11C-Hydroxytryptophan Uptake and Metabolism in Endocrine and Exocrine Pancreas

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Determination of the residual β-cell mass using noninvasive tools might help to follow up the efficacy of new treatments in both type 1 (insulin-dependent) and type 2 (non–insulin-dependent) diabetes mellitus, including islet transplantation. β-cells are neuroendocrine cells that can take up and metabolize the serotonin precursor 5-hydroxytryptophan. The serotonin pathway may therefore be an attractive target for the development of an imaging tracer for residual β-cell mass. The aim of this study was to evaluate the uptake mechanism and the retention of the PET tracer 11C-hydroxytryptophan in endocrine and exocrine pancreas in vitro and in vivo. Methods: The exocrine human pancreas carcinoma cell line (PANC-1) and the endocrine human insulinoma cell line (CM) were applied for in vitro 11C-hydroxytryptophan accumulation/efflux experiments and blocking studies using inhibitors of key enzymes and transporters involved in the serotonin pathway. Animal experiments were performed on normal Wistar rats and on rats pretreated with the monoamine oxidase (MAO) inhibitor clorgyline. After intravenous injection of 11C-hydroxytryptophan, a 60-min dynamic PET scan was acquired followed by an ex vivo biodistribution study. Autoradiography and hematoxylin–eosin staining were performed on the dissected pancreas to localize the radioactivity within the pancreatic tissue. Results: 11C-hydroxytryptophan accumulated rapidly in both endocrine CM cells and exocrine PANC-1 cells. In the exocrine cells, a rapid efflux of radioactivity was observed, whereas most radioactivity remained trapped in the endocrine cells. PET images showed clear accumulation of 11C-hydroxytryptophan in the pancreas in both animal groups, but with a significant 3-fold higher retention of the radiopharmaceutical in clorgyline-treated animals. Ex vivo biodistribution studies confirmed the results obtained by PET. Autoradiographs did not discriminate between the exocrine and endocrine pancreas in control animals, whereas autoradiographs showed intense radioactive spots colocalized with the islets of Langerhans in clorgyline-treated animals. Conclusion: 11C-hydroxytryptophan is trapped in β-cells but not in exocrine pancreatic cells. β-cell selectivity can be strongly enhanced by inhibition of MAO-A. This observation offers perspectives for the development of a more selective PET tracer for β-cell mass, based on an 11C-hydroxytryptophan derivative with increased resistance toward degradation by MAO-A.

Key Words: PET imaging; [11C]HTP; serotonin pathway; beta cells; exocrine pancreas

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The islets of Langerhans represent only 2%–3% of the total pancreas and consist of about 80% β-cells. In both type 1 and type 2 diabetes, dysregulation of glucose homeostasis and β-cell function and loss of β-cells are responsible of hyperglycemia (1). β-cells are neuroendocrine cells arising from precursor cells of the embryologic neural crest (2,3). Pancreatic β-cells regulate a large variety of body functions through paracrine and endocrine actions via dedicated amines and peptides, including the neurotransmitter serotonin. As a consequence, β-cells have an efficient mechanism for the uptake and metabolism of the amine precursors L-3,4-dihydroxyphenylalanine and 5-hydroxytryptophan through specific receptors (4,5).

5-hydroxytryptophan and its precursor tryptophan are taken up in β-cells by the L-type amino acid transporter (LAT). Tryptophan is converted to 5-hydroxytryptophan by tryptophan hydroxylase (TPH). Subsequently, 5-hydroxytryptophan is converted to serotonin by aromatic L-amino acid decarboxylase (AADC). Serotonin can then be transported into vesicles (secretory β-granules) by the vesicular monoamine transporter 2 (VMAT2) and released in the extracellular space together with insulin. Serotonin can be taken up again by the β-cells via the serotonin transporter or can bind serotonin receptors. Eventually, serotonin is degraded to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase (MAO). 5-HIAA leaves the cell and enters the extracellular space by passive diffusion (Fig. 1) (6,7).

