Effect of Dose and Specific Activity on Tissue Distribution of Indium-111-Pentetreotide in Rats

Wout A.P. Breeman, Dik J. Kwekkeboom, Peter P.M. Kooij, Willem H. Bakker, Leo J. Hofland, Theo J. Visser, Geert J. Ensing, Steven W.J. Lamberts and Eric P. Krenning

Departments of Nuclear Medicine, Internal Medicine III, University Hospital Dijkzigt, Rotterdam, The Netherlands and Mallinckrodt Medical, Petten, The Netherlands

To increase the target-to-background ratio in receptor scintigraphy, we hypothesized that receptor scintigraphy is best performed using the lowest possible mass with the highest possible specific radioactivity of the radioligand. **Methods:** Rats were injected with 2 or 10 μg of unlabeled octreotide or 2 or 10 μg of ^{111}In-pentetreotide. Scintigraphic images were then obtained from 10 min before to 20 min after the ^{111}In injection. **Results:** In some instances, there was a significant increase in ^{111}In uptake in somatostatin receptor-positive organs. In others, there was a significant decrease. Since no significant differences were found in background radioactivity in the percent dose uptake of ^{111}In in receptor-negative organs, these data indicate that target-to-background ratios can be increased by the administration of nonradiolabeled peptides under select conditions. **Conclusion:** The uptake of ^{111}In-pentetreotide in somatostatin receptor-positive organs results in a tissue-specific bell-shaped function of the injected mass of the radiopharmaceutical. This curve may also apply to somatostatin receptor-positive tumors, the visualization of which may be enhanced by optimizing the mass of ^{111}In-pentetreotide.

Key Words: indium-111-pentetreotide; somatostatin receptor imaging; peptide scintigraphy

J Nucl Med 1995; 36:623–627

In a previous study, we reported scintigraphic visualization of somatostatin receptor-positive tumors in rats with ^{111}In-pentetreotide (*1*). In that study, the administered mass of pentetreotide varied between 0.5 and 1 μg and the radioactive dose was kept constant at 18.5 MBq ^{111}In. However, the nonradioactive composition of $^{111}InCl_3$ has improved since the first labeling of pentetreotide in 1987. Therefore, it was possible to increase the specific radioactivity 5-fold, up to 185 MBq ^{111}In per μg pentetreotide, thus allowing the administration of smaller masses of peptide with the same radioactive dose. We hypothesized that receptor scintigraphy shows an optimal target-to-background ratio at the lowest possible mass of peptide with the highest specific radioactivity, which could result in a more sensitive imaging technique.

In this study, we test this hypothesis and the effects of mass and specific radioactivity of ^{111}In-pentetreotide on percent dose uptake and specific binding in several organs in rats. Therapy with unlabeled octreotide may have a negative influence on the target-to-background ratio in ^{111}In-pentetreotide scintigraphy. Surprisingly, Dörr et al. recently reported improved visualization of carcinoid liver metastases with ^{111}In-pentetreotide scintigraphy during treatment with a subcutaneous dose of 600 μg per day of octreotide (*2,3*). The mechanism(s) and the effect(s) of pretreatment and/or concomitant therapy with unlabeled ligand on receptor scintigraphy have not yet been studied in detail. Somatostatin receptors are structurally related integral membrane glycoproteins. Recently, five different human somatostatin receptor types were cloned. All subtypes bind native somatostatin-14 (SS_{14}) and SS_{28} (prosomatostatin with 28 aminoacids) with high affinity, while their affinity for numerous somatostatin analogs differs considerably (*4–7*). Octreotide binds with high affinity to the SSTR2 (somatostatin receptor type 2) subtype, although this analog has a relatively low affinity for SSTR3 and SSTR5 and shows no binding to SSTR subtypes 1 and 4 (*4–7*). Pentetreotide scintigraphy is therefore based on the visualization of octreotide binding somatostatin receptors (octreotide receptors), most probably the SSTR2.

In this study, we also investigate the tissue distribution of ^{111}In in octreotide receptor-positive (i.e., pituitary, adrenal and pancreas) and octreotide receptor-negative tissues (i.e., kidneys, spleen, liver and soft tissue (thigh) muscle) (*1,8*) 24 hr after injection of 0.5 μg pentetreotide labeled with 3 MBq ^{111}In. At various time points, relative to the injection of indium, additional unlabeled octreotide or pentetreotide was administered intravenously.

