In Vivo Demonstration of Enzyme Activity in Endocrine Pancreatic Tumors: Decarboxylation of Carbon-11-DOPA to Carbon-11-Dopamine

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Methods: We used PET to characterize the uptake and decarboxylation of ^{11}C-L-DOPA in vivo in two patients with endocrine pancreatic tumors: one glucagonoma and one gastrinoma. **Results:** With L-DOPA labeled with ^{11}C in the beta position, in which the radioactive label follows the molecule through decarboxylation to dopamine, significant uptake was observed in the tumors. With L-DOPA labeled in the carboxyl group, in which the label is rapidly eliminated from the tissue as $^{11}CO_2$ if decarboxylation takes place, an almost complete lack of uptake is noted. **Conclusion:** This study shows that, using selective position labeling, an in vivo action of enzymatic activity can be observed with PET and that significant decarboxylation occurs in the tested endocrine pancreatic tumors. Also, marked retention of radioactivity occurs after treatment with somatostatin analogs. It is hypothesized that this is a reflection of a reduction of exocytosis which is induced by this treatment.

Key Words: PET; endocrine pancreatic tumors; aromatic amino acid decarboxylase; carbon-11-DOPA; somatostatin analogs

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Neuroendocrine tumors have been classified as APUD-omas, based on the observation by immunohistochemical methods that they had significant amine precursor uptake and decarboxylation capacities (*1,2*). This means that these cells take up amine precursors, such as the amino acids L-DOPA and 5-hydroxytryptophan, which are then decarboxylated to the amines dopamine or serotonin through the enzyme, aromatic amino acid decarboxylase (AADC). Finally, the created dopamine or serotonin are stored in secretory granulae.

Based on the expression of this biochemical pathway in neuroendocrine tumors, we hypothesised that we could visualize these tumors using PET with ^{11}C-labeled L-DOPA or 5-hydroxytryptophan. Indeed, we could demonstrate a very high uptake of these tracer substances in neuroendocrine tumors compared to normal surrounding tissues, thereby allowing an improved visualization for diagnostic purposes. Notably high uptake of ^{11}C-5-hydroxytryptophan was observed in carcinoid tumors (*3*) as well as high uptake of ^{11}C-L-DOPA in endocrine pancreatic tumors (*4*).

It has usually been accepted that PET with one radiotracer suffers from a principal limitation in that the recorded images will only represent the concentration of the radioactivity, independent of which molecule the radioactive label is attached to. Thus, it is generally not possible to deduce if the radioactivity in the tissue remains in the form of the original injected substance or if it has undergone metabolic or enzymatic transformation to another substance with PET. Usually, tissue sampling with extraction and chemical analysis of the labeled compounds can supply complementary information of the distribution of the chemical forms of tissue radioactivity.

With PET, it is generally possible to evaluate tracer binding to an enzyme, but it is seldom possible to evaluate the actions of the enzyme itself. By applying multitracer protocols and careful tracer design with respect to stereochemistry, position-specific labeling and multiple isotope labeling, new possibilities for further interpretation of the content of radioactivity are formed (*5,6*).

PET can thus investigate enzyme activity; this paper presents one such case. By selectively introducing the ^{11}C atom in different positions in the tracer molecule, the ^{11}C atom may selectively follow the tracer molecules' major part, or appear in the split-off subgroup. If this split-off subgroup has significantly different kinetics of elimination from the tissue compared to the main group, the tissue radioactivity will show different kinetics, depending on where the ^{11}C label is inserted.

In this study, we demonstrate that major differences are observed with the two labeling positions in the in vivo kinetics as measured with PET by alternatively placing the ^{11}C label in the beta position or in the carboxyl-group of L-DOPA. This allows demonstration of decarboxylation activity in vivo in endocrine pancreatic tumors.

MATERIALS AND METHODS

Patients

Two female patients with endocrine pancreatic tumors, a gastrinoma and a glucagonoma, respectively, were selected for PET investigations.

