
Crucial Role for Somatostatin Receptor Subtype 2 in Determining the Uptake of [¹¹¹In-DTPA-D-Phe¹]Octreotide in Somatostatin Receptor–Positive Organs

Leo J. Hofland, PhD¹; Steven W.J. Lamberts, PhD, MD¹; P. Martin van Hagen, PhD, MD¹; Jean-Claude Reubi, PhD, MD²; James Schaeffer, PhD³; Marlijn Waaijers¹; Peter M. van Koetsveld¹; Ananth Srinivasan, PhD⁴; Eric P. Krenning, PhD, MD⁵; and Wout A.P. Breeman, PhD⁵

¹Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; ²Division of Cell Biology and Experimental Cancer Research, Institute of Pathology, University of Bern, Bern, Switzerland; ³Merck Laboratories, New York, New York; ⁴Mallinckrodt Discovery, St. Louis, Missouri; and ⁵Department of Nuclear Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

Human somatostatin (SS) receptor (sst)–positive tumors can be visualized by gamma camera scintigraphy after the injection of [¹¹¹In-diethylenetriaminepentaacetic acid (DTPA)-D-Phe¹] octreotide. Uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide is dependent on sst-mediated internalization of the radioligand by the tumor cells. Human sst-positive tumors frequently express multiple sst subtypes. In vitro studies have demonstrated that the 5 sst subtypes (sst_{1–5}) differentially internalize sst-bound ligand. The present study was performed to evaluate the role of sst₂ in vivo in determining the uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide, as well as of the more “universal” ligand [¹¹¹In-DTPA]SS-14, by sst-positive organs expressing multiple sst subtypes. **Methods:** Wild-type and sst₂ knockout mice (*n* = 4 per treatment group) were injected intravenously with 1 MBq (0.1 μg) [¹¹¹In-DTPA-D-Phe¹]octreotide or [¹¹¹In-DTPA]SS-14. After 24 h, the animals were sacrificed and radioactivity in the organs under investigation was determined. In addition, the sst subtype messenger RNA (mRNA) expression pattern in these organs was determined by reverse transcriptase polymerase chain reaction (RT-PCR) analysis. **Results:** RT-PCR analysis demonstrated the presence of all 5 sst subtype mRNAs in the adrenals and pituitary of wild-type mice but no sst₂ in the knockout mice. The thymus expressed mRNA for sst₂ and sst₄ mRNA in wild-type mice, whereas no sst₂ was detected in knockout mice. In wild-type mice, the in vivo uptake values (in percentage injected dose per gram of tissue) of [¹¹¹In-DTPA-D-Phe¹]octreotide for the pituitary, adrenals, pancreas, and thymus amounted to 1.2 ± 0.2, 0.26 ± 0.03, 0.18 ± 0.03, and 0.30 ± 0.05, respectively, in wild-type mice. Compared with wild-type mice, sst₂ knockout mice had dramatically lower uptake values in these organs—lower by 97%, 83%, 96%, and 94%, respectively (*P* < 0.01 vs. wild type). Comparable differences in the uptake of radioactivity between wild-type and knockout mice were found

using [¹¹¹In-DTPA]SS-14 as the radiotracer. Interestingly, in some organs expressing sst₂ mRNA (liver, muscle, and peripheral blood mononuclear cells), no specific binding of [¹¹¹In-DTPA-D-Phe¹]octreotide or [¹¹¹In-DTPA]SS-14 to sst in vivo was found, suggesting that the sst₂ protein expression level was very low in these tissues. **Conclusion:** The uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide and [¹¹¹In-DTPA]SS-14 in sst-positive organs is determined predominantly by sst₂.

Key Words: [¹¹¹In-DTPA-D-Phe¹]octreotide; uptake; sst₂; knockout; mouse

J Nucl Med 2003; 44:1315–1321

Human somatostatin (SS) receptors (sst) are expressed in a variety of organ systems, including the brain, anterior pituitary gland, pancreas, thyroid gland, and gastrointestinal tract (1). Tumors arising from sst-positive cells in these organs contain a high density of ssts (2–5). This high density of ssts on human tumors forms the basis for the successful and wide use of radiolabeled SS-analogs in the detection of human sst-positive tumors and their metastases by sst scintigraphy (4,6). Moreover, preliminary phase I clinical trials using the β-radiation–emitting SS-analogs, such as [⁹⁰Y-dodecanetetraacetic acid (DOTA),Tyr³]octreotide and [⁹⁰Y-DOTA]lanreotide for sst-targeted radiotherapy of neuroendocrine tumors have been performed with promising results (7–12). The long residence of tumor-associated radioactivity after the administration of radiolabeled SS-analogs suggests that the radioligand is internalized after binding to its sst. In vitro studies have indeed confirmed sst-mediated internalization of radiolabeled SS-analogs by sst-expressing cell lines and primary cultures of human sst-positive tumors (13–17). Internalization of [¹¹¹In-diethylenetriaminepentaacetic acid (DTPA)-D-Phe¹]octreotide in vivo is also evidenced by our observations in rats, in

