Articles

Comparison of Four ^{64}Cu-Labeled Somatostatin Analogues in Vitro and in a Tumor-Bearing Rat Model: Evaluation of New Derivatives for Positron Emission Tomography Imaging and Targeted Radiotherapy[||]

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Previous studies have shown that modification of the somatostatin analogue octreotide (OC), by substitution of tyrosine for phenylalanine at position 3 and of a C-terminal carboxylic acid for an alcohol, to give Tyr^3-octreotate (Y3-TATE) improved uptake of the peptide in somatostatin receptor-positive tissues. To determine which substitution best accounts for increased target tissue uptake, the peptides containing single modifications, Tyr^3-octreotide (Y3-OC) and octreotate (TATE), were synthesized. These peptides were conjugated to the macrocyclic chelating agent 1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (TETA) and radiolabeled with ^{64}Cu(II). The in vitro receptor binding, in vitro tumor cell uptake, and in vivo distribution properties of ^{64}Cu-labeled TETA-Y3-OC and TETA-TATE were compared to those of [^{64}Cu]TETA-OC and [^{64}Cu]TETA-Y3-TATE. Cu-TETA-TATE (IC_{50} = 0.297 ± 0.0055 nM) and Cu-TETA-Y3-TATE (IC_{50} = 0.308 ± 0.0375 nM) displayed significantly higher binding affinity to somatostatin receptors on CA20948 rat pancreatic tumor membranes than Cu-TETA-Y3-OC (IC_{50} = 0.397 ± 0.0206 nM) and Cu-TETA-OC (IC_{50} = 0.498 ± 0.039 nM). Similarly, the uptakes of [^{64}Cu]TETA-Y3-TATE (60.75 ± 1.21%) and [^{64}Cu]TETA-TATE (55.62 ± 0.16%) into AR42J rat pancreatic tumor cells over a 2-h time period were higher than those of [^{64}Cu]-TETA-Y3-OC (47.20 ± 1.20%) and [^{64}Cu]TETA-OC (34.07 ± 2.24%). The in vitro results suggest that the C-terminal carboxylate may contribute more to enhanced receptor binding and tumor cell uptake than the substitution at the 3-position. Biodistributions in CA20948 tumor-bearing rats showed receptor-mediated uptake of the ^{64}Cu-labeled peptides in somatostatin-rich tissues, including the pituitary, adrenals, pancreas, and tumor. The structure−activity relationships of the four ^{64}Cu-labeled peptides did not show consistent trends in all target tissues, but [^{64}-Cu]TETA-Y3-TATE exhibited tumor uptake 1.75−3.5 times higher than the other derivatives at 4 h postinjection. The greater tumor retention of [^{64}Cu]TETA-Y3-TATE justifies the selection of this agent for future PET imaging and targeted radiotherapy studies.

Introduction

The targeting of somatostatin receptors with radiolabeled peptides has led to the development of agents for both diagnostic imaging and radiotherapy of cancer. Octreotide (OC), an 8-amino acid analogue of somatostatin, has been radiolabeled and used to image somatostatin receptor-positive tumors in humans by positron emission tomography (PET) and single photon emission computed tomography (SPECT). For these purposes, somatostatin analogues have been labeled with a number of β^+- and γ-emitting radionuclides, including ^{111}In, ^{123}I, ^{99m}Tc, ^{68}Ga, ^{64}Cu, ^{18}F, and ^{86}Y.[1−8] In the United States and Europe, ^{111}In-DTPA-OC (In-111 Pentetreotide) is approved for routine clinical use in the diagnosis of neuroendocrine cancer. In addition, widespread interest in targeted radiotherapy has led to the labeling of somatostatin analogues with a variety of cytotoxic radionuclides. For example, [^{161}Tb]DTPA-OC,[9] [^{90}Y]DTPA-OC,[10] [^{188}Re]RC-160,[11] [^{90}Y]DOTA-Tyr^3-OC,[12,13] [^{64}Cu]TETA-OC,[14] and [^{64}Cu]TETA-Tyr^3-TATE[15] are being evaluated for radiotherapeutic efficacy in animal models and clinical trials.

Copper-64 ($t_{1/2}$ = 12.7 h, β^+ = 0.655 MeV (19.3%), β^- = 0.573 MeV (39.6%)) is an attractive radionuclide for both PET imaging and radiotherapy. Large quantities of high-specific activity ^{64}Cu can be produced on demand using a biomedical cyclotron.[16] The applications of ^{64}Cu for PET imaging and targeted radiotherapy through attachment to biologically active molecules have been reviewed.[17] The first ^{64}Cu-labeled somatostatin ana-

[||] Abbreviations: DTPA, diethylenetriaminepentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; TETA, 1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; Y3, tyrosine-3; OC, octreotide; TATE, octreotate; MALDI FTMS, matrix-assisted laser desorption−ionization Fourier transform mass spectrometry.

