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Evaluation in vitro and in rats of ^{161}Tb-DTPA-octreotide, a somatostatin analogue with potential for intraoperative scanning and radiotherapy

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Abstract. The characteristics of terbium-161 diethylene triamine penta-acetic acid (DTPA) labelled octreotide with respect to specific binding to somatostatin (octreotide) receptors on rat brain cortex membranes, biological activity, uptake and excretion by isolated perfused rat livers and metabolism in vivo in normal and tumour-bearing rats were determined and compared to those of indium-111 DTPA-octreotide. The results of the binding studies demonstrate that ^{161}Tb-DTPA-octreotide is a high-affinity radioligand for somatostatin receptors, with an affinity comparable to that of ^{111}In-DTPA-octreotide. Rat growth hormone secretion inhibition experiments showed that ^{161}Tb-DTPA-octreotide has a similar potency to ^{111}In-DTPA-octreotide. ^{161}Tb-DTPA-octreotide appeared to be taken up even less by the isolated perfused rat liver than ^{111}In-DTPA-octreotide, as almost no tracer disappeared from the perfusion medium. Furthermore, hardly any radioactivity was found in the liver, and excretion into the bile was negligible. The biodistribution studies showed that for octreotide receptor-positive organs, such as pancreas and adrenals, uptake of ^{161}Tb-DTPA-octreotide is lower then that of ^{111}In-DTPA-octreotide. However, as the clearance from the blood of the former compound is faster than that of the latter, the tissue /blood ratio is higher in the case of ^{161}Tb-DTPA-octreotide than with ^{111}In-DTPA-octreotide. Furthermore, these studies demonstrated that the uptake of ^{161}Tb-DTPA-octreotide by the renal tubular cells after glomerular filtration can be reduced by administration of lysine or sodium maleate. Increase in urine production before and during the experiment had no effect on the kidney uptake of ^{161}Tb-DTPA-octreotide. Finally, it appeared that a maximal labelling efficiency of ^{161}Tb-DTPA-octreotide is essential, as with decreasing efficiency the uptake in the octreotide receptor-positive organs decreased, whereas non-specific uptake in the other organs was increased. It is concluded that, on the basis of the favourable physical characteristics of ^{161}Tb combined with the in vitro and in vivo studies performed with ^{161}Tb-DTPA-octreotide, the latter is a promising radiopharmaceutical for both intraoperative scanning and radiotherapy. Studies in patients need to be performed now to see whether ^{161}Tb-DTPA-octreotide can indeed open new therapeutic applications for patients bearing octreotide receptor-positive tumours.

Key words: Terbium-161 diethylene triamine penta-acetic acid labelled octreotide – Rats – Receptors binding – Liver perfusion – In vivo distribution

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Introduction

Indium-111 diethylene triamine penta-acetic acid labelled octreotide (OcreoScan) is a radiopharamaceutical that binds to the somatostatin receptor present in certain tissues. It is being used for scintigraphic imaging of somatostatin receptor-positive lesions, such as gastro-intestinal pancreatic tumours, neuroblastoma, phaeochromocytoma, breast cancer, Hodgkin's lymphoma and small cell lung cancer [1].

Since the availability of this and other radiolabelled somatostatin analogues for in vivo imaging, the range of diagnostic applications under study is rapidly increased. One example is the intraoperative scanning using gamma rays emitting radiolabelled somatostatin analogues. This technique is used to guide surgery with respect to the localisation of small somatostatin receptor-positive tumours using a small hand-held probe [2, 3]. Another field of application that is under examination is the potential use of radiolabelled peptide for radiotherapy.

However, for these two purposes, ^{111}In will not be the radionuclide of first choice. While its characteristics are favourable for scintigraphy (gamma rays of 174 and 247 keV), they are not optimal for intraoperative scanning because of the high background radiation due to the relatively hard gamma rays emitted by ^{111}In. Furthermore, for radiotherapy a beta particle-emitting radionuclide is more suitable.

We have now investigated whether DTPA-octreotide labelled with terbium-161 is suitable for the above-mentioned applications. The characteristics of ^{161}Tb are quite ideal for intraoperative scanning: low-energy gamma rays (with peaks of 46–48 and 74 keV) and a half-life of 6.91 days. As for the potential use for radiotherapy, ^{161}Tb emits beta rays of 135, 154 and 180 keV. Therefore, the characteristics of ^{161}Tb-DTPA-octreotide with respect to specific binding to somatostatin (octreotide) receptors on rat brain cortex membranes, biological activity, handling in isolated perfused rat livers and metabolism in vivo and normal and tumour-bearing rats were determined and compared to those of ^{111}In-DTPA-octreotide [4–6].

It appeared from our experiments that, like ^{111}In-DTPA-octreotide, ^{161}Tb-DTPA-octreotide was mostly cleared from the body via the kidneys. In previous studies, we found that in humans a significant amount of ^{111}In-DTPA-octreotide accumulates in the renal parenchyma (about 7% of the injected dose, 4 h after injections) because of re-uptake by the tubular cells after glomerular filtration [7]. This renal retention reduces both the scintigraphic sensitivity for detection of small tumours in the abdomen and the potential use of the radiolabelled peptide for radiotherapy [8]. Therefore, we recently performed studies to determine whether renal uptake of ^{111}In-DTPA-octreotide could be reduced by inhibiting tubular re-uptake in vivo in the rat [6]. In the present study, we tested the effects of increased urinary flow and administration of lysine (400 mg/kg) or of sodium maleate (400 mg/kg) on tubular reabsorption of ^{161}Tb-DTPA-octreotide in vivo in rats.

