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A new radiolabelled somatostatin analogue [^{111}In-DTPA-D-Phe^1]RC-160: preparation, biological activity, receptor scintigraphy in rats and comparison with [^{111}In-DTPA-D-Phe^1]octreotide

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Abstract. We have evaluated the potential usefulness of indium-111 labelled [DTPA-D-Phe^1]RC-160, derived from the octapeptide somatostatin analogue RC-160, as a radiopharmaceutical for the in vivo detection of somatostatin receptor-positive tumours. For this purpose ^{111}In- and ^{115}In-labelled [DTPA-D-Phe^1]RC-160 was tested for its biological activity, and applied for somatostatin receptor scintigraphy in vivo to rats bearing the transplantable rat pancreatic tumour CA20948, which expresses somatostatin receptors. We previously described the development of the ^{111}In-labelled somatostatin analogue [DTPA-D-Phe^1]octreotide and its use in the in vivo visualization of somatostatin receptor-positive tumours in rats and in humans. Like [^{111}In-DTPA-D-Phe^1]octreotide, [^{111}In-DTPA-D-Phe^1]RC-160 showed uptake in and specific binding in vivo to somatostatin receptor-positive organs and tumours, and the tumours were clearly visualized by gamma camera scintigraphy. However, as compared to [^{111}In-DTPA-D-Phe^1]octreotide, blood radioactivity (background) was higher, resulting in a lower tumour to blood (background) ratio. Using this animal model we therefore conclude that [^{111}In-DTPA-D-Phe^1]RC-160 has no advantage over [^{111}In-DTPA-D-Phe^1]octreotide as a radiopharmaceutical in the visualization of somatostatin receptors which bind both analogues. However, recent reports suggest the existence of different somatostatin receptor subtypes on some human cancers, which differentially bind RC-160 and not octreotide. These tumours include cancers of the breast, ovary, exocrine pancreas, prostate and colon. [^{111}In-DTPA-D-Phe^1]RC-160 might be of interest for future use in such cancer patients as a radiopharmaceutical for imaging somatostatin receptor-positive tumours, which do not bind octreotide.

Key words: Radioindium labelled RC-160 – Somatostatin – Specific binding – Tumour imager – Radiopharmaceutical – Peptide

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Introduction

High numbers of high-affinity somatostatin receptors for both native somatostatin (for structures, see Fig. 1) and the synthetic octapeptide octreotide (Sandostatin), have been detected on most neuro-endocrine tumours, like endocrine pancreatic tumours and carcinoids [1–4]. We, and also others, have recently described the visualization of somatostatin receptor-positive tumours in vivo after the intravenous administration of [^{123}I-Tyr^3]octreotide [5–12] and [^{111}In-DTPA-D-Phe^1]octreotide [13–20]. Several reports have also been published on the in vitro binding to somatostatin receptors of another somatostatin analogue, the octapeptide RC-160 [21–23]. It has been reported that RC-160 has a higher affinity than octreotide for somatostatin receptors in human breast, ovarian, exocrine pancreatic, prostatic and colonic cancers [21–23]. A phase 1 clinical trial with RC-160 in patients with advanced exocrine pancreatic cancer suggests that RC-160 is well tolerated at doses up to 1500 μg/day [24–25]. The possibility of RC-160 binding to a somatostatin receptor subtype, which does not bind octreo-

Somatostatin

Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys

Octreotide

D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol)

Tyr^3-octreotide

D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr(ol)

[^{111}In-DTPA-D-Phe^1]octreotide

^{111}In-DTPA-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol)

RC-160

D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2

[^{111}In-DTPA-D-Phe^1]RC-160

^{111}In-DTPA-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2

Fig. 1. Structural formulae of native somatostatin, octreotide, Tyr^3-octreotide, [^{111}In-DTPA-D-Phe^1]octreotide, RC-160 and [^{111}In-DTPA-D-Phe^1]RC-160