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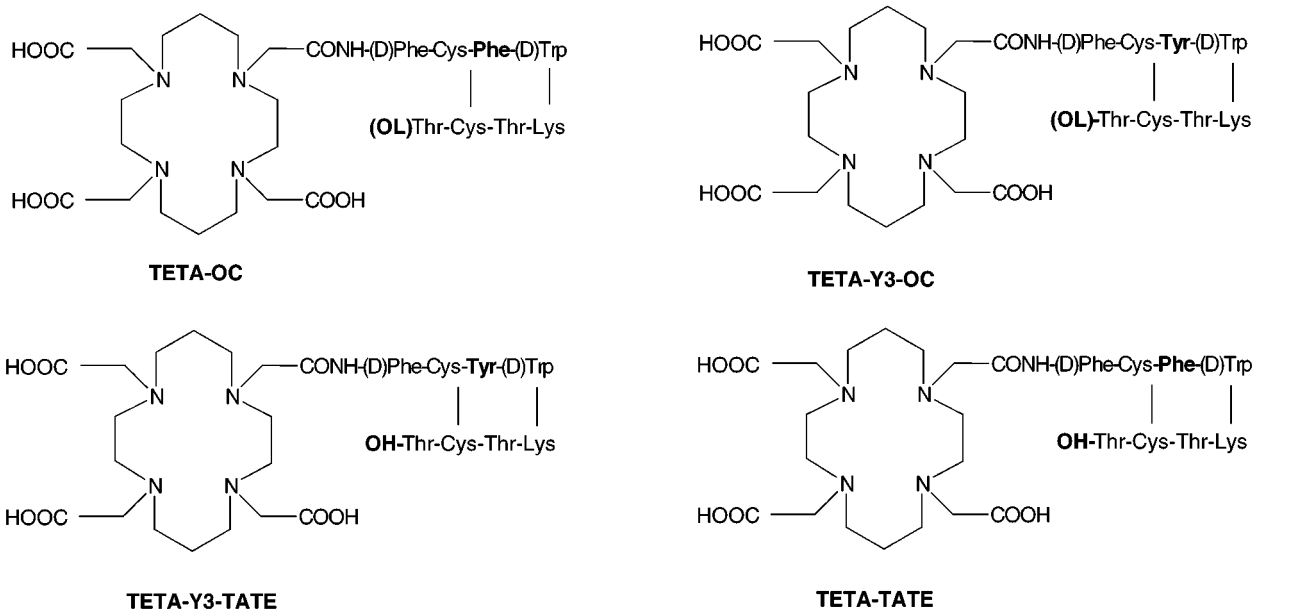

Figure 1. Structures of TETA-OC, TETA-Y3-OC, TETA-Y3-TATE, and TETA-TATE.

logue, [^{64}Cu]TETA-D-Phe^1-octreotide ([^{64}Cu]TETA-OC), displayed high-affinity somatostatin receptor binding in vitro and in vivo.[7] Evaluation of this agent in eight neuroendocrine cancer patients showed that PET imaging with [^{64}Cu]TETA-OC detected more lesions than γ-scintigraphy using [^{111}In]DTPA-OC.[18] Subsequent evaluation of the therapeutic efficacy of [^{64}Cu]TETA-OC has demonstrated growth inhibition of somatostatin receptor-positive tumors in rats at doses exhibiting minimal toxicity.[14]

Studies have shown that subtle modification of the octreotide peptide leads to improved uptake in receptor-rich tissues. The substitutions of a tyrosine (Y) for phenylalanine (F) in the 3-position and of a C-terminal carboxylic acid for an alcohol improved uptake of the peptide in adrenals, pancreas, pituitary, and tumor.[19−21] These findings were confirmed by our own studies, where [^{64}Cu]TETA-D-Phe^1-Tyr^3-octreotate ([^{64}Cu]TETA-Y3-TATE) demonstrated significantly greater uptake than [^{64}Cu]TETA-OC in the somatostatin-rich tissues of two tumor-bearing animal models.[22]

In the current investigation, we examined the effects of single modifications to the octreotide peptide on target tissue uptake, to determine which alteration best accounts for the improvements observed with [^{64}Cu]TETA-Y3-TATE. The substitution of the tyrosine for phenylalanine in the 3-position afforded the peptide Tyr^3-octreotide (Y3-OC), and changing the C-terminus from an alcohol to a carboxylic acid produced the analogue Phe^3-octreotate (TATE). Both of these peptides were subsequently conjugated to TETA (1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) and radiolabeled with ^{64}Cu. We studied receptor-mediated uptake of these two peptides in vitro and in a tumor-bearing animal model and compared the results to those obtained with ^{64}Cu-labeled TETA-OC and TETA-Y3-TATE.

Results

Synthesis and Radiolabeling of Peptides. OC, Y3-OC, TATE, and Y3-TATE (Figure 1) were synthesized by the solid-phase Fmoc method and conjugated with the 1-hydroxybenzotriazole ester of tri-*tert*-butyl TETA on the resin. It should be noted that while these peptides have what is termed TETA conjugated to them, the TETA in use is actually a monoamide derivative of TETA wherein one of the carboxylates has been used to form an amide bond with the peptide. After reversed-phase HPLC, all peptide conjugates were isolated in 90−96% purity. The exact masses of the peptides were confirmed by high-resolution MALDI FTMS, which showed errors of 0.2−6 ppm between observed and calculated values. The ^{64}Cu-labeled peptides were obtained in >98% radiochemical purity, as determined by radio thin-layer chromatography (radio-TLC), in specific activities ranging from 0.5 to 2.5 mCi/μg (18.5−92.5 MBq/μg).