MATERIALS AND METHODS

Radiolabeling and Quality Control

Pentetreotide and $^{111}InCl_3$ (DRN 4901, 370 MBq/ml in HCl, pH 1.5–1.9) were obtained from Mallinckrodt (Petten, The Nether-

Received Mar. 2, 1994; revision accepted Sept. 13, 1994.

For correspondence or reprints contact: W.A.P. Breeman, Department of Nuclear Medicine, University Hospital Dijkzigt, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

lands). Thirty minutes after the start of pentetreotide labeling with ^{111}In (specific radioactivity 185 MBq ^{111}In pentetreotide per μg[DTPA-D-Phe1]octreotide molar excess of 5- to 10-fold of peptide over ^{111}In), the labeling efficiency was over 98%. Quality control tests were performed consecutively as described earlier (*9*). Although additional groups of peptides may participate in ^{111}In complexation, we refer to the radiolabeled product as ^{111}In-pentetreotide.

Tissue Distribution and Specific Binding of Pentetreotide

One hundred twenty-three male Wistar rats (240–260 g) were used in three separate experiments. Rats were anesthetized with ether, and the radiopharmaceutical and/or additional peptides were injected into the dorsal vein of the penis and/or a sublingual vein. The injection volume was kept constant at 0.5 ml per rat. The radioactivity was measured in a dose calibrator. In order to study nonspecific binding, the rats were injected subcutaneously with 1 mg octreotide in 1 ml 0.05 *M* acetic acid in 154 m*M* NaCl 45 min before the ^{111}In-pentetreotide (*10*) injection. Specific binding was defined as the difference between tissue uptake of radioactivity in control rats (total binding) and that in animals treated with excess unlabeled peptide (nonspecific binding), expressed as percent of injected radioactivity per gram tissue (*10*). The ratio of percent dose uptake in tissue over soft tissue (thigh) and tissue over blood were calculated for each rat. The rats were killed 24 hr after administration of ^{111}In-pentetreotide. Blood was collected and the octreotide receptor-positive as well as negative tissues were isolated. Tissue and blood radioactivity were determined using an LKB-1282-Compugamma system (*10*).

Experiment A: Effects of Varying the Dose and Specific Activity of ^{111}In on Specific Binding

Experiments were performed with 18 groups of three male Wistar rats (240–260 g). Nine groups of three rats each were injected with 0.02, 0.1 or 0.5 μg pentetreotide with specific activities of 18.5, 55.5 or 185 MBq ^{111}In per μg pentetreotide. Consequently, the radioactive dose varied between 0.37 and 92.5 MBq per rat. Nonspecific binding in tissue was determined in nine parallel groups of three rats each, injected with 1 mg octreotide 45 min before the different doses of ^{111}In-pentetreotide.

Experiment B: Effects of Varying Mass of Pentetreotide at a Constant Radioactive Dose of ^{111}In

Because both the dose and mass had varied at the same time in experiment A, we also investigated the effect of varying mass at a constant radioactive dose. Experiments were performed with five groups of three male Wistar rats (240–260 g) injected with 0.02, 0.1, 0.5, 5 or 50 μg pentetreotide labeled with 3 MBq ^{111}In. Consequently, the specific radioactivity varied between 150 and 0.06 MBq ^{111}In per μg pentetreotide.

Experiment C: Effects of Intravenous Injection of Octreotide or Pentetreotide

Sixteen groups of three male Wistar rats (240–260 g) were each injected with 2 or 10 μg octreotide or [DTPA-D-Phe1]pentetreotide at −10, 0, 10 and 20 min relative to the injection of 0.5 μg pentetreotide labeled with 3 MBq ^{111}In. A group of six rats injected with indium only was used as controls. The percent dose uptake per gram tissues in treated animals is expressed as the percentage of that in the control animals.

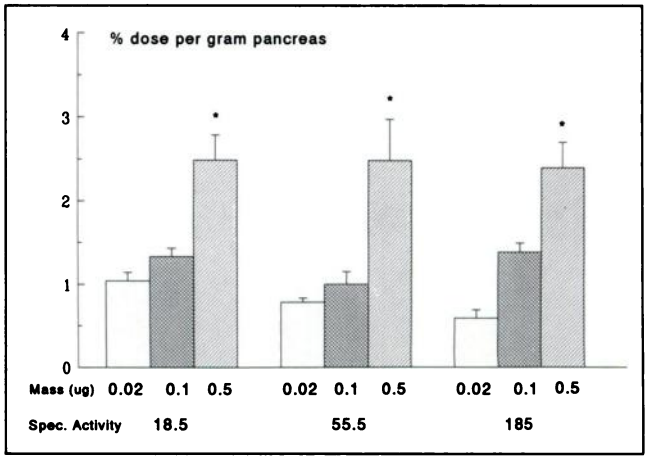

FIGURE 1. Effects of varying mass of pentetreotide (0.02, 0.1, 0.5 μg) and specific activity (18.5, 55.5, 185 MBq per μg) on specific binding of ^{111}In-pentetreotide expressed as %ID of radioactivity per gram tissue 24 hr after injection (n = 3, mean + s.d.). *p < 0.05; significantly different from 0.02 μg.