tide, for example on human exocrine pancreatic adenocarcinomas [26], offers a potential advantage of RC-160 over octreotide as radiolabelled tumour tracer. Furthermore, RC-160 and radioiodinated RC-160 may, in contrast to octreotide [27], also pass the blood-brain barrier [28–30]. This could represent a benefit in visualizing somatostatin receptor-positive brain tumours with an intact blood-brain barrier. We recently reported [30] our results with tumour visualization with ^{123}I-RC-160 in tumour-bearing rats and concluded that, despite the fact that ^{123}I-RC-160 shows high-affinity binding to various somatostatin receptor-positive tissues and tumours, no advantage over [^{123}I-Tyr^3]octreotide has been found in binding to the somatostatin receptor-positive tissues and tumours studied so far. Therefore, we compared [^{111}In-DTPA-D-Phe^1]RC-160 with [^{111}In-DTPA-D-Phe^1]octreotide (for structure see Fig. 1), as radiopharmaceuticals for scintigraphy in normal rats and in rats bearing the transplantable pancreatic somatostatin receptor-positive tumour CA20948 [26, 31, 32].

Materials and methods

Preparation and purification of the radiopharmaceuticals. RC-160 and [DTPA-D-Phe^1]RC-160 were purchased from Sanbio (Uden, The Netherlands). [DTPA-D-Phe^1]octreotide and $^{111}InCl_3$ (DRN 4901) were obtained from Mallinckrodt (Petten, The Netherlands). The radiolabelling of [DTPA-D-Phe^1]octreotide and [DTPA-D-Phe^1]RC-160 with ^{111}In and consecutive quality control were performed as described previously [33]. Although it is not excluded that additional groups of the peptides participate in ^{111}In complexation, the labelled products are referred to as [^{111}In-DTPA-D-Phe^1]RC-160 and [^{111}In-DTPA-D-Phe^1]octreotide. The radiochemical purity of the radiolabelled somatostatin analogues was greater than 95 %. All chemicals used were of the highest purity available.

Animals and tumours. Male Lewis rats (240–260 g) were inoculated in both upper hind legs with the transplantable rat pancreatic tumour CA20948, wich has previously been shown to possess somatostatin receptors [26]. Male Lewis rats (240–260 g) not bearing the CA20948 tumour were used as control rats.

For injection and scintigraphy the rats were anaesthetized with ether.

In order to study specific binding, the rats were injected subcutaneously with 1 mg RC-160 or 1 mg octreotide in 1 ml 0.05 *M* acetic acid containing 154 m*M* NaCl or solvent. Forty-five minutes later the rats were injected with either [^{111}In-DTPA-D-Phe^1]RC-160 or [^{111}In-DTPA-D-Phe^1]octreotide. Specific binding was defined as the difference between the tissue uptake in tumour-inoculated rats and that in similar animals treated with excess of unlabelled peptide, expressed as percentages of the injected radioactivity per gram tissue (mean ± SD).

The tracer agents were injected into the dorsal vein of the penis. The dose was 18.5 MBq (0.5 µg) for both analogues, measured in a dose calibrator (VDC-202, Veenstra, Joure, The Netherlands). The rats were sacrificed 2, 4, 24, 48 or 72 h after administration of the radiolabelled somatostatin analogue. The concentration of radioactivity in various tissues, urine and blood was subsequently measured. [^{111}In-DTPA-D-Phe^1]octreotide and [^{111}In-DTPA-D-Phe^1]RC-160 binds to SEP-PAK C_{18} stationary phase and is, using the separation technique as described by Bakker [6], only eluted with ethanol, while ^{111}In-DTPA does not retain on SEP-PAK C_{18} columns. Radioactivity in plasma and urine samples which is also eluted with ethanol from the SEP-PAK C_{18} column is termed peptide-bound radioactivity, but is not further characterized.

The radioactivity in blood and urine was analysed as described previously [6].

Biological activity and radioligand binding studies. [In-DTPA-D-Phe^1]octreotide (labelled with natural indium, i.e. a mixture of 4.23% non-radioactive ^{113}In and 95.77% ^{115}In, $T_{½}$=6.10^{14} years, hereafter referred to as ^{115}In) and [^{115}In-DTPA-D-Phe^1]RC-160 were prepared by mixing the respective [DTPA-D-Phe^1]-somatostatin analogues (stock concentration of 10^{-4} *M*) with 10^{-3} *M* $^{115}InCl_3$ (Aldrich) in 0.05 *M* acetic acid, to an 80-fold molar excess ratio of indium over peptide. The biological activity of [DTPA-D-Phe^1]octreotide, [^{115}In-DTPA-D-Phe^1]octreotide, [DTPA-D-Phe^1]RC-160 and [^{115}In-DTPA-D-Phe^1]RC-160 was assessed by measuring potency to inhibit the secretion of rat growth hormone from cultured rat pituitary cells. The preparation of dispersed female rat anterior pituitary cells and cell culture conditions have been described previously [34]. Receptor binding assays were carried out using [^{125}I-Tyr^3]octreotide as described previously [35].