We also investigated the influence of the labelling efficiency of DTPA-octreotide with ^{161}Tb as well as of rat body mass (age) on organ distribution in rats.

Materials and methods

Radiolabelling and quality control of the radiopharmaceutical. [DTPA-D-Phe^1]octreotide and $^{161}TbCl_3$ were obtained from Mallinckrodt Medical B.V. (Petten, The Netherlands). The radiolabelling (with 4 MBq) of [DTPA-D-Phe^1]octreotide was performed essentially as described for ^{111}In-DTPA-octreotide [4], except that 20 µg DTPA-octreotide instead of 10 µg and acetate instead of citrate buffer were used. Only when labelling efficiency was ≥95% was the radiolabelled product used for experiments, unless otherwise stated. The ^{161}Tb was produced at the Free University of Brussels, Belgium, from gadolinium-160 that was irradiated with neutrons. The first product after this reaction, ^{161}Gd, decays to ^{161}Tb (half-life 3.6 min). The latter is chemically separated from Gd.

Highly purified and fatty acid-free bovine serum albumin (BSA; Boseral) was a product of Organon Teknika (Oss, The Netherlands). All other reagents were of the highest purity commercially available.

In vitro receptor binding studies. Receptor binding assays were carried out using ^{161}Tb-DTPA-octreotide as described previously for ^{111}In-DTPA-octreotide [4] using rat brain cortex membranes. Binding curves and IC_{50} for displacement of ^{161}Tb- DTPA-octreotide by unlabelled DTPA-octreotide or ^{159}Tb- DTPA-octreotide were calculated using the computer fitting program of Graphpad (ISI software, Philadelphia, Pa., USA).

Biological activity. The biological activity of stable ^{159}Tb- DTPA-octreotide was assessed by measuring its potency to inhibit the secretion of rat growth hormone (rGH) from rat pituitary cells as described previously [4].

Isolated perfused rat liver studies. Livers of male Wistar rats (200–250 g) were isolated and perfused in a recirculating system at 37° C as described previously. [9]. The magnetically stirred perfusion medium used in all experiments was 150 ml Krebs-Ringer buffer (118 mmol/l NaCl, 5 mmol/l KCl, 1.1 mmol/l $MgSO_4$, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l KH_2PO_4, and 25 mmol/l $NaHCO_3$) supplemented with 10 m*M* glucose and 1% BSA. The pH of the medium was maintained at 7.43 by gassing with carbogen (95% O_2 and 5% CO_2, 400 ml/min). The function of the liver was monitored by its outer appearance, measurement of hydrostatic pressure necessary to maintain a perfusion medium flow of 40 ml/min, bile flow, and pH of the perfusion medium. Livers were preperfused for 30 min. The experiment was started by addition of 370 kBq of tracer to the stirred medium in the central reservoir. Subsequently, 0.5 ml medium samples were taken at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50 and 60 min from a smaller medium reservoir, the height of which determines the hydrostatic pressure. Bile samples were collected at 10-min intervals. The samples were stored at –20° C until analysis. The chemical status of the radionuclide in medium and bile samples was analysed as a function of time using instant thin-layer chromatography (ITLC-SG, Gelman Sciences, Ann Arbor, Mich., USA) in 1 M acetate buffer (pH 5) (not shown).

Tissue distribution and specific binding of ^{161}Tb- DTPA-octreotide in normal and tumour-bearing rats. Normal male Wistar or Lewis rats or tumour-bearing male Lewis rats (200–250 g, unless otherwise stated) were placed in metabolic cages 24 h prior to the experiment. Tumour-bearing rats carried the transplantable rat pancreatic tumour CA20948, which has previously been shown to possess somatostatin receptors [10], in both upper hind legs. Metabolic cages were used to collect all (radioactive) urine produced during the experiment to follow the urinary clearance of the radioactive compound. The rats were fed with normal rat chow (Hope Farms, Woerden, The Netherlands) or with 35 g/day of rat chow suspended in water (40-g pellets in 100 ml water). Drinking water was always available ad libitum. At t=0 h, rats were injected with 0.2 MBq (0.5 µg) ^{161}Tb- DTPA-octreotide (radiochemical purity>95%) into the dorsal vein of the penis (injected volume 200 µl) during ether anaesthesia. The radioactivity was measured in a dose calibrator (VDC-202, Veenstra, Joure, The Netherlands). In order to determine non-specific binding of the radiopharmaceutical, rats were injected subcutaneously with 0.5 mg octreotide (Sandoz, Basel, Switzerland) in 1 ml 0.05 *M* acetic acid in 154 mmol/l NaCl, 40 min before injection of ^{161}Tb- DTPA-octreotide.

Twenty hours after injection of the radiolabelled product, rats were sacrificed (ether) and organs were isolated from the rats and subsequently analysed. The tissue distribution of the ^{161}Tb-labelled somatostatin analogue was studied by measurement of radioactivity in isolated organs as well as of blood samples, using a LKB-1282-Compu-gammasystem.

Possible renal re-uptake blockers tested. Lysine (400 mg/kg) and sodium maleate (400 mg/kg) were administered intravenously to the rats at physiological pH in volumes of 200 µl immediately before ^{161}Tb- DTPA-octreotide injection.