Statistical Analysis

One-way analysis of variance (ANOVA) was used. Means were compared using Bonferroni's t-test or the Newman-Keuls method (*11*). A p value of <0.05 was considered significant.

RESULTS

Experiment A

Significant specific tissue binding of ^{111}In-pentetreotide was observed in the octreotide receptor-positive anterior pituitary gland, adrenals, and pancreas, but not in the octreotide receptor-negative liver, spleen, kidneys or soft tissue (*1*) (data not shown).

Figure 1 shows the effects of mass and specific activity on the specific binding of ^{111}In-pentetreotide in the pancreas. A significantly higher percent dose uptake was observed at an increasing mass in the range of 0.02, 0.1 and 0.5 μg of pentetreotide independent of the specific radioactivity, varying between 18.5 and 185 MBq ^{111}In per μg pentetreotide. Under these conditions, the pancreas-to-soft tissue ratio showed a similar pattern as depicted in Figure 1 (data not shown). Similar patterns in specific binding, although not statistically significant, were found for the adrenals and the anterior pituitary gland (data not shown).

Experiment B

At a constant radioactive dose of 3 MBq ^{111}In-pentetreotide, there was a biphasic response, i.e., an initial increase followed by a decrease, in percent dose uptake in the octreotide receptor-positive organs when the mass of injected pentetreotide was increased from 0.02 to 50 μg. The optimum was 0.5 μg for the anterior pituitary gland (although not significantly different from the other masses), 5 μg for the pancreas and 0.5 μg for the adrenals (Fig. 2). Remarkably, the percent dose uptake in the adrenals is still strongly increased at 50 μg compared with 0.02 μg peptide in contrast to the pituitary and pancreas. The corresponding tissue-to-soft tissue ratio showed similar profiles (data

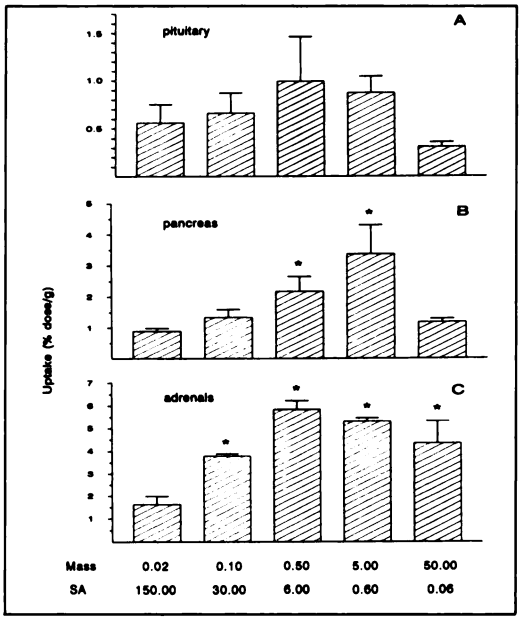

FIGURE 2. Effects of varying mass of pentetreotide labeled with a constant amount (3 MBq) of ^{111}In on 24-hr uptake of activity in anterior pituitary (A), pancreas (B) and adrenals (C) expressed in %ID of radioactivity per gram tissue, (n = 3, mean + s.d.). *p < 0.05; significantly different from 0.02 μg.

not shown). No significant differences were found in the ratio of activity in the liver, spleen, blood or kidney versus soft tissue (data not shown).

Experiment C

As shown in Figure 3A, the administration of 2 or 10 μg octreotide or 2 or 10 μg pentetreotide 10 min before administration of 0.5 μg ^{111}In-pentetreotide labeled resulted in significantly lower percent dose uptake values in the octreotide receptor-positive anterior pituitary gland. Coinjection of 10 μg pentetreotide or 2 or 10 μg octreotide with ^{111}In also significantly lowered the percent dose uptake in the pituitary. Coinjection of 2 μg pentetreotide, however, had no effect. A significant decrease in percent dose uptake was observed with 2 or 10 μg octreotide 10 min after ^{111}In injection, and 20 min after ^{111}In injection with 2 μg octreotide but not with 10 μg octreotide.