In contrast to β-cells, the serotonin pathway is not active in exocrine pancreatic cells. This difference between exocrine and endocrine cells in the pancreas may be exploited to develop novel probes for imaging of β-cells, which could...
be of interest for detecting type 1 diabetes early, monitoring the fate of β-cells after islet transplantation, and evaluating the efficacy of new drugs. We hypothesized that a labeled precursor or metabolite of the serotonin pathway could be a suitable imaging probe for β-cells. The radiolabeled serotonin precursor 11C-hydroxytryptophan is clinically used as a diagnostic PET tracer for neuroendocrine tumors (8,9). However, whether 11C-hydroxytryptophan also selectively accumulates in β-cells remains unknown.

The aim of this study was to establish the molecular mechanism of 11C-hydroxytryptophan uptake in β-cells, to investigate the selectivity of the tracer for β-cells over exocrine pancreas, and to assess the possibilities of using this tracer as a potential probe for noninvasive β-cell imaging.

MATERIALS AND METHODS

Chemicals
All chemicals were obtained from commercial suppliers (Sigma-Aldrich, Fluka, and Merck) and used without further purification. A solution was prepared by adding 5.6 mM d-glucose, 0.49 mM MgCl2, and 0.68 mM CaCl2 (GMC) to phosphate-buffered saline (PBS) (140 mM NaCl, 9.0 mM Na2HPO4, and 1.3 mM KH2PO4). The pH of the resulting solution was adjusted to 7.4 using sodium hydroxide.

11C-hydroxytryptophan was synthesized as described by Neels et al. in 15% ± 12% radiochemical yield (10). Radiochemical purity was greater than 99%, and average specific activity was 30 TBq/mmol.

Cell Culture
Cell experiments were performed with the exocrine human pancreas carcinoma cell line (PANC-1) and the endocrine human insulinoma cell line (CM). The cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum, penicillin (100 IU/mL), streptomycin (100 μg/mL), and for CM cells 2 mM L-glutamine.

The cells were grown in a humidified atmosphere containing 5% CO2 at 37°C and subcultured every 3–4 d.

One day before an experiment, the cells were plated at a density of 0.5–1.0 million in 6-well plates. On the day of the experiment, the cells were washed 3 times with 2 mL of warm PBS (37°C) and incubated for 1 h with 1 mL of PBS-GMC to deplete internal amino acid pools.

In Vitro Accumulation and Efflux
To each well were added 0.5–2 MBq of 11C-hydroxytryptophan in 50–70 μL of saline. After 5, 15, 30, or 45 min of incubation, the buffer was removed and the cells were washed 3 times with 2 mL of ice-cold PBS. The cells were harvested by the addition of 200 μL of trypsin and resuspended in 800 μL of growth medium. Subsequently, the cells were counted using an automatic γ-counter (LKGB–Compugamma CS 1282; LKB Wallac). Tracer accumulation was expressed as percentage dose/106 cells. The number of cells was determined by the trypan blue exclusion assay. Results are presented as the mean of 3 experiments (in duplicate) ± SD.

Blocking and Competition
Several competitors and inhibitors were used to study the uptake mechanism of 11C-hydroxytryptophan: 5-hydroxytryptophan (competitive inhibitor), 0.25 mM amino-2-norbornanecarboxylic acid (BCH, inhibitor of LAT), 0.08 mM carbidopa (peripheral inhibitor of AADC), 0.1 mM pargyline (nonselective MAO inhibitor), 0.1 mM clorgyline (selective MAO-A inhibitor), 200 μM tetrabenazine (VMAT2 inhibitor), and 500 μM para-
chlorophenylalanine (PCPA, inhibitor of TPH). In addition, combina-
tions of PCPA and carbidopa, and of PCPA and clorgyline, were used to perform double-blocking experiments.

All inhibitors were added to the cells 1 h before the addition of the tracer to ensure binding of the inhibitor to its target, except for 5-hydroxytryptophan, which was added together with 11C-hydroxytryptophan. The cells were incubated for 15 min with 11C-hydroxytryptophan, the buffer was removed, and the cells were washed 3 times with 2 mL of cold PBS. The cells were harvested by the addition of 200 µL of trypsin and resuspended in 800 µL of medium. Activity in the cells was measured with the automatic γ-counter. Tracer accumulation was expressed as a percentage relative to control cells that were not exposed to the inhibitor.