Received Feb. 11, 2002; revision accepted Mar. 28, 2003.
For correspondence or reprints contact: Leo J. Hofland, PhD, Department of Internal Medicine (Room Bd240), Erasmus Medical Center, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.
E-mail: L.hofland@erasmusmc.nl

which uptake of radioactivity in sst-positive organs, such as the pituitary gland and the pancreas, after the injection of the radiopharmaceutical can be prevented by injection of an excess of unlabeled octreotide up to 10 min after injection but not 20 min after injection. At that time, all radioactivity present in the sst-positive tissues probably reflects internalized radioligand (18). This process of sst-mediated internalization of radioisotopes coupled to SS-analogs will bring the radioisotope closer to its target, that is, the nucleus of the tumor cell. The currently used radiotherapeutics [⁹⁰Y-DOTA,Tyr³]octreotide, [⁹⁰Y-DOTA]lanreotide, and [¹⁷⁷Lu-DOTA,Tyr³]octreotate (19) bind with high affinity to sst subtype 2 (sst₂), with moderate affinity to sst subtype 5 (sst₅), and with low affinity to sst subtype 3 (sst₃). The binding affinity to sst subtypes 1 (sst₁) and 4 (sst₄) is very low (20). The majority of human sst-positive tumors frequently express multiple sst subtypes (3,5). The expression of multiple sst subtypes by human tumors makes it important to evaluate which sst subtypes are responsible for the uptake of radioactivity after the injection of radiolabeled SS-analogs. On the basis of the high binding affinity of [⁹⁰Y-DOTA,Tyr³]octreotide and [⁹⁰Y-DOTA]lanreotide to sst₂ (20), as well as on the basis of a correlation between the relative uptake values of [¹¹¹In-DTPA-D-Phe¹]octreotide and sst₂ messenger RNA (mRNA) expression (21,22), it can be suggested that this sst subtype plays an important role. Nevertheless, the involvement of sst₅ and sst₃ cannot be fully excluded. In fact, *in vitro* studies have demonstrated that both sst₅ and sst₃ internalize radiolabeled SS more efficiently than does sst₂ (23). In order to determine whether sst₅ and sst₃ play a role in sst-mediated uptake of radiolabeled octreotide as well, we performed tissue distribution studies of uptake of radioactivity in sst-positive tissues after the injection of [¹¹¹In-DTPA-D-Phe¹]octreotide in wild-type and sst₂ knockout mice. Uptake values were compared with the sst subtype expression pattern in these organs. For comparison, [¹¹¹In-DTPA]SS-14 was used as a “universal” radiotracer.

MATERIALS AND METHODS

Animals

C57 black wild-type and sst₂ knockout mice were kindly provided by Dr. James Schaeffer (Merck Laboratories). Establishment of these mice was described in detail previously (24). The animals were bred and maintained under specific pathogen-free conditions with free access to γ -irradiated pellet food (Hope Farms) and water acidified to pH 2.2 in the animal facilities of the Erasmus University Rotterdam. The experimental protocol was approved by the Animal Welfare Committee of the Erasmus Medical Center.

Preparation of Organs for RNA Isolation

After the mice were killed by CO₂ asphyxiation, peripheral blood was collected by cardiac puncture into polypropylene tubes containing 100 international units of heparin (Leo Pharmaceuticals B.V.). Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient (density, 1.07 kg/L; Pharmacia) centrifugation according to the manufacturer’s protocol. PBMC from 2 mice were

pooled, and aliquots of 10⁶ cells were collected. The aliquots were lysed as described in detail elsewhere (25) and were stored at –80°C until RNA isolation. Within minutes after the mice were killed, the following organs were removed and snap-frozen in liquid nitrogen: brain, pituitary, pancreas, adrenals, thymus, spleen, liver, kidneys, and soft tissue (muscle). These organs were then stored at –80°C until further processing. For RNA isolation, the tissues were ground to powder, while frozen, using a mortar and pestle. Tissue powder was stored in ribonuclease-free containers at –80°C.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Studies

RT-PCR was performed as described previously (25). Briefly, poly A⁺ mRNA was isolated using Dynabeads Oligo (dT)₂₅ (DynaL AS) from powdered tissue (10 mg) or 10⁶ cells. Complementary DNA (cDNA) was synthesized using the poly A⁺ mRNA captured on the Dynabeads Oligo (dT)₂₅ as solid-phase and first-strand primer. Twenty microliters of the cDNA were used for each amplification by PCR using primer sets specific for mouse sst₁₋₅, for SS, and for hypoxanthine-guanine phosphoribosyl transferase (hprt) as a control, as listed in Table 1. Mouse genomic DNA served as a positive control for sst₁₋₅. For SS and hprt, cDNA of mouse brain RNA was used. The PCR was performed in a DNA thermal cycler with a heated lid (Perkin Elmer Cetus Instruments). After an initial denaturation at 94°C for 5 min, the samples were subjected to 40 cycles of denaturation at 94°C for 1 min, annealing for 2 min at 60°C, and extension for 1 min at 72°C. After a final extension for 10 min at 72°C, 10- μ L aliquots of the resulting PCR products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide. The identities of the products were confirmed by direct sequencing using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) according to the manufacturer’s protocol.

sst-Binding Studies

Determination of the binding affinity profiles of SS-28, SS-14, and [DTPA]SS-14 for sst₁₋₅ was performed by sst autoradiography on cryostat sections of membrane pellets of cells stably expressing sst₁ or sst₅ (CHO-K1) and sst₂, sst₃, and sst₄ (CCL39 cells), as described in