Statistical analysis. Results are expressed as mean±SD of groups of at least three rats. Statistical evaluation was performed using one-way analysis of variance followed by comparison among class means and Student's *t*-test, corrected for multiple pairwise comparisons between means.

Results

In vitro receptor binding studies

Rat brain cortex membranes were used to study the binding of ^{161}Tb-DTPA-octreotide to octreotide receptors in the presence of increasing amounts of ^{159}Tb-DTPA-octreotide or unlabelled DTPA-octreotide (Fig. 1a). The binding of ^{161}Tb-DTPA-octreotide decreased in the presence of increasing concentrations of both compounds, indicating that the binding process was saturable and specific. It is also shown that DTPA-octreotide competes somewhat better than ^{159}Tb-DTPA-octreotide. The IC_{50} values appeared to be in the nanomolar range, similar to that found for ^{111}In-DTPA-octreotide [4].

Biological activity

Figure 1b shows the effects of DTPA-octreotide and of ^{159}Tb-DTPA-octreotide on the secretion of rGH by cultured rat pituitary cells. The radiolabelled somatostatin analogue significantly inhibited rGH secretion in a dose-dependent manner, similar to ^{111}In-DTPA-octreotide [4].

Isolated perfused rat liver studies

Table 1 shows that after 1 h perfusion of isolated rat livers with ^{111}In-DTPA-octreotide, 2.3% of the dose was excreted in the bile, 1.7% was present in the liver, whereas 96% was still present in the perfusion medium. The data obtained with ^{161}Tb-DTPA-octreotide show even less liver clearance: after 60 min of perfusion, <0.1% of the dose was excreted in the bile, and 1.4% of the dose was present in the liver.

Tissue distribution and specific binding of ^{161}Tb-DTPA-octreotide in normal and tumour-bearing rats in vivo

In Table 2 organ distribution of ^{161}Tb-DTPA-octreotide and ^{111}In-DTPA-octreotide in normal rats is shown, expressed as % of the injected dose (ID), 20 h after administration. It was found that there were no significant differences in Lewis or Wistar rats with regard to distribution of ^{161}Tb-DTPA-octreotide (not shown). ^{161}Tb-DTPA-octreotide is cleared even faster from the blood than ^{111}In-DTPA-octreotide: 20 h after injection only 0.0004% ID was found in the blood (P<0.001 vs ^{111}In-

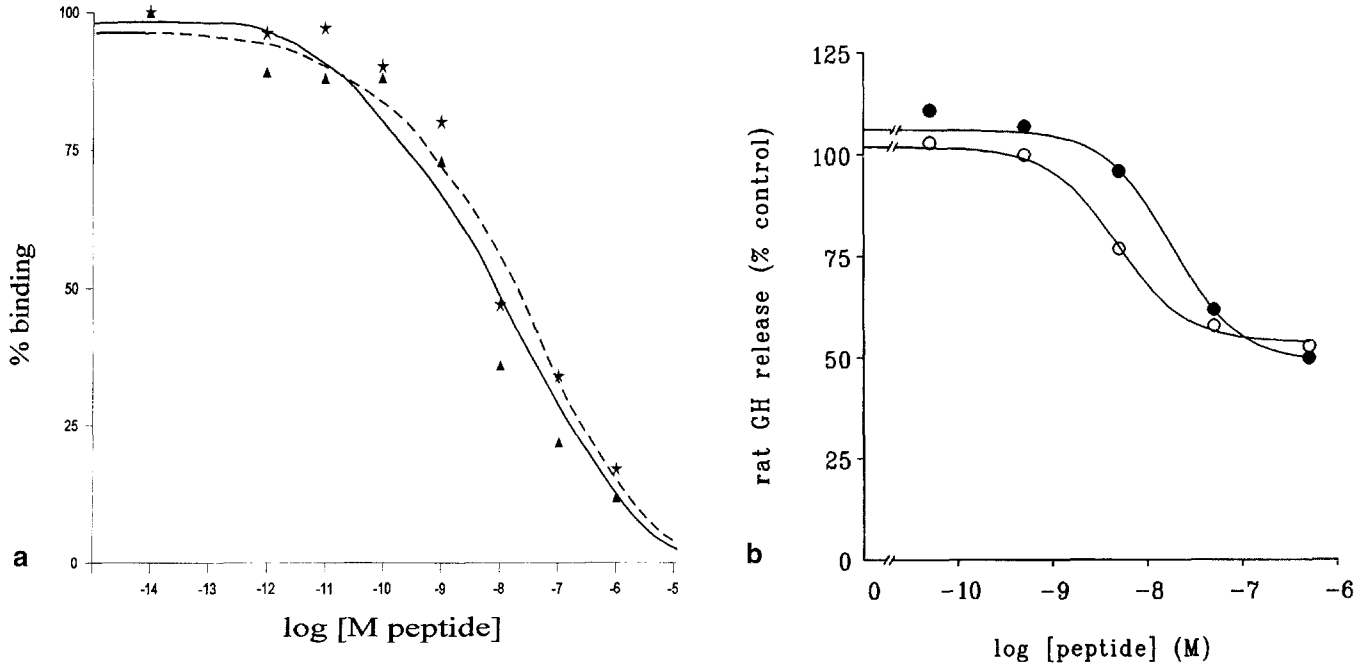

Fig. 1. a Binding of ^{161}Tb-DTPA-octreotide to rat brain cortex membranes in the presence of increasing concentrations of DTPA-octreotide (▲) or ^{159}Tb-DTPA-octreotide (★), expressed as the percentage of binding in the absence of competing compound. **b** Effects of DTPA-octreotide (○) and of ^{159}Tb-DTPA-octreotide (●) on secretion of rGH by cultured rat pituitary cells