Patient 1. This patient is a 73-yr-old woman with a history of diarrhea and dyspepsia which improved on and required continuous treatment with H2-receptor blockers or omeprazol. In 1992, elevated levels of serum gastrin (62 pmole/liter; n < 55), pancreatic polypeptide (PP) (895 ng/liter; n < 70), plasma chromogranin A (1800 μg/liter; n < 350) were found, as well as an increased basal acid secretion, which supported the diagnosis of gastrinoma. Radiological examination with CT, ultrasonography and angiography revealed a primary tumor in the head of the pancreas and lymph node metastases. A biopsy from the tumor in the pancreas showed chromogranin A-positive but Grimelius-negative tumor cells, which indicated that there was an endocrine tumor in the pancreas. The patient also underwent octreotide scintigraphy which revealed somatostatin receptors in the tumors. The patient was treated with the somatostatin analog somatuline (Henri Beaufour Institute, Washington, DC).

A pretreatment PET study with ^{11}C-L-DOPA was performed in October 1992. Two weeks after this study, the patient was started on subcutaneous injections of somatuline four times daily, starting with 750 μg/day, then escalating weekly up to 12,000 μg/day after 6 wk. The patient was maintained on this dose for more than 1 yr. Six months after the start of somatuline treatment, a second PET

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study was performed with ^{11}C-L-DOPA. Both of these studies were performed with L-DOPA labeled in the beta-position. Hormonal measurements at 6 mo showed decreased serum gastrin (23 pmole/liter) and plasma chromogranin A (340 ng/liter), whereas PP was unchanged. On the same day as the follow-up study, a repeat study was performed with ^{11}C-L-DOPA labeled in the carboxy position. The patient underwent surgery in December 1993, 14 mo after start of somatuline treatment. The primary tumor in the head of the pancreas (the uncinate process) was enucleated and multiple local lymph node metastases were excised. Histopathology showed a Grimelius, chromogranin A, PP, glucagon, insulin and somatostatin-positive tumor, but stainings against gastrin and vasoactive intestinal polypeptide (VIP) were negative.

Patient 2. This patient is a 58-yr-old woman who had a history of recurrent migrating skin lesions, stomatitis and conjunctivitis since 1985. In 1991, glucosuria and anemia were demonstrated. In 1992 grossly elevated levels of plasma glucagon (3200 pg/ml; $n < 140$) were found and glucagonoma syndrome was diagnosed.

Hormonal screening showed increased levels of serum insulin (23 mU/liter; $n < 20$), proinsulin (115 pmole/liter; $n < 9.8$) and plasma chromogranin A (960 μg/liter; $n < 350$) in addition to elevated plasma glucagon. CT, ultrasonography and angiography showed a large primary tumor in the head of the pancreas with a diameter of 6 cm, and small cystic and partly calcified lesions in the liver, from which biopsies showed Grimelius, chromogranin A, glucagon and insulin-positive tumor cells. Octreotide scintigraphy showed somatostatin receptors both in the pancreatic tumor and hepatic lesions. Hence, it was decided to start the patient on somatuline treatment according to the same schedule as in the previous patient.

A pretreatment PET study with ^{11}C-L-DOPA was performed and the patient was started on somatuline therapy. After 6 mo of treatment, a follow-up PET study was made and later on the same day the patient was reexamined with ^{11}C-L-DOPA labeled in the carboxy group. At 6 mo, plasma glucagon had decreased to 326 pg/ml, chromogranin A to 326 μg/liter, whereas serum pro-insulin had increased up to 153 pmole/liter. The patient's skin lesions had already disappeared after 6 wk of treatment.

The patient continued with somatuline treatment for more than 1 yr and underwent surgery with resection of the primary tumor in the pancreatic head in January 1994. Histopathology showed a chromogranin A-positive tumor with an adjacent local lymph node metastasis and calcified hyalinized stroma.

PET

The patients fasted for 4 hr prior to imaging. The PET studies were performed with a whole-body PET camera that simultaneously generates 15 tomographic slices with a separation of 6.5 mm and an in-plane resolution of about 5 mm (*7*). The total axial coverage of the slices was 10 cm. The patient was placed on the couch and the examination area was selected with a laser to cover the primary tumor. A transmission scan was made using an external rotating ^{68}Ge rod for subsequent attenuation correction.