Receptor Binding Assays. The displacement of [^{64}Cu]TETA-OC by the natural copper complexes of TETA-OC, TETA-Y3-TATE, TETA-Y3-OC, and TETA-TATE on rat CA20948 pancreatic tumor cell membranes is shown by the curves presented in Figure 2. All four unlabeled conjugates bound specifically to somatostatin receptors with high affinities. IC_{50} values were 0.308 ± 0.0375 nM for Cu-TETA-Y3-TATE, 0.397 ± 0.0206 nM for Cu-TETA-Y3-OC, and 0.297 ± 0.0055 nM for Cu-TETA-TATE. The value for Cu-TETA-OC, was previously reported to be 0.498 ± 0.039 nM.[14]

AR42J Cell Uptake Studies. The uptakes of ^{64}Cu-labeled TETA-Y3-TATE, TETA-Y3-OC, TETA-OC and TETA-TATE into AR42J rat pancreatic tumor cells during a 2-h incubation at 37 °C are shown in Figure 3. The somatostatin receptor density (B_{max}) on AR42J cells was previously determined by our group to be 148.8 fmol/mg of protein.[23] Thus, under the conditions employed, cell uptake was measured at a 10-fold molar excess of somatostatin receptor to peptide. At 15 min, accumulation of [^{64}Cu]TETA-OC in AR42J cells was 10.23 ± 2.38% of the total activity administered, with uptake increasing to 34.07 ± 2.24% at 2 h. Uptakes of ^{64}Cu-labeled TETA-Y3-OC and TETA-TATE were similar at 15 min (22.77 ± 2.38% and 20.36 ± 1.89%,

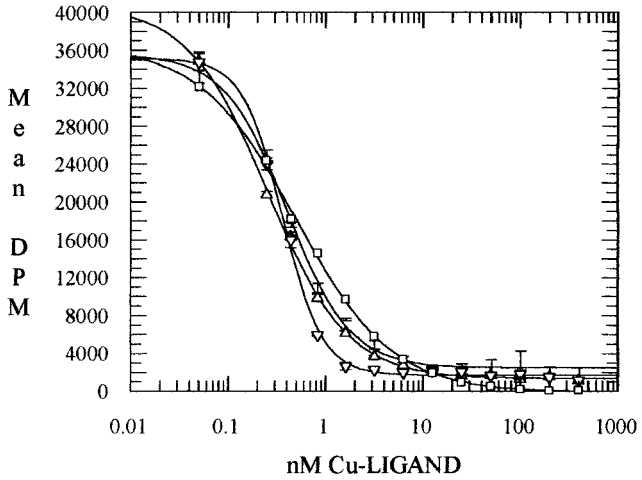

Figure 2. Displacement curves of [^{64}Cu]TETA-OC from CA20948 rat pancreatic tumor cell membranes. Results represent the mean of quadruplicate measurements using ^{nat}Cu-TETA-OC (□), ^{nat}Cu-TETA-Y3-TATE (△), ^{nat}Cu-TETA-Y3-OC (—), or ^{nat}Cu-TETA-TATE (▽).

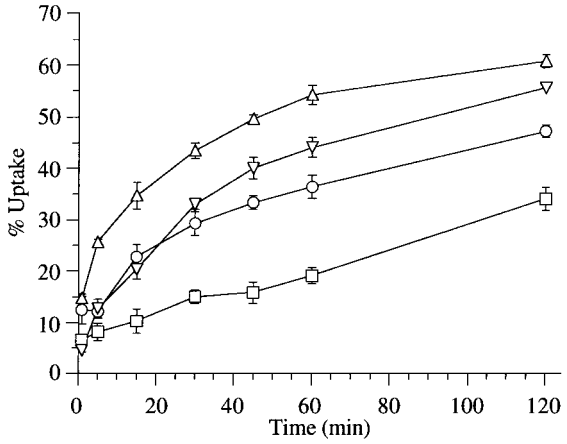

Figure 3. Percentage uptake of [^{64}Cu]TETA-OC (□), [^{64}Cu]-TETA-Y3-TATE (△), [^{64}Cu]TETA-Y3-OC (○), and [^{64}Cu]TETA-TATE (▽) into AR42J cells over time.

respectively) but differed significantly at 2 h (47.20 ± 1.20% and 55.62 ± 0.16%, respectively). Both [^{64}Cu]-TETA-Y3-OC and [^{64}Cu]TETA-TATE exhibited significantly greater accumulation in AR42J cells than [^{64}Cu]-TETA-OC at all time points. Uptake of [^{64}Cu]TETA-Y3-TATE was 34.68 ± 2.53% after 15 min and continued to increase to 60.75 ± 1.21% at 2 h. Over the 2-h experimental period, [^{64}Cu]TETA-Y3-TATE showed the greatest accumulation of the four analogues in AR42J cells. Compared to the other derivatives, the increased uptake of [^{64}Cu]TETA-Y3-TATE was statistically significant at all time points, with the exception of [^{64}Cu]-TETA-TATE at 2 h.