**In Vivo Pancreatic Accumulation**

All animal experiments were performed according to the Dutch Regulations for Animal Welfare. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Groningen. Animals were housed at the Central Animal Facility of the University Medical Center Groningen. After arrival, healthy male Wistar rats (Charles River) were allowed to acclimatize for at least 7 d. They were housed in Makrolon (Bayer) cages on a layer of wood shavings in a room with a constant temperature (21°C ± 2°C) and a 12 h:12 h light: dark regime (lights on at 7:00 AM). Commercial chow and tap water were available ad libitum.

A total of 11 Wistar rats were used for the in vivo study. As a control group, nontreated Wistar rats (n = 5) were anesthetized with 2% isoflurane in medical air and injected intravenously via the penile vein with a dose of approximately 50 MBq of 11C-hydroxytryptophan. The rats were placed supine in the camera with the abdomen in the field of view. A dynamic PET scan (microPET Focus 220; Siemens Medical Solutions USA, Inc.) was acquired for 60 min. After completion of the emission scan, a transmission scan of 515 s with a 57Co point source was obtained. After completion of the emission scan, the pancreas was dissected and immediately frozen in liquid nitrogen. Pancreas slices of 60 µm were prepared immediately, using a cryotome. Tissue slices were exposed to a storage phosphor screen (multisensitive screens; Packard) overnight in a closed film cassette. The screens were read the following day using a Cyclone storage phosphor imager (Perkin Elmer). Images were analyzed using OptiQuant software.

The percentage of β-cell–specific uptake in the islets of Langerhans was calculated using the following formula: [100 × (radioactivity in the islet of Langerhans – exocrine background)/exocrine background]. The same pancreatic sections were subsequently stained (hematoxylin–eosin) for light microscopy examination.

**Statistical Analysis**

All data are expressed as mean ± SD. Differences between groups were analyzed with the 2-sided unpaired Student test and were considered statistically significant when P was less than 0.05.

**RESULTS**

**In Vitro Accumulation and Efflux**

Accumulation of 11C-hydroxytryptophan in CM and PANC-1 cells, incubated in PBS-GMC, was rapid. At 15 min, tracer accumulation was 90% of maximum uptake in CM cells and 70% of maximum uptake in PANC-1 cells. In both cell lines, maximum uptake was already reached within 30 min (CM, 32% ± 8% uptake/10^6 cells; PANC-1, 43% ± 5% uptake/10^6 cells). At later time points (from 30 to 60 min), no significant changes in tracer uptake were observed (Fig. 2A).

Figure 2B shows the efflux kinetics of 11C-hydroxytryptophan from PANC-1 cells and CM cells. More than 35% of activity is released from PANC-1 cells within 15 min. After 60 min, PANC-1 cells contained only 22% ± 5% of the original activity. In contrast, only about 30% of the tracer was washed out of CM cells between 0 and 15 min. After this initial phase, the remaining tracer was trapped inside the cells. At 60 min, 59% ± 3% of cellular radioactivity remained trapped inside the cells.

**FIGURE 2.** (A) 11C-hydroxytryptophan accumulation in PANC-1 cells vs. CM cells measured in amino acid–free PBS-GMC. Results are represented as mean ± SD. (B) 11C-hydroxytryptophan efflux in PANC-1 and CM cells. Results are expressed as percentage of maximum intracellular radioactivity (mean ± SD).
Blocking and Competition

Blocking experiments were performed using competitors and inhibitors for enzymes and transporters involved in the serotonin pathway. Figure 3 shows the cellular accumulation of $^{11}$C-hydroxytryptophan in CM and PANC-1 cells in single- and double-blocking experiments.

5-hydroxytryptophan almost completely inhibited the accumulation of $^{11}$C-hydroxytryptophan in both cell types to 1.4% ± 0.5% of the control value in PANC-1 and 2.5% ± 1.6% in CM cells, indicating that $^{11}$C-hydroxytryptophan uptake can be saturated and is mediated by transporters.

The LAT inhibitor BCH reduced tracer accumulation to 15% ± 4% ($P = 0.0015$) and 55% ± 7% ($P = 0.014$) of the control value in PANC-1 cells and in CM cells, respectively.