There was significantly higher percent dose uptake values in the pancreas after administration of 2 μg [DTPA-D-Phe1]pentetreotide at 0 or 20 min or of 10 μg [DTPA-D-Phe1]pentetreotide at −10, 0 or 10 min relative to the injection of ^{111}In (Fig. 3B). Figure 3B also shows a significantly lower percent dose uptake of radioactivity in the pancreas after the administration of 10 μg octreotide at −10, 0, and 10 min, but not at 20 min postinjection of ^{111}In.

The percent dose uptake in the adrenals was significantly lower after administration of 10 μg pentetreotide at −10, 0 and 20 min relative to the ^{111}In injection (Fig. 3C). After administration of 2 μg pentetreotide 10 and 20 min after the ^{111}In injection, however, there was a significantly higher percent dose uptake in the adrenals. There was also significantly lower adrenal percent dose uptake after administration of 2 or 10 μg octreotide at −10, 0 and 10, but not at 20 min postinjection of ^{111}In.

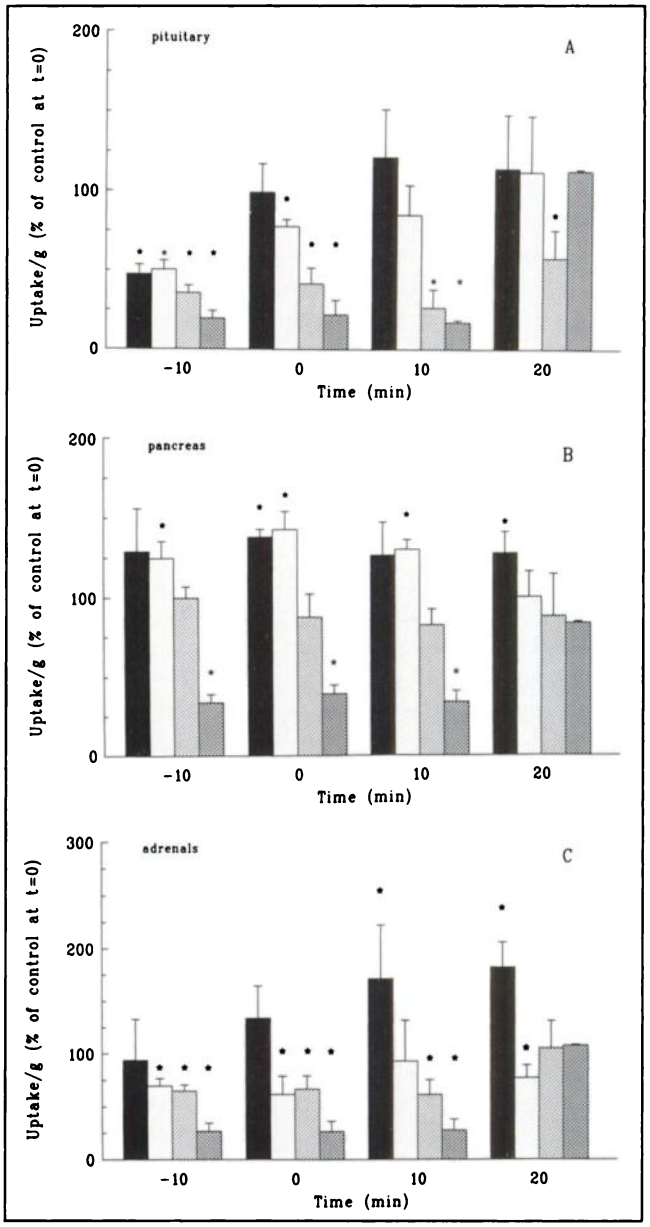

FIGURE 3. Effects of intravenous administration of 2 (black bar) and 10 (open bar) μg pentetreotide and 2 (hatched) and 10 (cross-hatched) μg octreotide at indicated time intervals relative to the injection of 0.5 μg (3MBq) ^{111}In-pentetreotide on 24-hr uptake of activity in anterior pituitary (A), pancreas (B) and adrenals (C). Values are expressed as %ID/g tissue (n = 3) relative to that in control rats (100%, n = 6). The control values (in %ID/g tissue) were 0.90 ± 0.20 for the pituitary, 2.2 ± 0.3 for the pancreas and 5.0 ± 1.1 for the adrenals. *p < 0.05; significantly different from control.