TABLE 1
Primers Used for RT-PCR Analysis

Specificity	Primer	Sequence (5'-3')	Expected size (bp)
sst ₁	M1-F817	-gtgatgatgggtggatggt-	259
	M1-R1075	-tcagggcagtgccatagtag-	
sst ₂	M2-F694	-atcatcaaggtgaagtcctctg-	376
	M2-R1069	-gggtctcctggtctcatt-	
sst ₃	M3-F732	-ctcgtgtcagtggttacagg-	297
	M3-R1028	-cgacgtgatggtcttagcag-	
sst ₄	M4-F1317	-ctaggactggctccaaggac-	299
	M4-R1615	-agaccgacacagagggaac-	
sst ₅	M5-F722	-gctcagaacgcaaggtgact-	268
	M5-R989	-gcacccacacaccgtatc-	
Somatostatin	MSS15F	-gatgtgtcctgcccgtct-	290
	MSS304R	-ccattgtctgggttcgagt-	
hprt	MHP513F	-tgttggatacagccagactt-	228
	MHP740R	-ggccacaggactagaacacc-	

Position vs. stopcodon.

detail previously (20). Briefly, 20- μ m-thick cryostat sections were incubated with [125 I-Leu 8 ,D-Trp 22 ,Tyr 25]SS-28 using increasing concentrations of unlabeled peptide ranging from 0.1 to 1,000 nmol/L. Concentrations resulting in 50% inhibition of specific binding (IC_{50}) were calculated after quantification of the autoradiographic signal, as described in detail elsewhere (26).

Radiolabeling of [DTPA-D-Phe 1]Octreotide and [DTPA,D-Ala 1 ,D-Trp 8 ,Tyr 11]SS-14 ([DTPA]SS-14)

[DTPA-D-Phe 1]octreotide, [DTPA]SS-14, and 111 InCl $_3$ (DRN 4901, 370 MBq/mL in HCl, pH 1.5–1.9) were obtained from Mallinckrodt. [DTPA]SS-14 was synthesized by one of us. [DTPA-D-Phe 1]octreotide and [DTPA]SS-14 were 111 In-labeled (labeling efficiency > 98%) as described in detail previously (27). Quality controls were performed as described elsewhere (27).

[111 In-DTPA-D-Phe 1]Octreotide Tissue Distribution Studies

Male mice under ether anesthesia were injected via the penis vein with 1 MBq (0.1 μ g) of [111 In-DTPA-D-Phe 1]octreotide or [111 In-DTPA]SS-14. This had been previously determined as being the optimal mass of peptide resulting in the highest uptake values (not shown). To determine specific binding to sst in vivo, a second group of mice was injected with the radiopharmaceutical in combination with 100 μ g of unlabeled octreotide (Novartis Pharma) or SS-14 (Sigma Chemical Co.), respectively. The injection volume was kept constant at 0.2 mL per mouse. The mice were sacrificed by CO $_2$ asphyxiation 24 h after injection of the radiopharmaceutical. Within minutes after the mice were killed, peripheral blood and organs of interest were removed, weighed, and collected into polyurethane tubes. Afterward, tissue and blood radioactivity was counted using an LKB-1282-Compugamma system (Wallac). Specific binding was determined by calculating the difference between uptake of radioactivity in the absence of competitor (total binding) and uptake of radioactivity in the presence of 100 μ g of unlabeled octreotide or SS-14 (nonspecific binding), as described previously (28). Statistical analysis was performed using ANOVA followed by the Newman-Keuls multiple-comparison test. A P value of <0.05 was considered statistically significant.

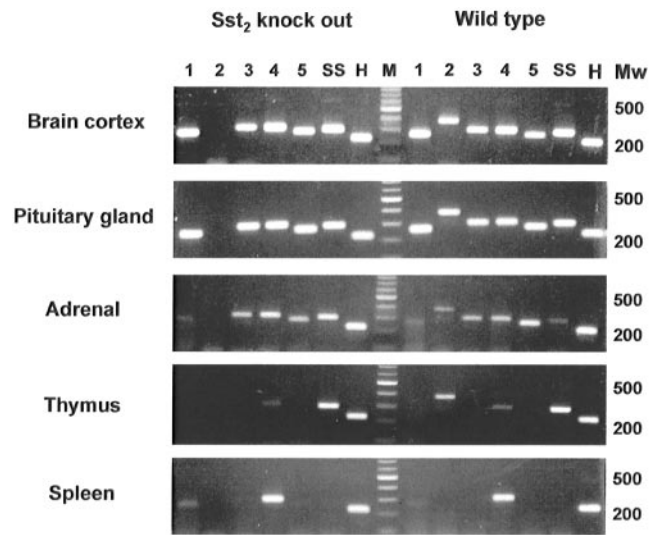


FIGURE 1. Expression of SS and sst $_{1-5}$ mRNAs in different tissues of wild-type and sst $_2$ knockout mice, as determined by RT-PCR. All mock-reverse-transcribed samples and all controls with no added template showed no PCR products. Marker lanes (M) contain 100-bp DNA ladder. H = hprt; Mw = molecular weight.