PCPA did not have a significant effect on $^{11}$C-hydroxytryptophan accumulation in PANC-1 cells (115% ± 26%) but significantly decreased tracer accumulation in CM cells (34% ± 3%, $P = 0.0016$).

The AADC inhibitor carbidopa did not significantly affect the accumulation of $^{11}$C-hydroxytryptophan in either cell line (119% ± 22%, $P = 0.31$, and 114% ± 24%, $P = 0.45$, for PANC-1 and CM, respectively).

Pargyline, clorgyline, and tetrabenzine, which are inhibitors of MAO and VMAT2 and thus involved in the metabolism and excretion of serotonin, had only a minor effect on tracer accumulation in PANC-1 cells (120% ± 26%, $P = 0.37$) but significantly decreased tracer accumulation in CM cells (24% ± 8%, $P = 0.001$). However, the effect of a combination of carbidopa and PCPA was not significantly different from the effect of PCPA alone. The second double-blocking experiment involved a combination of PCPA and clorgyline. No significant differences in tracer accumulation were observed in either cell line ($P = 0.43$ and 0.19 for PANC-1 and CM cells, respectively). Apparently, the opposing effects of PCPA and clorgyline in CM cells counteracted each other in the double-blocking experiments, resulting in a net effect of zero.

Results of the single- and double-blocking experiments are summarized in Table 1.

PET

Small-animal PET with $^{11}$C-hydroxytryptophan could clearly visualize the pancreas in normal Wistar rats (Fig. 4A). The images also showed a high $^{11}$C-hydroxytryptophan uptake in the kidney, which can be ascribed to renal excretion of the radiopharmaceutical. These results are in agreement with previous publications (10). Figure 4B shows a typical PET image of the abdomen in a clorgyline-treated rat. As in control animals, a high uptake of $^{11}$C-hydroxytryptophan was observed in the pancreas and kidneys. Pancreatic uptake of $^{11}$C-hydroxytryptophan was about 3-fold higher in clorgyline-treated animals than in the control group. Time–activity curves, showing tracer kinetics in the pancreas, liver, kidney, and heart, are presented in Figure 5. Time–activity curves of the pancreas of untreated and clorgyline-treated rats (Fig. 5A) show that $^{11}$C-hydroxytryptophan was largely retained in the pancreas of clorgyline-treated animals, whereas the tracer was cleared from the pancreas in nontreated Wistar rats. At intervals greater than 15 min, there was a significant difference between treated and nontreated animals ($P < 0.05$).

Time–activity curves of the heart, kidney, and liver of untreated and clorgyline-treated rats are displayed in Figures 5B, 5C, and 5D, respectively. The time–activity curves showed fast washout of $^{11}$C-hydroxytryptophan from these nontarget organs, except for the liver in clorgyline-treated animals, which showed retention of radioactivity. In contrast to the pancreas, these nontarget organs did not show any significant differences in time–activity curves between untreated and clorgyline-treated animals.

Ex Vivo Biodistribution

Results of the ex vivo biodistribution of $^{11}$C-hydroxytryptophan in normal Wistar rats and in rats pretreated with clorgyline are presented in Table 2. In the control group, the kidneys showed the highest radiotracer uptake
(SUV, 5.34 ± 1.97). There was relatively low uptake in the spleen, stomach, and liver (SUV, 1.82 ± 0.75, 1.69 ± 1.09, and 1.15 ± 0.53, respectively) and moderate uptake in the pancreas and duodenum (SUV, 2.91 ± 1.24 and 2.06 ± 1.32, respectively). Radioactivity in all other organs was below the plasma level. In the clorgyline-treated group, the pancreas showed the highest radiotracer uptake (SUV, 9.73 ± 2.07). Pancreatic tracer uptake in clorgyline-treated rats was significantly higher than that in control animals (3.3-fold, \(P = 0.0002\)). The other organs of clorgyline-treated animals showed a tracer distribution similar to that in control animals.

**Autoradiography**

To investigate the cellular localization of radioactivity within the pancreas, autoradiography was performed on untreated and clorgyline-treated rats. Autoradiography showed a homogeneous distribution of radioactivity in the pancreas of untreated rats, with no distinction between endocrine and exocrine pancreas (Fig. 6A). In contrast, the pancreas of clorgyline-treated animals displayed a heterogeneous distribution of radioactivity, with clear spots with high tracer accumulation (Fig. 6B). Hematoxylin–eosin staining demonstrated that the radioactive spots colocated with the islets of Langerhans.