Animal Biodistribution Studies. The uptakes of [^{64}Cu]TETA-Y3-OC and [^{64}Cu]TETA-TATE in pancreas, adrenals, liver, and tumor are shown in Figure 4. For comparison, previously published biodistribution data for [^{64}Cu]TETA-Y3-TATE[22] and [^{64}Cu]TETA-OC[7] are also presented in Figure 4. The results represent biodistributions performed with a similar mass of each radiolabeled peptide (5−8 ng). The results of blocking experiments, using either Y3-TATE or OC to compete with the receptor-mediated uptake of [^{64}Cu]TETA-Y3-TATE, are shown in Figure 5.

Both [^{64}Cu]TETA-Y3-OC and [^{64}Cu]TETA-TATE displayed rapid blood clearance after 1 h. The nontarget organs, e.g., kidney, brain, and liver, showed similar uptake for all four peptide conjugates, with no significant differences. The receptor-rich tissues (adrenals, pancreas, pituitary, and tumor) did not show any significant difference in uptakes between [^{64}Cu]TETA-Y3-OC and [^{64}Cu]TETA-TATE (adrenals, 8.01 ± 1.61% ID/g vs 5.93 ± 1.20% ID/g; pancreas, 4.45 ± 0.96% ID/g vs 5.13 ± 0.92% ID/g; pituitary, 3.41 ± 0.76% ID/g vs 3.69 ± 0.80% ID/g; tumor, 2.17 ± 0.66% ID/g vs 1.76 ± 1.15% ID/g, respectively).

[^{64}Cu]TETA-Y3-TATE had higher uptake in all receptor-rich tissues (except adrenals) than did the other analogues at 1 h (adrenals, 9.07 ± 1.24% ID/g; pancreas, 9.35 ± 1.66% ID/g; pituitary, 6.47 ± 1.77% ID/g; tumor, 2.37 ± 0.44% ID/g) ($p < 0.001$). The trend of adrenal uptakes revealed that ^{64}Cu-labeled TETA-Y3-OC and TETA-Y3-TATE had higher accumulation at 1 and 4 h postinjection than the corresponding Phe^3 analogues. With the exception of the tumor, [^{64}Cu]TETA-Y3-TATE, [^{64}Cu]TETA-Y3-OC, and [^{64}Cu]TETA-TATE all demostrated at least 2-fold higher uptake than [^{64}Cu]TETA-OC in receptor-positive organs. At 1 h, tumor uptakes of [^{64}Cu]TETA-Y3-OC and [^{64}Cu]TETA-TATE were similar to the values obtained with [^{64}Cu]TETA-Y3-TATE and [^{64}Cu]TETA-OC. However, at 4 h, the tumor uptake of [^{64}Cu]TETA-Y3-TATE (2.22 ± 0.26% ID/g) was significantly higher than that of [^{64}Cu]TETA-Y3-OC (1.28 ± 0.25% ID/g) and [^{64}Cu]TETA-TATE (0.63 ± 0.52% ID/g), as well as the tumor uptake of [^{64}Cu]TETA-OC at 3 h (0.63 ± 0.05% ID/g).

In the ligand competition experiments, more than 90% of the uptake of [^{64}Cu]TETA-Y3-TATE in somatostatin-rich tissues was blocked with a co-injection of either unlabeled Y3-TATE or unlabeled OC. At 1 h, co-injection of Y3-TATE decreased the pancreatic uptake of [^{64}Cu]TETA-Y3-TATE significantly more than co-injection of OC (0.15 ± 0.02% ID/g vs 0.76 ± 0.13% ID/g, respectively) ($p < 0.005$). The same trend is seen in the adrenals (0.17 ± 0.02% ID/g for Y3-TATE and 0.26 ± 0.09% ID/g for OC) and the tumor (0.22 ± 0.02% ID/g for Y3-TATE and 0.64 ± 0.10% ID/g for OC) at 1 h postinjection. Interestingly, the bone also shows receptor-mediated uptake of [^{64}Cu]TETA-Y3-TATE. Using Y3-TATE as the blocking agent, bone uptake was decreased from 0.61 ± 0.08% ID/g to 0.09 ± 0.02% ID/g at 1 h; a blocking dose of OC decreased the bone uptake to 0.13 ± 0.02% ID/g at the same time point. Co-injection with blocking peptides did not have a significant effect on uptake in nontarget organs.

Discussion

[^{64}Cu]TETA-OC is currently being investigated for clinical PET imaging of neuroendocrine cancer.[18] Preliminary results with this compound are encouraging in that more tumors have been visualized with this agent than with [^{111}In]DTPA-OC. [^{64}Cu]TETA-OC has also been evaluated for targeted radiotherapy in a tumor-bearing rat model.[14] However, it suffers from the disadvantages of less than optimal blood clearance and rapid tumor clearance. On the basis of previous results obtained with ^{111}In-labeled octreotide analogues,[20,21] we have evaluated [^{64}Cu]TETA-Y3-TATE in vitro and in