Carbon-11-L-DOPA was injected intravenously as a bolus in a dose of 120–560 (average 328) MBq. Immediately following the injection, a dynamic imaging sequence was started with 14 scans covering a total examination time of 45 min. The examination was thus subdivided into five 1-, 3- and 5-min frames as well as one 10-min frame. Quantitative images were reconstructed with attenuation correction from the transmission scan (Figs. 1, 2).

During the examination, 12 blood samples were withdrawn from a vein on the hand, heated with hot water pads and plasma radioactivity concentration was determined in a well counter cross-calibrated with the PET camera. The blood samples were taken at 0.5, 1, 2, 3, 5, 7, 10, 15, 20, 25, 30 and 40 min postinjection. At 5, 15, 30 and 40 min postinjection, separate blood samples were taken to analyze fractions of unchanged tracer and labeled metabolites.

A liquid chromatographic system was used to analyze ^{11}C-L-DOPA and radiolabeled metabolites. Before injection onto the analysis system, the plasma proteins were precipitated with perchloric acid. The sample was centrifuged and a standard solution containing L-DOPA, dopamine, methyl-dopa, DOPAC and HVA (homovanillic acid) was added to the resulting supernatant to allow recording of the UV signal. The sample was then injected onto the separation system and fractions corresponding to the UV signal of the compounds were collected and measured for radioactivity.

The synthesis of carboxy- and beta-^{11}C-labeled L-DOPA was performed according to previously published procedures (*8,9*). Racemic 1- or 3-^{11}C-labeled alanine were synthesized by conventional organic synthetic methods and then the appropriate alanine was used in a one-pot multi-enzymatic synthesis giving the labeled L-DOPA. After HPLC purification, pH adjustment and sterile filtration, the solution was ready for intravenous injection.

Data Analysis

In the images from one examination, regions of interest were outlined to represent solid tumor tissue, normal liver and kidney cortex. For each tissue type, regions were outlined in 7–10 slices and added together to represent a volume of interest. The same volume of interest was transposed to the different examinations in the same individual, with slight translations of position to compen-