To assess the specificity of \(^{11}\text{C}\)-hydroxytryptophan accumulation in the islets, tracer accumulation in the endocrine and exocrine pancreas was quantified by ROI analysis. Twelve to thirteen regions of interest for both the endocrine and the exocrine part of the pancreas were drawn for each pancreas. Figure 7 shows the percentage of specific \(\beta\)-cell uptake in the control group and in clorgyline-treated animals. The percentage of \(\beta\)-cell-specific uptake was 8% ± 22% and 108% ± 32% in the untreated and clorgyline-treated animals, respectively (\(P < 0.000001\)).

**DISCUSSION**

The primary objective of this study was to investigate the feasibility of \(^{11}\text{C}\)-hydroxytryptophan as a PET tracer for noninvasive \(\beta\)-cell imaging. One of the most important prerequisites of a good PET tracer for \(\beta\)-cell imaging is the ability to discriminate between the endocrine portion of the pancreas and the surrounding exocrine tissue.

Therefore, we evaluated the differences in tracer uptake and tracer retention between endocrine and exocrine cell lines using PANC-1 as an exocrine cell line and CM as a \(\beta\)-cell line. The CM and PANC-1 cell lines used in these experiments are immortalized cells that are able to proliferate indefinitely in culture. These immortalized cells may have different phenotypes and consequently some properties different from primary cells. However, these immortalized cells are much easier to work with than primary cells and do not require the sacrifice of laboratory animals, but the results should be interpreted with caution.

Cellular accumulation of \(^{11}\text{C}\)-hydroxytryptophan in both cell lines was rapid and plateaued within 30 min. There was no selectivity in tracer uptake rate for \(\beta\)-cells over exocrine cells. It is well know that \(^{11}\text{C}\)-hydroxytryptophan is taken up by various cells via the LAT. Thus far, 4 subtypes of LAT have been identified, but the substrate selectivity and the tissue expression profile of these transporters are still a matter of debate. Within the pancreas, LAT1 is exclusively present in the islets of Langerhans. However, high tracer uptake was observed in both the endocrine and the

### TABLE 1

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Target enzyme/transporters</th>
<th>(^{11}\text{C})-hydroxytryptophan uptake</th>
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<tr>
<td>5-hydroxytryptophan</td>
<td>LAT</td>
<td>Strongly decreased</td>
</tr>
<tr>
<td>BCH</td>
<td>LAT</td>
<td>Strongly decreased</td>
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<tr>
<td>PCPA</td>
<td>TPH</td>
<td>Unchanged</td>
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<tr>
<td>Carbidopa</td>
<td>AADC</td>
<td>Unchanged</td>
</tr>
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<td>Pargyline</td>
<td>MAO-A/B</td>
<td>Unchanged</td>
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<tr>
<td>Clorgyline</td>
<td>MAO-A</td>
<td>Mildly increased</td>
</tr>
<tr>
<td>Tetrabenazine</td>
<td>VMAT2</td>
<td>Unchanged</td>
</tr>
<tr>
<td>PCPA + carbidopa</td>
<td>TPH + AADC</td>
<td>Unchanged</td>
</tr>
<tr>
<td>PCPA + clorgyline</td>
<td>TPH + MAO-A</td>
<td>Unchanged</td>
</tr>
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</table>

**FIGURE 4.** PET images of control and clorgyline-treated animals 60 min after intravenous injection of \(~50\) MBq of \(^{11}\text{C}\)-hydroxytryptophan. Coronal sections of abdomen of control rat (A) and clorgyline-treated animal (B). P = pancreas; H = heart; L = liver; K = kidney.
Because uptake is fast in both cell lines and equilibrium is rapidly reached, selectivity in the uptake phase is unlikely. However, this experiment does not give any information about retention of the tracer inside the cell, since the cells are constantly exposed to the tracer. Therefore, analysis of the efflux and retention of 11C-hydroxytryptophan in both cell lines was performed. These studies demonstrated that the efflux kinetics of 11C-hydroxytryptophan are faster from PANC-1 cells than from CM cells and that tracer retention is lower. At 60 min, about 60% of the tracer was still trapped in the CM cells. In contrast, in PANC-1 cells only 20% of the tracer stayed inside the cells after 60 min.