Since the percent dose uptake in all measured octreotide receptor-negative tissues was unaffected by intravenous administration of 2 or 10 μg octreotide or 2 or 10 μg pentetreotide, the calculated ratio of percent dose uptake in octreotide receptor-positive tissue versus blood or versus soft tissue was similar to the results presented in Figure 3 (data not shown).

DISCUSSION

We wanted to determine whether varying the specific activity of ^{111}In-pentetreotide resulted in changes in the specific and nonspecific binding of either octreotide receptor-positive or octreotide receptor-negative tissues, and, consequently, in altered target-to-background ratios. We evaluated these parameters by varying the mass and radioactive dose of ^{111}In-pentetreotide (A). Unexpectedly, we found that the lowest possible mass of pentetreotide for maximum specific radioactivity was not optimal, but rather, that specific binding to octreotide receptor-positive tissue increased at a higher mass of the radiopharmaceutical with an optimum in the low microgram range, depending on the octreotide receptor-positive tissue under study. Since this might also apply to octreotide receptor-positive tumors, an extra parameter has become available to increase the target-to-background ratio, and hence, the sensitivity to detect such tumors. This was further substantiated by the findings that nonspecific binding in the tissues studied did not change with the dose or mass of injected radiopharmaceutical.

Next, we further evaluated the effects of varying mass with a constant radioactive dose of ^{111}In-pentetreotide on the activity uptake in the octreotide receptor-positive and octreotide receptor-negative tissues (B). We found optimal activity uptake in the anterior pituitary gland, the pancreas and the adrenals. It remains to be established, however, which peptide mass would be optimal for uptake of ^{111}In after the administration of ^{111}In-pentetreotide in different octreotide receptor-positive organs and tumors in humans. The reason for these differences in tissue uptake depending on the injected mass of pentetreotide has to do with the availability of the radiopharmaceutical to its receptor as well as the processes following the binding of the radiopharmaceutical to its receptor. Relevant factors for receptor accessibility include the capacity of the radiopharmaceutical to pass biomembranes, competition by endogenous somatostatin and the rate of tissue blood perfusion. The production of somatostatin in the pancreas, for instance, may contribute to the relatively high optimal dose of 5 μg of ^{111}In-pentetreotide for uptake in the pancreas. This is in contrast to the optimal dose of 0.5 μg for the highly perfused adrenal, which does not produce somatostatin. Other factors include the dissociation constant between the radiopharmaceutical and the receptor, the mode of administration that might influence the concentration and exposure time of receptor to the radiopharmaceutical, the rate of internalization of the ligand-receptor complex and the rate of reexpression and/or upregulation of the receptor. All of these parameters illustrate the dynamics and the complexity of the ligand-receptor binding process, particularly in vivo (*12*).

Finally, we evaluated the effects of the intravenous administration of 2 or 10 μg pentetreotide or 2 or 10 μg octreotide at various time intervals relative to the injection of ^{111}In-pentetreotide on tissue uptake of ^{111}In. In vitro findings also suggest that the optimal ratio between specific and nonspecific binding of peptides to the somatostatin receptor-containing cells is not necessarily highest at the lowest ligand concentration. Presky et al. found an increase in the number of somatostatin receptors on GH_4C_1 pituitary cells 24 hr after treatment with somatostatin (*13*). Our experiments were also performed because we recently observed rapid, increased internalization of [^{125}I Tyr3]octreotide in normal and tumor pituitary cells by the simultaneous addition of a nanomolar concentration of unlabeled octreotide [Hofland LJ, *unpublished data*]. Dörr et al. reported improved visualization of carcinoid liver metastases in patients by ^{111}In-pentetreotide scintigraphy during treatment with a subcutaneous dose of 600 μg of octreotide per day (*2,3*). These patient data are in accordance with our animal data.

In all the octreotide receptor-positive organs, we found a significantly lowered percent dose uptake of radioactivity when 10 μg octreotide were administered at −10, 0 and 10 min, but not at 20 min postinjection of the radiopharmaceutical. This may be an indication of the limited exposure time of indium to its receptor as well as the binding rate of the radioligand to its receptor and the subsequent internalization of the peptide-receptor complex. The amount of radioactivity in the octreotide receptor-positive tissues is stable, since no significant differences are found between 4 and 24 hr after radiopharmaceutical injection (*8*). The effects of the administration of octreotide on the inhibition of percent uptake of ^{111}In-pentetreotide in the octreotide receptor-positive tissues were more pronounced than the effects of pentetreotide administration. This may be due to the difference in affinity between the two somatostatin analogs for the receptor, which is ≈5-fold lower for pentetreotide than for octreotide (*9*). Furthermore, possible differences may be due to variances in distribution and metabolism.