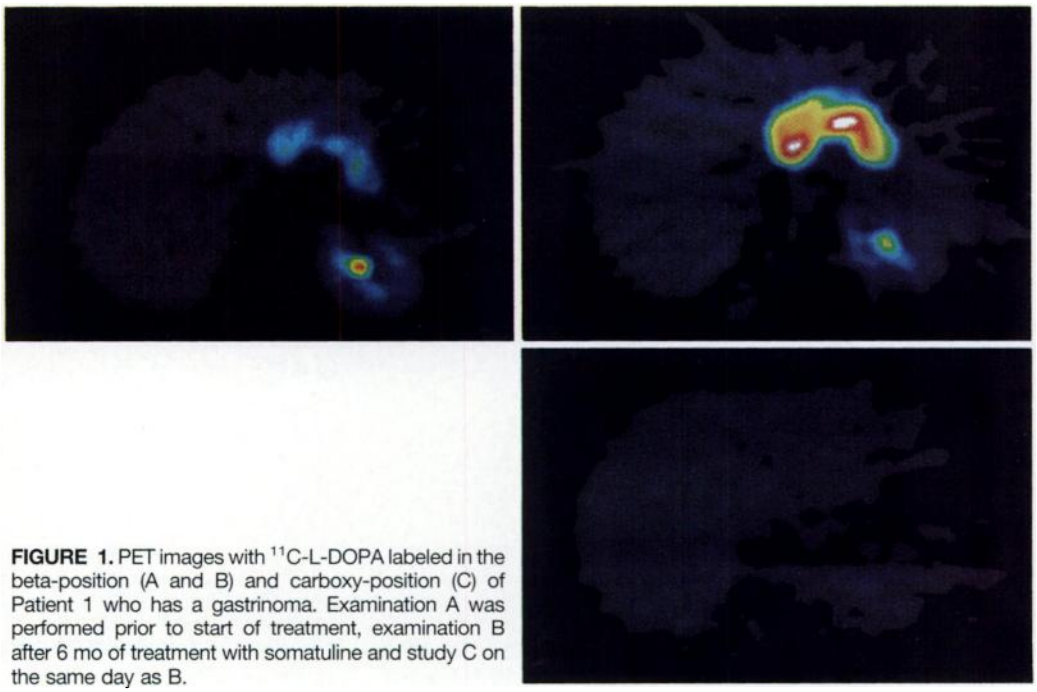

FIGURE 1. PET images with ^{11}C-L-DOPA labeled in the beta-position (A and B) and carboxy-position (C) of Patient 1 who has a gastrinoma. Examination A was performed prior to start of treatment, examination B after 6 mo of treatment with somatuline and study C on the same day as B.

sate for the fact that the patient was not in exactly the same position in each follow-up examination. The image display program was activated to generate time-activity curves for each tissue and each separate examination. The time-activity curves were re-normalized to represent the standard uptake values (SUV) by dividing by the ratio of injected activity and body weight (Fig. 3).

Analyses were made to represent organ tracer uptake at three different occasions in each patient:

1. Before treatment, examination with ^{11}C-L-DOPA labeled in the beta-position.
2. After somatuline treatment, examination with ^{11}C-L-DOPA labeled in the beta-position.
3. On the same day, an examination with ^{11}C-L-DOPA labeled in the carboxyl group.

The time-activity curves, together with plasma radioactivity values corrected for metabolites at different times after injection, were used to generate Patlak graphs (*10*) with:

$$Y = Ci/Cp$$

plotted against

$$X = \int Cp(t)\, dt/Cp(t),$$

where Ci = tissue concentration and Cp = plasma concentration corrected for metabolites.

This graphical representation reshapes the kinetic curves to simulate a situation in which the blood radioactivity kinetics are constant with a value of 1. In this representation, a tracer which is equilibrated will show a graph with constant values, whereas a tracer which is trapped shows a linear increase with time.

The percent of ^{11}C-L-DOPA taken up by the tissue, which has been decarboxylated, was assessed by the expression:

$$DCX = 100 \times (1 - DOC/DOP),$$

where DOC = tracer concentration with carboxyl-labeling and DOP = tracer concentration with beta-position labeling.

In each experiment, the tracer concentrations were normalized to the SUV and the percentage of decarboxylated ^{11}C-L-DOPA was plotted versus the time after injection (Fig. 4).

RESULTS

When ^{11}C-L-DOPA labeled in the beta-position was used, the PET images (Figs. 1, 2) demonstrated high accumulation in the kidneys, initially in the cortex and in the later images in the pelvis. High uptake was also observed in the tumors, especially after treatment with somatuline. When using ^{11}C-L-DOPA labeled in the carboxyl-group, relatively low, uniform uptake was observed in the whole abdomen.

The kinetic curves for the uptake of ^{11}C-L-DOPA (Fig. 3) showed a rapid increase in the tumor during the first few min in one patient, followed by a decrease with time and then a plateau in one patient. The accumulation in the kidney cortex showed a similar pattern with high initial uptake in each patient's study, followed by a marked decrease with time. The liver showed a moderate initial uptake which was followed by a slow decrease with time. The SUV values were around 6 initially and decreased to about 4 for beta-labeled and to about 2 for carboxyl-labeled L-DOPA. After treatment with somatuline, tumor uptake became markedly higher (approximately doubled when compared to the pretreatment uptake). In the kidney, no significant changes were observed after treatment, and there was a slight decrease after treatment in the liver.

In all tissues, a similar kinetic pattern was observed after injection of ^{11}C-L-DOPA labeled in the carboxyl-group. The uptake rapidly increased to a sharp peak 2 min postinjection, followed by a rapid decrease with time.

In the Patlak graphical representation, the tumor in Patient 1 showed a gradual increase with time using ^{11}C-L-DOPA labeled in the beta-position, with a curved pattern which, after somatuline treatment, converted to an almost linear time course. In the other patient, a slightly increasing time course was observed after the first min. After somatuline treatment, markedly higher uptake was noted with a continued increase with time.