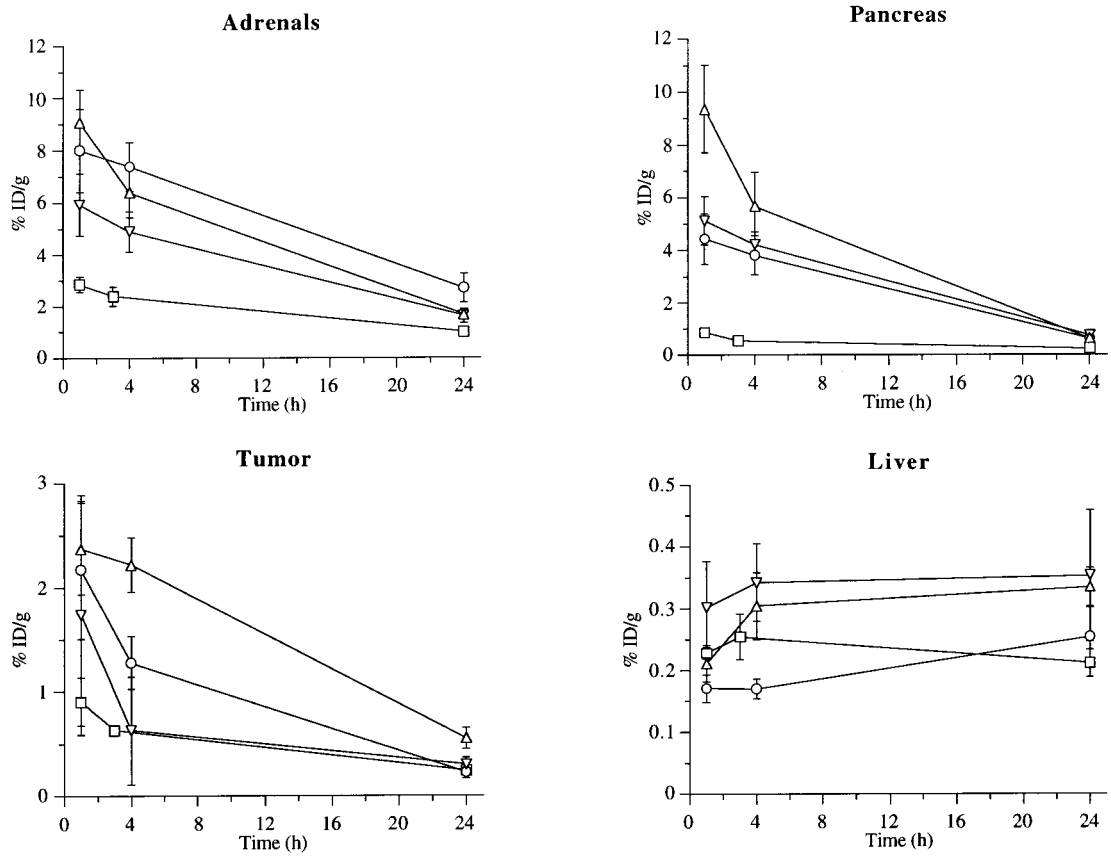

Figure 4. Uptake in selected organs of [^{64}Cu]TETA-OC (□), [^{64}Cu]TETA-Y3-TATE (△), [^{64}Cu]TETA-Y3-OC (○), and [^{64}Cu]TETA-TATE (▽) in Lewis rats bearing CA20948 rat pancreatic tumors. Standard deviations (SD) are indicated; all data were corrected for radiodecay. Note differences in *y*-axis scales.

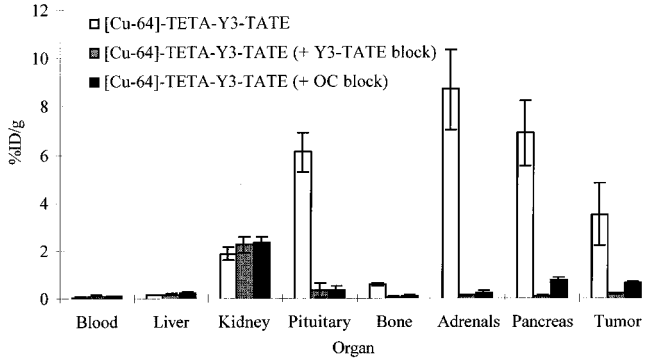

Figure 5. Biodistributions at 1 h of [^{64}Cu]TETA-Y3-TATE, [^{64}Cu]TETA-Y3-TATE co-injected with 150 μg of Y3-TATE, and [^{64}Cu]TETA-Y3-TATE co-injected with 150 μg of OC in Lewis rats bearing CA20948 rat pancreatic tumors. Standard deviations (SD) are indicated; all data were corrected for radiodecay.

two animal models as a potential agent for PET imaging[22] and targeted radiotherapy.[15] [^{64}Cu]TETA-Y3-TATE demonstrated rapid blood clearance in CA20948-bearing Lewis rats, with tumor uptake twice that of [^{64}Cu]TETA-OC. Moreover, tumor:blood ratios were over 4-fold higher at 1 h for [^{64}Cu]TETA-Y3-TATE.

[^{64}Cu]TETA-Y3-TATE differs from the parent compound, [^{64}Cu]TETA-OC, by the substitutions of tyrosine for phenylalanine in the 3-position and a C-terminal carboxylic acid for an alcohol. The current study was undertaken to determine how these modifications contribute to the increase in uptake of [^{64}Cu]TETA-Y3-TATE in receptor-rich tissues. Two peptides, TETA-TATE and TETA-Y3-OC, were synthesized, radiolabeled with ^{64}Cu, and evaluated in Lewis rats bearing CA20948 pancreatic tumors. Compared to the parent peptide OC, TETA-Y3-OC contains the substitution of tyrosine in the 3-position, while TETA-TATE incorporates the change in C-terminus from an alcohol to an acid.