RESULTS

mRNA Expression in Cells and Tissues

Table 2 shows the mRNA expression of sst $_{1-5}$ and SS in the various organs. All 5 sst subtypes were expressed in the brain, pituitary gland, and adrenals, whereas the knockout mice lacked sst $_2$ mRNA expression (Fig. 1; Table 2). In addition, the thymus expressed sst $_2$ and sst $_4$ mRNAs (Fig. 1; Table 2). Table 2 shows that sst $_1$ and sst $_4$ mRNAs are expressed in the spleen and kidney; sst $_1$, sst $_2$, sst $_4$, and sst $_5$ mRNAs in the liver; and sst $_1$, sst $_2$, and sst $_4$ mRNAs in muscle and PBMC. Neither of these organs showed sst $_2$ mRNA expression in the knockout mice. Compared with wild-type mice, the sst $_2$ knockout mice expressed no compensatory sst mRNAs. SS mRNA expression

TABLE 2
sst and SS mRNA Expression Pattern in Mouse Tissues as Determined by RT-PCR

Tissue	sst mRNA										SS mRNA	
	sst $_1$		sst $_2$		sst $_3$		sst $_4$		sst $_5$		wt	ko
	wt	ko	wt	ko	wt	ko	wt	ko	wt	ko	wt	ko
Brain cortex	+	+	+	–	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	–	+	+	+	+	+	+	+	+
Adrenals	+	+	+	–	+	+	+	+	+	+	+	+
Thymus	–	–	+	–	–	–	+	+	–	–	+	+
Spleen	+	+	–	–	–	–	+	+	–	–	–	–
Liver	+	+	+	–	–	–	+	+	+	+	–	–
Kidneys	+	+	–	–	–	–	+	+	–	–	–	–
Soft tissue	+	+	+	–	–	–	+	+	–	–	+	+
Blood (PBMC)	+	+	+	–	–	–	+	+	–	–	–	–

wt = wild-type mice; ko = sst $_2$ knockout mice.

TABLE 3
Tissue Distribution of [¹¹¹In-DTPA-D-Phe¹]Octreotide in Wild-Type and sst₂ Knockout Mice

Tissue	Wild type		sst ₂ knockout	
	Without blockade	With blockade	Without blockade	With blockade
Pituitary gland	1.2 ± 0.2	0.25 ± 0.04*	0.033 ± 0.037 [†]	0.19 ± 0.03
Adrenals	0.26 ± 0.03	0.12 ± 0.04*	0.045 ± 0.020 [†]	0.11 ± 0.03 [‡]
Thymus	0.30 ± 0.05	0.061 ± 0.020*	0.017 ± 0.003 [†]	0.039 ± 0.010*
Pancreas	0.18 ± 0.03	0.035 ± 0.008*	0.007 ± 0.002 [†]	0.014 ± 0.005
Spleen	0.048 ± 0.008	0.074 ± 0.021	0.075 ± 0.006	0.072 ± 0.016
Liver	0.090 ± 0.007	0.19 ± 0.05 [‡]	0.11 ± 0.01	0.15 ± 0.03
Kidneys	1.4 ± 0.3	1.9 ± 0.4	2.4 ± 0.5	2.3 ± 1.1
Soft tissue	0.004 ± 0.001	0.010 ± 0.006	0.003 ± 0.000	0.009 ± 0.002
Blood	0.004 ± 0.001	0.10 ± 0.00	0.005 ± 0.001	0.011 ± 0.004 [‡]

**P* < 0.01 for [¹¹¹In-DTPA-D-Phe¹]octreotide vs. [¹¹¹In-DTPA-D-Phe¹]octreotide with blockade.

[†]*P* < 0.01 for [¹¹¹In-DTPA-D-Phe¹]octreotide wild type vs. [¹¹¹In-DTPA-D-Phe¹]octreotide sst₂ knockout.

[‡]*P* < 0.05 for [¹¹¹In-DTPA-D-Phe¹]octreotide vs. [¹¹¹In-DTPA-D-Phe¹]octreotide with blockade.

Values are mean ± SD percentage injected dose per gram of tissue (*n* = 4 animals per group). Blockade means coinjection of 100 μg octreotide with [¹¹¹In-DTPA-D-Phe¹]octreotide.

was detected in the brain cortex, pituitary gland, adrenals, thymus, and muscle (soft tissue).

Biodistribution of [¹¹¹In-DTPA-D-Phe¹]Octreotide

Table 3 shows the uptake values of radioactivity after the injection of [¹¹¹In-DTPA-D-Phe¹]octreotide (percentage injected dose per gram of tissue) in wild-type and sst₂ knockout mice. Specific binding to sst as determined by a more than 50% displacement of uptake in the presence of excess unlabeled octreotide was found in the pituitary gland, adrenals, pancreas, and thymus. Interestingly, despite the presence of sst₂ mRNAs, as detected by RT-PCR in the liver, soft tissue, and blood, these organs did not show specific binding of [¹¹¹In-DTPA-D-Phe¹]octreotide. In sst₂ knockout mice, uptake values in the pituitary gland, adrenals, pancreas, and thymus were considerably reduced—by 97%, 83%, 96%, and 94%, respectively (*P* < 0.01 vs. wild type). Figure 2A shows the uptake of radioactivity in the pituitary gland, adrenals, pancreas, and thymus of wild-type and knockout mice. No specific binding was found in the spleen, liver, kidneys, soft tissue, or blood (Table 3). In some of these organs, uptake was even slightly higher when 100 μg of unlabeled octreotide was coinjected with the radiopharmaceutical.