Binding curves and IC_{50} for displacement of [^{125}I-Tyr^3]octreotide binding by unlabelled peptide were calculated from two experiments (triplicate determinations) using the computer fitting program of Graphpad (ISI software, Philadelphia, Pa., USA).

Data acquisition and analysis. All results are expressed as the mean ± SD. One-way analysis of variance (ANOVA), was used for statistical analysis. For the comparison of means the Newman-Keuls or Bonferroni *t*-test was applied [36]. A P value of <0.05 was considered significant. The tissue distribution and metabolism of the ^{111}In-labelled somatostatin analogues in vivo were studied by gamma camera scintigraphy (Rota-II, Siemens) [13] and measurement of ^{111}In in isolated organs was performed using an LKB-1282-Compugammasystem.

The ratio of tissue-binding values of tissue over soft tissue (thigh) and the radioactivity concentration ratio of tumour over soft tissue were calculated for each individual organ and for the tumours.

Results

Quality control, radiolabelling and preparation of the radiopharmaceutical

Amino acid analysis of RC-160 and [^{111}In-DTPA-D-Phe^1]RC-160 yielded a peptide content of more than 95% of the correct amino acid composition. The identity of the peptides was confirmed by fast atom bombardment mass spectrometry, showing a molecular weight of 1131 and 1507 daltons for RC-160 and [DTPA-D-Phe^1]RC-160, respectively.

More than 95% efficiency of labelling, tested by instant thin-layer chromatography and confirmed by high-performance liquid chromatography, was assured when a molar excess of five- to tenfold of peptide over ^{111}In was used, resulting in the formation of [^{111}In-DTPA-D-Phe^1]RC-160 with a specific activity of 150 MBq ^{111}In per µg [DTPA-D-Phe^1]RC-160. The [^{111}In-DTPA-D-Phe^1]RC-160 was stable for at least 4 h after preparation, and for longer than 24 h if stabilized by either dilution, with for instance saline, or addition of a quencher, such as gentisic acid.

Receptor binding studies and biological activity

Figure 2 shows binding of [^{125}I-Tyr^3]octreotide to rat brain cortex membranes in the presence of increasing concentrations of unlabelled [DTPA-D-Phe^1]octreotide or [DTPA-D-Phe^1]RC-160, with IC_{50} values of 20 and 30 n*M*, respectively. In Fig. 3 the effects of [^{115}In-DTPA-D-Phe^1]octreotide, [DTPA-D-Phe^1]octreotide, [^{115}In-DTPA-D-Phe^1]RC-160 and [DTPA-D-Phe^1]RC-160 on the secretion of rat growth hormone by cultured rat anterior pituitary cells are shown. There was a dose-dependent inhibition of basal rat growth hormone release by these cells, with IC_{50} values of 3, 8, 5 and 15 n*M*, respectively, for the four aforementioned compounds. There were no significant differences between the effects of the two ^{115}In-labelled and the two non-labelled somatostatin analogues ($P > 0.05$, Student's *t*-test). Trivalent indium ions, up to a concentration of $8 \cdot 10^{-5}$ *M*, did not influence rat growth hormone assay (data not shown).