This experiment clearly showed that tracer retention, rather than uptake, is the main difference between these two cell types. In β-cells, 5-hydroxytryptophan is taken up, rapidly metabolized, and trapped inside the cells, whereas in PANC-1 the tracer is rapidly taken up but also readily released. Several reports show that the first metabolite of 5-hydroxytryptophan, serotonin, is stored with insulin in secretory β-granules (7). Serotonin is an important regulator of the autocrine and paracrine functions of insulin. Alternatively, serotonin can be converted to 5-HIAA, which can passively diffuse from the cells into the extracellular space. In our study, the radioactivity that is retained in CM cells after 60 min likely represents serotonin stored in the vesicles. In contrast, serotonin in PANC-1 cannot be stored in the vesicles, because these cells lack an efficient vesicular system.

To prove this hypothesis, we performed blocking studies. BCH is a nonspecific inhibitor of the LAT family. BCH strongly reduces the accumulation of 11C-hydroxytryptophan in both cell lines, but to a larger extent in PANC-1 than in CM cells. Since the blocking is not complete in CM cells, transporters other than LAT, such as the A and ASC transporter systems, appear also to be involved in the transport of 5-hydroxytryptophan in these cells.

The TPH inhibitor PCPA does not have an effect on 11C-hydroxytryptophan accumulation in PANC-1 cells but reduces accumulation in CM cells. TPH is the rate-limiting enzyme in serotonin production. The structure of PCPA is

<table>
<thead>
<tr>
<th>Table 2: Ex Vivo Biodistribution of 11C-Hydroxytryptophan</th>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>(n = 5)</td>
</tr>
<tr>
<td>Bone</td>
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<td>Cerebellum</td>
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<td>Cerebrum</td>
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<td>Colon</td>
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<td>Duodenum</td>
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<td>Fat</td>
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<td>Kidney</td>
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<td>Pancreas</td>
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<td>Plasma</td>
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<td>Red blood cells</td>
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<td>Spleen</td>
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<td>Stomach</td>
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*P < 0.01.
similar to phenylalanine, a large neutral aromatic amino acid. A plausible explanation for the inhibition of $^{11}$C-hydroxytryptophan accumulation in CM cells by PCPA could therefore be that PCPA competes with $^{11}$C-hydroxytryptophan for a transporter system, as it was shown to compete for tryptophan transport. The inhibition experiment with BCH demonstrated that $^{11}$C-hydroxytryptophan is exclusively taken up by LAT in PANC-1 cells, but a second transporter system is likely involved in CM cells. If PCPA inhibits the uptake of $^{11}$C-hydroxytryptophan via a second unknown transporter system but not via LAT, this would explain why PCPA reduces accumulation of the tracer in CM cells but not in PANC-1 cells.

AADC is the enzyme that converts 5-hydroxytryptophan into serotonin. The enzyme is present in both the endocrine and the exocrine pancreas. A previous study by Neels et al. showed that inhibition of AADC with carbidopa had no significant effect on the in vitro uptake of $^{11}$C-hydroxytryptophan in BON cells, but remarkably they found an increase in $^{11}$C-hydroxytryptophan tumor uptake in vivo in tumor-bearing rats (9). In this study, we acquired similar results. In both endocrine CM and exocrine PANC-1 cells, we did not observe any effect of inhibition of AADC with carbidopa on cellular uptake of $^{11}$C-hydroxytryptophan. In a parallel study, however, we noticed a 3.3-fold increase in tracer uptake in the pancreas in rats pretreated with carbidopa compared with untreated control animals (unpublished results). This discrepancy between the in vitro and in vivo effects of carbidopa could be due to effects of carbidopa on metabolism of the tracer in vivo. Orlefors et al. showed that administration of carbidopa improves the tumor uptake of $^{11}$C-hydroxytryptophan and attributed this effect to a reduction of the conversion of 5-hydroxytryptophan into serotonin by the kidneys (11). Consequently, the administration of carbidopa leads to a higher concentration of parental tracer in the plasma and thus to an enhanced availability of the tracer. A similar explanation could also be proposed for our results. Through the administration of carbidopa, metabolism of $^{11}$C-hydroxytryptophan by the kidneys will be reduced and the availability of the tracer will be enhanced. In the in vitro experiments, metabolism of the tracer in the medium was negligible, and thus availability of the tracer is not affected by the presence or absence of carbidopa.