Biodistribution of [¹¹¹In-DTPA]SS-14

We also investigated tissue distribution after the injection of 1 MBq of the more universal ligand [¹¹¹In-DTPA]SS-14 in wild-type and sst₂ knockout mice. The [DTPA]SS-14 molecule showed high-affinity binding to sst₂, sst₃, and sst₄—affinity comparable to that of SS-28 and SS-14. The binding affinity of [DTPA]SS-14 to sst₁ and sst₅ was slightly lower (Table 4). Using this radioligand, we observed a very low uptake of radioactivity in the knockout mice, comparable with the findings using [¹¹¹In-DTPA-D-Phe¹]octreotide. Table 5 shows the uptake values after the

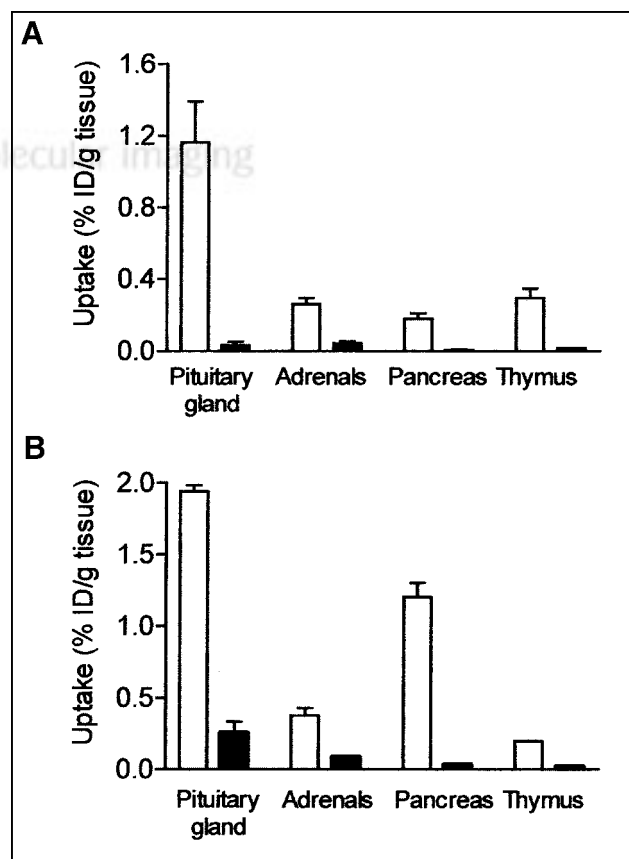


FIGURE 2. Uptake values of radioactivity after injection of [¹¹¹In-DTPA-D-Phe¹]octreotide (A) and [¹¹¹In-DTPA]SS-14 (B), expressed as percentage injected dose per gram of tissue (% ID/gram) in pituitary gland, adrenals, pancreas, and thymus of wild-type mice (white bars) and sst₂ knockout mice (black bars) (*n* = 4).

TABLE 4
Binding Affinity Profiles of SS-28, SS-14, and [DTPA]SS-14 for Human sst₁₋₅ Receptors

Compound	Displacement of binding of [¹²⁵ I-LTT]SS-28				
	sst ₁	sst ₂	sst ₃	sst ₄	sst ₅
SS-28	2.5 ± 0.3	2.1 ± 0.3	2.5 ± 0.2	2.2 ± 0.3	2.1 ± 0.4
SS-14	2.1 ± 0.4	0.6 ± 0.1	3.9 ± 1.3	1.9 ± 0.6	11.0 ± 3.5
[DTPA]SS-14	35 ± 1.6	3.9 ± 0.8	7.5 ± 1.3	5.5 ± 1.1	16.0 ± 2.0
[DTPA]octreotide*	>10,000	12 ± 2	376 ± 84	>1,000	299 ± 50

*Data are from (20).

Values are mean ± SEM of 3 experiments and represent IC₅₀ values (in nmol/L) of displacement of binding of [¹²⁵I-Leu⁸,D-Trp²²,Tyr²⁵]SS-28 to cryostat sections of membrane pellets of cells stably expressing sst₁ or sst₅ (CHO-K1) and sst₂, sst₃, and sst₄ (CCL39 cells), as described in detail previously (20).

injection of [¹¹¹In-DTPA]SS-14. Uptake values were significantly reduced in the pituitary gland (86% decrease), adrenals (76% decrease), pancreas (97% decrease), and thymus (88% decrease) of knockout mice. Figure 2B compares radioactivity uptake in the pituitary gland, adrenals, pancreas, and thymus. No specific binding of [DTPA]SS-14 was found in the spleen, liver, kidneys, soft tissue, and blood of either wild-type or sst₂ knockout mice (Table 5), although these organs expressed mRNAs of several sst subtypes to which [DTPA]SS-14 binds with high affinity (Table 4). In agreement with the observations with [¹¹¹In-DTPA-D-Phe¹]octreotide, we found that the uptake values in organs with a very low uptake of radioactivity, as well as in sst₂ knockout mice, were even slightly higher when an excess amount of 100 μg of unlabeled SS-14 was coinjected with the radiopharmaceutical.

DISCUSSION

Human sst-positive tumors show a high uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide with sst scintigraphy (6). The majority of human neuroendocrine sst-positive tumors express

multiple sst subtypes (3,5,29). The role of the individual sst in determining the uptake of radiolabeled SS-analogs is still unclear, however. Because [¹¹¹In-DTPA-D-Phe¹]octreotide is an sst₂-preferring ligand (20), it is assumed that this sst subtype plays an important role in determining the uptake of radioactivity by sst-positive tumors via internalization of this radioligand (30). However, some studies have demonstrated significant uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide in tumors lacking sst₂. sst scintigraphy revealed tumors in patients with thyroid tumors, which lacked sst₂ mRNA expression. However, analysis of sst subtype expression demonstrated the presence of the 4 other subtypes (31,32). Moreover, we recently described low but significant uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide in a patient with a thymoma (33). In vitro studies demonstrated the absence of sst_{2A}, sst_{2B}, and sst₅ and a predominant expression of sst₃ in the thymoma tissue (33). These data suggest that sst subtypes other than sst₂ could be involved in the uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide in vivo.