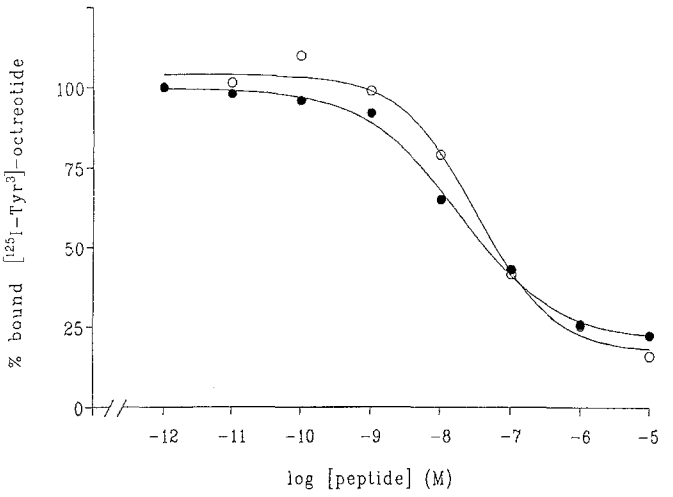

Fig. 2. Binding of [^{125}I-Tyr^3]octreotide to rat brain cortex membranes in the presence of increasing concentrations of [DTPA-D-Phe^1]RC-160 (•) and [DTPA-D-Phe^1]octreotide (○),expressed as the percentage of binding in the absence of competing compounds (n=3, maximal SD < 10%). No significant differences were found at equimolar concentrations

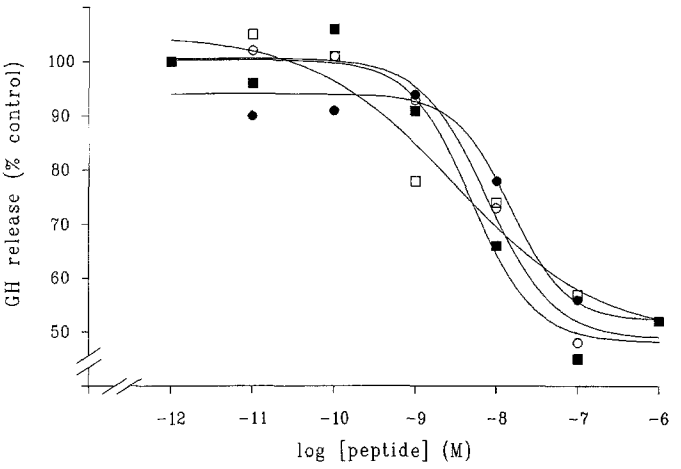

Fig. 3. Effects of [^{115}In-DTPA-D-Phe^1]RC-160 (■), [DTPA-D-Phe^1]RC-160(•), [^{115}In-DTPA-D-Phe^1]octreotide (□) and [DTPA-D-Phe^1]octreotide (○) on the secretion of rat growth hormone (*GH*) from cultured rat pituitary cells (n=4, maximal SD < 15%). No significant differences were found at equimolar concentrations

Tissue distribution and specific binding

The radioactivities measured in the isolated tumour and organs 24 h after injection of [^{111}In-DTPA-D-Phe^1]octreotide and 24, 48 and 72 h after injection of [^{111}In-DTPA-

Table 1. Tissue distribution (% injected dose per gram tissue, mean ± SD) and tissue ratios in tumour-bearing rats ($n \geq 4$) at indicated time intervals after intravenous administration of 0.5 μg of ^{111}In-labelled somatostatin analogue

Tissue	In-octreotide 24 h	In-RC 24 h	In-RC 48 h	In-RC 72h
Adrenals	2.2 ±0.4	7.1 ±2.6	0.87 ±0.12	0.73 ± 0.03
Pancreas	0.92 ±0.08	0.97 ±0.28	0.42 ±0.14	0.46 ± 0.14
Tumours	0.20 ±0.02	0.69 ±0.12	0.21 ±0.08	0.20 ± 0.08
Pituitary	0.11 ±0.02	0.35 ±0.02	0.11 ±0.02	0.13 ± 0.01
Brain cortex	0.0011±0.0001	0.011±0.001	0.0015±0.0002	0.0016± 0.0002
Kidneys	2.9 ±0.3	5.3 ±0.8	12 ±1	12 ± 2
Liver	0.031 ±0.006	0.74 ±0.04	0.22 ±0.03	0.26 ± 0.07
Spleen	0.033 ±0.018	0.93 ±0.08	0.36 ±0.08	0.30 ± 0.04
Intestines	0.05 ±0.01	0.37 ±0.09	0.12 ±0.03	0.18 ± 0.04
Soft tissue	0.0022±0.0003	0.046±0.003	0.011 ±0.004	0.013 ± 0.001
Blood	0.0035±0.0006	0.16 ±0.02	0.0067±0.0006	0.0061± 0.0012
In toto	13 ±2	50 ±4	51 ±5	49 ± 2
Tumours vs soft tissue	97 ±9	12 ±3	25 ±9	13 ± 7
Tumours vs blood	58 ±9	3.6 ±0.8	32 ±8	30 ±11