MAO consists of 2 subtypes, MAO-A and MAO-B, which are involved in the degradation of serotonin. MAO-A and MAO-B are both present in islets, whereas MAO-A is the predominant subtype in exocrine pancreatic tissue. Inhibition of MAO is expected to increase trapping of radioactivity because of suppressed formation of radio-labeled 5-HIAA, a substance that can diffuse out of the cell. Pargyline (MAO-A and MAO-B inhibitor) and clorgyline (MAO-A inhibitor) slightly increased the accumulation of $^{11}$C-hydroxytryptophan in PANC-1 cells but strongly increased the tracer accumulation in CM cells.

VMAT, a specific transporter located in vesicular membranes, mediates the loading of monoamines into these vesicles. Until now, only 2 subtypes have been characterized. VMAT1 is generally expressed in enterochromaffin cells, whereas VMAT2 is specifically expressed in pancreatic β-cells. Exocrine tissue does not express VMAT. Tetraabenazine, a specific inhibitor of VMAT2, did not have any effect on accumulation of $^{11}$C-hydroxytryptophan in PANC-1 cells but increased accumulation in CM cells, probably because release of $^{11}$C-serotonin from the secretory vesicles was prevented. These results suggest that $^{11}$C-hydroxytryptophan is metabolized in CM cells and subsequently stored in the vesicles and released into the extracellular space. Therefore, inhibition of uptake in vesicles will suppress the release of serotonin. In PANC-1 cells, on the other hand, the serotonin

![FIGURE 6. Autoradiograph and hematoxylin–eosin staining of pancreas slices of untreated rat (A) and clorgyline-treated rat (B).](image)

![FIGURE 7. Percentage specific uptake in islets compared with exocrine pancreas in control and clorgyline-treated animals. **P < 0.01.](image)
pathway does not seem to be involved in the uptake and release of $^{11}$C-hydroxytryptophan.

To translate our findings from the in vitro to in vivo studies, experiments were performed on normal Wistar rats and on clorgyline-treated animals. Clorgyline was the inhibitor of choice for the animal experiments, because MAO-A inhibition caused the greatest increase of $^{11}$C-hydroxytryptophan accumulation in the endocrine cell line, resulting in the largest difference between endocrine and exocrine cells. Clorgyline treatment was hypothesized to reduce the degradation of serotonin into 5-HIAA. Preclinical evaluation of $^{11}$C-hydroxytryptophan in normal Wistar rats was performed to investigate the uptake and retention of the tracer under normal conditions. The control group showed a high accumulation of $^{11}$C-hydroxytryptophan in the pancreas (SUV, 2.91 ± 1.24). Pretreatment of the animals with clorgyline, however, resulted in a strong increase in radioactivity accumulation in the pancreas (SUV, 9.73 ± 2.07). Time–activity curves showed substantial retention of radioactivity in the pancreas of clorgyline-treated animals, whereas radioactivity was cleared from the pancreas of controls. In contrast, the time–activity curves of the heart and the kidney in both untreated and clorgyline-treated animals showed a rapid washout of radioactivity without any significant differences between the 2 experimental groups. Although differences in hepatic accumulation of radioactivity between the 2 groups were not statistically significant at any time, the shape of the time–activity curve of the liver of clorgyline-treated animals was markedly different from that of controls. Whereas radioactivity is retained in the liver of clorgyline-treated animals, it is cleared from the liver of untreated animals. This discrepancy can be explained by the expression of MAO-A in the liver, where the enzyme is involved in the deamination of norepinephrine, epinephrine, serotonin, and dopamine (12).