With ^{11}C-L-DOPA labeled in the carboxyl-group, low uptake values were observed which increased slightly with time.

The percentage of decarboxylated tracer in the tumor (Fig. 4) rapidly increased to 80% within the first 5 min, and increased slowly thereafter to about 90%. In the kidney, the percentage that had been decarboxylated was about 80% after 5 min and decreased slightly to 60%–70% thereafter. Initially negative values (−20%–−60%) were observed in the liver, followed by a gradual increase to 20%–40%.

Plasma hormone levels showed a significant decrease after initiation of somatuline treatment (Fig. 5).

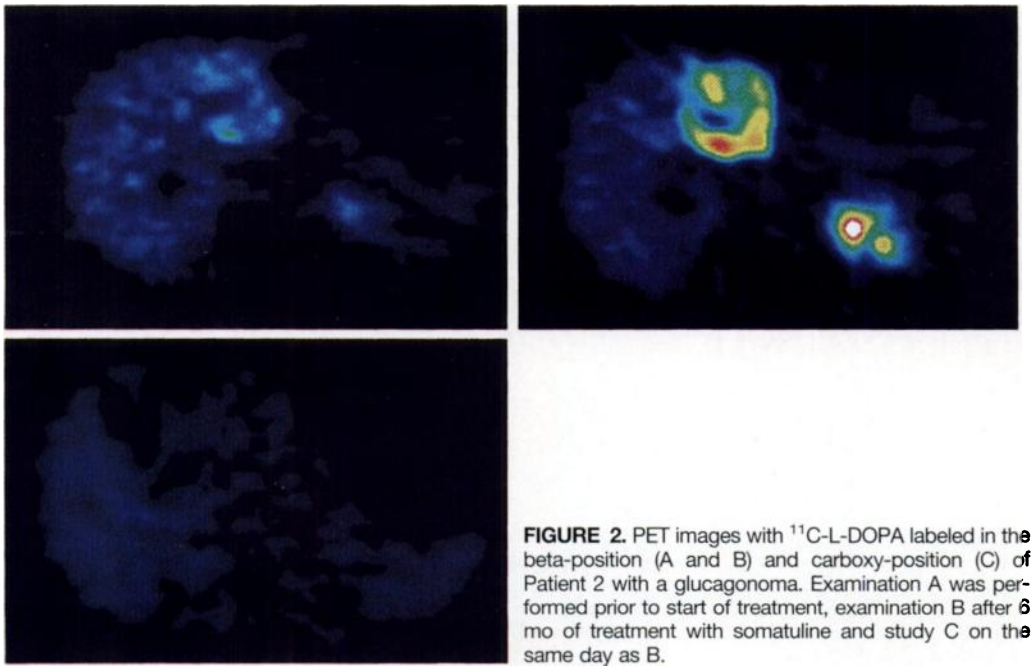

FIGURE 2. PET images with ^{11}C-L-DOPA labeled in the beta-position (A and B) and carboxy-position (C) of Patient 2 with a glucagonoma. Examination A was performed prior to start of treatment, examination B after 6 mo of treatment with somatuline and study C on the same day as B.

DISCUSSION

We used PET to assess ^{11}C-L-DOPA uptake and decarboxylation in endocrine pancreatic tumors. Three different features of the tumors are apparent from our study:

1. These endocrine pancreatic tumors have an extensive uptake of L-DOPA.
2. L-DOPA uptake is rapidly decarboxylated to dopamine.
3. Somatuline treatment greatly enhances tracer concentration in the tumor tissue.