In-RC, [^{111}In-DTPA-D-Phe^1]RC-160; In-octreotide, [^{111}In-DTPA-D-Phe^1]octreotide

Table 2. Specific binding in somatostatin receptor-positive organs and tumours (percentage injected dose per gram tissue, mean ± SD) in tumour-bearing rats ($n \geq 4$), at indicated time intervals after intravenous administration of 0.5 μg of ^{111}In-labelled somatostatin analogue

Tissue	In-RC 4 h	In-RC 24 h	In-octreotide 4 h	In-octreotide 24 h
Adrenals	5.0 ±0.3	6.1 ±2.6	2.1 ±0.5	1.8 ±0.4
Pancreas	0.44±0.22	0.56±0.28	0.47±0.20	0.44±0.15
Tumours	0.30±0.14	0.35±0.12	0.21±0.09	0.19±0.05
Pituitary	0.40±0.05	0.23±0.02	0.16±0.06	0.12±0.02
Brain cortex	<0.001	<0.001	<0.001	<0.001

Except for the brain cortex, all organs and tumours had specific binding significantly different from zero, $P < 0.05$
In-RC, [^{111}In-DTPA-D-Phe^1]RC-160; In-octreotide, [^{111}In-DTPA-D-Phe^1]octreotide

D-Phe^1]RC-160 are shown in Table 1. Between 24 and 48 h after injection of [^{111}In-DTPA-D-Phe^1]RC-160 there was a decrease of radioactivity in most tissues, accompanied by an increase of radioactivity in rat kidney. The uptakes in the kidney, in the somatostatin receptor-positive tissues and in the tumour were higher for [^{111}In-DTPA-D-Phe^1]RC-160 than for[^{111}In-DTPA-D-Phe^1]octreotide. The ratios of tumour over blood and tumour over soft tissue for [^{111}In-DTPA-D-Phe^1]RC-160 were significantly lower than those found for [^{111}In-DTPA-D-Phe^1]octreotide, mainly because of the very low uptake of radioactivity in soft tissue after injection of [^{111}In-DTPA-D-Phe^1]octreotide (Table 1). Table 1 also shows that even 72 h after injection of [^{111}In-DTPA-D-Phe^1]RC-160 the remaining radioactivity in blood is still twice the value 24 h after injection of [^{111}In-DTPA-D-Phe^1]octreotide, 0.0061% vs 0.0035% injected dose per gram. For soft tissue the relative difference is even higher, 0.013% vs 0.002% injected dose per gram (Table 1).

Despite the decrease in radioactivity in most somatostatin receptor-negative tissues, i.e. in the majority of tissues, total body radioactivity does not decrease between 24 and 48 h after the injection of [^{111}In-DTPA-D-Phe^1]RC-160, due to an increase of radioactivity in the kidneys (Table 1). This results in a significantly higher body retention of radioactivity after injection of [^{111}In-DTPA-D-Phe^1]RC-160 than with [^{111}In-DTPA-D-Phe^1]octreotide. This higher concentration of radioactivity in the kidneys after [^{111}In-DTPA-D-Phe^1]RC-160 is striking, since renal excretion is also the predominant route for clearance of [^{111}In-DTPA-D-Phe^1]octreotide. For instance about 50% of injected [^{111}In-DTPA-D-Phe^1]octreotide is excreted via the kidneys in the first 30 min after injection, as described previously by Bakker et al. [13].

In Table 2 the specific binding at several time intervals after injection of [^{111}In-DTPA-D-Phe^1]RC-160 and [^{111}In-DTPA-D-Phe^1]octreotide is given for the somatostatin receptor-positive tissues and tumour. There were no significant differences in binding of either peptide to these sites. Also, no specific binding of either analogue was found in the brain, suggesting that they were unable to pass the blood-brain barrier.