 Autoradiography in control animals showed a homogeneous distribution of radioactivity in the pancreas without any discrimination between endocrine and exocrine tissue. Thus, the high tracer uptake in the pancreas of control animals combined with the homogeneous distribution of the radioactivity throughout the pancreas indicates that in control animals the background signal in exocrine pancreas is high and might obscure the specific uptake in β-cells. In contrast, pancreatic tracer uptake was significantly increased in clorgyline-treated animals and radioactivity was mainly localized in the islets of Langerhans. Autoradiographic studies conducted by Gershon and Ross (4) indicate that more time may be required to enhance the endocrine pancreas–to–exocrine pancreas ratio in control animals. They evaluated the ex vivo localization of $^3$H-$^5$hydroxytryptophan and $^3$H-serotonin in the exocrine and endocrine pancreas. In the exocrine pancreas, $^3$H-$^5$hydroxytryptophan uptake was homogeneously distributed. After 2 h, the radioactive metabolite (serotonin) was transferred to the acinar duct. The endocrine pancreas showed a completely different pattern in uptake and secretion of labeled serotonin. All the islets of Langerhans analyzed were labeled with radioactivity ($^3$H-serotonin), which persisted inside the cells for more than 24 h.

After treatment of the animals with clorgyline, the enzyme MAO-A in the pancreas was inhibited. Since the serotonin pathway is active in the endocrine pancreas, inhibition of MAO-A leads to an increased retention of radioactive serotonin inside the β-cells. Radioactive serotonin is no longer degraded to 5-HIAA but stored inside the secretory vesicles via VMAT2. In the absence of stimuli, serotonin will not be released from the β-cells. Consequently, a higher concentration of radioactivity can be detected in the endocrine pancreas. Inhibition of MAO does not affect retention of the tracer in the exocrine pancreas because $^{11}$C-hydroxytryptophan is not metabolized via the serotonin pathway in these cells.

$^{11}$C-hydroxytryptophan, however, is metabolized not only in the pancreas but also in plasma. The main metabolite found in plasma is $^{11}$C-HIAA. Lindner et al. reported that treatment of rats with clorgyline increased the percentage of intact $^{11}$C-hydroxytryptophan in plasma from 63% to 90% at 40 min after tracer injection, whereas the plasma-to-blood radioactivity ratio decreased from 1.19 to 0.73 (13). This effect was ascribed to inhibition of MAO-A in the blood platelets, resulting in reduced production of $^{11}$C-HIAA and increased storage of $^{11}$C-serotonin in platelets. These data imply that the effect of plasma metabolites on radioactivity accumulation in the pancreas of clorgyline-treated animals is likely negligible. However, accumulation of $^{11}$C-HIAA from plasma into the pancreas of control animals cannot be completely excluded. In theory, pancreatic uptake (if any) of $^{11}$C-HIAA from plasma could then even be partially responsible for the lack of selective uptake in the islets of Langerhans of controls.

Taken together, this study provided valuable information for further development of PET tracers that allow β-cell imaging in the pancreas. To overcome the problem of a high background signal, a 5-hydroxytryptophan analog labeled with an isotope with a longer half-life could be applied. This would allow imaging at later time points when most radioactivity from exocrine tissue has been cleared. In addition, if a 5-hydroxytryptophan analog could be developed that is resistant against degradation by MAO, pretreatment with MAO inhibitors would no longer be required.

CONCLUSION

Our findings indicate that the uptake and metabolism of $^{11}$C-hydroxytryptophan in endocrine and exocrine pancreas proceed via different pathways. In β-cells, $^{11}$C-hydroxytryptophan trapping is strongly dependent on the serotonin pathway. Our data suggest that pretreatment with the MAO-A inhibitor clorgyline can improve the discrimination between islets of Langerhans and the surrounding exocrine tissue. However, noninvasive imaging of β-cells with $^{11}$C-hydroxytryptophan does not appear to be a feasible approach, because of the relatively high background signal
from the exocrine pancreas. On the other hand, 11C-hydroxytryptophan could be a suitable tracer for islet transplantation imaging, where the islets will be transplanted in liver or in a subcutaneous layer. In those tissues, 11C-hydroxytryptophan uptake is negligible, and therefore the signal-to-background ratio should allow easy detection of the transplanted islets.

DISCLOSURE STATEMENT

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11C-Hydroxytryptophan Uptake and Metabolism in Endocrine and Exocrine Pancreas

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