In the present study, we evaluated the role of sst₂ in determining the uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide

TABLE 5
Tissue Distribution of [¹¹¹In-DTPA]SS-14 in Wild-Type and sst₂ Knockout Mice

Tissue	Wild type		sst ₂ knockout	
	Without blockade	With blockade	Without blockade	With blockade
Pituitary gland	1.9 ± 0.1	0.24 ± 0.06*	0.26 ± 0.16†	0.43 ± 0.21
Adrenals	0.36 ± 0.03	0.16 ± 0.04*	0.088 ± 0.010†	0.63 ± 0.38*
Thymus	0.20 ± 0.00	0.079 ± 0.006*	0.025 ± 0.005†	0.33 ± 0.19*
Pancreas	1.2 ± 0.1	0.050 ± 0.008*	0.034 ± 0.011†	0.21 ± 0.15*
Spleen	0.081 ± 0.014	0.12 ± 0.02	0.065 ± 0.005	0.32 ± 0.22*
Liver	0.076 ± 0.009	0.14 ± 0.03	0.077 ± 0.020	0.44 ± 0.20*
Kidneys	19.3 ± 4.8	18.6 ± 2.8	20.5 ± 3.2	22.6 ± 6.6
Soft tissue	0.006 ± 0.001	0.023 ± 0.004*	0.010 ± 0.004	0.085 ± 0.045*
Blood	0.011 ± 0.001	0.036 ± 0.007*	0.005 ± 0.001	0.011 ± 0.002*

*P < 0.01 for [¹¹¹In-DTPA]SS-14 vs. [¹¹¹In-DTPA]SS-14 with blockade.

†P < 0.01 for [¹¹¹In-DTPA]SS-14 wild type vs. [¹¹¹In-DTPA]SS-14 sst₂ knockout.

Values are mean ± SD percentage injected dose per gram of tissue (n = 4 animals per group). Blockade means coinjection of 100 μg SS-14 with [¹¹¹In-DTPA]SS-14.

by using wild-type and *sst*₂ knockout mice (24). In addition, we used a universal ligand, [¹¹¹In-DTPA]SS-14, which binds with a relatively high affinity to the 5 *sst* subtypes, to investigate the role of the other *sst* subtypes in this respect. RT-PCR analysis of several organs of interest, that is, the pituitary gland, adrenals, thymus, spleen, liver, kidneys, soft tissue, and blood, showed that all but the spleen and kidney expressed *sst*₂ mRNA in wild-type animals whereas the *sst*₂ knockout animals lacked *sst*₂ mRNA. In addition, the other 4 *sst* subtype mRNAs were variably expressed in these tissues. With respect to the *sst*₁, *sst*₃, *sst*₄, and *sst*₅ expression patterns, no qualitative differences between wild-type and *sst*₂ knockout mice were observed, suggesting that no compensatory *sst* mRNAs are expressed after knockout of *sst*₂ expression. However, it cannot be excluded that there are changes in *sst* subtype expression levels in *sst*₂ knockout mice, compared with wild-type animals.

After the injection of [¹¹¹In-DTPA-D-Phe¹]octreotide, we found that uptake of radioactivity was virtually absent in *sst*-positive organs in *sst*₂ knockout mice, compared with the significant uptake of radioactivity in the pituitary gland, adrenals, thymus, and pancreas in the wild-type animals. This suggests a crucial role for *sst*₂ in determining the uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide in normal *sst*-positive organs. In these organs, *sst*₃ and *sst*₅ apparently do not play a role in this respect, although [¹¹¹In-DTPA-D-Phe¹]octreotide has a relatively high binding affinity to *sst*₅. In order to evaluate the role of the other *sst* subtypes in the *sst*-mediated uptake in vivo, we used a universal ligand, that is, [¹¹¹In-DTPA]SS-14, in this mouse model as well. Surprisingly, significant uptake of radioactivity in *sst*-expressing organs was found only in wild-type mice, not in *sst*₂ knockout mice. This finding indicates that *sst*₂ is the most important, if not the crucial, *sst* subtype for the uptake of radiolabeled SS and SS-analogs in vivo in mice. Data on humans need to be proved, however. A possible explanation may be that the *sst*₂, via physical interaction (e.g., heterodimerization) with one or more of the other *sst* subtypes, has a modulatory effect on *sst*-mediated internalization of these *sst* subtypes. Such heterodimerization of *sst* subtypes in vitro has previously been shown to modify the capacity of a particular *sst* subtype to internalize *sst*-bound agonists (34,35). In addition, in line with the observed crucial role of *sst*₂, it has been shown that uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide correlates with *sst*₂ expression levels at the mRNA (22) as well as with *sst*₂ protein level (21) in neuroblastoma and carcinoid tumors, respectively.