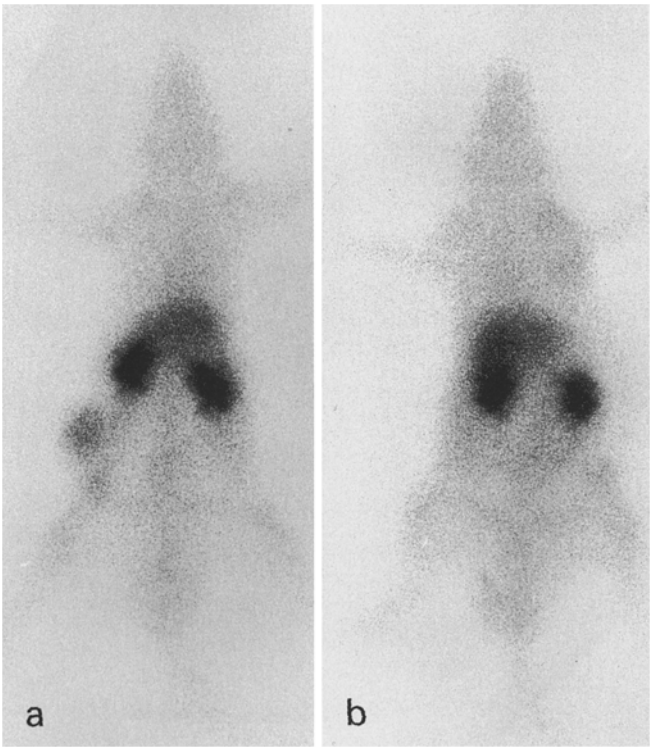

Fig. 4a,b. Static posterior images in rats with unilateral implanted somatostatin receptor-positive CA20948 tumour, located at the upper part of the right hind leg, 24 h after injection of [^{111}In-DTPA-D-Phe^1]RC-160. Note the normal uptake of radioactivity in liver and kidneys in both images. There is accumulation of radioactivity in the tumour (**a**, no preteatment); note the absence of accumulation of radioactivity in the tumour in **b**. In **b** the rat was pretreated s.c. with 1 mg RC-160 45 min prior to the injection with [^{111}In-DTPA-D-Phe^1]RC-160 in order to saturate the somatostatin receptors

Metabolism

SEP-PAK C_{18} columns were used for measurement of peptide-bound radioactivity in blood and urine of four control rats at 4 h and 24 h after the injection of [^{111}In-DTPA-D-Phe^1]RC-160. The percentages of peptide-bound radioactivity in blood were 23 ± 2 at 4 h p.i. and 18 ± 1 at 24 h p.i. HPLC analysis of the ethanol fractions of the SEP-PAK C_{18} columns of these blood samples confirmed that more than 90% of this peptide-bound ^{111}In was intact [^{111}In-DTPA-D-Phe^1]RC-160 and at 24 h p.i. this figure had dropped below 50%. The nature of the metabolites of both ^{111}In-labelled radiopharmaceuticals was not further investigated. The percentage of peptide-bound ^{111}In in urine samples was 73 ± 4 at 4 h and 75 ± 2 at 24 h after injection of [^{111}In-DTPA-D-Phe^1]RC-160. In the urine samples from two control rats 24 h after injection of [^{111}In-DTPA-D-Phe^1]octreotide, when most of the radioactivity had already been excreted, more than 90% of the ^{111}In was not peptide-bound, as described previously by Bakker et al. (13).

Imaging

Dynamic images during the first 20 min after injection of [^{111}In-DTPA-D-Phe^1]RC-160 or [^{111}In-DTPA-D-Phe^1]octreotide showed a rapid distribution of radioactivity over the whole body. Pretreatment with excess unlabelled RC-160 or octreotide did not affect the blood clearance. Immediately after injection of [^{111}In-DTPA-D-Phe^1]RC-160 or [^{111}In-DTPA-D-Phe^1]octreotide, an increasing amount of radioactivity was measured over the tumours.