Table 1. Radioactivity, expressed as % dose, in perfusion medium, bile and liver after 60 min of perfusion with ^{111}In-DTPA-octreotide (In-oc) or ^{161}Tb-DTPA-octreotide (Tb-oc)

	Medium	Bile	Liver
In-oc	95.9±0.9	2.3±0.2	1.7±0.2
Tb-oc	99.6±1.2*	<0.1*	1.4±0.1

* $P<0.001$ versus ^{111}In-DTPA-octreotide

Table 2. Distribution of 0.2 MBq (0.5 μg) ^{111}In-DTPA-octreotide and ^{161}Tb-DTPA-octreotide in several organs of control rats, expressed as % ID/g tissue, 20 h after administration

Organ	^{111}In-DTPA-octreotide (% ID/g)	^{161}Tb-DTPA-octreotide (% ID/g)
Blood	0.002±0.0003	0.0004±0.0001*
Kidneys	1.52±0.15	1.51±0.11
Liver	0.061±0.012	0.13±0.01*
Pancreas	0.52±0.12	0.23±0.03*
Spleen	0.03±0.005	0.02±0.001*
Adrenals	0.76±0.10	0.184±0.02*

* $P<0.001$ versus ^{111}In-DTPA-octreotide

DTPA-octreotide). Kidney uptake of ^{161}Tb-DTPA-octreotide and ^{111}In-DTPA-octreotide was similarly high (1.5% ID). Liver uptake of ^{161}Tb-DTPA-octreotide was higher than that of ^{111}In-DTPA-octreotide ($P<0.001$), whereas the reverse was true for spleen, pancreas, and adrenals ($P<0.001$), the latter two organs possessing octreotide receptors. Table 3 shows that uptake of ^{161}Tb-DTPA-octreotide and ^{111}In-DTPA-octreotide in pancreas and adrenals represents mostly specific binding to the

Table 3. Effect of pretreatment (40 min) with 0.5 mg unlabelled octreotide on the distribution of ^{161}Tb-DTPA-octreotide and ^{111}In-DTPA-octreotide in the pancreas and adrenals (organs containing somatostatin receptors) of normal rats, expressed as % of control (=same tracer without pretreatment with unlabelled octreotide)

Organ	^{111}In-DTPA-octreotide (% control)	^{161}Tb-DTPA-octreotide (% control)
Pancreas	1.92±0.20%*	4.34±0.18%*
Adrenals	1.63±0.22%*	4.52±0.84%*

* $P<0.001$ versus control

octreotide receptors, as uptake in these organs was decreased almost completely ($P<0.001$) by pretreatment of the rats with 0.5 mg unlabelled octreotide.

In Fig. 2 organ distribution of ^{161}Tb-DTPA-octreotide, with or without pretreatment with unlabelled octreotide, is depicted in tumour-bearing rats 20 h after administration of ^{161}Tb-DTPA-octreotide. The distribution in the tumour-bearing rats is the same as that seen in normal rats (Table 2). Uptake in colon, adrenals, pancreas, pituitary and tumours appeared to represent mostly specific binding to octreotide receptors, as a major decrease was observed after pretreatment with unlabelled octreotide ($P<0.001$).

Influence of renal re-uptake blockers

In Fig. 3 organ distribution of ^{161}Tb-DTPA-octreotide, 20 h after administration, is shown in the presence or absence of lysine (400 mg/kg) or sodium maleate (400 mg/kg), both potent blockers of the renal reabsorption process of ^{111}In-DTPA-octreotide in the proximal tubuli

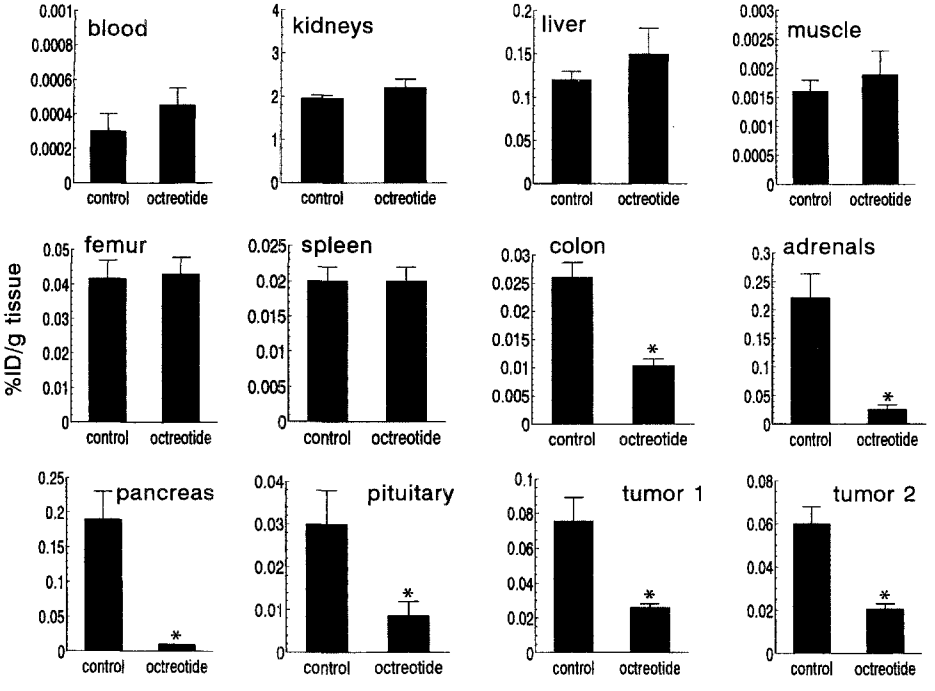

Fig. 2. Organ distribution of radioactivity [with (octreotide) or without (control) 40 min pretreatment with 0.5 mg unlabelled octreotide] in tumour-bearing rats, 20 h after administration of ^{161}Tb-DTPA-octreotide