Although we did not perform quantitative measurements of *sst* subtype mRNA levels, wild-type and *sst*₂ knockout mice have been shown to express comparable *sst*₁, *sst*₃, *sst*₄, and *sst*₅ mRNA levels in the brain (36). How can we explain, then, the uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide in particular patients with tumors lacking *sst*₂ expression? One possible explanation is a relatively high expression level of one of the other *sst* subtypes (e.g., *sst*₃ or *sst*₅), which could be involved on the basis of its binding, though

at low affinity, to [¹¹¹In-DTPA-D-Phe¹]octreotide (20). A high expression level, in combination with the high capacity of human *sst*₃ and *sst*₅ to internalize after agonist binding (23), could explain the (low) tumoral uptake of radioactivity after the injection of [¹¹¹In-DTPA-D-Phe¹]octreotide in patients with *sst*₂-negative tumors (32,33). Moreover, other factors, including the capacity of the cell to retain internalized radioactivity, can play a role as well.

In our present study, we observed that several organs, that is, the spleen, liver, kidneys, soft tissue, and blood, do not show specific binding in vivo of either [¹¹¹In-DTPA]SS-14 or [¹¹¹In-DTPA-D-Phe¹]octreotide, irrespective of the presence of *sst*₂ mRNA or mRNAs of one or more of the other *sst* subtypes. In some of these organs, uptake values were even slightly higher when 100 μg of unlabeled octreotide were coinjected with the radiopharmaceutical. Because this effect was also observed in the *sst*₂ knockout mice, this slight increased uptake might be due to a systemic effect of the pharmacologic amount of unlabeled octreotide, for example, via *sst*₅. Apparently, such a systemic effect is evident only when the uptake values are very low. The absence of specific binding in the spleen, liver, kidneys, soft tissue, and blood is unlikely to be caused by endogenous SS, competing with binding of the radioligand for *sst* plays a role, since these organs do not express a significant amount of SS mRNA. This indicates that, in these organs, *sst* protein levels are probably not high enough to result in specific binding and uptake of radioactivity in vivo. It also means that care should be taken, when drawing conclusions on the significance of the expression of a particular *sst* subtype, to consider its involvement in the complex processes of *sst*-mediated uptake of radiolabeled SS-analogs in vivo.

CONCLUSION

The present study demonstrates that the expression of *sst*₂ is crucial for the uptake of [¹¹¹In-DTPA-D-Phe¹]octreotide, as well as of a radiolabeled universal SS-ligand, in normal *sst*-positive organs in vivo. In addition, the expression of *sst*₂ mRNA, as determined by RT-PCR, does not necessarily result in a significant uptake of radioactivity in all organs, suggesting that *sst*₂ protein levels in these organs are too low to result in specific binding in vivo. This may also explain why in humans the spleen, but not the thymus, is visualized through *sst* scintigraphy whereas both organs express *sst*₂ mRNAs.

REFERENCES

1. Patel YC. Somatostatin and its receptor family. *Front Neuroendocrinol.* 1999; 20:157-198.
2. Reubi JC, Schaer JC, Laissue JA, Waser B. Somatostatin receptors and their subtypes in human tumors and in peritumoral vessels. *Metabolism.* 1996;45:39-41.
3. Hoffland LJ, Lamberts SW. Somatostatin analogs and receptors: diagnostic and therapeutic applications. *Cancer Treat Res.* 1997;89:365-382.
4. Lamberts SW, Krenning EP, Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. *Endocr Rev.* 1991;12:450-482.
5. Patel YC. Molecular pharmacology of somatostatin receptor subtypes. *J Endocrinol Invest.* 1997;20:348-367.