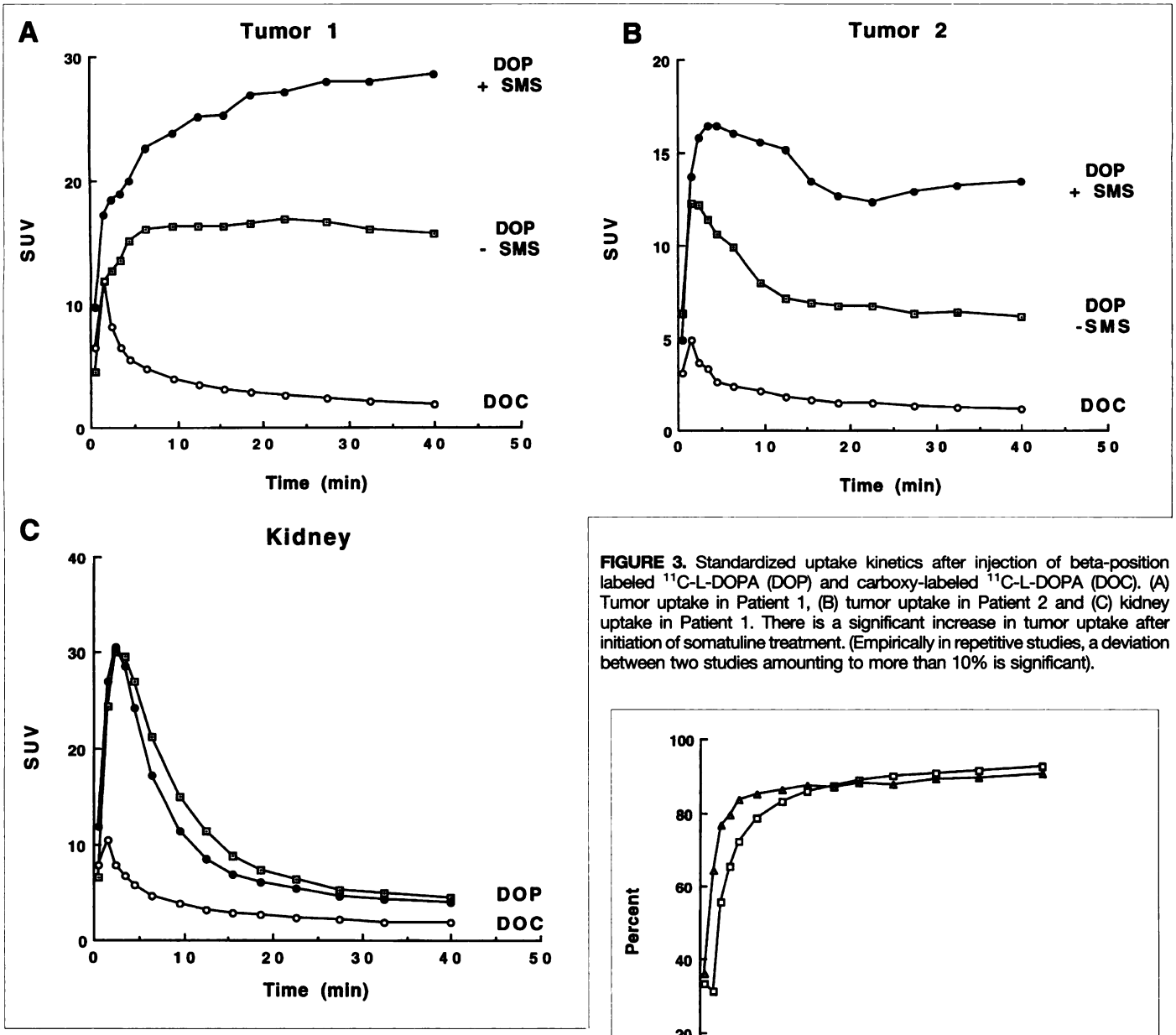

FIGURE 3. Standardized uptake kinetics after injection of beta-position labeled ^{11}C-L-DOPA (DOP) and carboxy-labeled ^{11}C-L-DOPA (DOC). (A) Tumor uptake in Patient 1, (B) tumor uptake in Patient 2 and (C) kidney uptake in Patient 1. There is a significant increase in tumor uptake after initiation of somatuline treatment. (Empirically in repetitive studies, a deviation between two studies amounting to more than 10% is significant).

FIGURE 4. Decarboxylation of ^{11}C-L-DOPA in the tumors of Patient 1 (□) and Patient 2 (△), as calculated from the ratios of carboxy-labeled and beta-position labeled ^{11}C-L-DOPA.