In vitro receptor binding studies showed that all peptides evaluated bound specifically to somatostatin receptors on CA20948 membranes with high relative affinities. The parent compound, Cu-TETA-OC, had the lowest affintity for the receptor, while Cu-TETA-Y3-TATE and Cu-TETA-TATE had the highest affinities. Cu-TETA-Y3-OC exhibited a lower affinity for the receptor than the TATE derivatives, but its IC_{50} value was still significantly lower than that of Cu-TETA-OC. These results suggest that the C-terminal modification may contribute more to high-affinity receptor binding than the substitution at position 3.

The AR42J rat pancreatic carcinoma cell line is also known to express somatostatin receptors both in vitro and in vivo.[24,25] To evaluate and compare the cellular uptake of the radiolabeled peptides in vitro, the AR42J cell line was utilized. Under the conditions employed, the mass of each peptide added was identical, and the somatostatin receptor concentration was 10-fold greater than the peptide concentration. Therefore the results obtained are a direct comparison of the accumulation rates of the analogues and likely represent a combination of membrane binding, internalization, and cellular retention of the compounds. The data revealed that [^{64}Cu]TETA-Y3-TATE had the highest uptake in AR42J cells, followed by [^{64}Cu]TETA-TATE, [^{64}Cu]TETA-Y3-

OC, and [^{64}Cu]TETA-OC in descending order. As in the case of the receptor binding studies, these results showed that the C-terminal carboxyl modification makes a greater contribution to increased cell uptake than the substitution at position 3.

The results of the cell uptake studies are in agreement with the findings of de Jong et al.,[20,26] who reported that the amounts of ^{111}In-labeled somatostatin analogues internalized into CA20948 and AR42J cells followed the trend DTPA-Y3-TATE > DTPA-Y3-OC > DOTA-Y3-OC > DTPA-OC. While those studies distinguished between internalized and membrane-bound ligand, they did not include DTPA- or DOTA-TATE derivatives, so the individual contributions of the C-terminal and 3-position modifications could not be assessed. The studies by de Jong et al. were conducted at peptide concentrations as low as 100 pM. The results described here were obtained at peptide concentrations of exactly 30 pM to give a receptor:ligand molar ratio of 10:1. Receptor excess is desirable for comparison of cellular uptakes because it mimics the physiological conditions of tumor targeting in vivo.

The rat biodistribution studies clearly demonstrated that the uptakes of [^{64}Cu]TETA-Y3-TATE, [^{64}Cu]TETA-Y3-OC, and [^{64}Cu]TETA-TATE in receptor-positive normal tissues are significantly higher at 1 and 4 h than that of [^{64}Cu]TETA-OC. The tyrosine-substituted analogues, [^{64}Cu]TETA-Y3-TATE and [^{64}Cu]TETA-Y3-OC, showed higher uptake in the adrenals than the corresponding phenylalanine-substituted derivatives. This finding suggests that the presence of the tyrosine residue may be responsible for increased adrenal uptake, possibly a result of the increased hydrophilicities of these peptides. In the pancreas and pituitary, [^{64}Cu]-TETA-Y3-TATE showed the highest uptakes at 1 h, while [^{64}Cu]TETA-TATE and [^{64}Cu]TETA-Y3-OC had similar intermediate uptakes, and [^{64}Cu]TETA-OC exhibited much lower uptakes than the other three analogues. In these target tissues, the combination of C-terminal and residue 3 modifications may have a synergistic effect on uptake. These observations are consistent with the findings of de Jong et al.,[20,21] who showed increased target tissue uptake with ^{111}In-labeled DTPA-Y3-OC and DTPA-Y3-TATE derivatives.

The in vivo ligand competition experiments demonstrated that uptake of [^{64}Cu]TETA-Y3-TATE is receptor-mediated in all target tissues. Moreover, Y3-TATE was generally more effective as a blocking agent than OC, a finding which may be attributable to its higher affinity for somatostatin receptors or differences in internalization rates or uptake kinetics. The same ligand competition effect was also observed in bone, suggesting that bone uptake of [^{64}Cu]TETA-Y3-TATE was also receptor-mediated.

Tumor uptakes of the four ^{64}Cu-labeled octreotide analogues at 1 h were more similar than the uptakes in other target tissues. At this time point, [^{64}Cu]TETA-OC had the lowest tumor uptake. While [^{64}Cu]TETA-Y3-TATE had the highest accumulation in tumor at 1 h, this value was not significantly different than those obtained with ^{64}Cu-labeled TETA-TATE and TETA-Y3-OC. However, at 4 h postinjection, tumor uptake of [^{64}Cu]TETA-Y3-TATE was 1.75−3.5 times higher than those of the other analogues. The longer residence time of [^{64}Cu]TETA-Y3-TATE in the tumor may increase its efficacy for targeted radiotherapy and justify future therapy studies using this agent.

It is evident from these investigations that modification of the 3-position amino acid and alteration of the C-terminus both contribute to increased target tissue uptake of ^{64}Cu-labeled octreotide analogues. While the structure−activity relationships of these four analogues do not show consistent uptake trends in all target tissues that identify the superior compound, the greater accumulation and retention of [^{64}Cu]TETA-Y3-TATE in tumor provide a rationale to select this agent for future targeted radiotherapy studies. We are continuing to evaluate the therapeutic efficacy of [^{64}Cu]TETA-Y3-TATE in the CA20948 rat model in preparation for clinical trials.