6. Krenning EP, Kwekkeboom DJ, Bakker WH, et al. Somatostatin receptor scintigraphy with [¹¹¹In-DTPA-D-Phe1]- and [¹²³I-Tyr3]-octreotide: the Rotterdam experience with more than 1000 patients. *Eur J Nucl Med.* 1993;20:716–731.
7. Krenning EP, de Jong M, Kooij PP, et al. Radiolabelled somatostatin analogue(s) for peptide receptor scintigraphy and radionuclide therapy. *Ann Oncol.* 1999;10:S23–S29.
8. Otte A, Herrmann R, Heppeler A, et al. Yttrium-90 DOTATOC: first clinical results. *Eur J Nucl Med.* 1999;26:1439–1447.
9. Virgolini I, Traub T, Novotny C, et al. New trends in peptide receptor radioligands. *Q J Nucl Med.* 2001;45:153–159.
10. Otte A, Mueller-Brand J, Dellas S, et al. Yttrium-90-labelled somatostatin-analogue for cancer treatment [letter]. *Lancet.* 1998;351:417–418.
11. Paganelli G, Zoboli S, Cremonesi M, et al. Receptor-mediated radiotherapy with ⁹⁰Y-DOTA-D-Phe1-Tyr3-octreotide. *Eur J Nucl Med.* 2001;28:426–434.
12. Paganelli G, Zoboli S, Cremonesi M, Macke HR, Chinol M. Receptor-mediated radionuclide therapy with ⁹⁰Y-DOTA-D-Phe1-Tyr3-octreotide: preliminary report in cancer patients. *Cancer Biother Radiopharm.* 1999;14:477–483.
13. Hofland LJ, Breeman WA, Krenning EP, et al. Internalization of [DOTA degrees, ¹²⁵I-Tyr3]octreotide by somatostatin receptor-positive cells in vitro and in vivo: implications for somatostatin receptor-targeted radio-guided surgery. *Proc Assoc Am Physicians.* 1999;111:63–69.
14. Andersson P, Forsell-Aronsson E, Johanson V, et al. Internalization of indium-111 into human neuroendocrine tumor cells after incubation with indium-111-DTPA-D-Phe1-octreotide. *J Nucl Med.* 1996;37:2002–2006.
15. Hofland LJ, van Koetsveld PM, Waaijers M, et al. Internalization of the radioiodinated somatostatin analog [¹²⁵I-Tyr3]octreotide by mouse and human pituitary tumor cells: increase by unlabeled octreotide. *Endocrinology.* 1995;136:3698–3706.
16. Koenig JA, Edwardson JM, Humphrey PP. Somatostatin receptors in Neuro2A neuroblastoma cells: ligand internalization. *Br J Pharmacol.* 1997;120:52–59.
17. De Jong M, Bernard BF, De Bruin E, et al. Internalization of radiolabelled [DTPA0]octreotide and [DOTA0, Tyr3]octreotide: peptides for somatostatin receptor-targeted scintigraphy and radionuclide therapy. *Nucl Med Commun.* 1998;19:283–288.
18. Breeman WA, Kwekkeboom DJ, Kooij PP, et al. Effect of dose and specific activity on tissue distribution of indium-111-pentetreotide in rats. *J Nucl Med.* 1995;36:623–627.
19. Kwekkeboom DJ, Bakker WH, Kooij PP, et al. [¹⁷⁷Lu-DOTAOTyr3]octreotate: comparison with [¹¹¹In-DTPA]octreotide in patients. *Eur J Nucl Med.* 2001;28:1319–1325.
20. Reubi JC, Schar JC, Waser B, et al. Affinity profiles for human somatostatin receptor subtypes SST1–SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. *Eur J Nucl Med.* 2000;27:273–282.
21. Janson ET, Stridsberg M, Gobl A, Westlin JE, Oberg K. Determination of somatostatin receptor subtype 2 in carcinoid tumors by immunohistochemical investigation with somatostatin receptor subtype 2 antibodies. *Cancer Res.* 1998;58:2375–2378.
22. Briganti V, Sestini R, Orlando C, et al. Imaging of somatostatin receptors by indium-111-pentetreotide correlates with quantitative determination of somatostatin receptor type 2 gene expression in neuroblastoma tumors. *Clin Cancer Res.* 1997;3:2385–2391.
23. Hukovic N, Panetta R, Kumar U, Patel YC. Agonist-dependent regulation of cloned human somatostatin receptor types 1–5 (hSSTR1–5): subtype selective internalization or upregulation. *Endocrinology.* 1996;137:4046–4049.
24. Zheng H, Bailey A, Jiang MH, et al. Somatostatin receptor subtype 2 knockout mice are refractory to growth hormone-negative feedback on arcuate neurons. *Mol Endocrinol.* 1997;11:1709–1717.
25. Ferone D, van Hagen PM, van Koetsveld PM, et al. In vitro characterization of somatostatin receptors in the human thymus and effects of somatostatin and octreotide on cultured thymic epithelial cells. *Endocrinology.* 1999;140:373–380.
26. Reubi JC, Kvols LK, Waser B, et al. Detection of somatostatin receptors in surgical and percutaneous needle biopsy samples of carcinoids and islet cell carcinomas. *Cancer Res.* 1990;50:5969–5977.
27. Bakker WH, Krenning EP, Reubi JC, et al. In vivo application of [¹¹¹In-DTPA-D-Phe1]-octreotide for detection of somatostatin receptor-positive tumors in rats. *Life Sci.* 1991;49:1593–1601.
28. Breeman WA, de Jong M, Bernard B, et al. Tissue distribution and metabolism of radioiodinated DTPA0, D-Tyr1 and Tyr3 derivatives of octreotide in rats. *Anticancer Res.* 1998;18:83–89.
29. Reubi JC, Schaefer JC, Markwalder R, et al. Distribution of somatostatin receptors in normal and neoplastic human tissues: recent advances and potential relevance. *Yale J Biol Med.* 1997;70:471–479.
30. Breeman WA, de Jong M, Kwekkeboom DJ, et al. Somatostatin receptor-mediated imaging and therapy: basic science, current knowledge, limitations and future perspectives. *Eur J Nucl Med.* 2001;28:1421–1429.
31. Kolby L, Wangberg B, Ahlman H, et al. Somatostatin receptor subtypes, octreotide scintigraphy, and clinical response to octreotide treatment in patients with neuroendocrine tumors. *World J Surg.* 1998;22:679–683.
32. Forsell-Aronsson EB, Nilsson O, Bejgard SA, et al. ¹¹¹In-DTPA-D-Phe1-octreotide binding and somatostatin receptor subtypes in thyroid tumors. *J Nucl Med.* 2000;41:636–642.
33. Ferone D, van Hagen MP, Kwekkeboom DJ, et al. Somatostatin receptor subtypes in human thymoma and inhibition of cell proliferation by octreotide in vitro. *J Clin Endocrinol Metab.* 2000;85:1719–1726.
34. Rocheville M, Lange DC, Kumar U, et al. Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. *J Biol Chem.* 2000;275:7862–7869.
35. Pfeiffer M, Koch T, Schroder H, et al. Homo- and heterodimerization of somatostatin receptor subtypes: inactivation of sst(3) receptor function by heterodimerization with sst(2A). *J Biol Chem.* 2001;276:14027–14036.
36. Viollet C, Vaillend C, Videau C, et al. Involvement of sst2 somatostatin receptor in locomotor, exploratory activity and emotional reactivity in mice. *Eur J Neurosci.* 2000;12:3761–3770.