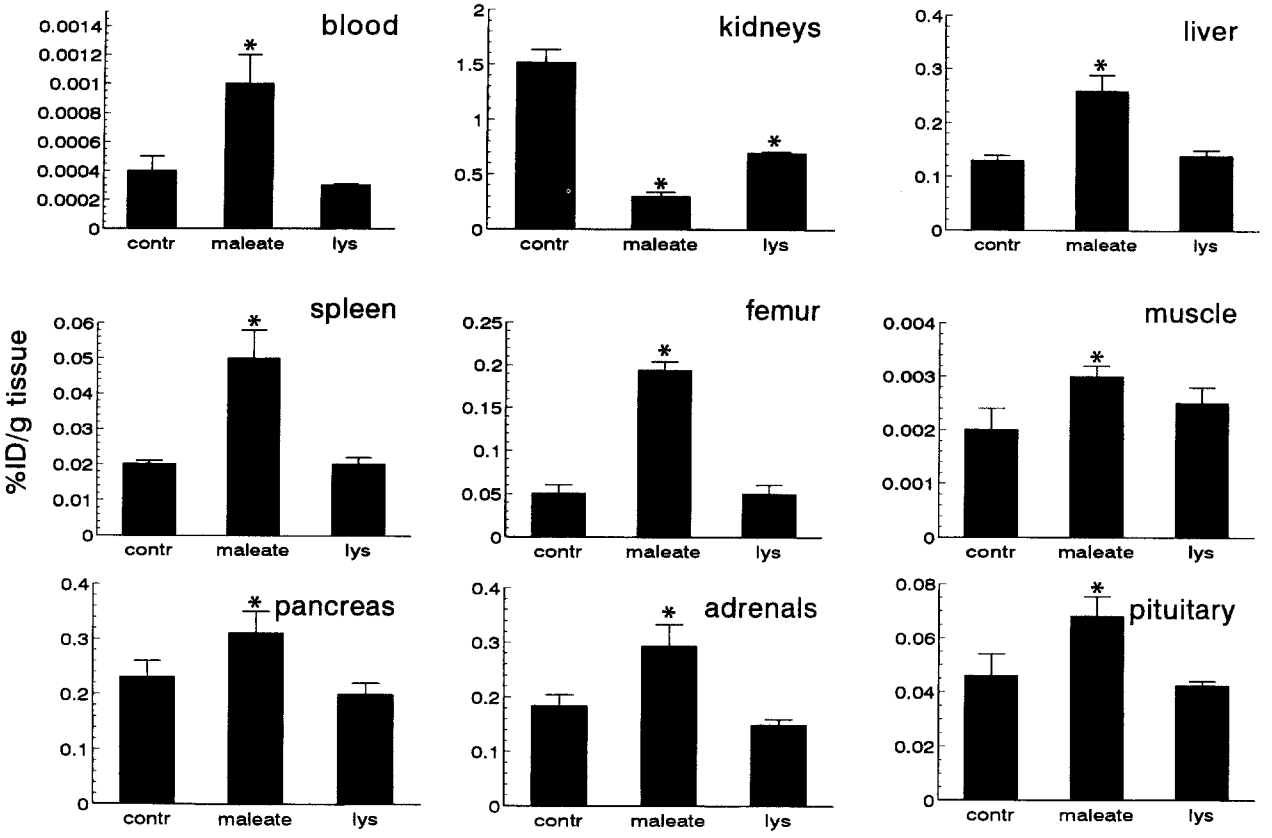

Fig. 3. Influence of sodium maleate (400 mg/kg) or lysine (*lys*; 400 mg/kg) on organ distribution of radioactivity in control rats, 20 h after administration of ^{161}Tb-DTPA-octreotide. * $P<0.001$ versus control

[6]. Also for ^{161}Tb-DTPA-octreotide, kidney uptake is inhibited significantly by both lysine and sodium maleate, the latter being about twice as potent as the former, showing that also ^{161}Tb-DTPA-octreotide is reabsorbed into the proximal tubular cells of the kidney after glomerular filtration. After administration of lysine, uptake of label in all other organs is not different from the control situation, whereas after sodium maleate administration uptake of ^{161}Tb-DTPA-octreotide is significanty increased compared to control ($P<0.001$).

In Fig. 4, the influence of urinary flow on tubular reabsorption of ^{161}Tb-DTPA-octreotide is depicted. It is shown that rats that eat dry rat chow (points on the left in the figure) produce less urine (range 5.1–7.5 ml/20 h) than rats that eat food suspended in water (points on the right in the figure; range 12.8–23.1 ml/20 h). However, this increase in urine production on the day of the experiment did not influence the amount of radioactivity in the kidneys. This was also true when the increase in urine production was induced by change in diet 48 h before the administration of ^{161}Tb-DTPA-octreotide (not shown).