From digital static images obtained 24, 48 and 72 h after injection of [^{111}In-DTPA-D-Phe^1]RC-160, whole-body retention was found to be approximately 50% of the injected dose after 48 h and 72 h, and this retention was predominantly accounted for by radioactivity in the kidneys (see also Table 1). Figure 4 presents static analog images of two rats with unilateral somatostatin receptor-positive CA20948 tumours, 24 h after the injection of [^{111}In-DTPA-D-Phe^1]RC-160. Accumulation of radioactivity in the tumour and kidneys was observed. The uptake of radioactivity by the tumour was successfully inhibited by the pretreatment of unlabelled RC-160 (Fig. 4).

Discussion

Like octreotide, RC-160 is a somatostatin analogue with potent hormone secretion-inhibitory and antiproliferative characteristics in vivo and in vitro. However, discrepancies with octreotide have been described with regard to binding to a number of human cancers, such as breast, ovarian, exocrine pancreas, prostate, and colon carcinomas [21–23]. Because radiolabelled RC-160 could have advantages over radiolabelled octreotide for the in vivo detection of some of these somatostatin receptor-positive tumours, at first we evaluated radioiodinated RC-160 as a radiopharmaceutical. These studies revealed some major drawbacks of ^{123}I-RC-160 as compared to [^{123}I-Tyr^3]octreotide. The main disadvantage of ^{123}I-RC-160 was its relatively low tumour to background ratio, implying poorer in vivo tumour detection [30]. In analogy with the development of [^{111}In-DTPA-D-Phe^1]octreotide we therefore evaluated [^{111}In-DTPA-D-Phe^1]RC-160. Since for use in animal models no somatostatin receptor-positive tumours are available displaying binding specificity for RC-160 and not for octreotide, we investigated both somatostatin analogues in the CA20948 tumour model.

The radiolabelling of [DTPA-D-Phe^1]RC-160 with (high-quality) ^{111}In is a simple single-step procedure with a high efficiency of labelling (> 95%) and does not require special skills or equipment. We found no significant differences in biological activity between [^{115}In-DTPA-D-Phe^1]RC-160 and [^{115}In-DTPA-D-Phe^1]octreotide and the two non-labelled [DTPA-D-Phe^1]-somatostatin analogues, as measured by inhibition of rat growth hor-

mone secretion. Recently, however, we found that the non-DTPA-conjugated RC-160 was significantly more potent in inhibiting hormone release by normal and tumourous pituitary cells [37]. Therefore, a direct comparison between somatostatin analogues with their DTPA-conjugated counterparts should be made with care. The results in the binding studies demonstrated that both non-labelled [DTPA-D-Phe^1]-somatostatin analogues are high-affinity and selective ligands for the somatostatin receptor. This was also demonstrated in the in vivo experiments after injection of [^{111}In-DTPA-D-Phe^1]octreotide and [^{111}In-DTPA-D-Phe^1]RC-160, in which uptake and specific binding in somatostatin receptor-positive tissues and tumours were found. Also, the somatostatin receptor-positive tumours were clearly visualized by gamma camera scintigraphy. However, we did find differences in metabolism and clearance of radioactivity from the blood compartment, soft tissue and other somatostatin receptor-negative tissues. A higher radioactivity concentration and a slower clearance from these tissues were demonstrated for [^{111}In-DTPA-D-Phe^1]RC-160. The difference in uptake of radioactivity in the liver and excretion in the intestines after injection of [^{111}In-DTPA-D-Phe^1]RC-160 or [^{111}In-DTPA-D-Phe^1]octreotide was probably due to a difference in handling of the two analogues by the liver [38]. In the perfused rat liver uptake of radioactivity from the medium was 20% of the dose in the liver during the first hour of perfusion with [^{111}In-DTPA-D-Phe^1]RC-160, but 2% of the dose for [^{111}In-DTPA-D-Phe^1]octreotide. Less than 1% of the dose of both radiolabelled somatostatin analogues was excreted in the bile, where they were in the peptide-bound form.