Since L-DOPA is an amino acid, it is probably transported across the tumor cell membrane in a manner analogous to other amino acids; i.e., by the L-transport system, which acts by facilitated exchange transport, by the A-transport system which is unidirectional and energy dependent or by another less well characterized transport system (*11*). This transport, together with tissue blood flow, are the main factors since they govern the immediate uptake after tracer injection. Based on the rapid and high uptake during the first few minutes, it is feasible to assume that both tissue blood flow and amino acid transport are enhanced in these tumors compared to liver and muscle tissue. An even higher initial accumulation is observed in the kidney cortex.

Merely by considering the kinetics of the uptake of ^{11}C-L-DOPA, it is not possible to assess the intracellular fate of the incorporated tracer. Continued uptake or lack of washout can occur if the tracer is decarboxylated to dopamine, which has a poor backdiffusion to the extracellular space. The same may also be the case with other metabolites that are formed intracellularly, such as DOPAC, methyl-DOPA or homovanillic acid. Also DOPA itself may have a slow backdiffusion. A comparison between the studies performed with ^{11}C-L-DOPA labeled in the beta-position and with ^{11}C-L-DOPA labeled in the carboxyl-group, however, gives a clear manifestation that most of the incorporated tracer is rapidly decarboxylated to dopamine. In this decarboxylation process, using ^{11}C-L-DOPA labeled in the carboxyl-group, the ^{11}C-label is cleaved off as $^{11}C\text{-}CO_2$ which in turn is equilibrated with the extracellular space and the blood pool very rapidly.

As an estimate of the percentage of accumulated tracer which is decarboxylated, the ratio of the studies with carboxyl-labeled and beta-position labeled ^{11}C-L-DOPA was formed. The deduced data demonstrate that the major portion of the tracer is rapidly decarboxylated to dopamine. Thus, more than 80% is decarboxylated within about 5 min. This fraction may be even