In summary, the results of this third experiment indicate that the injection of variable amounts of pentetreotide or octreotide at various time points relative to the injection of ^{111}In may be a means of increasing the target-to-background ratio, depending upon the octreotide receptor-positive tissue being studied. This mechanism may be used to increase the target-to-background ratio in somatostatin receptor imaging in humans. Preliminary findings in humans indicate that a specific activity higher than 220 MBq ^{111}In per 5 μg pentetreotide will lead to decreased scintigraphic quality of significantly reduced tumor uptake (*14*).

CONCLUSION

In contrast to the hypothesis that the percentage uptake of pentetreotide in octreotide receptor-positive tissues is optimal at the lowest possible dose of maximum specific radioactivity, we found that it is a bell-shaped function of the injected mass which is optimal between 0.5–5 μg ^{111}In-pentetreotide. This indicates that the sensitivity of the detection of somatostatin receptor-positive tumors by receptor scintigraphy may be improved by varying the mass of

radiopharmaceutical, which has now been confirmed in patients.

ACKNOWLEDGMENTS

The authors thank Marieke Steeneken, Thijs van Aken, Margreet Vlastuin, Ina Loeve and Vincent-Elvis Versendaal for expert technical assistance.

REFERENCES

1. Bakker WH, Krenning EP, J-C Reubi, et al. In vivo application of [^{111}In-DTPA-D-Phe1]octreotide for detection of somatostatin receptor-positive tumors in rats. *Life Sci* 1991;49:1593–1601.
2. Dörr U, Wurm K, Höring E, et al. Diagnostic reliability of somatostatin receptor scintigraphy during continuous treatment with different somatostatin analogs. *Hormone Metabol Res* 1992;27:36–43.
3. Dörr U, Räth U, Sautter-Bihl M-L, et al. Improved visualization of carcinoid liver metastases by indium-111 pentetreotide scintigraphy following treatment with cold somatostatin analogue. *Eur J Nucl Med* 1993;20:431–433.
4. Bell GI, Riesine T. Molecular biology of somatostatin receptors. *Trends Neurosci* 1993;16:34–38.
5. Yamada Y, Kagimoto S, Kubota A, et al. Cloning, functional expression and pharmacological characterization of a fourth (hSSTR4) and a fifth (hSSTR5) human somatostatin receptor subtype. *Biochem Biophys Res Commun* 1993;195:844–852.
6. Bruno JF, Berelowitz M. Somatostatin receptors: orphan that found family and function. *Mol Cell Neurosci* 1993;4:307–309.
7. Kaupmann K, Bruns C, Hoyer D, et al. Distribution and second messenger coupling of four somatostatin receptor subtypes expressed in brain. *FEBS* 1993;331:53–59.
8. Breeman WAP, Hofland LJ, van der Pluijm M, et al. A new radiolabeled somatostatin analogue [^{111}In-DTPA-D-Phe1]RC-160: preparation, biological activity, receptor scintigraphy in rats and comparison with [^{111}In-DTPA-D-Phe1]octreotide. *Eur J Nucl Med* 1994;21:328–335.
9. Bakker WH, Albert R, Bruns C, et al. [Indium-111-DTPA-D-Phe1]octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: synthesis, radiolabeling and in vitro validation. *Life Sci* 1991;49:1583–1591.
10. Breeman WAP, Hofland LJ, Bakker WH, et al. Radioiodinated somatostatin analogue RC-160: preparation, biological activity, in vivo application in rats and comparison with [^{123}I-Tyr^3]octreotide. *Eur J Nucl Med* 1993;20:1089–1094.
11. Snedecor GW, Cochran WG. *Statistical methods*, ed. 7. Ames, IA: Iowa State University Press; 1980.
12. Fischmann AJ, Babich JW, Strauss HW. A ticket to ride. *J Nucl Med* 1993;34:2253–2264.
13. Presky DH, Schonbrunn A. Somatostatin pretreatment increases the number of somatostatin receptors on GH_4C_1 pituitary cells and does not reduce cellular responsiveness to somatostatin. *J Biol Chem* 1988;263:714–721.
14. Kooij PPM, Kwekkeboom DJ, Breeman WAP, et al. The effects of specific activity on tissue distribution of [^{111}In-DTPA-D-Phe1]octreotide in humans. *J Nucl Med* 1994;35:226P.