Experimental Section

Materials. ^{64}Cu was produced on a biomedical cyclotron at Washington University School of Medicine by previously reported methods.[16] All chemicals, unless otherwise stated, were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). All solutions were prepared using ultrapure water (18 MΩ-cm resistivity). Thin-layer chromatography was performed using Whatman MKC$_{18}$F reversed-phase TLC plates with 10% ammonium acetate:methanol (30:70) as the mobile phase. Radio-TLC detection was accomplished using a BIOSCAN System 200 imaging scanner (Washington, DC). Radioactive samples were counted on a Beckman 8000 γ counter (Irvine, CA). Adult male Lewis rats (230−290 g) were purchased from Harlan Sprague−Dawley, Inc. (Indianapolis, IN). The rat pancreatic tumor CA20948[27] was obtained from the Tumor Bank at Biomeasure, Inc. (Hopkinton, MA) and was maintained by serial passage in animals.

Peptide Synthesis. Solid-phase peptide synthesis (SPPS) was performed on an Applied Biosystems model 432A "synergy" peptide synthesizer employing the Fmoc (9-fluorenylmethoxycarbonyl) method. Instrument protocol required 25 μmol of subsequent Fmoc-protected amino acids activated by a combination of 1-hydroxybenzotriazole (HOBt) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). The Fmoc-protected amino acids were purchased commercially unless otherwise stated; the prepacked amino acids were obtained from Perkin-Elmer (Norwalk, CT), while those unavailable in prepacked form, such as the D-amino acids and Fmoc-Cys(Acm), were supplied by BACHEM Bioscience, Inc. (King of Prussia, PA) or Novabiochem (San Diego, CA). Tri-*tert*-butyl TETA was synthesized internally by a modification of the published procedure.[28] Exact mass measurements on the peptide conjugates were performed by Mass Consortium (San Diego, CA), using an IonSpec Fourier transform ion cyclotron mass spectrometer with a 4.7-T superconducting magnet. Samples in 2,5-dihydroxybenzoic acid matrix were irradiated with a nitrogen laser (LaserScience, Inc.) operated at 337 nm.

The synthesis of TETA-Y3-TATE, TETA-OC, TETA-Y3-OC, and TETA-TATE was accomplished by previously reported methods.[22] The peptide conjugates were purified by reversed-phase HPLC, using a Vydac Protein & Peptide C$_{18}$ column (2.2 × 25 cm) and a linear gradient from 10% to 70% solvent B (solvent A, 0.1% TFA; solvent B, 0.1% TFA/90% CH_3CN) over 40 min at a flow rate of 10 mL/min. Detection was accomplished at 230 nm. Pure fractions were identified by analytical HPLC using two diverse systems: system A, HPLC on a Vydac diphenyl (219TP54) column (0.46 × 25 cm) and a linear gradient from 2% to 98% solvent B (solvent A, 0.1% TFA; solvent B, 0.1% TFA/CH_3CN) over 100 min at a flow rate of 1 mL/min, with detection at 214 and 280 nm; system B, reversed-phase HPLC on a Vydac Protein & Peptide C$_{18}$ column (0.46 × 25 cm), with detection at 214 nm. For TETA-Y3-TATE, TETA-Y3-OC, and TETA-TATE, analytical reversed-phase HPLC was performed using a solvent gradient starting with

0% solvent B for 2 min, followed by a linear gradient from 0% to 70% solvent B (solvent A, 0.1% TFA/5% CH_3CN; solvent B, 0.1% TFA/90% CH_3CN) over 15 min at a flow rate of 0.5 mL/min. For TETA-OC, analytical reversed-phase HPLC was carried out using a linear gradient from 5% to 70% solvent B (solvent A, 0.1% TFA; solvent B, 0.1% TFA/90% CH_3CN) over 15 min at a flow rate of 2 mL/min. The peptides were also analyzed by high-resolution MALDI FTMS. TETA-Y3-TATE: HPLC retention times = 33.0 min (system A), 11.2 min (system B); MALDI FTMS *m/z* calcd for $C_{67}H_{95}N_{14}O_{19}S_2$ $(M + H)^+$ = 1463.6339, found 1463.6343. TETA-OC: HPLC retention times = 35.9 min (system A), 10.8 min (system B); MALDI FTMS *m/z* calcd for $C_{67}H_{97}N_{14}O_{17}S_2$ $(M + H)^+$ = 1433.6598, found 1433.6609. TETA-Y3-OC: HPLC retention times = 32.5 min (system A), 11.1 min (system B); MALDI FTMS *m/z* calcd for $C_{67}H_{97}N_{14}O_{18}S_2$ $(M + H)^+$ = 1449.6547, found 1449.6646. TETA-TATE: HPLC retention times = 36.3 min (system A), 12.3 min (system B); MALDI FTMS *m/z* calcd for $C_{67}H_{95}N_{14}O_{18}S_2$ $(M + H)^+$ = 1447.6390, found 1447.6404.