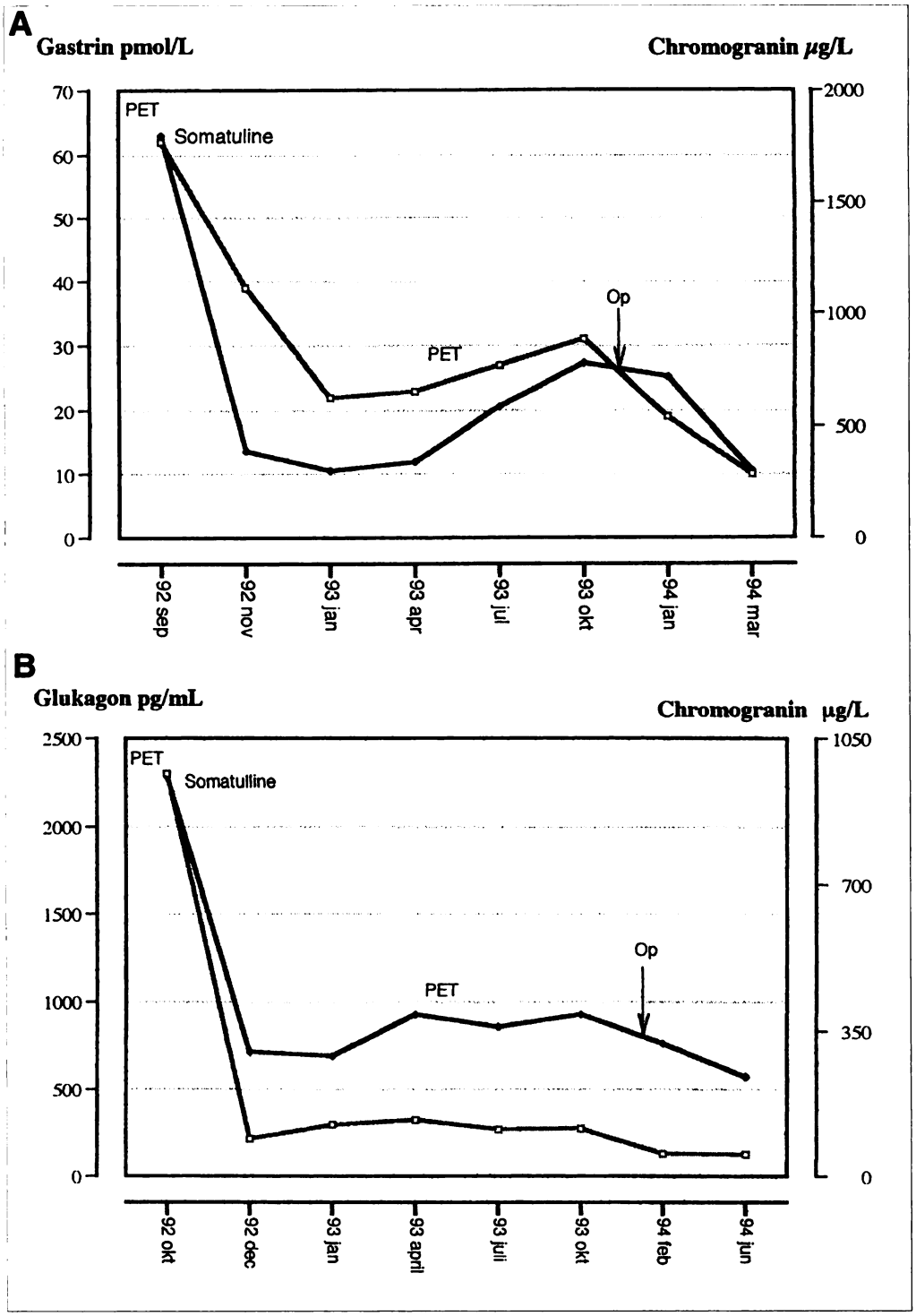

FIGURE 5. Circulating hormone levels in Patients 1 (A) and 2 (B) before and during somatuline treatment. Darker line = chromogranin A.

higher if adequate corrections are made for CO_2 distribution in blood and tissue. Such a correction would necessitate a determination of CO_2 in blood, which was not performed in this study.

Extremely marked increase in the uptake of ^{11}C-L-DOPA was observed in both patients after somatuline treatment. Increases were also observed in SUV values and in the ratio of tumor-to-liver radioactivity concentration, which amounted to a factor of 2–3. This increase occurs simultaneously with a clinically beneficial therapeutic effect, with marked reduction of otherwise highly elevated serum or plasma hormonal levels. This observation may have two implications. First, it might be possible to enhance tumor delineation with ^{11}C-L-DOPA by giving the patient an injection with a somatostatin analogue first. Second, this observation might explain some of the actions of somatostatin analogs in endocrine pancreatic tumors.

The routes of turnover, metabolism and modulatory systems of ^{11}C-L-DOPA in endocrine pancreatic tumor cells can be described as follows. The amine precursor ^{11}C-L-DOPA is transported into the cell through an amino acid transport system and is rapidly decarboxylated to ^{11}C-dopamine. Carbon-11-dopamine is stored mainly in one type of secretory granula which then constitutes a large pool of ^{11}C radioactivity. In endocrine-active tumors, ^{11}C-dopamine is constantly released from the secretory granula, thereby diminishing the content

of the ^{11}C label. Somatostatin analogs reduce the hormone synthesis by a mechanism affecting mRNA and transcription (*12,13*), as well as by inhibiting the release (*14*) of both the granula containing dopamine and the granula (if different) containing hormones but without major effects on amino acid transport or decarboxylation. The result is an inhibited release of ^{11}C-dopamine and, hence, enhanced concentration of radioactivity in the tumor. This increase of radioactivity in the tumor after somatostatin analog treatment thus occurs simultaneously with a significant reduction of hormone levels due to inhibition of exocytosis.

CONCLUSION

Although in vitro techniques will remain as the most important methods for evaluating mechanistic aspects of cellular physiology and biochemistry, new developments in the synthesis of PET tracers are making it possible to discern in vivo biochemistry. Thus, the quantitative and qualitative importance of a certain enzyme route or receptor activation can be assessed under more complicated but more intriguing in vivo conditions.

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