The presence of the C-terminal amino acid tryptophan in RC-160 (see Fig. 1) enhances the lipophilicity of the molecule, and this may explain the enhanced uptake of [^{111}In-DTPA-D-Phe^1]RC-160 and/or its metabolites in the liver and its reduced clearance from tissues and blood. The ratios of tumour over blood and tumour over soft tissue for [^{111}In-DTPA-D-Phe^1]RC-160 are lower than those for [^{111}In-DTPA-D-Phe^1]octreotide. Although the uptake of radioactivity in the tumour after [^{111}In-DTPA-D-Phe^1]RC-160 experiments is higher, the clearance of radioactivity from the blood compartment and soft tissue is much slower than for [^{111}In-DTPA-D-Phe^1]octreotide. This implies a poorer visualization of tumours when the former somatostatin analogue is used. The lack of uptake in brain cortex suggests inability of both [DTPA-D-Phe^1]-somatostatin analogues to penetrate an intact blood-brain barrier. This contrasts with earlier data on RC-160 and radioiodinated RC-160 [28–30] and implies that [^{111}In-DTPA-D-Phe^1]RC-160 is most probably not suitable for visualizing human brain tumours with an intact blood-brain barrier. However, because mention has been made of somatostatin receptor-positive tumours with other subtypes which do not bind (labelled) octreotide, we want to hypothesize that [^{111}In-DTPA-D-Phe^1]RC-160 might be applicable in the field of nuclear medicine in the visualization of some human cancers. We currently investigate patients with a negative [^{111}In-DTPA-D-Phe^1]octreotide gamma camera scintigraphy who have tumours which do not bind octreotide, but belong to the group of tumours which Schally et al. suggested to have a high affinity for RC-160 [21–23]. The therapeutic possibilities of [^{131}I-Tyr^3]octreotide are low [7], since the residence time of ^{131}I in the tumour is short. This is because radioiodinated [Tyr^3]octreotide is rapidly metabolized with release of ^{131}I in the circulation, as has been demonstrated previously in rats [6, 30] and patients [7].

Since the residence time in/on the tumour cells of somatostatin analogues with a DTPA group appears prolonged, it seems worthwhile to investigate whether DTPA peptides labelled with ^{131}I or other β-emitting radionuclides would be suitable for radiotherapy. Therefore, [^{131}I-Tyr^3][DTPA-D-Phe^1]octreotide or [^{131}I-Tyr^3][DTPA-D-Phe^1]RC-160 could open new therapeutic applications for patients bearing somatostatin receptor-positive tumours. However, whether the residence time of the radioiodinated somatostatin analogues with a DTPA group is also prolonged needs to be established, since deiodination of the molecule will then hamper the therapeutic application.

In conclusion, [^{111}In-DTPA-D-Phe^1]RC-160 does not seem to have advantages over [^{111}In-DTPA-D-Phe^1]octreotide as a radiopharmaceutical for somatostatin receptor scintigraphy, despite the fact that [^{111}In-DTPA-D-Phe^1]RC-160 shows specific high-affinity binding to various somatostatin receptor-positive organs. In contrast to radioiodinated [Tyr^3]RC-160 and RC-160, which do pass the blood-brain barrier, our experiments show that [^{111}In-DTPA-D-Phe^1]RC-160 and [^{111}In-DTPA-D-Phe^1]octreotide do not pass the blood-brain barrier. In comparison with [^{111}In-DTPA-D-Phe^1]octreotide the main disadvantage of [^{111}In-DTPA-D-Phe^1]RC-160 (as we recently also showed for ^{123}I-RC-160) is its relatively low tumour to blood (background) ratio, implying poorer in vivo tumour detection. However, previous studies suggest that RC-160 might bind to several human cancer types which do not bind octreotride. If the existence of different somatostatin receptor subtypes is confirmed, RC-160 and, in spite of its disadvantages, [^{111}In-DTPA-D-Phe^1]RC-160 may open new diagnostic and/or therapeutic applications for patients bearing such tumours. Further studies in patients need to be performed, with special attention to patients with a negative [^{111}In-DTPA-D-Phe^1]octreotide gamma camera scintigraphy scan and/or patients with tumours with a higher affinity for RC-160. Since the DTPA group appears to prolong the residence time of somatostatin analogues in/on the tumour cells, it seems worthwhile to investigate whether DTPA peptides labelled with ^{131}I or other β-emitting radionuclides would be suitable for radiotherapy. Consequently, [^{131}I-Tyr^3][DTPA-D-Phe^1]octreotide or [^{131}I-Tyr^3][DTPA-D-Phe^1]RC-160 could open new therapeutic applications for patients bearing somatostatin receptor-positive tumours.

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