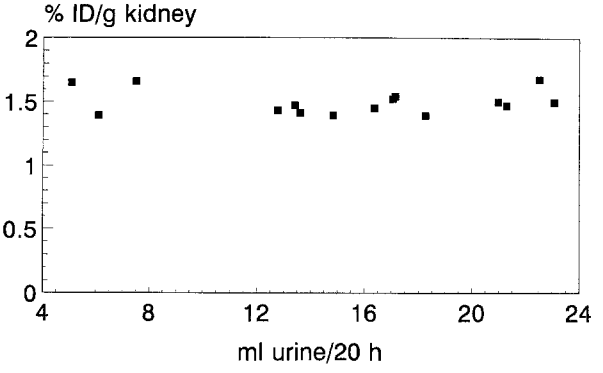

Fig. 4. Influence of urinary flow during the experiment on kidney uptake of radioactivity in control rats, 20 h after administration of ^{161}Tb-DTPA-octreotide

Influence of labelling efficiency on organ distribution of ^{161}Tb-DTPA-octreotide

In Fig. 5 it is shown that a maximal labelling efficiency is very important in achieving an optimal organ distribution of ^{161}Tb-DTPA-octreotide. A labelling efficiency of 100% means that all radioactivity administered to the rats was in the form of ^{161}Tb-DTPA-octreotide, whereas 0% means that it consisted of $^{161}TbCl_3$, in the absence of DTPA-octreotide. For organs not possessing octreotide receptors, such as liver, spleen, femur and muscle, the uptake increased significantly with decreasing labelling efficiency ($P<0.001$ vs 100% labelling). In the kidneys, uptake is about the same for all situations, showing that the terbium not bound to octreotide is taken up with about the same efficiency as ^{161}Tb-DTPA-octreotide.

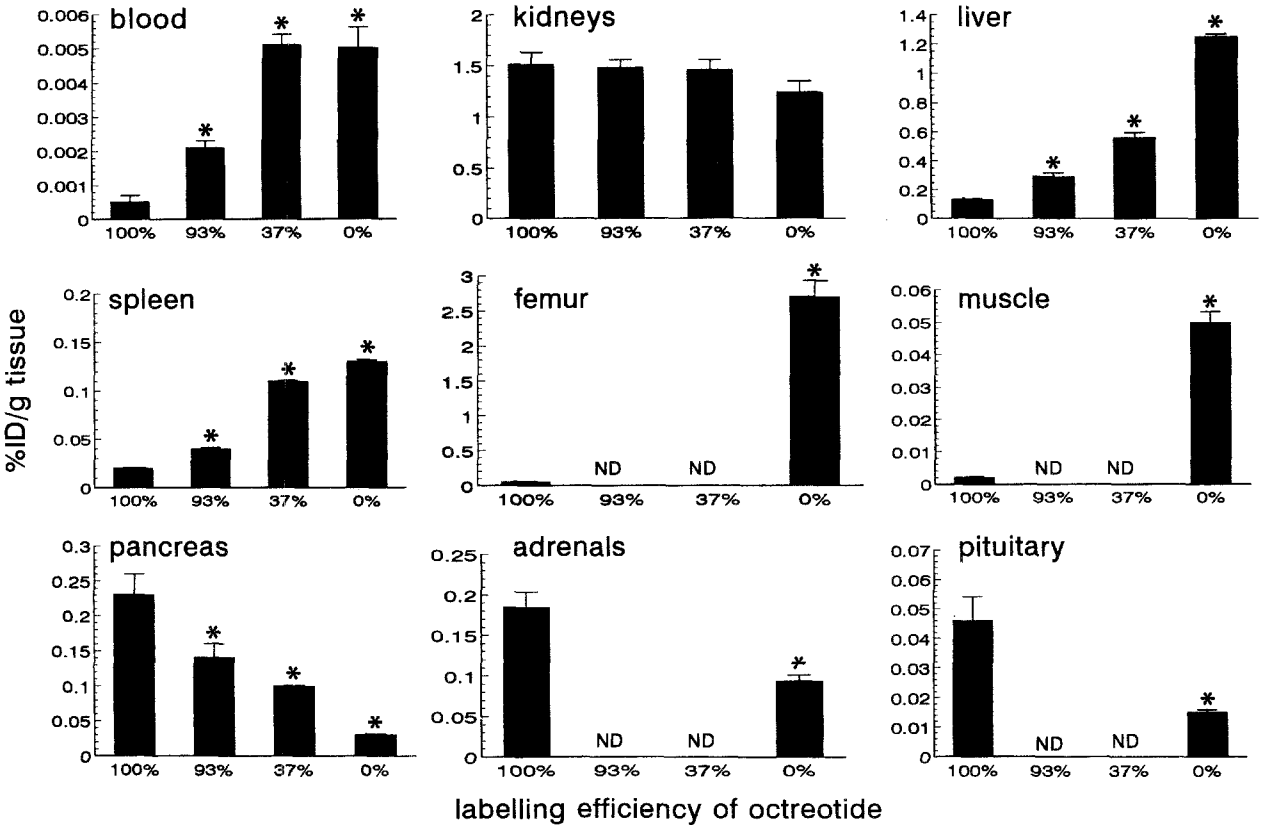

Fig. 5. Influence of labelling efficiency of ^{161}Tb-DTPA-octreotide on organ distribution in control rats, 20 h after administration of radiopharmaceutical. * $P<0.001$ versus control. *ND*, Not determined

