed that accumulation of the serotonin receptor tracer [^{11}C]5-HTP is reduced in the pancreas of type 1 diabetic patients as compared with healthy volunteers, implying that this tracer could also be a useful noninvasive marker to determine the total mass of endocrine cells in the pancreas. Since the serotonin receptor is expressed in all endocrine cells, a combination of [^{11}C]5-HTP imaging of serotonin activity and ^{111}In-exendin imaging could provide useful complementary information about the β-cell and total endocrine mass as well as changes in endocrine cell conformation during the development of diabetes.

In conclusion, we have demonstrated that the uptake of ^{111}In-exendin correlated with BCM and not with α-cell mass. The present data clearly indicate the specificity of

Figure 3—Correlation between the pancreatic uptake of ^{111}In-exendin and the absolute BCM (*A*); uptake on *y*-axis in percentage of the injected dose per gram of tissue (% ID/g), BCM in milligrams on *x*-axis as determined by morphometric analysis after immuno-histochemical staining with anti-insulin antibody. The correlation as determined by Pearson test is *r* = 0.82. No correlation between tracer uptake and absolute α-cell mass was observed (*B*); Pearson *r* = 0.18. A negative correlation (Pearson *r* = −0.76) was shown between the pancreatic ^{111}In-exendin uptake and the relative α-cell fraction calculated by dividing the α-cell mass by the total endocrine mass (*C*). Circles, control rats; squares, rats treated with 45 mg/kg alloxan; triangles, rats treated with 60 mg/kg alloxan.

[111]In-exendin for β-cells and a negligible influence of the α-cells on pancreatic ^{111}In-exendin uptake. This observation further supports the idea that ^{111}In-exendin uptake may indeed be a measure for BCM in vivo.

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