Radiolabeling of Peptide Conjugates. The conjugated peptides were labeled with ^{64}Cu(II) according to previously reported methods for the preparation of [^{64}Cu]TETA-OC[7] and [^{64}Cu]TETA-Y3-TATE.[22] Briefly, 1−5 mCi (37−185 MBq) of ^{64}Cu in 0.1 M ammonium acetate, pH 5.5, was added to 1−10 μg of the peptide conjugate in 0.1 M ammonium acetate, pH 5.5. Gentisic acid (1 mg/mL) was added to the labeling mixture to counteract the effects of radiolysis. The solution was incubated for 1 h at room temperature. The radiolabeled peptide was purified on a C-18 SepPak cartridge, using 100% ethanol as the elution solvent, and radiochemical purity was determined by radio-TLC.

Receptor Binding Assays. The receptor binding assays were performed using [^{64}Cu]TETA-OC on membranes obtained from CA20948 tumors harvested from euthanized rats. The competing ligands, ^{nat}Cu-TETA-OC, ^{nat}Cu-TETA-Y3-TATE, ^{nat}Cu-TETA-Y3-OC, and ^{nat}Cu-TETA-TATE, were prepared by the reaction of high-purity natural copper acetate, using the same procedure described above for preparation of the ^{64}Cu-labeled peptides. Purity of the final products were confirmed by HPLC, using the same method described for purification of the TETA conjugates. IC_{50} values were determined according to previously published methods,[14] using the Millipore MultiScreen assay system (Bedford, MA). Data analysis was performed using the programs GraFit (Erithacus Software, U.K.), LIGAND (NIH, Bethesda, MD), and GraphPad PRISM (San Diego, CA). Each data point represents the mean of four experimental values.

Cell Uptake Studies. The apparatus and procedures for the cell uptake experiments are based on previously described methods.[29,30] Briefly, the AR42J cell line was maintained by serial passage in monolayers in Dulbecco's modified Eagle's media (DMEM), supplemented with 10% fetal bovine serum, in a humidified 5% CO_2 atmosphere at 37 °C. Viability of the cells and cell numbers were measured by trypan blue exclusion procedures using a hemacytometer. The cell viability before and after the experiments was determined to be >95% in all cases. Cells were harvested from monolayers with cell dissociation solution (Sigma Chemical Co., St. Louis, MO) and resuspended in fresh DMEM media at a concentration of 2×10^6 cells/mL. An aliqout of 0.3 pmol of the radiolabeled peptide (1.11 μCi of [^{64}Cu]TETA-OC, 2.13 μCi of [^{64}Cu]TETA-Y3-TATE, 1.94 μCi of [^{64}Cu]TETA-Y3-OC, or 1.93 μCi of [^{64}Cu]TETA-TATE) was added to 10 mL of cells, which were incubated at 37 °C with continuous agitation. At 1, 5, 15, 30, 45, 60, and 120 min triplicate 200-μL aliquots were removed and placed in ice. The cells were immediately isolated by centrifugation, and the percent uptake of the compound into the cells was calculated as described.[30]

Animal Biodistribution Studies. Using a 21G Trocar, the somatostatin receptor-positive rat pancreatic tumor CA20948 (1-mm^3 piece) was implanted subcutaneously into the nape of the neck of male Lewis rats (230−290 g). The tumors were allowed to grow for 10 days, until approximately 4 g in size. The ^{64}Cu-labeled peptide conjugate (5.4 μCi, 5 ng) was injected intravenously via the tail vein into CA20948 tumor-bearing Lewis rats. Animals were euthanized at 1, 4, and 24 h postinjection. The tumor, blood, lung, liver, spleen, kidney, muscle, fat, heart, brain, pituitary, bone, adrenals, pancreas, stomach, small intestine, upper large intestine, and lower large intestine were removed, drained of blood, weighed, and counted in a γ counter. By comparison with a standard representing the injected dose per animal, the samples were corrected for radioactive decay, to calculate percent injected dose per gram (% ID/g) of tissue and percent injected dose per organ (% ID/organ).

Ligand Competition Experiments. [^{64}Cu]TETA-Y3-TATE (5.4 μCi, 5 ng) was injected intravenously via the tail vein into CA20948-bearing Lewis rats. Two additional groups of animals were co-injected with [^{64}Cu]TETA-Y3-TATE (5.4 μCi, 5 ng) and either 150 μg of unlabeled Y3-TATE or 150 μg of unlabeled OC. All three groups of animals were sacrificed at 1 h postinjection, after which biodistributions were obtained as described above.

Statistical Methods. To compare differences between the ^{64}Cu-labeled peptides, a Student's *t*-test was performed. Differences at the 95% confidence level ($p < 0.05$) were considered significant.

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Supporting Information Available: Tables of mean percent injected dose per gram (% ID/g) of [^{64}Cu]TETA-Y3-OC, [^{64}Cu]TETA-TATE, and [^{64}Cu]TETA-Y3-TATE with two blocking agents and percent injected dose per organ (% ID/organ) with standard deviations for 13 tissues and 3 time points evaluated and also synthesis of mono-*N*-(carboxymethyl)-tris-*N*,*N*,*N*-(*tert*-butyloxycarbonylmethyl)cyclam (tri-*tert*-butyl TETA) from cyclam. This information is available free of charge via the Internet at http://pubs.acs.org.

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