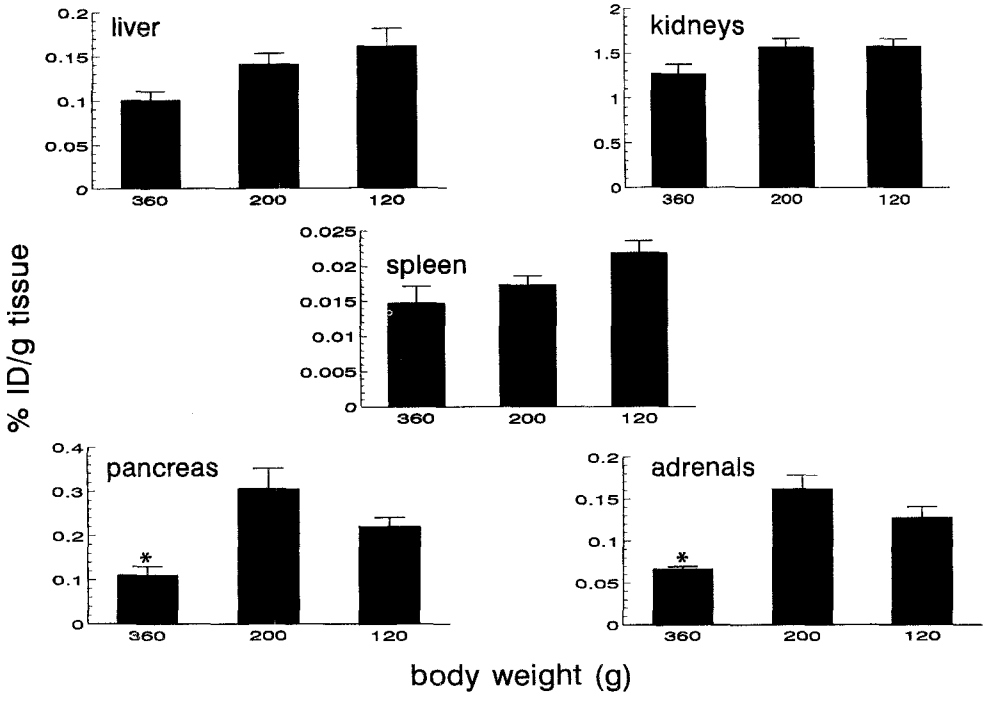

Fig. 6. Influence of rat body mass on organ distribution of radioactivity in control rats, 20 h after administration of ^{161}Tb–DTPA-octreotide. * $P<0.001$ versus control (=200-g group)

However, for octreotide receptor-possessing organs, such as pancreas, adrenals and pituitary, it holds that a decrease in labelling efficiency resulted in a significant decrease in uptake of ^{161}Tb-DTPA-octreotide in these organs.

Influence of body mass on organ distribution of ^{161}Tb-DTPA-octreotide

In Fig. 6 the influence of body mass on the organ distribution of ^{161}Tb-DTPA-octreotide is shown, 20 h after ad-

ministration. Three groups of rats with a body mass of 360, 200 and 120 g were tested. It is shown that no significant differences were found in the uptake of ^{161}Tb-DTPA-octreotide, expressed as % ID/g tissue, between the three groups with regard to liver, kidneys and spleen, organs that do not possess octreotide receptors. However, in pancreas and adrenals a significantly lower uptake was found in the 360-g group compared to the control (200-g) group.

Discussion

The results of the binding studies demonstrate that ^{161}Tb-DTPA-octreotide is a high-affinity radioligand for somatostatin receptors, with an affinity comparable to that of ^{111}In- DTPA-octreotide [4]. This was also demonstrated in the in vivo experiments in control and tumour-bearing rats after the injection of ^{161}Tb- DTPA-octreotide, in which uptake and specific binding in somatostatin receptor-positive tissues and tumour were found. Somatostatin receptors are structurally related integral membrane glycoproteins. Recently, five different somatostatin receptor types have been cloned. All subtypes bind native somatostatin-14 (SS_{14}) and SS_{28} (pro-somatostatin with 28 amino acids) with high affinity, while their affinity for numerous somatostatin analogues varies considerably [11–14]. Octreotide binds with high affinity to the SSTR2 (somatostatin receptor type 2) subtype, while this analogue has a relatively low affinity for SSTR3 and SSTR5 and shows no binding to SSTR subtypes 1 and 4 [11–14]. Octreotide scintigraphy is therefore based on the visualization of octreotide-binding somatostatin receptors (octreotide receptors), most probably the SSTR2.

Rat growth hormone secretion inhibition experiments showed that ^{161}Tb- DTPA-octreotide has a similar potency to ^{111}In- DTPA-octreotide [4].

The perfused rat liver is very suitable for the investigation of several parameters of liver uptake and excretion kinetics, such as the disappearance from the perfusion medium, appearance of degradation products and biliary excretion. ^{161}Tb- DTPA-octreotide was taken up less by the isolated perfused rat liver than was ^{111}In-DTPA-octreotide, as almost no tracer disappeared from the perfusion medium. Furthermore, hardly any radioactivity was found in the liver, and excretion into the bile was negligible. In order to find a tumour by intraoperative scanning in vivo, the specific activity expressed in counts per unit of area must exceed the local background radiation. As ^{161}Tb- DTPA-octreotide is not cleared via the liver and thus causes no accumulation of radioactivity in biliary and digestive tract, this radiopharmaceutical would be suitable for scanning of tumour receptor accumulation in the upper abdominal region, where many of the small endocrine gastro-entero-pancreatic target tumours are located.

However, the in vivo liver uptake is not in agreement with the liver perfusion studies; in vivo we find a higher liver uptake of ^{161}Tb- DTPA-octreotide than of ^{111}In-DTPA-octreotide, 20 h after administration. At present, the reason for this discrepancy between in vivo and in vitro findings is not clear and is currently being investigated. It could be explained by less stable intact ^{161}Tb-DTPA-octreotide after in vivo administration than ^{111}In-DTPA-octreotide, so degradation products of ^{161}Tb-DTPA-octreotide could cause the higher liver uptake found in vivo. However, the product is stable in serum for 24 h as measured with Sephadex PD-10 chromatography (not shown), so this explanation is not likely.

Our findings in Table 2 show that for octreotide receptor-positive organs, such as pancreas and adrenals, uptake of ^{161}Tb- DTPA-octreotide is lower than that of ^{111}In- DTPA-octreotide. However, as the clearance from the blood of the former compound is faster than that of the latter, the tissue/blood ratio is higher with ^{161}Tb-DTPA-octreotide than with ^{111}In- DTPA-octreotide. In the case of tumours this is a favourable situation for intraoperative scanning.

As for the high uptake in the kidneys: small peptides in the blood plasma are filtered through the glomerular capillaries in the kidneys and subsequently reabsorbed almost completely (≥90%) by the proximal tubular cells via carrier-mediated endocytosis. This is also the case for the radiolabelled octapeptides ^{111}In- DTPA-octreotide and ^{161}Tb- DTPA-octreotide. After the subsequent degradation process that takes place in the lysosomes of the tubular cells, their labelled degradation products are "trapped" in the lysosomes [15], causing a high dose of radioactivity in the kidneys. This study in vivo in rats demonstrates that the uptake of ^{161}Tb- DTPA-octreotide by the renal tubular cells after glomerular filtration can be reduced by administration of lysine or sodium maleate. As for lysine, the membranes of renal tubular cells contain negatively charged sites, to which positively charged residues of peptides or proteins are thought to bind [16]. An inhibition of this binding process may explain the effects of administration of the positively charged amino acid lysine on ^{111}In- DTPA-octreotide reuptake [6, 17, 18]. The most pronounced effect on tubular re-uptake of ^{161}Tb- DTPA-octreotide in this study was exerted by sodium maleate (Fig. 3). Sodium maleate has been used to create and study renal tubular dysfunction comparable to the human Fanconi's syndrome [19, 20], resulting in aminoaciduria and proteinuria. This compound forms maleyl-CoA by reacting with succinyl-CoA, thereby reducing the cellular CoA supply and inhibiting the citirc acid cycle in tubular cells [21]. The resulting reduced ATP supply or the reaction of the maleyl-CoA with membrane proteins may inhibit a variety of renal transport systems, including peptide re-absorption [21]. The inhibitory effect of sodium maleate, as found in this study, thus indicates that the re-absorption process of ^{161}Tb- DTPA-octreotide into the renal tubular cells is energy dependent. The increase in the uptake of ^{161}Tb- DTPA-octreotide in all organs by sodium maleate (except for the kidneys), as shown in Fig. 3, may be ex-

plained by the inhibitory effect of this compound on the renal glomerular filtration rate [22].

Increase in urine production before and during the experiment had no effect on the kidney uptake of ^{161}Tb-DTPA-octreotide (Fig. 4). The increase in urine production was induced by feeding the rats with chow suspended in water. However, re-uptake of peptides in the renal tubules is not influenced by an increased urinary flow through the kidneys. The results found here for ^{161}Tb-DTPA-octreotide are in agreement with those for ^{111}In-DTPA-octreotide [6].

Figure 5 shows that a maximal labelling efficiency of ^{161}Tb-DTPA-octreotide is essential, as with decreasing efficiency the uptake in the octreotide receptor-positive organs decreases, whereas non-specific uptake in the other organs is increased. Even when labelling efficiency was 93%, the effects on organ distribution were significantly different ($P<0.001$) compared with 100% labelling efficiency. This decreased specific binding in combination with increased non-specific binding is, or course, very unfavourable for both intraoperative scanning and radiotherapy.

The effect of rat body mass on ^{161}Tb- DTPA-octreotide organ distribution is shown in Fig. 6. As rats gain weight during their whole life, increase in body mass is paralleled by an increase in age and development. Since the same dose of ^{161}Tb- DTPA-octreotide was administered to the different groups (with widely different distribution volumes), it is not surprising that uptake in all organs studied is highest in the low-body-mass rats, while uptake is lowest is the high-body-mass animals. However, for adrenals and pancreas, both octreotide receptor-positive organs, the decrease in uptake in the 360-g group was much more pronounced than in the octreotide receptor-negative organs. This finding may point to developmental changes in expression of the octreotide receptor in these organs, as has been found for other somatostatin receptor-positive organs, such as the rat brain and the rat visual system [23, 24].

In conclusion: Based on the characteristics of ^{161}Tb (low-energy gamma rays, hard beta rays, and a half-life of nearly 7 days) combined with the in vitro binding studies, biological acticity, and in vivo organ distribution of ^{161}Tb- DTPA-octreotide, the latter may be considered a promising radiopharmaceutical for both intraoperative scanning and radiotherapy. Further studies in patients need to be performed now to see whether ^{161}Tb- DTPA-octreotide can indeed open new therapeutic applications for patients bearing octreotide receptor-positive tumours.

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